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Exploration of the contribution of isotope ratio mass spectrometry to the investigation of explosives : A study of black powders and ammonium nitrate fertilisers

Natacha Gentile

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Exploration of the contribution of isotope ratio mass spectrometry to the investigation of explosives

A study of black powders and ammonium nitrate fertilisers

Thèse de doctorat

Natacha Gentile

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IMPRIMATUR

A l'issue de la soutenance de thèse, le Jury autorise l'impression de la thèse de Mme Natacha GENTILE. candidate au doctorat en science forensique, intitulée

« Exploration of the contribution of isotope ratio mass spectrometry to the investigation of explosives : a study of black powders and ammonium nitrate fertilisers »

Le Président du Jury

Margot

Professeur Pierre Margot

Lausanne, le 18 décembre 2013

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Contents

List of abbreviations

% C	carbon content in weight %
% CR	percentage of samples correctly classified
% ER	percentage of samples wrongly classified
% FN	percentage of false negatives
% FP	percentage of false positives
% N	nitrogen content in weight %
% O	oxygen content in weight %
% TN	percentage of true negatives
% TP	percentage of true positives
N	number of measurements, including those with outlying values
A1	black powder control sample
AN	ammonium nitrate
AN std	ammonium nitrate standard
ANFO	ammonium nitrate fuel oil
APCI	atmospheric pressure chemical ionisation
ATF	Bureau of Alcohol, Tobacco, Firearms and Explosives
AUC	area under the curve
BP	black powder
BSIA	bulk stable isotope analysis
BTEX	benzene, toluene, ethylbenzene and xylene
CBD	cannabidiol
CBN	cannabinol
CDT	Canyon Diabolo Troilite
CE	capillary electrophoresis
CF	continuous flow
CRM	certified reference material
CS	control sample

CSI	crime scene investigation
CSIA	compound specific isotope analysis
DA	discriminant analysis
DCCNa	sodium dichloroisocyanate
DDT	deflagration to detonation transition
DI	dual inlet
DNA	deoxyribonucleic acid
DR	dynamic range
DSC	differential scanning calorimetry
EA	elemental analyser
EA-IRMS	elemental analysis isotope ratio mass spectrometry
ESI-MS	electrospray ionisation-mass spectrometry
EU	European Union
FBI	Federal Bureau of Investigation
FEL	Forensic Explosives Laboraltory
FIRMS	forensic isotope ratio mass spectrometry network Ltd
FTIR	Fourier transform infrared spectroscopy
GBL	gamma-butyrolactone
GC	gas chromatography
GC-C-IRMS	gas chromatography combustion isotope ratio mass spectrometry
GHB	gamma hydroxybutyric acid
HCA	hierarchical clustering analysis
HMX	high melting explosive; 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclohexane
HPLC	high-performance liquid chromatography
HR-ICP-MS	high resolution inductively coupled plasma mass spectrometry
HS-SDME	headspace single-drop microextraction
HTR	high temperature reduction
IAEA	International Atomic Energy Agency
IC	ion chromatography

IED	improvised explosive device
inter	intervariability
intra	intravariability
IR	infrared spectroscopy
IRA	Provisional Irish Republican Army
IRMM	Institute for Reference Materials and Measurements
IRMS	isotope ratio mass spectrometry
IRMS1	a Carlo Erba 1110 elemental analyser coupled with a Conflo II interface to a Delta S isotope ratio mass spectrometer
IRMS2	a Carlo Erba 1110 elemental analyser coupled with a Conflo II interface to a DeltaPlus XL isotope ratio mass spectrometer
ISO	International Organisation for Standardisation
IT	identical treatment
ITMS	ion trap-mass spectrometer
L5	ammonium nitrate fertiliser control sample
LA	laser ablation
LC	liquid chromatography
LD1	first discriminant function
LD2	second discriminant function
LDA	linear discriminant analysis
LR	likelihood ratio
LS	laboratory standard
LTR	low temperature reduction
MDA	3,4-methylenedioxyamphetamine
MDMA	3,4-methylenedioxy-N-methylamphetamine
MIX	an in-house working standard made of nitrate potassium and carbon
MS	mass spectrometry
MTBE	methyl tert butyl ether
n	number of measurements after exclusion of outliers
NIST	National Institute of Standards and Technology

ONUDC	United Nations Office on Drugs and Crime
OOB	out-of-bag
P2P	1-phenyl-2-propanone
PAH	polycyclic aromatic hydrocarbons
PC1	first principal component
PC2	second principal component
PCA	principal component analysis
PCB	polychlorinated biphenyls
PCR	polymerase chain reaction
PDB	Pee Dee Belemnite
PETN	pentaerythritol tetranitrate
PMK	piperonyl methyl ketone
PSI	Paul Scherrer Institut
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
Py	pyrolysis
Q-Q plot	quantile-quantile plot
R	R software
RBF	radial basis function
RDX	research department explosive; 1,3,5-trinitro-1,3,5-triazacyclohexane
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RF	random forests
ROC	receiver operating characteristics
SD	standard deviation
SEM-EDS	scanning electron-energy dispersive X-ray spectroscopy
SFE	supercritical fluid extraction
SLAP	Standard Light Antarctic Precipitation
SLE	supported liquid extraction

SNIF-NMR	specific natural isotope fractionation-nuclear magnetic resonance
SPE	solid phase extraction
SPME	solid phase micro extraction
Std1	Standard 1, an organic laboratory standard
Std2	Standard 2, an organic laboratory standard
STR	short tandem repeat
STU	scent transfer unit
SVMs	support vector machines
TATB	1,3,5-triamino-2,4,6-trinitrobenzene
TATP	triacetone triperoxide
TC	thermal conversion
THC	δ-9-tetrahydrocannabinol
TLC	thin layer chromatography
TNT	2,4,6-trinitrotoluene
TOF-SIMS	time-of-flight secondary ion mass spectrometry
TWGFEX	technical working group of fire and explosives
UK	United Kingdom
UN	urea nitrate
USA	United States of America
USGS	United States Geological Survey
UVF	Ulster Volunteer Force
VBIED	vehicle-borne improvised explosive device
VSMOW	Vienna Standard Mean Ocean Water
WFD	Wissenschaftlicher Forschungsdienst
WS	working standard
XRD	X-ray diffraction

Résumé

Le nombre croissant d'attentats à la bombe impliquant des engins explosifs artisanaux, ainsi que la nature des charges explosives, constituent une préoccupation majeure pour les autorités d'application de la loi et les organismes de sécurité. Les substances utilisées dans les charges explosives sont souvent des produits du quotidien, détournés de leurs applications licites. Par conséquent, réduire ou limiter l'accessibilité de ces produits dans un but de prévention est difficile. Le nitrate d'ammonium, employé dans l'agriculture comme engrais, est utilisé dans des petits et grands engins explosifs artisanaux. La poudre noire, initialement dédiée à la chasse et au tir sportif, est fréquemment utilisée comme charge explosive dans les *pipe bombs*, qui causent des dommages importants.

Si les développements des techniques d'analyse des explosifs n'ont cessé de repousser les limites de détection, leur contribution réelle à l'investigation des explosifs est limitée en termes de discrimination de sources. Une nouvelle technologie qui donne des résultats prometteurs a fait son apparition en science forensique: la spectrométrie de masse à rapport isotopique (IRMS). Sa première application en science forensique remonte à 1979. Liu *et al.* ont analysé des plants de cannabis provenant de différents pays [Liu *et al.* 1979]. Cette étude préliminaire, basée sur quelques analyses, a mis en évidence le potentiel de l'IRMS à discriminer des spécimens provenant de sources différentes. Trente ans plus tard, l'intérêt marqué pour cette nouvelle technologie en science forensique se traduit par un nombre florissant de publications. Les innombrables applications de l'IRMS à une large gamme de matériaux et de substances attestent de son succès et suggèrent que la technique est prête à être utilisée en science forensique. Cependant, de nombreuses études sont caractérisées par un manque de méthodologie et de données fondamentales. Elles ont été menées sans définir les hypothèses de recherche et en appliquant cette technique de façon exploratoire sur un échantillonnage restreint. Cette manière de procéder ne permet souvent pas au chercheur de répondre à un certain nombre de questions, tels que: est-ce que deux spécimens proviennent de la même source, qu'entend-on par source ou encore quelle est l'intravariabilité d'une substance? La production de résultats positifs a prévalu au détriment des fondamentaux de science forensique.

Cette recherche s'est attachée à évaluer la contribution réelle de l'information isotopique dans les investigations en matière d'explosifs. Plus particulièrement, cette évaluation s'est basée sur un échantillonnage constitué de poudres noires et d'engrais à base de nitrate d'ammonium provenant de sources connues. La méthodologie développée dans ce travail a permis non seulement de mettre en évidence des éléments cruciaux relatifs à la méthode d'analyse elle-même, mais également d'évaluer la variabilité de l'information isotopique d'un point de vue longitudinal et transversal. Dans un premier temps, l'évolution du profil en fonction du temps a été étudiée. Dans un second temps, la variabilité du profil des poudres noires et des engrais à base de nitrate d'ammonium au sein d'une même source et entre différentes sources a été évaluée. La contribution de cette information dans le cadre des investigations d'explosifs a ensuite été discutée et évaluée.

Le premier chapitre fournit un cadre de connaissances générales sur les engins explosifs artisanaux. Le deuxième chapitre présente les deux substances d'intérêt de ce travail, à savoir les poudres noires et les engrais à base de nitrate d'ammonium, leur origine, fabrication et caractéristiques. L'analyse des explosifs inorganiques et ses limitations actuelles sont présentées dans le chapitre 3. Le chapitre 4 présente les principes de la spectrométrie à rapport isotopique et fournit un état de l'art de ses applications en science forensique. Le cinquième chapitre expose le but de la recherche et les détails relatif au matériel et aux méthodes. Le chapitre 6 présente l'évaluation des méthodes analytiques et met en lumière des éléments fondamentaux. La stabilité du profile isotopique en fonction des conditions environnementales est présentée et discutée dans le chapitre 7. Les résultats sur la discrimination des sources de poudres noires sont exposés dans le chapitre 8. Le chapitre 9 présente les résultats concernant la discrimination de sources des engrais à base de nitrate d'ammonium. Le chapitre 10 offre une discussion générale sur les résultats et la contribution aux investigations en matière d'explosifs. Finalement, une conclusion sur le travail est fournie dans le chapitre 11.

Foreword

The increasing number of bomb attacks involving improvised explosive devices, as well as the nature of the explosives, give rise to concern among safety and law enforcement agencies. The substances used in explosive charges are often everyday products diverted from their primary licit applications. Thus, reducing or limiting their accessibility for prevention purposes is difficult. Ammonium nitrate, employed in agriculture as a fertiliser, is used worldwide in small and large homemade bombs. Black powder, dedicated to hunting and shooting sports, is used illegally as a filling in pipe bombs causing extensive damage.

If the main developments of instrumental techniques in explosive analysis have been constantly pushing the limits of detection, their actual contribution to the investigation of explosives in terms of source discrimination is limited. Forensic science has seen the emergence of a new technology, isotope ratio mass spectrometry (IRMS), that shows promising results. Its very first application in forensic science dates back to 1979. Liu et al. analysed cannabis plants coming from different countries [Liu *et al.* 1979]. This preliminary study highlighted its potential to discriminate specimens coming from different sources. Thirty years later, the keen interest in this new technology has given rise to a flourishing number of publications in forensic science. The countless applications of IRMS to a wide range of materials and substances attest to its success and suggest that the technique is ready to be used in forensic science. However, many studies are characterised by a lack of methodology and fundamental data. They have been undertaken in a top-down approach, applying this technique in an exploratory manner on a restricted sampling. This manner of procedure often does not allow the researcher to answer a number of questions, such as: do the specimens come from the same source, what do we mean by source or what is the inherent variability of a substance? The production of positive results has prevailed at the expense of forensic fundamentals.

This research focused on the evaluation of the contribution of the information provided by isotopic analysis to the investigation of explosives. More specifically, this evaluation was based on a sampling of black powders and ammonium nitrate fertilisers coming from known sources. Not only has the methodology developed in this work enabled us to highlight crucial elements inherent to the methods themselves, but also to evaluate both the longitudinal and transversal variabilities of the information. First, the study of the variability of the profile over time was undertaken. Secondly, the variability of black powders and ammonium nitrate fertilisers within the same source and between different sources was evaluated. The contribution of this information to the investigation of explosives was then evaluated and discussed.

The first chapter provides a general framework of knowledge on improvised explosive devices. The second chapter presents the two substances of interest of this work, black powders and ammonium nitrate fertilisers, their origin, manufacture and characteristics. The forensic analysis of inorganic explosives and its current limitations are presented in Chapter 3. Chapter 4 introduces the principles of isotope ratio mass spectrometry and provides a review of its applications in forensic science. The fifth chapter exposes the purpose of the research and details the materials and methods. Chapter 6 introduces the evaluation of the methods and highlights fundamental elements. The stability of the isotopic profile according to environmental conditions is presented and discussed in Chapter 7. The results about the discrimination of sources of black powders are exposed in Chapter 8. Chapter 9 presents the results concerning the source discrimination of ammonium nitrate fertilisers. Chapter 10 discusses the results and the contribution to the investigation of explosives. Finally, a general conclusion of the work is provided in Chapter 11.

Chapter 1

Improvised explosive devices

Contents

1 General notions

1.1 What is an explosive substance?

The limit between explosive and non explosive substances is not evident [Médard 1987]. Depending on the jurisdiction, the legal definition of explosive and explosion may be significant. The Swiss legislation defines explosives very broadly, based on the criterion of danger and destructive power. This general approach allows a better and more thorough integration of new substances with explosive properties into law and, more particularly, improvised mixtures that include ingredients whose use is legal.

1.1.1 Legal framework

In Switzerland, explosive substances are regulated by the Federal Act of 25 March 1977 on the Explosible Substances and the Ordinance of 26 March 1980 on the Explosible Substances. Both legislations govern the law enforcement applications, trade and transport of explosive substances and pyrotechnical devices, their use and destruction, as well as the security norms concerning their handling, storage and transportation.

According to article 4 of Federal Act on the Explosible Substances, explosive materials include explosives as well as means of ignition: slow-burning fuses, detonators, distant ignition wires and detonating cords are thus considered as explosives.

Explosives are defined broadly by article 5 of this Act (in effect as of 12 December 2008) as[1]:

> "des composés chimiques purs ou des mélanges de tels composés dont l'explosion peut être provoquée par allumage, par action mécanique ou d'une autre manière et qui, même en quantité relativement faible, sont dangereux en raison de leur pouvoir destructif, soit en charge libre, soit après bourrage.
>
> Ne sont pas considérés comme explosifs :
>
> a. les gaz explosibles, les vapeurs de combustibles liquides et les autres substances qui n'explosent qu'après avoir été mélangées avec de l'air;
>
> b. les adjuvants utilisés dans la fabrication des produits chimiques ou les produits en cours d'élaboration présentant un risque d'explosion, qui est toutefois éliminé avant l'achèvement de la fabrication;
>
> c. les produits et les préparations explosibles fabriqués et mis dans le commerce à d'autres fins qu'à des tirs de mines."

Thus, although black powder and ammonium nitrate fertilisers fulfil the definition of an explosive given by article 5, their original application (shooting for black powder and promoting plant growth for fertiliser) is legal according to the paragraph c of article 5. Nonetheless, the use of one of these substances as an explosive—for example the manufacture of a pipe bomb to threaten its neighbour or the use of

---

[1]

> (free translation) pure chemical compounds or mixtures of such compounds, whose explosion may be caused by ignition, mechanical action or any other means, and which, even in relatively small quantities, are dangerous because of their destructive power, either as a free charge or after filling.
>
> Are not considered as explosives:
>
> a. explosible gases, vapours of liquid fuels and other substances which explode only when mixed with air;
>
> b. adjuvants used in the manufacture of chemicals or intermediate products involving a risk of explosion, which is however removed before the completion of manufacture;
>
> c. explosible products and preparations manufactured and traded for other purposes than blasting.

ammonium nitrate fertiliser mixed with fuel by a farmer to blow up a structure—is illegal.

Finally, the legislation delineates another category: pyrotechnic devices. They are defined by article 7 as[2]:

> "des produits prêts à l'emploi, comprenant un élément explosif ou un dispositif d'allumage, qui
>
> d. ne servent pas à des fins de destruction, mais à d'autres fins d'ordre industriel, technique ou agricole, tels que moyens de signalisation, fusées météorologiques, cartouches servant à la soudure ou à la trempe des métaux, ou
>
> e. sont destinés au simple divertissement comme les pièces d'artifices."

From a legislative perspective, primary explosives or initiating materials, which are extremely sensitive and powerful—such as mercury fulminate, lead azide—and explosive elements used in the manufacture of ammunition and pyrotechnics all belong to this designation. The notion of explosives includes both pure explosives such as nitroglycerin, pentaerythritol tetranitrate (PETN), as well as mixtures of explosives, including black powder, nitroglycerin, ammonium nitrate or chlorate based explosives [Wissenschaftlicher Forschungs Dienst 1990].

1.1.2 Chemical conditions

From a chemical point of view, an explosive substance is a system which, under the influence of an external stimuli (mechanical shock, friction, mechanical or electrical spark, or heat shockwave) is likely to undergo a chemical decomposition, releasing large volumes of hot gas at high temperature in a very short time [Vennin & Chesneau 1914, Médard 1987, Quinchon 1987]. This reaction of decomposition is generally exothermic. Explosive substances contain chemical groups called explosophores, which produce energy and gaseous reaction products [Calzia 1969, Bircher 2004].

The explosive character is closely related to the nature of the chemical bonds, the composition and arrangement of the atoms in a molecule. Among others the electronic structure and the electronegativity of the atoms defines the chemical

---

[2]

> (free translation) products ready for use, including an explosive or an ignition device, which
>
> a. are not used for destruction purposes but for other industrial, technical or agricultural purposes, such as signalisation means, meteorological rockets, cartridges used for soldering or hardening of metals, or
>
> b. are intended for entertainment such as fireworks.

stability of a bond. In order to release a large amount of energy, oxygen must be present in the molecule in a form by which it is attached to another atom than carbon or hydrogen [Yallop 1980, Médard 1987]. One of the most frequent explosophore groups in civil and military explosives is the nitro group —NO2. It is divided into sub-groups according to the atom to which it is attached to (nitro-compounds —C—NO_2 ; nitrate esters compounds —O—NO_2; nitramine group —N—NO2). Other groups known for their explosive properties are [Calzia 1969, Yinon & Zitrin 1993]:

- chlorates and perchlorates -O—Cl;
- peroxides and ozonides -O—O—;
- diazoic derivatives N = N and azides N≡N;
- chloramine-N—Cl;
- fulminates C = N and cyanic compounds C≡N, and
- acetylides C≡C.

An explosive substance is composed of an oxidiser (oxidising agent) and a fuel (reducing agent). Organic explosives contain both the oxidiser and the fuel jointly in the same molecule. On the contrary, in an inorganic composition, the oxidiser and the fuel are separate molecules and are brought together to form an explosive mixture (for example, the well known association of chlorate (carrying oxygen) and sugar (fuel)).

1.2 What is an explosion?

Médard (1987) defines the explosion as[3]:

> "une transformation rapide d'un système matériel, donnant lieu à une forte émission de gaz ; ceux-ci, s'ils se forment à l'air libre en un temps très court ou s'ils sont produits en vase clos, sont sous une pression plus ou moins élevée et peuvent, en se détendant produire des effets mécaniques divers, et en particulier un bruit plus ou moins fort[4]".

In theory, one distinguishes homogeneous and heterogeneous explosions. A homogeneous explosion simultaneously affects the entire mass. At any point of the system, the temperature is uniform and the reaction rate is identical. In practice, since the reaction is not instantaneous, all explosions are heterogeneous: initiated in one point, the reaction of decomposition takes place in a very thin area (fractions of

---

[3] (free translation) a rapid transformation of a material system, giving rise to a strong emission of gas; these, if they are formed in a very short time in a confined or unconfined environment, are under a more or less elevated pressure and can produce, through their expansion, various mechanical effects, and particularly, a more or less loud sound.

[4] Sound is however not an essential characteristic of the explosion.

a millimeter) separating the volume that has not reacted yet (initial state) from the one where the reaction products are at high temperature (final state). This reaction propagates as a wave from one to another, without using external oxygen [Médard 1987].

There are two types of heterogeneous explosion: deflagration and detonation. They can be differentiated by their propagation speed and the transport mode of the reaction wave[5].

1.2.1 Deflagration

Deflagration is a rapid combustion reaction, whose propagation speed is smaller than 1000 m/s. This speed is dependent on the pressure exerted by the combustion gas on the original system [Quinchon 1987].

The chemical reaction of explosive decomposition is slower than the thermal transfer. The explosion is transmitted from one particle to neighbouring particles through thermal conduction, convection and radiation. Within the reaction zone, the specific volume, the pressure and the temperature vary continuously, from the initial region (with material which has not yet reacted) to the final region (containing the reaction products) [Calzia 1969].

A pressure wave propagating in the environment may create more or less important mechanical effects. The reaction products have a propagation direction opposed to the direction of travel of the wave [Calzia 1969, Médard 1987].

1.2.2 Detonation

Detonation generally propagates at a speed between 1'000 and 10'000 m/s. The propagation velocity of detonation, constant in a given medium, is characteristic of each explosive system[6] and can be tabulated. Indeed, it only depends on the volumic mass of the explosive [Quinchon 1987].

The chemical reaction of decomposition, which involves a rearrangement of the chemical bonds, is faster than the thermal transfer itself and leads to the development of a shock wave. The shock wave, through which the specific volume, the pressure and temperature are strongly discontinuous, transmits the explosion into the explosive material [Calzia 1969, Médard 1987]. The explosion results from the sudden decomposition of a small quantity of explosive, due either to a localised shock or a rapid heating to the point of decomposition. This causes an instantaneous release of gas, which abruptly compresses the adjacent layer of explosive and operates as a new shock gradually reproducible [Vennin & Chesneau 1914, Quinchon 1987].

---

[5] Deflagration to Detonation Transition (DDT) is an intermediate phenomena. Under certain conditions, the reaction rate of a deflagration can accelerate and turn into detonation.

[6] For example, the reaction rate of 2,4,6-trinitrotoluene (TNT) is 6970 m/s [Federoff *et al.* 1962].

The shock wave is transmitted to the surrounding environment with significant breaking effects and is able to initiate further charges at distance. The degradation products are propagated in the same transport direction as the wave [Calzia 1969].

1.3 What is an improvised explosive device (IED)?

There are different classification systems of explosives, which offer a categorisation by their chemical function or between primary and secondary explosives. Other classifications distinguish powder from explosives, or pure substance from mixtures[7] [Calzia 1969, Yallop 1980, Quinchon 1987, Akhavan 2004]. Another categorisation based on the use of the explosive distinguishes three broad classes: military, civil and non-conventional explosives. For the purpose of the present description, this categorisation will be retained and further developed.

1.3.1 Military explosives

Military explosives encompass nitro explosives (pentaerythritol tetranitrate (PETN) or 2,4,6-trinitrotoluene (TNT)) as well as pyrotechnic or propulsion systems[8]. These explosives are used by all armed services, the army, navy and air force and are not sold commercially. Their access is limited and military storage areas are generally secured. Their synthesis from chemical precursors is also possible but it requires extensive knowledge of chemistry and usually requires several steps [Wissenschaftlicher Forschungs Dienst 1990, Les Nations Unies 2002]. However, a supply is possible through the recovery of surplus in post-conflict environments or through the black market. In Israel, Iraq, Afghanistan or former Yougoslavia, for example, thefts from stockpiles of military explosives are the main source of explosives used illegally in improvised explosive devices [National Research Council 1998b, Wilkinson *et al.* 2008]. In parallel, the existence of explosives traffics and their relations with networks of illicit drugs and arms traffics have been demonstrated [Les Nations Unies 2002].

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[7] There is however a classification mode that needs to be used with caution, which differentiates low and high explosives. Although widely used, it does not constitute an absolute cataloguing. Many parameters govern the decomposition of explosives: confinement, particle size, air gaps between the grains of powder are all parameters that can affect the reaction rate. For example, some works consider smokeless powders as low explosives. Nevertheless, depending on the conditions of confinement and the initiator system used, the charge may undergo detonation [Vennin & Chesneau 1914, Calzia 1969, Beveridge 1998, Oxley *et al.* 2001]

[8] Among the most popular are single-base powders (nitrocellulose), double-base powders (nitrocellulose and nitroglycerin).

1.3.2 Civil explosives

Civil explosives are mainly used in mining and construction. Over the last fifty years, the market of these explosives has fundamentally changed. Dynamite, which was commonly used for destruction works has been replaced by explosives based on ammonium nitrate, such as ammonium nitrate fuel oil (ANFO) or, more recently, by emulsion and water gel explosives [Peterson *et al.* 1983, Bender *et al.* 1993, Midkiff & Walters 1993, National Research Council 1998b]. Dynamites are however still employed in some countries, such as Spain. In Switzerland, explosives dedicated to civil use are sold in specialty stores and are delivered to the buyer only upon presentation of an acquisition permit [Wissenschaftlicher Forschungs Dienst 1990]. Due to their short shelf life, manufacturers and sellers store relatively small stocks of civil explosives. The risks of theft or misappropriation are therefore reduced [Wissenschaftlicher Forschungs Dienst 1990, United Nations 1999, Les Nations Unies 2002]. Moreover, trade regulation laws on civil explosives are very strict in some countries, like Great Britain, Northern Ireland or Israel; the attacks involving such explosives are exceptional. Smuggling remains nevertheless a potential source of supply [National Research Council 1998b].

1.3.3 Non-conventional explosives

Besides these two vast families of explosives, literature and practice defines non-conventional explosives, also known as “Improvised Explosive Devices” (IEDs). The North Atlantic Treaty Organization broadly defines an improvised explosive device as [North Atlantic Treaty Organization 2010]:

> "A device placed or fabricated in an improvised manner incorporating destructive, lethal, noxious, pyrotechnic or incendiary chemicals and designed to destroy, incapacitate, harass or distract. It may incorporate military stores, but is normally devised from non-military components."

The improvised nature of a device involves the fabrication or modification, either of all the components of a device, or only a part of them. In post-conflict countries, where countless ammunition and explosive stockpiles are left unsecured, these conventional military explosives are adapted to constitute new IEDs [Wilkinson *et al.* 2008]. In other countries, because the accessibility to traditional explosives is more difficult, the explosive charge is very often homemade. Detonators or hand held igniters are the most difficult compounds of the bomb to acquire. They can also be homemade—recipes are manifold—yet the danger associated with their handling considerably limits their implementation. Some powerful organic explosives, such as PETN or RDX (research department explosive; 1,3,5-trinitro-1,3,5-triazacyclohexane), are sometimes artisanal. However, the complexity of the synthesis of these explosives, combined with the controlled access to the necessary chemical precursors, makes their domestic manufacture more occasional [National Research Council 1998b, Les Nations Unies 2002]. The peroxide-based explosives are however an exception. An example is triacetone triperoxide (TATP), an unpredictable explosive, whose sensitivity and power have often been the cause of serious accidents. TATP is a primary explosive and can be synthesised from readily available starting materials, using recipes freely available on the Internet. This is probably at the origin of the increase of cases in the 90's[9] [Marret 2006]. Other organic substances, such as single-base (nitrocellulose) and double-base propellants (nitrocellulose and nitroglycerin), are available in armourers and, as a consequence of their accessibility, are frequently used as fillers in pipe bombs.

Inorganic explosive charges, made from the physical mixture of an oxidiser and a fuel, are frequently found in explosive attacks. Among the most common are the association between alkali chlorate and sugar or between acid and aluminium [McPherson 1979, Wissenschaftlicher Forschungs Dienst 1990, Calisti 1997, Grau 2005]. It also includes ready-to-use compositions which are easily diverted from their legal use. It is particularly the case of pyrotechnic compositions[10], present in signalling devices or fireworks [Conkling 1985].

This research is focused on the investigation of inorganic explosive charges. Therefore, improvised charges made from organic explosives such as smokeless powders or TATP will not be further considered in this work.

The state of the art and the definition of the research problem that follow are orientated towards the inorganic explosive charges that can be easily made from everyday products.

2 Statistical overview

By nature, acts involving the illicit use of explosives are a highly sensitive issue, often connected—truly or not—to terrorism. As a result, public statistics and information about explosive devices are rare. In addition to the absence of national centralised databases, the lack of relevant and statistically valid data as well as exchange of information on this subject were, among others, observed by the United Nations and the National Research Council [National Research Council 1998b, United Nations 1999].

As stated by another study of the United Nations on the manufacture and

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[9] Peroxide-based explosives were in particular implemented in the London bombings in July 2005. TATP was also found in the shoe of Richard Reid in December 2001, a British who had planned to blow up a plane travelling from Paris to Miami.

[10] A pyrotechnic composition is a mixture which produces an emission of smoke, light, heat and/or sound during its thermal degradation.

the illicit traffic of explosives, the questionnaire (answered by 35 states out of 52) noted that the majority of the participants compiled statistics, but did not make them public. While others were limiting their diffusion due to technical, security or other reasons, 10 of them reported the publication of at least summaries of statistics. Two states, however, had no statistics on incidents related to explosives [Les Nations Unies 2002]. No further detail is provided on the countries concerned.

Although fragmentary and concerning different periods, published data are nonetheless presented hereafter in order to provide a broad overview on the use and type of improvised explosives.

2.1 In Switzerland

Despite a low number of bomb attacks since 1960, period in which Switzerland recorded its first terrorist attack [National Research Council 1998b], the entry into force of the legislation controlling the trade, transport and storage of explosives in 1977 nevertheless reduced the illegal use of explosives. The following statistics from 1989 to 1994 (**Table 1.1**) and from 1995 to 2004 (**Figure 1.1**) reflect the low number of bombings[11].

Although outdated, data presented in **Table 1.1** are the only detailed and published information on the illicit use of explosives in Switzerland[12]. The figures demonstrate the rare cases involving civil and military explosives. More recent data from 1995 to 2009 provided by the Swiss federal police also reflect this phenomenon, where bombings seldom involve commercial explosives but mostly improvised explosive charges. This trend is also confirmed by the low occurrence of thefts of commercial explosives (**Figure 1.2**).

Presently, the charges of IEDs are mainly inorganic and are composed of ammonium nitrate-sugar or pyrotechnic mixtures. Some cases, though, reveal the use of organic explosives (Swiss or foreign military grenades, modified by the addition of wounding components and TATP) [Grau 2005]. Overall, the use of explosives in Switzerland remains exceptional.

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[11] A solved case is a case in which the author was identified

[12] No comment is provided to understand the imbalance between the number of total bombings and of occurrences of explosives used or of the targets. The number of total bombings might correspond to the number of bombs that effectively exploded.

Bombings	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**
Total bombings	10	10	5	31	23	7
Persons killed	0	0	0	0	1	0
Energetic material used						
Flammable liquid	0	1	0	1	0	0
Dynamite						
Untagged	3	4	4	4	5	1
Tagged	0	2	3	2	2	1
Black powder						
Untagged	7	7	9	15	13	10
Tagged	0	0	1	0	0	0
Smokeless powder	2	0	1	3	1	0
Pyrotechnics	20	19	26	3	4	2
Chemicals	5	12	1	4	1	0
Blasting agents	2	0	1	0	0	0
Military explosives (including grenades)	4	4	2	3	3	3
Target						
Residential	8	9	7	2	4	2
Commercial	10	7	11	7	8	2
Vehicles	1	2	3	5	6	2
Mailboxes	13	15	1	3	1	2
Utilities	7	14	11	13	5	1
Form of explosive						
Pipe bomb	10	10	6	9	9	7
Dynamite sticks	1	5	4	4	0	0
Bottles	1	2	1	3	1	3

Table 1.1. Number of cases involving the illegal use of explosives in Switzerland from 1989 to 1994 (adapted from [National Research Council 1998b] p.204).

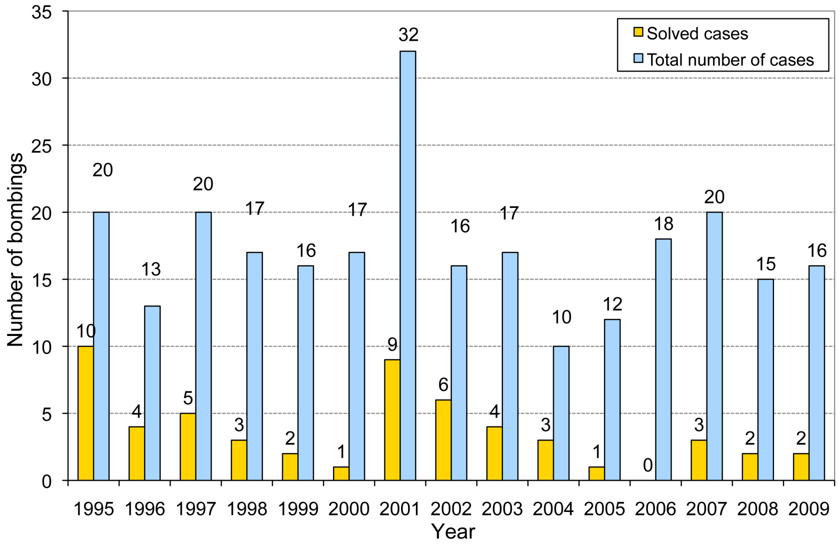

Figure 1.1. Statistics on bombings in Switzerland from 1995 to 2009, (adapted from [Grau 2010]).

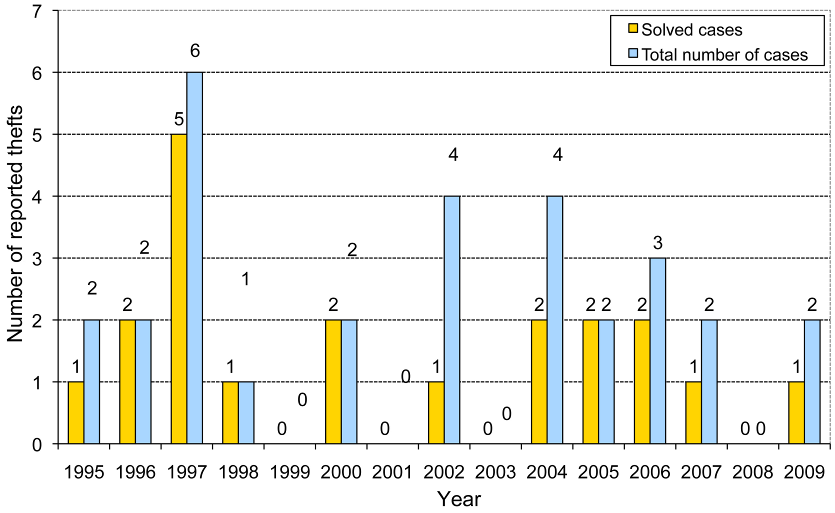

Figure 1.2. Statistics on reported thefts of commercial explosives in Switzerland from 1995 to 2009 (adapted from [Grau 2010]).

2.2 In foreign countries

In the United States of America (USA), the number of crimes involving the use of explosives has tripled between 1985 and 1995 (**Table 1.2**). This is partly attributed to the increased use of pipe bombs [National Research Council 1998a, National Research Council 1998b, Turlure 2005]. According to Oxley et al. (2001), over 10'000 bombings and attempts which occurred between 1993 and 1997, more than 30 % of the explosive devices were pipe bombs loaded with black or smokeless powder [Oxley *et al.* 2001, Turlure 2005].

Cases	**1985**	**1986**	**1987**	**1988**	**1989**	**1990**	**1991**	**1992**	**1993**	**1994**	**1995**
Actual	575	580	600	593	641	931	1551	1911	1880	1916	1562
Attempted	113	101	102	161	243	254	395	384	375	522	417
Total	688	681	702	754	884	1185	1946	2295	2255	2438	1979

Table 1.2. Number of cases involving the illegal use of explosives from 1985 to 1995 in the United States of America (source: [National Research Council 1998b] p.35).

Detailed data about bombings in 1998 published by the Bomb Data Centre confirm the trend observed in Switzerland: a large majority of non-conventional explosives are made of inorganic chemicals, mostly improvised mixtures, followed by pyrotechnic mixtures and black and smokeless powders (**Figure 1.3**). Organic compositions (dynamite, ANFO, etc.) represent less than 2 % of the total number of devices used in bombings and recovered in searches [Gadson *et al.* 1998].

In Canada, after a peak of activity in the period from 1990 to 1995, bombings and attempted bombings have decreased and are at present fairly stable (**Figure 1.4**)[13] [Royal Canadian Mounted Police 2010].

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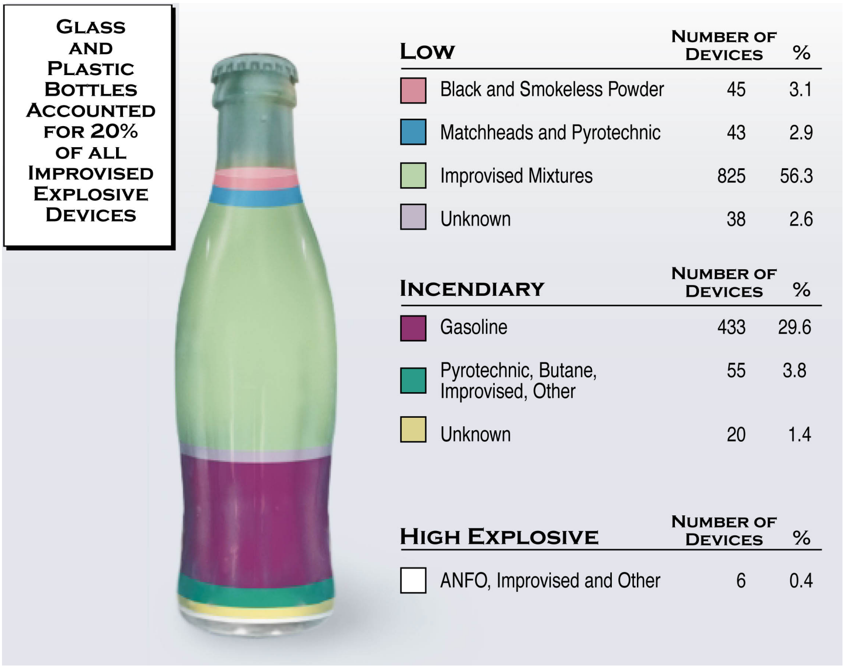
[13] the number of devices include integral devices, as well as parts or components of a device (partial bomb). The recovery of IEDs comprises incidents where armed, dumped or stolen explosive materials were recovered.


GLASS AND PLASTIC BOTTLES ACCOUNTED FOR 20% OF ALL IMPROVISED EXPLOSIVE DEVICES

LOW	NUMBER OF DEVICES	%
Black and Smokeless Powder	45	3.1
Matchheads and Pyrotechnic	43	2.9
Improvised Mixtures	825	56.3
Unknown	38	2.6

INCENDIARY	NUMBER OF DEVICES	%
Gasoline	433	29.6
Pyrotechnic, Butane, Improvised, Other	55	3.8
Unknown	20	1.4

HIGH EXPLOSIVE	NUMBER OF DEVICES	%
ANFO, Improvised and Other	6	0.4

Figure 1.3. Illustrated statistics of year 1998 published by the Bomb Data Center (source: [Gadson *et al.* 1998]).

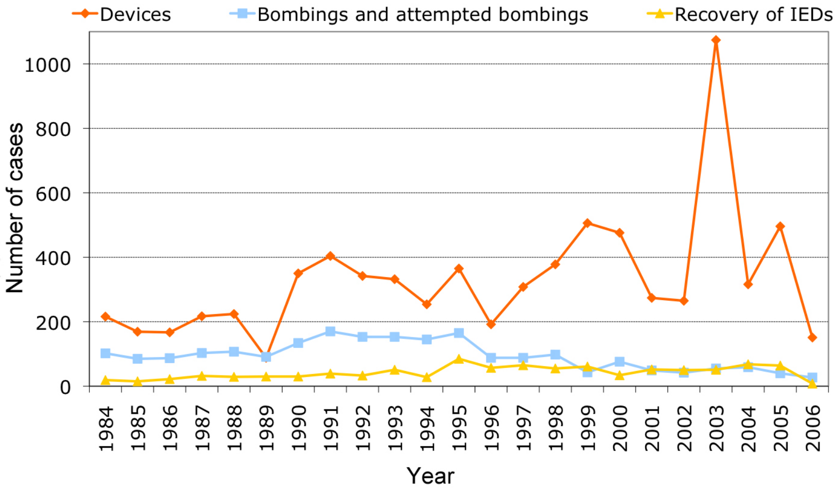


Figure 1.4. Selected statistics concerning the unlawful use of explosives in Canada from 1984 to 2006. Data based on the incidents reported by the Canadian police services to the Bomb Data Centre (adapted from [Royal Canadian Mounted Police 2010]).

2.3 Targets, authors and motivation

In Switzerland, acts involving the illegal use of explosives generally concern minor offences. However, the damage caused by these types of offences are significant and costly. Targets are often telephone booths, mailboxes and safes. They result more often from irresponsible acts committed by young people or are means of perpetration of other offences (such as thefts) than premeditated or revenge acts [Fedpol 2002, Grau 2005].

In the USA, bomb attacks are primarily directed towards private residential properties[14] (55 %), followed by vehicles (8 %), educational facilities (6 %) and commercial business (4 %). Bombings and accidental incidents often involve young offenders (37 %), gangs (3 %) and people from illicit drug networks (1 %). Cases attributed to organised crime and terrorist groups represent less than 1 %. Among the reasons of the illegal use of explosives, bullying and vandalism arrive first (41 %), followed by experimentation and personal animosity (7 %) [National Research Council 1998b, Gadson *et al.* 1998].

On the other hand, as reported by the United Nations (2002, 2010) and Turlure (2005), the use of explosives is widespread among extremist political groups, terrorists, criminals and is in close relation with the drugs cartels [Les Nations Unies 2002, Turlure 2005, United Nations Office on Drugs and Crime 2010].

3 Technical constitution of an IED

The term improvised explosive device includes elementary devices, such as a pipe bomb, as well as larger bombs, such as a car bomb, controlled by a complex electronic system for example, or a device including a mechanism to prevent its defusing. Depending on the intentions of the author (extensive loss of life in general public and massive destruction of infrastructure or targeted attacks on person or institution), the location, delivery and explosive power of the device will vary considerably depending on the expected impact [Wilkinson *et al.* 2008].

The basic components of any bomb typically consist of an explosive charge, which is enclosed or not in an envelope (container), and a firing system which includes a trigger mechanism and a initiator device.

3.1 Envelope

The envelope or the container of an explosive device has several functions. For explosives that require it, it is intended to provide the necessary confinement to

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[14] based on statistics from 1998.

produce an explosion. The presence of an enclosure around the charge is a *sine qua non* condition to ensure a rapid rise in gas pressure and cause the rupture of the envelope for explosive substances which react by rapid combustion. It also allows the concealment and transport of the device (as suitcase, parcel or vehicle, etc.). Finally, it can be selected, modified or manufactured in order to generate wounding fragments [Calisti 1997, National Research Council 1998a, National Research Council 1998b, Fanning 2002].

The most common explosive device is the pipe bomb. Its most rudimentary construction consists in a pipe, usually steel or polyvinyl chloride (PVC), filled with an explosive and closed at the ends. One end is pierced with a hole to allow the passage of the initiator device to the charge. The popularity of these devices in the USA is considerable [National Research Council 1998b].

The materials used as a container are available in hardware or plumbing stores. In fact, any container can potentially serve as an envelope (jerrycan, bottle, condiment tube, fire extinguisher, etc.). The choice of the envelope, defined by its thickness and rigidity, will also be dictated by the desired effects. In the USA, tube in cardboard or paper, glass or bottle plastic and metal or plastic pipes are the most common containers involved in IEDs [Gadson *et al.* 1998].

The envelope may also be absent, although this applies preferentially to organic military or civil explosives, which, by their nature, do not need to be confined.

3.2 Firing system

The firing system comprises a trigger mechanism and a initiator device. As demonstrated by the immense variety of available systems in the literature, with some basic knowledge of electricity, original firing systems can be easily implemented.

3.2.1 Trigger mechanism

The trigger mechanism is designed to manage the action of the initiator device at the moment and under the conditions wanted by the designer. Controlled by an arming device, it constitutes an installation security for the author, when the arming is delayed. There are three types of mechanisms: instant, delayed or remote trigger [Stoffel 1977, Calisti 1997].

Instant trigger mechanism The instant trigger mechanism can be:

- mechanical: this category includes all mechanisms that involve the action of a physical force (such as pressure, traction or relaxation). Booby traps[15] often involve such trigger mechanisms and are based on the action (or interaction) of a person with a supposed inoffensive environment (pulling a door, opening a box, etc.).

The mercury tilt switch system, used in the attacks of some planes or vehicles, is also part of this category. **Figure 1.5** illustrates the mechanism. The mercury tilt switch system is an open electrical circuit, which incorporates, into its system, a mercury drop. Under the action of the moving forces of the aircraft or the vehicle (up or down for example), the latter will move into the direction of the electrical circuit and will close it, allowing the passage of the current to an electric or electronic detonator.

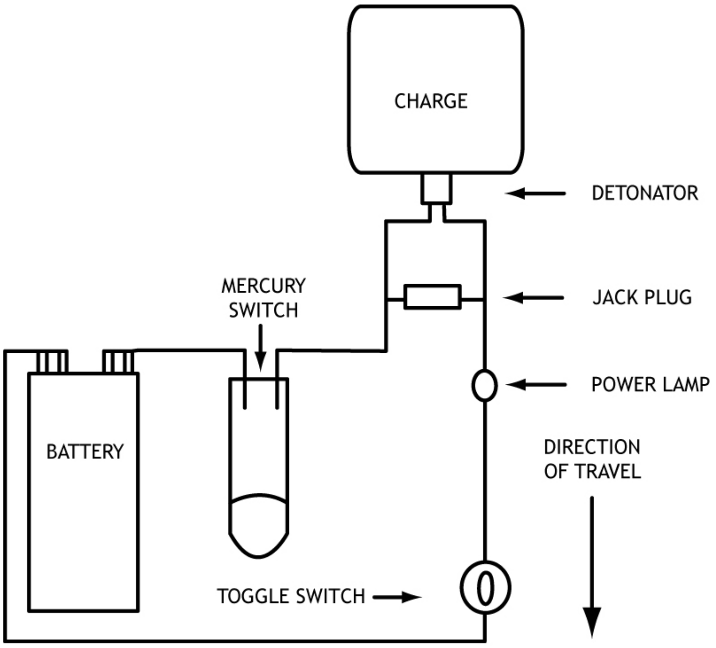

Figure 1.5. Example of an instant trigger mechanism by traction (source: adapted from [Harber 1990] p.51).

- electric: the closure of the electric circuit is obtained for example through the variation of a magnetic field.
- electronic: the electric circuit closes under the influence of a variation of lighting (photosensitive cells), noise (decibels sensitive device), etc.

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[15] A booby trap is "a device designed, constructed or adapted to kill or injure, which functions when a person disturbs or approaches an apparently harmless object or performs an apparently safe act" [North Atlantic Treaty Organization 2010].

Delayed trigger mechanism The delay of initiation of the device can be obtained:

- chemically: by chemical reaction or by wear and tear of the material by a corrosive chemical (strong acid in contact with metal for example);
- electrically: using clocks, watches, exploiting variations of pressure (altimeter) or temperature (bimetallic, thermometer (**Figure 1.6**)), etc.
- electronically: by the discharge of a condenser or through electrolytic cells for example.

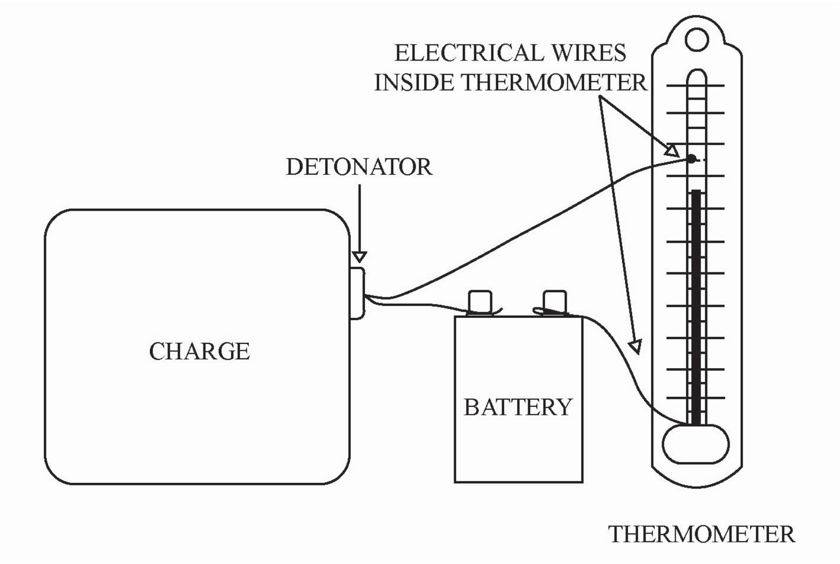

Figure 1.6. Illustration of a trigger mechanism, based on the variation of temperature (source: adapted from [Lecker 1996] p.9).

Remote trigger mechanism The trigger system can be controlled by shooting line, radio, telephone, etc. According to the intentions of the authors, several trigger mechanisms can be combined in one single bomb, in order to ensure the activation of the charge in case a system would fail to function [Wissenschaftlicher Forschungs Dienst 1990, Calisti 1997].

3.2.2 Initiator device

The initiator device, designed to cause the explosion of the charge, is the portion of the system which, one way or another, brings the energy required for the activation of the reactive components of the device. For compositions sensitive to spark or flame, the initiator device is sufficient in itself [Wissenschaftlicher Forschungs Dienst 1990].

Depending on the effects they produce, initiator devices can be classified into two categories [Calisti 1997]: according to a combustion-deflagration or a detonation reaction.

Based on a combustion-deflagration reaction, initiator device can be:

- mechanical: primers and percussion caps;
- pyrotechnic: matches, slow fuses, etc.;
- chemical: by exploiting the exothermic properties of some chemical reactions (such as the association of acid and alkali chlorate, or water and metallic sodium;
- electrical: electric igniters. This ignition system is based on the heating caused by the passage of current through a resistance and its conductive elements, heating which supplies the necessary energy to initiate the explosive system.

  A common electric initiator is the broken glass envelope of a light bulb filled with a small amount of explosive mixture. The ignition system is then placed inside the non-conventional explosive charge. Once the circuit closed, the current flows through the filament bulb. The released heat then ignites the nearby mixture, which in turn makes the charge explode.

  In addition to this non-conventional initiator, various materials can be used as initiator device: wires or ropes, sometimes soaked with flammable liquids, toilet paper, paper towel arranged in torch or hygienic pads, can serve as means of ignition.

The second category of initiator devices is based on a detonation reaction. It includes pyrotechnic electric and percussion detonators. Detonating cord also belongs to this class.

3.3 Explosive charge

The explosive charge is the active component of the device. Its nature and mass are chosen depending on the desired effect. Improvised compositions usually assemble an oxidiser and a fuel.

The presence of a booster, placed downstream of the main charge, provides the activation energy required to ignite the active components. This additional explosive charge is made of organic explosives.

The enhancement of the destructive effects can be obtained, as a result of the explosion, either by the elements of the container itself, or by materials incorporated into the bomb as nails, screws, pieces of metal or glass, etc. The destructive effects can also be increased by the presence of an incendiary charge. An additive is also often added to the flammable liquid, so that the mixture adheres to the target surface and lengthens the persistence of fire. Such formulations include egg white, wax or sodium hydroxide for example.

3.3.1 Evolution of the explosive charge due to legislation

In 1993, Oxley drew attention to the likely evolution of the charges' nature in bombings [Oxley 1993]. Indeed, if the introduction of legislation successfully reduced the illicit use of traditional explosives, it only moved the problem to another category of products. When terrorists and other bomb makers were first confronted with legislative regulations, they turned to chemicals with a lower explosive power, but easier to obtain [McPherson 1979, National Research Council 1998b, Marret 2006]. In Northern Ireland, for example, the explosive charges in the early 70s consisted of dynamite or similar commercial explosives. Following the implemented laws, terrorists have turned to agricultural processing products. As a result, improvised explosive compositions have gradually replaced the illegal use of traditional military or civil charges [National Research Council 1998b, Grau 2005].

3.3.2 Regulation of chemical precursors

Chemical precursors are chemicals needed for explosive manufacture, which do not necessarily have an explosive character[16]. Most substances are readily available in shops, drug or garden stores, or for more specific products, can be purchased on the Internet [United Nations 1999].

As part of the fight against the illicit use of explosives, several measures have been considered to limit or monitor the access to these precursors, particularly to sodium chlorate and ammonium nitrate. The measures currently implemented consist in restricting the access to precursors and desensitising the pure substances by introducing additives. These are commonly based on calcium carbonate (limestone, dolomite, chalk) for ammonium nitrate (also known as calcium ammonium nitrate (CAN)), and chloride-based calcium, a flame suppressor for sodium chlorate.

In Northern Ireland, to contend with the changing tactics of the bomb makers, regulatory statutes were enacted in 1972 to restrict the access to main oxidants (pure ammonium nitrate, sodium chlorate and chlorite, nitrobenzene[17],

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[16] The main target precursors are: ammonium and potassium nitrate, sodium nitrate, nitromethane, concentrated nitric acid, concentrated hydrogen peroxide, sodium and potassium chlorate, potassium perchlorate, as well as chemicals widely used, such as acetone, ammoniac, benzene, butane, glycol ethylene glycol, glycerine, iodine, methane, perchloric and sulphuric acid, urea, toluene, lead, mercury and silver.

[17] Nitrobenzene is not an oxidant, but it was often used in IEDs in Northern Ireland with ammonium nitrate.

sodium and potassium nitrate). Therefore, most of the common oxidants are only available in their modified form, except under license, and even in these conditions, in small quantities. On the other hand, 84 % purity sodium chlorate and 79 % purity ammonium nitrate are available on the Irish market and can be bought without license [McPherson 1979, National Research Council 1998b]. Similar regulatory measures have been implemented in South Africa, and more recently in France, for weed killer preparations containing sodium chlorate [Journal Officiel de la République Française 2004].

Despite a significant reduction of their use in bomb attacks, these substances are still used, especially in large size bombings. The presence of additives did not long hampered bomb enthusiasts, who either used them as is in large scale bombings or rapidly learned to separate additives from the target substances [United Nations 1999].

3.3.3 Extension of the sources of information

Nature of the sources Along with the evolution of the charges' nature, recipes for the fabrication of homemade explosives have become widespread and accessible to all [Anonymous 1982, United Nations 1999, Les Nations Unies 2002, Marret 2006].

Information for the manufacture of improvised explosive charges was initially available in textbooks, such as *Kitchen improvised explosives, CIA Field expedient methods for explosives preparations, Explosives and propellants from commonly available materials, The anarchist arsenal: improvised incendiary techniques and explosives* or *The anarchist cookbook* [Powell 1971, Anonymous 1977, Anonymous 1982, Anonymous a, Harber 1990].

Subsequently, the exchange of this information has been greatly enhanced by the development of the Internet in the 1990s. The expansion of digital data, associated to the proliferation of peer-to-peer networks, resulted in an increase of the type and number of available information sources. Fully digitised books, simple texts or videos entirely dedicated to the manufacture of improvised compositions, can easily be downloaded on the Internet.

The European Community has become aware of the situation. In its communication to the European Parliament and the Council of the 6 November 2007 on enhancing the security of explosives, the European Commission proposes in its action plan harmonising the criminal sanctions for diffusing information on bomb making over Internet, as a preventive measure [Council of the European Union 2008, Commision des Communautés Européennes 2007].

Content and conveyed information Recipes for the production of improvised explosives not only indicate the tools, process and techniques required to assembly the device, some documents also cite suppliers where the starting materials can be obtained [Anonymous 2004]. Similarly, procedures to extract the necessary chemicals from household products, drugs or desensitised precursors are therein explained.

Knowledge needed to perform these recipes can vary considerably. While fundamental notions of chemistry and a relatively basic material are most of the time sufficient, the manufacture of sophisticated explosive devices may require extensive knowledge of electricity or electronics.

Scientific literature that combines data on the characteristics of explosives (sensitivity, velocity, chemical properties, etc.) are also available on the Internet (*The chemistry of powder and explosives, Encyclopedia of explosives and related items, High explosives and propellants, etc.*) [Davis 1942, Federoff *et al.* 1962, Fordham 1980]. Furthermore, it is interesting to note that forensic investigative methods are also very well documented. Some of these documents (*White resistance manual*) report potential methods of explosives investigation[18] and the measures to take in order to avoid leaving traces [Anonymous b]. While these documents are still somewhat elementary for the specialist, they draw nevertheless the attention of some authors to the traces they may leave and the degree of individuality they may achieve. Fingerprints and DNA (deoxyribonucleic acid) are obviously firstly mentioned. More surprising is the report of tool marks possibly left during the assembly of a bomb or the potential transfer of explosive particles on clothing. This kind of awareness is also disseminated by the popular CSI (crime scene investigation) series and derivates [Durnal 2010].

3.3.4 Common chemical precursors and explosive compositions

The oxidisers and fuels which may compose an improvised mixture are presented below. Often, a component plays several roles and is used for example both as an oxidant and a dye or as a fuel and a binder [Calzia 1969].

Oxidisers Oxidants are mostly solid, oxygen-rich, ionic compounds. Oxygen is supplied by anions, containing generally high-energy bonds, such as Cl—O or N—O [Conkling 1985]. Because of the electronegativity of these atoms, the most frequent anions identified in improvised charges are precisely nitrate, chlorate and perchlorate [Phillips & Hiley 1999, Russell 2000, Miller *et al.* 2001, Kuila *et al.* 2005]. Main chemical precursors are [Yallop 1980, Conkling 1985]:

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[18] the terms "explosives investigations", as reported in this research, are not limited to the explosive substances themselves but encompass all investigations undertaken in relation with a bombing event or attempt.

- potassium[19] , sodium[20] or ammonium nitrates. Because of the hygroscopic nature of sodium, its use in black powder or commercial pyrotechnic mixtures is less frequent;
- sodium and potassium chlorates. The decomposition of chlorate being exothermic, mixtures which contain such substance require a very low input of external energy. The extreme sensitivity to friction and heat of these compositions makes their use rarer than the same nitrate-based compounds. Despite many accidents provoked by their handling, they remain widely used;
- potassium, sodium or ammonium perchlorate. Less risky, perchlorate has partly replaced chlorate in pyrotechnic devices. Because of its higher melting point, it is less sensitive to heat, shock and friction than chlorate. However, perchlorate can react very violently, especially in the presence of aluminium.

Peroxides (BaO_2, PbO_2), oxides (Fe_3O_4, Fe_2O_3, MnO_2, Pb_3O_4, PbO), sodium or calcium sulphates, oxalates ($Sr(COO)_2$) may also provide oxygen. They are rather found in pyrotechnic compositions [Calzia 1969].

Fuels Among the wide variety of fuels, there are three categories: metals, non-metals, and organic fuels [Conkling 1985].

Metals Metallic elements are generally excellent fuels. Due to their resistance to oxidation and moisture, aluminium and magnesium are the metals most frequently employed [Kuila *et al.* 2005]. Zinc or zinc-aluminium alloys are also widely used.

Non-metals In principle, these compounds are stable to air and moisture. The best known non-metal fuel, sulphur, is part of the composition of gunpowder. In addition to its role as a fuel, sulphur is also used to lower the ignition temperature of some mixtures and make them more reactive. Silica is also present in some pyrotechnic and delayed ignition compositions. Boron, red phosphorous, or arsenic sulphide are also observed in improvised explosive compositions [Kuila *et al.* 2005].

Organic fuels Among the huge variety of potential organic fuels, the most employed are sugars (lactose, sucrose, starch), cellulose, some polymers (PVC) or epoxy resins and hydrocarbons (gasoline, kerosene). The use of nitrocellulose or dextrin may also occur.

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[19] Also known as saltpetre, potassium nitrate is the main component of black powder. It is the most ancient oxidant used in explosive compositions.

[20] It is not unusual to detect the presence of sodium nitrate in homemade compositions.

The possible number of explosive mixtures from available oxidants and fuels is therefore immense. In practice, however, these mixtures are usually restricted to some formulations well known to bomb makers.

Most common inorganic explosive compositions The most common inorganic explosive charges encountered in IEDs are:

- ammonium nitrate mixed with a fuel, such as diesel fuel, kerosene or nitromethane. These ingredients may be in the form of prills containing jointly industrial grade ammonium nitrate and fuel, also known as ANFO [21]. It may also result from the association of both components—technical or fertiliser grade ammonium nitrate and fuel—directly on site. One of the major bombings that involved such composition was the bombing of Alfred P. Murrah's building in Oklahoma City in April 1995[22]. Ammonium nitrate fuel was also involved in the four bombings in Istanbul in November 2003. This substance is still widely used in worldwide bomb attacks, especially in truck or car bomb, as evidenced by the discovery of 250 tons of ammonium nitrate fertiliser along with 2000 bomb-making devices dissimulated in a cache in 2009, in Kandahar, in Afghanistan[23] [Global Security 2009] and by the Norwegian bomb attack perpetrated by Breivik against a government building in 2011.
- another ammonium nitrate derivative is the common mixture of ammonium nitrate, fuel and aluminium, as it increases the detonability of pulverised fertiliser grade ammonium nitrate [Anonymous 1980, Kopp 2008]. Other substances often combined with ammonium nitrate or potassium nitrate are coal dust, molasses and sugar [Cullum *et al.* 2000, Phillips *et al.* 2000].
- urea nitrate, which is sold in the form of a fertiliser or which can be made from urea based fertiliser and nitric acid. This substance has been used extensively in bombings in Israel, in Pakistan, in North and South America and, in particular, was the main charge of the World Trade Center's first bombing in New York in 1993 [Taylor 2005, Almog *et al.* 2007].
- mixtures of potassium chlorate[24] or sodium chlorate associated with sugar. Other fuels often combined with chlorates are wax, Vaseline and aluminium powder [Kopp 2008]. Sulphur and potassium chlorate are also frequently used as improvised explosive initiators [Joint Homeland Security Assessment 2007].

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[21] ANFO is a commercial blasting agent used in quarrying.

[22] The charge involved approximately 2500 kg of high grade ammonium nitrate fertiliser, ANFO, Tovex and nitromethane.

[23] The use, production, storage and sale of ammonium nitrate fertiliser was recently banned in Afghanistan and in Pakistan. The latter is believed to supply Afghanistan with large amounts of ammonium nitrate fertiliser [Cullison & Trofimov , IRIN 2010]

[24] The main charge used in one of the Bali bombings was a mixture of potassium chlorate, sulphur and aluminium, boosted by TNT [Royds *et al.* 2005].

- mixtures of aluminium and potassium or sodium perchlorate, which can be extracted from swimming pool cleaner products or calcium hypochlorite [Kopp 2008].
- pyrotechnic mixtures that encompass compositions of fireworks, including black powder and flash powders. Flash powders are pyrotechnic mixtures that emit a bright light. The most common formulations contain aluminium, sulphur and potassium chlorate or perchlorate. It is not infrequent they contain sodium or potassium nitrate [Meyers 1978].
- combinations of these formulations, such as the mixture of black powder, chlorate and sugar, often employed in Corsica attacks [Martinez 2005].

Most of these substances can easily be obtained in school laboratories, shops, from agricultural suppliers or extracted from household products or pyrotechnics [United Nations 1999].

CHAPTER 2

Black powder and ammonium nitrate

Contents

This chapter provides an overview on black powders and ammonium nitrates, their characteristics, uses and the way they are manufactured and distributed. Investigations carried out between late 2006 and early 2007 offer an insight of the market situation at that period. The production and distribution network of these substances is outlined within the limits of available information.

1 Black powder

1.1 Introduction

Black powder (BP), formerly synonym of gunpowder, is a mixture of potassium nitrate, sulphur and charcoal. It is recognised as the ancestor of all explosive compositions. Whether its discovery arose from the research of an elixir of life or from an accidental manipulation during the separation of gold from silver, the finding of black powder was attributed to Chinese alchemists in 220 BC. In the

13th century, black powder was introduced in Europe for the first time by Roger Bacon, an English monk. The energetic properties of this composition were then studied by a German monk, Berthold Schwartz. By the end of the century, the explosive mixture was generally adopted in Europe as a military component in small guns and hand grenades [Akhavan 2004].

Its appearance in Switzerland was mentioned for the first time in 1371. In the 15th century, Switzerland started to produce its own black powder in states of Bern, Zurich and Luzern with a hand made mixing process. In the 16th century, a mechanical process using water-driven corn mills displaced the homemade production method. The intimate mix of raw materials under heavy mills along with the integration of a corning step produced a more homogenous black powder. At the end of the 19th century, Switzerland used black powder as a civilian explosive for the first time. Although there were three black powder mills in Switzerland in 1850[1], Aubonne is nowadays the only plant where black powder is still produced [Berger 2004].

1.2 Current legal and illegal uses

Beside its use as a blasting agent in the mining industry and its military applications, black powder is primarily employed for sporting and shooting. The followers of such activities purchase it in retail trade for ammunition reloading and muzzle loading firearms. Black powder is also the basic compound of pyrotechnics, either as a delay constituent, a propelling or bursting charge or for lighting effects in fireworks [National Research Council 1998a].

Deviated from its legitimate applications, black powder is used as an improvised explosive charge, generally in small-scale bombings (pipe bombs) rather than large bombs. Because of its chemical characteristics, black powder needs to be confined to produce an explosion. Thus, typical containers used for that purpose are metal pipes, plastic or glass tubes and bottles.

Although size may vary, a typical pipe bomb of 20 to 30 cm length contains 300 to 500 g of black powder. Owing to the price of such substance (about 15 CHF for a one pound canister), to the particular constraints regarding safe handling and the necessary confinement to explode, black powder is not cost-effective for large-scale bombings [National Research Council 1998a].

Black powder can be easily purchased in retail outlets—in the USA, in gun shops, hardware stores, shooting clubs and over the Internet ; in Switzerland, essentially in armourers—without any restriction and control [National Research Council 1998a, Morier 2010, Rodriguez 2013]. More specifi-

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[1] One in Worblaufen, a second in Chur and a third in Aubonne

cally in Switzerland, only the name and the address of the purchaser is asked [Morier 2010]. Another way of procurement is by taking fireworks apart and extracting the filling, although these manipulations are more arduous and highly hazardous.

1.3 Physical and chemical properties

Depending on its application, there are different types of black powder: military, civilian and recreational black powder for sporting and shooting. Each type of black powder exists in different granulations, which correspond to the various grades of the product. The producing countries have adopted their own grade system. As an example , “F”or “Fg” is an American coding system in the manufacture of shooting black powder that corresponds to intervals of size and of number of corns per gram (1Fg to 4Fg). An equivalent designation exists in Switzerland (n°1 to 5) with n°5 (equivalent to 1Fg in the anglo-american system) being the coarser (indicatively between 1.200 and 1.600 mm) and n°1 (or 4Fg), the finest (between 0.250 and 0.500 mm) [Haag 2001, Poudrerie d’Aubonne 2003].

The ignition temperature of black powder ranges between 280°C and 400°C [Russell 2000]. Black powder is chemically stable under normal ambient conditions [Whiting 1970, Nobel Explosifs 2006]. However, due to its sensitivity, careful manipulation is needed. It must be kept away from heat, flames and sparks[2]. Any shock, friction and risk of electrical discharge must be avoided. The chemical characteristics of its constituents, the size of the grains, the proportions of the different constituents and its moisture content influence the reactivity, as well as the burning rate of the powder [Whiting 1970, Brown & Rugunanan 1989]. From a chemical point of view, because of its high reactivity, any contact with alkaline agents (bases, hydroxide, ammonia), amines, strong acids or oxidising agents should be avoided [Nobel Explosifs 2006].

1.4 Manufacturing process

Black powder results from the intimate mix of saltpetre (potassium nitrate), charcoal and elemental sulphur in approximate the proportions of 75:15:10 per cent. Charcoal, often produced from alder or buckthorn wood, acts as a fuel and potassium nitrate as an oxidising agent. Sulphur lowers the ignition temperature and operates as a fuel as well as a binding agent [Haag 2001]. Due to restrictions on transport and storage of black powder, especially in the USA, several black powder substitutes have been developed such as Pyrodex, Golden Powder and Clear Shot Powder. These substitutes are all derived from the usual formula and contain at

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[2] Indicatively, the minimum energy which characterises the sensitivity to electric spark of a powder with a grain size comprised between 0.36-0.57 mm is 26.4 mJ [Guoxiang & Changying 1982]

least nitrate potassium with additional chemicals such as potassium perchlorate, sodium benzoate, dicyanamide, ascorbic acid or starches [Haag 2001].

Although each producer has its personal and secret recipe, the principle of production does not differ much between companies. The manufacturing process does not involve any chemical reaction; it is based on the physical and intimate mix of the ingredients in predetermined proportions. A typical production circuit essentially includes the grinding and blending of raw materials, the compression and subsequent corning of the resulting blend and the sifting, polishing and coating of the grains. **Figure 2.1** describes the manufacturing process employed in Aubonne plant.

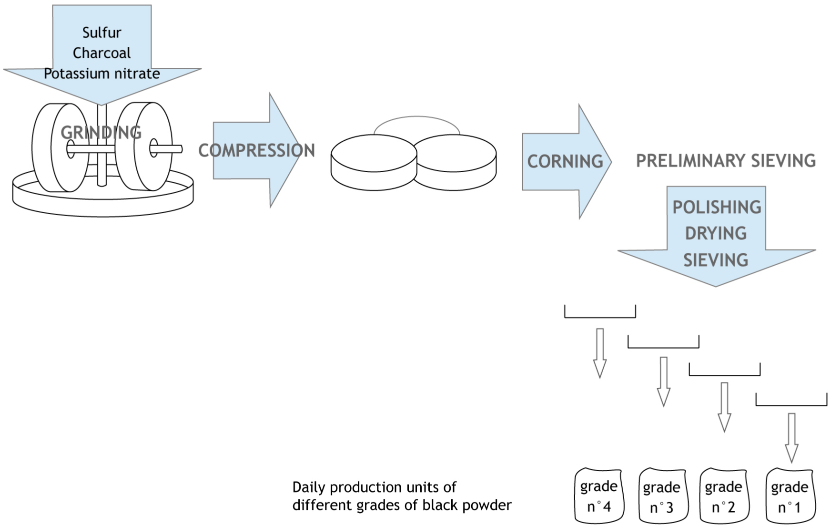

Figure 2.1. Manufacturing process of black powder, which consists essentially in the physical mix of raw materials. The proposed figure is based on the process used in Aubonne plant.

A load[3] of charcoal, sulphur and nitrate potassium undergo a first step called grinding. The substances are loaded with the addition of water and crushed together[4] under heavy mills. The milled powder is then compressed into blocks; the chunks are subsequently broken into large pieces. Through corning or granulating, material is laminated again into smaller granules. This step is followed by a preliminary screening of the grains size using sieves. The sharp grains of powder are then rounded in a wet polishing barrel which smoothes the surface of the grains by rubbing each other and removes the dust. To eliminate any residual moisture, the powder is dried

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[3] The load corresponds to the amount of potassium nitrate, sulphur and charcoal mixed in the usual proportions that can be worked at the same time in a ball mill

[4] This manufacturing process, which involves a ternary mix of the components, is less and less used because of the risks it involves. Other powderies, such as the manufactures of Vonges and Wano, produce black powder from two binary preparations previously mixed, namely a mixture of charcoal and sulfur and a mixture of nitrate potassium and wood charcoal (or potassium nitrate only). These are combined later in the manufacturing process of the powder [Ohse 2006].

in a dryer for 24 hours at 40°C. In order to make the grains more compact, they are dry polished and coated with graphite to protect them from moisture. The grains finally go through a long channel equipped with a series of sieves to sort the grains by size. This last selection produces the different grades of black powder [Paccolat 1997, Fiaux 2006]. An interesting feature of this manufacturing process is that two powders of the same type—if produced at the same time—but with different grain sizes (for example grade n°1 and grade n°2), are obtained from the same raw materials, as illustrated in **Figure 2.1**. Indeed, the distinction of grades is performed downstream from the initial mix of the three components .

At the end of the process, for each grade, the resulting product stands for one day-production. As the quantities under the mills are limited for practical and safety reasons, several day-productions are mixed together to produce one batch of black powder (**Figure 2.2**). Batches of black powder amounts oscillate approximately between one and five tons depending on the manufacturer [National Research Council 1998a, Fiaux 2006, Ohse 2006]. Beside the common production circuit, in USA, most companies recycle partially processed materials or unsatisfactory finished merchandise in the production cycle by incorporating them at the beginning of the process [National Research Council 1998a].

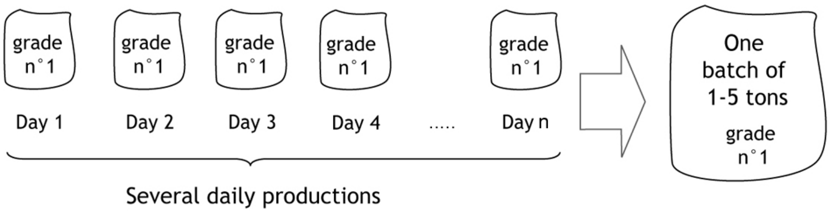

Figure 2.2. The final product, sold as one batch, results from the mix of several daily productions.

1.5 Production market and distribution systems

Published data on the global production market, distribution system and imported and exported quantities of black powder are currently almost inexistent. The information provided in this section is by far not exhaustive. Nonetheless, it helps to identify the different producers and the possible distribution ways of a black powder leaving the factory till its sale in retail outlets.

Because of its unpredictable behaviour and its extreme sensitivity to a wide range of stimuli, the manufacture of black powder implies significant risks and requires many precautions. For these security reasons, and because smokeless powders have partly superseded black powders, the number of black powder producers throughout the world is restricted, except in Asia (particularly in China) where production is more developed. Two major world manufacturers are GOEX

Inc. in the USA and Pernambuco Powder Factory in Brazil. Europe counts only very few powder factories. Six companies producing sporting and shooting black powder have been identified in Europe: WANO located in Liebenburg in Germany, the powdery of Aubonne in Switzerland, KIK Kamnik in Slovenia, Explosia in Pardubice in Czech Republic, Zaklady Produkcji Specjalnej in Pionki in Poland and the French manufacture of Vonges, which closed its black powder production site in 2008.

1.5.1 USA

Although American black powders are not part of the sampling of this research, details on the distribution system in the USA are provided hereafter in contrast to the Swiss market structure to illustrate the differences between a large and small scale market.

Except for quantities sold to foreign militaries and for exported products, most of the domestic production is sold on the American territory to domestic militaries, mining companies, commercial firms such as fireworks manufacturers, retailers and intermediates such as master distributors and wholesalers. Large quantities of the domestic production are sold to master distributors, which in turn sell it to wholesalers, before being dispensed to consumers in smaller quantities at numerous retail outlets [National Research Council 1998a].

In the distribution chain, black powder leaves the production site and is sold to intermediate distributors. It is either already packaged in canisters for direct retail sale, either it is delivered in bulk quantities to companies with repackaging facilities, which label it and sell it with their own trade name. It is worth mentioning that one batch of a manufacturer is not necessarily sold exclusively to a single distributor.

Imported black powder comes either in the form of canisters for direct sale to consumers or in bulk quantities for repackaging by companies. Fireworks, which also contain black powder, are generally imported as end products directly from China [National Research Council 1998a].

1.5.2 Switzerland

In Switzerland, the use and production of black powder are more restricted. The Swiss production reaches roughly a total of 60 tons per year, while the total annual European production is 2000 tons [Poudrerie d'Aubonne 2003]. The small Swiss production encompasses blasting powder, shooting powder and canon powder.

Mining black powder, whose annual production does not exceed 10 to 20 tons, is entirely used in Switzerland[5]. Additional powder is imported to cover the needs of the mining industry. This type of powder, whose storage and use requires a licence, is sold directly by the powder factory to owners of a mining licence or to retailers who have the right to sale explosives [Morier 2010].

Each year, 30 to 40 tons of shooting black powder are produced, of which 90 % are exported. In contrary to the American system, there is few, if any, intermediates for the sale and distribution of black powder in Switzerland. The shooting powder of Aubonne is packaged on site and distributed directly to armourers and retailers in 100 g and 1 kg containers. Black powder is never sold directly to individuals neither in Switzerland nor abroad. Additional shooting powder is generally not imported in Switzerland, except for some rare gun shops.

When exported, black powder is packaged for retail sale in 454 g, 500 g and 1 kg containers or in 5, 10, 11 or 25 kg bags for military supply or plant ammunition loading. All products which leave the Aubonne factory are traceable to the Swiss production market and are not further re-packaged [Morier 2010]. Very few Swiss black powder is used as a filling in fireworks. As for America, they are commonly imported from China as end products.

2 Ammonium nitrate

2.1 Introduction

Ammonium nitrate (AN) was first synthesised by J. R. Glauber in 1654 by mixing ammonium carbonate and nitric acid. It was not before 1867 that its explosive properties were exploited. Two Swedish chemists, C. J. Ohlsson and J. H. Norrbin, patented a mixture including ammonium nitrate and nitroglycerin as an explosive. A. Nobel purchased their invention shortly after and started to apply it in his explosive compositions. Because they were more stable, dynamite was rapidly replaced by ammonium nitrate based explosives. However, the hygroscopicity of ammonium nitrate remained a problematic issue. To overcome this property, chemists created various derivative compositions in which ammonium nitrate was blended or coated with inorganic compounds. The invention, in the 1950s, of ammonium nitrate fuel oil (ANFO) in the form of prills, and later, in the second part of the 20th century, of water-gel and emulsion explosives revolutionised the industry of civil blasting by displacing nitroglycerin based explosives with cheaper and safer products. Simultaneously with the emergence of prilled ANFO, AN prills

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[5] By law, any materials manufactured and intended to be used as explosives, such as dynamites, slurries and black powder for blasting, must be tagged. For forensic purpose, a sample of each tagged batch is sent to the Wissenschaftlicher Forschungsdienst (WFD) in Zurich [National Research Council, 1998b]. On the contrary, substances which are explosive, but whose application is not for use as explosive, do not need to be tagged.

suitable for farm use were introduced on the market around the 1950s. In contrast to blasting products which had good oil absorbency and nearly no coating, these fertilising prills were made specifically with a glassy and non porous surface in order to dissolve gradually in the fields [National Research Council 1998b, Akhavan 2004].

For a long time, the explosive properties of AN and the hazards related to its use and storage were not very well known. Numerous severe fires and accidental explosions occurred throughout the world. After several studies undertaken on its properties as well as on those of its mixtures with organic and inorganic substances, stricter regulations were enacted about the storage, use and transport of ammonium nitrate [Akhavan 2004].

However, these measures have not been sufficient, as attested by the catastrophic explosion that occurred in AZF plant in Toulouse in 2001. A warehouse containing 300 to 400 tons of downgraded AN exploded, as a result of the accidental dumping of a chlorine compound on a stock of moist AN [Tribunal de grande instance de Toulouse 2009].

2.2 Current legal and illegal uses

Depending on its legal application, ammonium nitrate differs by its physical form and can be found either as technical grade AN in explosive compositions or as fertiliser grade AN in the liquid or solid form [Institut National de Recherche et de Sécurité 2007].

Used as an explosive in blasting, quarrying or tunnelling, technical grade ammonium nitrate, which accounts for 20 % of the world ammonium nitrate consumption, is utilised in the following commercial compositions [National Research Council 1998b, Brusset *et al.* 2002, Suresh 2007].

- nitrated explosives, which contain about 80 % of ammonium nitrate and one or more other organic explosives such as TNT or PETN;
- slurries, whose composition is a combination of ammonium nitrate (30 to 70 %), calcium and sodium nitrate, water, sensitising and gelling agents, stabilisers, ethylene glycol and various other compounds such as oxidants, fuels and binding agents;
- emulsion explosives, which contain droplets of a solution of ammonium nitrate dispersed in oil. In addition of mineral nitrates (70 to 80 %) and oil (4 to 10 %), they also enclose between 8 to 15% of water;
- ammonium nitrate fuel oils (ANFOs), one of the cheapest explosives, which combine about 95 % AN and 5 % of fuel oil (liquid hydrocarbons). They can be either oiled and bagged in factory and transported to the site of use or they

can be prepared directly on site, by pouring and mixing AN followed by fuel oil in the whole. Sold in the form of small, porous and low density prills, its performance of detonation is directly related to the quality of AN, and more specifically to its porosity. The surface must be sufficiently porous in order to absorb the ideal quantity of fuel;

- heavy ANFOs which are the last generation of ammonium nitrate based explosives and are made of traditional ANFO coated with a slurry or an emulsion.

Unlike at the beginning of previous century, all these compositions are now considered as explosives in Switzerland and require a license to be purchased, transported, handled and stored [Loi fédérale sur les substances explosibles 1977, Ordonnance sur les explosifs].

Used as a fertiliser, this grade can be found in the form of urea-nitrate solutions or solid AN fertilisers. They can either contain one unique fertilising element (simple fertilisers) or more than one fertilising elements (composed fertilisers) such as a combination of nitrogen, phosphor and potassium (NPK). In this work, ammonium nitrate based fertilisers refer only to simple solid fertilisers, which contain only one fertilising element. Liquid as well as solid fertilisers combining several fertilising elements will not be addressed.

Solid AN fertilisers, which are the most commonly used, are commercialised in the form of non-porous and high density prills, also known in French as “ammonitrate"[6]. These products are expressly processed to decrease the absorbance of ammonium nitrate, rendering it more difficult to detonate. According to the CE 2003/2003 community legislation on fertilisers [Parlement Européen et Conseil de l'Union Européene 2003], they are classified into two categories depending on their nitrogen content:

- fertilisers with a nitrogen content below 28 %. They contain approximately 80 % of pure ammonium nitrate and 20 % of inert additives, such as kaolin, dolomite calcium carbonate or calcium sulphate, added in order to decrease the sensitivity to initiation. Incorporated during the production process, these fillers are integrated as a coating around the prills or directly into the mass of ammonium nitrate in the form of fine powder [Institut National de Recherche et de Sécurité 2007].
- fertilisers with a nitrogen content higher than 28 %. These products, which contain almost pure ammonium nitrate, need to undergo a detonation test and to fulfil several criteria (such as the proportion of combustible compounds and the rate of oil retention) to be commercialised in the European Community [Parlement Européen et Conseil de l'Union Européene 2003].

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[6] This designation includes all ammonium nitrate fertilisers with a minimum nitrogen content of 20 % [Parlement Européen et Conseil de l'Union Européene 2003]

Due to growing security concern, several measures were imposed to reduce the risks related to transport and storage of AN fertilisers. Fertiliser grade AN were even banned in several countries including China, Colombia, Algeria and the Philippines [Suresh 2007]. In the European community, harmonised safety requirements have been established not only to decrease the risks of accidental explosions, but also to impose a minimum control on high nitrogen content fertilisers [Parlement Européen et Conseil de l'Union Européene 2008]. Such requirements concern the non detonability of the substance in specific conditions, the physical characteristics and the allowed limits of chemical impurities in the product. In addition, the manufacturers have the obligation to keep records of their production sites. A modification of this legislation has been proposed to introduce, first, a system allowing the sale of such substances only to authorised persons, who should prove the agricultural use of the substance[7]. Secondly, the European commission would like to oblige the companies that sell these substances to inform the police of any suspect transactions [Commision des Communautés Européennes 2005].

Supplemental safety measures have been taken by adding high nitrogen content AN to the annex of dangerous substances of the regulation on registration, evaluation, authorisation and restriction of chemicals (REACH) of the European Union[8] [Parlement Européen et Conseil de l'Union Européenne 2009]. In 2010, the European parliament and council proposed a regulation on the marketing and use of explosives precursors, which was enacted in 2013. This regulation, which lists 8 chemical precursors requiring stricter controls, includes ammonium nitrate with more than 16 % nitrogen coming from ammonium nitrate [European parliament and council 2010, European parliament and council 2013].

Employed for the first time by the Provisional Irish Republican Army (IRA) in their bombing campaign, its use as an improvised explosive charge has never been effectively impeded. Despite the efforts to regulate the access to AN based products and to lower the nitrogen content of ammonium nitrate fertilisers by introducing inert additives, ammonium nitrate is still used in bomb attacks [National Research Council 1998b, Oxley *et al.* 2002]. Ammonium nitrate either comes from illegal supplies of ANFO (stolen stocks in mining careers or through illegal drugs networks) or from the legal acquisition of AN fertilisers used in combination with fuel. Fertilisers used in an explosive charge may be prepared in two ways:

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[7] Following Breivik's bomb attack in Oslo in 2011, the Norwegian Directorate for Civil Protection proposed more restrictive schemes to control the access to ammonium nitrate in 2012. However, current regulation has not included the proposed measures yet [Norwegian Directorate for Civil Protection 2012]

[8] From the 27th of July 2010, AN based substances containing more than 28 % nitrogen to be used as fertilisers can not be commercialised unless they fulfil the criteria of the CE 2003/2003 directive. Additionally, substances or mixture with more than 16 % nitrogen coming from AN can only be delivered to users and distributors of civil explosives with a licence or an authorisation, to farmers for agricultural activities or to persons with similar activities

- incorporated as it is in the form of prilled fertiliser, the fertiliser is combined with fuel to increase sensitivity and energy output. In order to optimise the absorbance power of ammonium nitrate, the fertiliser can be ground into a fine powder.
- pure ammonium nitrate is extracted from the fertiliser with a simple recipe based on the chemistry and solubility of the compounds [Joint Committee on Justice Equality Defence and Women's Rights 2004].

Contrary to the marketing of high nitrogen content fertilisers which are strictly regulated by the CE 2003/2003 community legislation, the second category of AN fertilisers follows the rules on free movement of goods and are largely available to the general public in gardening and do-it-yourself shops. Whereas the acquisition of these fertilisers requires to provide identity details and an address in some countries, there is no control on the identity of the purchaser and the quantities bought in other countries. In Switzerland, as in Germany, ammonium nitrate fertilisers with 27.5 % N are the most frequently used. Legislation on AN fertiliser in Switzerland is regulated by a set of texts, which follow the CE 2003/2003 legislation [Ordonnance sur les engrais , Ordonnance sur les produits chimiques , Ordonnance sur le livre des engrais]. As such, once the fertiliser has been approved for its marketing on the Swiss market, no further controls on the identity of the purchaser are performed.

Bomb makers have largely exploited the aspect on the legal possession of large amounts of AN fertilisers. As long as ammonium nitrate fertilisers are kept separate from hydrocarbon components, both substances are not considered as explosives. It is only when they are mixed together that the composition becomes illegal [Akhavan 2004, United Nations Office on Drugs and Crime 2010]. However, as highlighted by the digest of terrorist cases of the United Nations Office on Drugs and Crime (ONUDC), the possession of both substances, even kept separate in a storage facility, may be considered as preparatory acts of terrorism within the context of the case.

For a long time, the detonability of ammonium nitrate fertilisers has been a controversial subject. The incorporation of inert additives to AN fertilisers, and consequently the reduction from 34 % to 27.5 % nitrogen content was originally implemented in Northern Ireland and Republic of Ireland in 1972 as a countermeasure to thwart bomb makers. Based on the evidence that mixtures of AN with desensitising additives were more stable, the dilution of AN was thought to be sufficient to reduce its detonability and to render it more difficult to use in the preparation of explosives [National Research Council 1998b]. However, the attacks involving these substances did not decrease and different studies have confirmed that the modification of ammonium nitrate does not definitively prevent its detonation, though its explosive power is reduced [Phillips *et al.* 2000, Cullum *et al.* 2000, Monsfield *et al.* 2001, Oxley *et al.* 2002]. Even fertilisers with a nitrogen content as low as 16 % AN) mixed with fuel have been demonstrated to be detonable [Parlement Européen et Conseil de l'Union Européene 2008].

Following the 2001 explosion in Toulouse, the European Community imposed a detonation test for all AN containing more than 28 % nitrogen [Parlement Européen et Conseil de l'Union Européene 2003]. This test, undertaken under specific conditions (minimum dimensions of the material, material composition, booster, etc,), must be successfully passed, for the AN to be marketed. In reality, the detonability of ammonium nitrate prills depends on many parameters such as the size and diameter of the charge, prills diameter, porosity, temperature, moisture content, presence of contamination, etc. [Anonymous 1980, Kordek *et al.* 2005, Martinez 2007, Office Fédéral de l'Environnement 2011]. Therefore, the test imposed by the European Community evaluates the detonability risk of AN under the specific set of conditions of the detonability test [Parlement Européen et Conseil de l'Union Européene 2003]. However, even if an AN fertiliser passes the test, modifications of some parameters, for example, its granularity or its composition (mixed with other substances, such as metals) may modify its explosive properties, and therefore its detonability [Office Fédéral de l'Environnement 2011].

2.3 Physical and chemical properties

Pure ammonium nitrate is a white crystalline solid, soluble in water. With a low boiling point at 169.6°C [Meyer *et al.* 2002], ammonium nitrate possesses 5 stable crystalline forms according to temperature ranges (see **Table 2.1**) [Brown & McLaren 1962, Médard 1987]:

Transition temperature	Forms transition
-18 °C	$V \rightleftharpoons IV$
32 °C	$IV \rightleftharpoons III$
84 °C	$III \rightleftharpoons II$
125 °C	$II \rightleftharpoons I$

Table 2.1. Crystalline forms transition of AN and the temperature at which the transition occurs.

While transitions between forms I, II, IV and V take place in the dry solid and imply little crystal modification, transitions II to III and IV to III only occur in the presence of moisture and involve its dissolution and re-crystallisation. During storage, the transition IV to III is mostly responsible for the loss of quality of the product due to variations of the crystal volume [Brusset *et al.* 2002]. Additives are therefore used to repel moisture and prevent this degradation.

AN is very hygroscopic and its exposure to more than 50 % relative humidity not only leads to caking, but also to the formation of a saturated aqueous solution covering the crystals. Nevertheless, under appropriate conditions (i.e. less than 50 % relative humidity and 30°C), AN is stable at ambient pressure and can be stored for long periods of time [Institut National de Recherche et de Sécurité 2007].

The thermal decomposition of AN is a complex phenomenon, involving numerous consecutive reactions and phase equilibria. Decomposition of AN starts progressively above its boiling point and leads to its endothermic dissociation into ammonia and nitric acid and its exothermic decomposition into N_2O and H_2O. Around 300°C, AN decomposes rapidly and releases NH_4, HNO_3, N_2O and H_2O and nitrogen oxides (NO_x) [Médard 1987, Turcotte *et al.* 2003b].

With less than 0.2 % of organic components, pure ammonium nitrate is non flammable and insensitive to friction and mechanical shocks [Quinchon 1987]. AN is relatively insensitive and requires a detonator and a booster to be detonated. AN may also explode due to a heating source, such as a fire, but detonation is not immediate and may be initiated after a long heating time.

Its explosive sensitivity depends on numerous factors and primarily on particle size, density and the presence of inert material or on the contrary the presence of contaminations. Indeed, its contact or mixture with organic fuels, elemental sulphur, chlorinated derivates, alkali metals, metallic powders, oxidising impurities or acids lowers the critical temperature and accelerates reaction rates. Therefore, AN should not be mixed with such catalysing substances, as they can induce its decomposition and even lead to detonation, such as in the disastrous accidental explosion of AZF plant in 2001 [Marais 2002, Institut National de Recherche et de Sécurité 2007, Tribunal de grande instance de Toulouse 2009]. In the present case, the more likely cause retained by the Commission of experts was the reaction mechanism induced by the incompatible and hazardous mixture of moist AN and sodium dichloroisocyanate (DCCNa), a chlorine derivative used in disinfection (such as for cleaning swimming pool water), which was also produced on the site. In the presence of water or moisture, DCCNa produces hypochlorite acid (HOCl). When the mass of water exceeds that of DCCNa, the product maintains its disinfectant properties. Otherwise, the presence of 1 to 20% water eventuates in a slurry and give rise to secondary reactions. The intermediate HOCl, reacting with DCCNa and AN, produces trichloroamines (NCl_3), which decomposes spontaneously around 90°C [Tribunal de grande instance de Toulouse 2009, Martin 2012]. Thus, the likely causal chain expressed in the prosecution during the criminal trial of the case was the accidental dumping of DCCNa on a stock of moist downgraded AN, which resulted in the production of NCl_3, acting as a booster.

2.4 Manufacturing process

Ammonium nitrate is not present at natural abundance. It is manufactured from different synthesis methods with varying reaction pressures and temperatures. All are based on the process of neutralisation of nitric acid with ammonia and the evaporation of the solution. The following described hereafter is based on the manufacturing process used in Lonza plant in Visp (Switzerland).

Ammonia, obtained through the reaction of hydrogen with atmospheric nitrogen, is automatically injected in the reactor with concentrated nitric acid under a definite pressure. The energy released by this exothermic reaction of neutralisation is subsequently used to vaporise the resulting ammonium nitrate solution: excess of water is removed until the solution reaches a purity of 90 to 99 % according to the reaction conditions. This almost pure solution is then stored in a tank, before being mixed with additives. Additives are pulverised in a very fine powder and added in proportions of 25 to 30 % to the ammonium nitrate solution. The solution is then sprayed through a prilling tower. Dropping down from the top of the tower, AN droplets cool down and transform into hard pellets, which are dried and coated with wax to prevent from caking [National Research Council 1998b, Brusset *et al.* 2002, Joint Intelligence Task Force 2005, Kummer 2007]. The production is a 24 hours a day process. Each manufacturer adds its own additives and has its own products [Kopp 2007]. A particularity of Lonza manufacturing process is the production of different types of AN fertilisers from the same stock of AN solution. Different additives are then added to the solution according to the fertiliser produced.

2.5 Production market and distribution systems of AN fertilisers

Millions tons of ammonium nitrate are produced each year. Although its production decreased since urea nitrate conquered part of the market (due to its higher stability and lower production costs), available statistics estimated the world manufactured AN quantities to be more than 15 million metric tons in 2006 [Suresh 2007]. USA and Russia are the largest AN producers, assisted by Western Europe which also produces considerable amounts. While about 25 % of the global world AN production is exported, Russia remains the main exporter and supplies especially former soviet union countries, Latin America and Middle East [Market Publishers].

Accounting for over 30 % of global consumption, Western Europe is the major AN consumer, despite the restrictions imposed in some countries (Belgium, Germany, Ireland, Netherlands) related to its application in agriculture. European market is principally governed by Yara International, originally from the Norwegian company Norsk Hydro. Yara International is a world's leading supplier of fertilisers and produces more than 8 millions tons per year [Kopp 2007]. Despite the existence of several manufacturing companies, most of them are directly or indirectly related to Yara. Another independent leading producer is GPN in France, (formerly AZF-Grande Paroisse) belonging to the TOTAL Group.

Every year, about 130'000 tons of 27 % N AN fertilisers and 400 tons of 33 % N AN fertilisers are consumed on the Swiss market [Kopp 2007].

Switzerland has one single ammonium nitrate plant producing ammonium nitrate based fertilisers. Based in Visp, Lonza company provides the Swiss market with a variety of fertilisers made from one pure stock solution of ammonium nitrate and different additives. Lonza covers half of the needs of the national market. The rest of AN fertilisers sold on the Swiss market is manufactured in foreign countries by about 20 producers and is imported by Landor SA, the major importer of these substances in Switzerland [Kopp 2007].

Distribution of AN fertilisers is operated by truck, rail or barge, involving respectively shipments of 50, 100 or 1500 tons of fertilisers [National Research Council 1998b]. AN fertilisers generally leave the production plant in a bulk form and, unless the prilling plant has bagging facilities, AN fertilisers are bagged at subsequent points in the distribution chain. As an example, Landor imports AN from different manufacturers. AN fertilisers arrive generally in barges on the Rhine from seaports and European or overseas factories. A crane transfers the content of the barge into a huge storage hall. From there, fertilisers are transported to bagging facilities. They are generally bagged into 25 kg bags and brought to a warehouse, before being routed to retailers or outlets [Kopp 2007, Landor]. An additional characteristic of the distribution system is that a batch of AN fertiliser can be produced in June and delivered only 3 months later [Kopp 2007]. Additionally, according to the arrivals, fertilisers from different manufacturers are bagged with the same bag design, therefore the same packaging does not guarantee that AN comes from the same manufacturer.

3 Bombings involving black powder and AN fertilisers

Numerous bombings involving the use of black powder and ammonium nitrate have been perpetrated worldwide. To illustrate the current illicit use of these substances, **Table 2.2** lists some of these bombings, within the limits of available information[9].

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[9] For AN bombs, most references did not indicate whether it was AN fertiliser or another AN based explosives that was used

Date	Location	Target	Bomb details	Nb of fatalities and injured
19.04.1995	USA Oklahoma city	Alfred P. Murrah Federal Building	truck bomb containing 2500 kg AN fertilisers, 630 L nitromethane 160 kg Tovex and diesel fuel [Wikipedia 2011c] [National Research Council 1998b]	168 fatalities > 600 injured
25.07.1995 06.10.1995 17.10.1995	France Paris	metro, RER	potassium chlorate, sulphur BP, nails and bolts [SOS-attentats 2011]	10 fatalities 200 injured
03.12.1996	France Paris	RER	Gas canister containing sodium nitrate, BP, nuts sugar, nails and gasoline [Blog 90]	4 fatalities 100 injured
14.07.2003	Indonesia Jakarta	parliament building	Pipe bomb containing BP [Global terrorism database 2011a]	none
15.11.2003 20.11.2003	Turkey Istambul	2 synagogues	4 trucks containing 2500 kg AN fertilisers and fuel oil [Global Jihad 2007]	25 fatalities >300 injured
10.12.2004	Thailand	rail lines	AN fertiliser [Global terrorism database 2011b]	none
03.04.2005	Thailand Hat Yai, Songkhla	store airport hall	dynamite, fertiliser [Wikipedia 2011b]	2 fatalities 66 injured
07.04.2005	Egypt, Cairo	market	a 3.5 kg BP bomb [Global Jihad 2008]	4 fatalities 18 injured
11.07.2006	India Mumbai	railway	7 pressure cooker bombs with 2.5 kg of AN and RDX [Wikipedia 2011a]	209 fatalities 700 injured
21.07.2008	China Kunming	buses	AN based bus bombs [Global terrorism database 2011c]	3 fatalities 14 injured
25.07.2008	India Bangalore	business district	8 IEDs containing AN, bolts and nuts [Global terrorism database 2011d]	3 fatalities 20 injured
23.10.2008	Colombia, Bogota	unknown	6 bombs fitted with timed clocks, AN, sodium benzoate and potassium chlorate [Global terrorism database 2011e]	0 fatalities 6 injured
22.02.2009	Egypt, Cairo	market	BP, TNT pieces of metal, nails [Shenker 2011]	1 fatality 24 injured
15.07.2009	Indonesia Jakarta	hotels	BP, potassium nitrate silver carbon, screws and bolts [World Health Organisation 2009]	9 fatalities 55 injured
13.02.2010	India, Pune	bakery	AN fuel oil, RDX nuts and bolts [Rediff news]	17 fatalities 60 injured
28.05.2010	USA New York	unknown	attempted car bombing with 3 propane gas canisters gasoline and BP [The Australian 2011]	none
02.11.2010	Athens	international organisations embassies	8 mail bombs containing BP [The Telegraph 2011]	2 injured
28.04.2011	Morocco Marrakesh	tourist place	AN, TATP, nails [Reuters]	15 fatalities 20 injured
25.05.2011	India, New Delhi	court	AN [The Times of India 2011]	none
22.07.2011	Norway Oslo	government building	car containing 1 ton AN fertiliser fuel oil [Mala & Goodman 2011]	7 fatalities 209 injured
15.04.2013	USA Boston	marathon	BP, nails, ball bearings [Rodriguez 2013]	3 fatalities 183 injured

Table 2.2. Bombings involving the use of black powder and AN fertiliser.

Chapter 3

Forensic analysis of inorganic explosives

Contents

1 Introduction

In explosives investigation, the explosive device is the vector of various types of traces. Traces such as fingermarks or DNA may be present on the components of the explosive device. Additional traces, such as hair or fibres retained on adhesive tape or tool marks resulting from the construction of the device may also be observed. Besides, the explosive device conveys complementary dimensions of information through its various components. The following paragraphs present first the exploitation of traces related to the identification of the person in explosives investigations, and secondly, the exploitation of information inherent to an explosive device. The presentation of the different types of traces recovered and exploited in investigations is necessary to understand the relevance and the place held by the analysis of explosive substances.

1.1 Traces related to the identification of the person

Forensic investigations of explosives include pre-blast and post-blast aspects. The former involves intact, unexploded explosive material, while the latter concerns materials remaining after explosion.

Pre-blast investigations In pre-blast investigations, traces such as fingermarks or DNA, whose discrimination or individualisation potential is widely recognised, are commonly exploited in order to establish a link between the trace and a person or between several traces.

Some authors even investigated the possibility to recover fingerprint and DNA on the exterior and interior of vehicle-borne improvised explosive devices (VBIED), as well as on objects placed inside, following the use of a water-based render-safe tool [Ramasamy *et al.* 2011]. The recovery and identification of fingermarks were more successful on the exterior of VBIED than on the interior. Besides, DNA profiles could be recovered from hair and saliva samples deposited on most objects. These results indicate that, when the IED did not detonate during the process of defusing, the recovery and exploitation of fingermarks or DNA on different surfaces is still possible despite the deployment of render-safe tools.

However, as mentioned previously (see **page 20**), knowledge on the exploitation of forensic traces is disseminated in open source manuals on the manufacture of bombs or on television through CSI series [Anonymous b, Durnal 2010]. As bomb makers are getting more aware of traces allowing their identification, these types of traces may become less frequently encountered. Indeed, forensic awareness results in increasing detection avoidance strategies, such as wearing gloves to dissimulate their identity [Beauregard & Bouchard 2010].

Post-blast investigations In addition to precautions taken by the bomb maker, the mechanical and thermal effects caused by the explosion not only affect the possibility to recover traces, but also alter their integrity.

Fingermarks The possibility of revealing fingermarks on the components of an exploded explosive device has been very lately reported in literature. Given the heat and the extraordinary destructive force deployed by an explosion, the survival of traces are a priori low [Gardner 2010]. However, studies undertaken in fields involving analogous conditions, such as firearms or fire investigations, suggest the possibility to recover fingermarks from items submitted to very high temperatures. Several authors demonstrated that, on fire scenes, identifiable fingermarks could still be revealed, even on objects placed near the origin of the fire [Spawn 1994, Deans 2006, Moore *et al.* 2008, Bradshaw *et al.* 2008].

These conclusions are also supported by the results of Migron *et al.*, who revealed sebaceous fingermarks on brass fired cartridges [Migron *et al.* 1998]. Similarly, Zenger succeeded in revealing fingermarks on petrol bombs following passive headspace extraction of flammable vapours [Zenger 2004]. Nevertheless, fingermarks on Molotov coktails were irremediably affected by the gasoline - sulfuric acid mixture and could not be revealed anymore. Shelef *et al.* demonstrated that one third of fingermarks left on glass slides, bathed in accelerant and incendiarised could be recovered and were identifiable, after using toluene as a solvent for sonication [Shelef *et al.* 1996]. Stow and McGurry, who focused on petrol bombs debris, produced similar results, yet with a different method. They could recover most soot-covered or accelerant-contaminated fingermarks by washing traces with a 1 or 2 % NaOH solution [Stow & McGurry 2006].

Lanagan was the very first to publish in the public domain on the possibility to recover latent fingermarks from a vehicle detonated by two explosive devices, one containing Composition B, a mixture of RDX and TNT, and another containing an ammonium nitrate based dynamite [Lanagan 2006]. None of the fingermarks deposited on indoor and outdoor surfaces of the vehicle, as well as on items in the car could be detected, excepted for one fingermark that showed few ridge details. This information was however not exploitable.

These preliminary results are counterbalanced by the studies of Gardner and Sanders, who highlighted the possibility to recover fingermarks from post-blast debris [Gardner 2010, Sanders 2011]. Using reflective ultraviolet imaging systems, Gardner was able to locate one identifiable fingermark on a non porous surface. Shortly later, Sanders reported on the recovery of 5 fingermarks showing friction ridge details out of 42 control marks deposited on a total of 7 explosive devices, filled with different types of explosives.

Kuznetsov *et al.* also undertook few tests by exposing aluminium and steel plates with latent fingermarks at a short distance of a Composition B explosive charge [Kuznetsov *et al.* 2008]. Yet, no indication was provided on the quality of the obtained traces.

Using artificial solutions to mimick the chemical composition of fingerprints, some researchers recently found that amino and fatty acids are the only constituents to persist after explosion of IEDs. They developed and tested a dye reacting on these compounds and obtained promising results [Lewis *et al.* 2011a, Lewis *et al.* 2011b].

DNA The application of genetic analysis when the device has exploded is less popular. Although the degradation of genetic material seems thoretically unavoidable with the extreme conditions of an explosion, the recovery of DNA on fragments of an exploded device appears possible.

A study examined the feasibility to recover DNA from the remains of an exploded pipe bomb [Esslinger *et al.* 2004]. Twenty pipe bombs in metal and PVC were handled by 10 subjects. The profiles were determined by PCR / STR (Polymerase Chain Reaction / Short Tandem Repeat). These analyses allowed establishing a complete DNA profile and 3 partial profiles, matching those of the person who handled the bomb. Eight other matching profiles were detected, but their profile was below the threshold of identification. No influence of the material composing the pipe was noted on the chances to obtain a DNA profile. Yet, the degree of fragmentation of the device and the subsequent collection of these fragments are the variables that were reported to have a greater influence on the chances to recover a DNA profile. Overall, the greater the degree of fragmentation, the lower the chances to recover a DNA profile. DNA traces are more affected by the dispersion and fragmentation of the object due to explosion than the chemical process of the explosion itself.

Bille *et al.* studied the influence of 3 factors on the amount of DNA collected from exploded IEDs. Conducting their experiments on 6 pipe bombs, they evaluated the time between the collection and analysis of DNA, cyanoacrylate fuming and the location of biological material on the pipe bomb. After 1 week and 3 months from the day of collection, the quantity of recovered DNA significantly decreased to respectively 10 % and 1 % of the initial amount of deposited DNA. Cyanoacrylate fuming was reported not to affect the amount of collected DNA. Furthermore, larger amount of DNA were collected on the body of the pipes, rather than on their end caps [Bille *et al.* 2009].

The recent experiments of Berti *et al.*, which evaluated the possibility to recover saliva and touch evidences from exploded postal packages, confirmed that the quantity of DNA in the biological trace is determinant to obtain an exploitable profile [Berti *et al.* 2011]. Different quantities of biological material were deposited in the packages and filled with black powder, RDX or TNT. They highlighted that biological material containing a higher concentration of DNA, such as saliva, was more persistent than perspiration (touch evidences) and offered therefore greater chances to recover a profile. They also showed that STRs DNA typing could produce complete (over 16 detected loci) and partial (between 5 and 16 detected loci) biological profile when black powder and TNT had been employed. In contrast, the use of RDX hindered the obtainment of a full or exploitable DNA profile.

Hoffmann *et al.* focused their research on the secondary container, in which pipe bombs or IEDs are often packed [Hoffmann *et al.* 2012]. Eleven backpacks were used by volunteers for 11 days, before serving as containers for pipe bombs and being detonated. Partial and full DNA profiles of the handler were obtained from all swabs processed from different regions of the backpacks. Regions of the backpacks producing the most successful results were the top handle and the region close to the neck.

Alternatively, Foran *et al.* reported the possibility to identify the bomb assembler of an exploded pipe bomb through the recovery and analysis of mitochondrial DNA (mtDNA). Despite its weaker individualisation power compared to nuclear DNA, mtDNA demonstrated its potential of information in assigning half of the 36 exploded bombs to the correct handler [Foran *et al.* 2009].

Human scent

A few studies introduced human scent as a new possibility to link an explosion site or an explosive device with a person [Stockham *et al.* 2004b, Stockham *et al.* 2004a]. Stockham *et al.* collected human scent from post-blast debris of 6 explosive devices on pads using a Scent Transfer Unit (STU). Based on scent absorbed onto gauze pad, bloodhound dogs trailed and correctly identifed the target person in 66 % of the experiments, with no false positive.

Curran *et al.* used human scent specific canines to locate and identify individuals who had been in contact with improvised explosive devices. Despite some offset between their experiments and the conditions of real bombing case involving numerous people and, possibly, injured persons, the authors confirmed the potential evidentiary value of human odour in explosives investigation first highlighted by Stockham *et al.* [Curran *et al.* 2010].

This new type of trace was used in the investigations of a bombing casework in Pennsylvania [Meserve & King]. The human scent of the post-blast debris of an exploded package was presented to 2 bloodhounds. The dogs trailed a scent from the mailbox where the package was found to the building of the main suspect. A search at his home revealed the presence of many materials similar to that of the exploded device. Nonetheless, the admissibility of human scent evidence in California courts, and more specifically the use of a STU as an intermediate mean to collect scent, has been controversial as shown in two murder cases [Stockham *et al.* 2004a, Court of Appeal of the state of California 2004]. Considering this type of evidence was based on new scientific techniques and principles, human scent evidence did not meet the requirements of the Kelly-Frye rule[1] and was discarded from the panel of evidence.

Human scent identification or tracing is already often used in the scene processing of different crimes. Its exploitation in bomb scene investigation in an intelligence perspective appears as a promising and natural evolution of the field.

In summary, while not hindered in pre-blast investigations, the recovery of traces related to persons identification in post-blast investigations is altered by the effects of explosion. Nevertheless, these studies have highlighted the possibility to retrieve information from such traces after the use of render-safe tool or even in post-blast investigations. Although in many cases explosion somehow modifies the trace in such a way that it is no more detected, in some cases information is still present with a varying degree of quality.

Exploitation The first exploitation of these traces that springs to mind is their use as evidence for conviction. Through their potential of individualisation, traces such as fingerprint or DNA profile may provide the element that establishes the identity of the person who handled the bomb or its components. This nominative approach, in which the closeness between the trace and the material of a suspect is evaluated in a one to one comparison, designs the prosecutorial use of the trace [Barclay 2009]. This evidence-based culture prejudices the use of latent and imperfect information and leads investigators to exploit only high quality traces. This way of thinking can even affect the first steps of investigations, as emphasised by the results of Strom and Hickman. They showed that the lack of processing and exploitation of collected traces by law enforcement agencies was largely due to the absence of a specific suspect [Strom & Hickman 2010]. This strong interest in evidence production occults the generation of forensic intelligence which may be useful at different levels [Ribaux *et al.* 2010, Margot 2011c, Delémont *et al.* 2013]. Some authors have named these levels as tactical, operational and strategical, depending on the context in which intelligence is applied. It is worth noting though that, according to the country or the continent, the denomination and the number of levels can vary [Ratcliffe 2007, United Nations Office on Drugs and Crime 2011].

The first level, which is the most common form of intelligence, assists investigators and officers on *case-specific* activities. This approach is often arrests-focused and oriented towards the resolution of the particular case. Intelligence produced by the information extracted from the trace can support investigators in providing new leads, help identify potential suspects or uncover elements that assist the police officers in solving the case. The intelligence generated at this level is used in a proactive or reactive way in the context of the specific case.

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[1] This rule requires firstly the new technique or method to have gained general acceptance in its field. Secondly, the testifying person needs to be a qualified expert on the subject and thirdly, the correct scientific procedures must have been used.

The detection of series or repetitions can also assist in solving the specific case. The analysis of several traces or cases may result in the detection of a new series of connected cases previously identified as isolated, increase of the number of events associated with a series of bombings or confirm existing links between events established by other traces (manufacturing mode, DNA, etc.) [Rossy *et al.* 2013]. Rossy highlighted the complementarity of the different types of traces, as they do not provide the same level of information. Thus, the exploitation and combination of all traces are essential first steps that may reveal different dimensions of the criminal activity. The potential connections highlighted between cases can be based on the characteristics of the bomb (manufacturing mode, parts of the IED, the explosive substance), other traces conveyed by the IED (DNA, fingerprints, human scent, etc.) or the environment of the bombing (type of target, place, type of bomb, etc.). The intelligence generated from the repetitions or series is used in the context of the specific case in an operational perspective, with the aim to solve the case [Ribaux 2014].

In a broader dimension, intelligence is produced from a process of *generalisation* to anticipate and prevent crime [Ribaux 2014]. The detection and analysis of series or patterns, or the resolution or actions undertaken in one specific case or in a series of cases can generate knowledge that can be used to take decisions in order to reduce or anticipate crime. This can ultimately result in increasing security systems or surveillance in specific environments for a given period of time. Actions derived from such intelligence can be the deployment of resources to protect citizens or a specific area of the city, expand surveillance (increasing patrols, video cameras, etc.) or reduce the vulnerability of targets, for example, by diffusing information campaigns.

The actions derived from intelligence and the character of prevention can extend on a longer temporal scale. In the long term, the understanding of the criminal phenomenon, behaviour and environment engenders a form of general knowledge on which legislation, safety organisations or politics can rely on to prevent crime on a larger scale. The detection of new criminal trends or the identification of trafficking networks or routes can lead law enforcement agencies to take a number of measures. Such measures can be the restriction of the access to specific elements (detonators) or substances (precursors, substances such as ammonium nitrate, etc.) by enacting regulations on their marketing and use. An example is the control implemented in Ireland in the 1970s to reduce the use of military explosives or the regulation of the European parliament and council to control the marketing and use of explosives precursors [European parliament and council 2013]. Another possibility of actions directly derived from such dimension of intelligence is the reduction of criminal opportunities by implementing stricter safety measures in particular places, such as in airports. The increased security controls and restrictions on traveling with liquids in all airports throughout the world were applied to reduce the risk of bomb attacks on planes, based on the knowledge gained from similar events[2].

The generation of intelligence goes through the exploration and exploitation of all dimensions of information conveyed by a trace. Although the search of profiles or traces in national databases is one of the usual form of generation of intelligence provision from a DNA trace or fingermark, the output of database searches are often perceived as an "evidence provider" against a suspect rather than as an "intelligence generator" [Cole 2010, Margot 2011c]. Indeed, the contribution of other dimensions of information of such traces in investigations remains often unseen. By nature, the effects of explosion have the propensity to alter traces and therefore, to leave partial or imperfect traces. Still, the interesting study of Hicks *et al.* demonstrates the investigative potential of partial DNA profiles based on six loci or less [Hicks *et al.* 2010, Delémont *et al.* 2013].

Thus, even partial traces recovered from a bombing scene can play a major role in the reduction of the number of possible sources, the elimination of suspects or at a broader level in crime prevention [Barclay 2009].

1.2 Information conveyed by an explosive device

The information conveyed by an explosive device and its debris, and more precisely, the exploitation of this information directly relates to the notion of source of the objects or substances involved in the device and to the notion of action (the manufacture, transport, disposal and triggering of the bomb). The concept of common source in explosives investigation has been hardly discussed in literature. Despite numerous publications on the explosive charge itself, very few, if any, report on the multiple dimensions of information conveyed by an explosive device.

Information can be extracted from:

- the characteristics related to the components and the way elements have been assembled or the features pertaining to the mode of construction of the device;
- the physical and chemical characteristics of the explosive substance;
- the presence of traces on devices that arise from a common source (such as the same trace on two different explosive devices).

Exploitation These dimensions of information may be exploited at different levels. The way the different constituents (clock, fuse, etc.) have been assembled, the physical and chemical characteristics of the explosive substance or its residues, as well as the simultaneous presence of a trace on distinct devices provide information

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[2] In 2006, the British police foiled a plot planning to blow up 10 transatlantic flights flying from the UK to North America with peroxide based explosives concealed in soda drinks. Following this operation, all liquids and gels in the cabin were banned [Casciani].

on the explosive device as a whole. Therefore, the concept of inference of putative common source does not strictly concern the explosive substance alone, but also the whole explosive device. These elements may be exploited according to two distinct forms of reasoning [Barclay 2009]:

- an open process, in which the information conveyed by the construction of the IED, the explosive substance or the simultaneous presence of a trace on several devices is considered as an *investigation tool* and generates intelligence to assist inquiry. With a variable degree of certainty, forms of intelligence can be indications on the sequence of actions, or the *modus operandi* used by the bombers, indications on places where the components or substances have been bought or indications on the existence of a potential link between two or more devices. When used in the context, intelligence provision can greatly contribute to the investigative phase, by providing indications on the sequence of events of the bomb attack for instance.
- a closed process, in which the information extracted from the explosive substance or the components of the device is compared to a specific source. The degree of similarity or closeness is usually evaluated with the purpose to demonstrate a common source and to be used as evidence in court.

The following sections present the information extracted from the construction of an IED and from the explosive substance based on published works. The exploitation of these information sources are however not much documented in the public domain of scientific literature. The association of bombings to the sensitive issue of terrorism certainly prevents from communicating or publishing on the subject. Thus, literature reported below on the exploitation of information of IEDs certainly does not reflect the knowledge put into practice.

1.2.1 Construction of the IED

Information derived from the particular characteristics of manufacture of the explosive device, the rarity of a component or the similarity of construction of devices may be exploited in a prosecutorial or investigative purposes.

In the perspective of evidence demonstration in court, the existence of a link between two cases is very difficult to prove, based solely on the mode of construction of the explosive device, especially if no other evidence supports this hypothesis.

In United States v. Trenkler, two bombings perpetrated a few years apart involved explosive devices with numerous resemblances. Some of the components used and the mode of construction of the devices were similar. The debates of the Court illustrate the difficulty in evaluating such similarities in the absence of any other corroborative evidence [United States v. Trenkler 1995].

Considering the prosecutorial use of information, there is a general offset between the expectations of the courts, interested in the identity of the perpetrator, and the technical and scientific information extracted from the explosive device and presented by the expert. As attested by the Public Hearings on the Barron report in relation to the investigation of the 1974 Dublin and Monaghan bombings, one key point of the debates of February 2004 was the attempt to attribute these bombings to the IRA or the Ulster Volunteer Force (UVF) terrorist groups on the basis of their mode of construction, debris and chemical composition [Joint Committee on Justice Equality Defence and Women's Rights 2004].

As the evidentiary value of similarities between two devices is not evident to evaluate, information extracted from the construction of the device is more interesting in an intelligence purpose to assist inquiry, especially when there are no or limited leads or when a large number of possible sources is concerned at the beginning.

The type and characteristics of the components found on the scene may provide indications on an action, the sequence of events of the bombing or its preparatory acts, such as how the bomb was triggered or the identity of the VBIED used in a bombing. In the bomb attack against Rafiq Hariri in Beyrouth in 2005, due to the size and violence of the explosion, the only remains of the IED found on the crime scene were parts of the vehicle supposed to contain the explosive charge. Information conveyed by these debris allowed the identification of the vehicle and, in conjunction with other elements of the inquiry, corroborated that the explosive device was contained in a Mitsubushi van stolen in Japan [United Nations 2005, Nations Unies 2007].

Similarly, the rarity of a component of the explosive device, its particular characteristics of manufacture or the levels of skills or knowledge necessary to build the explosive system may significantly reduce the number of potential sources and accelerate the investigative phase of inquiry [Messler 1983, Barclay 2009].

Furthermore, devices exhibiting a similar physical aspect, sharing similar components or on which the same traces have been found may provide indications that events are linked. In light of the circumstances of the case, such intelligence support in the investigative phase of inquiry may guide the investigators towards new hypotheses. In the Omagh bombing in August 1998, remains of the timer and electric circuit that were part of the fertiliser based explosives car bomb were found. The similarity between these components and those of a dozen of other bombings with the same construction mode allowed establishing a link between these cases. Additional links could be established between other bombing cases with different construction modes, but with a timer displaying an identical serial number [Margot 2011a, Margot 2011b]. However, the elements of the case were insufficient according to the trier-of-fact to link the suspect to any of the attacks

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1.2.2 Explosive substance

Information provided by the physical and chemical characteristics of the explosive substance is systematically exploited in investigations. The evidentiary value of these characteristics depends on their frequency (rarity), which is directly related to the market structure (production, distribution and features related to its use).

Physical characteristics Haag studied the physical and chemical properties of black powder substitutes. He highlighted the possibility to differentiate them by their physical characteristics, despite their similar chemical composition [Haag 2001].

Differentiation of specimens on the basis of their physical characteristics requires nonetheless the collection and accumulation of data of various samples. Both the FBI (Federal Bureau of Investigation) Explosives and Chemistry Units Laboratory and the ATF (Bureau of Alcohol, Tobacco, Firearms and Explosives) National Laboratory Centre stores samples and collects data on the physical dimensions and chemical composition of different commercial black and smokeless powders [National Research Council 1998a]. Yet, the rarity of scientific literature on the use of such databases as investigatory tool does not allow perceiving their concrete use and actual benefits in law enforcement activities.

Chemical characteristics Whether in the form of bulk explosive[3], traces[4] or residues[5], the chemical characteristics of explosive substances have been measured by a variety of analytical techniques. The analysis of explosives and their residues has always been a prosperous field of publications. The reference book of Yinon and Zitrin (1993) confirms it with the compilation of an extraordinary number of articles [Yinon & Zitrin 1993].

One of the fundamental steps underlying forensic investigation is the recognition of information conveyed by a material and its potential exploitation, before

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[3] *Bulk explosive* or *bulk material* refers to an explosive substance which has not reacted. It is generally present in visible amounts to the naked eye. Bulk material may be recovered during a search or result from the dysfunction of an explosive device.

[4] *Traces of explosives* define explosives particles that did not explode. They are present in such quantities that they are invisible to the eye or hardly noticeable. They may be vapours or particles of explosives detected on the surface of a bag at an airport, for example.

[5] *Residues* are defined by the French dictionary le Littré as any "material remaining after a chemical operation, which can often still be used". Residues of explosives result from the chemical degradation of the explosive. They include products of reaction and also intact particles of explosives. Ordinarily, residues are particles not visible to the eye and whose presence must be established by sensitive analytical techniques.

generating intelligence or acting as evidence. A crucial question concerns the exploitability of information conveyed by pre-blast and post-blast material. In the context of explosives investigation, it is therefore possible to formulate a first hypothesis:

> Hypothesis I
>
> The search, collection and analysis of bulk explosives, traces or residues may provide useful information for the investigative process.

The following sections will discuss this hypothesis by establishing the state of the art in the analysis of inorganic explosives, taking account of both pre-blast and post-blast situations. However, since the research problem is more complex for residues than for bulk explosives, this chapter is mainly focused on explosives residues.

2 On-site collection

2.1 Debris of explosive device

In most cases, fragments of components of the explosive device are found on the site of explosion. Parts of alarm, batteries, electrical or electronic components or fragments of detonator may be dispersed over the site. Other components, such as remains of black powder fuse or tape may also be recovered.

In addition to the information they provide on the physical constitution of the device, these components are a potential source of explosives residues [Hoffman & Byall 1974]. The envelope and the firing system being in the close vicinity of the explosive charge, debris of these material are very likely to carry explosive residues. Thus, it is important to collect any fragment that was part of the device. However, the difficulty of this task on the scene must be emphasised, given the disturbance of the site.

2.2 Explosive residues

When objects are not portable, swabs are performed on surfaces close to the explosive charge, within the possible limits. In order to collect the organic and inorganic components of the charge, the target surfaces are successively wiped with dry swabs, swabs impregnated with distilled water, as well as with organic solvents[6]. Organic solvents used for this task vary from one laboratory to another. However, the most common are acetone, methanol (for swabs on surfaces with a special coating, such as varnish or paint), ethanol and methyl tert butyl ether (MTBE).

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[6] Brushes or vacuum cleaner may be use as sampling tools

Sampling in and around the crater must also be collected and stored in sealed, vapours-tight containers [Hoffman & Byall 1974]. A recent paper addressed the loss and exchange (cross-contamination) of compounds from dynamites stored in polyethylene bags [Sáiz *et al.* 2011]. Although the study concerns organic explosives, it is worth mentioning it, as it is the first study that examines such issue.

2.3 Blanks

The collection of blanks (controls) on the site of explosion is crucial to ensure that the compounds detected in the analysis do not come from the background or from a contamination of the site. The difficulty related to this type of sampling is the delineation of an area free from debris or residues of the explosion. In addition, these blanks must be packed and stored in order to avoid contamination by other samples and to preserve their integrity.

3 Physical examinations and treatments of specimens

3.1 Bulk explosives

Visual examinations enable the scientist to determine the homogeneity and morphology of the material [Hoffman & Byall 1974]. When several components compose the charge, they are separated. Mechanical separation is preferable, because solvent extraction, especially with water, can sometimes change the chemical composition of the mixture. Indeed, the chemical constituents recovered after water evaporation may no longer correspond to those that have been dissolved [Beveridge *et al.* 1975, Beveridge *et al.* 1983].

3.2 Explosive residues

Material collected on the site is examined, sorted and analysed in order to identify the type of explosive used and the possible components of the device [Calisti *et al.* 1998]. When the quality and quantity of material allow it, the device is reconstructed. Otherwise, the nature of the fragments may provide an idea of the type of device used.

Visual examinations allow on one hand to identify the characteristics of some components (number, packaging, particular mechanisms etc.) and, on the other hand, to recover other traces (fibers, tool marks, etc.). The debris are then sorted according to the appropriate method of treatment. The method of treatment is determined by the nature of the support (plastic, metal, wood, etc.), the surface coating (presence of varnish, paint, etc.), the appearance of finish (smooth, porous, rough) and the matrix (presence of grease, oil, resins, etc.). Based on these parameters, debris are treated by brushing, dipping, washing with spray or rubbing with swabs impregnated of solvents. Residues collected on swabs and brushes are finally collected in two solutions, one organic and one aqueous solutions.

Organic constituents are extracted with acetone, methanol, MTBE or ethanol [7]. The mineral constituents are extracted with distilled water, which can be heated, according to the practice of the laboratory [Calisti *et al.* 1998]. Yet, this procedure seems to cause an increase of background noise, due to the increased solubility of interferences [Reutter & Buechele 1983, Beveridge 1998].

A new method was proposed to recover simultaneously organic and inorganic species in an ethanol - water mixture (50: 50) [Warren *et al.* 1999]. The extraction is performed with the same solution and applied directly onto a solid phase extraction system (SPE). Although the technique is simple and effective, results showed a general decrease in the recovery rate of organic analytes, accompanied by the loss of some inorganic species.

While solutions of clean specimens are simply filtered, the extraction step is combined to a process of concentration and purification for dirty specimens. SPE is the most common used technique in the presence of a complex matrix [Doyle 2013]. Supercritical fluid extraction (SFE) and solid phase micro extraction (SPME) are also used in some laboratories.

4 Analysis of inorganic explosives

4.1 Analysis of bulk explosives

Analytical techniques used for the identification of oxidisers and fuels are mainly the same for bulk explosives and explosives residues. The following methods are usually employed: microchemical tests, X-ray diffraction (XRD), scanning electron-energy dispersive X-ray spectroscopy (SEM-EDS), Fourier transform infrared spectroscopy (FTIR), ion chromatography (IC), capillary electrophoresis (CE) and gas chromatography (GC) [Bender 1998]. The application of these analytical techniques is detailed in the following section.

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[7] A study has shown the ability to perform the extraction with distilled water, followed by solid phase extraction (SPE) [Thompson *et al.* 1999]. Except for traces of explosives on clothing, this method seems to offer a better selectivity for complex matrices.

The fundamental difference lying between the analysis of bulk material and residues is the possibility to quantify the different components of the former. It is then possible to compare the qualitative and quantitative composition of an explosive charge with that of a material of a comparison, seized during a search for example. However, a proper interpretation of such results is not possible in the absence of adequate knowledge that is further discussed.

4.2 Analysis of explosive residues

4.2.1 Origin and nature of residues

During the decomposition of an explosive, three types of chemical species are observed:

- gaseous decomposition products, such as CO_2, CO, H_2O, NH_3, CH_4, etc. ;
- solid decomposition products, such as chlorides and perchlorates for a chlorate based explosive;
- particles of explosives that have not reacted during the explosion. In theory, the molecules of an explosive are entirely converted into reaction products. However, practice has shown that even after detonation, intact particles of explosive are always recovered. It seems that the detonation shockwave scatters a fraction of the exterior of the charge, before it reacts. In the case of inorganic charges, residues left by the explosion are found in greater quantities. Indeed, during deflagration, the rise in gas pressure leads to the rupture of the envelope, before the entire mixture has reacted [Yallop 1980, Kelleher 2002]. It is the existence of particles of explosive, still intact after explosion, which allows the forensic exploitation of residues.

Therefore, the presence of intact particles of explosives is a prerequisite for the identification of the chemical nature of the explosive. In addition, the detection of some reaction products may corroborate the conclusions of the analysis. It is important to note that only the qualitative composition can be determined. As all the residues are not entirely recovered, the quantitative aspect of the different compounds appears less exploitable. In practice however, quantitative data are collected, reported and adduced as evidence [Doyle 2013].

4.2.2 Applicable techniques

To comply with the variety of constituents of an explosive charge, implemented analytical techniques must not only be manifold but also complementary. It is recognised that a single analysis technique is insufficient to identify a compound [Phillips & Hiley 1999, Technical Working Group for Fire and Explosives 2004, Technical Working Group for Fire and Explosives 2007]. The American Technical

Working Group of Fire and Explosives (TWGFEX) developed recommendations on the minimum number of analytical methods necessary to the identification of various explosive substances in pre-blast and post-blast investigations. A categorisation of the techniques is also given according to the degree of identification they permit. The number of required methods depends therefore on the complementarity of each technique[8].

Microchemical tests or spot tests Despite the amount of material that some spot tests require, these are still used as preliminary indicative tests for their rapidity and low cost [Parker *et al.* 1975, Abramovich-Bar *et al.* 1993, Almog 2006]. The best known are the Nessler reagent for the detection of ammonium ion, the silver nitrate reagent for the detection of chloride, barium chloride to test for the presence of sulphate and the pyridine-based test for sulphur. Test strips are also used by some laboratories to test for the presence of nitrate, nitrite and chloride. These tests provide indication on the potential presence of analytes, they do not allow the identification of a substance. They are poorly selective, and as such, a positive reaction may be induced by several substances. Thus, their results always require to be confirmed with analytical techniques. Due to their indicative nature, they may greatly contribute in investigations by eliminating potential sources or by orienting analyses. However, they are unreliable and inadequate to be used as evidence to infer the source of an explosive.

In the Birmingham Six case, the Griess test was applied on swabs taken from each of the suspects [Woffinden 1987]. A positive reaction of the test was obtained for two of suspects. These results were however not confirmed by the subsequent TLC (thin layer chromatography) and GC-MS (gas chromatography-mass spectrometry) analyses. A misinterpretation of the results of the forensic tests and analyses, as well as inappropriate expert testimony, in conjunction with other highly questionable elements, led to a serious miscarriage of justice[9].

Infrared spectroscopy (IR) Infrared spectroscopy is mainly employed to identify inorganic oxidising agents and organic compounds (sulphur, sugars) in bulk explosives. Despite its difficulty in detecting trace anions, IR is sometimes applied to the analysis of residues, for example to identify the presence of thiocyanate SCN^- in black powder residues [Miller & Wilkins 1952, Beveridge *et al.* 1975].

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[8] For example, the identification of ions requires the application of at least two analytical techniques.

[9] In this case, the suspects had been playing cards in the moments preceding their arrest. Nitrocellulose, which enters in the composition of many paints and varnishes, may produce false positive with the Griess test. The varnish covering the cards may well have reacted with the test. Although the police said the test did not react to cards, there was a request to submit the pack of cards to additional independent tests. However, the pack was not found and notified as mislaid. The outcome of such a case demonstrates the inappropriateness to use the results of indicative tests for source inference.

The X-ray diffraction (XRD) XRD allows the identification of the crystalline structure of a compound. With the advantage of being non-destructive, this technique provides excellent results for pure compounds. In contrast, the analysis of explosive residues is more challenging if the contribution of the matrix is unknown to the operator [Tamiri 2000].

Scanning electron microscope coupled to energy dispersive X-ray spectroscopy (SEM/EDS) This technique serves mainly to identify metal fuels and sulphur. It allows scanning quickly the composition of an explosive mixture and provides the elemental composition of some particles. It must be supplemented by another technique, such as FTIR, CE or IC. Pyrotechnic compositions can be analysed by SEM/EDS. By observing the specific morphology of residue particles, Kosanke et al. reported the successful differentiation of pyrotechnic reaction residues from unrelated material [Phillips 2001, Kosanke *et al.* 2003, Kosanke *et al.* 2006].

It is important here to emphasize the priority of initially analysing a sample that is in a solid form by methods such as XRD, IR or SEM-EDS, prior to their dissolution in a liquid. Indeed, according to the ions originally present, the subsequent drying of residues contained in aqueous solutions can result in a chemical reduction of the compounds. By analysing explosive residues by IC before and after XRD analysis, Reutter and Buechele observed that almost half of chlorate originally present in the residues had been reduced into chloride [Reutter & Buechele 1983].

Ionic species analysis: ion chromatography and capillary electrophoresis Ion chromatography (IC), and more recently, capillary electrophoresis (CE) are, to date, the two most successful and most used analytical methods for the analysis of inorganic explosives and their residues. Both techniques are considered as complementary, because their separation mechanisms are different: CE is based on differences in ion electrophoretic migrations, while separation of IC is mainly based on differences in ion interactions with a stationary phase [Haddad 1997]. Whatever the method, cations and anions are usually analysed separately. The results can thus confirm the presence of a cation or an anion, but not the presence of the whole molecule of oxidiser or fuel [Bender 1998].

Some authors studied the composition of inorganic explosives residues in order to improve knowledge on the ionic chemical profile [Beveridge *et al.* 1983, Hargadon & McCord 1992, McCord *et al.* 1994, Klapec & Ng 2001, Kuila *et al.* 2005, Hopper & McCord 2005, Technical Working Group for Fire and Explosives 2007]. The following table (**Table 3.1**) shows some commercial and homemade compositions frequently encountered, as well as ions that may be observed with ionic analyses. Ordinarily, only a portion of the ions is detected in the ionic profile of residues; all ions of a formulation are rarely observed.

Explosive substance	**Common composition**	**Detected ions**
Black powder	KNO_3, C, S	NO_3^-, NO_2^-, SO_4^{2-} HS^-, SCN^- OCN^-, HCO_3^- CO_3^{2-}, S^{2-}, $S_2O_3^{2-}$, K^+
Pyrodex black powder	KNO_3, C, S, $KClO_4$ C_6H_5COONa $NH_2C(NH)NHCN$	NO_3^-, NO_2^-, SO_4^{2-}, HS^-, SCN^- OCN^-, HCO_3^-, CO_3^{2-}, Cl^-, ClO_4^- ClO_3^-, $C_6H_5CO^-$, $N(CN)^{2-}$, K^+, Na^+
Flash powders	$KClO_4$, S, Al or Mg	SO_4^{2-}, HS^-, Cl^-, ClO_4^-, ClO_3^-, K^+
Chlorate-sugar	(K^+ or Na^+)ClO_3^-, $C_{12}H_{22}O_{11}$	Cl^-, ClO_3^-, ClO_4^-, K^+ or Na^+
ANFO	NH_4NO_3, hydrocarbon	NH_4^+, NO_3^-, NO_2^-, HCO_3^-

Table 3.1. Ionic composition of residues of common explosive mixtures.

Ion chromatography (IC) Ions are partitioned between an ion exchange resin (stationary phase) and the ionic aqueous medium (mobile phase). The detection of ions is obtained by a conductivity or an indirect UV/visible detector. IC is not only used for the analysis of inorganic residues, but also for that of sugars. The main advantage of ion chromatography is its great resolution and limit of detection [Woolfson-Bartfeld *et al.* 1990, Abramovich-Bar *et al.* 1993, Doyle *et al.* 2000, Klassen 2002].

Dicinoski *et al.* detailed a short review up to 2006 on the analysis of inorganic explosives by IC [Dicinoski *et al.* 2006]. The simultaneous determination of inorganic explosives using a portable IC with two columns connected in series was reported by Meng *et al.* [Meng *et al.* 2008]. Johns *et al.* described an IC method for the separation and detection of 18 anions and 12 cations often encountered in inorganic compositions. The analysis of specimens from controlled detonation of explosive devices were used to validate its application [Johns *et al.* 2008]. Recently, Lang and Boyle optimised an IC-MS method to determine ascorbic acid and its degradation products in intact black powder substitutes and their residues [Lang & Boyle 2009]. Lastly, Tyrrell *et al.* proposed a coupled reversed-phase and ion chromatographic system for the simultaneous identification of 10 target anions and 9 organic compounds through one single sample injection [Tyrrell *et al.* 2011]. A solvent mixture of acetonitrile/Milli-Q water (50:50) appeared as the best compromise for the extraction of analytes from swabs.

Capillary electrophoresis (CE) Capillary electrophoresis is based on the migration of charged species in a sample solution under the influence of an electric field. Inorganic ions are usually detected by indirect UV/VIS, with separate runs for anions and cations. The advantages of capillary electrophoresis is a short time of analysis (15 minutes or less) and the ease of sample preparation [Hargadon & McCord 1992, Doyle & McCord 1998, Kishi *et al.* 1998, Miller *et al.* 2001]. In addition to the full method validation described for 10 target anions, Sarazin *et al.* also studied potential matrix effects. No significant matrix effect was observed, when residues were extracted from soil, metal or cloth [Sarazin *et al.* 2010c].

The simultaneous analysis of cations and anions by CE with a single electrolyte was the logical ensuing improvement. Via dual end injection, one portion of the solution is sequentially introduced at both ends of the capillary. The analytes migrate against each other, towards the centre of the capillary. They are separated by a single analysis and detected by a fixed or removable system [Kuban & Karlberg 1998, Kuban & Kuban 2002, Kuban *et al.* 2002, Hopper *et al.* 2005, Sarazin *et al.* 2010b].

A major technological breakthrough was the miniaturisation of CE technology, a particularly attractive feature for field portable instruments. Wang *et al.* analysed cations and anions separately using microfluidic systems imprinted on microchip and reduced the time of analysis to less than one minute [Wang *et al.* 2002]. With the same technology, they further developed a dual opposite injection microchip, allowing the simultaneous separation and detection of both organic and inorganic cations and anions in about 3 minutes [Wang *et al.* 2003]. Hutchinson *et al.* reported the separate analysis of 11 target cations and 15 target anions in explosive residues with a portable CE [Hutchinson *et al.* 2007, Hutchinson *et al.* 2008]. They successfully tested their method with field controlled detonations of IEDs.

Detailed reviews of the technological evolution of CE in the analysis of inorganic explosives are provided by Pumera and Sarazin *et al.* [Pumera 2006, Pumera 2008, Sarazin *et al.* 2010a].

Liquid chromatography (LC) Gapeev and Yinon successfully identified the following inorganic oxidants in explosive mixtures and residues by electrospray ionisation-mass spectrometry (ESI-MS): $NaMnO_4$, NH_4ClO_4, $KClO_3$, $NaBrO_3$. In positive mode, cations are identified by their "ionic envelope", a series of multicharged molecular ions, centred on the cation positively charged and containing the entire molecule of the oxidiser. The MS/MS analysis of the results produced by ESI-MS allowed them confirming the composition of the ions in series [Gapeev & Yinon 2004]. This method of chemical ionisation was also used by Zhao and Yinon to identify the molecule of ammonium nitrate [Zhao & Yinon 2001] and several oxidisers (NaNO3, KNO3, (NH4)2SO4, K2SO4, NaClO3, NH4ClO4, NaClO4) present in explosive formulations [Zhao & Yinon 2002]. Urea nitrate was also characterised with ESI and atmospheric pressure chemical ionisation (APCI) [Tamiri 2005, Almog *et al.* 2007]. Tamiri *et al.* proposed the use of crown ethers to isolate urea nitrate in post blast residues before analysing the adducts formed by APCI [Tamiri *et al.* 2009]. Recently, Cummins *et al.* analysed bulk, as well as post-blast residues of black powders via a porous graphitic column using high-performance liquid chromatography (HPLC)-ESI-MS [Cummins *et al.* 2011]. Anions are separated as a result of the temporary dipole moment induced in the stationary phase by the proximity of the charged anion.

These results represent a significant advance in the analysis of inorganic explosives: this techniques allows the identification of the whole oxidising agent's molecule and appears therefore as an excellent complementary method to IC or CE.

Gas chromatography (GC) Some tests were performed to identify ions by GC-MS. Inorganic ions are either derivatised or incorporated into molecules to allow their identification by GC-MS. Despite encouraging results, these types of analyses only permit the identification of specific ions. Some ions such as chlorate, perchlorate or sulphate can not be detected [Funazo *et al.* 1985, Chen *et al.* 1987, Sakayanagi *et al.* 2006]. GC-MS is preferably employed for the analysis of reducing agents, such as sugars or hydrocarbons. Sugars contained in mixtures or in explosive residues can be analysed by GC-MS after derivatisation with an agent of trimethylsilyl type [Beveridge *et al.* 1983, Nowicki & Pauling 1988]. Glucose and fructose are often identified in residues by this way. It seems however that these monosaccharides are not reaction products of sucrose, but rather result from its hydrolysis when dissolved in solution. Alternatively, sugars can also be analysed by TLC.

Brown *et al.* analysed ammonium nitrate by SPME-GC-MS after derivatisation with ethyl or butylchloroformate [Brown *et al.* 2004]. This method, particularly suitable for ANFO type explosives, allows detecting ammonium nitrate and fuel with the same technology. Bradley *et al.* reported on the identification of elemental sulfur contained both in pre and post-blast inorganic explosive mixtures [Bradley 2005]. Routon *et al.* successfully discriminated commercial Pyrodex and Triple Seven black powders by GC-MS [Routon *et al.* 2011]. Both powders have the same appearance and the same oxidisers. However, they have different organic fuels. The analysis of the trimethylsilyl derivatives of their organic fuels enabled the authors to differentiate the two commercial powders.

Other techniques Prest *et al.* reported the use of a isotachophoresis chip to determine chloride, chlorate and perchlorate in inorganic explosive residues [Prest *et al.* 2008]. Mahoney *et al.* analysed specimens of black and smokeless powder using time-of-flight secondary ion mass spectrometry (TOF-SIMS) [Mahoney *et al.* 2006]. With this technique, Pyrodex could be differentiated from the five different grain size of Geox samples (1-4Fg). The Geox samples could however not be further differentiated.

4.2.3 Note on the preservation of evidence

During investigation, inorganic explosive residues are likely to deteriorate between the moment of explosion, their collection and their analysis. With IC, Reutter and Buechele observed the high volatility of ammonium nitrate, through the drastic loss of ammonium.

Aqueous extracts must be analysed as quickly as possible, because some inorganic constituents may be rapidly hydrolysed.

The whole specimen of residues is extracted in aqueous and organic solutions. In general, there is no material left for a cross-examination. Moreover, aqueous solutions can not be kept for a second analysis (cross-examination). Reutter and Buechele found that, even stored in a refrigerator in sealed vials, the concentrations of ammonium, nitrite and nitrate decreased significantly over time [Reutter & Buechele 1983]. The article does not mention the rapidity with which these concentrations were altered. Tests undertaken by the WFD showed that the solutions of samples should be analysed within 15 days from the dissolution or extraction time of the specimen [Schlatter 2005].

5 Interpretation of results

Results interpretation of explosive residues is an essential step in the inference of the hypothetical composition of the explosive charge[10]. In addition to the qualitative aspect, some authors have also mentioned the exploitation of semi-quantitative data to differentiate two explosives. These aspects are discussed below.

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[10] In the absence of explosive residues, it seems however that a hypothesis about the type of explosive of an IED may be formulated. Oxley documented the fragmentation characteristics of pipe bombs in order to identify the nature of the energetic material in the absence of chemical residues [Oxley *et al.* 2001]. The influence of the pipe size, the firing system, the type of charge and its quantity on fragmentation were studied. The explosive power and the nature of the explosive charge were clearly reflected in the number, size and appearance of the pipe fragments. This evaluation of the nature of the explosive however requires the trained eye of a specialist. The microstructure and hardness of fragments of exploded pipe bombs were examined in two other metallographic studies [Walsh *et al.* 2003, Dawson *et al.* 2004]. The physical and microscopic appearance of the material (fractures, deformation, etc.) very often provides reliable evidence of explosives involvement [Doyle 2013].

5.1 Inference of the type of explosive

Currently, the identification of all the compounds involved in an explosive mixture is generally not achievable with the application of one single method of analysis. The composition of the explosive charge is determined by gathering and interpreting the results obtained by the different analytical techniques.

Bulk explosives (pre-blast) Despite the high selectivity of IC and EC, these two techniques do not provide structural information on the molecule. They only allow the identification of ions, not the identification of the whole molecule. The potential chemical constituents of the explosive charge are inferred mainly from ions observed in the anionic and cationic profiles. This task is often complicated by the presence of several anions and cations that are, each, compatible with a certain number of oxidisers. These results are compared with fuels and other detected substances in order to infer the composition of the explosive mixture. It would be interesting to confront the results obtained by this method of interpretation for the inference of the type of explosive with those of a more objective approach, using Bayesian networks. For bulk material, the use of methods such as IR or XRD are a valuable help for the identification of the compounds.

Explosive residues (post-blast) Contrary to what is reported by Dicinoski *et al.* [Dicinoski *et al.* 2006], and as also demonstrated by Kuila *et al.* [Kuila *et al.* 2005], the identification of inorganic explosives is not unequivocal. Tamiri *et al.* and Almog *et al.* also highlighted the ambiguity residing in the identification of these substances [Almog *et al.* 2007, Tamiri *et al.* 2009]. They discussed the case of urea nitrate. The identification of this compound is uneasy, due first to its hydrolysis and transformation into urea and nitric acid in the presence of water, secondly to the legitimate presence of urea and/or nitrate in some cases (some AN fertilisers do contain urea as well) and third, to the formation of artefact urea nitrate during the analytical process according to reactions between urea, nitrate salt and a source of protons. Therefore, the identification of urea and nitrate by CE or IC does not necessarily imply the presence of urea nitrate.

Interpreting results requires to take into account not only the selectivity and limit of detection of each method, but also the combination of anions and cations detected, their concentration, their frequency in the environment and potential interferences [Murray 1998].

5.1.1 Background components

Only two publications have been dedicated to the examination of background levels of inorganic substances in the environment [Walker *et al.* 2001, Lahoda *et al.* 2008].

Walker *et al.* collected samples in England during winter and summer on vehicles, houses, hotels, the exterior walls of buildings, roads and traffic signs. Most of the target ions were found in all tested areas: chlorides, sulphates, nitrates and phosphates were the most frequent and abundant, unlike fluoride, nitrite and chlorate, which were detected in lower concentrations.

Sodium and calcium were present in almost all samples. Potassium and magnesium were found in half of the samples. Ammonium was not detected in many samples, but was present in significant amounts in samples collected from walls. Glucose, fructose and sucrose were particularly present in samples collected inside houses, hotels and cars. During winter, detected levels of sodium and chloride were significantly more important than in summer; this phenomenon was attributed to the salt shed in winter against frost.

On the contrary, perchlorate and thiocyanate were not observed in any of the 71 samples analysed. On the basis of these results, their presence in residues has therefore a higher evidentiary value than those of ions, such as sulphate or nitrate.

The results of Lahoda *et al.* support these findings. They analysed over 300 samples collected in 28 cities of the United States of America. Samples were collected in autos, airports, public and government buildings, hospitals, hotels, mailboxes, outdoor locations, parking meters, post offices, pay phones, restaurants, transportation centres and sports arena.

The more common ions encountered in the environment were potassium, sodium, calcium, chloride, nitrate and sulphate. Ammonium, nitrite, chlorate, perchlorate, cyanate and thiocyanate were rarely observed.

These studies provided the recovered quantities for each ions. However, these figures are indicative, as factors such as the collection mode, the type of swabs used and the extraction method all impact on the recovery rate of ions and vary between laboratories. Nevertheless, these contributions fill an important gap in literature and are a first step towards a better interpretation of results. Further published research is however needed to confirm and expand information on background levels of target ions.

5.1.2 Sampling of blanks

The presence of relatively frequent inorganic ions in the environment highlights the importance of control samples (blanks). Essential to interpretation of results, appropriate control samples are difficult to collect from the scene: they need to be made sufficiently close to the site, though in places out of reach of explosion projections. However, the force of the explosion is often difficult to estimate, making the position of blanks delicate to choose. Many surfaces, even far from the epicentre, may be covered with residues.

A mathematical model of distribution, which ignores the mass of the charge, predicts that beyond a radius of 60 meters from the epicentre, the residues concentration decreases rapidly to zero [Kelleher 2002]. The author was not able to test the accuracy of the equation. However, three other studies on the explosion of large improvised explosive devices have effectively demonstrated that the quantity of residues found at about 60 meters was very low or almost equal to zero [Cullum *et al.* 2000, Phillips *et al.* 2000, Monsfield *et al.* 2001].

These studies also demonstrated that the collection of blanks on the back of surfaces exposed to the charge is not appropriate: they are likely to present as much residues as the side which is directly exposed to the direction of the blast.

5.1.3 Confirmation of the first hypothesis

In pre-blast investigation, the discovery of an improvised explosive device may raise several questions. The judge will primarily be interested in the identity of the substance composing the charge and whether it is an explosive[11]. Literature has shown that analytical techniques provide information on the chemical nature of inorganic explosive charges, which may be useful to the investigations.

As part of post-blast investigations, the type of device implemented and the nature of the charge are questions with which the expert is usually confronted. Whatever the propagation speed of the reaction (deflagration or detonation), intact particles of explosives remain after the explosion. Their presence in residues allow identifying the type of explosive involved. These elements confirm the initial hypothesis:

> Hypothesis I
>
> The search, collection and analysis of bulk explosives, traces or residues may provide useful information for the investigative process.

Traces of explosives found on a suspect, (i.e. particles of explosives on clothing or hands) or bulk explosives found in a house during a search may strengthen or discredit his connection in an activity involving explosives. However, the analytical information extracted from this material pertain only to the chemical nature of the explosive. This leads to the formulation of the second hypothesis:

> Hypothesis II

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[11] Additionally, he may also be interested in knowing if there is a link between two cases, if the detected DNA profile allows identifying the bomb maker or in a pre-blast case, the reality and risks associated with the threat.

> The chemical information obtained by current techniques allows reaching a conclusion on the chemical nature of the explosive and to infer the type of explosive used. However, the exploitation of this analytical information does not allow differentiating between two specimens of explosives with the same chemical nature but of different sources.

5.2 Differentiation of explosives and explosive residues of the same chemical nature

5.2.1 In pre-blast investigations

The differentiation of two explosive substances with the same chemical nature is currently possible from a qualitative point of view, i.e. if the detected compounds are different. It is for example possible to distinguish a common black powder from a Pyrodex black powder substitute, which contains additional sodium benzoate and dicyanamide compounds.

Dicinoski *et al.* reported a real case in which intact explosives were differentiated on the basis of semi-quantitative data [Dicinoski *et al.* 2006]. The perpetrator of an attempted murder case used an IED with a slurry explosive. Using relative concentrations of the slurry compounds, the FBI scientists matched the composition of the slurry with that of a commercial slurry. On the basis of this information, the police arrested a suspect which had access to this specific slurry.

The use of semi-quantitative profiles in a one-to-one comparison emphasises the feasibility of such an approach. It is tempting to use quantitative or semi-quantitative data in order to differentiate intact explosives of the same nature or to infer a common source. However, for the differences or similarities to be properly exploited, data on quantitative variations of these compounds in explosives are required from different manufacturers, brand and production batches, as well as data on variations within a manufacturer, brand and batch.

5.2.2 In post-blast investigations

Some authors who studied the composition of inorganic explosive residues also attempted to exploit semi quantitative information of the ionic profile in order to differentiate residues from two explosives of the same chemical nature (e.g. residues of two black powders) [McCord *et al.* 1994, Hopper & McCord 2005].

However, the interpretation of such results requires prior knowledge about:

- the influence of the conditions of explosion on the formation of residues[12];
- the adherence and persistence of these residues in the environment and on different surfaces;
- the efficiency of collection of the compounds or ions by the sampling and extraction methods.

In regard to the efficiency of collection and extraction, one single study provides data on the efficiency of sampling by cotton swab saturated with a mixture of isopropyl alcohol - water (70: 30) [Jung 2004]. The author showed that the method used to extract inorganic ions from swabs resulted in a high percentage of recovery oscillating between 84 and 95%. On the contrary, the rate of inorganic oxidants collected on hands with wet cotton swabs ranged between 7 and 27%, with higher rates of recovery at low concentrations.

In addition, the use of semi-quantitative data implies that the components of the residues are distributed evenly across the site. However, if we consider three different samples collected after an explosion, a sample of soil from the crater, some fragments of the device and various pieces of plastic of the envelope, the relative amount of ions may be different from one sample to another. Some ions may be present in traces in a sample, while not being detected in the two others.

Theory describes that the distribution of residues follows a law which is a function of the inverse square of the distance. The practice, however, is different. Many parameters influence the distribution of residues, including the position of the charge and its geometric shape, the presence of wind, air resistance, the adhesion of residues to surfaces, the positive and negative pulses of the shock wave, etc. [Cullum *et al.* 2000, Phillips *et al.* 2000, Kelleher 2002].

Furthermore, high concentrations of residues are not only found in the vicinity of the centre of the explosion, they can also be recovered at large distances from the crater [Kelleher 2002]. The distribution of residues does not simply follow a law equal to the inverse square of the distance.

This phenomenon was confirmed by two studies implementing a number of improvised explosive devices of very large size (454 and 2268 kg). The mixtures were composed of ammonium nitrate / sugar, ammonium nitrate and calcium carbonate / sugar, TNT or ANFO. The results showed that, on certain surfaces, the detected amount of some ions, such as nitrate and ammonium increased, while moving away from the original location of the charge. In addition, the quantities of materials recovered on witness plates located at the same distance but in different directions, varied considerably [Cullum *et al.* 2000, Phillips *et al.* 2000].

Moreover, water used to fight fires developing subsequently to explosion and atmospheric moisture may alter post-blast residues, adding therefore a supplementary

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[12] The formation of residues depends on numerous conditions related to the explosion, particularly the temperature, the pressure, the type of container, the degree of confinement, etc.

dimension of complexity in their interpretation [Royds *et al.* 2005].

Thus, the exploitation of semi-quantitative data may perhaps offer the possibility to differentiate residues of the same type of explosives. However, current knowledge does not allow a correct interpretation of results.

5.2.3 Confirmation of the second hypothesis

The following scenario summarises the situation. Following a bomb attack, explosive residues are collected on the site of the explosion. In parallel, investigations lead to the seizure of bulk explosive at the suspect's home. Analysed with routine analytical techniques, they are respectively identified as black powder and residues of black powder. Currently, the hypothesis of a common source can not be ruled out. Their comparison only allow determining that the seized explosive is likely to produce the same residues as those collected on the site.

Unless there are unusual or characteristic substances, the degree of the analytical link is limited to the identification of the type of explosive involved. In other words, the link is qualitative, not quantitative. These elements confirm the second hypothesis:

> Hypothesis II
>
> The chemical information obtained by current techniques allows reaching a conclusion on the chemical nature of the explosive and to infer the type of explosive used. However, the exploitation of this analytical information does not allow differentiating between two specimens of explosives with the same chemical nature but of different sources.

The use of semi-quantitative data to persuasively differentiate explosives of the same type, whether in pre-blast or post-blast investigations, is not conceivable in the present context of knowledge.

To date, no routine analytical method allows inferring a common source, or even distinguishing between two specimens of explosives or between explosive residues with the same chemical nature but coming from different sources.

6 Legitimacy of the research

One of the major concerns in forensic science is the identification and, more particularly, the establishment of the identity of source. The concept of identification in forensic science involves a classification procedure, whereby an object of a category is classified in a smaller or narrower class. This identification process uses a concept of reduction of the initial population and may, if the number of possible sources equals one, lead to individualisation. This reduction to a person or a single object can be observed for some traces such as fingerprints, DNA or shoe traces. Individualisation is described by Kirk as the essence of forensic science [Kirk 1963].

> "The real aim of all forensic science is to establish individuality, or to approach it as closely as the present state of the science allows." ([Kirk 1963], p. 236)

For some types of traces, current analytical techniques in forensic science allow, in an ever more sensitive way, identifying the class to which a specimen belongs. However, this qualitative identification only allows the distinction of specimens with different chemical species, i.e. belonging to separate classes. This is especially true for paints, fibres, soils, weathered flammable liquids, whose degree of identification is limited to the chemical nature of the substance.

As demonstrated by the first two hypotheses of this work, this has also been confirmed for inorganic explosives. Despite the growing number of improvised charges, few studies have actually contributed to the evolution of their investigation in forensic science: most studies have focused on the application or the comparison of one analytical method with another. Several analytical techniques are available to identify the type of explosive used. Nevertheless, while the limit of detection and selectivity of these techniques have improved over the years, the level of information obtained remains equivalent. They do not provide sufficient information to differentiate specimens of the same chemical nature.

6.1 Isotope ratio mass spectrometry

Isotope ratio mass spectrometry is widely used in geochemistry to determine the origin and formation of rocks, the activity of geological processes or the climate of past times [Ghosh & Brand 2003]. It is also extensively used in ecology to trace the activity of plants [Ammann *et al.* 1999, Saurer *et al.* 2004] or in archeology to reconstruct climate or ingested food of individuals [Longinelli 1984, O'Connell & Hedges 1999b, Sharp *et al.* 2003]. Its many and varied applications, especially in food authentication [Kelly 2003, Forstel 2007], have demonstrated its ability to distinguish substances coming from different origins.

The number of publications on the use of stable isotopes in a source inference perspective attests to the increasing popularity of isotope ratio mass spectrometry (IRMS) in forensic science. Exploratory studies on TNT and cannabis were among the very first forensic applications [Nissenbaum 1975, Liu *et al.* 1979]. Subsequent applications to natural and synthetic illicit drugs [Mas *et al.* 1995, Ehleringer *et al.* 2000, Denton *et al.* 2001, Palhol *et al.* 2003, West *et al.* 2009], organic and inorganic explosives [Pierrini *et al.* 2007, Lock & Meier-Augenstein 2008, Benson *et al.* 2009a, Benson *et al.* 2009b], petroleum hydrocarbons [Mansuy *et al.* 1997, Smallwood *et al.* 2002, Li *et al.* 2009], and to various other forensic materials [Sharp *et al.* 2003, Meier-Augenstein & Fraser 2008] have confirmed its potential of discrimination. This higher feature appears therefore very attractive as a complementary technique to conventional routine methods.

Besides, the collection and exploitation of traces with high individualisation power in post-blast investigations is not optimal due to the nature of explosion. Thus, these limitations encourage the use of additional techniques, with a high discrimination power, that can reveal supplementary information. The isotopic profile of an explosive substance–whether it concerns bulk explosive, traces or residues–is only one part of the information conveyed by a trace. This isotopic information and its usefulness in the investigation process need to be investigated and evaluated.

In the investigative phase of the inquiry, the isotopic information extracted from the trace may be used to generate intelligence and assist the police in the inquiry [Brodeur & Ouellet 2005, Barclay 2009, Delémont *et al.* 2013, Margot 2011c]. From a practical point of view, the forensic intelligence generated from the isotopic profile of a trace may :

- assist the police to reduce the number of possible sources. In an investigative framework, the exploitation of the isotopic information in conjunction with circumstantial elements or other elements of the inquiry, can greatly accelerate the investigative phase by eliminating non-corresponding or irrelevant sources of explosive material and therefore by excluding possible suspects.
- provide new investigative leads that can be explored by law enforcement agencies. This can be particularly useful for investigators, when very limited information has been gathered through investigation, or in cases where the police has no clue to orientate their inquiry or no information about potential suspects. It can be used to build up alternative propositions related to actions in connection with the bombing. Indeed, it could help investigators in obtaining more details on the sequence of events (reconstruction) that preceded the triggering of the bomb, i.e. where or when the explosive substance have been bought. For example, the isotopic profile of black powder contained in an unexploded bomb has a similar profile with traces of black powder found in a car, parked in the vicinity of the attempted bombing. This can provide information on the possible earlier presence of the IED in the vehicle and lead the investigator to hypothesise that the vehicle has been used to transport the device, thus providing them with new leads to investigate. Furthermore, it

can also offer valuable intelligence, by linking two explosives devices used in distinct bombings on the basis of the isotopic profile of the explosive charge and corroborate additional similarities observed between the two devices.

In that sense, the use of isotopic information for intelligence purpose should ideally produce a low level of false negatives to ensure that the source of the trace is not missed and is included in the possibilities or the selected "candidates".

Conversely, the use of isotopic information as evidence in court for inculpatory or, more rarely but still, exculpatory purposes is highly promoted by some authors [Daeid Nic *et al.* 2010, Ehleringer & Matheson 2010]. It is also one of the aim of the Forensic Isotope Ratio Mass Spectrometry (FIRMS) network to set up a structure to promulgate the use of isotopic ratios as evidence in court [Carter, J. F. and Barwick, V. J. (Eds) 2011]. Nevertheless, this approach imposes certain requirements. In a prosecutorial framework, the use of information is generally oriented towards a given suspect. The end point of this approach being the use of evidence in trial, the prosecutorial purpose requires a comparative process between the evidentiary and the comparison material producing a low level of false positives. Thus, the number of unrelated "objects" (objects that did not produce the trace) but displaying the same degree of closeness as the source of the trace should be very limited.

It is the aim of this work to evaluate how the isotopic information contributes to explosives investigation and, in that sense, how it can be exploited in the different dimensions of the investigation process. By revealing the global contribution - the added value - of this type of information to the investigation process, we will be able to elicit either its use as a tool to orientate investigations or its precious value as evidence to present in court or both aspects.

Chapter 4

Isotope ratio mass spectrometry (IRMS)

Contents

1 General notions

Elements constituting the molecules of a material involve several stable isotopes. Isotopes are chemical elements with the same atomic number but different mass numbers. They possess therefore the same number of protons, but a different number of neutrons.

An isotope is expressed through the following notation: ${}^{n}X$, with n being the mass number of the atom X. For example, carbon has three natural isotopes: carbon-12 (${}^{12}C$) with 6 neutrons and carbon-13 (${}^{13}C$) with 7 neutrons. The last isotope, carbon-14 (${}^{14}C$) with 8 neutrons, is a radioactive isotope.

For most of the elements of Mendeleev periodic table, several isotopes exist at the natural state. Only 21 elements have one single stable isotope, while some, such as Sn, having as many as 10 isotopes [Krouse 2000, Coplen & Holden 2011]. More than 3000 isotopes are currently known, but most are not observable in natural samples. The majority of the isotopes are radioactive and have an extremely short lifespan. Only 237 are stable, that is, the structure of their nucleus does not change over time without external energy supply [Platzner 1997]. The study of isotopes distribution generally concerns stable isotopes only, because the natural abundance of radioactive isotopes is very low. This work only focus on light stable isotopes.

1.1 Stable isotopes

Isotopes of a given element have identical chemical properties, since they have the same electronic structure. However, they have different physical properties [Krouse 2000]. The difference of mass between two molecules involving different isotopes leads to changes in bonds energy, and therefore changes in the physical properties of these molecules (melting temperature, vapour pressure, equilibrium constants, etc.). These differences in properties induce isotopic fractionations and are at the origin of the distribution of isotopes.

The different isotopic compositions of a molecule for a given element are called isotopomers [PAC 1994]. **Figure 4.1** illustrates the different isotopomers of the molecule of ethanol, each including one heavy isotope.

$${}^{2}H{}^{1}H_2{}^{12}C-{}^{12}C{}^{1}H_2-{}^{16}O{}^{1}H \qquad {}^{1}H_3{}^{12}C-{}^{12}C{}^{1}H{}^{2}H-{}^{16}O{}^{1}H \qquad {}^{1}H_3{}^{12}C-{}^{12}C{}^{1}H_2-{}^{16}O{}^{2}H$$

$${}^{1}H_3{}^{13}C-{}^{12}C{}^{1}H_2-{}^{16}O{}^{1}H \qquad {}^{1}H_3{}^{12}C-{}^{13}C{}^{1}H_2-{}^{16}O{}^{1}H$$

$${}^{1}H_3{}^{12}C-{}^{12}C{}^{1}H_2-{}^{17}O{}^{1}H \qquad {}^{1}H_3{}^{12}C-{}^{12}C{}^{1}H_2-{}^{18}O{}^{1}H$$

Figure 4.1. Isotopomers of ethanol (Source: [Remaud & Akoka]).

1.2 Isotope ratios and abundances

The isotopic composition of an element in a chemical object or a given molecule can be expressed by its isotope ratio or its isotopic abundance.

The *isotope ratio R* is by convention the quotient of the amount of the minor isotope on the amount of the major isotope. For example, the isotope ratio of carbon-13, ^{13}R, is determined by ^{13}C/ ^{12}C. However, these raw isotope ratios are not used as such: they are expressed against an international reference material or standard (see section 1.3).

The *isotopic abundance A* is the relative atomic proportion of a given isotope. For example, the isotopic abundance of carbon-13 is ^{13}C/ (^{12}C+^{13}C).

While the number of atoms of stable isotopes is constant in the biosphere, the natural abundance of these isotopes varies between the different constituents of the biosphere (air, organisms, vegetal, mineral and organic matters, etc.). Light isotopes are abundant, in contrast to heavy isotopes which are rarer. The relative abundances of isotopes of the principal elements are reported in **Table 4.1**:

Elements	**Isotope**	**Relative abundance (%)**
Carbon	^{12}C	98.93
	^{13}C	1.07
Nitrogen	^{14}N	99.632
	^{15}N	0.368
Oxygen	^{16}O	99.757
	^{17}O	0.038
	^{18}O	0.205
Hydrogen	^{1}H	99.9885
	^{2}H	0.0115

Table 4.1. Relative abundance of isotopes of the main elements (source: [National Institute of Standards and Technology 2010]).

1.3 Expression to international standards

Variations in isotope ratios being naturally small, isotope ratios are generally expressed against a reference value to facilitate their handling. By convention, the isotopic deviation, or more commonly the isotope value, noted as δ-value, expresses in per mil (‰) the deviation of the isotope ratio of the sample from the primary reference material (also commonly referred to as international standard):

$$\delta = [(R_s - R_{ref})/R_{ref}] * 1000 \text{ ‰}$$

where R_s and R_{ref} are, respectively, the isotope ratios of the sample and of the reference.

For example, the measurement of the isotope ratio of ^{13}C in a sample is:

$$\delta^{13}C_s = [((^{13}C/^{12}C)_s - (^{13}C/^{12}C)_{ref})/(^{13}C/^{12}C)_{ref}] * 1000\ ‰.$$

Values can be positive or negative. Negative values indicate a smaller abundance of the heavy isotope in the sample than in the reference.

Nature encompasses quantities of tanks with a relatively constant isotopic composition, which is characteristic of a geographical location, a formation mode (for rocks) or growth conditions (for plants). **Figures 4.2** to **4.5** illustrate the variations of δ^2H, $\delta^{13}C$, $\delta^{15}N$ and $\delta^{18}O$ isotopic composition of various materials.

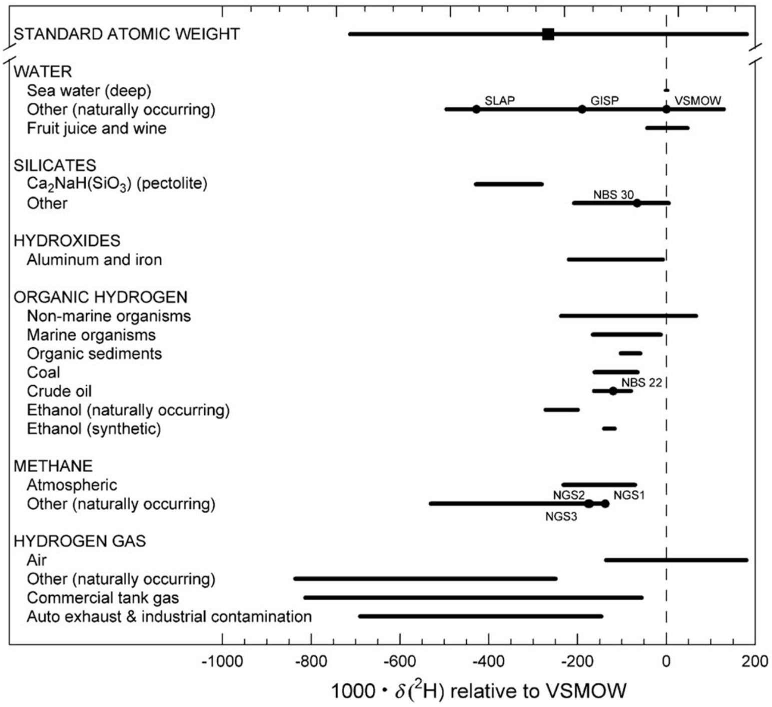

Figure 4.2. Variations of δ^2H isotopic composition of various materials (source: adapted from [Coplen *et al.* 2002a], p.1992).

Because isotope ratio values rely on a conventional scale defined by primary reference materials, the use of reliable and traceable reference materials and standards is decisive to ensure the comparability of results. Initially, the choice of reference materials was focused on large and relatively homogeneous terrestrial reservoirs to represent international standards. By definition, the isotopic value δ of these

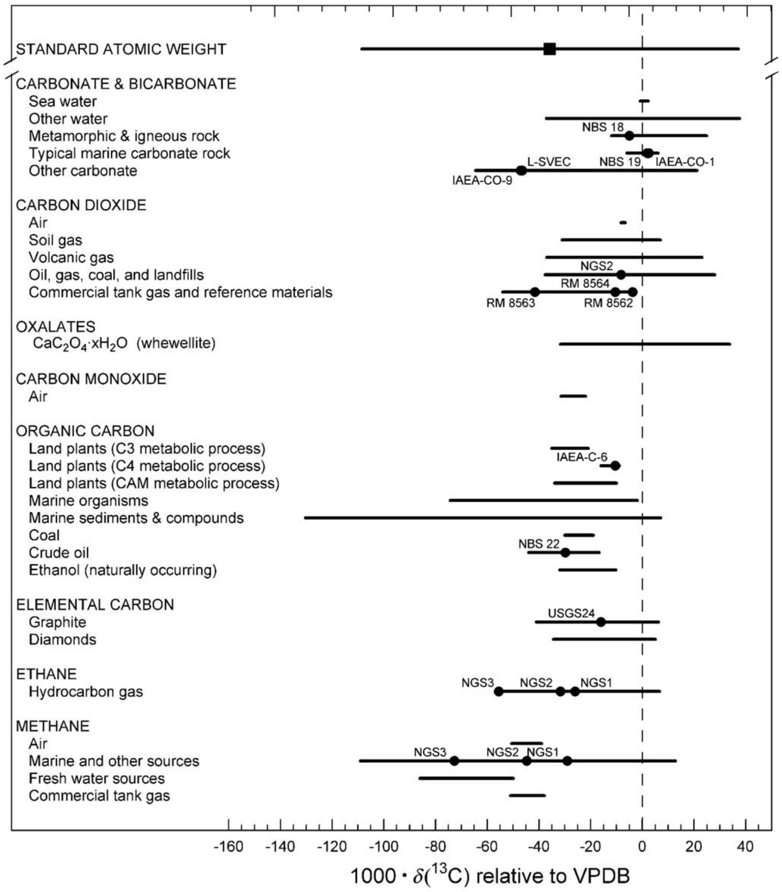

Figure 4.3. Variations of δ^{13}C isotopic composition of various materials (source: adapted from [Coplen *et al.* 2002a], p.1996).

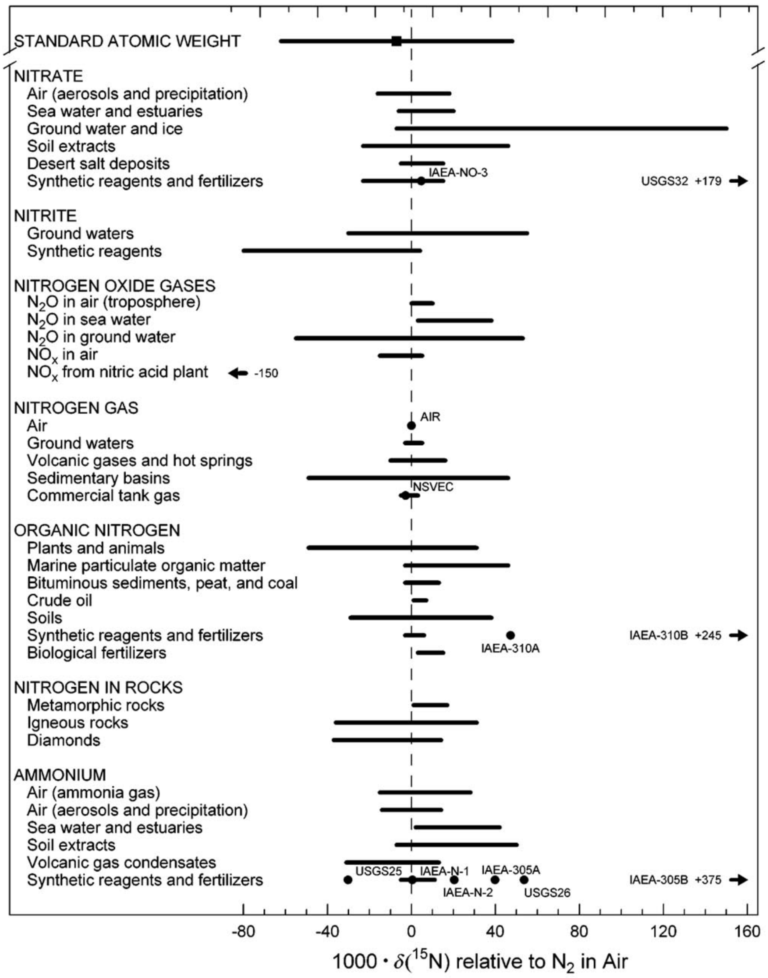

Figure 4.4. Variations of δ^{15}N isotopic composition of various materials (source: adapted from [Coplen *et al.* 2002a], p.1997).

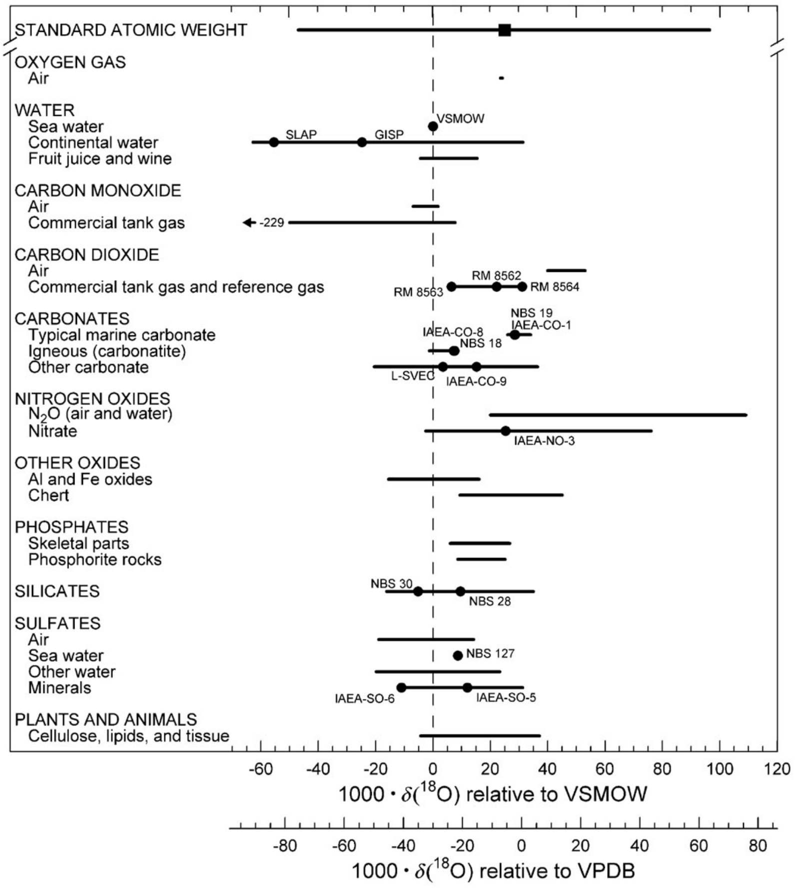

Figure 4.5. Variations of $\delta^{18}O$ isotopic composition of various materials (source: adapted from [Coplen *et al.* 2002a], p.1999).

standards is 0 ‰. **Table 4.2** hereafter displays the absolute isotope ratios of the primary reference materials and their uncertainties [Gröning 2004].

Elements	δ	**Name**	**Isotope ratio**	**Absolute value of the isotope ratio**
Carbon	$\delta^{13}C$	PDB (Pee Dee Belemnite) calcium carbonate	$^{13}C/^{12}C$	$(11237.2 \pm 30) * 10^{-6}$
Nitrogen	$\delta^{15}N$	NSVEC[1] atmospheric nitrogen	$^{15}N/^{14}N$	$(3663 \pm 0.5) * 10^{-6}$
Oxygen	$\delta^{18}O$	VSMOW (Vienna Standard Mean Ocean Water) oceanic water	$^{18}O/^{16}O$	$(2005.20 \pm 0.45) * 10^{-6}$
Hydrogen	$\delta^{2}H$	VSMOW	$^{2}H/^{1}H$	$(155.60 \pm 0.12) * 10^{-6}$
Sulphur	$\delta^{34}S$	CDT (Canyon Diabolo Troilite) meteorite material	$^{34}S/^{32}S$	0.04500

Table 4.2. Absolute isotope ratios of international standards.

The main drawback of these primary certified reference materials is their limited availability. As a result, other secondary reference materials have emerged over time, such as the Standard Light Antarctic Precipitation (SLAP) to assist the VSMOW and the carbonate NBS-19 for PDB. Mainly distributed by the International Atomic Energy Agency (IAEA), the National Institute of Standards and Technology (NIST) and the Institute for Reference Materials and Measurements (IRMM), stable isotope reference materials are used to calibrate laboratory equipment and laboratory standards used in routine analysis. In order to preserve their availability, laboratories are impelled to develop their own laboratory standards or find other certified reference materials for routine analysis [Gröning 2004, Jardine & Cunjak 2005]. In practice, measurements of the sample are made against a working or laboratory standard (ws), which itself is precisely calibrated against a certified reference material (CRM) [De Groot 2004].

As pointed out by Gröning [Gröning 2004], there is palpable divergence between the ISO (International Organisation for Standardisation) norm on international vocabulary of metrology and the common terminology referring to standards and reference material used in the stable isotope community, in addition to terms adapted from one author to another. The terms used in this work are based on the nomenclature given by Gröning; the broad term “standard” comprises reference material, certified reference material, internal and laboratory standard as well as reference gas.

1.4 Isotopic fractionation

As previously mentioned, the difference in mass between two isotopes leads to an isotopic selection in the physico-chemical processes, for some molecules' properties depend on the mass of its atoms. Thus, the isotope ratio may vary between the initial and final states. This phenomena is known as isotopic fractionation[2] [Krouse 2000, Hoefs 2009]. The factor of isotopic fractionation of an element is defined by α

$$\alpha_{a-b} = R_a/R_b \tag{4.1}$$

with R_a and R_b being the respective isotope ratios of the element considered in the starting reactant and in the product.

Fractionation depends on the relative masses between the isotopes. The bigger the difference, the more important the isotopic fractionation. Isotopic fractionation may occur during biological, chemical or physical processes. According to the reactions involved, fractionation can be kinetic or thermodynamic.

1.4.1 Kinetic fractionation

Kinetic fractionations are observable during chemical reactions involving the formation and rupture of bonds. They result from the differences in vibrational energy of bonds involving isotopes of the same element. This difference in binding energy is at the origin of differences in reaction speed between light and heavy isotopes. The bonds of lighter isotopes is more easily and quickly broken than those involving heavy isotopes. Thus, during a chemical reaction, molecules containing the heavier isotopes react slightly slower than those containing the lighter isotopes. Molecules containing the lighter isotopes are therefore more reactive.

This isotopic selection will enrich the reaction products with light isotopes and the starting reactants with heavy isotopes; the isotopic fractionation coefficient is then greater than 1. In the opposite case (the reaction products are enriched with heavy isotopes and the starting substrates with light isotopes), the fractionation coefficient is less than 1.

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[2] Isotope fractionation is also commonly referred to as isotope effect. Nevertheless, some authors differentiate isotope fractionation, which describes the differences in isotopic compositions of compounds and isotope effect, which pertains to a mass-dependant process. For sake of clarity, this terminology will be used in this work.

1.4.2 Thermodynamic fractionation

Thermodynamic fractionations occur during reversible exchange reactions. Molecules with different isotope ratios are partitioned between two compounds or two phases. These thermodynamic isotopic effects are due to the different physical properties of the isotopes, properties which are also related to the vibrational binding energy. These exchange reactions do not involve the formation or the breaking of bonds. Identically to kinetic fractionations, the value of the fractionation coefficient indicates the isotopic enrichment of the reactant or the product. For example, when water evaporates, the evaporation of $H_2{}^{18}O$ is slower than that of $H_2{}^{16}O$. Water steam will then be enriched with $H_2{}^{16}O$ compared to condensed phase of water.

Whatever the type of fractionation, it is inversely dependent on temperature. Isotopic fractionation is more important at low temperatures than at high temperatures. Knowledge of isotopic fractionations models associated with the chemical and biochemical processes enables identifying the phenomena responsible for fractionation and to explain the observed isotopic variations.

2 Instrumentation: the isotope ratio mass spectrometer

The isotope ratio mass spectrometer measures the isotope ratio of solid, liquid or gaseous samples and compares it to that of a reference gas calibrated against an international standard [Platzner 1997]. This high precision technology allows the measurement of isotope ratios with four to six significant digits. Isotope ratio commonly measured are $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{2}H/^{1}H$ from the pure gases CO_2, N_2 , CO and H_2.

A sample preparation technique is usually placed upstream of the isotope ratio mass spectrometer to convert the sample into gaseous products.

The isotope ratio mass spectrometer consists of an admission system, an ionisation source, an analyser to separate the ions, and a detector [Platzner 1997, Krouse 2000, Brand 2004].

2.1 Sample preparation techniques - gas inlet system

Whatever the form of the sample, it needs to be converted into simple gas before analysis. However, the sample may be in a relatively complex matrix or may not be analysable as it is. The compounds must first be isolated or extracted by physical and/or chemical techniques, before being introduced as a gas into the isotope ratio mass spectrometer. Thus, the admission or inlet system treats pure gases (mainly CO_2, N_2, CO and H_2 but sometimes O_2, SO_2, N_2O, CH_3Cl).

2.1.1 Dual inlet isotope ratio mass spectrometry (DI-IRMS)

The dual inlet system, first developed by Nier and later by McKinney in the 1950s, involves two volume gas reservoirs made to store, in one volume, the calibrated gas of known isotopic composition and in the second, the sample gas. A change-over valve enables the alternative introduction of the reference gas and the sample gas into the vacuum chamber of the source. This off-line sample preparation system is mainly used for relatively pure substances, such as water or gas specimens. The off-line preparation is time consuming with a high risk of contamination. This construction, known as Dual Inlet (DI) system enables high accuracy measurements. Improvements made in recent decades have reduced the problems of maintaining vacuum, isotopic fractionation and contamination [Brand 1996, Brand 2004].

2.1.2 Continuous flow isotope ratio mass spectrometry (CF-IRMS)

Another system emerged in the 1960's and 1970's, the continuous flow mode (CF) which allows the online preparation and conversion of organic and inorganic specimens in a gas form suitable for IRMS [Preston 1992, Krouse 2000]. After the on-line preparation of the sample by elemental analyser (EA), gas chromatography (GC) or more recently liquid chromatography (LC), and the purification of the sample gas by means of a variety of traps and molecular sieves, it is injected into the flow of a carrier gas, usually helium, which transports it to the ion source. The system continuously measures the gas sample flowing into the source and compares it to reference gas pulses introduced into the carrier gas flow. The coupling of an instrumental method to a CF-IRMS system with an autosampler allows performing simpler and faster analyses and requires less sample.

Several continuous flow systems interfaced to an isotope ratio mass spectrometer enable the isotopic analysis of solid, liquid and gas samples. Depending on the presence of a separation technique prior to isotopic analysis or not, we can distinguish two categories of analysis [Platzner 1997]:

- the compound specific isotope analysis, abbreviated CSIA. Capillary gas or liquid chromatography interfaced to IRMS allows the isotopic measurement of selected compounds of the sample. The most common method is GC-CF-IRMS (gas chromatography continuous flow IRMS), already developed at the end of the 1970's and commonly used nowadays. The eluted compounds, first separated by gas chromatography, are introduced in an oxidation furnace and quantitatively converted into CO_2, NO_x and H_2O. LC-CF-IRMS (liquid chromatography-CF- IRMS) also belongs to this category. However, it has been rarely employed in forensic science.

- the isotopic analysis of the whole sample, also known as bulk stable isotope analysis (BSIA). The measured δ value reflects the isotopic composition of the totality of the components of the mixture. Sample preparation is performed using an elemental analyser (EA) for organic or inorganic specimens. An EA-IRMS was the instrumentation used in this research.

Elemental analyser

Elemental analyser is based on flash combustion, in order to ensure the complete combustion of the sample and to produce CO_2, N_2, CO or H_2 gas. The simple gas reflects the isotopic composition of each molecule containing the target element. After combustion, the gas is purified using chemical gas traps to remove oxygen, water and nitrogen oxides. Gaseous products are subsequently separated from each other by a GC column, before being introduced into the ion source [Preston 1992, Brenna *et al.* 1997].

Figure 4.6 illustrates the different components of an elemental analyser coupled to an isotope ratio mass spectrometer for the analysis of carbon and nitrogen.

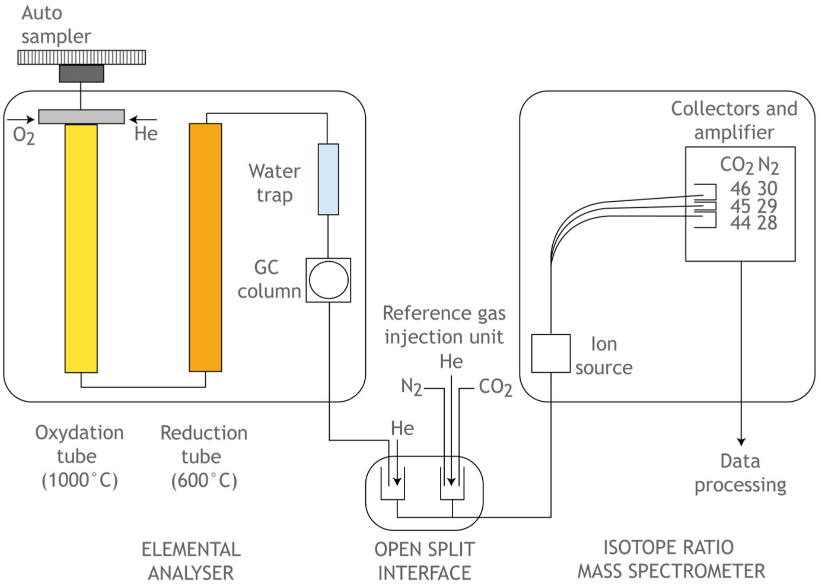

Figure 4.6. Illustration of the different components of an EA-IRMS (source: adapted from [Limén & Marty 2001, Remaud & Akoka]).

Flash combustion Placed in an autosampler, the sample packed in a tin or silver capsule, is dropped into the combustion reactor, held at a temperature around 1000°C. The combustion reactor, also known as the oxidation tube, contains an oxidant such as copper, chromate or cobalt oxides. The combustion, which should take place as fast as possible, is performed in an excess of oxygen and in a helium atmosphere. The sample, packed in an oxidisable metal, undergoes a flash combustion reaching temperatures of 1700-1800°C [Pella & Colombo 1973]. The gases resulting from the reaction are first transported to a reduction reactor to remove oxygen and to convert nitrous oxides into N_2. They are then directed to a trap to remove water from the reaction products. The remaining gaseous products are separated on a chromatographic column, before being introduced into the IRMS via an open split interface. Elements generally analysed are C, N and S [Brenna *et al.* 1997]. For the analysis of O and H, a thermal conversion system (TC) is incorporated to the elemental analyser. The analysis of these two elements is based on the pyrolysis of the sample between 1100 and 1300°C in presence of carbon [Kornexl *et al.* 1999].

Pyrolysis The measurement of oxygen is based on the reduction of oxides by carbon into CO or CO_2, also known as carbon reduction. Pyrolysis is the separation of a complex material by heat in the absence of other partners reaction (in inert atmosphere). The original reaction, commonly known as Schütze/Unterzaucher procedure in the elemental analysis of oxygen, has evolved and the reaction is now undertaken with glassy carbon instead of diamonds or graphite. Online recent methods require the fast and complete conversion of all oxygen bearing compounds into CO [Werner 2003, Saurer & Siegwolf 2004].

2.2 Ion source

The molecules of the pure gas are introduced into the source, where they are ionised by electron impact. Electrons are released from the heating of a tungsten or rhenium filament. They are ensuingly accelerated in an electric field, until they acquire an energy between 50 and 150 eV at their arrival in the ionisation chamber [Brand 2004]. The collision of the electrons with the molecules of simple gas causes their ionisation. Eventually extracted from the ionisation chamber, the ions are accelerated by means of high potential (typically 2 to 5 kV) and reach the analyser in the form of an ion beam [Preston 1992, Platzner 1997].

2.3 Mass analyser

The ions enter the analyser, a single magnetic sector, through the entrance slit. Under the action of a uniform magnetic field, ions are deflected perpendicular to their flight direction and follow circular paths according to their mass: ions are separated according to their mass-to-charge ratio (m/z) [Brand 2004].

2.4 Ion beam detector and data system

Ions arrive on an array of Faraday cups, one for each ion beam of interest, set to collect the different masses (masses 44, 45 and 46 for CO_2 or 28, 29 and 30 for N_2 and CO). An amplifier connected to the collectors eventually increases the electrical signals which depend on the amount of collected ions. These signals are then transmitted to a computer where data are analysed and the ratios of isotopic masses (45/44 and 46/44 for CO_2, 29/28 and 30/28 for N_2 and CO) are determined and compared to those of the reference gas [Preston 1992, Platzner 1997, Brand 2004].

3 Applications in forensic science

The discovery that stable isotope ratios were not constant in nature was primarily exploited in geosciences. Applications focused on the study of geological processes, such as the determination of the origin and formation of fluids and rocks, climate reconstruction or the detection of environmental changes [Ghosh & Brand 2003]. Light stable isotope ratios being the most abundant on earth, the isotopic analysis of hydrogen, carbon, nitrogen, oxygen and sulphur found a variety of other applications in biogeochemistry [Bowen 2010], ecology [Ammann *et al.* 1999, Saurer *et al.* 2004], archeology [Longinelli 1984], hydrology, medicine, etc. The applications developed in forensic science all have a common approach: the exploitation of isotope ratios measurements in order to infer the source of a substance, a material or a living creature. These types of applications are depicted in the following sections.

Applications of IRMS in forensic science were reviewed by Benson *et al.* in a concise paper presenting the theory, instrumentation and forensic applications up to 2006 [Benson *et al.* 2006]. More recently, Meier-Augenstein published an overview of the theory and principles underlying stable isotopes in addition to many details on technical aspects (instrumentation, isotopic calibration, guidance to set up a CF-IRMS laboratory, etc.) and general considerations for the forensic application of IRMS. He also surveyed different forensic applications illustrated by the main ongoing research in the fields and some case studies [Meier-Augenstein 2007, Meier-Augenstein 2010].

This section provides a systematic and thorough overview of forensic applications based on stable isotopes. Through this survey, it is meant to offer a critical review on the state of the art of IRMS used as a tool for source inference in forensic science. Most of the following subsections report the results obtained by the different groups of authors[3]. Likewise, values have been reported without their standard deviation as they are provided as an indication for discussion. For some fields, results are summarised in tables. When δ values were not reported or not explicitly disclosed in the studies, figures or references followed by * signify

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[3] δ values are reported as mentioned in the studies, without rounding numbers

estimated δ values extracted from plot or graph and provided as an estimation for the review.

3.1 Food authentication

A major application of stable isotope ratios measurement is food analysis. Several authors reviewed the numerous studies undertaken in food authentication [Rossmann 2001, Kelly 2003, Kelly 2005, Forstel 2007, Gonzalvez *et al.* 2009, Primrose *et al.* 2010]. Routinely used in wine quality control, IRMS has been mainly applied in the detection of adulterants and the assignment of the regional origin of food. The technology enables for example the detection of the addition of water and organic components, such as sugars or acids in fruit juices or the determination of the presence of external sugars in wine and honeys [Gensler *et al.* 1995, Yunianta *et al.* 1995].

Authenticity control isotope analyses also focus on the determination of the geographical origin of caffeine, honey, wine, fruit juices, raw materials, etc. The distribution of stable isotopes in both organic and inorganic materials are characteristic of geographical or regional locations. For the determination of the geographical origin, isotopic analyses of light elements, mostly hydrogen and oxygen, indicative of climatic characteristics, are often supplemented by heavier trace element isotope analysis, the most common being strontium [Kelly 2005, Gonzalvez *et al.* 2009, Pilgrim *et al.* 2010]. Recently, stable isotope analysis has proven to aid in determining the production method of food. An increasingly popular application is the δ^{15}N analysis to differentiate organically grown food and vegetables grown with synthetic fertilisers [Forstel 2007, Primrose *et al.* 2010].

Animal derived products may also be subject to food authentication by stable isotope measurements. Considering food chain and food webs, animal tissues and products of primary and secondary consumers, i.e. herbivores and carnivores, necessarily inherit their isotopic signature from the isotopic composition of their diet (food and water intake). Based on the isotopic values of plant materials, meteoric and ground water, as well as on isotopic fractionations induced by metabolisms, the geographical and regional provenance of animal products, such as milk, meat, etc. may be determined through stable isotope analysis [Rossmann 2001, Boner & Förstel 2004, Nakashita *et al.* 2008]. Moreover, the particular isotopic signature of animal derived products may be indicative of local agricultural practices or specific animal diets, and hence be used for authentication.

3.2 Wildlife forensics

As mentioned above for the provenance determination of animal products, the isotopic composition of animal tissues reflects their local food and water intake [DeNiro & Epstein 1978, DeNiro & Epstein 1981, Schoeninger & DeNiro 1984]. The isotopic composition of ingested materials is transferred to consumers through food chain. Wildlife forensics exploits this feature to gain information on diet and provenance of feeding to track migration patterns and movements of animals [Hobson 1999]. Depending on the turnover rate of the target tissue (feather, hair, nail, liver, muscle, blood or bone collagen), stable isotope ratios can provide information related to a period of time ranging from weeks to months or years.

Mostly δ^{13}C, δ^{15}N, δ^{34}S accompanied by δ^{87}Sr are measured to infer the origin of animals. Besides, δ^{18}O, and especially δ^{2}H, provide further geographical information at continental scale, as they vary according to geographic locations [Bowen *et al.* 2005]. Although stable isotopes have also been used to detect and follow migration patterns of exotic animals such as elephants [Cerling *et al.* 2006], most studies concern migratory movements of birds [Chamberlain *et al.* 1997, Hobson *et al.* 2004, Kelly *et al.* 2008].

3.3 Pharmaceuticals authentication

Although less popular than food analysis, IRMS has also been evaluated in pharmaceuticals authentication. Individual batches of active pharmaceutical ingredients analysed by IRMS are characterised by specific isotopic signatures [Jasper 2004]. The use of combined stable isotope ratios allows differentiating genuine drugs from counterfeit pharmaceutical tablets [Santamaria-Fernandez *et al.* 2009].

3.4 Environmental forensics

The application of stable isotope ratios in environmental contamination studies, either in a risk prevention perspective or to determine the liable party, have been the subject of innumerable publications. Compounds of interest comprise polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), n-alkanes, as well as lighter compounds such as benzene, toluene, ethylbenzene and xylene (BTEX), methyl tert-butyl ether (MTBE), nitrate and organophosphate compounds. Stable isotope ratios measurements, mainly carbon, have been exploited to trace the source of contaminants or to differentiate sources of chemicals [O'Malley *et al.* 1994, O'Malley *et al.* 1996, Harrington *et al.* 1999, Wang *et al.* 1999, Budzinski *et al.* 2003, Boyd & Coffin 2004, Boyd *et al.* 2006]. The analysis of other elements, such as hydrogen, nitrogen, oxygen, chlorine or sulphur were progressively reported in publications to trace sources of contaminants or to evaluate their fate in environment [Smallwood *et al.* 2001, Wang & Huang 2001, Silva *et al.* 2002, Wang *et al.* 2004, Kuder *et al.* 2009].

Other research focused on the identification of degradation reactions, as well as non-degradative processes, such as volatilisation or dilution which may induce isotope fractionation [O'Malley *et al.* 1994, Carpentier *et al.* 1996, Mansuy *et al.* 1997, Harrington *et al.* 1999, Diegor *et al.* 2000, Coffin *et al.* 2001, Wang & Huang 2001, Morasch *et al.* 2002, Kuder *et al.* 2009, Li *et al.* 2009, Shin & Lee 2010]. Two comprehensive reviews on environmental forensic studies expose the ins and outs of CSIA in environmental forensics [Schmidt *et al.* 2004, Philp 2007].

3.5 Doping

The isotopic analysis of steroids was reported for the first time in literature in 1994 [Becchi *et al.* 1994]. The potential of GC-C-IRMS (gas chromatography combustion isotope ratio mass spectrometry) technology to distinguish between endogenous and exogenous (synthetic) testosterone and its metabolites was presented. Since then, literature on carbon isotope ratio measurements of urinary steroids has expanded and the technology has become the method of choice in the fight against doping [Cawley & Flenker 2008, Flenker *et al.* 2008, Piper *et al.* 2008, Cawley *et al.* 2009, Piper *et al.* 2009, Saudan *et al.* 2009, Cawley *et al.* 2010].

Over the years, a considerable list of endogenous steroids of interest has been progressively investigated by IRMS, several reference populations studies were undertaken, the influence of various factors such as diet or gender on δ^{13}C value was also evaluated. Meanwhile, hydrogen isotope ratios aroused growing interest and generated varied research. An excellent and comprehensive review recently published by Piper *et al.* sets the state of the art, through the referencing and comments of these numerous studies. Alternatively it also discusses method validation in IRMS [Piper *et al.* 2011].

A noteworthy feature of the use of stable isotope ratios in the fight against doping is the evidentiary aspect of the results as they may be employed in trials. This prosecutorial aspect necessarily implies to work with validated methods as well as to be able to justify any results, as attested by the record of the Court of Arbitration for Sport in the case of Floyd Landis v. USADA [Court of Arbitration for Sport 2008]. These implications certainly led to the numerous studies on influencing factors and reference populations.

3.6 Illicit drugs

3.6.1 Natural illicit drugs

Most studies focusing on natural illicit drugs are based on the postulate that stable isotope ratios distribution in plant materials reflects the environment in which the plant grows (climatic conditions such as humidity, temperature, amount of light, ambient carbon dioxide, etc.) and its metabolic factors [Denton *et al.* 2001, Galimov *et al.* 2005]. On this theoretical basis, isotope ratios may therefore be useful indicators of the geographical origin of specimens of natural illicit drugs.

Cannabis Liu *et al.* analysed 6 specimens of Cannabis sativa grown in different geographical places in the USA by DI-IRMS [Liu *et al.* 1979]. The analysis of leaves and flowers of male and female plants showed δ^{13}C variations between specimens (-34.15 ‰ to -27.82 ‰), which suggested the possibility to differentiate specimens from different origins. These variations oscillated however in a narrow range, requiring therefore further investigations. The authors concluded that the combination of IRMS with other instrumental techniques may increase the discrimination power of the sequence of used techniques.

The FIRMS network (forensic isotope ratio mass spectrometry network Ltd) reported the prosecutorial use of carbon and nitrogen isotopic profile of cannabis as evidence in the Supreme Court of the Northern territory in the 1980's in a case opposing R v. Thomas Ivan Brettschneider [Forensic Isotope Ratio Mass Spectrometry 2003a]. δ^{13}C and δ^{15}N values of specimens of cannabis seized from a truck and cannabis from a plantation could not be differentiated at 95 % level confidence.

Denton *et al.* were the first to publish fundamental data on cannabis isotopic intravariability, as well as a large study on the relationship between carbon and nitrogen stable isotope ratios of cannabis plants and the environmental and growth conditions [Denton *et al.* 2001]. They analysed around 150 cannabis leaves seized from 66 crop sites in Australia, Papua New Guinea and Thailand mainly grown outdoor. Sampling lower, middle and upper leaves of plants coming from 3 selected crops, they evaluated the bulk δ^{13}C and δ^{15}N within-plant variations. For specimens coming from 2 crops, upper leaves were more enriched than middle and lower leaves by 1 to 2 ‰ for δ^{13}C and more than 1 ‰ for δ^{15}N. Similarly, on 9 crop sites, the range of variations observed within a crop was 2.1‰ for δ^{13}C and 6.7 ‰ for δ^{15}N. Although 70 % of the specimens exhibited a δ^{13}C value between -29 and -27 ‰, the δ^{13}C values were distributed on a wide range extending from -36.4 ‰ to -25.0 ‰. δ^{15}N values ranged from -1.0 ‰ to +15.8 ‰. Specimens from the 3 countries could not be differentiated on the basis of their isotopic profile. However, they noted significant differences in δ^{13}C values between indoor (-36 ‰ to -27 ‰) and outdoor grown cannabis plants (-30 ‰ and -25 ‰), corroborating the values obtained by Liu *et al.* for indoor and outdoor specimens. Using controlled environment experiments, they observed that low and well-watered plants showed significant different δ^{13}C and δ^{15}N values. They also related the δ^{15}N value of cannabis plant to the type of fertiliser used (industrial fertiliser versus animal manure) and concluded by the example of a casework. Although the identification of the country of origin of the crop was not possible, differences in crop conditions (indoor/outdoor, watering and fertiliser) seemed to offer the possibility to differentiate specimens.

Galimov *et al.* analysed the leaves of 8 cannabis specimens coming from Russia and Ukraine by EA-IRMS [Galimov *et al.* 2005]. δ^{13}C values varied from -28.38 ‰ to -26.43 ‰. δ^{15}N fluctuated on a wider range from -3.17 ‰ to +9.65 ‰.

Shibuya *et al.* evaluated the potential of δ^{13}C and δ^{15}N of marijuana to trace its geographical origin [Shibuya *et al.* 2006]. 90 specimens seized by law enforcement on 3 outdoor production sites in different geographical locations in Brasil were analysed by EA-IRMS. They evaluated the variability of δ value obtained from seeds, twigs, leaves and from the whole specimen and observed no significant difference. Analysing the whole specimen, they used k-means to classify specimens coming from different regions. Specimens from dry regions showed a different isotopic profile than those grown in humid and semi-humid conditions. The isotopic composition suggested they had been seized in the same region where they had been grown. They mentioned the use of additional analytical techniques (GC-MS, ICP-MS, etc.) to bring supplementary information.

Shibuya *et al.* pursued their efforts in 2 other researches on the geographical sourcing of marijuana [Shibuya *et al.* 2007a, Shibuya *et al.* 2007b]. The first publication reported the use of a linear discriminant analysis (LDA) model built on 150 specimens of known geographical origin to classify 76 specimens seized in Sao Paulo and analysed by EA-IRMS [Shibuya *et al.* 2007a]. The δ^{13}C and δ^{15}N values of specimens from dry regions ranged from -30 to -24 ‰ and from -4 to +11 ‰ respectively, with most of the samples distributed between -28 to -25 ‰ for C and -1 to +5‰ for N. Specimens of humid regions displayed δ^{13}C and δ^{15}N values from -32 to -25 ‰ and +2 to +10 ‰, respectively. The isotopic profile showed that most of them had grown in humid regions. Besides, the results also confirmed the existence of traffic routes between the Northeast and the Midwest regions of the country, close to Paraguay. The second publication used stable isotope data in addition to inorganic constituents measured by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) to verify existing differences between specimens of cannabis grown in different regions of Brasil [Shibuya *et al.* 2007b]. Applying hierarchical clustering analysis (HCA) and LDA, δ^{13}C and δ^{15}N were eventually found to be the most discriminant parameters in their model.

West *et al.* analysed 508 specimens of marijuana, from 30 counties of the USA, with additional specimens from USA borders, Colombia and Mexico [West *et al.* 2009]. Carbon and nitrogen isotope ratios were measured in duplicates by EA-IRMS, mainly from leaf material. For 10 outdoor grown specimens, they also analysed inflorescence and leaves separately. δ^{13}C and δ^{15}N values showed significant differences (respectively, 0.6 ‰ and 1.1 ‰) between the two groups. Specimens from the USA showed a wide range of δ^{13}C values (from -51.8 ‰ to -20.3 ‰) and δ^{15}N values (from -7.9 ‰ to +29.5 ‰). δ^{13}C average values showed significant differences between plants grown indoor (-33.1 ± 6.5 ‰) and outdoor (-28.2 ± 1.7 ‰), while δ^{15}N showed not to be affected by this factor, but rather by the fertiliser type. The authors suggested therefore that stable isotope ratios indicated cultivation methods rather than regional differences. Based on theoretical fractionation models of plants reported in literature, the authors proposed an interpretation framework. δ^{13}C and δ^{15}N were located on a bivariate plot with identification thresholds of δ value to categorise seized specimens of cannabis (indoor/outdoor; organic/inorganic fertiliser)[4]. Most specimens of known growth environment fell within the corresponding area.

Booth *et al.* investigated whether Marijuana seized in Alaska, from unknown grow location, reflected the isotopic profile of Marijuana grown in different regions of Alaska [Booth *et al.* 2010]. δ^{13}C, δ^{15}N, δ^{18}O and δ^{2}H were analysed by EA-IRMS from leave material of 56 specimens of known grow locations and 122 specimens of unknown origin. δ^{13}C and δ^{15}N values of known specimens showed a wide variations range, respectively from -53.8 ‰ to -26.4 ‰ for carbon and from -12.5 ‰ to +12.1 ‰ for nitrogen. In addition to the wide variations observed among specimens for δ^{18}O (from +10.4 to +37.0 ‰) and δ^{2}H (from -203.1 to -136.7 ‰), they also noticed significant variability *within* specimens for δ^{18}O (up to 11 ‰) and for δ^{2}H (up to 40 ‰). Using the same identification thresholds as West *et al.*, they observed that in plants from known and unknown origin, most specimens had grown indoor and had been given inorganic fertilisers. Finally, the comparison of seized specimens from known and unknown origin showed close corresponding δ values, suggesting a local traffic between production and consumption. They could however not exclude non-Alaskan sources.

Hurley *et al.* tested three models based on carbon and hydrogen stable isotope to predict the geographic origin and growth environment of marijuana specimens [Hurley *et al.* 2010a]. The first model combined δ^{2}H of zones defined by source water precipitation described in literature and by average δ^{2}H values of over 500 specimens of marijuana leaves coming from these different zones. In the second model, USA, Canada and Mexico were geographically divided into 5 large zones.

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[4] specimens with δ^{13}C value less than -32 ‰ has likely grown indoor, while specimens with δ^{13}C value higher than -29 ‰ is likely to have been grown outdoor. A δ^{13}C value between -32 ‰ and -29 ‰ indicate the plant has either grown indoor or in the shade. For δ^{15}N, plants with a value above 7 ‰ have likely been fertilised with organic fertilisers

The last model was based on the aforementioned δ^{13}C identification thresholds to determine indoor or outdoor growth environment. The models were blind tested with 60 specimens of known origin and growth environment. 60 % of the specimens were correctly assigned with the first model versus 67 % with the second and 87 % with the last model. Rightly, the authors indicated that the models were most powerful when additional information on likely sources was available.

The same group of authors analysed the δ^{13}C and δ^{2}H values of leaf material from 628 specimens seized from retailers [Hurley *et al.* 2010b]. Data were evaluated using their models to provide information on the domestic market of cannabis and predict the geographical origin and growth conditions. Results showed that most of the seized cannabis originated from the seizure region. While about 11 % of seized specimens clearly showed to have grown indoor, a larger part (32 %), comprised in the middle zone, indicated they were either grown in the shade or indoor.

Comparing 5 cannabis specimens by BSIA and CSIA, Muccio *et al.* were the first to report the specific δ^{13}C value of THC (Δ-9-tetrahydrocannabinol) , cannabinol (CBN) and cannabidiol (CBD) of specimens [Muccio *et al.* 2012]. However, 4 of the 5 specimens came from old seizures (more than 10 years old) and one from a fresh grown plant. Thus, as THC is degraded into CBN and CBD over time, comparison of δ^{13}C of compounds was difficult as specimens were of unknown origin and of different age. The results mainly showed that the bulk δ^{13}C value was on average 2‰ more depleted than that of CBN or CBD and suggested that isotopic fractionation occurs during the formation of these compounds.

Table 4.3 summarises the range of bulk isotopic values of cannabis reported in the different studies.

Cocaine Finnigan analysed the δ^{13}C and δ^{15}N of 6 different shipment seizures of cocaine by EA-IRMS [Finnigan MAT 1995]. The δ^{13}C and δ^{15}N variations, varying from -35.5 to -33.5 ‰* and from -13 to -5 ‰* respectively, showed the specimens were separated into 3 clusters.

The isotopic profile of 4 specimens of cocaine of unknown origin were measured by means of CSIA for δ^{13}C and BSIA for δ^{2}H and δ^{15}N [Ihle & Schmidt 1996]. While δ^{13}C value was similar for the 4 specimens, δ^{2}H and more specifically δ^{15}N offered additional discrimination and permitted their differentiation.

Ehleringer *et al.* analysed the bulk isotopic composition of 28 cocaine specimens coming from 4 presumed geographical locations [Ehleringer *et al.* 1999]. δ^{13}C and δ^{15}N variations as much as 0.6 ‰ and 7.1 ‰ respectively were measured. According to the authors, this range of variations allowed a differentiation of the 4

Authors	**Material analysed**	**Number specimens**	**Origin**	**δ^{13}C values [‰]**	**δ^{15}N values [‰]**	**δ^{18}O values [‰]**	**δ^{2}H values [‰]**
[Liu *et al.* 1979]	leaves flowers	6	USA	-34.15 to -27.82	-	-	-
[Denton *et al.* 2001]	leaves	150	Australia Papua Thailand	-36.4 to -25.0	-1.0 to +15.8	-	-
[Galimov *et al.* 2005]	leaves	8	Russia Ukraine	-28.38 to -26.43	-3.17 to +9.65	-	-
[Shibuya *et al.* 2006]*	whole plant	90	Brasil	-32 to -23	-4 to +10	-	-
[Shibuya *et al.* 2007a]	whole plant	50 104 76	Brasil H[a] Brasil D[b] unknown	-32 to -25 -30 to -24 -30 to -26*	+2 to +10 -4 to +11 0 to +8*	-	-
[West *et al.* 2009]	leaves	508 3 2 41	USA Colombia Mexico unknown	-51.8 to -20.3	-7.9 to +29.5	-	-
[Booth *et al.* 2010]	leaves	56 122	Alaska unknown	-53.8 to -26.4 -61.8 to -24.6	-12.5 to +12.1 -5.0 to +14.7	+10.4 to +37.0 +10.0 to +34.5	-203.1 to -136.7 -214.6 to -107.5
[Hurley *et al.* 2010a]	leaves	764	North America	- 35.1 to -28.2 [c]	-	-	-160 to -125[c]
[Muccio *et al.* 2012]	flowers	5	unknown	- 34.2 to -27.2	-	-	-

Table 4.3. Summary of data on the bulk isotopic analysis of cannabis in literature.

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[a] humid regions

[b] dry regions

[c] reported mean values

growing regions (Bolivia, Peru, Ecuador and Colombia).

Later, Ehleringer *et al.* investigated the δ^{13}C and δ^{15}N variations of 200 samples of coca leaves coming from five growing regions of the Andean Ridge [Ehleringer *et al.* 2000]. δ^{13}C varied from -32.4 to -25.3 ‰ and δ^{15}N from +0.1 ‰ to +13.0 ‰. Combined to the content of some alkaloids extracted from the same leaves, the isotopic information allowed the authors to correctly identify the country of origin of 96 % of the samples.

Casale *et al.* investigated the effect of the South American manufacturing processes on the isotopic profile of cocaine, with 10 chemists converting Peruvian cocaine base in controlled settings [Casale *et al.* 2005]. Using different Colombian, Peruvian and Bolivian conversion methods, the processing of cocaine base to cocaine hydrochloride (HCl) did not significantly affect the carbon isotope ratio. However, the δ^{15}N value showed a positive shift ranging from 0.8 to 3.7 ‰, related to the fraction of cocaine that precipitated. They experimented successive precipitations for reaction with less than 100 % yield and demonstrated the resulting progressive depleted values cocaine HCl precipitates.

Galimov *et al.* compared the δ^{13}C and δ^{15}N values of 15 samples of cocaine from different departments of Colombia obtained by a single EA-IRMS analysis and GC-C-IRMS analysis [Galimov *et al.* 2005]. As GC-C-IRMS showed the presence of impurities in some specimens, they ruled out from comparison bulk isotopic results of specimens containing impurities, considering them as outliers. Thus, the reported averages of 10 pure cocaine specimens were -35.5 ± 0.3 ‰ for δ^{13}C and -4.8 ± 1.2 ‰ for δ^{15}N obtained by EA-IRMS. They finally concluded that EA-IRMS could be applied on pure specimens and GC-C-IRMS on specimens containing impurities.

Sewenig *et al.* undertook a large scale study on the isotopic analysis of 132 specimens sampled from a 1.2 ton seizure of cocaine [Sewenig *et al.* 2007]. This allowed for the first time the observation of the bulk isotopic variations of carbon and nitrogen of cocaine bricks in a big seizure and to confront the isotopic profile of cocaine bricks to physical characteristics, such as the imprinted logo. Despite the absence of link between the logo and the isotopic profile, this study exhibited the limited variations in δ^{13}C (-35.4 ‰ to -33.9 ‰) and the wide span of δ^{15}N values (-17.4 ‰ to -1.8 ‰). Except for two specimens with very negative values (-17.4 ‰ and -15.1 ‰), most of the specimens had a δ^{15}N value typical from samples coming from Colombia (-5.7 ‰ to -3.2 ‰). The range of δ^{15}N values around -5 ‰ is attributed to growth conditions, in particular soil, fertiliser and land-use history, while the very depleted δ^{15}N value are supposed to come from successive precipitation of cocaine–HCl. The δ^{13}C variations is thought to derive from different humidity conditions.

Muccio *et al.* reported on the use of GC with concurrent single quadrupole and IRMS spectrometers as detectors [Muccio & Jackson 2011]. They demonstrated the performance of their system on 6 cocaine samples of which 3 were cocaine standards. They highlighted the discriminatory power of the technique with the differentiation of 3 samples out of 6 based on the δ^{13}C values and emphasized the usefulness of the combined detectors for the identification of the compounds.

Table 4.4 summarises the range of isotopic values of cocaine reported in the different studies.

Authors	**Method**	**Number specimens**	**Origin**	**δ^{13}C values [‰]**	**δ^{15}N values [‰]**
[Finnigan MAT 1995]*	EA-IRMS	6	unknown	-35.5 to -33.5	-13 to -5
[Ihle & Schmidt 1996]	EA-IRMS GC-IRMS	4	unknown	-34.4 to -33.6	-13.7 to -5.4
[Ehleringer *et al.* 1999]*	EA-IRMS	28	Bolivia Peru Ecuador Colombia	-34.8 to -33.5	-13 to -4
[Casale *et al.* 2005]	EA-IRMS	10	Peru	-34.8 to -34.4	-10.3 to -6.6
[Galimov *et al.* 2005]	EA-IRMS GC-IRMS	15	Colombia	-39.92 to -30.85 -37.31 to -34.54	-10.36 to -2.19 -10.50 to -1.64
[Sewenig *et al.* 2007]	EA-IRMS	132	unknown	-35.4 to -33.9	-17.4 to -1.8
[Muccio & Jackson 2011]*	GC-IRMS	6	commercial standards unknown	-37 to -35	-

Table 4.4. Summary of data on the isotopic analysis of cocaine reported in literature.

Heroin Desage *et al.* analysed the δ^{13}C value of heroin specimens coming from Turkey, India, Niger, Pakistan and Thailand as well as from unknown origin by GC-C-IRMS [Desage *et al.* 1991]. Using t-test, they observed significant differences between specimens of heroin from different countries with δ^{13}C values ranging from -33.561 to -31.568 ‰.

Finnigan analysed the δ^{13}C and δ^{15}N of 4 different shipment seizures of heroin by EA-IRMS [Finnigan MAT 1995]. While limited variations was observed for carbon isotope ratios (from -33 to -31 ‰*), the δ^{15}N values were distributed on a wider range (from -9 to -1 ‰ *), suggesting that the plant growth conditions involved different nitrogen sources.

Dautraix *et al.* reported on the δ^{13}C analysis of acetaminophen by EA-IRMS and GC-C-IRMS, when mixed with heroin [Dautraix *et al.* 1996]. They verified that the nitrogen contained in the compounds would not interfere with the analysis. Due to transacetylation between acetominophen and heroin, they observed a constant and reproducible isotope fractionation of about 4 ‰ that could be corrected for.

A preliminary study involving 5 specimens of heroin of unknown origin highlighted the discriminatory power of δ^{13}C and δ^{15}N variables [Ihle & Schmidt 1996]. The combination of δ^{13}C determined by GC-IRMS and bulk δ^{15}N measurement allowed the differentiation of all 5 specimens.

Besacier *et al.* used GC-C-IRMS to supplement their analytical procedure of heroin specimens (i.e. qualitative, quantitative and trace impurities analysis) [Besacier *et al.* 1997a]. The δ^{13}C values of acetylcodeine and diacetylmorphine were measured in triplicate on 9 specimens coming from 5 packages of heroin. In addition to confirming the homogeneity of the packages, this procedure allowed them to observe 3 groups among the specimens by means of biplots.

Besacier *et al.* investigated then the isotopic effect of heroin deacetylation and morphine acetylation by acetic anhydride on the δ^{13}C value of the resulting product, respectively morphine and diacteylmorphine [Besacier *et al.* 1997b]. 31 specimens of heroin of presumed geographical origin were analysed by GC-C-IRMS. The isotopic fractionation of -3.3 ‰ caused by morphine acetylation, as well as the δ^{13}C variability measured for heroin coming from the same geographical source suggested that carbon isotope ratio was influenced by both factors. Because the carbon isotope ratio of heroin depends on the geographical origin of the opium poppy, as well as on the source of the acetic anhydride used to convert morphine to diacetylmorphine, the authors indicated that the δ^{13}C value was inappropriate for origin assignment of heroin. However, the deacetylation of 17 heroin specimens allowed recovering the original δ^{13}C value of morphine, the natural precursor. The δ^{13}C value of morphine, reflecting the environmental conditions of growth, could provide an indication on the geographical origin of the heroin specimens. Nevertheless, the closeness of values may limit the discriminating power of such variable.

Later, Besacier *et al.* analysed the δ^{15}N values of diacetylmorphine and caffeine in heroin specimens [Besacier *et al.* 1999]. Given the narrow range of delta values (-4.34 to +0.40 ‰), the authors could not determine the geographic origin of heroin with certainty. However, they mention the potential of isotopic information to trace cutting agents added at each stage of the distribution chain.

Ehleringer *et al.* performed bulk isotopic analysis on 76 heroin specimens and their associated extracted morphine [Ehleringer *et al.* 1999]. With a variations range of 2.4 ‰ and 3.1 ‰ for δ^{13}C and δ^{15}N respectively, each region of origin of heroin (Mexico, Southwest Asia, Southeast Asia and South America) could be statistically differentiated. For deacetylated heroin, the combined δ^{13}C and δ^{15}N values of the resulting morphine even improved the geographical distinction of the specimens.

In a casework refered in [Phillips *et al.* 2003a], the isotopic profile of heroin traces recovered in a vehicle was compared to those of a bulk seizure. The isotopic profiles of the traces and the bulk material were different, leading to the conclusion that the trace did not originate from the bulk material.

Carter *et al.* reported the analysis of 5 seized heroin specimens by GC-MS, EA and TC/EA-IRMS [Carter *et al.* 2005]. Combining the heroin purity, the chemical profile, the isotopic composition of heroin and of cling film wrapping the specimens, the authors concluded that 2 out of the 5 heroin specimens had been prepared with the same supply of heroin, diluted with the same cutting agent and wrapped in the same packaging material.

Casale *et al.* also investigated the influence of the Columbian manufacturing process used in South America on the isotopic profile of heroin specimens [Casale *et al.* 2005]. Like Besacier *et al.* [Besacier *et al.* 1997b], the authors noted that the production of kilograms of heroin base from the acetylation of morphine base induced a carbon kinetic isotopic fractionation (-1.5 ‰). This shift, however smaller than the one found by Besacier *et al.* (-3.3 ‰) who worked on a milligram scale, suggests there is a scale factor. Successive precipitations of heroine base showed there was limited δ^{15}N fractionation in the first two precipitates, while the third precipitate of heroin base had a significant depleted δ^{15}N value. The conversion of heroin base to heroin HCl did not induce a carbon isotopic fractionation, in contrast to δ^{15}N values which showed significant and variable isotopic enrichment. In addition, the δ^{13}C value of the original morphine base was compared to that of deacetylated heroin HCl. This experiment revealed that the δ^{13}C values of the deacetylated products were close to those of original raw morphine starting material, confirming thus the results of Besacier *et al.* [Besacier *et al.* 1997b], and the possibility of being indicative of a given geographical region. Furthermore, the authors proposed an equation to calculate the approximate δ^{13}C value of the acetic anhydre used to produce Colombian heroin.

Galimov *et al.* performed the single analysis of 9 specimens of heroin and one specimen of morphine coming from Colombia, Korea and Afghanistan by EA-IRMS and GC-C-IRMS [Galimov *et al.* 2005]. Carbon isotope ratios varied on a wide range from -38.35 ‰ to -32.74 ‰, while nitrogen isotope ratios ranged from -6.69 ‰ to +7.15 ‰. Comparing their data with values published by Desage *et al.*, they found that the δ^{13}C values of their specimens were significantly different from heroin produced in Niger, Pakistan and Thailand, but not significantly different from Turkish heroin.

Idoine *et al.* determined the δ^{13}C, δ^{15}N, δ^{18}O and δ^{2}H values of 14 seized heroin specimens by EA-IRMS [Idoine *et al.* 2005]. They also measured δ^{13}C and δ^{2}H by GC-C-IRMS. Compared to δ^{13}C, δ^{15}N and δ^{18}O of heroin, δ^{2}H values showed the largest variability. However, as heroin could be diluted with cutting agents, they concluded that EA-IRMS was not suitable for tracing a distribution chain, unlike GC-C-IRMS, which allowed the analysis of separate compounds. δ^{13}C of diacetylmorphine measured by GC-C-IRMS indicated 2 potential geographic origin, while δ^{13}C of caffeine suggested different cutting events.

Zhang *et al.* analysed the δ^{13}C value of 10 seized specimens of heroin assumed to come from different regions and their respective hydrolysed fraction, morphine, by GC-C-IRMS [Zhang *et al.* 2005]. In order to obtain morphine, 4 heroin specimens were hydrolysed under acidic conditions and 9 under alkaline conditions. δ^{13}C values of heroin oscillated between -37.51 to -35.23 ‰, whereas those of morphine were more enriched with values from -34.67 to -32.58 ‰. Through experiments on the acetylation of morphine and the reverse conversion of the resulting heroin into morphine, the authors observed no isotopic fractionation of the δ^{13}C values of morphine before and after the synthesis. This confirms the conclusions of precedent works [Besacier *et al.* 1997b, Casale *et al.* 2005] on the potential of the δ^{13}C value of morphine to infer the geographical origin of heroin specimens. The δ^{13}C values of both heroin and its extracted morphine provides information on the acetylating reagent.

Casale *et al.* analysed 20 heroin HCl samples seized on the cargo vessel Pong Su Australia. The very rare impurities profile of the specimens raised the issue of a possible new region of origin or new process [Casale *et al.* 2006]. The bulk δ^{13}C and δ^{15}N of the specimens were determined to see if the values could correspond to the isotopic profile of specimens of known geographical origin (Southeast Asia, Southwest Asia, South America and Mexico). In addition, the carbon isotope ratios of diacetylmorphine and morphine were also measured by GC-C-IRMS. However, the results confirmed previous impurity analyses: the heroin specimens had an isotopic distinct signature from the common known regions.

Meier-Augenstein reported a pilot study on the oxygen and hydrogen analysis of heroin specimens [Meier-Augenstein 2010]. The first results showed the wide variability of δ^{18}O and δ^{2}H values ranging respectively from +11.5 to +32.1 ‰ and -179 to -80 ‰, highlighting further possibilities to discriminate specimens especially with hydrogen analysis.

Table 4.5 summarises the range of bulk and compound specific[5] isotopic values of heroin reported in literature.

In essence Most studies on the isotopic analysis of natural illicit drugs have focused on the determination of the geographical origin of the specimens. The bulk δ^{13}C and δ^{15}N values are the most common parameters measured by IRMS for cannabis, heroine and cocaine. Growing interest is now focused on δ^{18}O and δ^{2}H values. As they are related to precipitation and humidity, they may offer new possibilities to assist in differentiating the geographical origin of plant-derived products. However, inference of the geographical source based on stable isotopes is not always evident.

For cannabis, the distinction of geographical regions according to the natural δ^{13}C and δ^{15}N isotopic variations is hindered by the wide range of growth conditions of indoor and outdoor crops. Whereas the natural isotopic variations of outdoor grown cannabis are well documented, research on cannabis known to have grown indoor is more limited. Indoor cultivation conditions often impose a set of unnatural conditions (hydroponic system, growing conditions, forced light cycles, over-ventilation, moist environment, optimum nutrients distribution, etc.), stressing the plant, in order to accelerate growth and increase the yield of active substance. In contrast, outdoor crops, although hidden in the shade or dissimulated among flora to prevent from aerial identifications, reflect more natural environment. As a result of these cultivation methods, carbon and nitrogen stable isotopes of seized cannabis specimens are not traceable to a geographic origin, but rather indicative of a growth environment as attested by the results of current studies. δ^{13}C value may indicate indoor or outdoor growth, although there is an overlap between the two, preventing samples in the middle zone from a categorical classification. According to empirical data of forensic studies, cannabis plants which had been grown outdoor displayed δ^{13}C value between -32 and -23, whereas plants known to have grown indoor showed values between -36 to -27 ‰. Even more depleted values as much as -61.8 ‰ have been measured and have been attributed to presumed indoor grown plants [Booth *et al.* 2010], due to additional CO_2 cylinder supply. Noteworthy is the fact that data on indoor grown specimens are fewer than those on outdoor plants. Depending on the soil conditions and the biosynthetic mechanisms, nitrogen isotope ratio is related to the source of nitrogen of the plant, mainly influenced by the type of fertiliser used. Organic and inorganic

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[5] When GC-C-IRMS was used, values reported in the table are for diacetylmorphine.

Authors	**Method**	**Number specimens**	**Origin**	δ^{13}**C values [‰]**	δ^{15}**N values [‰]**	δ^{18}**O values [‰]**	δ^{2}**H values [‰]**
[Desage *et al.* 1991]	GC-IRMS	unknown	Turkey India, Niger Pakistan Thailand	-33.561 to -31.568	-	-	-
[Finnigan MAT 1995]*	EA-IRMS	4	unknown	-33 to -31	-9 to -1	-	-
[Dautraix *et al.* 1996]	GC-IRMS	1	unknown	-32.81	-	-	-
[Ihle & Schmidt 1996]	EA-IRMS GC-IRMS	5	unknown	-34.1 to -31.4	-3.6 to +1.7		
[Besacier *et al.* 1997a]	GC-IRMS	9	unknown	-34.97 to -31.16	-	-	-
[Besacier *et al.* 1997b]	GC-IRMS	31	India, Africa Lebanon Turkey, Syria Thailand Pakistan	-34.97 to -28.06	-	-	-
[Besacier *et al.* 1999]	EA-IRMS	20	India, Turkey Lebanon Thailand	-	-4.34 to +0.40	-	-
[Ehleringer *et al.* 1999]*	EA-IRMS	76	Mexico S-E Asia S-W Asia South America	-34 to -29	-2 to +3	-	-
[Carter *et al.* 2005]	EA-IRMS	5	unknown	-33.4 to -31.9	-8.3 to -3.0	+18.3 to +19.7	-173.6 to -143.5
[Casale *et al.* 2005]	EA-IRMS	12	Colombia	-32.4 to -27.5	-12.2 to +4.8[a]	-	-
[Galimov *et al.* 2005]	EA-IRMS GC-IRMS	3 6	Colombia Korea Afghanistan	-35.11 to -33.26 -38.35 to -32.74	-6.69 to +1.29 -2.93 to +7.15	-	-
[Idoine *et al.* 2005]*	EA-IRMS GC-IRMS	14	unknown	-34 to -29 -40.71 to -32.60	-9.0 to -2.5	+15 to +20	-200 to -110 -203 to -158
[Zhang *et al.* 2005]	GC-IRMS	10	unknown	-37.51 to -35.23	-	-	-
[Casale *et al.* 2006]	EA-IRMS GC-IRMS	20	unknown	-32.8 -32.3	-2.5 to -0.5 -	-	-
[Meier-Augenstein 2010]	EA-IRMS	unknown	unknown	-	-	+11.5 to +32.1	-179 to -80

Table 4.5. Summary of data on the isotopic analysis of heroin reported in literature.

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[a] Heroin base and HCl precipitates were considered

fertilisers fed plants can be differentiated on their δ^{15}N value. In addition, the δ^{15}N variations between specimens indicate that many different inorganic fertilisers are used. Moreover, ongoing research on hydrogen stable isotope may provide additional elements for regional or geographic origin determination [Hurley *et al.* 2010a].

As hydroponic and indoor cultures have largely supplanted outdoor crops in many countries to limit visibility and to increase marijuana yields, there is a tangible need for knowledge on the isotopic variability of indoor grown cannabis. Since cannabis plants reflects their growth environment, a relevant question in a case-to-case comparison pertains to the possibility to discriminate specimens grown under different sets of indoor conditions. Indeed, the isotopic variations of the different nutrients administered to plants (non-atmonpheric CO_2 from gas cylinders or fermentation CO_2 generators, fertiliser and water supply) as well as the influence of conditions such as light exposure, humidity, temperature and ventilation conditions, may contribute to create an isotopic signature reflecting a given set of indoor growth conditions, differentiable from other sets of growth conditions. This is of course a hypothesis that needs to be investigated. In that perspective, additional data on intravariability are heavily needed. Some studies have shown that different parts of the cannabis plant exhibit different bulk isotopic profile. While no significant isotopic differences were noticed between leaves, twigs and seeds [Shibuya *et al.* 2006], variations of isotopic composition were observed between leaves and flowers [Liu *et al.* 1979, West *et al.* 2009]. Moreover, significant variations in carbon and nitrogen isotope ratios [Denton *et al.* 2001], as well as in oxygen and hydrogen isotope ratios [Booth *et al.* 2010] were observed within plant leaves. Light, ventilation and CO_2 supply may affect within plant variability and result in isotopic stratification for dense crops. Finally, question pertaining to the influence of the matureness (grow stage) of the plant on the isotopic profile of the different tissues should be investigated.

For illicit drugs requiring chemical processing, research has shown that stable isotopes may be used to infer their geographical origin. Cocaine from different geographical growing regions exhibited differentiable isotopic profiles, suggesting that stable isotopes are connected to the geographical location of coca leaves. The influence of the chemical extraction of cocaine alkaloids and the conversion of cocaine paste to cocaine base is however unknown. Interestingly, as highlighted by Ehleringer *et al.* [Ehleringer *et al.* 1999, Ehleringer *et al.* 2000], the bulk δ^{13}C and δ^{15}N values of cocaine are significantly more depleted (-3 to -8 ‰) than bulk plant material.

The geographic origin of heroin may be revealed through the δ^{13}C value of morphine, while the δ^{13}C value of heroin provides information on the acetylating reagent. The study of Casale *et al.* provides possible explanations on the origin of δ^{15}N variations through the manufacturing process of heroin [Casale *et al.* 2005]. At present, it appears however difficult to relate the nitrogen isotopic variations to a specific level of source.

Moreover, most research analysed the bulk isotope values of illicit drugs. Natural impurities as well as cutting agents such as phenacetin or lidocaine in cocaine, or paracetamol and caffeine in heroin, contribute to the bulk value, reflecting thus an average of the isotopic composition.

An indisputable fact is that specimens from seizures have different isotopic profiles [Carter *et al.* 2005, Sewenig *et al.* 2007]. The origin of such variations is currently unknown and could remain it for a while. However, an exciting perspective is the comparison of the isotopic profile with the natural and production impurities drug profile, as both are connected to growth environment and chemical processing. The association and exploitation of different types of variables provide information on different levels of sources for investigative purpose.

3.6.2 Synthetic illicit drugs

Unlike natural illicit drugs where IRMS technology may assist in ascertaining the geographical origin of the plant-derived product, isotopic variations in synthetic illicit drugs are expected to originate both from the precursors and reagents used and the synthetic reactions (or synthetic pathways). The measurement of stable isotopes is therefore used in a source inference perspective to establish a link between a precursor and a synthetic illicit drug or between two seizures. It is also used to determine the synthetic pathway used to manufacture the illicit drug.

Amphetamine-type stimulants - ecstasy Mas *et al.* analysed the δ^{13}C values of MDMA (3,4-methylenedioxy-N-methylamphetamine) contained in 16 confiscated ecstasy tablets by GC-C-IRMS [Mas *et al.* 1995]. Although discrimination was limited, δ^{13}C varied from -29.3 to -27.2 ‰ allowing the distinction of 4 groups of specimens. The analysis of bulk δ^{15}N value provided further discrimination. However, as some specimens were found to contain also amphetamine and caffeine, the nitrogen bulk value also encompassed the contribution of these molecules.

Carter *et al.* measured the isotopic profile of 50 seized ecstasy tablets, containing either pure MDA (3,4-methylenedioxyamphetamine) or MDMA, from 5 batches [Carter *et al.* 2002b]. While δ^{13}C and δ^{2}H obtained by GC-C-IRMS offered little discrimination, the bulk δ^{15}N values showed wider variability (from -4.0 ‰ to +20.9 ‰), most probably coming from the synthetic route involved in the manufacturing process. The ^{2}H site-specific analysis determined by site-specific natural isotope fractionation-nuclear magnetic resonance (SNIF-NMR) revealed significant differences between batches, denoting promising possibilities for further differentiation.

The same leading authors investigated the sources of δ^{13}C and δ^{15}N variations in amphetamines, through the reverse conversion of MDMA into its reciprocal ketone, as well as the synthesis of 4 batches of methamphetamine from phenylacetone [Carter *et al.* 2002a]. These experiments proved that the differences observed between the starting materials and the final products originated, somewhat partly, from isotopic fractionation induced by the reductive amination step of the manufacturing process.

Measuring δ^{13}C and δ^{15}N by GC-C-IRMS, Palhol *et al.* confirmed the limited δ^{13}C variations (-24 to -28 ‰) of MDMA contained seized tablets [Palhol *et al.* 2002, Palhol *et al.* 2003]. Typical of natural products derived from vegetation, these values were close to those of precursors, (safrole and isosafrole). However, the δ^{15}N values of MDMA, with a wide range of variations (from -16 to +19 ‰), allowed further discrimination and confirmed the similarity observed between the logos, physical parameters and MDMA content of some tablets. As the subsequent chemical profiling of the tablets corroborated the established links, the authors assumed that the tablets had been produced in the same clandestine lab. They also noted that the small range of δ^{15}N variations measured in precursors (-5.8 to 5.5 ‰) did not correspond to the large ones observed in tablets, indicating the occurrence of isotopic fractionation during synthesis. This was confirmed by the synthesis of MDMA through reductive amination and Leuckart reaction, which induced negative and positive isotopic shift respectively.

Focusing on δ^{15}N, Palhol *et al.* further investigated the potential of IRMS to link seized ecstasy tablets through a multivariate statistical analysis [Palhol *et al.* 2004]. Combining the physical parameters of 106 tablets (logo, mass, size, form, content, etc.) and the δ^{15}N values, they applied principal component analysis (PCA) followed by hierarchical cluster analysis (HCA). With the δ^{15}N value and the MDMA content as the most discriminant parameters, the statistical analysis assembled the specimens into 5 groups, most probably with similar synthetic pathway. Again, the chemical profile analysis of the tablets confirmed the established links.

Carter *et al.* related the use of the isotopic composition of ecstasy tablets from 2 seizures as an investigative tool in a casework [Carter *et al.* 2005]. The two MDMA tablets seizures, found in the same place, had visually similar characteristics. Two tablets of each seizure were analysed for δ^{13}C and δ^{15}N by EA-IRMS. Tablets of the first seizure, with δ^{13}C and δ^{15}N values of -28.85 ‰ ±0.47 and 7.61 ‰ ±0.05 respectively were found indistinguishable at 95 % confidence from tablets of the second seizure, with δ^{13}C and δ^{15}N values of -28.87 ‰ ±0.18 and 6.86 ‰ ±0.07 respectively. Considering their background study [Carter *et al.* 2002b], the authors concluded that they originated from the same source [Forensic Isotope Ratio Mass Spectrometry 2003a]. On the presentation of such evidence, the defendant pleaded guilty and the case went eventually to trial in Nottingham Crown Court in 2002

[Forensic Isotope Ratio Mass Spectrometry 2003b].

Billault *et al.* synthesised 45 samples of MDMA under controlled conditions according to 5 synthetic routes involving different nitrogen precursors [Billault *et al.* 2007]. δ^{13}C and δ^{15}N were analysed by EA-IRMS in order to see if the isotopic profile could provide indications on the synthetic route and the precursor employed. The δ^{13}C value of MDMA, ranging from -30.4 to -26.0 ‰, showed to be influenced by the number of steps involved in the synthesis. For nitrogen, precursors with δ^{15}N values fluctuating from -17.9 to +26.4 ‰ resulted in MDMA with δ^{15}N extending from -8.5 to +17.4 ‰. Although the δ^{15}N value of MDMA was clearly influenced by that of nitrogen sources, the synthetic pathway and reaction conditions, distinct correlation patterns between the isotopic profile and the synthetic route could not be highlighted. Indeed, the δ^{15}N values were spread on a large range and were not reproducible for synthesis involving 4 steps. They mentioned that 2 specimens with similar δ^{13}C and δ^{15}N values may well have resulted from different synthetic pathways, given the large interval of variations and the significant differences induced by a modification of synthesis conditions. However, the authors proposed the combination of other analytical data with stable isotopes to determine the synthetic route of MDMA specimens.

De Korompay *et al.* proposed the use of supported liquid extraction (SLE) cartridges to extract MDMA contained in ecstasy tablets, before the δ^{13}C and δ^{15}N analysis of pure MDMA by EA-IRMS [de Korompay *et al.* 2008]. On a set of 4 batches of 10 tablets each, the authors tested different conditions (organic eluent, volume of eluent, concentrations of aqueous base) and observed no significant isotopic fractionation after the extraction step. The method was reported to be highly reproducible and not influenced by the tablet matrix.

Neumann, as cited in [Wakelin *et al.* 2008], reported the use of isotopic evidence in the Regional Superior Court judgement of a case concerning a large MDMA clandestine laboratory. The isotopic analysis of MDMA samples proved that at least 2 batches of MDMA had been produced by the laboratory.

Buchanan *et al.* synthesised 18 samples of MDMA following 3 reductive amination routes [Buchanan *et al.* 2008]. Producing a large batch of piperonyl methyl ketone (PMK) from a bottle of safrole, the authors varied the reducing agents and subsequently analysed the bulk δ^{13}C, δ^{15}N and δ^{2}H values of the resulting MDMA. δ^{13}C varied little (by 1.6 ‰), as the carbon came from the same batch of safrole for all 18 samples. With a variation range of 27.4 ‰, δ^{15}N values showed that only one particular synthetic route was isotopically distinct from the 2 others. They demonstrated that isotopic fractionation during the synthetic process was mainly responsible for the resulting δ^{15}N values. HCA showed that, even used alone, δ^{2}H data, varying from -93.9 to +6.3 ‰, allowed differentiating all samples according to their synthetic route. While controlled and constant reaction conditions produced reproducible δ values within some synthetic routes, other showed significant variability witihin the same synthetic process. Because of this variability despite careful controlled conditions, the authors concluded that this variable was not adequate to determine the synthetic route and that it could only be used for discriminating samples from different production batches.

Using factorial design, Buchanan *et al.* investigated the influence of 5 synthetic reaction conditions related to the Pt/H_2 reductive amination synthesis of MDMA on its bulk $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$ and $\delta^{2}H$ values [Buchanan *et al.* 2010]. However, modifying the reaction conditions led to significant isotopic variations within the 32 synthesised batches. Even stable isotopes supposed to remain unchanged and close to the value of the starting material, such as $\delta^{13}C$ and $\delta^{18}O$, were subject to isotopic fractionation. Indeed, isotope ratios were highly dependant on the reaction conditions and reaction efficiency. The authors highlighted the importance of such knowledge to understand the origin of isotopic variations between specimens. Besides, they stressed the complexity of the situation for the interpretation of isotopic data.

The ability of organic impurities profiling and stable isotope analysis to discriminate between MDMA samples of known origin were assessed in a recent paper [Buchanan *et al.* 2011]. The organic impurities obtained by GC-MS and the bulk isotopic profile of samples previously synthesised under different controlled conditions were evaluated in combination with diverse pre-treatments and pattern recognition techniques (HCA, PCA, discriminant analysis (DA)). Neither GC-MS impurities, nor IRMS data on their own allowed full discrimination of the synthetic route or batch of starting material. However, the application of DA to the data set showed the best results over PCA or HCA. A major drawback of DA is that it requires the operator to determine the number of groups to be used for classification, which is an unknown parameter for MDMA of unknown source. Although it has been undertaken in details and with scrutiny, such studies lead to the question: are the police really interested in knowing the synthetic route employed to produce the seized MDMA specimens? This information is obviously relevant. However, it is worth noting that such data set conveys in reality a higher dimension of information which could be exploited at another level of source.

Amphetamine-type stimulants - methamphetamine and precursors The growing concern that arose some years ago on methamphetamine engendered several studies on that particular type of drugs.

Kurashima *et al.* measured the carbon and nitrogen isotope ratio of 13 ephedrine samples coming from biosynthetic, semi-synthetic and synthetic origins by EA-IRMS [Kurashima *et al.* 2004]. $\delta^{13}C$ values enabled differentiating semisynthetic ephedrine (-23.1 to -22.0 ‰) from biosynthetic (-31.1 to -26.0 ‰) and synthetic

(-29.2 to -28.0 ‰) ones. Using δ^{15}N values the authors achieved further discrimination between synthetic ephedrine (-10.5 to -10.0 ‰) and semisynthetic or biosynthetic ephedrine (3.8 to 10.6 ‰). δ^{13}C and δ^{15}N values of methamphetamine synthesised from the different ephedrine samples showed similar delta values to the ephedrine. The additional analysis of 25 methamphetamine specimens, of which 21 came from police seizures, confirmed the potential of IRMS to highlight differences between groups of specimens.

Supplementing the research of Kurashima *et al.*, Makino *et al.* analysed pseudoephedrine, another precursor of methamphetamine, manufactured from L-ephedrine [Makino *et al.* 2005]. The pseudoephedrine samples from 6 manufacturers could be differentiated based on their δ^{13}C and δ^{15}N values. The obtained values gave indications on the origin of the ephedrine used for the manufacture of methamphetamine.

Iwata *et al.* evaluated the concomitant use of impurity profiling and carbon and nitrogen isotopic analysis of seized methamphetamine by EA-IRMS [Iwata *et al.* 2008]. Using Euclidean distance, the degree of similarity of 39 methamphetamine specimens was first evaluated on the basis of investigative information and impurity profiling. δ^{13}C varied by 4.5 ‰ (from -29.4 ‰ to -24.9 ‰) and δ^{15}N values by 8.3 ‰ (from -2.3 ‰ to + 5.9 ‰). The exploitation of isotopic data in addition to impurity profiling and investigative information confirmed the pre-established links between different cases.

Pursuing their research on the identification of the manufacturing processes of ephedrine through δ^{13}C and δ^{15}N data, Kurashima *et al.* extended their research by analysing hydrogen isotope ratios of 27 legal ephedrine and pseudoephedrine samples, 2 seized specimens of ephedrine and 25 methamphetamine specimens seized in Japan [Kurashima *et al.* 2009]. Besides, 7 samples of methamphetamine were also synthesised from the set of precursors. δ^{2}H values obtained by TC/EA-IRMS allowed differentiating between biosynthetic (-193 and -151 ‰) and synthetic (-73 and -30 ‰) or semi-synthetic (-74 and +243 ‰) manufacturing processes of ephedrine. Moreover, hydrogen isotope fractionation occurring during the synthesis of methamphetamine did not preclude to the correct inference of the type of ephedrine used. The subsequent combination of carbon, nitrogen and hydrogen isotopic values offered a clearer distinction of ephedrines according to their manufacturing process. This ternary system also provided indications on the potential origin of the ephedrine used for the synthesis of the 25 seized methamphetamine.

The results of Collins *et al.* published the same year [Collins *et al.* 2009] partly confirmed the results of the group of Kurashima *et al.*. Collins *et al.* reported the carbon, nitrogen and hydrogen analysis of methamphetamine synthesised from ephedrine and pseudoephedrine of natural and semi-synthetic origin. While δ^{13}C, δ^{15}N and δ^{2}H values of precursors were not subject to isotopic fractionation during synthesis of methamphetamine, they observed that methamphetamine synthesised from natural ephedrine showed negative values and those synthesised from semi-synthetic ephedrine displayed positive values only. Collins *et al.* finally concluded that the use of bulk analysis for determining the precursors origin of methamphetamine might only be successful with high purity methamphetamine specimens.

Iwata *et al.* analysed 3 seized packages of methamphetamine specimens by GC-MS and EA-IRMS [Iwata *et al.* 2010]. Despite a similar impurity profile by GC-MS, the isotopic analysis of crystals showed different signatures. While δ^{13}C values were constant between specimens (-25.14 to -24.65 ‰), the δ^{15}N values of the specimens showed considerable intravariability, with variations by more than 12‰ according to the crystal analysed. The authors assumed therefore that packages of methamphetamine contained crystals coming from different "batches". Based on these results, the authors defended the crystal profiling approach by IRMS versus the average isotopic data obtained by the common homogenisation of the specimen. It appears however difficult, from their work, to understand the level of hypothesis and the definition of source considered. Nevertheless, it highlights the significance of the representativeness of the sampling when using IRMS.

Through their study on isotopic fractionation during precipitation of methamphetamine, David *et al.* offered a possible explanation for the results obtained by Iwata *et al.* [David *et al.* 2010a]. David *et al.* measured the bulk δ^{13}C, δ^{15}N and δ^{2}H values of 8 specimens of methamphetamine synthesised from ephedrine. The authors reported the isotopic fractionation of δ^{15}N and δ^{2}H induced by the multiple precipitation steps necessary to produce methamphetamine HCl sample, resulting in precipitates becoming gradually more depleted in heavy isotopes. This phenomenon was also related by Casale *et al.* for δ^{15}N value of cocaine and heroin [Casale *et al.* 2005]. David et *et al.* highlighted that, even specimens produced with the same precursor may present a wide intravariability. Using subsequently HCA on isotopic data of 38 methamphetamine specimens from 4 seizures, they speculated on the origin of the specimens and mixed up the possibility to infer the synthetic or non-synthetic origin of the specimens and the inference of origin of the same criminal group.

By means of experimental design, David *et al.* investigated the influence of changes in reaction conditions during the synthesis of methamphetamine on their isotopic profile [David *et al.* 2010b]. 53 samples of methamphetamine were analysed by EA, TC/EA-IRMS and SNIF-NMR of which 48 were synthesised under controlled conditions using variations of the hydriodic acid/red phosphorus synthetic route and 5 under uncontrolled conditions. δ^{13}C was not significantly influenced by reaction conditions, emphasising the possibility to link a precursor and its product by means of their δ^{13}C value. Experimental designs showed that δ^{13}C and δ^{2}H values of the precursors were the main determinants of carbon and hydrogen isotopic composition of methamphetamine. In contrast, results on δ^{15}N values required further investigations.

A recent study [Salouros *et al.* 2012] investigating the influence of different synthesis conditions on the δ^{13}C, δ^{15}N and δ^{2}H of methamphetamine confirmed the previous results of David *et al.*. The different variations of synthetic conditions of the Nagai and Emde routes did not alter the isotopic profile of the resulting methamphetamine. Thus, the ephedrine and pseudoephedrine used to produce methamphetamine may be linked through their isotopic profile.

Seven samples of seized methamphetamine were analysed for their organic impurities by GC-MS and their bulk δ^{13}C and δ^{15}N [Tsujikawa *et al.* 2012]. Except for two specimens that produced similar profile and were therefore classified in the same group with both instrumental technique, the five remaining specimens were not classified in the same way on the basis of the results of the two techniques. Nevertheless, the authors report the enhanced discrimination of the additional use of IRMS.

Schneiders *et al.* analysed 7 seized specimens of 1-phenyl-2-propanone (P2P), an amphetamine-type stimulant precursor, coming from 4 caseworks. The specimens displayed different isotopic profiles with δ^{13}C values oscillating between -32.1 and -27.3 ‰ and δ^{2}H values between -196 to -85 ‰ [Schneiders *et al.* 2009]. In order to interpret the observed differences, the authors evaluated the isotopic variations within and between 9 batches of one manufacturer of P2P produced over 5 years. δ^{13}C was found to vary from -31.6 to -28.0 ‰, δ^{2}H ranged from -71.3 to -53.3 ‰ and δ^{18}O from 10.3 to 21.5 ‰. Although δ^{2}H determination increased discrimination between batches, the isotopic heterogeneity of batches observed within this manufacturer hampered the interpretation of isotopic variations of the specimens.

Collins *et al.* measured the bulk δ^{2}H value of amphetamine produced from natural and industrial natural benzaldheyde and nitroethane, as well as non-benzaldehyde-derived amphetamine [Collins *et al.* 2010]. The few analyses showed the possibility to discriminate amphetamine according to their precursor and synthetic route on the basis of δ^{2}H data. The authors presented their method as an alternative for determining the synthetic pathway of amphetamine specimens, in the absence of route-specific impurities.

Other synthetic illicit drugs Marclay *et al.* demonstrated the potential of GC-C-IRMS for tracing gamma-butyrolactone (GBL), a chemical precursor of gamma hydroxybutyric acid (GHB), through the high repeatability of δ^{13}C measurements on samples of GBL batches and the wide intervariability (from -45.5 to -23.1 ‰) observed between 19 GBL from different sources [Marclay *et al.* 2010].

The authors also distillated some bulk GBL, in order to investigate if a purification step in the production of GBL could explain the wide range of δ^{13}C values observed among the specimens. This step only induced a small isotope fractionation of 0.8 ‰, leaving open questions about the origin of δ^{13}C variations in GBL.

Concerning the detection of GHB in body fluids, Saudan *et al.* proposed to determine the δ^{13}C value of urinary GHB as an alternative to the concentration cut off limit to differentiate endogenous or exogenous GHB [Saudan *et al.* 2007]. δ^{13}C values of endogenous GHB were distributed on a narrow range (-27.0 to -23.5 ‰) in contrast to exogenous GHB with δ^{13}C values from -42.1 to -32.1 ‰.

Marclay *et al.* studied the δ^{13}C value of urinary GHB following the ingestion of prescripted Xyrem^{TM}, a pharmaceutical preparation containing GHB, administered to 6 individuals [Marclay *et al.* 2011]. They investigated the occurrence of potential isotopic fractionation induced by the metabolism in the body. The comparison of the δ^{13}C values of urinary GHB and that of the marketed GHB varied on a very small range (from -25.06 ‰ to -24.81 ‰). Statistical tests did not highlight significant differences between the δ^{13}C value of the administered GHB and urinary GHB, indicating that the metabolism of GHB did not induce isotopic fractionation. Thus, carbon isotope ratio of GHB may be used for example to establish a link between GHB found in the house of a suspect and the urinary GHB of a person. Interestingly, according to the study of Saudan *et al.* [Saudan *et al.* 2007], the isotopic value of administered GHB reported in this study would have constituted a false negative, as the value falls within the narrow range of endogenous GHB [Saudan *et al.* 2009].

In essence From current literature on synthetic illicit drugs, there are two main trends. The first one encompasses studies, whose sampling is based on drugs of known sources. They focused mainly on the possibility to differentiate drugs according to the synthetic routes and the precursors employed or, for ephedrine, according to their bio or synthetic origin. Conversely, the second category of studies involved specimens seized by law enforcement, and therefore of unknown "source". Studies in this category mostly concern caseworks or case-to-case comparisons. As specimens are of unknown source, the definition of the notion of source in these studies have always been smartly passed around by a generic expression such as "specimens came from the same source".

From research conducted on synthesised drugs, it appears that the isotopic profile varies within a synthetic route despite controlled conditions [Buchanan *et al.* 2008]. Modifications of the reaction conditions within a synthetic route induce significant isotopic differences between batches of MDMA produced with the same batch of precursors and reagents [Buchanan *et al.* 2010]. This was considered as a complex issue by Buchanan *et al.*, hindering interpretation step.

At present the definition of the source in the isotopic analysis of synthetic drugs has been mainly focused on the synthetic route and the precursors used. However, Buchanan *et al.* showed the isotopic profile was of limited use to infer the synthetic route of the drug. In light of these results, studies involving specimens of unknown sources and concluding on the common source of some synthetic drug exhibits may well be unsettled, and in search of a new meaning of "source".

From a police point of view, the high variability and poor reproducibility of the isotopic profile within a synthetic route may be perceived differently. Indeed, the wide variability of the multi-element isotopic profile may not be traceable to a specific synthetic route, but may offer the possibility to differentiate production batches of MDMA, meaning that the "poor" reproducibility of synthetic conditions may constitute a certain degree of "specificity" of the batch. This hypothetical "specificity" is supported by the fact that illicit synthetic drugs may be manufactured in a less controlled manner than scientific bench experiments, as rightly mentioned by Buchanan *et al.*. These uncontrolled conditions would induce an even wider variability and lead to production batches with variable isotopic profiles. This hypothesis needs obviously to be studied and evaluated through inter and intravariability of production batches. On that point, measured δ values were reported with small standard deviation for triplicate analysis, providing a preliminary indication on the isotopic homogeneity of the batches. Yet, these experiments were conducted on a small-scale and do not replace a thorough evaluation of batch intravariability.

The evaluation of the isotopic variability at such level of source would not indicate that specimens with similar isotopic profile had been synthesised following the same synthetic route (which is of limited use to the police), but could indicate that they had come from the same production batch. This hypothesis necessitates of course to be evaluated and, in order to assess the false positive and false negative rates, require fundamental data on inter and intravariability of production batches.

Intervariability is commonly well measured as it easily shows the discrimination power of the technique. In contrast, intravariability data are cruelly missing for both samples of known and unknown sources, suggesting that variability within a specimen or within a production batch could be overlooked. This lack of knowledge may lead to underestimation of inherent variability and overestimation of the signification of apparent different isotopic values, prejudicing interpretation of isotopic data.

Alternatively, the fundamental aspect of sampling is also raised through the paper of Schneider *et al.*. Their results highlight the significance of sampling on interpretation and questions the notion of sampling representativeness when using IRMS [Schneiders *et al.* 2009].

Finally, CSIA analysis appears more adequate and more specific to deal with the complex matrix of illicit drugs, as they often contain numerous compounds (impurities, active substance, cutting agents). Except for pure illicit drugs, bulk analysis offers a less transparent result.

If the complementarity of IRMS to other techniques has been justly mentioned by Meier-Augenstein, a most peculiar and worrying issue is the far-fetched apparition of DR (dynamic range) notion to evaluate the evidentiary value of the isotopic profile of an ecstasy specimen stored in a database [Meier-Augenstein 2010]. Based on a multivariate data set (δ^{2}H, δ^{13}C, δ^{15}N, δ^{18}O) of seized ecstasy tablets, the author defines the "Dynamic Range (DR, unitless) of the δ value as the isotopic range for a given suite of samples divided by the typical standard deviation of a replicate measurement of the same (e.g. 14‰/0.2‰=70)" (p.168 in [Meier-Augenstein 2010]). By multiplying the DR for each isotope, the final figure provides the probability for a random match. Not only does the system appears questionable, as it does not take into account the distribution of the population, but it also proposes the use of conservative figures for standard deviations (2, 0.5, 0.5 and 0.5 respectively for δ^{2}H, δ^{18}O, δ^{13}C and δ^{15}N values) "given the influence of sample (in)homogeneity on the error of measurement", meaning that the specimen homogeneity is not considered in such an approach.

3.7 Flammable liquids

Most publications reporting the isotopic analysis of petroleum products concern environmental forensics. Research about the isotopic analysis of flammable liquids within the frame of fire investigation is relatively limited. While source inference is the underlying objective in both fields, the research problem, sampling, method and factors influencing the isotope ratio are different. Therefore, only studies presenting a potential interest and relevance for fire investigation have been selected and are reported in this section.

Smallwood *et al.* analysed the δ^{2}H and δ^{13}C values of 3 samples of pure MTBE, an additive in gasoline, purchased from different distributors [Smallwood *et al.* 2001]. The authors tested 4 different methods to determine the carbon isotope ratio of MTBE. The bulk analysis of the samples produced indistinguishable δ^{13}C values. On the contrary, δ^{2}H values ranging from -56.8 ‰ to -36.9 ‰ suggested the possibility to differentiate specimens of MTBE. δ^{13}C value determined through direct injection of the neat samples onto GC-C-IRMS were 0.55 ‰ heavier than the bulk values. The headspace analysis of MTBE neat samples and of water samples showed significant isotopic enrichment of the vapour phase, i. e. an inverse isotope effect. Additionally, the δ^{13}C composition of MTBE of 10 gasoline samples collected at service stations located in 3 different areas of the USA was also determined.

δ^{13}C values of MTBE oscillated between -31.68 ‰ and -29.7 ‰, suggesting with this preliminary study, that GC-C-IRMS may be used to differentiate sources of MTBE.

Jasper *et al.* conducted an initial study on the analysis of gasoline by GC-C-IRMS [Jasper *et al.* 2001]. They analysed one sample of gasoline under 3 different conditions (1 unevaporated neat gasoline and 2 gasolines extracted from burnt carpet, evaporated at respectively 50 % and 90 %). The carbon isotopic composition of 14 specific compounds of gasoline was measured. Data of evaporated gasoline were compared to non-evaporated gasoline. The authors reported the enrichment of most analysed compounds in evaporated gasolines. However, according to the authors, they still showed a general correspondence with neat gasoline. Unfortunately, the methodology and moreover, the lack of data do not provide sufficient arguments to demonstrate the merit of the study.

Smallwood *et al.* reported the δ^{13}C values of 19 gasoline samples collected at several gas stations located in different areas in the USA [Smallwood *et al.* 2002]. The authors selected 16 compounds, each showing a wide range of variations between samples. Using t-test, most gasoline samples could be differentiated from each other based on their carbon isotope ratios. In addition, they investigated the effect of evaporation and water washing of gasoline samples on their isotopic profile. After one week evaporation, some compounds, such as naphtalene derivates, conserved their carbon isotope ratios. Similarly, only part of the compounds of the samples showed to be affected by water washing for one week. These preliminary results showed the potential of GC-C-IRMS to discriminate specimens of gasoline and suggested that all compounds in gasoline samples are not isotopically affected by weathering factors.

O'Sullivan and Kalin measured the bulk δ^2H and δ^{13}C values of a set of 28 gasoline samples, each coming from a different country [O'Sullivan & Kalin 2008]. δ^{13}C values ranged from -33.5 ‰ to -25.9 ‰, while δ^2H values were distributed on a considerable larger range, from -145.5 ‰ to -73.2 ‰. Combining both δ values, the authors could discriminate more than 76 % of the samples. The measurement of the same variables of 4 MTBE samples confirmed the additional discriminating power of hydrogen. Additionally, they determined the compound specific δ^{13}C value of 19 compounds present in all gasoline samples. Using t-test to evaluate the number of compounds significantly different between pairs of gasoline samples, they observed that, despite close values between certain compounds, the discrimination was effective. They also investigated the discriminating power of the isotopic composition of BTEX compounds only and found that the combination of the 6 compounds was sufficient to differentiate 99 % of the pairs of gasoline samples.

Li *et al.* proposed the analysis of 12 target gasoline range compounds by headspace single-drop microextraction (HS-SDME) coupled to GC-C-IRMS

[Li *et al.* 2011]. Comparing the δ^{13}C values of the 12 compounds obtained by direct injection onto GC-C-IRMS with those of the compounds dissolved in water and extracted by HS-SDME, they did not observe any isotope fractionation. The applicability of the method was demonstrated through the analysis of a crude oil. However, as specified by the authors, the method is more suitable for the extraction and analysis of hydrocarbons lighter than C_{13}.

3.8 Human geo-sourcing

Human geo-sourcing or human provenancing is based on the analysis and interpretation of stable isotope ratios in human tissues to determine the geographical origin and life history of a person [Meier-Augenstein 2007, Meier-Augenstein 2010].

Although the isotopic analysis of human tissues in forensic caseworks is rather recent, Katzenberg and Krouse were the first to evoke the possibility of using stable isotope variations in human tissues for forensic investigations [Katzenberg & Krouse 1989]. They highlighted the potential of combining the isotopic composition of different tissues and fluids to provide information on the geographical long-term residence and the recent travel of an individual. They did not apply it in practice to a forensic case, but already emphasised the use of isotopic information in a forensic intelligence perspective.

The first published case involving the exploitation of stable isotope analysis on human remains, the "Adam" case in London, only dates back to 2001 [O'Reilly 2007]. The application of human provenancing in forensic investigations was reported in two other caseworks [Rauch *et al.* 2007, Meier-Augenstein & Fraser 2008]. Three additional cases involving the isotopic analysis of human tissues were related by Meier-Augenstein and Kennedy *et al.* [Meier-Augenstein 2010, Kennedy *et al.* 2011]. The limited applications to caseworks, and the few rare forensic background studies on this topic may raise skepticism on the possibilities of such an application. However, a detailed scrutiny of literature reveals that the knowledge constituting the basis of this science derives from earth science works and from numerous applications in the study of ancient cultures remains. The relationship between the isotopic profile of human tissues and the diet or the environmental conditions of an individual has been much investigated and exploited in archaeology to extract information on paleodietary or paleoenvironmental conditions [Schoeninger *et al.* 1983, Longinelli 1984, Macko *et al.* 1999, O'Connell & Hedges 1999b, Lee-Thorp & Sponheimer 2003, Sharp *et al.* 2003, Bianucci *et al.* 2008, Richards & Trinkaus 2009].

As mentioned earlier, the relationship between the isotopic signature of animal tissues and its diet also prevail for any other organism involved in the food chain. As such, the isotopic analysis of its tissues and fluids may reveal its diet, and hence its dietary habits and geographical environment. Carbon, nitrogen, oxygen and hydrogen stable isotope ratios of human tissues reflect our food and water intake. While δ^{13}C and δ^{15}N are the mirrors of our food intake, δ^{18}O and δ^{2}H are indicative of the isotopic profile of the water we consume through food and beverages [Meier-Augenstein & Fraser 2008].

Reference data indicate that worldwide regions show different isotopic patterns, which can be used to differentiate geographical regions. In order to interpret isotopic values of human tissues, global databases, isotope maps, also known as isoscapes illustrating spatial distribution of isotopic variations [Bowen 2010, West Lab 2011], as well as prediction maps and models for isotope spatial pattern [Ehleringer *et al.* 2008, Van der Veer *et al.* 2009] have been developed.

One major element of exploitation of this information is based on the time at which the tissues of a human body are formed, as well as on their different turnover rates [Katzenberg & Krouse 1989, Rauch *et al.* 2007]. Indeed, skull bone has a much slower turnover than body fluids, hair and nails. As the isotopic composition of the tissue reflect diet and environment at the time of formation of the tissue, the combination of the isotopic signatures of several tissues reveals information on the geographical residence and trajectories of a person on long and short-term scale.

Probably because of the non-invasive character of its sampling, hair is the most studied medium from which isotopic information is extracted [Katzenberg & Krouse 1989, Macko *et al.* 1999, O'Connell & Hedges 1999a, O'Connell & Hedges 1999b, O'Connell & Hedges 2001, Bol & Pflieger 2002, Sharp *et al.* 2003, Fuller *et al.* 2005, Petzke *et al.* 2005, Fraser *et al.* 2006, Bol *et al.* 2007, Fraser & Meier-Augenstein 2007, O'Brien & Wooller 2007, Ehleringer *et al.* 2008, Fraser *et al.* 2008, Mützel Rauch *et al.* 2009, Thompson *et al.* 2010, Kennedy *et al.* 2011, Landwehr *et al.* 2011]. Other media studied for their isotopic composition are fingernails [O'Connell & Hedges 2001, Fraser *et al.* 2006, Fraser & Meier-Augenstein 2007, Fraser *et al.* 2008], teeth enamel [Lee-Thorp & Sponheimer 2003, Bell *et al.* 2006, Alkass *et al.* 2011, Holobinko *et al.* 2011] and bone collagen [Schoeninger *et al.* 1983, Longinelli 1984, O'Connell & Hedges 1999b, O'Connell & Hedges 2001, Lee-Thorp & Sponheimer 2003, Richards & Trinkaus 2009].

In addition to its possible application in disaster victim identification, counter terrorism investigations or illicit immigration identification, stable isotope ratios may help in the identification of a corpse, when other evidence such as fingerprints or DNA are not exploitable. The following applications demonstrate the potential of isotopic information to reconstruct the life history and geographical movements of an individual. The information conveyed by stable isotopes provides intelligence to assist investigations Such intelligence is complementary to the information given by other techniques and may allow for example focussing the search of the identity of a person in databases of a specific geographic area or in a given environment.

In 2001, the torso of a child[6] was discovered floating in the River Thames. In the absence of identifying features on the dismembered body, the isotopic analysis of bone was undertaken to provide information on the provenance of the victim. Several geographic regions were excluded, and after several on-site samplings in West Africa, a narrow geographical zone near Benin City was ascertained. Further isotopic analyses were conducted in order to evaluate the time spent by the victim in the UK (United Kingdom). However, as there was neither teeth, nor hair or nails, the scientists worked on skin and fat samples of the victim and concluded that the child experienced a significant change in diet 4 weeks prior to his death. Additional evidentiary elements suggested it was a ritual murder. The true identity of the child and his family has not been discovered yet.

In 2002, the decomposed human body of an unidentified person was discovered buried near an expressway in Germany. Rauch *et al.* analysed a bundle of hairs of 8 cm, parts of the occipital region of the skull, 3 teeth, as well as 2 samples of soil where the body was buried to check for potential contamination [Rauch *et al.* 2007]. The isotopic analyses of hydrogen, carbon, nitrogen, strontium and lead were undertaken. The δ^{13}C and δ^{15}N values of hair showed the person was omnivorous; δ^{2}H values suggested a non-coastal environment. Isotopic values of strontium of hair and skull did not help to precise a geographical, as they were very common for Germany and Europe. Finally, the high values of lead isotope ratios extracted from the teeth and skull corresponded to environment of the USA, Canada or the Balkans in Europe. As investigative information based on a dental work expertise provided strong indication of an Eastern-Europe origin, North America was excluded. Comparing their results with paleontology reference data, the authors concluded the person was born and spent his life in Romania. Using this information, police was able to formally identify the body through the DNA analysis of members of a family and arrested two men responsible for the death of the victim. The authors concluded on the need of basic research on the turnover-rates of the human tissues and of a database storing authentic reference data.

In the "Scissor sisters" case, Meier-Augenstein and Fraser exploited the carbon, nitrogen, oxygen and hydrogen isotope ratios to provide information on the life history and geographic origin of a dismembered and decapitated murder victim discovered in 2005 [Meier-Augenstein & Fraser 2008]. Focusing on fast growing tissues, the δ^{13}C, δ^{15}N, δ^{18}O and δ^{2}H values allowed reconstructing the life history of the person until 200 days before its death. In addition, supplementary δ^{18}O analysis of femural bone apatite provided information on the likely geographic origin of the person, as well as a time frame for his immigration into the country.

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[6] later named Adam to provide him an identity

Except for bone, the authors also analysed corresponding tissue samples of a man with known geographic life history (control sample). They interpreted results based on the control sample, as well as a longitudinal study involving the isotopic analysis of hair and nails of 20 persons [Fraser *et al.* 2006]. δ^{15}N value of nails (10.90 ‰) suggested the person had a diet rich in protein in contrast to the control sample (9.26 ‰), which came from an omnivore. Stable isotope results helped the investigators to focus their research on the victim's identity. δ^{13}C value enabled the exclusion of the North American of African

provenance of the victim. Nail and hair of the deceased showed indistinguishable hydrogen and oxygen stable isotope values from the control person, with δ^{2}H values well correlated with local tap water isotopic profile. From the length of submitted nails, they deduced the victim had lived for 7 months around Dublin. The δ^{18}O values of the inner and outer part of the femur, converted using two mathematical models, produced isotopic signatures close to five specific geographical parts in the world (East coat of Brazil, from the Winward Islands to Puerto Rico, the Horn of Africa, the United Arab Emirates and part of Oman and the West coast of India). These results provided intelligence for the investigations and led to several possible identities for the victim. Family cross-DNA analyses allowed the identification of the victim. The presumed geographic origin and life history of the person were confirmed by the police and corresponded to the information suggested by the isotopic signature of the tissues. Two sisters, that became the major suspects of the case, confessed having committed the crime and were convicted for murder and manslaughter.

Meier-Augenstein reported 2 other cases of human provenancing [Meier-Augenstein 2010]. The first case concerned an Asian young man that succumbed to multiple stab wounds in a hospital in the UK. The identity of the victim was established on the basis of a hit in the Interpol fingerprint database-. However, his entry in the country was not referenced anywhere. Stable isotope analysis was used to provide information on where the person had lived prior to death, and how long he had been living in the UK. The δ^{2}H, δ^{13}C, δ^{15}N and δ^{18}O values of a bundle of hair were determined by EA and TC/EA-IRMS. Both δ^{13}C and δ^{15}N values, reflecting diet, showed 3 significant changes in times, with sharp rises in δ^{15}N values, suggesting at least 3 distinct periods in which protein intake changed significantly. The δ^{2}H and δ^{18}O values of hair indicated allowed tracing back his trajectory and the length of his stay in Eastern and Central Europe, before arriving in UK. Parallel police investigations confirmed the life history reconstructed on the basis of stable isotopes of hair.

The second case involved multi stable isotope analysis on scalp hair and tooth enamel of unidentified remains of an adult male discovered in 2001 in Newfounland island, in Canada [Meier-Augenstein 2010]. δ^{2}H, δ^{13}C, δ^{15}N and δ^{34}S values of hair were determined, while δ^{13}C and δ^{18}O of tooth enamel were analysed, in combination with strontium isotope to provide information on the geographic location and life history of the person. The diet markers of hair, i.e. δ^{13}C and δ^{15}N values, showed for the last 19 months of his life, a diet consistent with North American diet. Corroborated by the δ^{34}S value of scalp hair, δ^{13}C value of tooth enamel showed that, from the age of 8 to 15 years old, the individual had a diet consistent with most North America people. δ^{2}H variations of scalp hair indicated movement between 4 different locations. However, the contrasting δ^{18}O value of tooth enamel did not correspond to the isotopic profile of local drinking water. Several regions could be excluded, leaving other parts of the USA and Canada as potential regions where the person had recently stayed during its last 19 months. The identity of the murdered victim had not been established yet at the time of the publication.

Kennedy *et al.* recently reported the isotopic analysis of human scalp hair of an unidentified woman found in Utah in 2000 [Kennedy *et al.* 2011]. Along the 26 cm sample of scalp hair, the authors found sequential variations of δ^{18}O values. Focusing on a specific δ^{18}O value appearing several times along the hair sample, the authors could restrict the corresponding geographic region to two locations, Utah and North Dakota. It is however not mentioned if the information was communicated or exploited by law enforcement.

Meier-Augenstein highlighted the limitations of such analysis in human provenancing, as even isotopic data in combination with information on diet, may not be sufficient to determine the precise region of origin of an individual. It is however interesting to examine the context and use of isotopic information from human tissue. The measurement of stable isotope ratios to infer the geographical provenance of individuals in a forensic perspective has been mainly undertaken in high profile cases, when other techniques could not establish the identity or provenance of the person. In the 6 cases reported above, the isotopic information was used to generate intelligence and to orientate investigations, rather than for its evidentiary value in court. Interpreted in the context and in conjunction with other elements, the presented cases attest to the potential of intelligence unveiled by isotopic information. Compared to other forensic applications, its main use as intelligence in the case of human provenancing is probably due to a larger awareness of the level of uncertainty associated with the trace.

3.9 Other applications

3.9.1 Paints

Finnigan MAT reported on the differentiation of 3 paints and 3 varnish based on the determination of δ^{13}C and δ^{15}N by EA-IRMS [Finnigan MAT 1995]. While δ^{13}C values varied only by 1 ‰, δ^{15}N allowed a clearer distinction with a range of

2.5 ‰ variations.

Reidy *et al.* investigated the discriminating power of δ^{13}C of 28 architectural white paints from unknown origin [Reidy *et al.* 2005]. Investigating first the homogeneity of a mono-layer of white paint, the authors noticed that the carbon isotopic composition varied by approximately 1.07 ‰ within the paint layer. They also evaluated the weathering of a 3-layers paint under controlled conditions and observed after 3 months a depletion of approximately 0.7 ‰, probably due to mass discrimination associated with solvent evaporation. The δ^{13}C values of the 28 paints, ranging from -23.31 ‰ to -31.01 ‰, allowed the authors to observe 4 different groups between the specimens. The authors concluded that isotopic variations provided further discrimination when their δ^{13}C value differed by more than 1.07 ‰ and should be interpreted in conjunction with data obtained with traditional techniques (Pyrolysis (Py)-GC-MS, FTIR and Raman). They also highlighted the need for further studies on heterogeneity and long-term weathering of paint exposed to sun and UV.

Farmer *et al.* applied likelihood ratios on stable isotope data (δ^{13}C, δ^{18}O, δ^{2}H) of a set of 51 white architectural paints from 8 brands bought in several outlets [Farmer *et al.* 2009b]. Likelihood ratios were calculated using the 51 isotopic profiles of white paints as the reference population. Pair-wise comparisons showed to produce only small false positive and false negative rates (2 % for both). However, as they mentioned that there seemed to be no relationship between paints from the same manufacturer or between paints with the same colour and outward aspect, it is therefore unclear what the LR values indicate without an explicit definition of the notion of source.

3.9.2 Soils

Croft and Pye studied the spatial and temporal δ^{13}C and δ^{15}N variations of soils [Croft & Pye 2003]. Over 2 years, 15 samples of soils were sampled every 3 months in a target zone. Reported averages of δ values for the first year oscillated between -26.64 to -25.52 ‰ for δ^{13}C and between 4.54 and 6.31 ‰ for δ^{15}N. For the second year, the sampling of the site did not show significant carbon and nitrogen isotopic variations (respectively from -26.93 to -26.74 ‰ and from 5.26 to 5.71 ‰). The authors also investigated primary transfer of two different sources of soils on shoe soles. They showed that there was no statistical difference between the soil collected on the shoe sole and the source soil and highlighted the ability of the method to differentiate different sources of soils.

The same group of authors published a similar study on the discrimination of sources of soil using IRMS in conjunction with colour, particle size and elemental composition determination [Croft & Pye 2004]. Five different soils sampled in five places in southern UK and 5 types of shoes were chosen to perform primary transfer experiments. The combination of the four measured parameters allowed the correct association of the soil collected on the shoe sole to its source. Carbon and nitrogen isotopic values provided a good discrimination between the 5 soil samples. The authors noted however the complexity to interpret nitrogen isotopic variations measured in soils, as the nitrogen composition may depend upon factors, such as fertilisers, according the period of time in the year.

Further research on the spatial variability of soil characteristics on 2 sites was undertaken by Pye *et al.* [Pye *et al.* 2006]. Major and trace element composition concentrations, carbon and nitrogen isotope ratios, particle size distribution and colour determinations were part of the evaluated parameters. The results were averaged from 9 samples made at each site. Considering all particle sizes categories, the isotopic variability ranges were comprised, at the first site, between -28.26 to -27.35 ‰ for δ^{13}C and between -2.35 to -0.64 ‰ for δ^{15}N. On the second site, δ^{13}C ranged from -27.83 to -23.00 ‰ and δ^{15}N from 1.88 to 4.80 ‰. Although each site could be distinguished on the basis of any of the measured parameters, the results of this study especially highlighted the high intravariability of the sites, which would easily be underestimated if overlooked. In addition, it confirms the high variability of nitrogen istopic composition in soils, pointed out by previous studies [Croft & Pye 2004, Croft & Pye 2003].

3.9.3 Fibers

A succinct pilot study focused on the bulk analysis of 7 un-dyed cotton fibre samples coming from Egypt, Argentina, Turkey and Uzbekistan [Daeid Nic *et al.* 2011]. δ^{13}C values of the samples were close to each other (-24.1 to -22.9 ‰). In contrast, δ^{18}O and especially δ^{2}H values allowed further discrimination between the 3 specimens coming from Egypt.

3.9.4 Packaging tapes

Van der Peijl, cited in [Phillips *et al.* 2003a], reported a pilot study on the carbon, nitrogen, oxygen and hydrogen isotopic comparison of packaging tapes. δ^{13}C and δ^{2}H bulk analysis enabled a good discrimination of the tapes. While all batches could not be differentiated with the analysis of the adhesive fraction of the tapes, a clearer differentiation between batches and even sub-batches was possible with the analysis of the backing material.

The isotopic analysis of 10 pressure-sensitive adhesive tapes collected by the laboratory is related by Carter *et al.* [Carter *et al.* 2004]. The sampling was composed of 5 tape rolls purchased from local suppliers and 5 other samples collected from parcels delivered to their laboratory. They analysed untreated tapes, as well as tapes treated with solvents to remove the adhesive and binder. δ^{13}C, δ^{18}O and δ^{2}H values were determined by EA and TC/EA-IRMS. Overall, the isotopic compositions of untreated tapes were more discriminating than the those of the treated ones. To evaluate the effect of storage in water, they immersed 2 parcels in water, mimicking the common practice of concealing packaged drugs in water tanks. The storage of tape in water did not modify their original isotopic composition, not even the δ^{2}H value.

Carter *et al.* reported the δ^{13}C analysis of masking tapes to establish a link between two cases involving the smuggling of money [Carter *et al.* 2005]. In the first case, sterling banknotes bound in masking tape and conditioned in parcels also covered with masking tape, were found concealed in suitcases. In the second case, a person was apprehended with banknotes wrapped with masking tape. Compared to those of 10 background samples collected from laboratory staff [Carter *et al.* 2004], the δ^{13}C value of the exhibits showed to be different from the "background" values (-27.3 to -24.3 ‰). The masking tape of the two cases were isotopically distinct, indicating that the two cases were not related.

Aziz *et al.* combined GC-MS and EA-IRMS techniques to discriminate 32 adhesive tapes of eight different colours coming from 8 manufacturers [Aziz *et al.* 2008]. The application of Linear Discriminant Analysis (LDA) to the relative abundance of alkylnaphtalenes measured by GC-MS allowed discriminating the manufacturers. Bulk carbon isotope analysis of the adhesive layer showed values comprised between -29.4 to -26.5 ‰, with variations up to 0.5 ‰ within tapes of a given manufacturer. The isotopic results corroborated the differentiation based on the GC-MS molecular profile. The closeness between the isotopic values of the adhesive moiety and those obtained by Carter *et al.* from the backing material might reflect the general use of petroleum derivatives in the production of adhesive and backing materials.

Horacek *et al.* measured the δ^{13}C and δ^{2}H values of 18 brown adhesive tapes manufactured in Korea from 11 brands [Horacek *et al.* 2008]. The glue and backing material of each tape was separately analysed. δ^{13}C and δ^{2}H values ranged respectively from -28.9 to -25.3 ‰ and from -83 to -52 ‰. δ^{13}C and δ^{2}H values of backing material oscillated respectively between -28.4 and -23.6 ‰ and from -110 to -88 ‰. Presenting the results in bivariate plots, the authors concluded that most samples could be unequivocally differentiated from other samples. With many overlapping measurements, the distinction is however not as clear as claimed.

Interested in black PVC tape backings, Dietz *et al.* analysed 87 tape rolls including 60 different products from 20 brands [Dietz *et al.* 2012]. With a very small intravariability within roll, δ^{13}C values showed to be mainly distributed between 2 populations and ranged from -41.3 ‰ to -23.5 ‰. Using a window of 0.3 ‰, the δ^{13}C values discriminated 93.7 % of all tape rolls. Combined with the routine physical and chemical examinations (FTIR, SEM, Py-CG/MS), δ^{13}C values increased the discriminatory power of the sequence of analyses to 98.9 %. Additional experiments showed that δ^{13}C value of these tapes was not significantly affected by the effect of explosion exposure. This makes δ^{13}C measurement of PVC tapes an interesting tool in explosives investigations.

3.9.5 Safety matches

Farmer *et al.* analysed the bulk δ^{13}C and δ^{2}H values of matches from 9 brands manufactured in EU (European Union), Czech Republic, India and USA. The collected data were used to evaluate the isotopic profiles of matches related to a criminal case. Matches found at a crime scene were compared to matches seized from a suspect [Farmer *et al.* 2005, Farmer *et al.* 2007]. The potential discrimination of matchsticks relies on the exploitation of existing isotopic variations between trees grown at different geographical locations. Evaluating intravariability within a match box, the δ^{13}C value of 3 matches coming from one box analysed in triplicate showed a wide intravariability of up to 6.0 ‰, other variations observed within one box were 1.5 and 2.5 ‰. A maximum intravariability of 5 ‰ for δ^{18}O and 17 ‰ for δ^{2}H was measured within one box. The additional measurement of δ^{18}O and δ^{2}H values provided further discrimination. The eventual isotopic comparison of the evidentiary matches and the seized material suggested different sources, as confirmed by the data obtained from the XRD analysis of match heads and the microscopic observations of wood.

Extending their work on safety matches, Farmer *et al.* investigated the potential of IRMS to discriminate burnt wooden matchsticks found at fire scenes [Farmer *et al.* 2009a]. From 27 match boxes of different brands, 10 matches were removed of each box, of which 5 unburnt served as comparison and 5 others were burnt for the experiments. The experiences were designed to see if the application of petrol on matches and the use of alternative extinguishers (water, powder, synthetic foams and CO_2) could alter the isotopic profile. Analysing the section of the unburnt wooden part of burnt matchsticks by EA and TC/EA-IRMS, they compared their isotopic profile to the unburnt matches. Overall, the δ^{13}C, δ^{18}O and δ^{2}H values of unburnt and burnt matches, with added petrol or extinguished by different means, were found to be statistically indistinguishable. The comparison of the isotopic profiles of 19 matches placed in mock fire training scenes with their respective unburnt match confirmed the results of the experiences. They highlighted the possibilities to perform isotopic analysis on burnt matches and concluded it could provide an indication on the geographical origin of the wood composing the matchstick.

3.9.6 Plastic

Idoine *et al.* measured the carbon, oxygen and hydrogen of 7 cling film coming from heroin seizures, as well as 15 retail cling film samples by EA-IRMS [Idoine *et al.* 2005]. The mutlivariate isotopic analysis of cling film samples allowed differentiating most of the samples, with δ^{13}C being the most discriminating variable.

Taylor *et al.* evaluated the discriminating power of IRMS combined to physical examinations of grip-seal plastic bags, commonly recovered in drug seizures [Taylor *et al.* 2008]. In an initial study, the authors sampled 16 grip-seal bags coming from different European countries and used for consumable items of analytical instruments. Besides, they also analysed six different batches of re-sealable food storage bags from the Tesco brand. They reported the complementarity of bulk δ^{13}C and δ^{2}H measurements to physical data such as size, birefringence and infrared spectroscopic measurements. They concluded that bags with different isotopic composition were likely to come from different manufacturers or production batches. They stressed however, that in case two bags were to have indistinguishable isotopic profiles, the probability that this match has arisen by chance should be considered.

Quirk *et al.* measured the δ^{13}C and δ^{2}H values of plastic composing two-ways radios [Quirk *et al.* 2009]. The plastic of 4 pairs of radio were preliminarily identified by IR and Raman spectroscopy. One radio of each pair was detonated. Both the detonated radio and the intact one were analysed by EA and TC-EA-IRMS. Using Pearson correlation to evaluate the similarity of δ values, the authors observed a close agreement between pre and postblast data, indicating the possibility to associate fragments of radio on an explosion scene with the radio used to transmit the detonation signal.

3.9.7 Miscellaneous

Van der Peijl *et al.* reported the use of IRMS technology in a safe burglary case [Van der Peijl *et al.* 2004]. The safe filling material sampled on the crime scene and the material recovered in a bag of a suspect were compared by ICP-MS , laser ablation (LA)-ICP-MS and IRMS. The carbon, oxygen and hydrogen isotopic analysis could not discriminate both materials.

The microbial application of IRMS in forensic science was largely initiated by the anthrax terrorist attacks in the USA in 2001, referred to as the Amerithrax case [The United States Department of Justice 2010]. These bio-weapons attacks, involving the mailing of envelopes containing spores of anthrax to news media offices and 2 US senators, killed 5 individuals and infected 17 other people [Ehleringer & Matheson 2010]. Stable isotope ratio analysis was used to provide potential information on the geographic location associated with the culture of the anthrax spores and on the medium used to culture anthrax.

Horita *et al.* reported that the isotopic composition of 2 strains of microbial agents grown under controlled conditions was predictable, as it reflects the media and substrates isotopic signature [Horita & Vass 2003]. Although reporting their results advantageously, it appears however difficult to establish a definite and unequivocal relationship between the nutrients and the δ^{2}H, δ^{13}C and δ^{15}N values. They concluded that the isotopic analysis of such organisms provide information to evaluate the growth conditions, the media waters and the organic substrates used for the culture.

Kreuzer-Martin *et al.* reported the bulk isotopic analysis of microorganisms in the perspective of source inference of a bioterrorism act [Kreuzer-Martin *et al.* 2003, Kreuzer-Martin *et al.* 2004a, Kreuzer-Martin *et al.* 2004b, Kreuzer-Martin *et al.* 2005, Kreuzer-Martin & Jarman 2007]. Evaluating the within-batch and between-batches δ^{13}C, δ^{15}N, δ^{18}O and δ^{2}H variations, their results showed that the isotopic profile could be used to differentiate different batches of microorganisms, grown under the same conditions. In addition to the comparison of the isotopic signature of spores, they confirmed the preliminary conclusions emitted by Horita *et al.* [Horita & Vass 2003]. They characterised the relationship existing between the isotopic profile of spores and their growth environment, highlighting the potential of stable isotopes as indicators of the growth medium and water supply used for the culture of a batch of organisms. δ^{2}H and δ^{18}O values of growing cells and spores were shown to be directly related to those of the media water, confirming that such stable isotope ratios may be useful to trace the geographical origin of microbial cultures [Kreuzer-Martin *et al.* 2003, Kreuzer-Martin & Jarman 2007], while carbon and nitrogen isotope ratios of the spores were related to the δ^{13}C and δ^{15}N values of the growth medium [Kreuzer-Martin *et al.* 2004a, Kreuzer-Martin *et al.* 2004b, Kreuzer-Martin *et al.* 2005, Kreuzer-Martin & Jarman 2007].

Roelofse and Horstmann measured the bulk carbon isotope ratio of a carbonate rock discovered in a container filled with nickel metal [Roelofse & Horstmann 2008]. A volume of nickel metal similar to that of the rock had disappeared and had been replaced by the rock. Stable isotopes were used in this case to determine in which country the theft had taken place. The isotopic data supported the hypothesis that the rock originated from Israel rather than South Africa.

Papesch and Horacek used δ^{18}O measurements to highlight a suspected mislabelling of a cheap beer brand with the label of a high priced premium beer [Papesch & Horacek 2009]. Confronted with the evidence of isotopic difference between beers, the suspects confessed the purchase of cheap beers and their re-labelling as high premium beers. The authors also tested this technology to assign the origin of water found in the diesel tank of a service station. The similar isotopic composition of water sampled in the tank and of local tap water indicated that the local tap water as the most probable source of contamination.

Founding their research on a series of arson incidents involving candles as an initiating device, Dogger *et al.* demonstrated the discriminating power of GC and IRMS through the analysis of 26 candles wax from caseworks, as well as the analysis of candles from 128 different boxes bought in shops [Dogger *et al.* 2009]. The authors concluded that the indistinguishable GC and IRMS profiles of some specimens provided strong evidence for a common source.

Van Es *et al.* evaluated the potential of LA-ICP-MS, IRMS and XRF to discriminate 25 paper samples from the European market [van Es *et al.* 2009]. The reported intravariability within one sheet was very small for δ^{13}C, δ^{18}O and δ^{2}H (respectively 0.06 ‰, 0.31 ‰ and 1.01 ‰). Differentiating 21 of the 25 papers, IRMS demonstrated a good discriminating power. All paper samples could be discriminated, combining either IRMS and XRF or IRMS and LA-ICP-MS.

In the Amerithrax case, the isotopic analysis of the cellulose of the envelopes used to mail the spores was also undertaken [The United States Department of Justice 2010, Ehleringer & Matheson 2010]. However, the results showed limited discrimination between envelopes.

Michalski *et al.* used δ^{15}N and δ^{18}O values to investigate the source of the high nitrate concentration found in the Main Lake depression of the Nevada desert that poisoned 71 wild horses [Michalski *et al.* 2010]. Isotopic data of the nitrate contained in the Main Lake depression, sediments and nearby water spring did not support the dumping of synthetic urea into water as the source of nitrate contamination. In contrast, δ^{15}N and δ^{18}O and δ^{17}O values suggested that horse's manure was the source of water nitrification. Accompanied by high evaporative conditions, this abnormal combination of circumstances led to the poisoning of the horses.

Recent studies have started to be interested in the differentiation of sources of poisons, such as sodium and potassium cyanide (NaCN and KCN). Kreuzer *et al.* determined the δ^{13}C and δ^{15}N values of 25 samples of NaCN and 40 of KCN, coming from 14 different suppliers [Kreuzer *et al.* 2012]. Although most samples produced repeatable measurements, some samples displayed a large isotopic intravariability. The authors found that this heterogeneity was accompanied by a lower % N yield and seem related with the partial degradation of cyanide into $NaCO_3$ in the presence of moisture and CO_2. It appears unclear why this phenomena affects only some samples and not others. Finally, the authors reported a true match rate higher than 95 % and a false match rate lower than 3 %.

In addition to the observation of δ^{13}C and δ^{15}N values of different KCN and

NaCN, Tea *et al.* undertook investigations on the way to extract KCN and NaCN from matrices, such as orange juice, yoghurt drink and medicine [Tea *et al.* 2012]. They found that the precipitation of cyanide into its cupric form (Cu2[Fe(CN)6]) resulted in limited isotopic fractionation and good repeatability of isotopic values. The δ^{13}C values oscillated on a large range (between -51.96 to -25.77 ‰) and δ^{15}N from -4.51 to +3.81‰. They concluded that δ^{13}C and δ^{15}N values are well dispersed with small overlap, though no further consideration is provided on the ability of the technique to discriminate the specimens.

Moran *et al.* showed a similar interest in the analysis of acid scavenger compounds used to stabilise nerve agents. The δ^{13}C, δ^{15}N and δ^{2}H values of 22 acid scavengers (N,N-diethylaniline, tributylamine, triethylamine) from 3 different suppliers and from inventories of chemical laboratories were widely spread on the isotopic scale [Moran *et al.* 2012]. Using standard deviation intervals to consider potential overlap, they concluded that 96 % of the triethylamine samples could be differentiated on the basis on the 3 isotopes and therefore that the isotopic profile could be used to distinguish between samples and to establish a link with potential precursors.

3.10 Explosives

This section presents the state of the art of the isotopic analysis of explosives, differentiating preblast and postblast studies, and organic and inorganic explosives. As some studies in the field often reported on several types of explosives at the same time, the categorisation between organic and inorganic will be respected as far as possible. Studies conducted on a small scale, presenting little information or limited results as well as unpublished results are referenced in the following paragraph.

Pilot studies In a preliminary short study, Finnigan MAT suggested that different lots of explosives could be differentiated [Finnigan MAT 1995]. Analysing 3 specimens of TNT from Germany, Russia and an unknown source by EA-IRMS, they showed a range of 3 ‰ variations for δ^{13}C and δ^{15}N. They concluded that further investigations were required to interpret the significance of these differences.

Wakelin reported on the bulk carbon, nitrogen, oxygen, sulphur and chlorine isotopic analysis of various types of explosives and substances found in improvised explosive charges [Wakelin 2001]. Preliminary analysis was undertaken on ammonium nitrate, sodium chlorate, nitromethane, sugar, black powder, TNT, plastic explosive, nitrocellulose, flashpowders and perchlorates. For each type of explosives, the results showed the existence of significant isotopic variations. Differentiation of explosives of the same type was possible by measuring only one element. However, a better discrimination was achieved when a combination of isotope ratios were measured. **Figures 4.7** and to **4.8** illustrate the variations of δ^{13}C, δ^{15}N, δ^{18}O and δ^{34}S values that were measured by Wakelin for specimens of black powder.

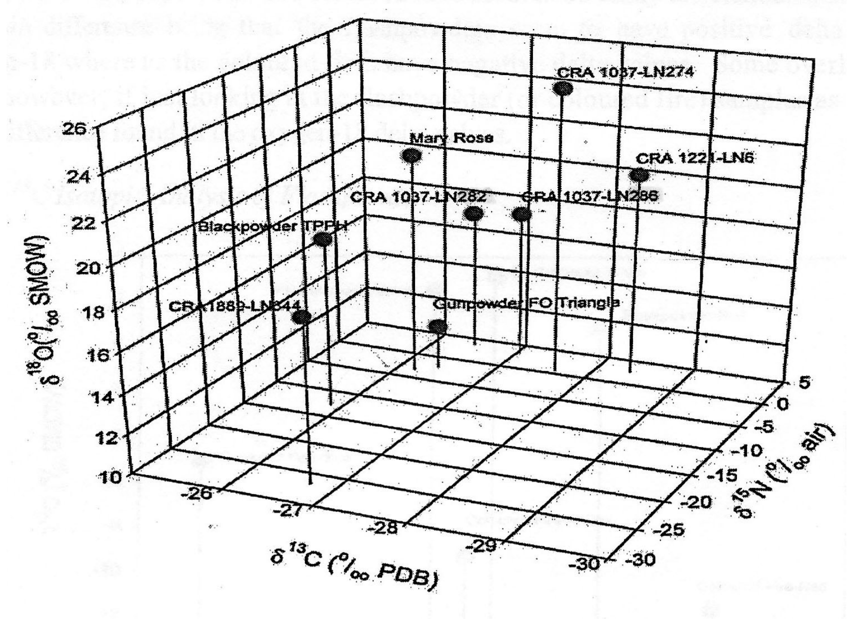

Figure 4.7. δ^{13}C, δ^{15}N and δ^{18}O of specimens of black powder (source: [Wakelin 2001], p.163).

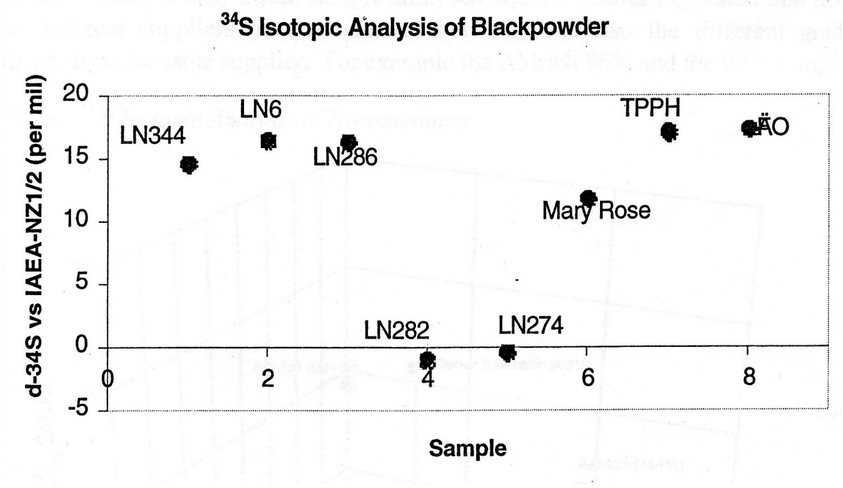

Figure 4.8. δ^{34}S of specimens of black powder (source: [Wakelin 2001], p.163).

Blind tests were also conducted in which all explosives were correctly identified on the basis of their isotopic ratios, except for flashpowders. The author concluded that not only different suppliers could be differentiated, but also different purity grades of the same manufacturer.

Belanger determined the δ^{13}C and δ^{15}N values of ammonium nitrate, black powder, TNT and plastic explosives samples [Belanger 2002]. Additional δ^{18}O and δ^{34}S analyses were also performed. These preliminary results demonstrated the existence of isotopic variations in carbon and nitrogen for all type of explosives. A range of 2.5 ‰ for δ^{13}C values and 30.4 ‰ for δ^{15}N values were reported for black powder. For ammonium nitrate, δ^{15}N values varied on a range of 5.3 ‰.

PETN, RDX, HMX (high melting explosive; 1,3,5,7-tetranitro-1,3,5,7-tetrazacyclohexane) and AN were analysed by Lott *et al.* [Lott *et al.* 2002]. The authors could successfully discriminate explosives coming from different sources. They also mentioned the evaluation of batch-to-batch and lot-to-lot variations, although further details regarding the nature of this approach and the obtained results were not provided.

As cited in Phillips *et al.*, Ehleringer mentioned the isotopic study of PETN samples [Phillips *et al.* 2003b]. He concluded that PETN samples produced by the same manufacturer resulted in similar isotopic values, which are isotopically distinguishable from other manufacturers. Data and results are however not reported in more details.

In a FIRMS newsletter [Forensic Isotope Ratio Mass Spectrometry 2003b], Beardah *et al.* related the application of IRMS to military high explosives, gunpowder and pyrotechic compositions. According to the authors, IRMS allowed them to make important links and to differentiate specimens of explosives.

Wakelin mentioned the bulk isotopic study of commercially manufactured RDX and home-made nitroglycerin [Wakelin 2004]. As reported by the author, the δ^{13}C, δ^{15}N and δ^{18}O values allowed differentiating specimens of explosives in many cases.

According to Pierrini, the Forensic Explosives Laboraltory (FEL) did some tests with bulk and explosive residues and observed an isotopic fractionation [Pierrini 2005]. Further details were not provided.

Brogden as cited in [Wakelin *et al.* 2008] reported on the Py-GC-MS and EA-IRMS analysis of 7 different smokeless powders containing several constituents. Using Py-GC-MS, they searched for differences in the relative ratios of the combustion products of smokeless powders and combined these results to δ^{13}C and δ^{15}N values of residues. The authors concluded that the use of both techniques allowed the detection, identification and determination of the source of smokeless powders. The possibility of linking unfired and fired powders was also discussed and viewed as a challenging issue.

Ehleringer and Lott as cited in [Wakelin *et al.* 2008] performed tests with TNT, RDX and PETN [Wakelin *et al.* 2008], as well as HMX [Ehleringer & Lott 2007].

They observed that despite a rough 1 ‰ enrichment, the bulk δ values of explosive residues were closely related to that of intact explosives.

3.10.1 Preblast studies

Organic explosives - nitro compounds The first publication on the use of isotopic ratios in the field of explosives is attributed to Nissenbaum [Nissenbaum 1975]. The carbon isotope ratio of 7 samples of TNT, coming each from different countries, were measured by DI-IRMS. The wide range of δ values (from -30.93 ‰ to -24.53 ‰) showed the potential of IRMS to differentiate specimens of TNT based on their carbon isotopic variations.

Pierrini *et al.* analysed the bulk δ^{13}C and δ^{15}N values of 26 seized Semtex samples, assumed to come from different sources [Pierrini *et al.* 2007]. Carbon isotopic variations oscillated between -36.53 ‰ to -24.76 ‰, while nitrogen isotopic values varied from -25.68 ‰ to -2.72 ‰. The authors evaluated the isotopic results in a likelihood ratio (LR) framework, using 3 methods: LR using a multivariate random-effects, LR using a kernel distribution for the distribution of between-group variability and LR using a multivariate Hotelling's T^2 distance and a kernel density estimate. While the two first methods produced about 11 % false positive, the third gave 5 % misleading evidence rate. The authors highlighted the necessity to have large stable isotope ratio databases to use this approach.

In order to evaluate the isotopic relationship existing between a precursor and the resulting product, Lock and Meier-Augenstein examined the isotopic fractionation induced by the synthesis of RDX from the starting material hexamine [Lock & Meier-Augenstein 2008, Meier-Augenstein 2010]. The bulk δ^{13}C and δ^{15}N values of 11 hexamine samples from 6 different manufacturers were determined. The δ^{13}C values oscillated between -46.18 to -34.51 ‰, while the δ^{15}N values ranged from -2.48 to +0.41 ‰. Five hexamine samples were selected to synthesise RDX following one synthetic pathway, with the same reaction conditions and reagents. First the δ^{13}C and δ^{15}N intravariability of 2 batches was evaluated, which was found to be small (less than 0.11 and 0.15 ‰, respectively). With an approximate reaction yield of 60 %, the resulting RDX was characterised by δ^{13}C and δ^{15}N values ranging from -38.34 to -26.23 ‰ and from -15.62 to -13.79 ‰, respectively. The carbon signature of RDX was found to be directly related to hexamine, while δ^{15}N value depended on hexamine, as well as on the nitric acid or sodium nitrite/nitric acid mixture. The δ^{18}O value of RDX, varying between -37.07 to -36.52 ‰, likely came from the nitric acid or sodium nitrite/nitric acid source involved in the reaction. Produced twice from the same hexamine material, the δ^{13}C, δ^{15}N and δ^{18}O values of the product were shown to be repeatable. For both carbon and nitrogen, a systematic and reproducible isotopic fractionation of +8.35 ‰ for carbon and -14.01 ‰ for nitrogen was measured as a result of the transformation of hexamine into RDX. Calculating the fractionation and enrichment factors, they highlighted the linear relationship between the reactant and product, and hence the possibility to predict the signature of the starting material from an RDX specimen.

The same authors also evaluated the intervariability of 14 hexamine samples coming from different suppliers, by applying statistical methods (HCA, ANOVA and Tukey comparison) to the data set (δ^{13}C, δ^{15}N, δ^{2}H) [Meier-Augenstein 2010].

Widory *et al.* analysed the bulk δ^{13}C, δ^{15}N, δ^{18}O and δ^{2}H values of 30 samples of PETN, TNT and AN based explosives [Widory *et al.* 2009]. The δ^{13}C values oscillated from -38.4 to -29.0 ‰ for PETN, from -29.9 to -23.5 ‰ for TNT and from -32.5 to -22.9 ‰ for AN based explosives. The δ^{15}N values varied from -16.3 to -5.1 ‰ for PETN, from -8.7 to +3.5 ‰ for TNT and from -2.4 to +0.8 ‰ for AN based explosives. The δ^{18}O values fluctuated between from +16.1 to +22.9 ‰ for PETN, from +16.5 to +19.2 ‰ for TNT and from +21.6 to +23.3 ‰ for AN based explosives. Finally, the δ^{2}H values ranged from -64 to +20 ‰ for PETN, from -103 to -60 ‰ for TNT and from -237 to -170 ‰ for AN based explosives. The combination of the elemental concentration and the isotopic signatures allowed the authors to distinguish the different categories of explosives (PETN, TNT and AN based explosives). Despite the large quantity of collected data, the relevance and forensic aspect of the study is largely questionable, when considering the cost and complexity of IRMS. The results obtained by the authors are mainly orientated towards the differentiation of the type of explosives, while other methods routinely employed allow the identification of the explosive.

Benson *et al.* undertook a preliminary study on the potential of IRMS to distinguish TATP and PETN samples coming from different sources [Benson *et al.* 2009b]. The δ^{13}C value of a first set of 14 TATP samples, synthesised under different conditions, showed to be distributed between 3 main groups, varying between -27 to -34.5 ‰*. Astonishingly, no correlation were observed between the reaction conditions (source of reagent, acid type, reaction and cooling temperatures) and the carbon isotope ratio. Four additional TATP samples were synthesised with different analytical grades, reagents mole ratios and reaction temperatures. These samples were highly enriched in ^{13}C with δ^{13}C values between -17.2 to -7.6 ‰. However, the difference of range of values for carbon could be attributed to the modification of the analytical method. The δ^{18}O and δ^{2}H values ranged from +20.2 to +32.5 ‰ and from -125.6 to -105.8 ‰, respectively. Considering δ^{13}C, δ^{18}O and δ^{2}H, the 4 samples of TATP could successfully be discriminated.

Bulk δ^{13}C and δ^{15}N were determined on 15 specimens of PETN coming from the filling of detonating cord or from boosters . The δ^{13}C values of the 15 specimens oscillated on a large range (between -48 to -3 ‰*) and the δ^{15}N values were distributed between -15 to +4 ‰*, corresponding to more extended ranges than those measured by Widory *et al.* (see **Table 4.7** for comparison). All the 15 PETN specimens could be differentiated on the basis of their δ^{13}C and δ^{15}N signature.

Tables 4.6 and **4.7** summarise the range of bulk isotopic values of TNT and PETN reported in different studies.

Organic explosives - peroxides In 2007, in the case opposing R. v. Ibrahim, Omar, Osman and Mohamed concerning the attempted London bombings on the 21st July 2005, one of the defendants stated that the hydrogen peroxide, used in the hydrogen peroxide-flour mixture, had an initial concentration of 70 %, but had been diluted with tap water to reach a concentration of 35 %. Based on the comparison of the isotopic composition of London tap water and the isotopic signature of the explosive charge, the expert said his argument was impossible [Royal Courts of Justice 2008, Ehleringer & Matheson 2010].

In the context of the London bombings in July 2005, Lock studied the δ^{18}O and δ^{2}H variations of 10 samples of hydrogen peroxide coming from 7 different suppliers, as mentioned in [Meier-Augenstein 2010]. The δ^{18}O and δ^{2}H values ranged from -1.90 to +13.43 ‰ and from -132.9 to -18.8 ‰, respectively. The use of HCA statistical analysis provided an insight of the similarity between samples and could group 2 samples coming from the same batch but from 2 different bottles. Probably in connection with the argument alleged by one of the defendants (see above [Royal Courts of Justice 2008]), they also investigated the possibility to link a diluted hydrogen peroxide solution with the original one and to provide information on the water used to dilute it. They diluted hydrogen peroxide of 60 % with deionised water and applied mathematical regression to predict the isotopic values of the original substance and water. By applying a polynomial regression for δ^{18}O values and a linear regression for δ^{2}H measured values in function of hydrogen peroxide concentration, the predicted δ^{18}O and δ^{2}H values (-8.54 ‰ and -46 ‰ respectively) of the water were close to the isotopic signature of the actual water (-6.58 ‰ and -47.5 ‰ respectively) used for dilution.

A recent study focused on the δ^{2}H and δ^{18}O analysis of 97 H_2O_2 from USA and Mexico, including four different grades [Barnette *et al.* 2011]. δ^{2}H and δ^{18}O values oscillated on a wide scale (230 ‰ and 23 ‰ respectively). Diluted H_2O_2 solutions were correlated with the Meteoric Water Line, which describes the δ^{18}O and δ^{2}H variations in water. The δ^{2}H values of H_2O_2 showed wide variations allowing the discrimination of bulk H_2O_2 samples.

Inorganic explosives Ader *et al.* focused on the analysis of weed killers, often diverted from their primary use and employed as oxidant in homemade explosive compositions [Ader *et al.* 2001]. They developed a method for the determination of δ^{37}Cl through the reduction of chlorate and perchlorate in chlorine. The measured

Authors	**Number specimens**	**Origin**	**δ^{13}C values [‰]**	**δ^{15}N values [‰]**	**δ^{18}O values [‰]**	**δ^{2}H values [‰]**
[Nissenbaum 1975]	7	UK, USA, Israel Italy, Canada Yugoslavia, Hungary	-30.93 to -24.53	-	-	-
[Finnigan MAT 1995]*	3	Germany Russia Unknown	-30 to -26	-0.5 to 1.5	-	-
[Coffin *et al.* 2001]	5	USA Croatia	-26.42 to -22.21	-5.36 to +9.64	-	-
[Widory *et al.* 2009]	14	Montenegro France	-29.9 to -23.5	-8.7 to +3.5	+16.5 to +19.2	-103 to -60

Table 4.6. Summary of reported results on the bulk isotopic analysis of TNT in literature.

Authors	**Number specimens**	**Origin**	δ^{13}**C values [‰]**	δ^{15}**N values [‰]**	δ^{18}**O values [‰]**	δ^{2}**H values [‰]**
[Benson *et al.* 2009b]* [a]	8 7	unknown	-25 to -3 * -48 to -28 *	-12 to +4 * -15 to -1 *	-	-
[Widory *et al.* 2009]	12	Montenegro France	-38.4 to -29.0	-16.3 to -5.1	+16.1 to +22.9	-64 to +20

Table 4.7. Summary of reported bulk isotopic values of PETN in literature.

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[a] Note that 6 of the specimens were reported to contain presumably PETN with TNT

δ^{37}Cl values of 4 chlorate and 3 perchlorate based compounds showed limited variability, comprised respectively between -0.34 and +0.42 ‰ and between +0.23 and +2.30 ‰. Interestingly, the isotopic differences between crystals of sodium chlorate, similarly to what has been reported by David *et al.* and Iwata *et al.* [David *et al.* 2010a, Iwata *et al.* 2010], are likely to originate from the isotopic effect associated with the crystallisation step. Thus, according to the size of the crystal and the time at which it was formed, a small crystal may well have a different isotope ratio than a larger crystal that nucleated earlier from the same solution.

Benson *et al.* evaluated the discrimination power of IRMS on different sources of ammonium nitrate [Benson *et al.* 2009a]. Ammonium nitrate prills were collected from 3 main Australian manufacturers during a 12 months period. In addition, 10 seized samples from the Philippines, 6 samples from Lithuania and 4 samples manufactured in Indonesia, China and the Philippines were analysed. Bulk δ^{15}N , δ^{18}O and δ^{2}H values were determined using EA and TC/EA-IRMS. By conducting such a study on several months, Benson *et al.* provided fundamental data on the δ^{15}N intravariability pertaining to each of the 3 Australian manufacturers. Two manufacturers showed large δ^{15}N variations along the period of analysis (from -2.3 to +2.0 ‰ for the first manufacturer and from -0.4 to +2.0 ‰ for the second one), while the third one showed a more limited range of values (from -1.4 to -0.4 ‰). As no changes in the manufacturing process of the suppliers seemed to have occurred, the results raise the question of the origin of isotopic variability measured within each manufacturer, in addition to issues on sampling and sampling representativeness for mass produced substances. Overall, the use of δ^{15}N value only offered limited discrimination between Australian manufacturers (from -2.3 to +2.0 ‰) and overseas AN sources (from -4.0 to +1.4 ‰*). These results corroborate the limited range of δ^{15}N variations found by Widory *et al.* and also reported in [Meier-Augenstein 2010] (see **Table 4.8** for comparison). δ^{18}O and δ^{2}H allowed the differentiation of Australian manufacturers (ranging from +13 to +20.5 ‰* and from -30 to +80 ‰* for O and H respectively), as well as overseas sources (from +12.5 to +21.5 ‰* and from -25 to +45 ‰* for δ^{18}O and δ^{2}H respectively). However, due to overlap, the differentiation between Australian AN prills and overseas prills was limited. The isotopic data could nevertheless provide investigative support.

Table 4.8 summarises the range of bulk isotopic values presented in the different papers.

The same leading authors presented the validation of the technique for the analysis of bulk δ^{15}N value in ammonium nitrate [Benson *et al.* 2010a]. They described the selection and characterisation of laboratory standards, the different parameters of method validation, uncertainty calculations as well as the suitability of laboratory standards and the sequence design.

Authors	**Number specimens**	**Origin**	δ^{15}**N values [‰]**	δ^{18}**O values [‰]**	δ^{2}**H values [‰]**
[Widory *et al.* 2009]	4	France commercial handcrafted	-2.4 to +0.8	+21.6 to +23.3	-237 to -170
[Benson *et al.* 2009a]*	79	Australia	-2.3 to +2.0	+13.0 to +20.5	-30 to +80
		overseas	-4 to +1.4	+12.5 to +21.5	-25 to +45
[Lock 2009] [Meier-Augenstein 2010]*	14	Australia, USA, Algeria, Lithuania Ukraine, Bulgaria, Georgia unknown	-1.8 to +2.2	+13.2 to +26.2	-257 to +20

Table 4.8. Summary of reported results on the isotopic analysis of ammonium nitrate in literature.

Aranda *et al.* explored the isotopic relationship existing between precursors and the resulting urea nitrate (UN) [Aranda IV *et al.* 2011]. The authors first developed a method to separate UN into its ionic compounds (urea and nitrate) for isotopic nitrogen analysis. The separation step did not alter the δ^{15}N values of urea and nitrate. δ^{13}C was measured from bulk UN, as the purification step induced a significant enrichment of the δ^{13}C value of the isolated urea. They synthesised 4 small batches (10g) and 3 large batches (25 kg) and compared the isotopic signature of the UN products to that of the reactants. The small and large batch scale, as well as temperature variations did not influence the isotopic signature of the resulting UN. The δ^{13}C values of the product UN was indistinguishable from the δ^{13}C value of the urea precursor and the δ^{15}N value of the nitrate isolated from UN was correlated to that of the nitrate reagent. Yet, the δ^{15}N value of the urea isolated from UN was slightly more enriched (+0.5 and +0.9‰) than the initial urea precursor. These results emphasised that UN isotopic signature reflect that of the precursors used for the synthesis. In addition to the analysis of 2 seized UN specimens, they listed carbon and nitrogen isotopic variations ranges for urea and nitric acid referenced in literature, highlighting thus the possibilities to differentiate UN specimens.

3.10.2 Postblast studies

As mentioned in [Anisichkin *et al.* 1988], the tagged atom method was early reported by McGuire *et al.* to study the detonation products of explosives. They tagged molecules of bis trinitroethyl adipate with ^{13}C and ^{18}O and compared the isotope ratios of the detonation products with those of the original explosive. They found that the detonation mechanism broke almost all intramolecular bonds and the resulting fragments recombined randomly in detonation products. Anisihkin *et al.* explored the gaseous and condensed products resulting from the detonation of comp B, a mixture of TNT and RDX, by tagging molecules of TNT with a methyl containing ^{13}C [Anisichkin *et al.* 1988]. Conducted on a small scale in bomb calorimeters, experiments showed that tag atoms were shared out among the different gaseous products as well as condensed carbon products.

McGuire *et al.* were the very first to report the isotopic analysis of postblast residues of HMX, TNT, octol, TATB (1,3,5-triamino-2,4,6-trinitrobenzene) and 3 plastic bonded explosives [McGuire *et al.* 1995]. Except for the δ^{13}C values of aromatic explosives which appeared almost unchanged, that of other explosives underwent isotopic fractionations upon detonation. δ^{2}H and δ^{15}N values were also affected by explosion. Globally, δ^{15}N values of postblast residues were more positive than the initial isotope ratio.

Benson *et al.* investigated the postblast δ^{15}N value of AN based explosives, by detonating 6 explosive devices in duplicates, with varying improvised and commercial charges and boosters [Benson *et al.* 2009a]. They observed a significant isotopic fractionation leading to highly enriched δ^{15}N values of residues. The rupture of the molecules in the explosion reaction, a phase transition in AN and the potential contamination of the explosive residues with environmental material during the explosion were considered as potential explanation of the observed isotopic fractionation.

Depending on the strength of the explosion, residues are collected on a variety of surfaces and, or extracted from samples of soil. Numerous environmental studies discuss the effect of external factors on the isotopic signature of an explosive. Three of them are mentioned hereafter to illustrate these factors.

With a direct impact on explosives investigations, an interesting study addressed the issue of the recovery and stability of the isotopic profile of TNT contained in soils [Coffin *et al.* 2001]. The δ^{13}C and δ^{15}N values of 5 TNT samples coming from different sources were determined using GC-ion trap-mass spectrometer (ITMS)-IRMS. The ranges of measured variations for δ^{13}C (from -26.42 to -22.21 ‰) and δ^{15}N (from -5.36 ‰ to +9.64 ‰) values demonstrated the discriminating power of IRMS. In addition to excellent recovery rates, the extraction step of TNT from soil using Soxhlet and SPE did not cause any isotopic fractionation. The authors investigated the potential isotopic fractionation induced by moisture and bacterial degradation of TNT, through four-weeks incubation experiments in two different soils involving 50 samples for each soil. In contrast to δ^{15}N which displayed no isotopic fractionation, δ^{13}C value increased from -27.03 ‰ to -25.46 ‰. This isotopic fractionation was accompanied by a decrease in TNT concentration.

Berg *et al.* presented a SPME-GC-IRMS method to determine the carbon and nitrogen isotopic signature of nitroaromatic compounds and anilines in water [Berg *et al.* 2007]. They described the nitrogen isotope fractionation occurring during microbial transformation processes of nitroaromatic compounds into anilines in water, soils, as well as in abiotic environment on surfaces comprising Fe(II).

Similarly, Bernstein *et al.* described the different isotope fractionation patterns of δ^{15}N and δ^{18}O induced by aerobic and anaerobic biodegradation of RDX in aqueous media [Bernstein *et al.* 2008].

In essence The number of short communications on the isotopic analysis of explosives demonstrate the numerous researchers exploring the subject. However, given the abbreviate format of the pilot studies, it is difficult to grasp the amount of work that has been done so far.

The isotopic profile of organic explosives has only been determined by bulk analysis, while CSIA would offer a finer analysis of the explosive composition, separating the explosive substance from additives, such as binders, stabilisers, etc and measuring only delta values of the target substances.

All studies reported the admirable capacity of IRMS to discriminate the limited number of samples involved. However, very few studies oriented their research towards the evaluation of intravariability of the explosive substance. Often, intravariability is mistakenly presented through the standard deviation of replicates of the specimen. This however does not reflect the variation of isotopic ratios in the material, but only the precision in measurement. Conversely, this simplification of the problem can only result in a misleading underestimation of the intravariability.

About inorganic explosives, several studies were interested in the analysis of AN. Despite the large variety of analysed AN fertilisers, the results showed limited ranges of δ variations. Moreover, data on intravariability studies show that the intravariability within a manufacturer could be large. As for other mass-produced substances, an evaluation of the homogeneity of the isotopic profile often show variations larger than expected. For these reasons, intravariability should not be overlooked but measured at the same time than intervariability.

A new axis of IRMS research focused on the possibility to predict the signature of the starting material [Lock & Meier-Augenstein 2008, Aranda IV *et al.* 2011]. However, given the results of Buchanan *et al.* in the application of IRMS to illicit drugs [Buchanan *et al.* 2010], it is to be feared that the reaction yield and conditions have a non negligible influence on the isotopic signature.

So far, there has been limited research in the analysis of post-blast residues, and their comparison with the initial explosive. Actual results show that explosion induces an isotopic fractionation. By contrast, there are many studies on external factors influencing the isotopic signature of explosives remaining in soil or water. This has a direct impact on explosives investigations if the isotopic analysis is once applied to residues.

3.11 Stepping back—Possibilities for improvements

Forensic applications of IRMS are undertaken in a source inference perspective. The technology is used to infer the geographical origin of samples in food authentication, wildlife forensics, natural illicit drugs or in human provenancing. It is used to detect adulteration in food authentication or to differentiate genuine drugs from counterfeit in pharmaceuticals. The comparison of isotopic profiles is also oriented towards the differentiation of samples coming from different manufacturers or from different brands (paints, explosives) or the establishment of links between specimens coming from different seizures (synthetic illicit drugs, paints, packaging tapes, plastic films, explosives). A recent application in synthetic illicit drugs and explosives is the comparison of the isotopic profile of a precursor and the resulting product in order to establish a link between them.

The numerous publications definitely show the potential of discrimination of IRMS compared to other techniques. However there is a major and recurrent obstacle to the interpretation of isotopic variations: the lack of definition of the source. Rare studies present the research problem in its context and offer a transparent definition of the source. This obviously impacts on the sampling, as the sampling is intimately connected with the research problem, and therefore with the identity of source. Additionally, the very small quantities of element analysed (<1 mg) question the notion of sampling representativeness when performing such analyses.

Moreover, interpretation of isotopic data is also hampered by the lack of data on intravariability. Much has been done in terms of intervariability, while intravariability was often neglected, probably because it was considered as useless. However, it is interesting to note that, everytime the isotopic intravariability of a substance, a batch or a manufacturer was evaluated, it often showed to be larger than expected [Benson *et al.* 2009a, Schneiders *et al.* 2009]. This lack of data on intravariability leads to an underestimation of the natural variability of a substance and prevents a correct interpretation. Conversely, the presentation of intervariability only gives a wrong impression on the discriminatory power of the technique.

Finally, the isotopic profile measured at a given time is often taken as granted. Very rare studies have considered factors which could induce a potential isotopic fractionation (weathering during storage, fractionation during sampling or sample preparation, etc.). Additional studies undertaken in a bottom-up approach, i.e. defining the research problem, establishing the relevant sampling, determining the factors that may possibly influence the isotope ratios, should provide more fundamental data for a valid contribution of the isotopic information in a forensic context [Gentile *et al.* 2011].

Chapter 5

Materials and methods

Contents

1 Purpose of the research

The potential high discriminatory power of IRMS technology over other techniques used in routine promotes it as a hopeful technique in forensic sciences. It may be tempting to infer a common source when the isotope ratio of two substances are similar or to differentiate two specimens with a different isotope ratio. However, observing that the analytical technique is able to discriminate specimens of different sources is not sufficient. It appears also primordial to assess the stability of isotope ratios before exploiting and interpreting any isotopic difference. The stability of the isotopic profile, and implicitly, the way it is influenced by storage and weathering, is part of the feature of constancy enunciated by Kwan in the selection of valuable characteristics for source inference [Kwan 1977]. It requires the properties characterising an object and its source to be stable over time or, at least, predictable. The assessment of this feature allows the interpretation of variations between specimens with more confidence.

The aim of this work is to evaluate the forensic utility of isotope analysis on a set of black powders and ammonium nitrate fertilisers. This assessment will first go through the examination of the stability of the isotopic profile of black powder and ammonium nitrate samples. Subsequently, it will appraise the discrimination power of the technique, through the performance of statistical methods used to classify samples according to their source. This approach should permit to appreciate the possibilities and limitations of the technique and highlight whether the isotopic information is adequate to be used in different dimensions, to gather intelligence and/or produce evidence for courts (see **Chapter 3**).

1.1 Stability of the isotopic profile

The interest to know the evolution of the isotopic profile or to be able to predict the isotopic stability of a specimen under typical storage conditions[1] or in the presence of given factors resides in the comparison of specimens of explosives which did not have the same history or were not seized and analysed at the same time.

The stability, or conversely, the weathering of a substance depends on the storage conditions, that is the environmental conditions, the storage time and the packaging. The weathering of a substance, like evaporation, oxidation, hydrolysis or biodegradation, either natural or accelerated, occurs by the interaction or the stress of a specimen with external factors, such as ultraviolet and visible wavelength, heat, moisture, aggressive pollutants, bacterial activities [Waterman & Adami 2005, Waterman *et al.* 2007]. These phenomena can induce mass discrimination and prevent the evidentiary trace to be correlated with its

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[1] typical storage conditions here mean the storage conditions under which the specimens or the samples are expected to be stored

source.

The effect of weathering processes on stable isotope ratios has been widely investigated in contaminants attenuation research [Morasch *et al.* 2002, Berg *et al.* 2007, Kuder *et al.* 2009, Shin & Lee 2010] and in source tracing studies of hydrocarbons and oil spills [Mansuy *et al.* 1997, Li *et al.* 2009]. Two comprehensive reviews of environmental forensic studies plainly expose the ins and outs of weathering effects [Schmidt *et al.* 2004, Philp 2007]. The sensitivity of the isotopic profile to weathering processes is however not exclusive to environmental materials. Nevertheless, few studies have addressed the issue of integrity of the isotopic profile of a specimen due to storage conditions or weathering. Smallwood *et al.* exposed gasoline samples to evaporation and water-washing for one week. Despite the carbon isotopic alteration of certain compounds, most of them were isotopically similar to neat samples [Smallwood *et al.* 2002]. The weathering of white paints under controlled conditions was investigated by Reidy *et al.* [Reidy *et al.* 2005]. After 3 months, the authors noticed a δ^{13}C depletion of 0.7 ‰ possibly associated with solvent evaporation from the paint. By exposing human hair to artificially isotopic light and heavy water, Sharp *et al.* noted the re-equilibration of a reproducible portion of exchangeable hydrogen with ambient atmospheric moisture, allowing the use of δ^{2}H hair samples for investigation of food and water intake [Sharp *et al.* 2003]. Benson exposed ammonium nitrate to moisture and surprisingly did not observe any significant change of the δ^{15}N, δ^{18}O and δ^{2}H values [Benson 2009].

Since it is known that kinetic and thermodynamic isotopic fractionations occur imperceptibly at the atomic level due to physico-chemical or chemical reactions, the evaluation of the constancy or evolution of analytes within a matrix under different conditions appears of particular relevance for a correct and reliable interpretation of isotopic variations.

Ammonium nitrate is known for its strong hygroscopicity. A potential modification of the δ^{18}O and δ^{2}H isotopic composition of a sample exposed to moisture must therefore be considered. Beside moisture, heat is another parameter often evaluated in weathering studies. As a result, the reactivity of the isotopic profile to moisture and heat will be assessed in this research. Nevertheless, given the results of the works of Sharp *et al.* and Benson, we can speculate that the isotopic profile fulfils the criteria of constancy of Kwan, as long as it does not undergo extreme and violent conditions such as explosion or fire. In view of this argument, the following hypothesis can be formulated.

> Hypothesis III
>
> Typical storage conditions and weathering through the exposure of the samples to moisture and heat do not significantly alter stable isotope ratios. The isotopic profile can thus be used in a

source inference or source discrimination perspective.

1.2 Discrimination power of the isotopic profile

As mentioned in our paper, the definition of the source is at the heart of the definition of the research problem [Gentile *et al.* 2011]. A review of published works on the forensic isotopic analysis of explosives reveals the frequent absence of the definition of the source. The common couple of terms "samples from different sources" generally hides a lack of definition of the source, that is an absence of hypotheses on which to interpret isotopic variations. Interpretation of the results is accordingly limited, as the meaning of similar or different isotopic profiles is difficult to express. In few papers, the concept of source is exposed as being the manufacturer or the supplier of a material [Phillips *et al.* 2003b, Benson *et al.* 2009a, Meier-Augenstein 2010], the purity grade of the same manufacturer [Wakelin 2001], the precursors used for the synthesis of the explosive [Lock & Meier-Augenstein 2008, Meier-Augenstein 2010, Aranda IV *et al.* 2011]. In others, the definition of the source is left in a grey area. This is especially the case when the sampling is based on specimens issued from seizures; the unknown origin of samples is often put aside. Yet, some authors working in illicit drug profiling of seized specimens were able to provide a consistent definition of the source in the context of their problem research [Esseiva *et al.* 2011]

In our case, from a police perspective, one of the aim of explosives investigations resides in establishing a link between the explosive collected on the scene, for example black powder enclosed in a pipe bomb and the explosive found at a suspect's house. This research problem delimits the source as the container or the commercial packaging from which the black powder was taken to fill the pipe bomb. The potential uniqueness of the source is directly correlated with the frequency of its characteristics, which is in turn highly dependent on the way the substance is manufactured and distributed. The manufacture of black powder gives valuable information on the prior frequency of isotopic characteristics. Raw materials are delivered in huge quantities to the powdery and are used to make several daily productions and batches. Even though the manufacturing process is not industrial, the manufacture follows a precise recipe and does not involve any chemical process which could create isotopic fractionations, providing daily productions with particular isotopic profile. Thus, as daily productions are generally all issued from the same raw materials and as the constitution of a batch only involves the physical mixture of daily productions, it is expected that the daily productions and the end product (the batch) will have similar isotopic profile. Furthermore, as the same arrival of raw materials are used to produce several batches as well as different grades of black powder at the same time, it appears logical that different products (black powder of different grades made up from the same raw materials) will have similar isotopic profiles. Given that each manufacturer is likely to have different sources of raw material procurement and their own secret manufacturing recipe, we can more likely expect different isotopic compositions between manufacturers. It is therefore possible to define the source at the manufacturer level and to consider the following hypothesis.

> Hypothesis IV.1
>
> The information extracted from the isotopic analysis of black powders allow differentiating distinct manufacturers.

For ammonium nitrate fertilisers, ammonium nitrate is synthesised from nitric acid and ammonia. There are however several synthesis methods. Each manufacturer uses its own synthesis method and supply of raw materials. Therefore, ammonium nitrate fertilisers produced by different manufacturers are expected to have different isotopic profiles. In the particular case of Lonza (see **Chapter 2**, **Section 2.4**), different additives are added to the same ammonium nitrate. In the case of bulk isotope analysis, these additives contribute to the isotope profile of the AN fertilisers. However, as they are added in proportions of ~20 %, their contribution to the bulk δ value of the fertiliser is limited, unless the isotopic profile of the additive is highly depleted or enriched. Thus, the different types of ammonium nitrate fertilisers manufactured by Lonza should have a similar isotopic profile. On the batch level, it is unlikely that several batches of production of the same type of AN fertiliser have significantly different isotopic profiles. Syntheses in industrialised processes are performed under controlled conditions, which logically preclude from the introduction of significant isotopic variations between batches. On the basis of these considerations, it is possible to enunciate the following proposition.

> Hypothesis IV.2
>
> The information extracted from the isotopic analysis of ammonium nitrate fertilisers allow differentiating distinct manufacturers.

2 Sampling

2.1 Black powders

Among the six companies known to produce sport or shooting black powder in Europe, only three of them accepted to provide samples of black powder. The sampling of this work is constituted of black powders from the powderies of Vonges (France), Wano (Germany) and Aubonne (Switzerland).

2.1.1 Powdery of Vonges

Closed at present, the site of Vonges used to produce about 70 tons of black powder per year, of which about 45 tons were for civilian black powder and 25 tons for sporting, shooting and the use of ancient weapons. One batch of black powder produced by the powdery corresponded to approximately 2500 kg. As the amount of binary mixture loaded under the mills is limited (70 kg) (see **Chapter 2**, **Section 1.4**), one batch resulted from the mix of several daily productions. Generally, one arrival of raw material led to the production of several batches of black powder. According to Martin, it could have been possible that one batch of black powder encompassed two arrivals of raw material, however this would have been rather seldom [Martin 2007].

The powdery of Vonges provided 15 samples coming from 5 different batches, produced between 2005 and 2007 of the same type of shooting black powder (see **Table 5.1**). For each batch, samples come from half kilos of black powder kept at the end of each batch production to ensure the follow up of the quality of the batches. Three samples of 2 g each were sampled for each batch, making a total of 15 samples. For V1 to V4, one analysis per sample was made, resulting in a total of 3 analyses per batch. For V5, the isotopic intravariability was evaluated with 3 analyses per samples, making a total of 9 analyses for this batch.

Type of powder	Year of production	Batch number	Reference number	Samples
Shooting powder PNF1	2005	32/05	V1	3 x 2 g
Shooting powder PNF1	2006	23/06	V2	3 x 2 g
Shooting powder PNF1	2006	34/06	V3	3 x 2 g
Shooting powder PNF1	2007	02/07	V4	3 x 2 g
Shooting powder PNF1	2007	07/07	V5	3 x 2 g

Table 5.1. Samples of black powder provided by the powdery of Vonges.

2.1.2 Powdery of Wano

The manufacture of Wano yields about 12 tons of sporting and shooting black powder per year. The production of one batch takes approximately one week and represents an amount of about 300 kg. Quality control tests are then undertaken either on the 300 kg batch or on an average quantity of 2 tons corresponding to the mixture of 10 small batches.

The powdery of Wano provided 24 samples of black powder coming from 8 different batches of production, manufactured over 5 years. For each batch, the samples are issued from kilos of black powder stored at the end of each production to ensure the follow up of the quality of the batches. Three samples of 5 g each were sampled for each batch, making a total of 24 samples. For W1 to W4 and W6 to W8, one analysis per sample was made, resulting in a total of 3 analyses per batch. For W5, the isotopic intravariability was evaluated with 3 analyses per samples, making a total of 9 analyses for this batch. The samples, listed in **Table 5.2**, are representative of the two types of black powder produced by this manufacturer: FFg powder, made from alder charcoal, is dedicated for shooting and PP powder, made from dogwood charcoal, is used for sporting. Each type of powder contains slightly different proportions of the 3 ingredients (charcoal, sulphur and nitrate potassium).

Type of powder	Year of production	Batch number	Reference number	Samples
Shooting powder FFg	2003	Batch 2003	W1	3 x 5 g
Shooting powder FFg	2004	Batch 2004	W2	3 x 5 g
Shooting powder FFg	2005	Batch 2005	W3	3 x 5 g
Shooting powder FFg	2006	Batch 2006	W4	3 x 5 g
Shooting powder FFg	2007	Batch 2007	W5	3 x 5 g
Sporting powder PP	2005	Batch 2005	W6	3 x 5 g
Sporting powder PP	2006	Batch 2006	W7	3 x 5 g
Sporting powder PP	2007	Batch 2007	W8	3 x 5 g

Table 5.2. Samples of black powder provided by the powdery of Wano.

2.1.3 Powdery of Aubonne

The 20 samples prepared by the powdery of Aubonne offered the possibility to evaluate the isotopic intravariability at the different levels of production (see **Table 5.3**). The powdery of Aubonne has a daily production of 300 kg of black powder, which are stored in 50 kg bags. As illustrated by **Figure 2.2**, **page 29**, each batch of black powder is composed by mixing several day-productions. When the total amount necessary for the constitution of one batch has been produced, generally 1.8 tons, the contents of the bags are dumped and mixed in a huge funnel. The end product resulting from this mix constitutes one batch. The black powder is then packaged in 100 g and 1 kg containers for armourers and retailers, or when exported, in 454 g, 500 g and 1 kg containers.

The sampling included samples of daily productions collected in different bags *before* they were mixed to form the final batch, as well as samples of the final batch itself (*after* the mixture). Note that the sampling of the daily productions only represent a restricted number of bags from all the bags mixed to constitute the final batch.

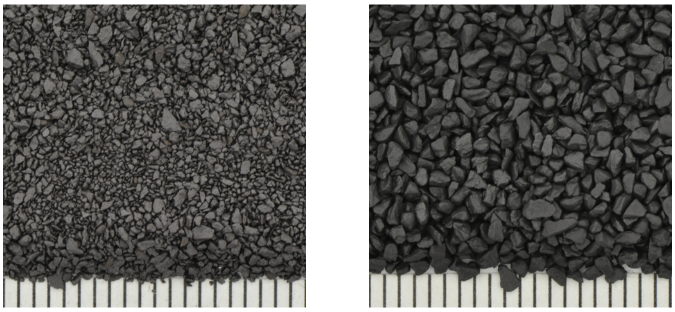
Type of powder	**Period of production**	**Batch number**	**Reference number**	**Samples**
Shooting powder n°2	July 2006	040.906	A1	1 x 1 kg
Shooting powder n°2	January 2007	290.107 batch	A2.0	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production A	A2.1	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production B	A2.2	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production C	A2.3	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production D	A2.4	1 x 100 g
Shooting powder n°2	October 2007	151.007 batch	A3.0	1 x 100 g
Shooting powder n°2	October 2007	151.007 day production A	A3.1	1 x 100 g
Shooting powder n°2	October 2007	151.007 day production B	A3.2	1 x 100 g
Shooting powder n°2	October 2007	151.007 day production C	A3.3	1 x 100 g
Shooting powder n°2	March 2008	170.308 batch	A4.0	1 x 100 g
Shooting powder n°2	March 2008	170.308 day production A	A4.1	1 x 100 g
Shooting powder n°2	March 2008	170.308 day production B	A4.2	1 x 100 g
Shooting powder n°2	2003	170.308 day production C	A4.3	1 x 100 g
Shooting powder n°2	October 2008	071.008 batch	A5.0	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production A	A5.1	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production B	A5.2	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production C	A5.3	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production D	A5.4	1 x 100 g
Mortar powder Böller n°5	unknown	-	A10	1 x 100 g

Table 5.3. Samples of black powder provided by the powdery of Aubonne.

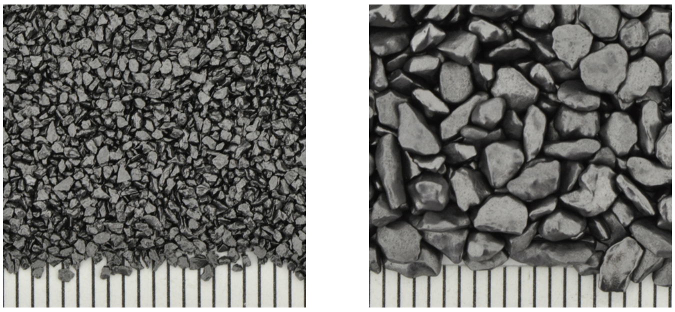
2.1.4 Physical aspects and preliminary anionic profile

Although the discriminatory power of the physical aspect of the samples and their ionic profile is not considered in this research, it is interesting to note that these features carry a significant layer of information, which may in some cases help in doing already a first selection between candidates.

Physical aspect The black powders produced by each manufacturer exhibit the same physical characteristics, except for sample A10, which has coarser grains than the other samples of Aubonne (A1 to A5.0). Yet, black powders of the three manufacturers have different physical aspect (see **Table 5.4**).



Sample V1 Sample W1



Sample A1 Sample A10

Table 5.4. Physical aspect (magnified 5x) of black powder samples from Vonges (V1), Wano (W1) and Aubonne (A1 and A10).

Anionic analyses The anionic analyses were made at the Wissenschaftlicher-ForschungsDienst of Zurich. The analyses of black powder samples showed the presence of a large peak of nitrate and a small amount of sulphate (for details on the IC method used for the determination of anions, see **Appendix 1**). All samples showed the same anionic composition, as illustrated in **Figures 5.1**, **5.2** and **5.3** by the results of analysis of black powders V1, W1 and A1 manufactured by Vonges, Wano and Aubonne respectively. Chloride, sulphate and the two unknown peaks came from the blank (deionised water).

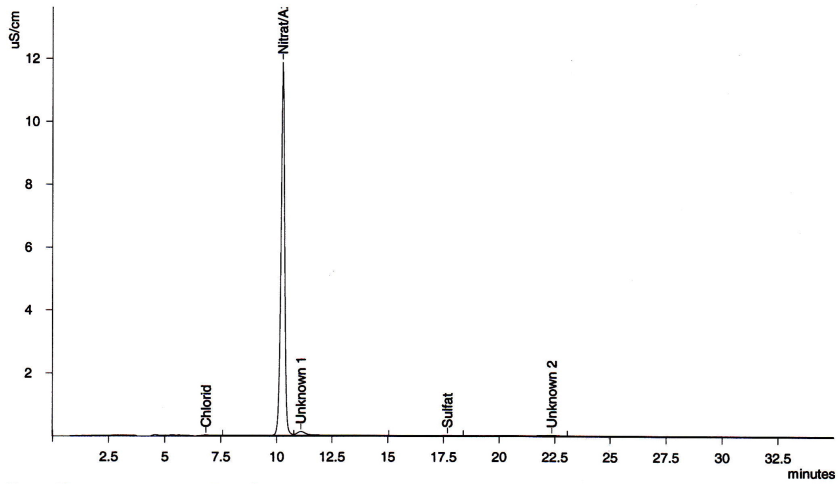


Figure 5.1. Anionic chromatogram of black powder V1 produced on the site of Vonges.

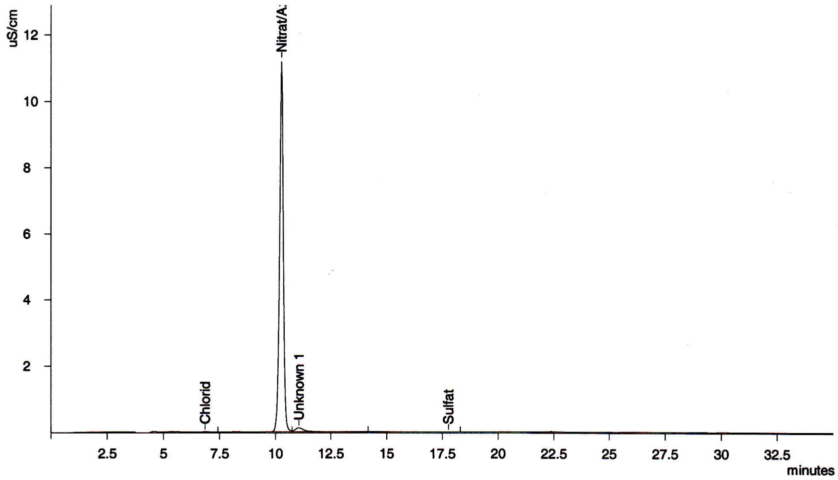


Figure 5.2. Anionic chromatogram of black powder W1 produced by the powdery of Wano.

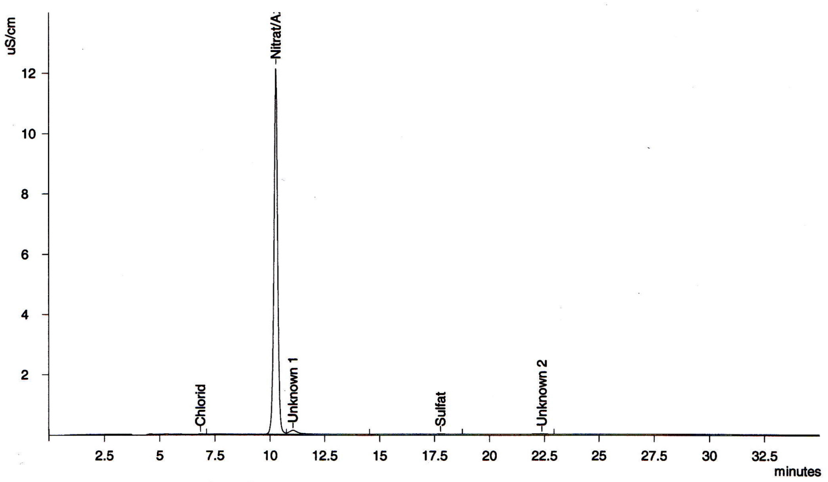


Figure 5.3. Anionic chromatogram of black powder A1 produced by the powdery of Aubonne.

The cationic analyses of the samples were not performed. However, given the composition of black powder, and on the basis of literature, we infer that we would have obtained similar cationic results between samples, with a unique peak of potassium.

The indistinguishable anionic profile of black powder samples illustrates the limits of the forensic analytical comparison of explosives (previously described in **Chapter 3**) when comparing two substances with the same chemical nature. However, the physical aspect of samples should not be neglected as the information conveyed by these features may already offer a first level of discrimination in preblast investigations.

2.2 Ammonium nitrate based fertilisers

Ammonium nitrate fertilisers sold in Switzerland mainly come from Landor, a Swiss importer of fertilisers based near Basel and Lonza, a Swiss manufacturer based in Visp. The sampling of this research encompass AN fertilisers manufactured by Lonza as well as fertilisers imported by Landor.

2.2.1 Landor

Landor (Birsfelden, Switzerland) imports ammonium nitrate based fertilisers from about 20 manufacturers, mostly European ones. This company provided a small sampling of fertilisers coming from their different manufacturers. It was not possible to obtain several samples from one batch for the evaluation of isotopic intravariability. Supplementary samples were sought without success. In addition, one 50 kg bag sold on the Swiss market and commercialised by Landor was included in the sampling although its manufacturer was unknown. As already mentioned in **Chapter 2**, **Section 2.4**, the bagging of ammonium nitrate in Landor precludes any identification of the manufacturer. Three samples were taken at different locations in the bag (L1.1 to L1.3) in order to evaluate the isotopic intravariability in a bag of ammonium nitrate fertiliser sold on the market. Samples obtained from Landor are listed in **Table 5.5**.

Fertiliser type Composition	**Period of production**	**Manufacturer Country**	**Reference number**	**Samples**
AN (27%N 9% Ca, 2.5% Mg)	before 2003	Unknown	L1.1-1.3	3 x 100 g
AN	March 2007	BASF Belgium	L2	1 x 500 g
AN	March 2007	Pec Rhin France	L3	1 x 500 g
AN	March 2007	DSM Stein Netherland	L4	1 x 500 g
AN	April 2007	Kemira Belgium	L5	1 x 500 g
AN (27%N, 2.5% Mg inert agents)	March 2007	Yara Sluiskil Netherland	L6	1 x 500 g
AN (27%N, 2.5% Mg inert agents)	April 2007	Yara Sluiskil Netherland	L7	1 x 500 g

Table 5.5. Samples of ammonium nitrate fertilisers obtained from Landor.

2.2.2 Lonza

A particularity of Lonza production consists in the manufacture of one huge batch of pure ammonium nitrate, from which different types of fertiliser are produced according to the additive mixed with it [Kummer 2007]. Lonza (Basel, Switzerland) provided 12 samples produced between spring 2006 and April 2007 (O1 to O6.3) (**Table 5.6**). For fertiliser O4 to O6, 3 samples of 100 g each could be collected in different places of the huge mass (several tons) of the stored fertiliser, in order to evaluate the intravariability of the batch.

Type of fertiliser Composition	Period of production	Manufacturer Country	Reference number	Samples
AN Borax	March 2007	Lonza Switzerland	O1	1 x 100 g
AN, Mg Dolomite	April 2007	Lonza Switzerland	O2	1 x 100 g
AN $CaCO_3$	March 2007	Lonza Switzerland	O3	1 x 100 g
AN $MgSO_4$	April 2007	Lonza Switzerland	O4.1-4.3	3 x 100 g in the batch
AN schiste	spring 2006	Lonza Switzerland	O5.1-5.3	3 x 100 g in the batch
AN schiste	spring 2007	Lonza Switzerland	O6.1-6.3	3 x 100 g in the batch

Table 5.6. Samples of ammonium nitrate fertilisers provided by Lonza.

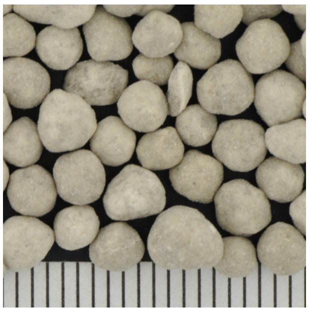
2.2.3 Physical aspect and preliminary anionic analyses

Physical aspect **Table 5.7** presents the visual aspect of the different ammonium nitrate fertilisers samples that consist of prills. It is interesting to note that several fertilisers already exhibited different physical characteristics.

Anionic analyses The anionic profile of ammonium nitrate fertilisers revealed mainly the presence of nitrate. The similarity between the anionic profile of ammonium nitrate fertilisers is illustrated by the following two **Figures 5.4** and **5.5** of samples L4 and O2. Chloride, sulphate and the two unknown peaks came from the blank (deionised water).

Despite a similar anionic composition preventing any possible discrimination, some ammonium nitrate fertilisers samples have distinct physical aspect from each others (colour, size, granularity), which could already be exploited in pre-blast investigations.

The cations analyses of the samples were not performed here. In addition to the ammonium cation, these analyses would probably have shown the presence of other cations coming from the additives, which could help in categorising AN fertilisers.

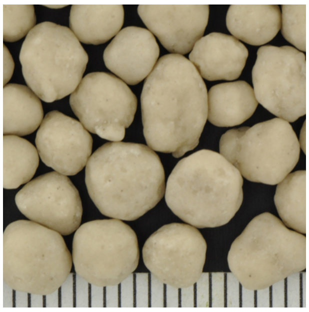

Sample L1


Sample L2

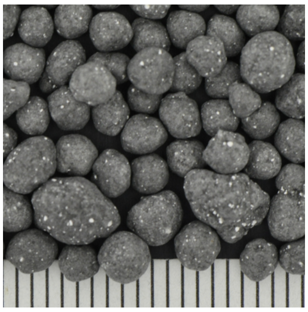

Sample L3


Sample L4

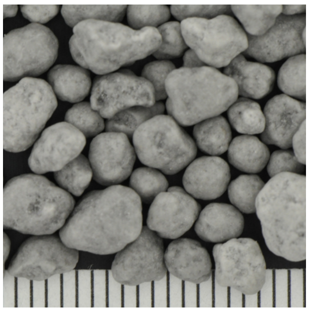

Sample L5


Sample L6


Sample L7


Sample O1


Sample O2


Sample O3


Sample O4


Sample O5

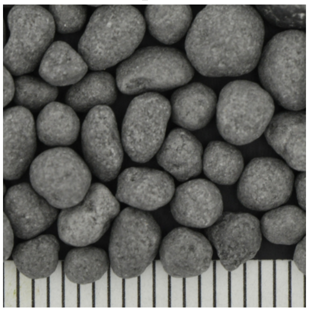

Sample O6

Table 5.7. Physical aspect of ammonium nitrate fertilisers (magnified 5x) imported by Landor (L1 to L7) and produced by Lonza (O1 to O6).

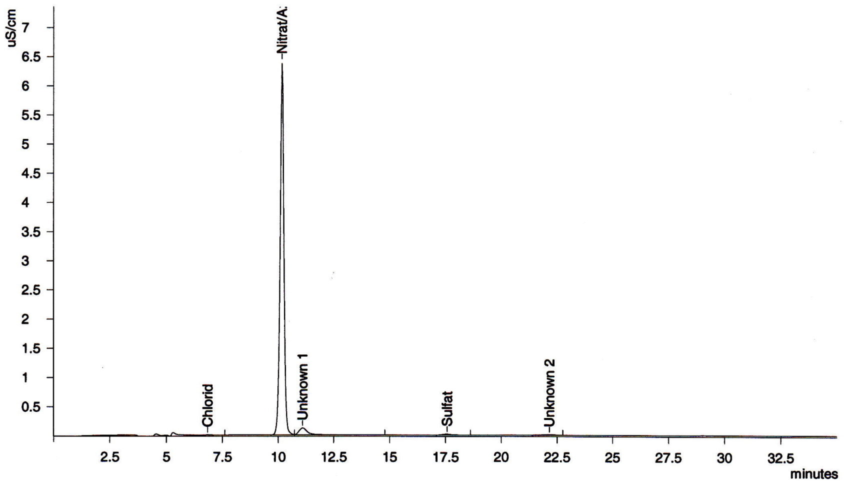


Figure 5.4. Anionic chromatogram of ammonium nitrate fertiliser L4 manufactured by DSM Stein in the Netherlands and imported by Landor.

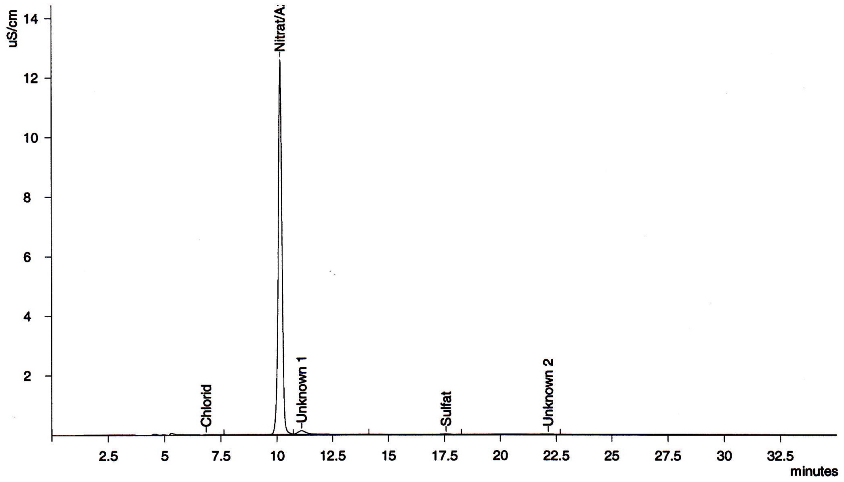


Figure 5.5. Anionic chromatogram of ammonium nitrate fertiliser O2 manufactured by Lonza.

3 Chemicals

Carbon dioxide ($>$ 99.998 %), nitrogen ($>$ 99.999 %), carbon monoxide ($>$ 99.997 %), helium ($>$ 99.9999 %) and oxygen gas ($>$ 99.995 %) were supplied by Messer (Lenzburg, Switzerland). Potassium nitrate (purum p.a. $>$ 99.0 %), ammonium nitrate ($\geqslant$ 99 %) and magnesium perchlorate ($>$ 98 %) were obtained from Fluka (Buchs, Switzerland). Carbon in the form of graphite powder (1-2 μm, 99.95 % purity) and vanadium (V) pentoxide (99.99 %) were supplied by Sigma-Aldrich (Buchs, Switzerland). Cellulose was obtained from Merck (Darmstadt, Germany). Chromium (III) oxide (Cr_2O_3), silvered cobaltous-cobaltic oxide (Ag CoO-Co_2O_3), 15 mm diameter quartz wool discs, reduced copper wire (0.5 mm), quartz chips, lead chromate, quartz glass tubes, ceramic tubes, tin and silver capsules for solids (3.3 x 5 mm) were purchased from Säntis Analytical AG (Teufen, Switzerland). A pre-packed CHNS reactor (n°99.0733.10) was purchased from Lüdi AG (Flawil, Switzerland). Glassy carbon grit (Sigradur G, 3150-4000 μm grit) was supplied by HTW GmbH (Thierhaupten, Germany) and Carbosorb by HEKAtech GmbH (Wegberg, Germany). 96-well Microtiter plates were acquired from Semadeni (Ostermundigen, Switzerland). USGS-24 (graphite), IAEA-CH-6 (sucrose), IAEA-CH-7 (polyethylene), IAEA-N-1 ($(NH_4)_2SO_4$), IAEA-N-2 ($(NH_4)_2SO_4$), IAEA-NO-3 (KNO_3) and IAEA-CH-3 (cellulose) were obtained from the International Atomic Energy Agency (IAEA, Vienna). Water with heavily depleted oxygen isotope ratio ($\delta^{18}O$ = -250 ‰) was produced in-house from a distillation column.

4 Standards selection and utilisation

Whatever the instrumentation, the fundamental principle underlying reliable stable isotope measurements is the principle of identical treatment (IT) of the reference material and the sample [Werner & Brand 2001]. By submitting the reference material and the sample to the same analytical pathway and same conditions, this procedure allows taking into account any potential isotope fractionation occurring in the analytical train of the instrumentation. This procedure is crucial, as the result of the sample are expressed against an international reference material.

Reference materials are first used to calibrate the equipment and laboratory standards. They can also be used to normalise raw data to the international reference scale (see below), although laboratory standards are usually employed for that purpose. In addition, reference materials can be used to monitor the quality of the IRMS and of the analysis method.

Through the observation of standards of known isotopic composition, quality control can be performed at different times in the analytical process. Before any analytical session, the repeated analyses of reference gas pulses allow checking the stability of the IRMS system. During chromatographic runs, the introduction of reference gas pulses with known isotopic composition, bracketing the retention time of the analytes as closely as possible, allows the control of the quality of the analyte response. Similarly, the daily analysis of laboratory standards or reference materials allows verifying the analytical response of the machine throughout the sequences of analysis, as well as to monitor accuracy and precision in a quality control perspective. In order to evaluate the quality of the response of the whole method, the standards should go through the full analyte preparation and instrumentation.

Concerning standards selection, the choice of appropriate laboratory standards and laboratory reference materials is guided by the following criteria [Werner & Brand 2001, Jardine & Cunjak 2005]:

- the material should be easy to handle during preparatory steps and replaceable if exhausted;
- it must be preferably a chemical compound available at high purity, non-hygroscopic and stable over a long period of time;
- it should have a low vapour pressure at room temperature and atmospheric pressure and a good solubility in high purity solvents (valid for CSIA);
- the material should have a δ value within the expected range of variations of the analytes;
- the elemental mole ratio and the chemical composition of the standard should be identical or similar to the material to be analysed for an accurate and precise measurement.

4.1 Laboratory standards, working standards and control samples

For carbon and nitrogen, two laboratory standards (LS) routinely used in the daily process of bulk sample analyses of the laboratory were analysed along with samples. Laboratory standards were plant based materials, routinely employed in the analytical sequences of bulk analyses of the laboratory. Although they have a chemical composition different from that of the target samples (nitrate), they have a well known δ value, intermediate precision and long-term stability. They are therefore useful indicators of the precision and accuracy of δ measurements. Standard 1 (Std1), (δ^{13}C = -28.5 ‰, 42.1 % C; δ^{15}N = 0.4 ‰, 2.1% N) originated from the mixture of plant materials. Standard 2 (Std2) (δ^{13}C = -33.0 ‰, 44.0 % C; δ^{15}N = 1.0 ‰, 2.4 % N) was made from catalpa leaves. The two laboratory standards were previously carefully calibrated against international reference materials USGS-24 (-16.05 ‰ ± 0.04), IAEA-CH-6 (-10.50 ‰ ± 0.03) and IAEA-CH-7 (-32.15 ‰ ± 0.05) for carbon, IAEA-N-1 (+0.4 ‰ ± 0.2), IAEA-N2 (+20.3 ‰ ± 0.2), IAEA-

NO-3 (+4.7 ‰ ± 0.2) for nitrogen. They have shown an overall precision through an everyday use over several years of less than 0.1‰ for carbon and 0.2 ‰ for nitrogen.

For oxygen, a cellulose standard routinely employed for the bulk analyses of oxygen in the laboratory was analysed along with samples. Its δ^{18}O was confirmed by ring tests results [Boettger *et al.* 2007] (δ^{18}O = 28.7 ‰, 49.3 % O) and by the measurement of IAEA-CH-3 (32.6 ‰). The overall intermediate precision estimated over several years of use is 0.3 ‰.

Given the importance of similarity-matrix feature, two working standards (WS), one for black powders and one for ammonium nitrate fertilisers, with a chemical composition and matrix similar to the samples, were selected and analysed in each sequence. For black powder, an in-house working standard (MIX) made of nitrate potassium and carbon was constituted in proportions of 75:25. Sulphur was not added to the mix for safety reasons and to avoid its deposition in the isotope ratio mass spectrometer. For ammonium nitrate fertilisers, the standard (AN std) consisted of pure ammonium nitrate. Their δ values were determined against laboratory standards by a series of 20 consecutive measurements.

The three laboratory standards (Std1, Std2 and cellulose) were used to normalise raw data to the international reference scale, while the working standards were inserted in the sequences to check the quality of the analytical response. For nitrogen, further investigations were performed on the basis of the observations presented in **Chapter 6** and led to the development of another method. For this second method, AN std and KNO_3 were used to normalise raw data to the international scale.

The carbon (% C) and nitrogen content (% N) (in weight %) were determined against the elemental composition of the laboratory standards. For the second determination of % N and the oxygen content (% O), they were calculated according to the working standard (either the AN std or the MIX), because of the observations presented in **Chapter 6**.

Each sequence also included a control sample (CS) analysed in duplicate or triplicate in the middle of the sequence. Samples A1 and L5 were arbitrarily chosen as control samples for black powders and ammonium nitrate fertilisers.

4.2 Sequence design

The strategy of standards utilisation for calibration, normalisation and quality control often varies from one laboratory to another. The number, type and frequency of analysed standards, the way samples, standards and blanks are positioned, the number of specimen replicates between standards and within each run all design the analytical sequence [Werner & Brand 2001, Jardine & Cunjak 2005]. While the frequency of the analysed standards is governed by the required precision, the number of standards and their position can influence the results.

Samples run in triplicates were analysed along with working and laboratory standards. Triplicates of working and laboratory standards were positioned at the beginning and at the end of each sequence (max. 98 positions for carbon and 49 positions for nitrogen and oxygen) as well as throughout the sequence, generally after 9 analyses of samples. Blanks were positioned at different places in the sequence to monitor any carryover effects. One working standard and one laboratory standard were employed in every sequence. A typical sequence design was as follow: 1x blank, 3x WS, 3x LS, 9 analyses of samples, 1x WS, 1x LS, 9 analyses of samples, 3x CS, 1x WS, 1x LS, 9 analyses of samples, 3x WS, 3x LS, 1x blank. On occasions, sequence design was modified to investigate particular effects. Due to various observations (see **Chapter 6**, sequence design was subsequently adapted to include double replicates of WS and LS during the sequence.

5 Sample preparation and storage

Prills of ammonium nitrate fertilisers were ground with a porcelain pestle and a mortar. Any handling of black powder was undertaken carefully, using an anti-electrstatic security system (ELME 157 kit 6090 wrist strap and benchmat, Distrelec, Nänikon, Switzerland). Given the hazards related to the grinding of black powder, preliminary analyses were performed to investigate whether grinding the samples influenced significantly the repeatability of the results. For these tests, black powder was ground with a wooden spoon and a porcelain mortar. Ground and unground black powder samples were analysed. A Sartorius Micro MC5 analytical microbalance (Sartorius, Goettingen, Germany) was used to weigh samples and standards for isotope analysis. Due to balance availability, a Mettler Toledo MT5 analytical balance (Mettler Toledo, Switzerland) was also used occasionally. Weighing of larger quantities for mixtures of substances was undertaken with a Mettler Toledo New Classic MF ML204. For carbon and nitrogen, samples and standards were weighed in 3.3 x 5 mm tin capsules for solids. Weighed masses were adjusted to produce a peak amplitude similar to that of the reference gas (about 4000 mV for m/z 44 and 2000 for m/z 28) whenever possible and contained the equivalent of approximately 220 μg of carbon and 100 μg of nitrogen respectively. For oxygen, samples and standards were weighed in silver capsules for solids. Weighed masses were adjusted to produce a peak amplitude similar to that of the reference gas (about 3000 mV for m/z 28) whenever possible and contained the equivalent of approximately 280 μg of oxygen. Replicates of analyses, generally 3, were taken from portions of 1 to 5 g each (depending on the available quantities) randomly taken in the sample.

Laboratory standards and samples were stored at ambient room temperature and enclosed in sealed glass containers. Prepared samples were placed in 96-well Microtiter plates and stored in the desiccator until analysis.

5.1 Storage experiments

The ageing of a material, mainly due to oxidation and hydrolysis, is occurring naturally through the interaction of environmental factors, such as ultraviolet and visible wavelength, heat, aggressive pollutants, moisture, etc. Ruled by Arrhenius' law, accelerated ageing is implemented by exposing the material to severe conditions, generally heat or moisture, for a definite period of time carefully selected [Waterman & Adami 2005, Waterman *et al.* 2007]. In order to observe the ageing effect on isotope ratios of black powder, three portions of about 150 g each, taken in one kilo of black powder (sample A1) produced in July 2006, were stored under different conditions. The first one was stored in a room under normal conditions (20°C, ~50% relative humidity) and the second one in another room under high moisture conditions (20°C, ~80% relative humidity). Electronic probes (ELPRO Ecolog, Buchs, Switzerland) were used to monitor the temperature and relative humidity under normal and high moisture conditions. The third portion was stored under elevated temperature in an oven (60°C, 0% relative humidity), whose temperature was controlled by a thermostat. For each of these 3 groups, samples were taken after periods of time t = 0, 1, 2, 3, 7, 9, 12, corresponding to the number of months elapsed between storage and sampling. Each collected sample was conditioned in glass vial sealed with Parafilm® and stored at ambient temperature in the dark to minimise possible interactions with light, heat and moisture in the air.

An additional storage experiment was undertaken with heavily depleted water with $\delta^{18}O$ = -250 ‰ in order to see the effect of short term storage on the $\delta^{18}O$ value of the samples. The cellulose standard, the MIX, the AN std, one black powder and one AN fertiliser samples were placed in a desiccator and exposed to vapours of heavily $\delta^{18}O$ depleted water at room temperature. This was performed by replacing the desiccant with a container filled with the depleted water. Samples were collected after 6, 12, 24 and 72 hours. Analyses were undertaken before the exposure experiment and immediately after collection, using the evacuation procedure with the Zero Blank autosampler. The standards and the samples exposed to moisture were analysed in triplicates along with the cellulose standard kept away from moisture interaction in a desiccator, in order to correct raw data to the international reference scale.

6 Isotope analysis methods

Analyses were performed at the Laboratory of Atmospheric Chemistry of the Paul Scherrer Institut (PSI) in Villigen in the stable isotopes research group. For black powder, carbon, nitrogen and oxygen isotope analyses were undertaken. For ammonium nitrate, nitrogen and oxygen isotope analyses were performed.

6.1 Carbon

6.1.1 Instrumentation

Carbon analyses were performed on a Carlo Erba 1110 elemental analyzer (EA) (Carlo Erba, Milano, Italy) coupled with a Conflo II interface (Finnigan MAT, Bremen, Germany) to a Delta S isotope ratio mass spectrometer (Finnigan MAT). Given instrument availability, part of the analyses were also carried out using the same EA coupled to a DeltaPlus XL isotope ratio mass spectrometer (Finnigan MAT). These two instruments are later referred to as IRMS1 and IRMS2 respectively. Interface parameters were He = 1.0 bar, reference gas (CO_2) = 1.0 bar.

6.1.2 Carbon method

The sample is dropped from the autosampler into a combustion reactor held at approximately 1020°C, consisting of a quartz tube filled with chromium (III) oxide (Cr_2O_3) and silvered cobaltous-cobaltic oxide (Ag CoO-Co_2O_3). For a certain period of time, lead chromate was also inserted in the reactor in order to trap sulphur. By means of a flash combustion, the sample is combusted in the presence of excess oxygen. The analytical cycle, lasting 70 seconds (s), was as follows. Oxygen was injected into the combustion reactor for 60 s from the beginning of the analytical cycle. The sample was dropped 18 s after the beginning of the cycle (analytical cycle set-up: cycle lasting 70 s, oxygen injection for 60 s, sample start and stop at 18 and 20 s, respectively). The gaseous products are carried in a helium flow (80 ml/min) to a reduction reactor packed with reduced copper wire, held at 650°C. This reduction step converts nitrogen oxides into N_2 and traps oxygen. Water is captured by a perchlorate magnesium trap. The resulting gases are separated by a Porapak Q GC column (4 m, 50°C) before entering the ion source. The molecules are then ionized and accelerated with a voltage of ~3040 V. A magnetic field deflects the ions and separates the molecules according to their mass-to-charge ratio (m/z) before reaching an array of Faraday cups monitoring masses 44, 45 and 46. The method length is 560 s. Raw data were processed with Isodat 2.0 software (Thermo Fisher Corporation, Bremen, Germany).

6.2 Nitrogen

6.2.1 Instrumentation

Nitrogen analyses were also performed on a Carlo Erba 1110 elemental analyzer (EA) (Carlo Erba) coupled with a Conflo II interface to a Delta S isotope ratio mass spectrometer (Finnigan MAT) (IRMS1). Due to instrument availability, part of the analyses were also performed using the same EA coupled to a DeltaPlus XL isotope ratio mass spectrometer (Finnigan MAT) (IRMS2). A Zero Blank autosampler (Costech Analytical Technologies, Valencia, CA, USA) was employed for nitrogen analyses in order to avoid possible interferences with atmospheric N_2 and moisture. Interface parameters were He = 0.8 bar, reference gas (N_2) = 0.8 bar.

6.2.2 Nitrogen method

Before the measurements are started, the autosampler is evacuated for 30 minutes then refilled with helium until a pressure of 0.5 bar is reached. The sample is dropped from the autosampler into a combustion reactor held at 1020°C, consisting of a quartz tube filled with chromium (III) oxide (Cr_2O_3) and silvered cobaltous-cobaltic oxide (Ag CoO-Co_2O_3). For a certain period of time, lead chromate was also inserted in the reactor in order to trap sulphur. By means of a flash combustion, the sample is combusted in the presence of excess oxygen. The analytical cycle, lasting 70 s, was as follows. Oxygen was injected into the combustion reactor for 60 s from the beginning of the analytical cycle. The sample was dropped 18 s after the beginning of the cycle (analytical cycle set-up: cycle lasting 70 s, oxygen injection for 60 s, sample start and stop at 18 and 20 s, respectively). The gaseous products are carried in a helium flow (80 ml/min) to a reduction reactor packed with reduced copper wire, held at 650°C. This reduction step converts nitrogen oxides into N_2 and traps oxygen. Water is captured by a perchlorate magnesium trap. The resulting gases are separated by a Porapak Q GC column (4 m, 50°C) before entering the ion source. The molecules are then ionized and accelerated with a voltage of $\sim$3040 V. A magnetic field deflects the ions and separates the molecules according to their mass-to-charge ratio (m/z) before reaching an array of Faraday cups monitoring masses 28, 29 and 30. The method length was extended to 600 s to ensure the total elution of CO peak for samples containing carbon. $\delta^{15}N$ was calculated with Isodat 2.0 software (Thermo Fisher Corporation).

Given the observations reported in the method evaluation chapter (**Chapter 6**), another method was subsequently used for the $\delta^{15}N$ measurements of samples. This method consisted in analysing the samples and standards without oxygen injection. Except for the exclusion of oxygen from the analytical cycle, all other parameters of the method remained unchanged.

6.3 Oxygen

6.3.1 Instrumentation

Oxygen stable isotope ratios were first measured by low temperature reduction (LTR) using a Carlo Erba 1108 EA (Carlo Erba) coupled with a Conflo II open split interface to a Delta Plus XP isotope ratio mass spectrometer (Finnigan MAT). Given the poor results obtained by LTR, analyses were subsequently performed by high temperature reduction (HTR) using a TC-EA coupled to a DeltaPlus XL isotope ratio mass spectrometer (Finnigan MAT) (IRMS2) [Leuenberger & Filot 2007]. A Zero Blank autosampler (Costech Analytical Technologies) was employed for oxygen analyses in order to avoid possible interferences with atmospheric O_2 and N_2. Interface parameters were He = 0.9 bar, reference gas (CO) = 0.8 bar.

6.3.2 Method

Before the measurements were started, the autosampler was evacuated for 30 minutes then refilled with helium until a pressure of 0.5 bar was reached. In HTR, the sample is dropped from the autosampler into a ceramic tube encompassing an inner glassy carbon tube held at 1420°C. This configuration of the reactor is expressly made to avoid contact between the oxygen of the sample and that of the ceramic. Carbon being reactive at such temperature, high blank values can be produced from the exchange reaction between ceramic and carbon [Kornexl *et al.* 1999, Saurer & Siegwolf 2004]. The sample is pyrolysed at high temperature, without the addition of oxygen. The analytical cycle, lasting 70 s, was as follows. The sample was dropped 10 s after the beginning of the cycle (analytical cycle set-up: cycle lasting 70 s, sample start and stop at 10 and 11 s, respectively). The gaseous products are carried in a helium flow (170 ml/min) to a water trap and a CO_2 trap. They are subsequently separated by a 2 m Porapak QS GC column held at 70°C (5 Å molecular sieve) before entering the ion source. The molecules are then ionized and accelerated with a voltage of ∼3040 V. A magnetic field deflects the ions and separates the molecules according to their mass-to-charge ratio (m/z) before reaching an array of Faraday cups monitoring masses 28, 29 and 30. The method length is 450 sec. The $\delta^{18}O$ of the resulting CO gas is calculated using the ratio of m/z 30 ($C^{18}O$) and 28 ($C^{16}O$) with Isodat 2.0 software (Thermo Fisher Corporation).

7 Normalisation to the international reference scale

Because each laboratory employs different certified or calibrated reference gas and standard materials, raw δ values need to be normalised to true δ values on the international reference scale. This "conversion" step is used to counter the effect of “scale compression” in IRMS instruments and ensures that data from different laboratories are comparable. In the absence of any limitations, normalisation requires the analysis of standards of known isotopic composition covering a wide range of δ values in order to draw the normalisation line [Sessions 2006]. Normalisation of raw data to the international reference scale can be undertaken: 1) relative to a reference gas with known isotope ratio (this procedure is however not consistent with the principle of identical treatment), 2) relative to a single reference material, 3) relative to two or more reference materials. Definitely, the normalisation strategy influences the final δ value. The contribution of Paul *et al.* offers an excellent review of the different normalisation strategies and their pitfalls [Paul *et al.* 2007]. Skrzypek *et al.* also investigated error propagation during the normalisation step, using different numbers of standards and of repetitions of these standards [Skrzypek *et al.* 2010]. The normalisation error decreases with an increasing number of standards and repetitions. To reduce significantly the error due to normalisation, they propose to use two or more standards covering the whole stable isotope scale. Although the two-point and multiple-point linear normalisation are preferred to single-point anchoring using the reference gas or a reference material, normalisation used in this research mainly involved a one-point normalisation. This is not disturbing as long as the δ value of the reference material is not significantly different from that of the specimens, as it is the case in this research.

Peak shape, amplitude, background, precision and accuracy of the δ values and elemental composition (%) were taken into consideration in the evaluation of the quality of the results. Laboratory standards were used to correct raw data to the international scale by means of a simple shift or a linear regression, whereas working standards were used to check the quality of the analyses. For δ^{15}N measurements obtained with the second method, one working standard was used for the correction of raw data and the control sample to check the quality of the analytical response. The % C, % N (in weight %) and % O were calculated on the basis of integrated peak areas of the major isotopes and determined against their theoretical % C, % N and % O.

8 Statistical analyses and treatments

8.1 Q-Q plot

Data distribution was evaluated using quantile-quantile plots, generally abbreviated Q-Q plots. This type of plot allows comparing two probability distributions on a graph. The quantiles[2] of each probability distribution are represented against each

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[2] a quantile is a value which separates the data in two sets: x % (x varying between 0 to 100) of the data from the rest (100-x %). The 25 %, 50 % and 75 % quantiles, also named quartiles, are the values used to construct boxplots.

other. In this research, the quantiles of the observed data were compared to those of a normal probability distribution. If the distribution of the observed data points is similar to the normal distribution, the points of the plot roughly lie on a straight line. Data are thus supposed to follow a normal distribution. Otherwise, deviations indicate that data are not distributed according to normal law [Millot 2011]. Q-Q plots were constructed with qqnorm function from the stats package in R.

8.2 Control charts

Control charts were used to monitor the quality of the analytical response. Upper and lower warning limits were set at μ, the mean or expected value ± 2 standard deviation (SD) and upper and lower action limits at $\mu \pm 3$ SD [Taverniers *et al.* 2004]. Data lying out of the action limits of the control charts were considered as outliers. Data showing the occurrence of an analytical problem (peak shape, background, retention time, etc.) were not considered either. Means and standard deviations were calculated for each variable after exclusion of outliers.

8.3 Comparison of values

The significance of the difference between two values was tested using ANOVA for parametric distributions and Kruskal Wallis test for non parametric distributions [Miller & Miller 2005].

8.4 Expanded uncertainty calculation

The expanded uncertainty U was calculated according to literature [Joint Committee for Guides in Metrology 2008, Joint Research Centre 2010] and using type A evaluation, that is estimated from repeated measured values. The calculations did not encompass all the measurements. They were made on a set of measurements randomly selected covering at least 3 to 4 days. Estimated for each instrument, the expanded uncertainty (coverage factor k = 2) associated with the measurement of each of the variable evaluated in the following chapter showed to be similar on both apparatus. Tables in **Chapter 6** only displays the largest expanded uncertainty estimated on the two instruments. Bias was also checked for the δ values of standards.

8.5 Descriptive statistics

Strip plots and boxplots were used to illustrate descriptive statistical analysis of data. Graphics were made using R software.

Strip plot Especially useful when the number of measurements per sample is small, strip plots, also known as dot plots, consist of univariate scatter plots, that represent the relationship between a categorical variable and a numerical variable [Sarkar 2008]. Strip plots were created using the lattice package in R.

Boxplot A well-known visualisation method for comparing the distribution of several series of measurements is the boxplot or box-and-whisker plot representation. The distribution of data in boxplots is characterised by 5 numbers: the median, the smallest and largest observations and the lower and upper quartiles. The first or lower quartile (Q_1) covers the lowest 25 % of data, the third or upper quartile (Q_3) covers the highest 25 % of data. The diagram shows the interquartile range (Q_3 - Q_1) delimited by the rectangle and the median, represented by a line crossing the box. The end of the whiskers indicate the lowest and highest values. Any observations smaller than Q_1, or larger than Q_3, by at least 1.5 time the interquartile range is considered as an outlier and represented by a separate dot on the plot [Miller & Miller 2005]. The boxplots were created using the graphics package of R.

8.6 Data preprocessing

Three different preprocesses were applied to the data set in order to scale data. The data set on which data preprocessing was undertaken was composed of raw elemental content (% C, % N, % O) and δ value (δ^{13}C, δ^{15}N, δ^{18}O) preliminarily normalised to the international scale. The three following transformations were tested [Brereton 2009]:

1. range scaling. The variable was scaled to a range between 0 and 1, with 0 and 1 corresponding to the minimum and maximum values respectively of the variable in the data set. The normalised value x_{norm} was calculated following the equation:
$$x_{\mathrm{norm}} = \frac{x_{\mathrm{ij}} - min_{\mathrm{j}}(x_{\mathrm{ij}})}{max_{\mathrm{j}}(x_{\mathrm{ij}}) - min_{\mathrm{j}}(x_{\mathrm{ij}})}$$
where $\mathrm{min_j(x_{ij}}$ is the minimum of variable j over all samples and $\mathrm{max_j(x_{ij}}$ the corresponding maximum. The advantage of this transformation is that it can deal with variables with a negative value, such as δ^{13}C value. In contrast, the addition of new data with values that are smaller or larger than the minimum and maximum of the data set require a complete range scaling;

2. standardisation or autoscaling. The value is mean centred and divided by the standard deviation, folllowing the equation:
$$x_{\mathrm{norm}} = \frac{x_{\mathrm{ij}} - \bar{x}_{\mathrm{j}}}{sd_{\mathrm{j}}}$$

where $\mathrm{sd_j}$ is the standard deviation of variable j. This transformation ensures that variables covering different ranges have a similar weight in the statistical analyses. Nevertheless, when the variable conveys little information, this data preprocessing is not recommended, as it can reduce the discrimination of groups;

3. scaling rows to constant total. The variable is divided by the sum of all variables of the sample:

$$x_{\mathrm{norm}} = \frac{x_{\mathrm{ij}}}{\sum_{j=1}^{J} x_{\mathrm{ij}}}$$

Negative values were converted to their absolute value for this preprocessing. One of the major drawback of this transformation is the influence of variables with large values.

8.7 Multivariate statistical methods

Data were evaluated using multivariate statistical methods to highlight the relevant information useful for interpretation. These methods involve exploratory approaches indicating the possible differentiation that can be observed in the data set and predictive approaches allowing the assignment of a sample in pre-determined classes. These are known as supervised and unsupervised (or exploratory) methods respectively [Booksh 2000, Lavine 2000, Miller & Miller 2005].

Exploratory or unsupervised methods analyse data without preliminary knowledge on classes to be expected. They highlight the structure of the data set and indicate possible groupings of samples. The exploratory methods applied to the data sets were the principal component analysis (PCA) and hierarchical clustering analysis (HCA) using the Euclidean distance. Distance measurement using the Pearson correlation coefficient was also assessed.

Supervised methods are based on a process in which the classes are already determined. The aim is to use the pre-classed objects to develop a classification model which enables a correct classification of an unknown object. There are numerous supervised classification methods. In this research, it was chosen to compare a linear model, linear discriminant analyses (LDA) with random forests (RF) and support vector machines (SVMs), two non-linear models.

Except for Pearson correlation coefficient which was calculated on Excel, R software was employed for the application of these statistical treatments.

8.7.1 Exploratory data analysis

Principal component analysis Principal component analysis (PCA) is a mathematical method, whose aim is to reduce the complexity of a data set while keeping most of its information. The variables are transformed into new variables, namely the principal components. Each principal component (PC) corresponds to a linear combination of the original variables. One of the properties of principal components is that they are non correlated, thus maximising the variance in the original data set. The first principal component (PC1) is the most informative dimension, PC2 is the second most informative and so forth for the following PCs. In most cases, the first two or three PCs are sufficient to account for most of the variations contained in the data set. The new coordinates of the samples projected on the PCs are called scores. The plot of the scores of the samples on the first PCs is often used for preliminary data visualisation. PCA is also useful to operate a selection between highly correlated variables or to reduce the dimensionality of the data set for further statistical treatments [Lavine 2000, Miller & Miller 2005, Brereton 2009]. The PCA function from the FactoMine package in R was used for this data analysis.

Hierarchical clustering analysis As its name suggests, hierarchical clustering analysis (HCA) is part of cluster analysis, whose aim is to discover groups within data. Cluster analysis is based on distance measurement between the points (or samples) of the data set, so that similar samples will lie close to each other and dissimilar ones will be separated by a larger distance. HCA using the Euclidean distance is one of the most commonly used clustering method for continuous variables. A similarity matrix is first created, by calculating the distance between all pairs of samples. A similarity value is then attributed to each distance between samples. The two samples with the highest similarity value are then combined and form a new point. After deleting the old data points from the matrix, the similarity matrix is re-computed to take into account the similarity between the new point and the rest of the data set, and so forth until all points have been linked. The clusters are displayed in the form of dendrograms. There are different methods to calculate the distance that separates a point from a cluster of points. HCA applied in this research uses the complete linkage method, which involves measuring the distance to the farthest point in the cluster [Lavine 2000, Miller & Miller 2005]. HCA was performed using the hclust function in R.

Distance measurement The degree of similarity between samples coming from the same source and from different sources was also evaluated with the measurement of Pearson correlation. The Pearson correlation coefficient P_{xy} varies between -1 and 1 and is defined as [Millot 2011]:

$$P_{\mathrm{xy}} = \frac{\frac{\sum_{i=1}^{n}(x_{\mathrm{i}}-m_{\mathrm{x}})(y_{\mathrm{i}}-m_{\mathrm{y}})}{n-1}}{sd_{\mathrm{x}}sd_{\mathrm{y}}}$$

where $\mathrm{x_i}$ is the value of observation i for variable x, $\mathrm{y_i}$ the value of observation i for variable y, $\mathrm{m_x}$ the mean of variable x, $\mathrm{m_y}$ the mean of variable y, n the number of values, $\mathrm{sd_x}$ the standard deviation of x, $\mathrm{sd_y}$, the standard deviation of y.

A macro developed on Excel and used at IPS to calculate the correlation matrix using Pearson coefficient was employed. The plots display the Pearson correlation coefficient multiplied by 100 in order to ease the reading.

8.7.2 Supervised classification methods

Linear discriminant analysis Linear discriminant analysis (LDA) creates several discriminant functions, which delimit linear boundaries separating the different classes. The functions, which are a linear combination of the original variables, are found by maximising the difference between classes meaning that samples in the same class will have a similar value of the discriminant functions and samples from different class will have very different values. The value of the function for a given sample corresponds to its projection on the discriminant function. The class prediction of a new observation is based on the distance between the sample and the class centroid [Miller & Miller 2005, Brereton 2009]. LDA was applied using the MASS package in R.

Random Forest Random forests (RF) is an ensemble of trees method, which predicts the class of samples based on the combined outputs of the trees in the forest. It is based on the selection of a random subset of descriptors (or variables) at each node and bagging on samples (bootstrap aggregating)[3] which yields the final classification result according to the most popular vote (majority voting) [Breiman 2001, Liaw & Wiener 2002, Svetnik *et al.* 2003]. In the training phase, the random forest model, defined by a number of trees n_{tree}, draws numerous bootstrap samples (training sets) from the original data set and, for each bootstrap sample, grows a tree to its maximum size. The trees are not pruned. During growing, unlike in standard trees where the best split is chosen among all variables, the tree is split at each node using the best split among a random subset of variables, defined by the m_{try} parameter. This variable selection process is reported to improve the model performance and avoid overfitting [Qi 2012]. In the training step, some samples will be left out of the bootstrap sample, and therefore of the tree construction. These observations constitute the Out-of-Bag (OOB) samples and are then used as test sets, during the training process, to evaluate the ensemble

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[3] bootstrap samples, i.e. randomly sample *with* replacement, are used to create several classifiers. The results of each tree are then combined to produce the final class prediction.

prediction performance, through the estimate of the OOB error rate. RF also measures the variable importance, which produces a ranking of the most and least important variables [Breiman 2001, Qi 2012].

The randomForest function from the randomForest package in R was used. The function is mainly characterised by 3 parameters [Svetnik *et al.* 2003]: n_{tree}, the number of trees in the forest, m_{try}, a selected number of descriptors (or variables) chosen at each node (it can vary from 1 to the maximum number of variables), the minimum node size, which corresponds to the minimum node size below which no split will occur. For classification, the default value is 1 in order to ensure the full development of the tree. The other two are the parameters to tune in order to optimise the model performance.

Support Vector Machines Support Vector Machines (SVMs) classifier is a supervised learning method used for two-class or multi-class classification. SVMs can handle linearly and non-linearly separable classes by creating complex curved boundaries. In contrast to other classification methods, which use all samples of the data set to define the boundaries of the classes, SVMs boundaries definition relies only on samples lying on the margins of the classes. These samples are called support vectors. The decision boundary between two classes, represented by a hyperplane, is set at equal distance from the extreme samples of the classes and with maximum margins of separation. For non-linearly separable classes, a simple hyperplan is not sufficient and SVMs use a trick to handle such situations. The kernel trick consists in mapping the data into a higher dimensional space, the feature space, by applying a kernel function. In this feature space, the classes are more easily separated by a linear boundary in the form of a hyperplane. The linear boundary, constructed in the feature space is projected back into the original variable space. This projection takes the form of a complex non-linear boundary in the input space, which optimally separates the classes [Brereton & Lloyd 2010, Brereton 2009]. In this research, the most common kernel function, the radial basis function (RBF), was used. γ, the RBF width, is one of the parameter to optimise, which may influence the results. SVMs are either hard-margin or soft margin based. Whereas misclassification is never admitted with hard-margin model resulting in perfectly separated classes, soft-margin SVMs allows classification errors and thus accept less complex model. In this research, soft-margin SVMs were applied. Directly related to these notions of hard and soft margins, C is the penalty error which controls the complexity of SVMs model. Large values of C renders the model more complex in order to minimise misclassification, while low values of C make the model more tolerant to classification errors. One of the major drawback of using high C values to favour a perfect separation of the classes is overfitting. This means that the model constructs perfect boundaries for the training set, but when evaluated on the test set, it yields very high classification errors.

For the application of this classifier to the data set, the svm function from the libsvm and e1701 packages was used in R. This function uses the one-against-one voting scheme, meaning that the unknown sample is confronted to pairwise classes and is assigned to the class in which it is most frequently classified [Karatzoglou *et al.* 2006].

8.7.3 Models optimisation

Evaluation of the performance of the classifiers can be misleading if the model is not optimised. Optimisation of LDA was undertaken by removing highly correlated variables or the less discriminant variables and see how it modified the performance of the model. The set of variables yielding the best performance of the model was chosen to create the final classification model.

For RF, different values of the two tuneable parameters were tested. The number of trees in the forest, n_{tree}, was set at 500, 1000 and 2000. The quality of the classification model was assessed through the Out-of-Bag error rate. The selected number of variables chosen at each node, m_{try}, was tested over a range oscillating between 1 to 6 for black powders and 1 to 4 for ammonium nitrate fertilisers. To evaluate this parameter, the best performance of the model determined the settings of the final model used for discriminating samples.

For SVMs model optimisation, the tune.svm function allows performing a grid search over specific ranges of γ, the RBF width, and C, the penalty parameter [Karatzoglou *et al.* 2006]. For both black powders and ammonium nitrate fertilisers, C was tested between 0.1 and 10000 and γ between 0.0001 and 100, for each training set, using a 10-fold cross-validation. The best classification performance were used for model validation and discrimination of the samples.

8.7.4 Models validation

From the overall data set, 2/3 of the observations (training set) were randomly chosen to build the classification model with the optimised parameters, while the 1/3 left was used as the test set (or validation set) to evaluate the performance of the model. As the number of observations was not evenly distributed between the classes, the training and test steps were repeated 100 times with samples randomly chosen for every iteration. This iterative process provides a more stable and robust estimate of the model performance [Brereton 2009].

In addition, 5 blind black powder and 4 blind ammonium nitrate samples, selected by an external operator, were each analysed in triplicate (N = 15 and N = 12, respectively). These blind BP and AN measurements were integrated to the model evaluation as new samples and classified on each iteration. Mean and median were calculated from the 100 classification results.

8.7.5 Performance assessment

One of the advantages of the sampling of this research is the information on the production of the sample (manufacturer, type and batch of AN and BP). The known "source" or class of the samples could therefore be used to evaluate the ability of the statistical method to correctly classify samples.

Performance assessment of distance measurement For two-class classification, typically for the determination of linked or unlinked samples based on distance or similarity measurement, the performance was assessed through the sensitivity and specificity of the model which in turn were calculated on the basis of the percentage of true positives (% TP) (linked samples correctly predicted), the percentage of false positives (% FP) (unlinked samples predicted as linked), the percentage of false negatives (% FN) (linked samples predicted as unlinked), the percentage of true negatives (% TN) (unlinked samples correctly predicted) (see **Table 5.8**) [Brereton 2009].

Predicted class True class	Linked	Unlinked
Linked	True positive (a)	False negative (b)
Unlinked	False positive (c)	True negative (d)

Table 5.8. Results of a two-class classification method.

Sensitivity is defined as $a/(a+b)$ and can be calculated from the % FN (= 1- % FN). Specificity is calculated from $d/(c+d)$ and can be derived from the % FP (= 1-% FP).

Well known in medical decision making, Receiver Operating Characteristics (ROC) curve is a visualisation tool, which allows monitoring the sensitivity and specificity of a two-class test and provides a response on its performance to predict the actual class of a sample. A ROC curve is a graph which plots the true positive rate (sensitivity) as a function of the false positive rate (1-specificity). The ROC space in which the plot is designed has the following particularities: the plot is divided by an upward diagonal (x = y) going from the lower left point (0,0) to the higher right point (1,1). The coordinates (0,1) reflect a perfect model with 100 % sensitivity and 0 % false positive (100 % specificity). The more northwest the classifying model is on the graph, the better is its performance. Classifiers which are close to or on the diagonal indicate that they perform as good as random guessing (one chance out of two). Classifiers which are below the diagonal (x = y) indicate that predicting the samples class using random guesses (50:50) produces better performance than the model. The interest of ROC curves is that they allow seeing the sensitivity and false positive rate at one glance, without having to choose a definite threshold and showing directly the whole performance of the model. In addition, they are insensitive to non-normal distribution of data, which makes them exploitable for any data set [Fawcett 2006, Hopley & van Schalkwyk].

Performance assessment of the classification model For multiclass classifications, the overall performance of the classifier is reported as the correct rate (% CR), i.e. the percentage of samples correctly classified, in contrast to the error rate (% ER = 1-CR) representing the percentage of samples wrongly classified [Brereton 2009, Liu *et al.* 2013]. These rates are calculated as follows:

$$\%\ \mathrm{CR} = \frac{\text{number of correctly classified samples}}{\text{total number of classified samples}} * 100\%$$

$$\%\ \mathrm{ER} = \frac{\text{number of wrongly classified samples}}{\text{total number of classified samples}} * 100\%$$

CHAPTER 6

Method evaluation

Contents

The isotope analysis of an element allows determining the δ value as well as the percentage of element (in weight %) contained in the substance. Each of these two variables were evaluated for carbon, nitrogen and oxygen. Quantile-quantile plots (Q-Q plots) and control charts presented in this chapter encompass all measurements (N), including outliers. Means and standard deviations, as well as expanded uncertainty were calculated after exclusion of outliers[1] (n).

1 Carbon analysis

Sample preparation Ground and unground samples of black powder were analysed in order to observe whether grinding the samples influenced significantly the repeatability of the results. **Table 6.1** summarises the results of this preliminary test and shows that ground black powder shows a slightly better repeatability of % C than unground black powder. As no significant difference was noted between

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[1] Data lying out of the action limits of the control charts were considered as outliers.

the % C and δ^{13}C values of ground and unground black powder, and for safety reasons, black powder samples were analysed without being ground.

	% C ± SD (%)	**δ^{13}C±SD (‰ vs VPDB)**
Unground black powder	9.2 ± 0.2	-27.2 ± 0.1
Ground black powder	9.2 ± 0.1	-27.2 ± 0.1

Table 6.1. Average and standard deviation (SD) of % C and δ^{13}C value of ground and unground black powder sample A1 obtained from 3 measurements.

Data distribution **Figures 6.1** and **6.2** illustrate the Q-Q plots of δ^{13}C and % C measurements obtained on a period of 20 months. The δ^{13}C and % C of the laboratory standards, working standards and the control samples appear to follow a normal distribution. The δ^{13}C distributions are mostly slightly left skewed, while the % C distributions are right skewed for the MIX and the control sample A1. Contrarily to steep tails, these flat deviations from normality can however be considered as benign [Reis & Judd 2000].

1.1 δ^{13}C determination

1.1.1 Laboratory standards

All δ^{13}C values of laboratory standards measured over 20 months on IRMS1 and IRMS2 instruments are plotted in the control charts displayed in **Figures 6.3** and **6.4**. Measurements show a good intermediate precision as demonstrated by the rare points lying out of the control limits (blue lines). The standard deviation of the δ^{13}C value measured on both instruments, as well as its expanded uncertainty U are smaller than 0.1 ‰ for both laboratory standards (see **Table 6.2**).

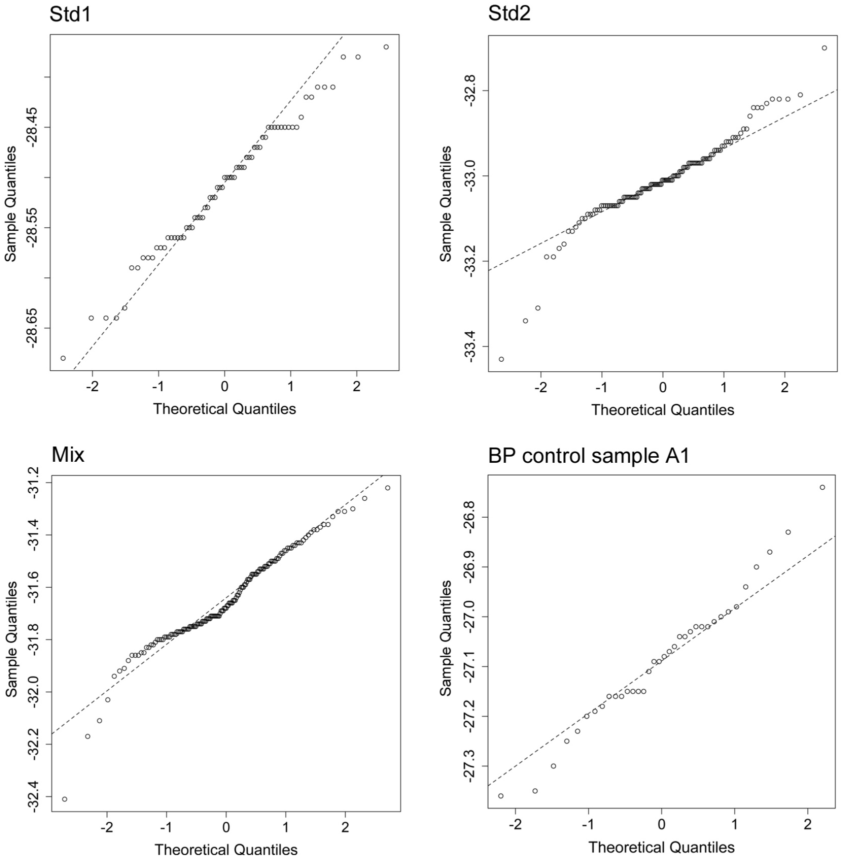


Figure 6.1. Q-Q plot of the δ^{13}C values for the different substances (Std1: N = 69, Std2: N = 124, Mix: N = 149, A1: N = 36).

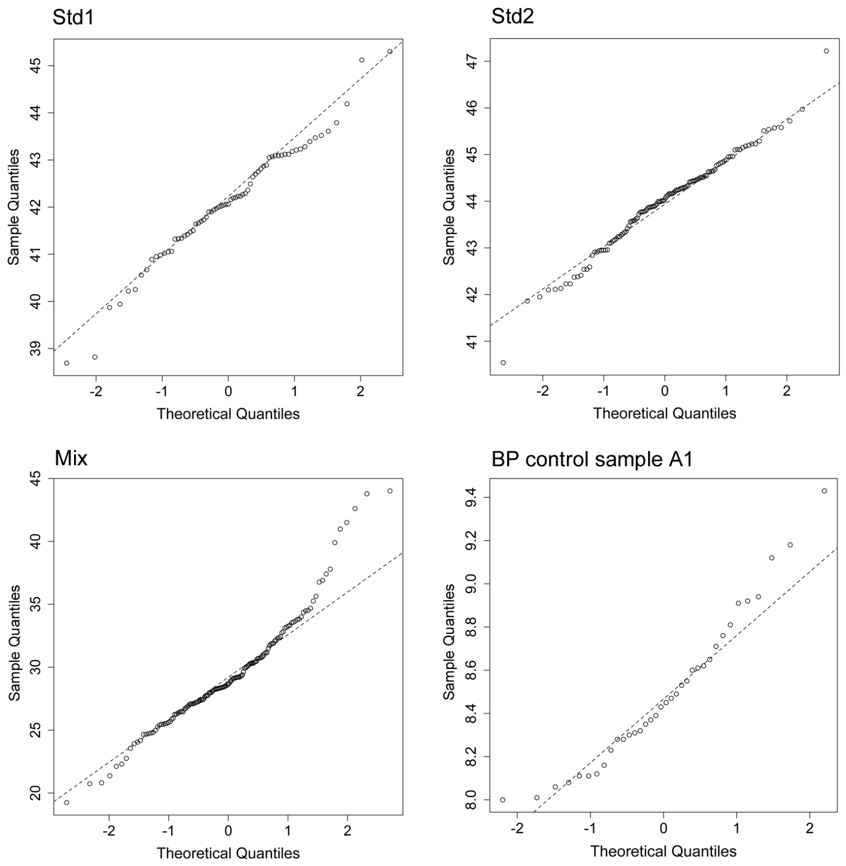


Figure 6.2. Q-Q plot of the % C for the different substances (Std1: $N = 69$, Std2: $N = 124$, Mix: $N = 149$, A1: $N = 36$).

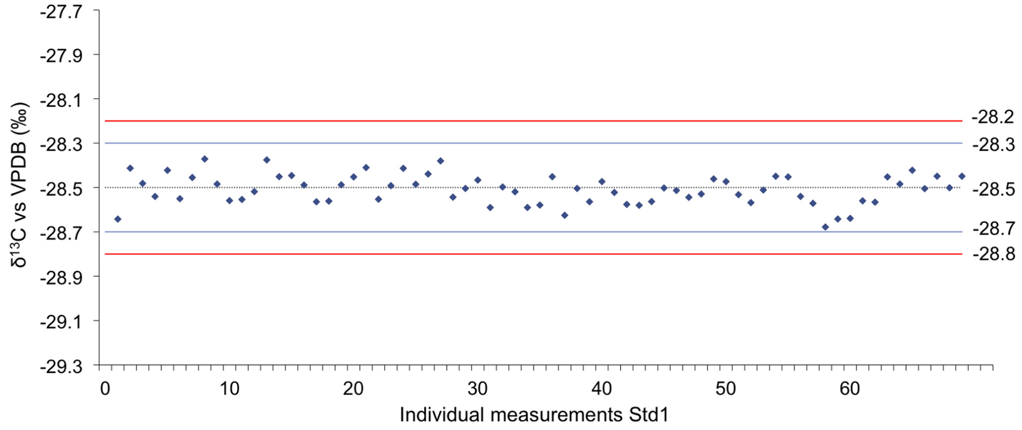


Figure 6.3. Control chart of the δ^{13}C value of Std1. All measurements are plotted ($N = 69$).

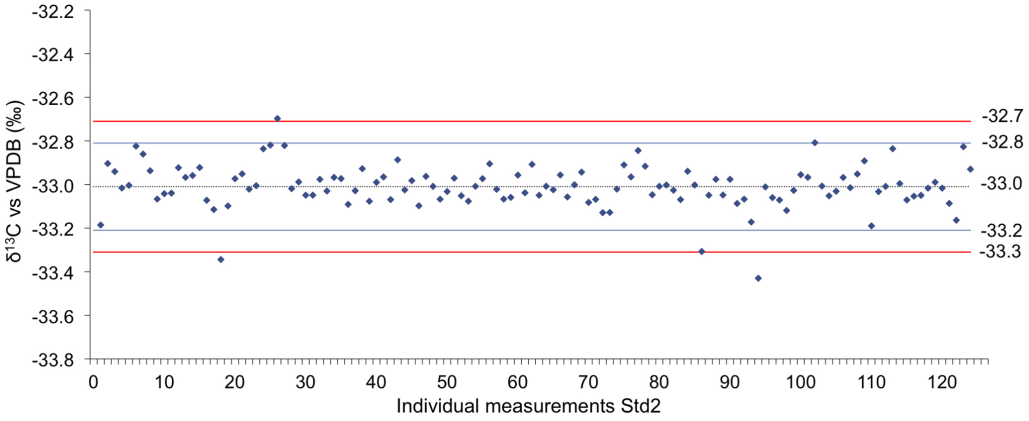


Figure 6.4. Control chart of the δ^{13}C value of Std2. All measurements are plotted (N = 124).

1.1.2 Working standard and control sample

Figure 6.5, illustrating the δ^{13}C value of the working standard MIX shows two groups of points: one with a mean value of -31.5 ‰ and another centred around -31.7 ‰. This slight but distinct shift of the δ^{13}C value of the MIX is significantly different ($p < 0.05$). However, it does not coincide with the change of instruments. Indeed, one sequence of measurements performed on IRMS2 has an average value similar to that obtained on IRMS1. Any maintenance would also have shifted the δ^{13}C value of the laboratory standards and BP control sample A1 (see **Figure 6.6**). Yet, none of them exhibited a similar shift.

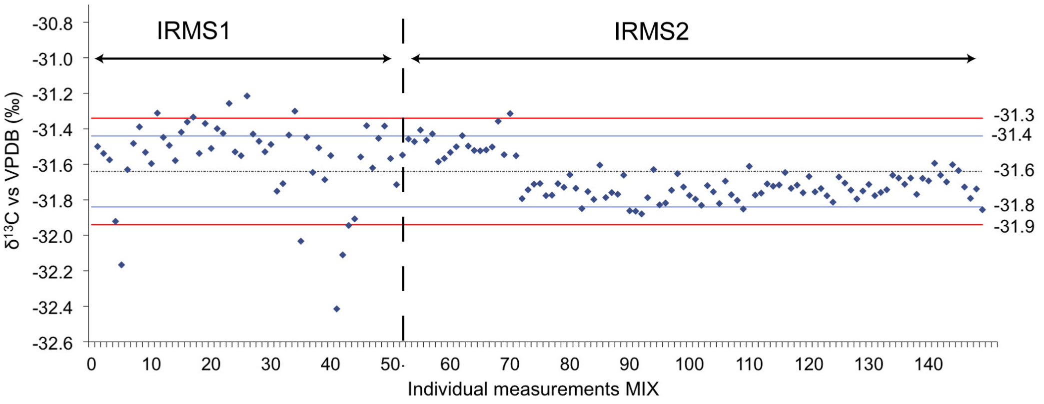


Figure 6.5. Control chart of the δ^{13}C value of the MIX. All measurements are plotted (N =149).

A possible explanation is the evolution of the δ^{13}C value of the MIX. However, in terms of time scale, 2 days separate the 2 groups of values. Despite different matrices, results presented in **Chapter 7** show that the δ^{13}C value of black powder does not vary significantly according to environmental conditions. The exact cause of this shift remains unknown. Nevertheless, as the control charts of the laboratory standards and control sample did not reflect any anomaly, no further reserve was considered for δ^{13}C measurements.

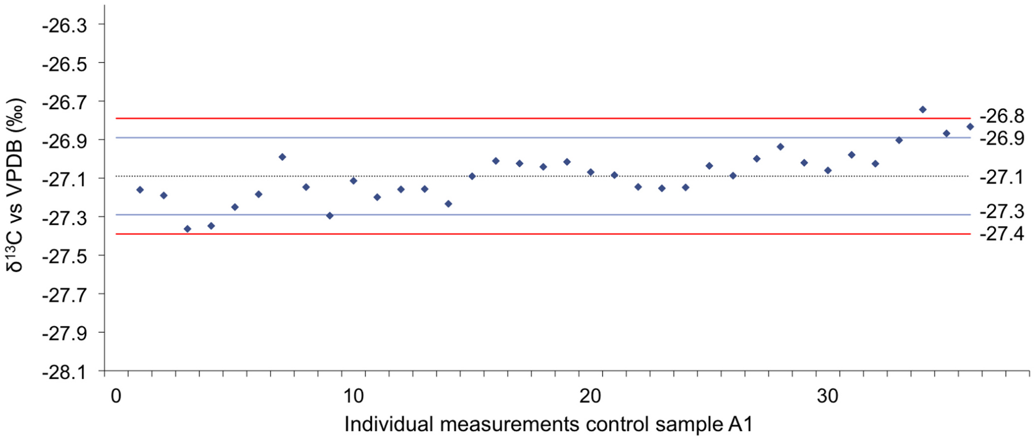


Figure 6.6. Control chart of the δ^{13}C value of the black powder control sample A1. All measurements are plotted ($N = 36$).

Table 6.2 summarises the δ^{13}C values obtained on both instruments for the laboratory and working standards, as well as the control sample, after exclusion of outlying values. Contrarily to the MIX, there is no significant difference between the δ^{13}C means of laboratory standards obtained on both instruments.

Substances	$\delta^{13}C_{\mathbf{IRMS1}}$ **(n)**	$\delta^{13}C_{\mathbf{IRMS2}}$ **(n)**	**known δ^{13}C value [‰]**	***U* [‰] (k=2)**
Std1	-28.5 ± 0.1 (12)	-28.5 ± 0.1 (57)	-28.5	0.1
Std2	-33.0 ± 0.1 (24)	-33.0 ± 0.1 (97)	-33.0	0.1
MIX	-31.5 ± 0.1 (41)	-31.7 ± 0.1 (96)	NA	0.2
A1 (BP)	-27.1 ± 0.1 (9)	-27.0 ± 0.1 (26)	NA	0.1

Table 6.2. Average δ^{13}C value, its standard deviation and the estimated expanded uncertainty *U* in ‰ vs VPDB, obtained on IRMS1 and IRMS2, after exclusion of outlying values.

1.2 Carbon content determination (% C)

1.2.1 Laboratory standards

Most % C measurements of laboratory standards measured over 20 months were within the control limits of the control chart (**Figures 6.7** and **6.8**).

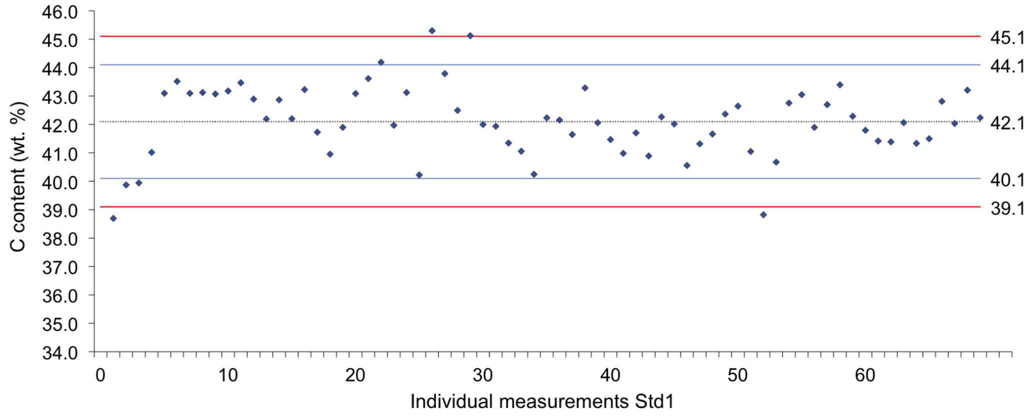


Figure 6.7. Control chart of the % C of Std1. All measurements are plotted ($N = 69$).

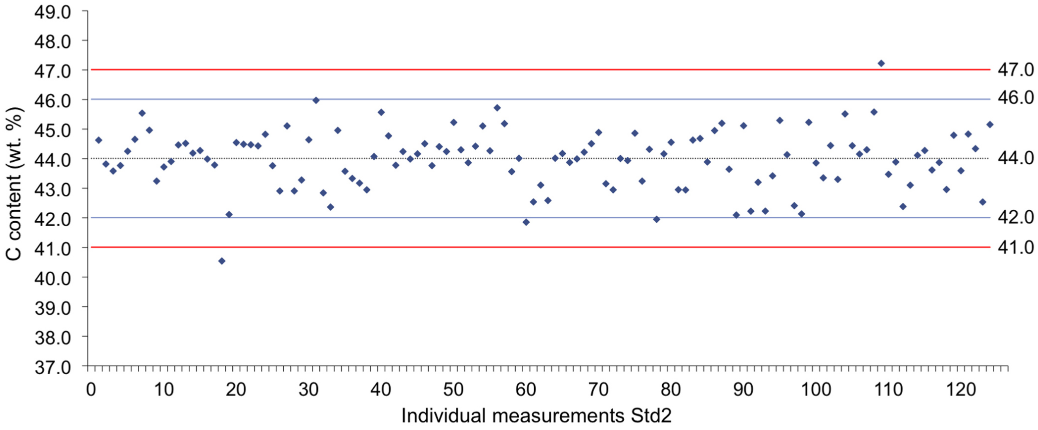


Figure 6.8. Control chart of the % C of Std2. All measurements are plotted ($N = 124$).

1.2.2 Working standard and control sample

The % C determination of the MIX presented more variability than the laboratory standards, as shown on **Figure 6.9**. In contrast, the % C of the control sample A1 was precise and showed very little variations (see **Figure 6.10**). This difference in variability is more related to the homogeneity of the substances than to an analytical factor. The compounds (C and KNO_3) of the homemade mixture MIX were carefully ground and mixed together. Nevertheless, the binary character of the mixture was noticed during weighing. **Table 6.3** summarises the % C of the standards and control sample.

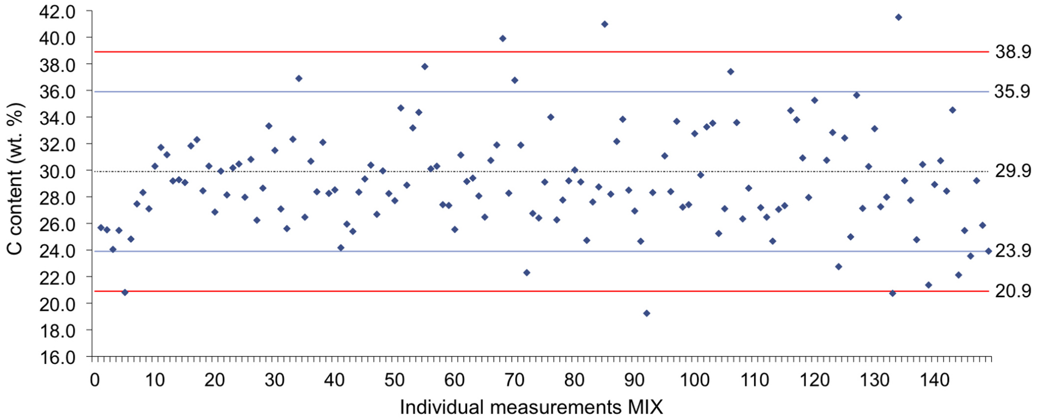


Figure 6.9. Control chart of the % C of the MIX. All measurements are plotted ($N = 149$).

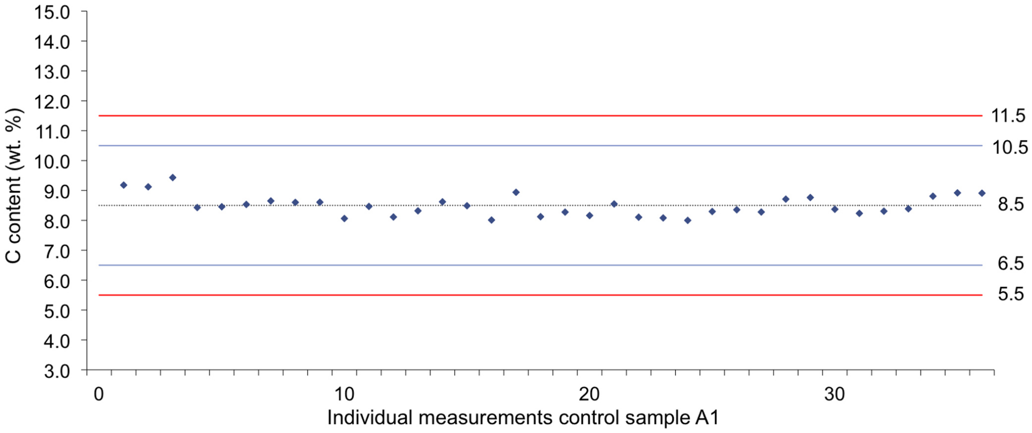


Figure 6.10. Control chart of the % C of the black powder control sample A1. All measurements are plotted ($N = 36$).

Substances	$\%C_{\mathbf{IRMS1}}$ **(n)**	$\%C_{\mathbf{IRMS2}}$ **(n)**	**theoretical % C**	$\boldsymbol{U}$ **[%] (k=2)**
Std1	42.4 ± 1.4 (11)	42.1 ± 0.9 (54)	42.1	1.1
Std2	44.2 ± 0.7 (24)	43.9 ± 1.0 (97)	44.0	1.0
MIX	29.0 ± 2.4 (45)	29.1 ± 3.7 (91)	25.0	3.2
A1 (BP)	8.8 ± 0.4 (9)	8.4 ± 0.3 (26)	NA	0.3

Table 6.3. Average % C, its standard deviation and the estimated expanded uncertainty *U* expressed in wt.% determined on IRMS1 and IRMS2 after exclusion of outlying values.

1.3 Discussion

Overall, very few outliers were observed for the determination of % C and δ^{13}C values. Although the repeatability of the carbon isotope ratio was excellent for all substances, an unexplained shift was noted for the working standard MIX. The magnitude of the shift (about 0.2 ‰) does not hamper interpretation of carbon isotope data, however it questions the origin of such difference. The origin of this shift is even more unexpected as none of the laboratory standards and control sample exhibited a similar behaviour. Concerning the % C determination, the uncertainty associated to the measurement seems to be related to the matrix of the analysed substance. Both vegetal powdery standards (Std1 and Std2) exhibited a similar expanded uncertainty (about 1.0 %). The MIX, which is a homemade mixture of powdery carbon and crystalline KNO_3, showed a larger range of expanded uncertainty, which likely reflects the binary texture of the two components. By contrast, the BP control sample A1 displayed very precise % C, suggesting the measurement of this feature could be used for differentiation of samples depending on the measured intravariability and intervariability of this variable. These results show the excellent repeatability of the δ^{13}C values and highlight the influence of the nature of the substances themselves on the % C determination.

2 Nitrogen analysis

2.1 First method evaluation

Sample preparation The results of the preliminary test investigating the influence of grinding black powder on the repeatability of the results are presented in **Table 6.4**. The repeatability between ground and unground black powders is similar. The mean δ^{15}N values are however significantly different ($p < 0.05$). This difference, which cannot be explained by the contamination of the wooden pestle as it contains very little nitrogen (less than 2 %), likely reflects the isotopic variability of the sample. For safety reasons, the nitrogen analyses of black powder were undertaken without grinding the samples.

	% N ± SD (%)	**δ^{15}N ± SD (‰ vs Air)**
Unground black powder	10.7 ± 0.1	0.9 ± 0.1
Ground black powder	10.8 ± 0.1	0.7 ± 0.1

Table 6.4. Average and standard deviation (SD) of % N and δ^{15}N value of ground and unground black powder sample A1 obtained from 3 measurements.

Data distribution As shown on the Q-Q plots illustrated in **Figures 6.11** and **6.12**, the distributions of the δ^{15}N values and % N obtained over 2 years are asymmetric for some substances. For both δ^{15}N values and % N, the distributions of the measurements of Std1 and Std2 display two slopes: a first flat slope below the median and a second very steep slope indicating a thick tail. Furthermore, except for the % N of the MIX for which the phenomenon is less visible, the MIX and the AN std also exhibit a two-slope distribution, with however a less thicker left tail. The long tails indicate numerous outliers, either on the right tail for the laboratory standards or the left tail for the nitrate based working standards. A departure from normality on the Q-Q plots of control samples A1 and L5 is less easy to diagnose, due to the lesser number of measurements.

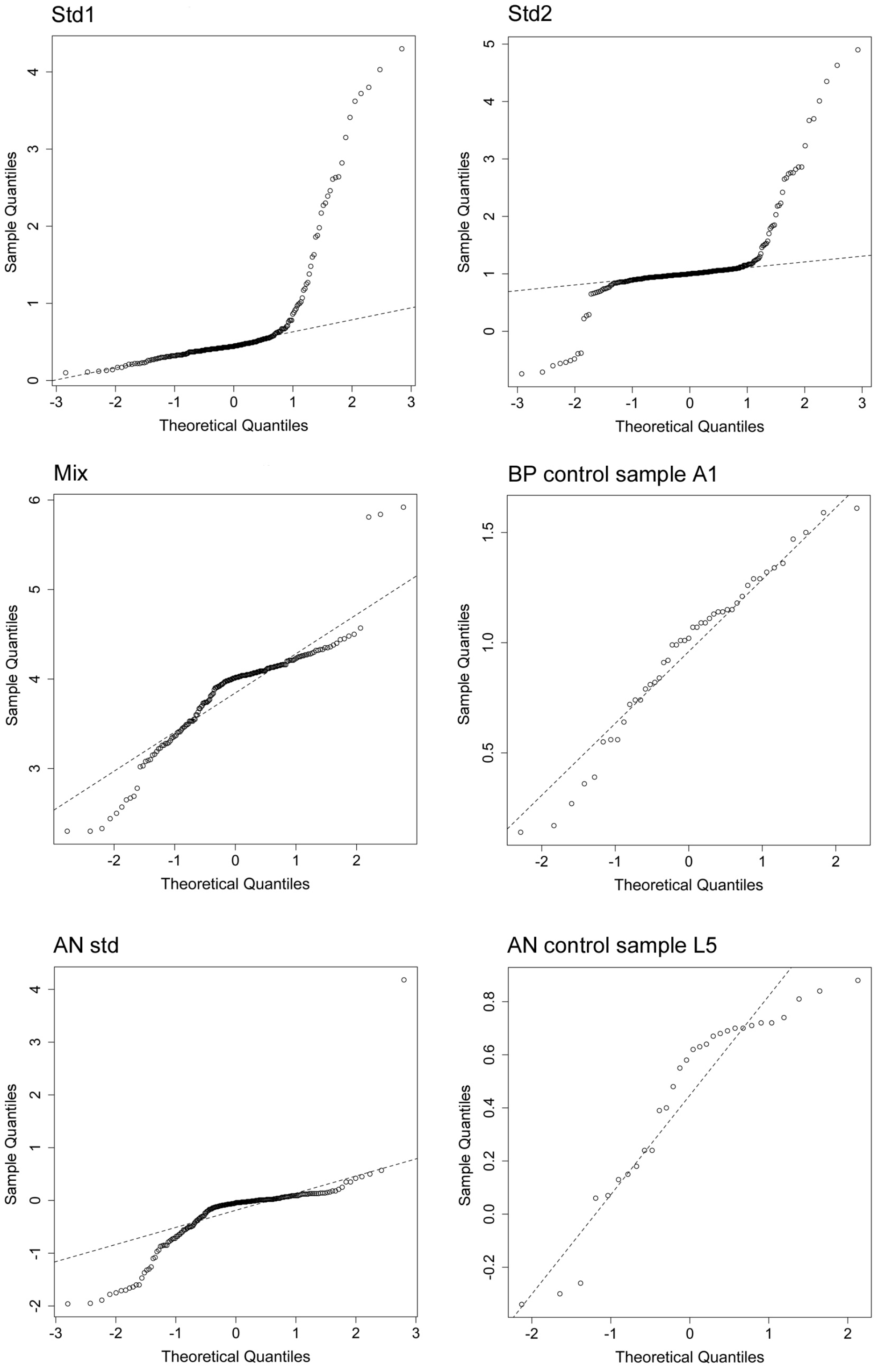


Figure 6.11. Q-Q plot of the δ^{15}N values for the different substances (Std1: $N = 223$, Std2: $N = 291$, Mix: $N = 180$, AN std: $N = 196$, A1: $N = 45$, L5: $N = 30$).

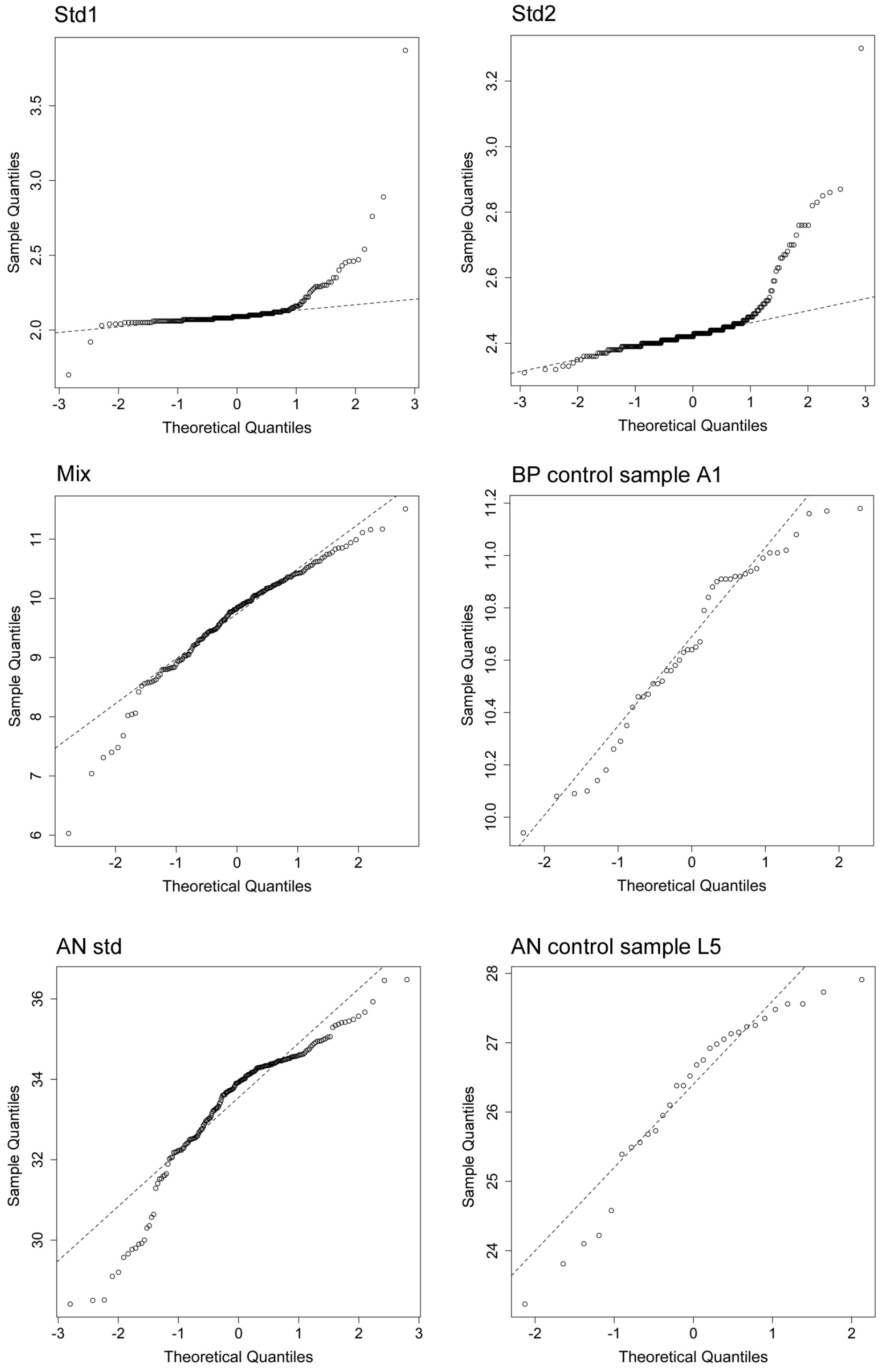


Figure 6.12. Q-Q plot of the % N for the different substances (Std1: $N = 223$, Std2: $N = 291$, Mix: $N = 180$, AN std: $N = 196$, A1: $N = 45$, L5: $N = 30$).

2.1.1 Unexpected behaviour of standards

The δ^{15}N value and % N of samples and standards obtained with the first nitrogen method was punctuated with numerous outliers and assorted with an unexpected behaviour of the standards.

Different effects on the δ^{15}N value were observed. **Figures 6.13**, **6.14** and **6.15** illustrate curious effects noticed along analytical sequences, such as variations of the δ^{15}N value of the standards between the beginning and the end of the sequence or sudden shifts of the δ^{15}N value in the middle of a sequence. This obviously impacts on the correction of raw data: depending on the set of standards chosen for the normalisation of raw data to the international scale, the selection clearly influences the δ^{15}N value of the samples. Various factors (maintenance, reagents quality, electronic instability) were considered. Notwithstanding the cause of these drifts and shifts of the δ^{15}N value remain unknown.

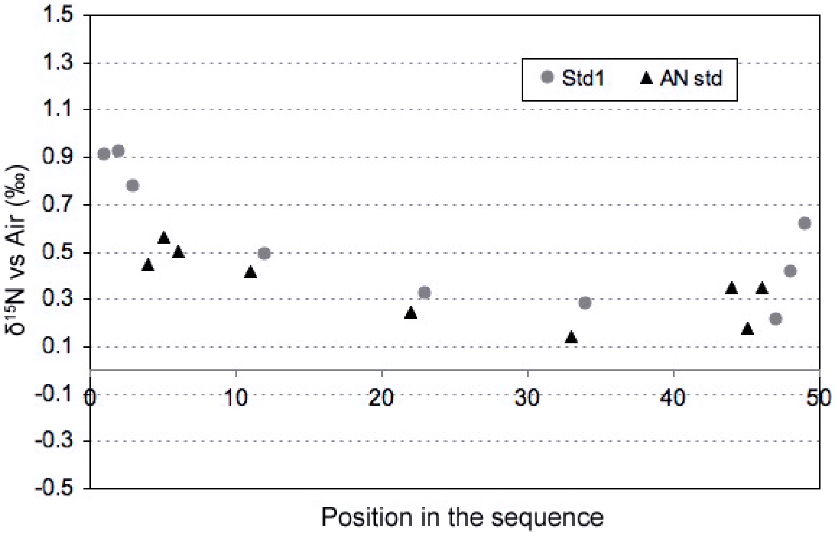


Figure 6.13. Results of δ^{15}N measurements for Std1 and AN std in a sequence of analyses. The δ^{15}N value and the isotopic difference between Std1 and AN std vary between the beginning and the end of the sequence. A difference of about 0.4 ‰ can be noticed at the beginning of the sequence, while it disappears further in the sequence, and follows, in addition, a downward trend.

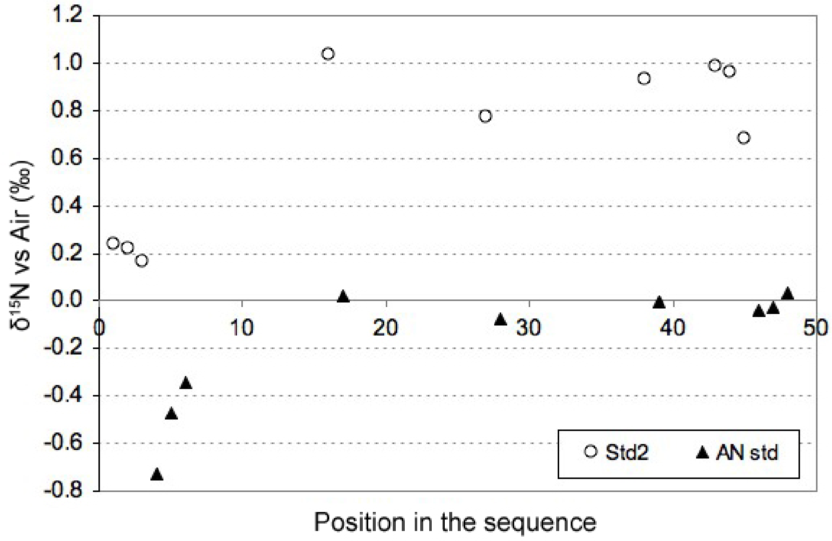


Figure 6.14. Results of δ^{15}N measurements for Std2 and AN std in a sequence of analyses. The isotopic difference is not always constant along the sequence. In addition, there is an unexpected shift of the δ^{15}N value during the sequence.

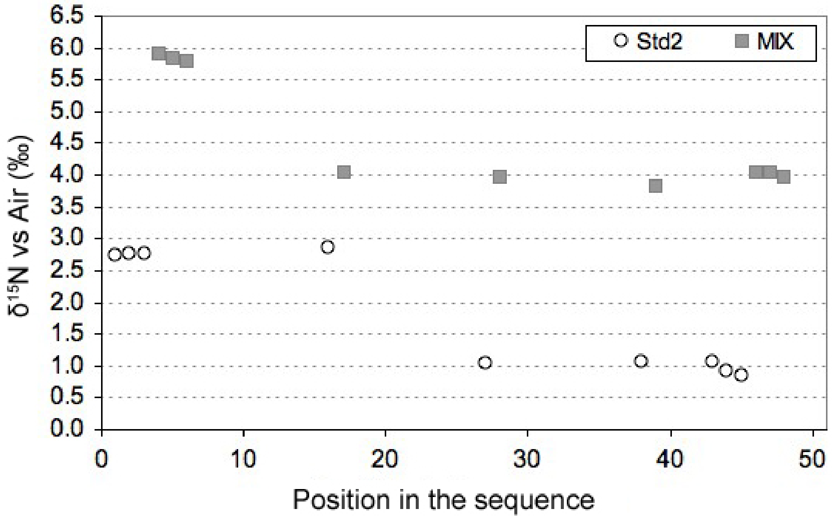


Figure 6.15. Results of δ^{15}N measurements for Std2 and the MIX working standard in a sequence of analyses. The δ^{15}N values of the laboratory and working standard undergo a sudden depletion of 1.8 ‰ in the middle of the sequence (position n°17). The isotopic difference (expected to be about 3 ‰) is maintained in the second part of the sequence though.

The use of control charts generally assumes a Gaussian distribution of data. Thus, the highly non normal distributions of the standards illustrated by the Q-Q plots would normally not favour the use of control charts. However, this type of representations consider all the measurements obtained over more than two years at once. Plotting these values in a control chart considering the chronological order reveals an interesting phenomenon. The unexpected behaviour of standards is observable from the control charts of Std1 and Std2, which show a sudden growing number of outlying points (see **Figures 6.16** and **6.17**). This phenomenon started in a sequence run on IRMS2 (see the red arrow in **Figures 6.16** and **6.17**) and was noticed through the δ^{15}N values of both laboratory standards. As the appearance of the phenomenon did not correspond to the change of instruments, this analytical problem was therefore not related to the use of IRMS2 itself.

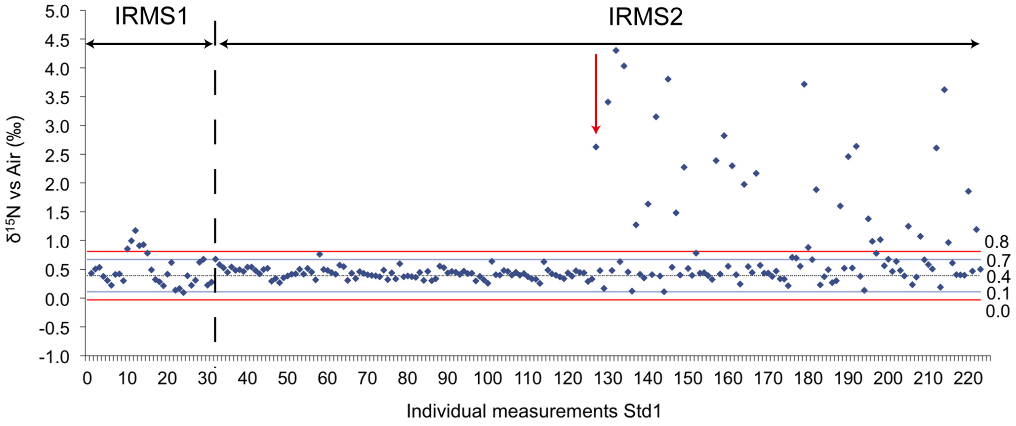


Figure 6.16. Control chart of the δ^{15}N value of Std1. All measurements are plotted ($N = 223$). The red arrow indicates the beginning of sequences with aberrant δ^{15}N values produced by a regular pattern (see phenomenon illustrated in **Figure 6.18**).

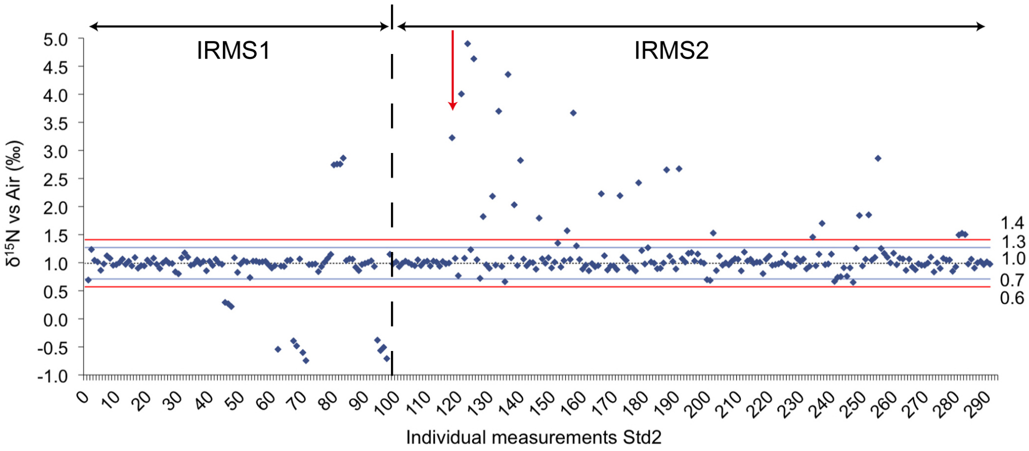


Figure 6.17. Control chart of the δ^{15}N value of Std2. All measurements are plotted ($N = 291$). The red arrow indicates the beginning of sequences with aberrant δ^{15}N values.

The outliers were not present on a regular basis, however a pattern could frequently be observed in the analytical sequences. **Figure 6.18** depicts the unexpected behaviour of laboratory standards, run alternatively with blanks and working standards in a sequence.

The δ^{15}N value of the first replicate of laboratory standard in the sequence (in position 7, 12, 25 and 40) following a nitrate sample, was always more enriched than the other replicates and increased along the sequence (Std2 in **Figure 6.18a**). δ^{15}N values of other replicates were not affected by this phenomenon. As the same effect was noticed for Std1 and Std2 analysed after AN std and KNO_3, this phenomenon appeared to be related to the type of substance analysed. The intensity of the phenomenon did not decrease, when analysing AN fertiliser containing less nitrogen (27 % N) than pure AN std (35 % N). Conversely, while the first δ^{15}N value of a series of AN std measurements was more depleted than the following, the δ^{15}N value tended to stabilise after a few replicates. Though, differences of up to 2 ‰ between nitrate replicates could be observed. Note that one AN std measurement stands apart from the other replicates, with a δ^{15}N value of 4.1 ‰.

In addition, the δ^{15}N values variations of laboratory standards (**Figure 6.18a**) seemed correlated with their % N deviations (**Figure 6.18b**). Whereas AN std samples and fertiliser exhibited a negative difference of % N of around 2 to 3 % below the expected value, the first laboratory standard analysed immediately after a nitrate based compound displayed a slight positive deviation from the expected value (**Figure 6.18b**). A zoom-in on the y scale shows more clearly this discreet positive deviation and uncovers even slight % N deviation of the second or third replicate positioned further in the sequence (**Figure 6.18c**).

Curiously, blanks did not allow detecting this phenomenon. Indeed, blanks run in position 4, 6, 10, 14 and 24 of the sequence (indicated by black arrows in **Figure 6.18a**) did not release any nitrogen. This supports the hypothesis that some nitrogen was retained in the EA and influenced the δ^{15}N value of other measured samples (memory effect). Finally, repeatability and intensity of the phenomenon were not constant from one period of time to another.

Since there was a correlation between the type of material analysed just before the standard and its δ^{15}N value and % N, it appeared reasonable that the IRMS was not at the source of these odd measurements. This phenomenon was likely caused by nitrogen remaining in the EA system, either in the combustion or in the reduction reactors, originating from a non-quantitative conversion of the nitrogen bearing compound into N_2, and carrying over to the subsequent analyses.

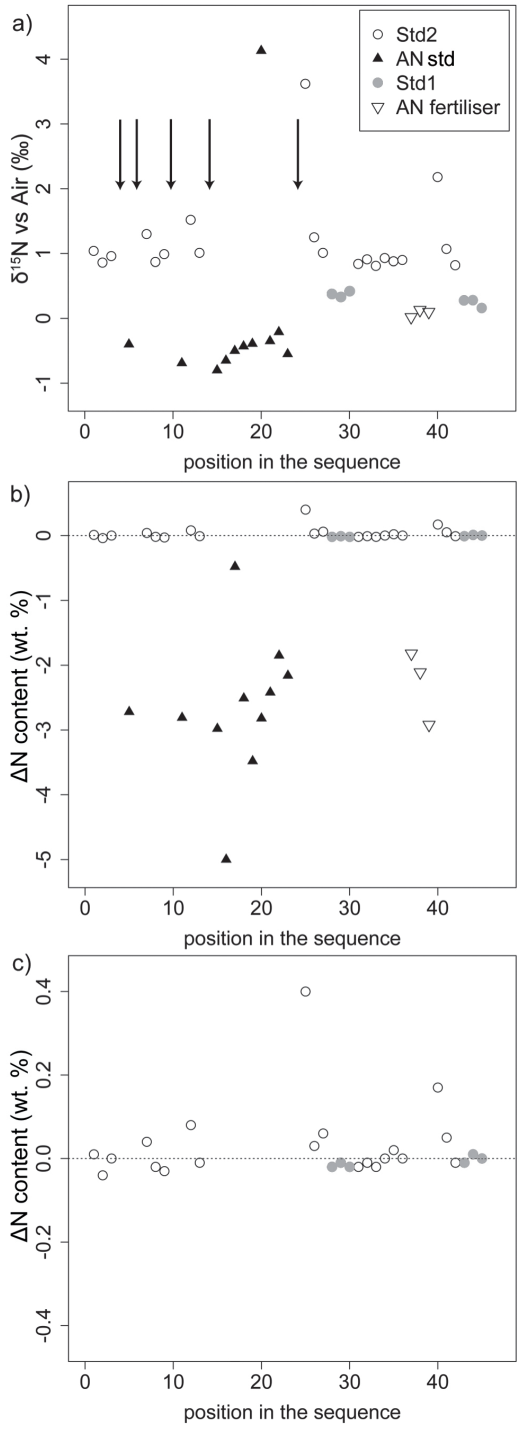


Figure 6.18. a) δ^{15}N value of AN std run alternatively in a sequence with laboratory standards Std1 and Std2, as well as blanks (their position is highlighted by black arrows). Note the odd value of the first replicate of a series of standards, as well as the increasing δ^{15}N values of AN std; b) Deviation of the nitrogen concentration (wt. %) of the standards from their known value. AN std and fertiliser display a difference of 2 to 3 % N with their expected value; c) Zoom in of the difference of % N in the same sequence. Laboratory standards with an odd δ^{15}N value also displayed a slight offset in their difference of % N with the expected value.

2.1.2 Peak shape

A close examination of the nitrogen peak of laboratory standards and AN std showed a difference in peak shape. The analysis of laboratory standards, Std1 and Std2, which are organic materials, showed a well eluted nitrogen peak, followed by CO peak (see **Figure 6.19**). Even data displaying an aberrant δ^{15}N value did not present any particularities.

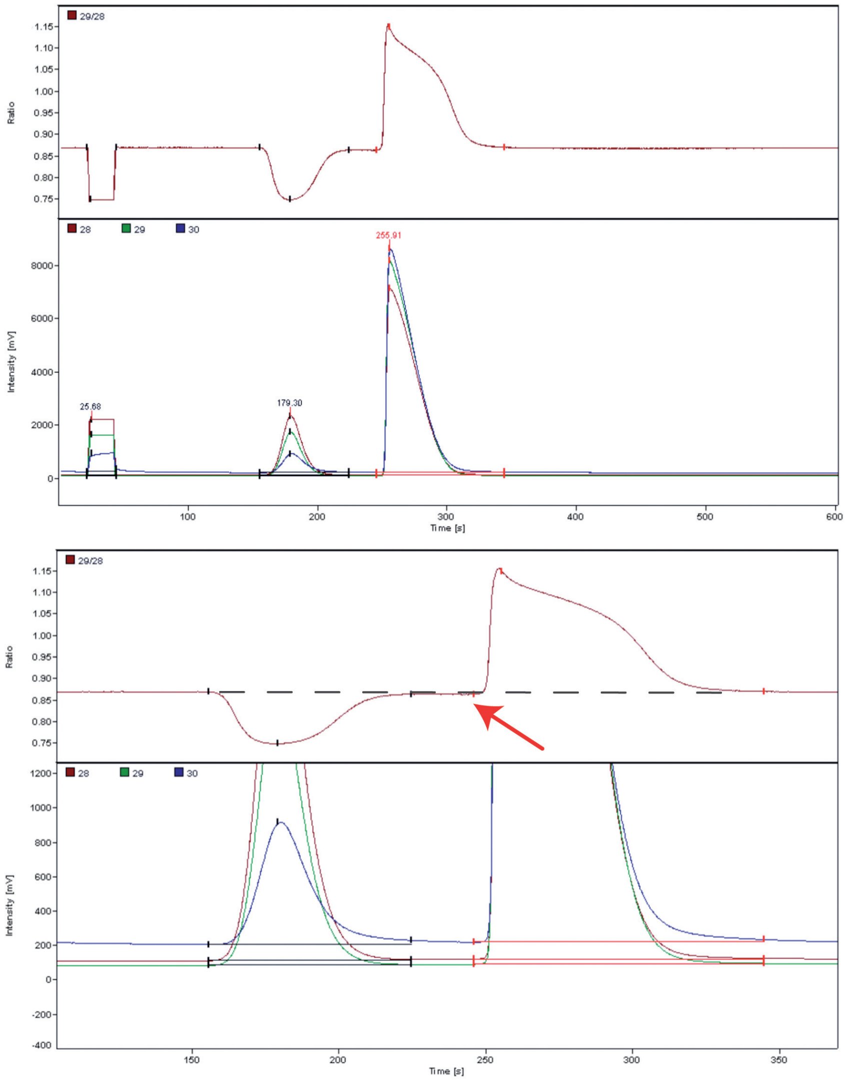
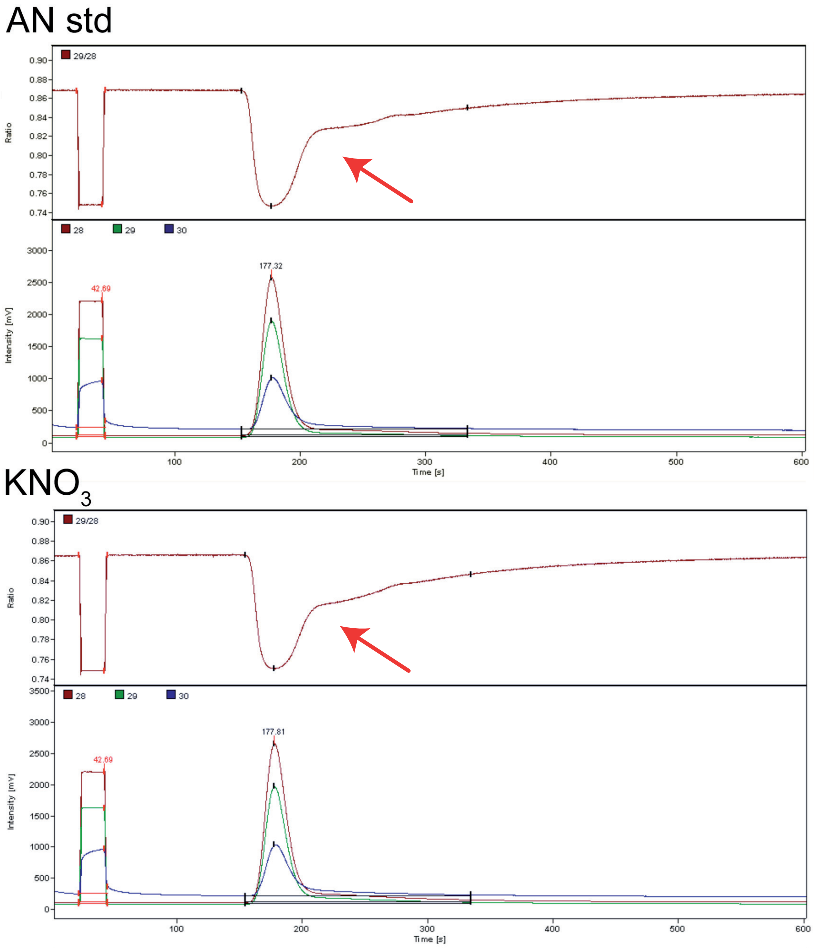


Figure 6.19. δ^{15}N measurement of Std2. Upper part of the figure: The first peak corresponds to the reference gas, the second peak to nitrogen and the last one to CO. Lower part: Zoom in on the baseline of the N_2 and CO peaks. Note the well eluted N_2 peak and the return of ratio 29/28 to baseline highlighted by the grey dotted line and the red arrow in the upper portion of part b)

On the contrary, AN std and KNO_3 most of the time exhibited a tailing shape (see **Figure 6.20**). This is highlighted by the 29/28 ion current ratio on the upper portions of parts a) and b), which had difficulties to return to the original baseline. The peak tailing indicates that either combustion or reduction (or both) was not performing optimally and lingered on. On rare occasions, the analysis of nitrates produced a well defined peak. As peak tailing was observed for nitrates, but not for the organic laboratory standards, we assumed that this phenomenon specifically pertained to the conversion of nitrate into N_2. Due to tailing, N_2 was not completely integrated as illustrated by the m/z 29/28 ratio in the upper part of **Figure 6.20**. This explains the lower measured % N of nitrates. Moreover, tailing led to variations in peak integration, which affected the repeatability of the δ^{15}N value.


AN std

KNO$_3$

Figure 6.20. δ^{15}N measurements of AN std and KNO_3. For both nitrate substances, the N_2 peak is tailing and the 29/28 ratio indicate a non-optimum combustion and/or reduction process (indicated by the red arrows).

Different actions were undertaken to get rid of the tailing phenomenon without success. Baking out the oven and the molecular sieve, cleaning of the combustion tube or regeneration of copper in the reduction oven, as well as replacements of the chemicals of both reactors did neither eliminate the problem nor help to determine which reactor was at the source of this behaviour.

2.1.3 Discussion

This unexpected behaviour of the values of the standards was not observed earlier and could not be related to any maintenance or manipulations. However, it is interesting to note that literature from different disciplines using IRMS provides hints that the quantitative conversion of nitrate based compounds into nitrogen presents difficulties. In forensic science, Aranda *et al.* needed to add activated charcoal to their nitrate sample and standards to support combustion by trapping evolving O_2 [Aranda IV *et al.* 2011]. Benson *et al.* noticed significantly more depleted values for the first ammonium nitrate standard run in a sequence [Benson 2009, Benson *et al.* 2010a]. Results from interlaboratory trials also showed that the analysis of nitrates led to unusually large variations [Böhlke & Coplen 1993, Benson *et al.* 2010b, Forensic Isotope Ratio Mass Spectrometry 2011]. In addition, such features have also been observed in the field of identification of sources of nitrate in water and soils. Some researches reported that the analysis of such analytes may produce irregular results. Silva *et al.* [Silva *et al.* 2000], followed by Spoelstra *et al.* [Spoelstra *et al.* 2004], overcame inconsistent δ^{15}N values, by adding 2mg of sucrose to nitrate salt samples on the advice of Micromass Elemental. This sugar addition method was initially described by Noguchi as an improvement of the micro Dumas method for nitrogen quantitative analysis of nitro and oxidation-resistant stable species [Noguchi 1951]. Noguchi observed that glucose had a beneficial catalytic effect on sample combustion. Yet, Borda and Hayward reported scattered % N values of nitrate esters, despite the addition of glucose to their samples [Borda & Hayward 1967]. Schindler and Knighton also reported on the incomplete recovery of nitrogen from nitrate when KNO_3 was analysed with the Dumas method [Schindler & Knighton 1999]. Although these earlier applications were related to the determination of % N using the micro Dumas combustion in its original version, they support the fact that the conversion of solid nitrates into pure N_2 may produce inconsistent results due to an incomplete decomposition or reduction processes. This may therefore affect the δ^{15}N value, when elemental analysis is coupled to IRMS. Eventually, numerous discussions among the community of isotope specialists highlight difficulties related to the isotope analysis of nitrates [ISOGEOCHEM].

2.2 Further investigations

A series of experiments were set up to investigate the phenomenon described in **Figure 6.18** and to try to understand its cause. These tests were unertaken using pure ammonium nitrate (AN std) and potassium nitrate (KNO_3) samples, the organic laboratory standards Std1 and Std2 as well as the international secondary reference materials IAEA-N1, N2 and NO-3 (see **Table 6.5**).

Substances	**Use as**	Composition	**% N (wt. %)**	**δ^{15}N ± SD (‰ vs Air)**
IAEA-N1	International reference material	Inorganic $(NH_4)_2SO_4$	21.2	0.4 ± 0.2
IAEA-N2	International reference material	Inorganic $(NH_4)_2SO_4$	21.2	20.3 ± 0.2
IAEA-NO-3	International reference material	Inorganic KNO_3	13.9	4.7 ± 0.4
Std1	Laboratory standard	Organic $C_xH_yN_z$	2.1	0.4 ± 0.2
Std2	Laboratory standard	Organic $C_xH_yN_z$	2.4	1.0 ± 0.2
Ammonium nitrate	sample	Inorganic $(NH_4)_2SO_4$	35.0	-
Potassium nitrate	sample	Inorganic KNO_3	13.9	-

Table 6.5. Characteristics of the international reference materials, laboratory standards and nitrates used in experiments 1 to 3, with the theoretical % N and known δ^{15}N values (δ^{15}N values of international reference materials were taken from [Böhlke & Coplen 1993]).

Experiment 1 evaluated the addition of graphite (C) and vanadium pentoxide (V_2O_5) in different proportions to nitrates as a possible solution to eliminate peak tailing [Aranda IV *et al.* 2011, ISOGEOCHEM]. Results showed that the use of these additives was not conclusive. Carbon led to biased δ^{15}N value of reference materials, because of the possible contribution of CO[2] (see **Figure 6.21**). V_2O_5 increased peak tailing and led to variable δ^{15}N values.

In experiment 2, another EA configuration involving an additional section of copper was used in order to see whether the tailing may originate from an incomplete reduction process. Although the use of this additional copper section

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[2] CO and N_2 have the same mass (28 for $^{12}C^{16}O$ and $^{14}N^{14}N$ and 29 for $^{13}C^{16}O$ and $^{15}N^{14}N$).

eliminated peak tailing, δ^{15}N values still showed poor repeatability.

Finally, in experiment 3, several method parameters (helium flow, sample delay, oxygen pressure and oxygen exclusion) were modified and their influence on the peak shape, δ^{15}N value and % N of the different substances were assessed.

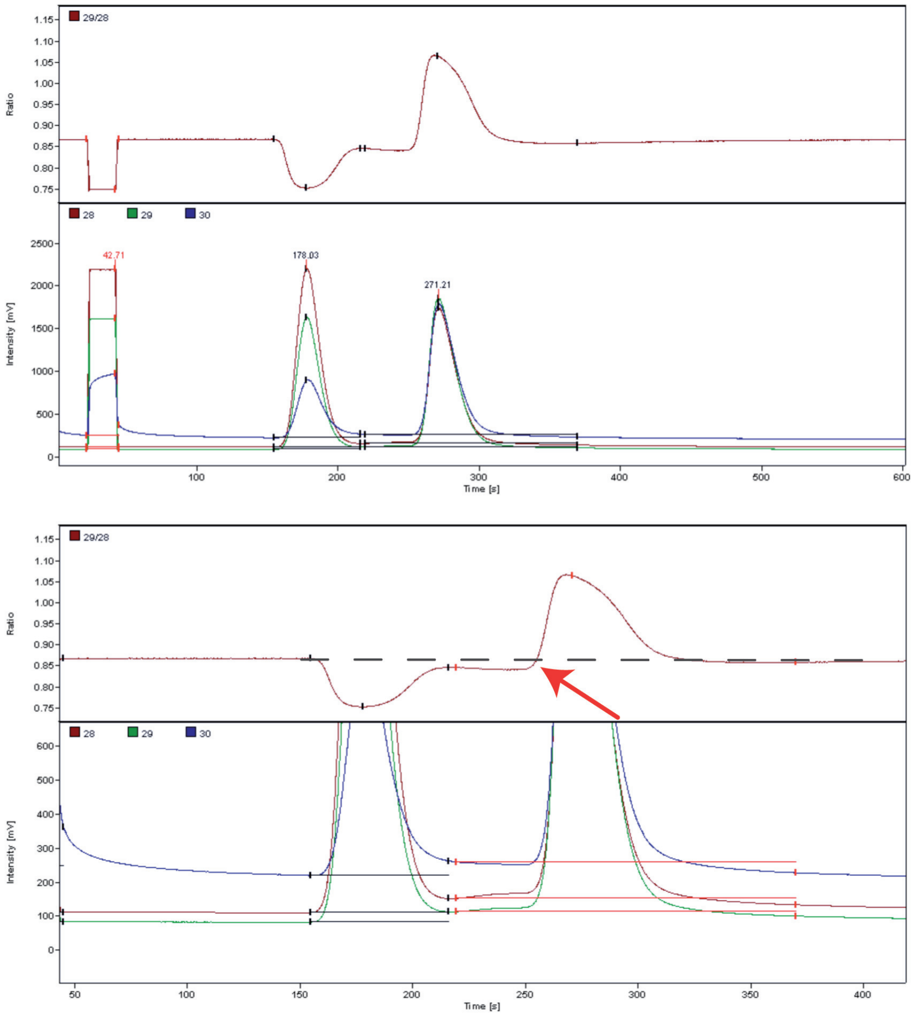

Figure 6.21. δ^{15}N measurement of the working standard MIX, which corresponds to a mixture of KNO_3 and C. On the upper part, the N_2 peak is not tailing and the ratio 29/28 shows a normal shape with an apparent return to baseline. However on the lower part of the figure, the zoom shows that the return to baseline of ion current ratio 29/28 is not perfect, as pointed out by the grey dotted line and the red arrow. This incomplete return to baseline is probably the reason for biased δ^{15}N values.

2.2.1 Variations of the method parameters and O_2 exclusion (experiment 3)

The sample delay (sample start and stop) was adapted in order to introduce the sample at different moments of the analytical cycle (sample start = 0, 5, 10, 18, 55, 62 seconds), while oxygen was injected for 60 seconds from the beginning of the analytical cycle. Finally, the exclusion of oxygen from the analytical cycle was also tested.

For IAEA-N1 and N2, the best results were obtained without injection of O_2 (see part a) in **Figure 6.22**) or when the O_2 concentration was at the lowest level (sample introduced at 0 or 5 s). **Table 6.6** reports the mean δ^{15}N value and standard deviation of the three international reference materials analysed with and without O_2. Under these conditions, the peak shape was symmetric and the m/z 29/28 ratio did not show any anomaly throughout repeated analyses. The intermediate repeatability of the δ^{15}N value for these compounds is excellent (0.1 ‰) and corresponds to the estimated expanded uncertainty ($<$ 0.1 ‰, k = 2).

Substances	**δ^{15}N value with O_2**	***N***	**δ^{15}N value without O_2**	***N***	**known δ^{15}N value**
IAEA-N1	0.4 ± 0.3	*67*	0.4 ± 0.1	*46*	0.4 ± 0.2
IAEA-N2	20.3 ± 0.3	*53*	20.3 ± 0.1	*50*	20.3 ± 0.2
IAEA-NO-3	4.5 ± 0.6	*71*	4.7 ± 0.2	*50*	4.7 ± 0.4

Table 6.6. δ^{15}N value vs Air (‰) and the standard deviation (SD) of international reference materials, analysed with and without O_2 injection. All measurements (*N*) are included in the calculated means and SD. Known δ^{15}N values of international reference materials were taken from [Böhlke & Coplen 1993].

Figure 6.22 compares the δ^{15}N values of IAEA-N1, N2 and AN std obtained a) with oxygen injection (analytical cycle = 70 s, oxygen = 60 s, sample start/stop = 18/20 s), b) with oxygen injection (analytical cycle = 70 s, oxygen = 60 s, sample start/stop = 0/2 and = 5/7 s) and c) without oxygen injection (analytical cycle = 70 s, sample start/stop = 18/20 s). The expected δ^{15}N value is represented by a central black dotted line. For AN std, the dotted line represents the mean value of measurements performed without oxygen. Upper and lower warning (in blue) and action lines (in red) are defined as 2SD and 3SD respectively.

For IAEA-NO-3 and nitrate based samples, only the exclusion of O_2 from the analytical cycle (analytical cycle = 70 s, sample start/stop = 18/20) gave a well eluted peak with a m/z 29/28 ratio returning perfectly to baseline, even after 10 replicates (**Figure 6.23**). These analytical conditions produced an excellent repeatability of the δ^{15}N value of 0.1 ‰, after exclusion of outliers when present.

The estimated expanded uncertainty (k = 2) associated with the δ^{15}N measurement of nitrates (IAEA-NO-3, KNO_3 and AN std samples) is 0.1‰.

As expected, only the organic laboratory standards showed inconsistent results when analysed without oxygen. The incomplete combustion of organic material produces CO which interferes with N_2 if not eliminated by an adequate trap ($^{12}C^{16}O$ with m/z 28 and $^{13}C^{16}O$ with m/z 29). The best results were obtained using the usual settings of the analytical cycle (analytical cycle= 70 s, oxygen = 60 s, sample start/stop = 18/20). These conditions favour the introduction of the organic sample in an oxygen rich environment and ensures the complete combustion of the organic sample.

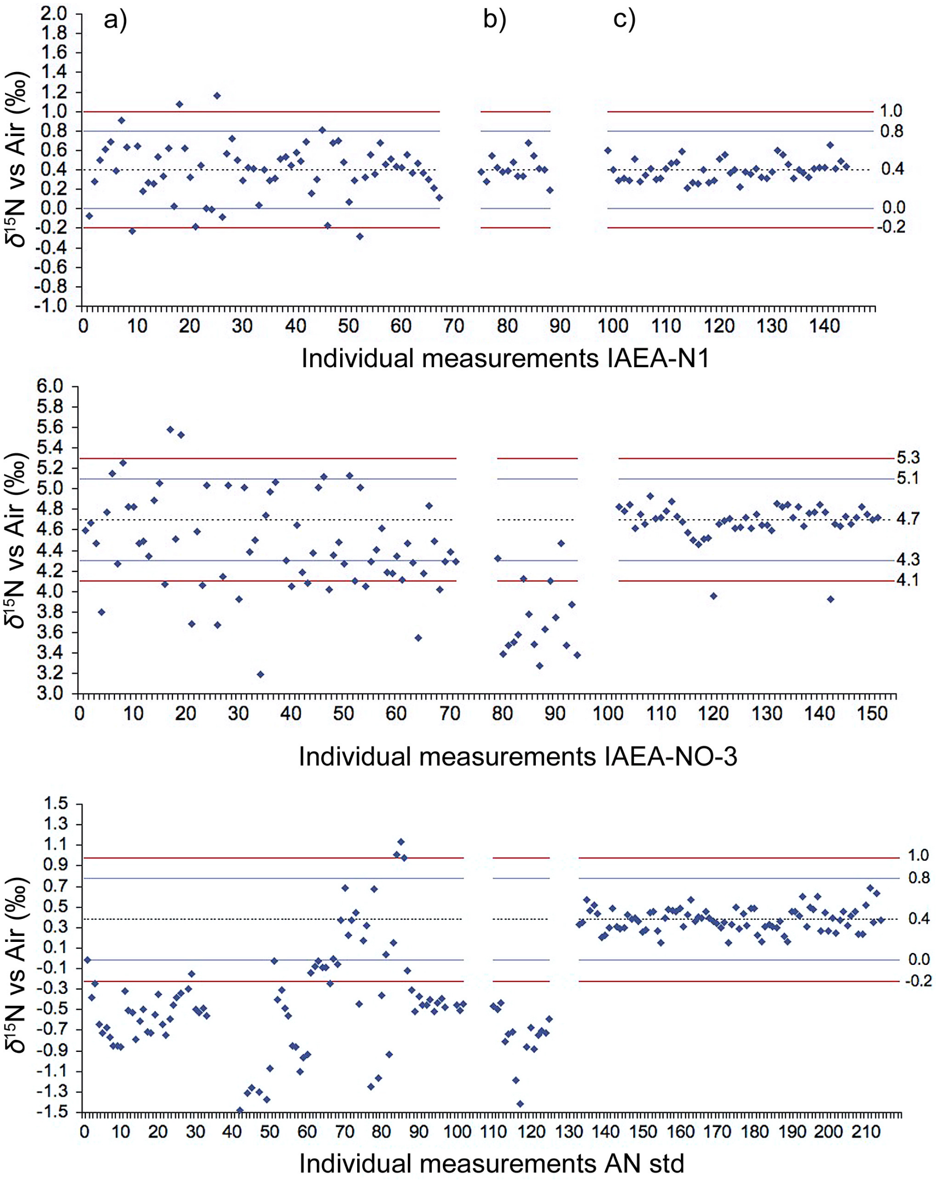

Figure 6.22. Control charts of the δ^{15}N measurements of IAEA-N1 ($(NH_4)_2SO_4$), IAEA-NO-3 (KNO_3) and AN std samples a) with oxygen injection (the O_2 concentration was at the highest level), b) with oxygen injection (the O_2 concentration was at the lowest level), c) without oxygen injection.

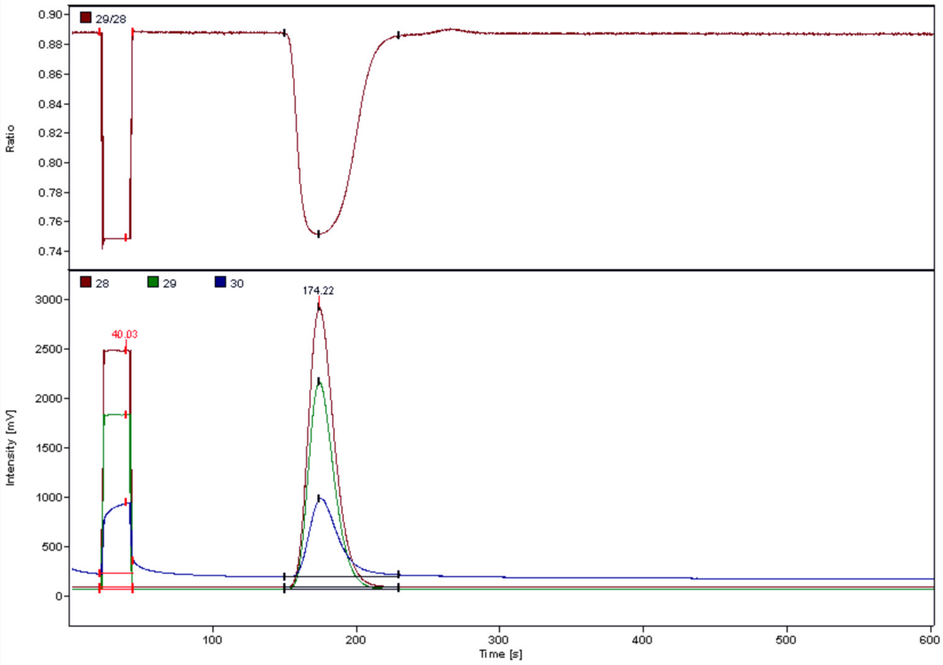

Figure 6.23. δ^{15}N measurement of IAEA-NO-3 (KNO_3) obtained without O_2 injection. The N_2 peak is well eluted and does not show any tailing. In addition, the m/z 29/28 ratio perfectly returns to the baseline even after 10 replicates.

The δ^{15}N results of the inorganic substances are supported by the measured % N. **Figure 6.24** presents the % N of IAEA-N1, N2, and AN std obtained a) with oxygen injection (analytical cycle = 70 s, oxygen = 60 s, sample start/stop = 18/20 s), b) with oxygen injection (analytical cycle = 70 s, oxygen = 60 s, sample start/stop = 0/2 and = 5/7 s), c) without oxygen injection (analytical cycle = 70 s, sample start/stop = 18/20 s). The expected % N is represented by a central black dotted line. Upper and lower warning lines (in blue) and action lines (in red) are defined as 2 SD and 3 SD respectively.

The % N values of IAEA-N1, N2 were improved when the reference material was introduced at 0 or 5 seconds after the beginning of the analytical cycle (with O_2 injection). Moreover, the results significantly improved when the substances were analysed without O_2, with a repeatability of 0.1 to 0.2 %. While the analysis of nitrates (IAEA-NO-3, KNO_3 and AN std) with O_2 always produced lower % N than expected, their analysis without oxygen yielded the expected % N with a satisfactory repeatability (0.3 to 0.6 % for nitrates after exclusion of outliers).

The results of this series of experiments are compiled in a paper published in 2013 in Analytical and Bionalytical Chemistry [Gentile *et al.* 2013]. It provides further details on the experiments, their results and the thermochemical developments.

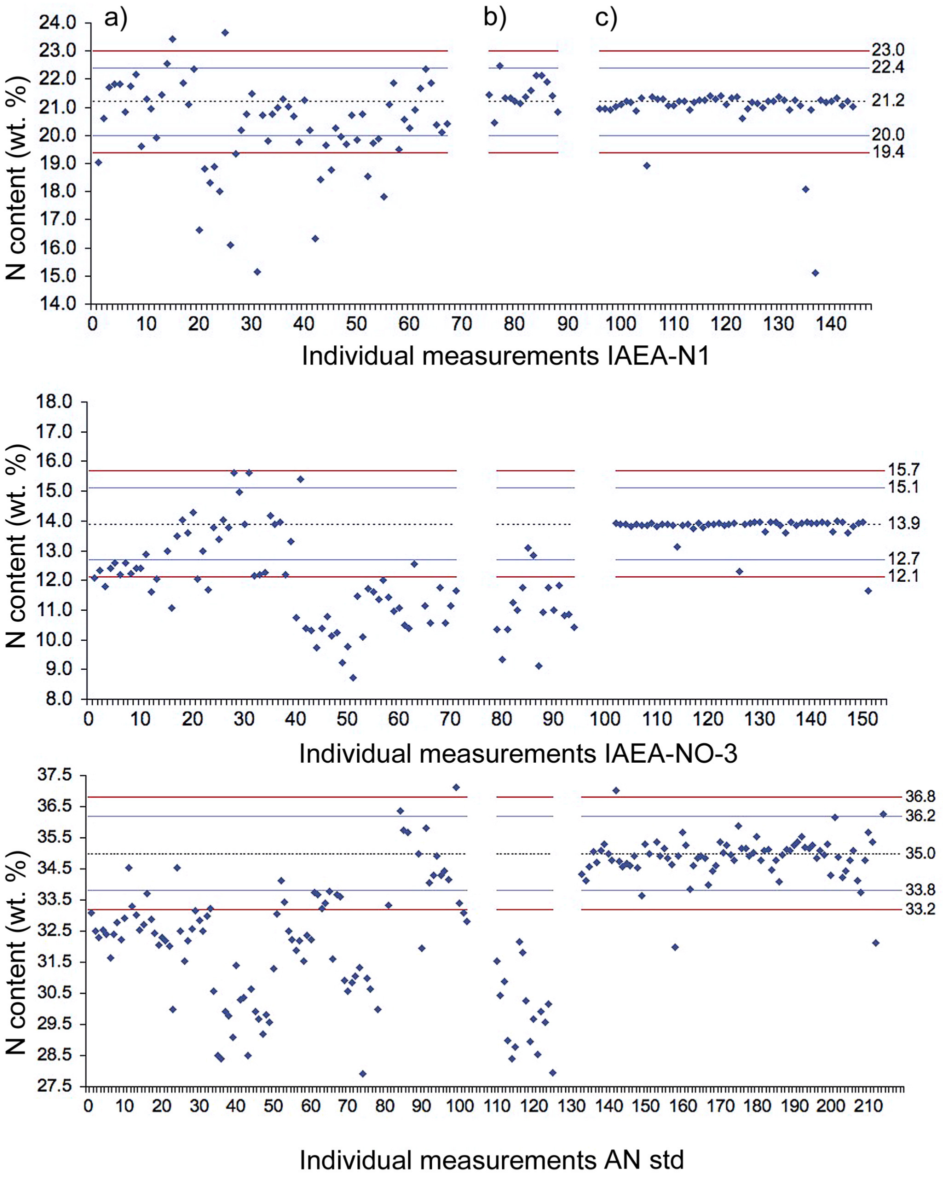

Figure 6.24. Control charts of the % N of IAEA-N1 ($(NH_4)_2SO_4$), IAEA-NO-3 (KNO_3) and AN std samples a) with oxygen injection (the O_2 concentration was at the highest level), b) with oxygen injection (the O_2 concentration was at the lowest level), c) without oxygen injection.

2.3 Second method evaluation

The second method (without oxygen injection) was evaluated through the measurements of the working standards (KNO_3 and AN std) and the control samples A1 and L5. Data presented in this subsection as well as the numbers reported in **Tables 6.7** and **6.8** result from a number of measurements obtained on a period of 2 months.

Data distribution The distributions of the δ^{15}N value and % N are presented in **Figures 6.25** and **6.26**. The δ^{15}N values of the different substances lie close to a line, suggesting data follow a normal distribution. Only control sample L5 seems to show two groups of points. Indeed, four points appear slightly isolated from the others. However, as the number of measurements is restricted, interpretation on normality is not straightforward.

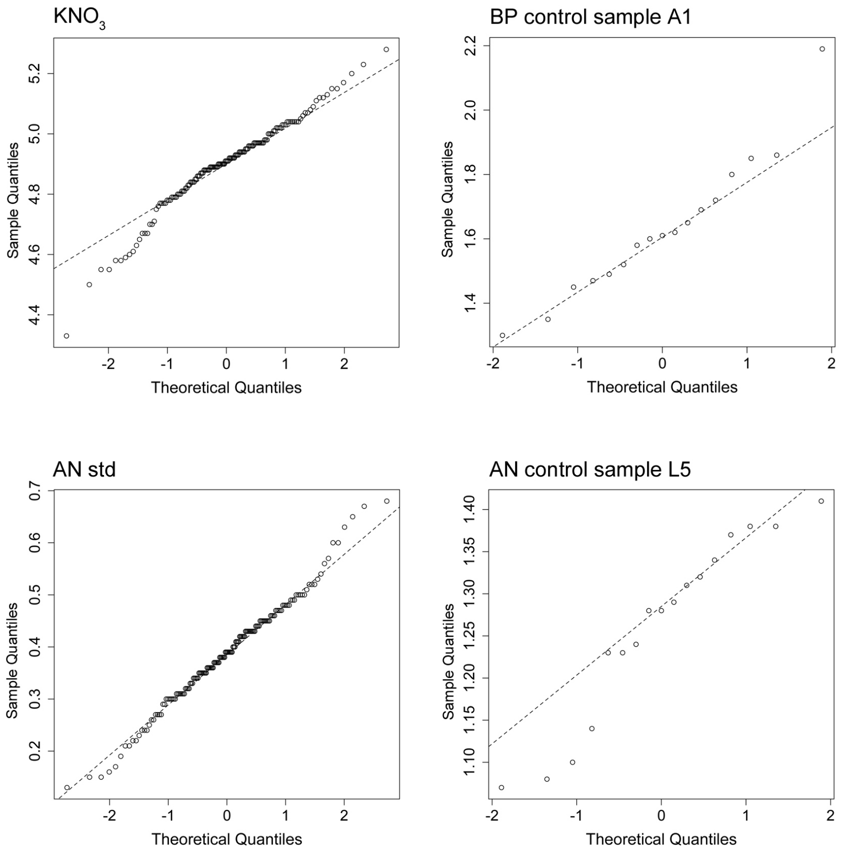

Figure 6.25. Distribution of the δ^{15}N values for the different substances (AN std: $N = 155$, KNO_3: $N = 149$, A1: $N = 17$, L5: $N = 17$).

Regarding the % N distributions, the AN std and KNO_3 exhibit a heavier left tail, while the right tail of the distributions slightly deviates. However, as explained by Reis and Judd, violation of the normality assumption should seriously be considered when a steep slope is observed at both end of the Q-Q plot [Reis & Judd 2000]. In reality, the sample preparation of the second method nitrogen is likely to be responsible for these data points with less nitrogen (see nitrogen content determination section). It does not preclude the use of control charts. Simply, more data will be discarded because of their smaller % N.

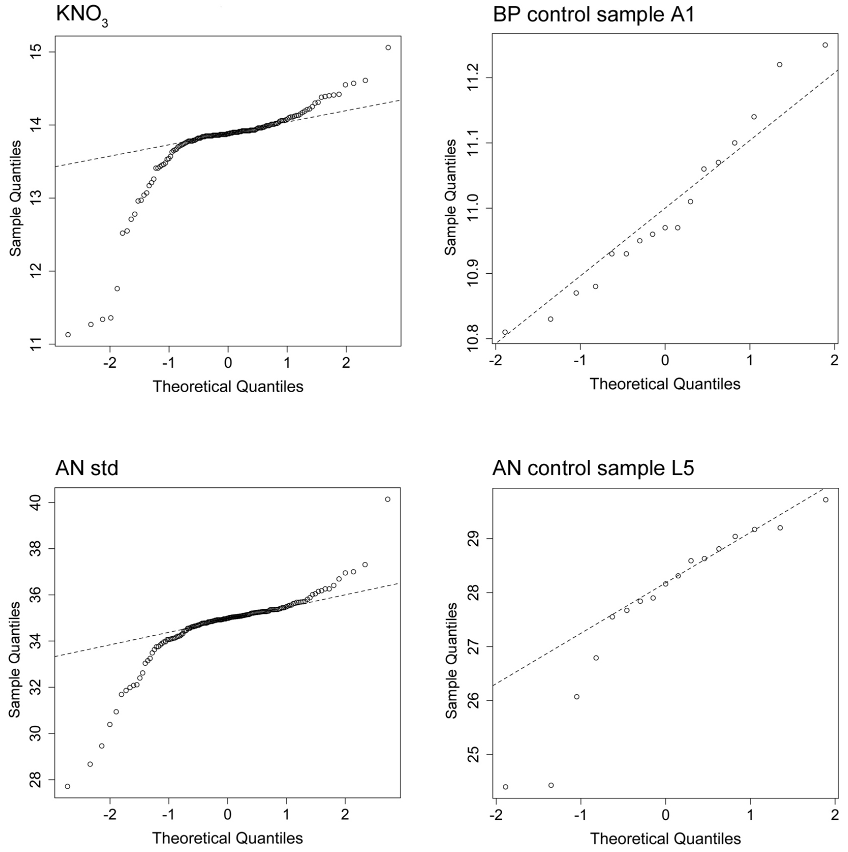

Figure 6.26. Distribution of % N for the different substances (AN std: $N = 155$, KNO_3: $N = 149$, A1: $N = 17$, L5: $N = 17$).

2.3.1 $\delta^{15}N$ determination

The control charts of AN std and KNO_3 show an excellent repeatability of $\delta^{15}N$ values (see **Figures 6.27** and **6.28**). Using this second nitrogen method, the estimated expanded uncertainty associated with $\delta^{15}N$ measurement of nitrates is 0.1 ‰ (see **Table 6.7**).

Both control samples A1 and L5 also displayed an excellent precision of the $\delta^{15}N$ value, as shown in **Figures 6.29** and **6.30**. The sequences of analyses did not include any organic standards (Std1 and Std2), but included only inorganic substances (IAEA-N1, IAEA-N2, KNO_3, AN std, black powder and AN fertiliser samples). **Table 6.7** summarises the δ^{15}N value measured for the working standards and control samples.

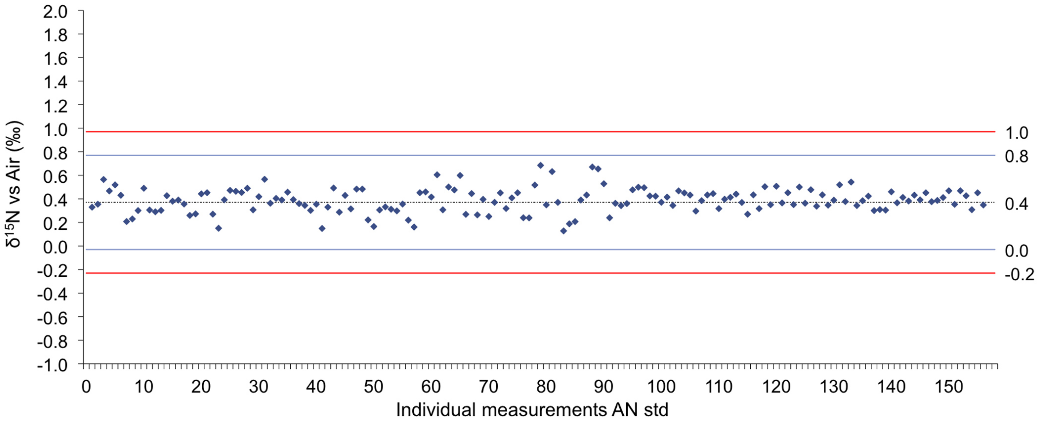

Figure 6.27. Control chart of the δ^{15}N value of AN standard. All measurements are plotted ($N = 155$).

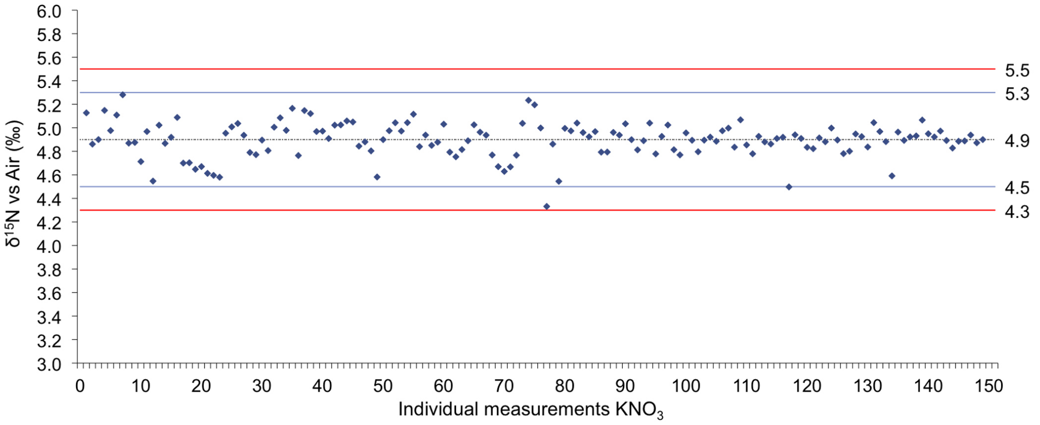

Figure 6.28. Control chart of the δ^{15}N value of KNO_3 standard. All measurements are plotted ($N = 149$).

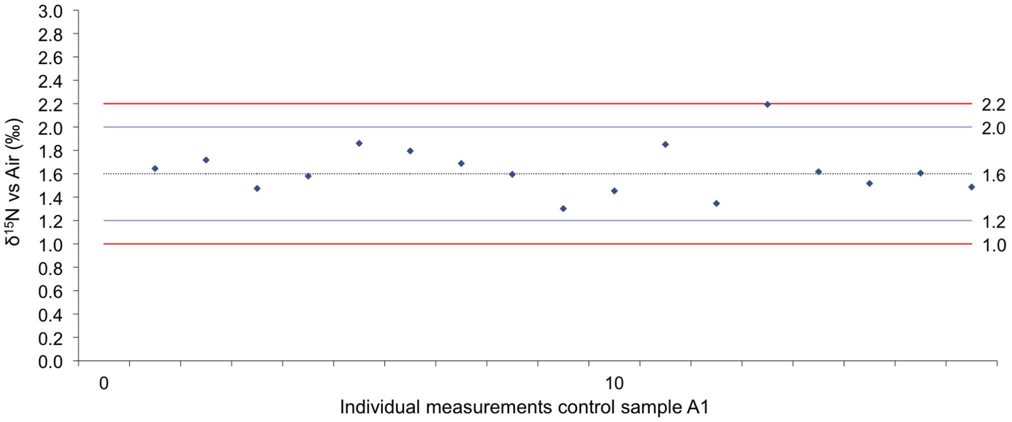

Figure 6.29. Control chart of the δ^{15}N value of the BP control sample A1. All measurements are plotted ($N = 17$).

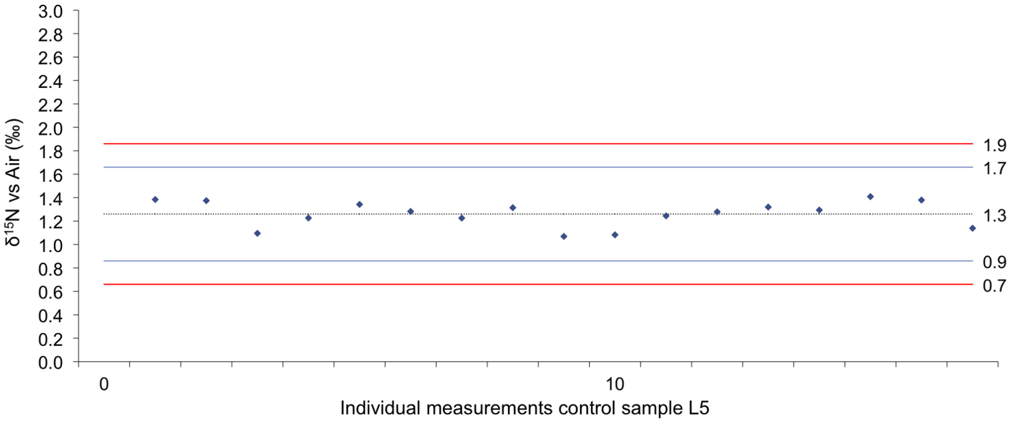

Figure 6.30. Control chart of the δ^{15}N value of the AN fertiliser control sample L5. All measurements are plotted ($N = 17$).

Substances	δ^{15}N value (n)	U [‰] (k=2)
AN std	0.4 ± 0.1 (155)	0.1
KNO_3	4.9 ± 0.2 (149)	0.1
A1 (BP)	1.6 ± 0.2 (17)	0.2
L5 (AN)	1.3 ± 0.1 (17)	0.1

Table 6.7. Average δ^{15}N value, its standard deviation and the associated expanded uncertainty U expressed in ‰ vs Air, measured with the second method, after exclusion of outlying values.

2.3.2 Nitrogen content determination (% N)

The control charts of both substances show a good agreement with the theoretical % N, except for some measurements of AN std (see **Figures 6.31** and **6.32**). The outlying values may come from the sample preparation. As only a very small mass of AN std is weighed (about 300 μg), small inaccuracies in weighing may affect the % N determination. Besides, as the tin capsule melts instead of undergoing a flash combustion, melted tin accumulates on the top of the reactor after dozens of samples and creates a plug of liquid tin which may prevent the total conversion of nitrate into nitrogen. In order to reduce the quantity of melted tin involved during analysis, the excess of unnecessary tin foil was removed from the capsule when packing the sample. Combined with a regular cleaning of the combustion reactor (every 50 samples), this way of preparing the sample improves both the lifetime of the reactor and the quality of the analyses.

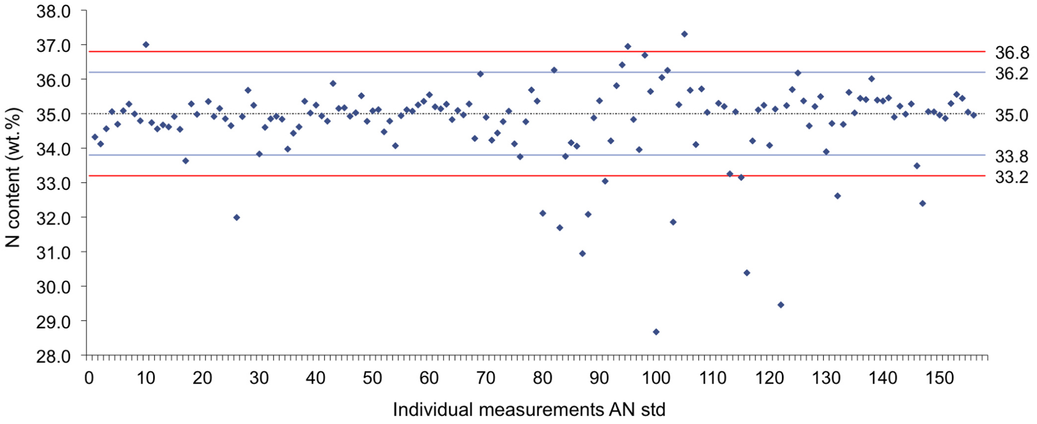

Figure 6.31. Control chart of the % N of AN std. All measurements are plotted ($N = 155$).

The % N of control samples A1 and L5 showed to be repeatable throughout se-

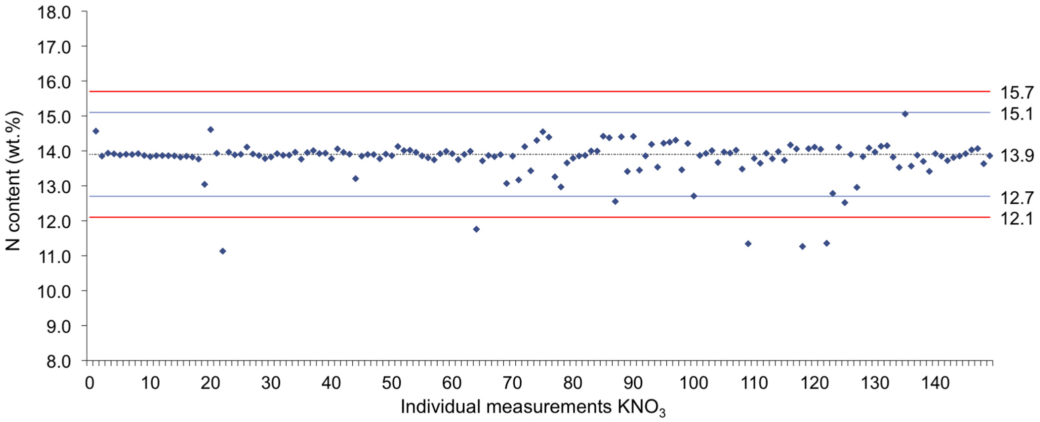

Figure 6.32. Control chart of the % N of KNO_3. All measurements are plotted ($N = 149$).

quences (**Figures 6.33** and **6.34**). This feature may therefore be exploited to discriminate samples if they show sufficient variability between them.

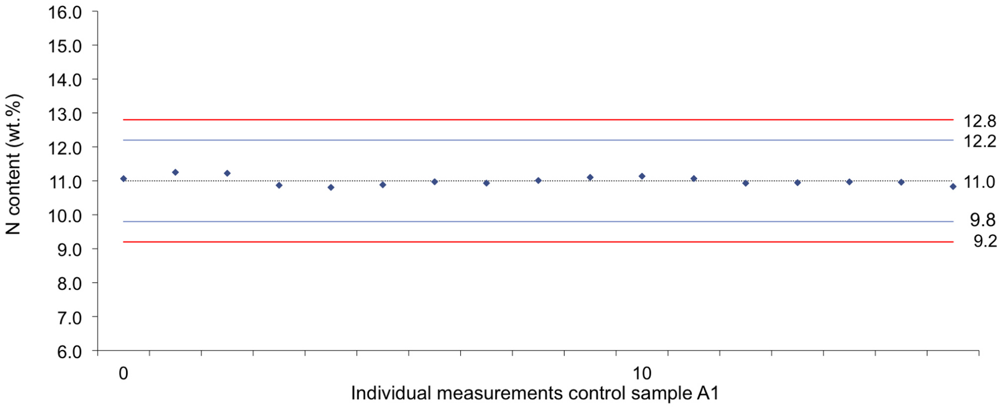

Figure 6.33. Control chart of the % N of BP control sample A1. All measurements are plotted ($N = 17$).

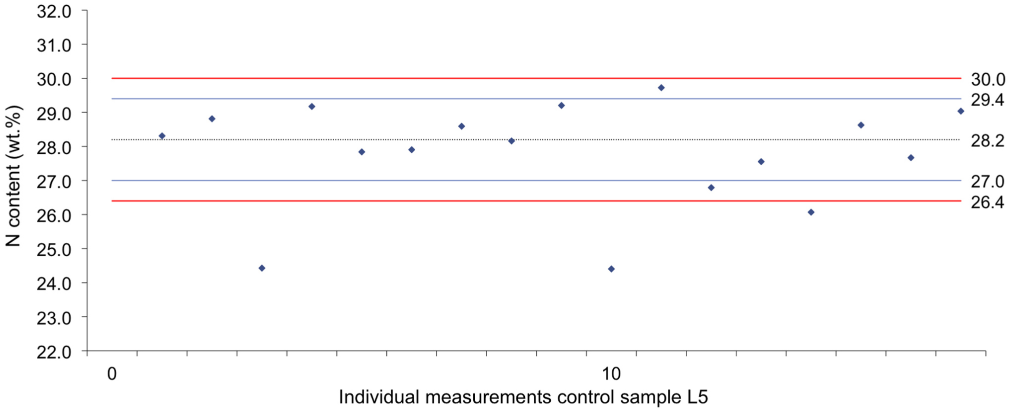

Figure 6.34. Control chart of the % N of AN control sample L5. All measurements are plotted ($N = 17$).

As summarised in **Table 6.8**, the determination of % N of nitrate working standard is associated with an uncertainty varying between 0.3 ‰ for KNO_3 and 0.5 ‰ for AN std.

Substances	**% N (n)**	**known % N**	***U* [%] (k=2)**
AN std	35.0 ± 0.6 (137)	35.0	0.5
KNO_3	13.8 ± 0.4 (145)	13.9	0.3
A1 (BP)	11.0 ± 0.1 (17)	*NA*	0.1
L5 (AN)	28.4 ± 0.8 (15)	*NA*	0.7

Table 6.8. Average % N, its standard deviation and the estimated expanded uncertainty *U* expressed in wt. %, after exclusion of outlying values.

2.4 Discussion

Reduction of nitrogen oxides and influence of oxygen In 1973, Pella and Colombo previously stated that the combustion of nitro-compounds generated large quantities of nitrogen oxides which might not be quantitatively reduced [Pella & Colombo 1973]. This was also confirmed by the outcome of experiment 2. The results showed that the tailing of the N_2 peak of nitrates came from an incomplete reduction. The authors also highlighted the influence of O_2 concentration on the determination of the % N of nitro compounds when performing elemental analysis. Nitro-compounds dropped under maximal oxygen concentration gave inaccurate % N values. Yet, lower oxygen concentration favoured the reduction of nitrogen oxides and produced more accurate % N values.

The results of experiment 3 demonstrated the importance of the influence of oxygen on the δ^{15}N measurements of nitrate (KNO_3 and AN std), but also on other inorganic compounds such as $(NH_4)_2SO_4$. Only the complete exclusion of oxygen from the analytical cycle led to excellent and repeatable results for nitrates.

The principle of the analytical technique is based on flash combustion, which is obtained through the sudden oxidation of tin in an oxygen rich environment. This exothermic reaction produces a bright flash and instantly transforms the sample into gaseous combustion products at around 1700°C. Gaseous products are then carried through the reduction reactor for the oxides to be reduced. The requirement of injecting oxygen in excess to create an oxygen rich environment is necessary for organic material, i.e. carbon-rich material, in order to ensure the complete oxidation of the sample. However, for highly oxidised inorganic substances such as nitrates where N assumes its highest possible oxidation number of +V, the injection of oxygen is not necessary when the sample is introduced and is, moreover, not advised. Indeed, under high temperature, nitrates decompose according to different equilibria but produces among others nitrogen oxides and oxygen. For example, crystalline KNO_3, which is a stable salt, decomposes according to the following high-temperature equilibrium: $2\,KNO_3 \rightleftharpoons K_2O + 2\,NO + \frac{3}{2}\,O_2$. The underlying equilibria and the thermochemical calculations are not further detailed here but can be found in the published results [Gentile *et al.* 2013].

The crucial point is that the decomposition of nitrate under high temperature *yields oxygen in the reaction of equilibrium.* Thus, the injection of extra (added) oxygen—as required by the analytical principle of EA-IRMS—when the sample is introduced in the elemental analyser shifts the equilibrium to the left, *favouring nitrogen in its high oxidation state (+V) at the expense of NO [N(+II)].* This phenomena is just a consequence of Le Chatelier's principle. The addition of O_2 in reactions of thermal decomposition of species that are already in their highest oxidation state leads to an increased extent of reaction products that will have to be reduced in the reducing part of the elemental analyser in order to monitor nitrogen as N_2. The thermochemical considerations related to the decomposition of nitrates in the presence of oxygen can be found in the manuscript published in Analytical and Bioanalytical Chemistry [Gentile *et al.* 2013].

Significance of the chemical similarity between analysed substances As demonstrated by the previous results, organic material on one hand and inorganic substances, such as $(NH_4)_2SO_4$ (IAEA-N1 and N2) and KNO_3 (IAEA-NO-3) on the other hand, should not be analysed using the same analytical procedure given their different chemical nature. Although the instrumental technique was developed to transform the sample into simple gases by means of flash combustion, thermodynamic considerations demonstrate that not all materials behave in the same way because of their different chemical nature. This aspect needs to be taken into consideration when using EA-IRMS and upholds the principle to use standards with a chemical structure and matrix as close as possible to the samples. This notion of similarity is underlined by De Bièvre (p.149): “Other things being equal, the more similar are the two items to be compared, the smaller becomes the uncertainty due to the measurement itself” [De Bièvre *et al.* 1993]. This sentence epitomises the significance to consider the chemical and physical properties of the unknown material during their passage through the instrumentation.

This issue on closeness becomes crucial, when selecting the right standard but also when calibrating the standards with international reference materials. This means that organic laboratory standards can not be used to calibrate inorganic standards for the nitrogen isotope analysis of inorganic samples. The chain of analysis must not only consider the same analytical processing between samples and standards, but also ensures that there is a chemical coherence when calibrating standards or measuring samples.

3 Oxygen analysis

Sample preparation **Table 6.9** summarises the results of the preliminary test, investigating the influence of grinding on the repeatability of the % O and δ^{18}O value. Although the repeatability of % O and δ^{18}O measurements was better with ground black powder, samples of black powder were analysed unground for safety reasons, the variations of 0.6 ‰ was considered as acceptable. The differences between % O andδ^{18}O means are not significantly different ($p > 0.05$).

	% O ± SD (%)	**δ^{18}O±SD (‰ vs VSMOW)**
Unground black powder	41.0 ± 0.3	23.4 ± 0.6
Ground black powder	40.6 ± 0.1	23.5 ± 0.2

Table 6.9. Average and standard deviation (SD) of % O and δ^{18}O value of ground and unground black powder sample A1 obtained from 3 measurements.

Data distribution Data presented in this section and the numbers reported in **Tables 6.10** and **6.11** result from a number of measurements collected on a period of 2 months.

The distributions of the δ^{18}O value and % O of the different standards and control samples are illustrated in **Figures 6.35** and **6.36**. The data seem to follow approximately a normal distribution for both variables. Except for the δ^{18}O value of the MIX and control sample A1, most distributions are slightly skewed at both ends. This is however not regarded as a serious drag on the normality assumption of data.

It is interesting to note that the AN std and the control sample L5 display more outliers than other substances. This is especially true for the % O variable. The reason for such outlying points is not known but could be related to the chemical nature of the substance, as both are ammonium nitrate.

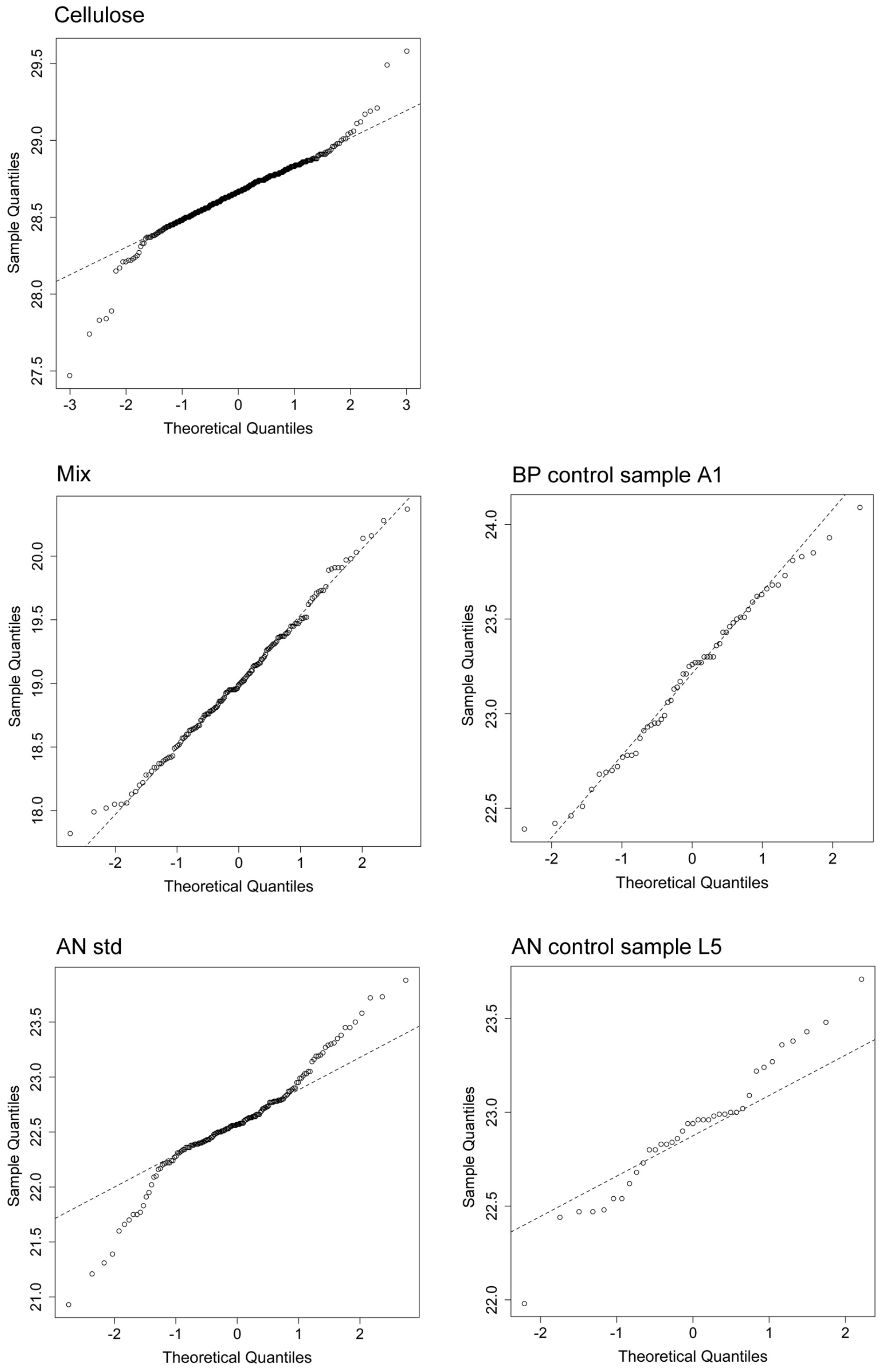

Figure 6.35. Q-Q plot of the δ^{18}O value of the different substances (Cellulose: $N = 374$, MIX: $N = 158$, AN std: $N = 166$, A1: $N = 59$, L5: $N = 37$).

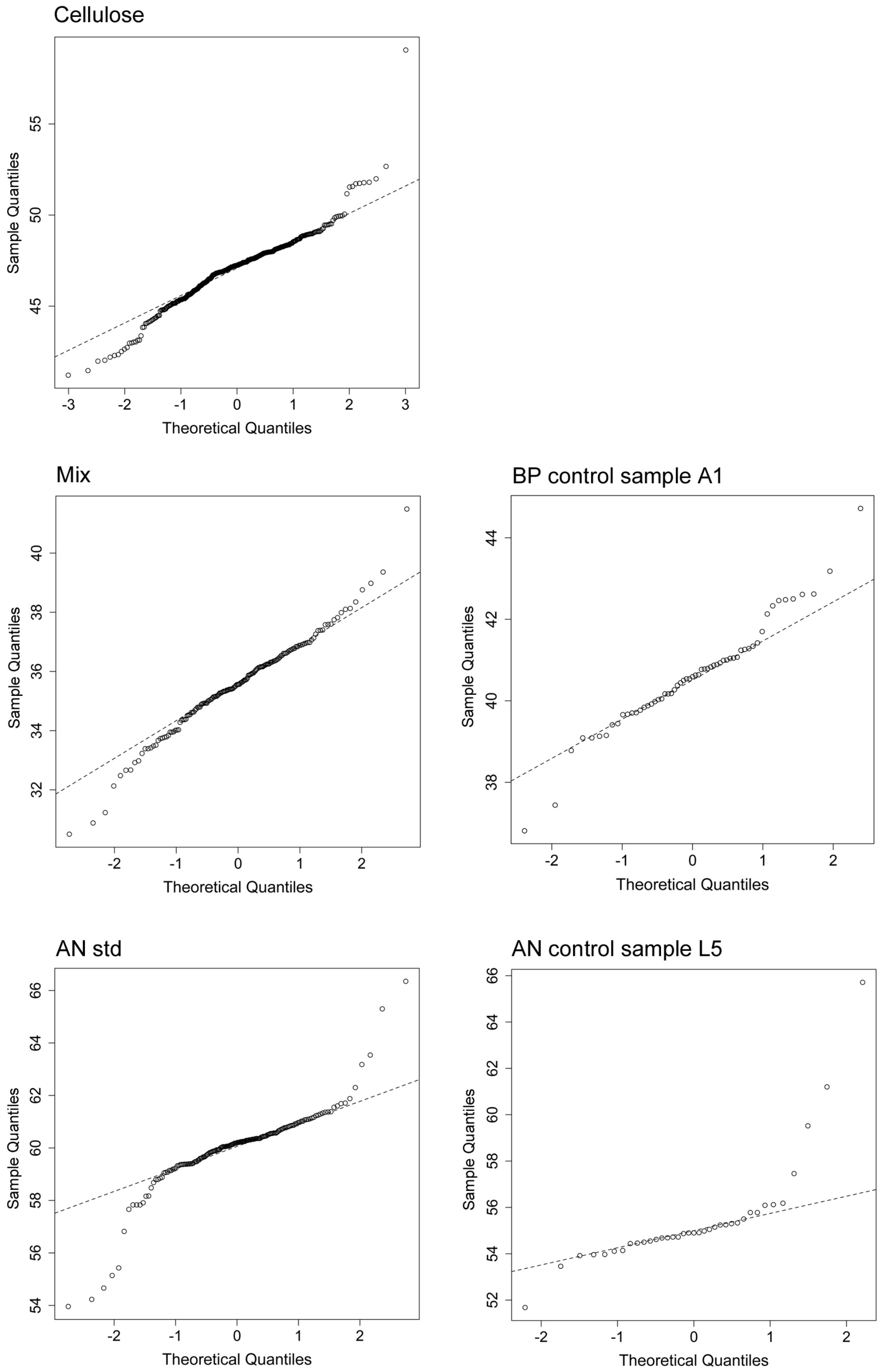

Figure 6.36. Q-Q plot of the % O of the different substances (Cellulose: N = 374, MIX: N = 158, AN std: N = 166, A1: N = 59, L5: N = 37).

3.1 δ^{18}O determination

Based on the practice of the laboratory, a standard deviation of 0.3 ‰ is an acceptable value for oxygen measurements. As shown on **Figure 6.37**, the intermediate precision of the δ^{18}O value of cellulose is very good (0.2 ‰). On the contrary, the δ^{18}O values of the MIX vary on a larger scale (see **Figure 6.38**). This range of isotopic variations likely comes from the isotopic heterogeneity of the matrix itself rather than from the analytical system, as the black powder control sample A1 also presents a similar range of variations (see **Figure 6.39**).

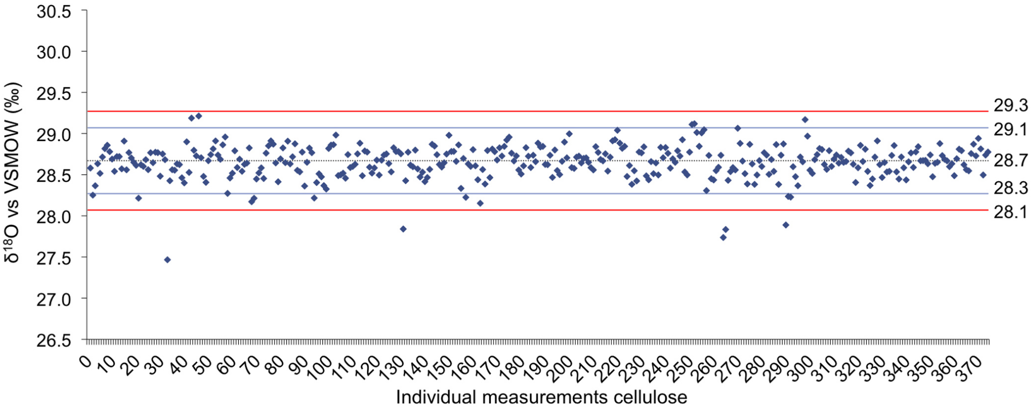

Figure 6.37. Control chart of the δ^{18}O value of cellulose. All measurements are plotted (N = 374).

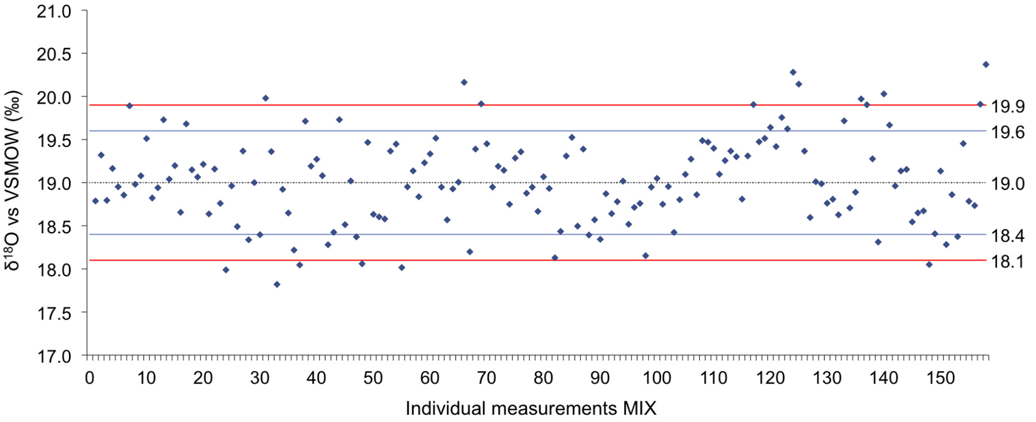

Figure 6.38. Control chart of the δ^{18}O value of the MIX. All measurements are plotted (N = 158).

The δ^{18}O value of AN standard showed a similar repeatability as cellulose at the beginning of the analyses. However, the variations increased throughout time (see **Figure 6.40**). Overall, the control sample L5 showed a repeatable δ^{18}O value throughout sequences (see **Figure 6.41**). A small upward trend is however observable at the end of the period of analysis. This trend was also noticed for the AN std and appears therefore due to the instrument.

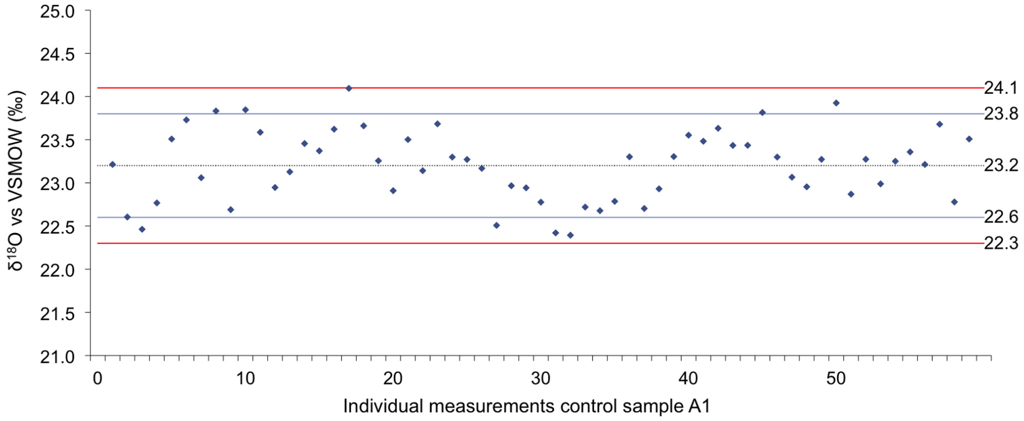

Figure 6.39. Control chart of the δ^{18}O value of the black powder control sample A1. All measurements are plotted ($N = 59$).

The ammonium nitrate fertiliser control sample L5 also showed an increase of its δ^{18}O value at the end of the period of analysis

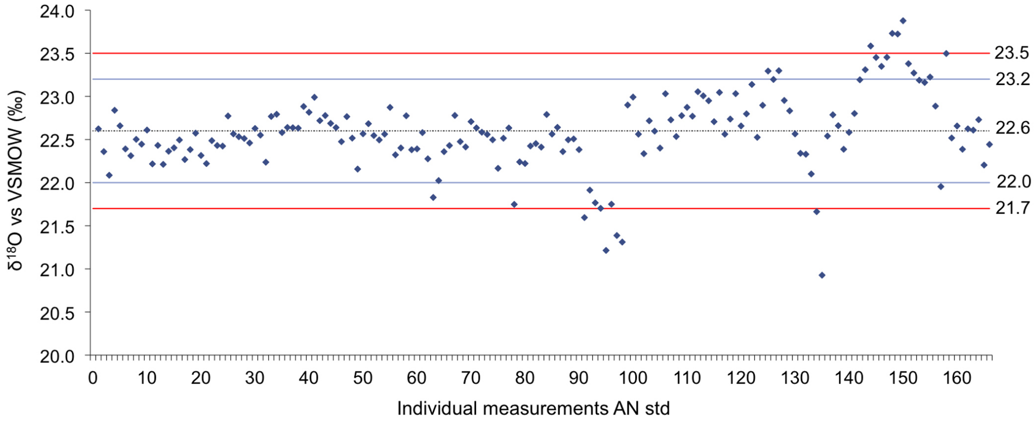

Figure 6.40. Control chart of the δ^{18}O value of the AN standard. All measurements are plotted ($N = 166$).

Table 6.10 summarises the δ^{18}O value and expanded uncertainty of laboratory and working standards, as well as control samples.

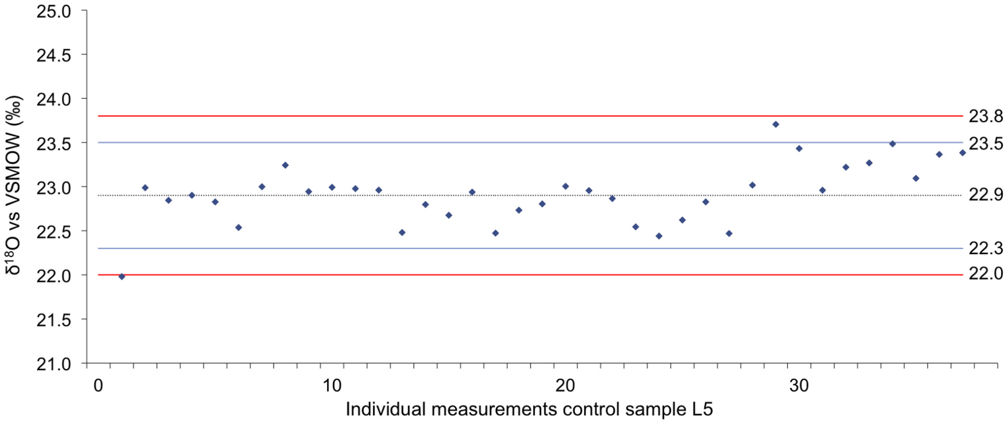

Figure 6.41. Control chart of the δ^{18}O value of the AN fertiliser control sample L5. All measurements are plotted ($N = 37$).

Substances	δ^{18}**O** **n**	**known** δ^{18}**O value** [‰]	U **[‰] (k=2)**
Cellulose	28.7 ± 0.2 367	28.7	0.2
MIX	19.0 ± 0.4 139	NA	0.3
AN std	22.6 ± 0.3 154	NA	0.3
A1 (BP)	23.2 ± 0.4 59	NA	0.4
L5 (AN)	22.9 ± 0.3 36	NA	0.4

Table 6.10. Average δ^{18}O value, its standard deviation and the expanded uncertainty U expressed in ‰ vs VSMOW. The number of measurements (n) does not encompass outlying values.

3.2 Oxygen content determination (% O)

The determination of the % O was first calculated on the basis of the cellulose standard. However, the mean % O obtained for the AN std and MIX (62.0 ± 2.0 and 37.9 ± 2.2, respectively) was higher than the theoretical value (60.0 % and 35.6 %). Such observation can result from:

- a non optimum conversion of oxygen from cellulose. The oxygen yield of cellulose being smaller, the calculated % O of the AN std and MIX relative to the theoretical % O of cellulose gives higher % O than expected.
- a contribution of N_2 to the CO peak, because both have mass m/z 28. This would lead to an apparent higher % O, which would actually be a contribution of N_2 and CO.

The % O was re-calculated relative to the % O of AN std and MIX. The control chart displayed in **Figure 6.42** presents all the measurements of the % O of cellulose corrected against the % O of the working standards. Note that most % O measurements are around 47 %, while the expected % O of cellulose is 49.3 %. This suggests that the conversion of cellulose is not complete.

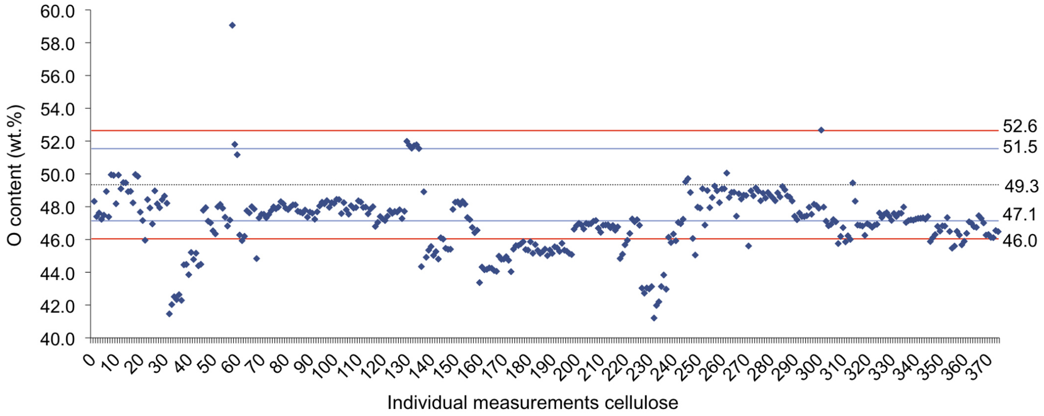

Figure 6.42. Control chart of the % O of cellulose. All measurements are plotted ($N = 374$).

Both the MIX and the AN std display a constant % O throughout sequences with a similar uncertainty (see **Table 6.11** and **Figures 6.43** and **6.44**). **Table 6.11** summarises the % O of the laboratory and working standards, as well as the control samples.

The % O of control samples A1 and L5 exhibit a limited variability throughout sequences, as exemplified by the control chart of control sample L5 (**Figure 6.45**).

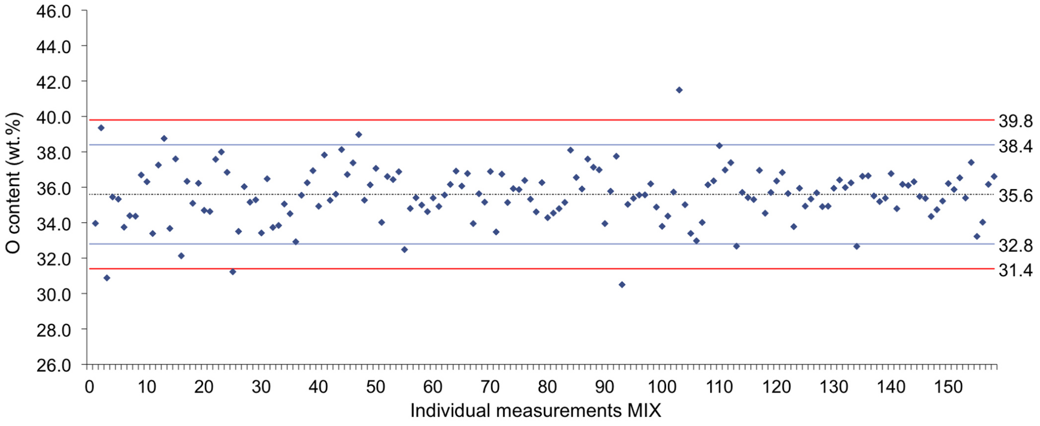

Figure 6.43. Control chart of the % O of the working standard MIX. All measurements are plotted ($N = 158$).

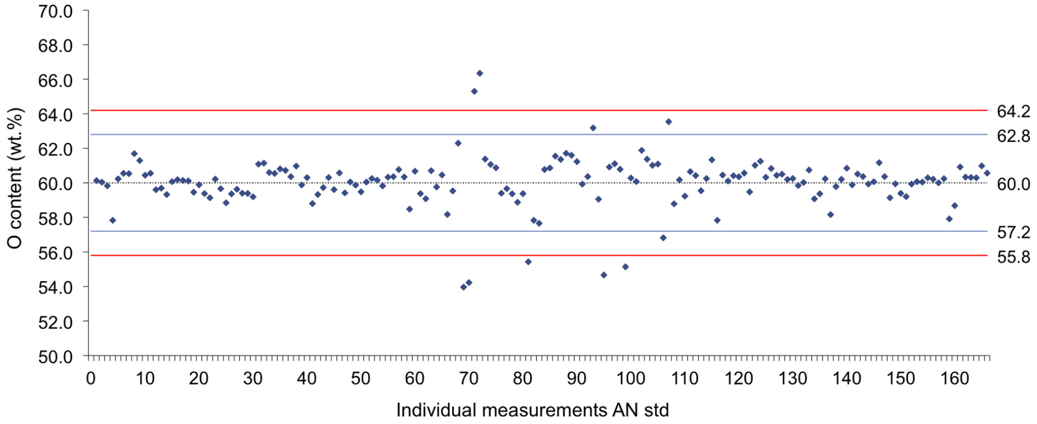

Figure 6.44. Control chart of the % O of AN std. All measurements are plotted ($N = 166$).

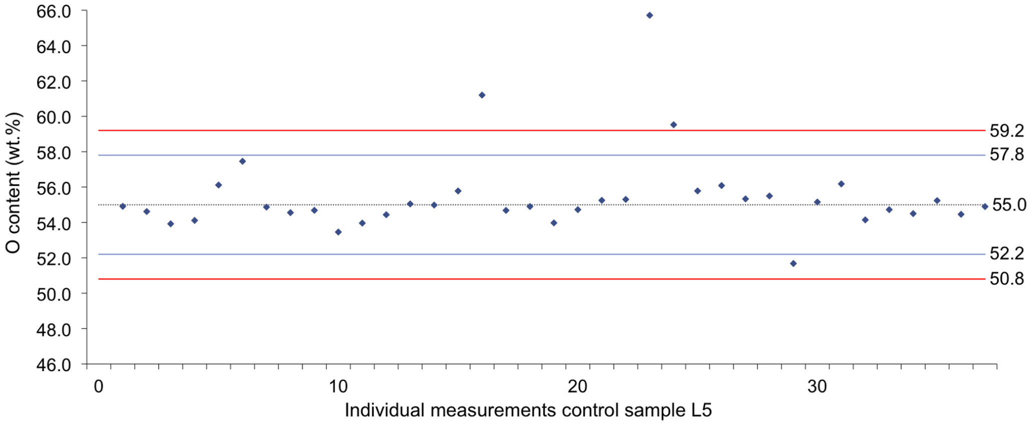

Figure 6.45. Control chart of the % O of the ammonium nitrate fertiliser control sample L5. All measurements are plotted ($N = 37$).

Substances	**% O** **n**	**known** **% O**	***U* [%]** **(k=2)**
Cellulose	47.9 ± 1.1 254	49.3	2.2
MIX	35.6 ± 1.4 154	35.6	1.5
AN std	60.1 ± 1.0 160	60.0	1.4
A1 (BP)	40.5 ± 1.3 58	*NA*	1.1
L5 (AN)	55.0 ± 1.1 35	*NA*	1.2

Table 6.11. Average % O and its estimated expanded uncertainty expressed in wt. %, after exclusion of outlying values.

3.3 Discussion

The $\delta^{18}O$ measurements of the samples and the standards were obtained with a reactor held at 1420°C. The % O measurements showed that the organic laboratory standard, cellulose, had a different oxygen yield that the inorganic working standards and target substances (nitrates). These results suggest different conversion processes according to the nature of the analysed substances.

Literature reports the complete conversion of both substances. On one hand, oxygen measurements of cellulose obtained with high temperature reduction (HTR) (or high temperature pyrolysis) are reported to be quantitative at about 1400°C, contrarily to low temperature reduction (LTR) which produces significant quantities of CO_2, water and carbon deposition in the reactor and directly affects the $\delta^{18}O$ value of the sample at about 1000°C [Leuenberger & Filot 2007]. On the other hand, the oxygen of nitrates is reported to convert completely or nearly completely to CO at high temperatures, with a CO yield of 95-100 % [Kornexl *et al.* 1999].

In nitrogen containing substances, nitrogen is known for being a source of inaccuracies in oxygen measurements. The high % N of some substances, such as nitrates, may interfere with oxygen measurements. The isobaric interference of N_2 with CO can occur when their chromatographic separation is not efficient [Accoe *et al.* 2008]. This in turn not only affects the $\delta^{18}O$ value but also the determination of the % O, as it is calculated based on peak areas. An interesting alternative suggested by Accoe *et al.* is the activation of the dilution of N_2 with Helium before it is introduced in the ion source. In addition, NO is also reported to form in the ion source, influencing the m/z ratio 30/28. In some ion sources, NO is formed by the reaction of N and O (coming from oxygen remaining in the source, from water or small leaks) on the hot surface of the filament [Gehre & Strauch 2003, Accoe *et al.* 2008].

From the obtained results, it is not possible to know whether the conversion of oxygen of cellulose is non quantitative or whether the higher yield of oxygen of nitrates is due to an interference of nitrogen of the sample. However, as the N_2 and CO peaks are well resolved, no element indicates that nitrogen is interfering with the analysis (see **Figure 6.46**).

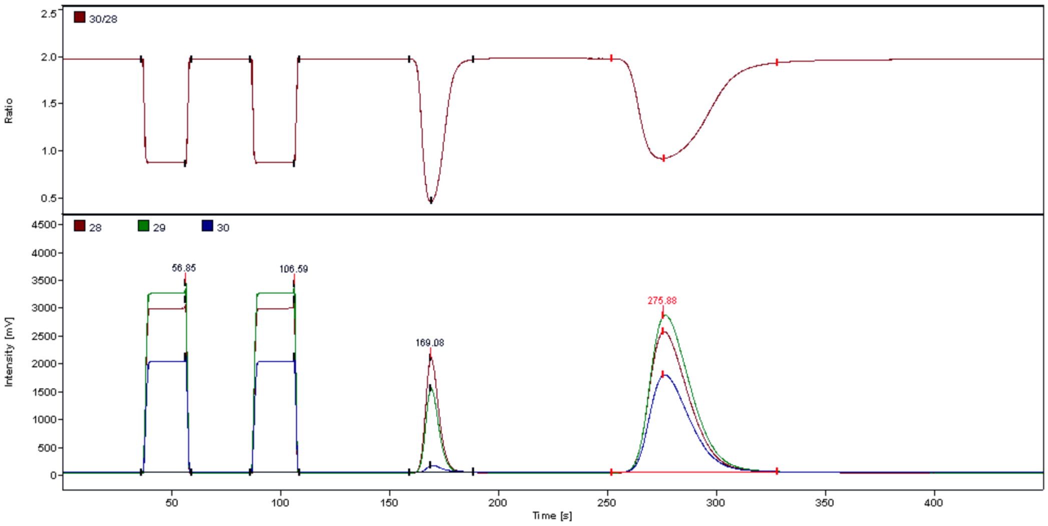

Figure 6.46. δ^{18}O measurement of ammonium nitrate fertiliser L1. The two pulses of reference gas are followed first by the N_2 peak and then the CO peak. The peaks are well separated and the ion current m/z 30 /28 ratio does not show any visible influence of N_2 on the baseline of the ratio.

The results of Benson are extremely interesting [Benson 2009]. She analysed different IAEA reference materials along with AN prills and observed that nitrates did not have the same pyrolysis reaction behaviour as NBS19—a limestone—and LSVEC—a lithium carbonate. On the contrary, USGS 35 (sodium nitrate), USGS34 (potassium nitrate) and the AN prills showed to react similarly. Her results bring attention to the different behaviour of the substances during the pyrolysis cycle, according to the chemical nature of sample.

From a chemical point of view, organic and inorganic substances cannot undergo the same reduction reaction. The oxygen of cellulose is converted to CO using the carbon coming from cellulose, while the oxygen of nitrate combines to the reactive carbon of the reactor to produce CO [Gehre & Strauch 2003]. These different thermodynamic processes of the formation of CO are probably at the origin of the difference in oxygen yield. More fundamental research is needed to investigate these reaction behaviours. As for nitrogen, this issue highlights the significance of using standards or certified reference materials with a similar chemical nature as that of the measured sample [Boettger *et al.* 2007, Benson 2009].

In addition, moisture and memory effects are also two significant issues in oxygen measurements. According to sample hygroscopicity, moisture may interfere with oxygen measurement if the sample is not free from water and may produce lower δ^{18}O values [Boettger *et al.* 2007]. Indeed, one of the major disadvantages of HTR is that the oxygen of H_2O is completely converted to CO [Kornexl *et al.* 1999, Gehre & Strauch 2003, Saurer & Siegwolf 2004, Boettger *et al.* 2007]. Besides, memory effects can arise from exchange processes between the sample gas and surface oxides of the reactor walls [Kornexl *et al.* 1999, Koziet 1997, Saurer & Siegwolf 2004], although the use of glassy carbon instead of elemental carbon already reduces this phenomenon [Koziet 1997, Werner *et al.* 1996].

4 Overview

Overall, tables and control charts have shown the good performances of the methods used for the determination of the δ^{13}C, δ^{15}N and δ^{18}O values. The measured δ values of laboratory standards did not present any bias and the intermediate precision of δ^{13}C, δ^{15}N and δ^{18}O was excellent (0.1‰, 0.1‰ and 0.3 ‰, respectively). Similarly, the δ values of control samples A1 (black powder) and L5 (ammonium nitrate fertiliser) showed a good repeatability throughout sequences with an intermediate precision as good as 0.1 ‰ for carbon, 0.1 ‰ for nitrogen and 0.3 ‰ for oxygen. In the same way, the % C, % N and % O of these control samples could be determined with an acceptable intermediate precision (up to 0.3 % for carbon, 0.7 % for nitrogen and 1.2 % for oxygen). Therefore, the combination of the isotope ratios and elemental contents may be useful in discriminating samples, if there is enough variability between sources.

At another level, the information extracted from the monitoring of standards and control samples have revealed interesting issues within more fundamental aspects. The main observations are:

1. an unexpected shift of the δ^{13}C value of one standard (see δ^{13}C value of the MIX), while the other control materials were not affected by this phenomenon;
2. when measuring δ^{15}N, the distinct behaviour of organic materials on one hand and inorganic substances (such as nitrates and ammonium suplhates) on the other hand, because of their different chemical nature;
3. the different oxygen yields of AN std, MIX and cellulose, indicating a different chemical behaviour during pyrolysis cycle.

These elements highlight an important issue in EA-IRMS, which is related to the instrumental technique itself. From a theoretical point of view, the bulk isotope analysis requires the conversion of the target element into simple gas before entering the IRMS. This conversion step is obtained at elevated temperatures through a flash combustion combined to a reduction step for carbon and nitrogen, and through pyrolysis for oxygen. In both cases, the conversion of the bulk material into simple gas is governed by high temperature reactions, i.e. thermochemistry. The thermochemical considerations developed in [Gentile *et al.* 2013] for the δ^{15}N measurement of organic and inorganic samples attest to the significance of the chemical nature of the substance on one hand, and to the influence of each parameters of the EA system on the other hand. While the cause of the shift of the δ^{13}C value of the standard remains unknown, it is likely that the different yields of oxygen (% O) for AN std and cellulose result from distinct conversion rates of oxygen, related to differences in thermochemical behaviour.

On the basis of the results presented in this chapter, the following recommendations can be made:

- One particularity of isotope analysis is the report of the δ value against an international reference scale. For that purpose, standards calibrated against international reference material are analysed along with sample. This specificity of IRMS renders the selection of standard crucial. As highlighted by the different behaviours of inorganic and organic standards during nitrogen analysis as well as the differences in oxygen yield between cellulose and nitrates, the feature of matrix similarity is critical when selecting adequate standards for sample measurements. As the analyses are operated at high temperatures to convert the bulk substance into simple gas, the thermochemistry underlying the analysis, and the results, are therefore dependent on the nature of the substance. Given that the value of the standard is used to report the results of the samples to the international reference scale, any chemical incoherence between the standard and the samples may bias the results, or at least increase the measurement uncertainty. Therefore, it is highly recommended to select a standard with a matrix as similar as possible to the matrix of the sample.
- When performing nitrogen isotope analysis, inorganic samples, and more specifically, inorganic species where N assumes its highest oxidation state– such as nitrates, should be analysed *without* oxygen injection. Although this is contrary to the usual way of processing sample when performing bulk isotope analysis, this method has proved to give better results than the traditional one, with excellent and repeatable δ^{15}N values and % N.

Chapter 7

Stability of the isotopic profile

Contents

This chapter addresses the stability of the isotopic profile of black powder and ammonium nitrate (AN) fertiliser considering the long term exposure to heat and moisture as well as the short term exposure to moisture.

In the long term exposure experiment, one black powder (sample A1) and one ammonium nitrate fertiliser (sample L5) were separated into three portions and exposed to three different types of environmental conditions for 12 months: at normal ambient conditions (20°C, ∼50 % relative humidity (r. h.)), at high relative humidity (20°C, ∼80 % r. h.) and at elevated temperature (60°C, 0 % r. h.). The influence of each environmental condition on the isotopic profile was observed.

In order to evaluate the influence of moisture on the δ^{18}O value in the short term, an additional experiment involving the exposure of the black powder sample A1 and the AN fertiliser sample L5 to water heavily depleted in ^{18}O (δ^{18}O = -250 ‰) was undertaken for 72 hours.

1 Long term exposure to heat and moisture

1.1 Black powder

1.1.1 Influence on carbon

The effect of storage conditions on the δ^{13}C value and carbon content (% C) is illustrated in **Figure 7.1** as a function of time. The measurements at t = 0 reflect the intravariability of the kg of black powder split into three portions.

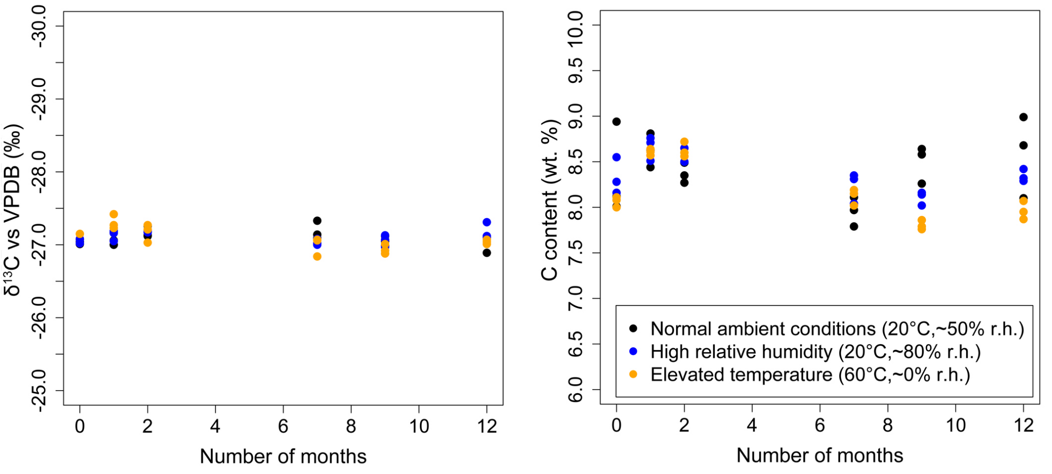

Figure 7.1. Evolution of the δ^{13}C value (left) of black powder sample A1 and its % C (in wt. %) (right) under different environmental conditions over a period of 12 months.

The δ^{13}C variations observed at t = 1 and t = 7 months are slightly higher than the estimated expanded uncertainty associated with the measurement (0.1 ‰), nevertheless they are within the range of variations monitored by the repeated analyses of control sample A1 (see **Figure 6.6**, **page 178**). As the time of exposure to heat and humidity increases, there is no significant difference between the δ^{13}C value of the three portions of black powder stored under different conditions. Over one year, the carbon isotope ratio is not affected by heat (up to 60°C) and moisture (up to 80 %).

A close look at the % C shows that the variations recorded over one year under different storage conditions correspond to the range of variations monitored in the Shewart chart of control sample A1 with a range of 1.4 % and a standard deviation of 0.4 % over 20 months (see **Figure 6.10**, **page 180**). The plot of the % C does not exhibit any trend which could indicate a further evolution or transformation (degradation) of the sample at this stage.

1.1.2 Influence on nitrogen

The δ^{15}N value and nitrogen content (% N) of the black powder sample A1 stored under different conditions exhibit a different pattern from that shown by carbon. The evolution of these two variables is illustrated in **Figure 7.2** as a function of time.

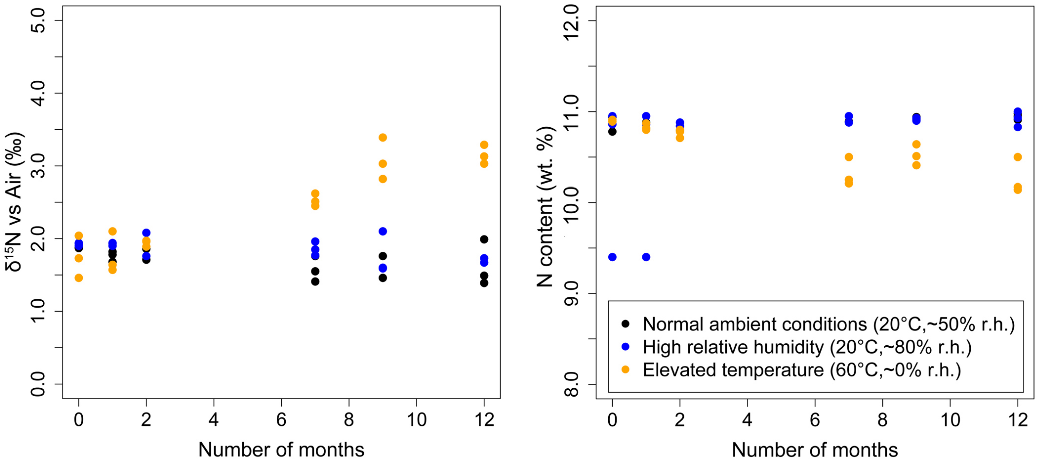

Figure 7.2. Evolution of the δ^{15}N value (left) of black powder sample A1 and its % N (in wt. %) (right) under different environmental conditions over a period of 12 months.

As for carbon, the δ^{15}N values measured at t = 0 provide an estimation of the intravariability of the sample. The nitrogen isotope ratios measured over one year under ambient conditions and high relative humidity (blue and black dots on the plot) correspond to the intravariability of the sample. Thus, there is no significant modification of the δ^{15}N value under these conditions. By contrast, the δ^{15}N value of the portion of black powder exposed to elevated temperature (orange dots) shows an enrichment of 1.4 ‰ after 12 months exposure (difference between means at t = 0 and t = 12). This enrichment seems to follow a linear relation as a function of time. The deviation of the enriched mean δ^{15}N value from its original value (mean δ^{15}N value at t = 0) is not covered by the estimated expanded uncertainty associated with the measurement (0.2 ‰) and is statistically significant ($p < 0.05$).

Under ambient and high relative humidity conditions, the % N was very stable over 12 months except for two outlying measurements (at t = 0 and t = 1). As attested by the excellent intermediate precision of the % N measurement of control sample A1 (see **Figure 6.29**, **page 203**) and the small associated expanded uncertainty (0.1 %), these two outlying points are not part of the intravariability of the sample. They likely come from the sample preparation, which was modified at some point for the second nitrogen method. Indeed, after sealing the tin capsule, the excess of tin foil was removed from the capsule in order to avoid the creation of a plug of liquid tin in the combustion reactor which could affect the repeatability of measurements. In some cases, few particles of material could come off with the removal of extra tin foil, resulting in a loss of the weighed sample, and therefore, in a diminished % N. Given these considerations, the significance of the evolution of the % N under elevated temperature can be fully appreciated. Exposed at 60°C, it showed a decline after 12 months (decrease of 0.6 % N between means at t = 0 and t = 12). This difference is statistically significant ($p < 0.05$) and is likely related to the enrichment of the $\delta^{15}N$ value. This nitrogen loss supports the hypothesis of the evaporation of a nitrogen-containing molecule under elevated temperature.

Thus, exposing black powder to a temperature of 60°C over one year induces an enrichment of the $\delta^{15}N$ value and, in parallel, a diminution of the % N (nitrogen loss). This isotopic fractionation is consistent with the evaporation of the lighter nitrogen-14-based molecules compared to the heavier ones, resulting in an isotopic enrichment of the black powder and combined with a loss in nitrogen.

1.1.3 Influence on oxygen

Figure 7.3 illustrates the influence of environmental conditions on the $\delta^{18}O$ value and oxygen content (% O) of black powder.

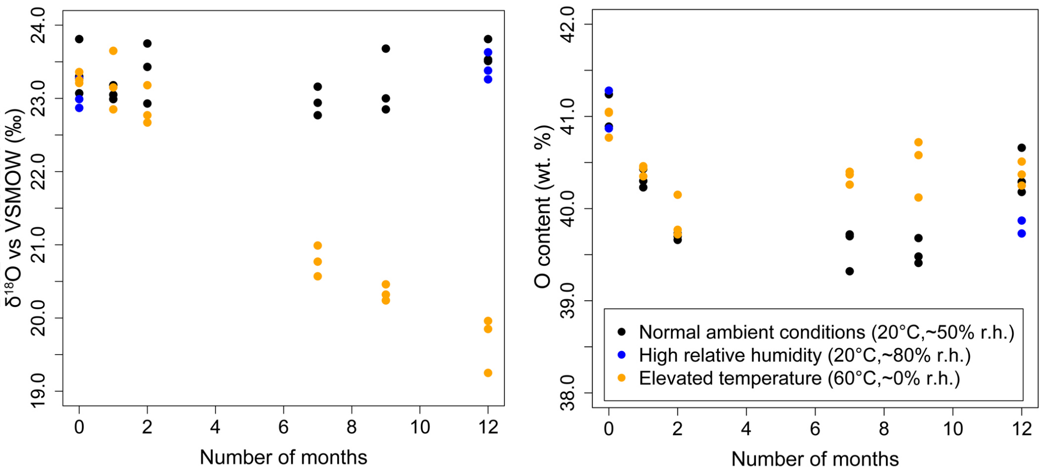

Figure 7.3. Evolution of the $\delta^{18}O$ value (left) of black powder sample A1 and its % O (in wt. %) (right) under different environmental conditions over a period of 12 months.

The $\delta^{18}O$ variations of the sample stored under normal ambient conditions (black dots on the plot) are within the range of variations observed in the Shewart chart of control sample A1 (see **Figure 6.39**, **page 213**). As all $\delta^{18}O$ measurements were performed one year after the end of the storage experiment, samples collected at t = 0 and t = 12 were analysed first in order to see whether there were isotopic differences. The analysis of black powder exposed to high relative humidity collected at t = 0 and t = 12 (blue dots) showed there was no difference between both samples. Their δ^{18}O values were within the range of variations of the sample stored under ambient conditions. Thus, high relative humidity does not affect the δ^{18}O value of black powder. For these reasons, the samples stored under high relative humidity and collected throughout the year were not further analysed.

Concerning the black powder exposed to elevated temperature, the δ^{18}O value linearly decreased as a function of time from 23.3 ± 0.1 ‰ at $t = 0$ to 19.7 ± 0.4 ‰ at $t = 12$. This difference of 3.6 ‰, which is statistically significant ($p < 0.05$), demonstrates that heat can induce a significant depletion of the δ^{18}O value of black powder. This depletion does not support a phenomenon of evaporation as normally such a phenomenon leads to the enrichment of the substrate. However, this issue is not always straightforward. For hydrocarbons compounds for example, isotopic fractionation is controversial. Shin and Lee described the significant enrichment of δ^{13}C of the remaining liquid of toluene and benzene after evaporation [Shin & Lee 2010], while others reported a slight depletion of the non distilled fractions of aromatic compounds [Harrington *et al.* 1999]. Thus, the evaporation of an oxygen-containing molecule resulting in a depletion of the δ^{18}O value of black powder cannot be excluded. Further experiments should be undertaken in order to better understand the cause of the observed depletion.

The examination of the evolution of the % O during storage does not reveal any significant trend. As attested by the repeated measurements of control sample A1 and the associated expanded uncertainty (1.1 %), the measurements of the % O under the different conditions just reflect the intravariability of the black powder sample. Thus, the % O data does not assist in unveiling the phenomenon responsible for the depletion of the δ^{18}O value.

Time equivalence of the observed changes under ambient conditions As both the δ^{15}N and δ^{18}O values of black powder were linearly affected by elevated temperature conditions, this may indicate that either two independent phenomena affect these elements during storage under elevated temperature or a common reaction involving nitrogen and oxygen at the same time occurs, such as one releasing NOx for example. Literature brings some light on the possible reactions occurring during the thermal analysis of black powder. According to different studies [Russell 2000, Turcotte *et al.* 2003a], the thermal decomposition of black powder is essentially driven by a pre-ignition reaction between S and KNO_3, followed by a main reaction between charcoal and KNO_3. It is interesting to note that the pre-ignition reaction is reported to be initiated by the energy released from the oxidation of organic materials originating from the charcoal. Some authors cite the reaction of S with volatile organic material and the formation of NO_2 from KNO_3 with organic material [Russell 2000]. Using various thermal techniques, Turcotte *et al.* studied in details the thermal analysis of black powder. They described first the rhombic to monocyclic sulphur phase transition at 108 °C, followed by the melting of sulphur at

133 °C and the KNO_3 rhombic to trigonal phase transition at 133 °C. The melting of KNO_3 occurred at around 330°C and a last broad exothermic peak corresponding to the oxidation of the charcoal by KNO_3 was seen between 350 and 450°C. The gaseous products were also detected by coupling FTIR and /or MS to thermal techniques. Except for water, the major gaseous products (CO_2, NO and N_2O) only started to be observed from 200°C. None of these reactions occured in the range of 60°C. Nevertheless, the authors noticed that when experiments were undertaken under an oxidative atmosphere (instead of an inert one) with a smaller heating rate and involving a larger sample size with larger grain size, the onset temperature of the reactions was lower of several tens of degrees. Furthermore, none of the studies undertook isothermal analyses of black powder. The thermal behaviour of black powder may be somehow different if studied in an isothermal mode or in a controlled temperature program. Interestingly, their results also showed that two black powders from different manufacturers may have different endotherms and exotherms. Indeed, because of the variability of its characteristics, a difference in charcoal may result in black powders with significant different thermal behaviours. **Figure 7.4** illustrates the thermal behaviour of a sample of black powder manufactured by Aubonne, analysed by differential scanning calorimetry (DSC).

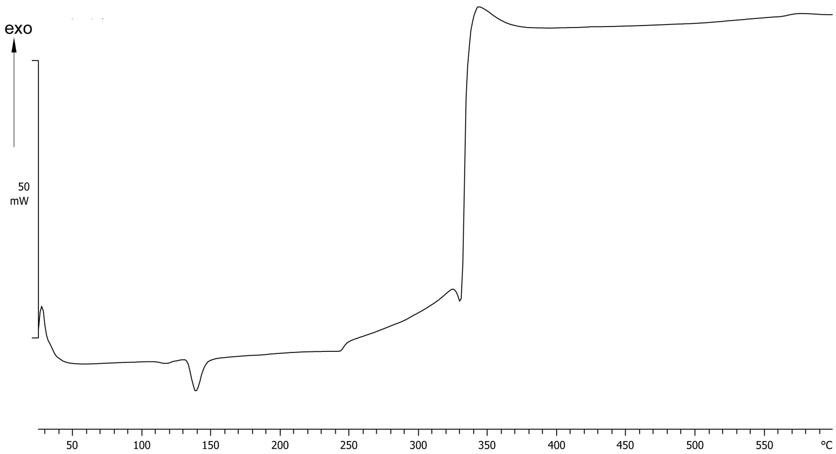

Figure 7.4. DSC curve of Aubonne black powder obtained with a heating rate of 70°C/min in air.

Eventually, a thermal analysis coupled to MS or FTIR of the specific black powder used in this storage experiment would assist in the identification of the released compounds and possibly provides hints on the phenomenon that takes place.

When a change (transformation or degradation of the product or its properties through the formation of new compounds or through the increase or decrease of individual compounds, etc.) is observed during accelerated ageing, a common procedure consists in calculating the time equivalence under "normal" storage conditions necessary to observe the same modification [Vogelsanger 2004]. For drugs, most reaction rates of the formation or degradation of a product follow the Arrhenius' law:

$$lnk = lnA - E_a/(R * T) \tag{7.1}$$

where k is the reaction rate, A is a the pre-exponentional factor (a constant specific to the substance which is proportional to the frequency of collisions between molecules), E_a is the activation energy, R is the gas constant and T is the temperature. It is however recognised that, in practice, many systems do not conform to Arrhenius kinetics because of various factors (phase transitions, pH shifts, uncontrolled relative humidity, complex reaction mechanisms and changes in Arrhenius parameters with temperature) [Waterman & Adami 2005]. In order to make sure that the reaction follows an Arrhenius behaviour, it is necessary to have several values of the reaction rate k determined with different temperatures. This also allows determining the corresponding action energy E_a and the pre-exponentional factor A.

In this experiment, the enrichment of the δ^{15}N value and the depletion of the δ^{18}O value were only observed under elevated temperature at 60°C. Therefore, given the limited data, it is not possible to verify whether the reaction(s) follow an Arrhenius kinetic. The equivalent time under normal storage conditions are provided here only as an indication, as the calculations were made with an estimation of the activation energy, assuming Arrhenius's kinetic. Literature provides different figures for the activation energy of black powder depending on its sulphur content (from 56 to 130 kJ/mol) [Russell 2000]. Based on these figures, time equivalence was calculated using Arrhenius equation. **Table 7.1** provides an estimation of the equivalent storage time of black powder under ambient temperature after which a 1.4 ‰ enrichment of δ^{15}N and a 3.6 ‰ depletion of δ^{18}O would be observed.

Activation energy E_a (kJ/mol)	**Time equivalent (years) storage at 25°C**
56	10
93	51
130	247

Table 7.1. Estimation of the equivalent storage time under normal ambient conditions of black powder after which a 1.4 ‰ enrichment and a 3.6 ‰ depletion would be observed for δ^{15}N and δ^{18}O values, respectively.

Considering the smaller and larger activation energies, the estimation of time equivalence oscillates between 10 and 247 years! This large range highlights the importance to have accurate E_a values, especially as E_a may be dependant on temperature, meaning that it can be different from one range of temperatures to another. Furthermore, these estimations should be considered with caution as the figures of E_a provided by literature are the activation energy measured for the *ignition* of black powder. Thus, it probably does not correspond to the activation energy producing the δ^{15}N enrichment or δ^{18}O depletion of black powder. The reaction(s) responsible for these isotopic fractionation are unknown; they may involve the re-arrangement of the elements and/ or a bond breakage of the molecule releasing N, O or NO. Without an empirical determination of E_a, it is impossible to associate it with a precise activation energy. Therefore, estimations presented in **Table 7.1** are indicative and should be confirmed by thorough experiments involving additional temperatures in order to determine the exact activation energy of the reaction(s). This approach would also permit to confirm or invalidate whether the reaction (s) follow an Arrhenius behaviour.

1.2 Ammonium nitrate fertiliser

Stored under high relative humidity, the portion of ammonium nitrate fertiliser rapidly showed signs of hygroscopicity. Although the prills are coated with a water repellant layer, white dots appeared at the surface of the prills after one month. Two months after the beginning of the experiment, the layer of prills in contact with the air became muddy and small crystals were visible on the sides of the receptacle. After five months, the prills were completely covered with water. Prills had transformed into a muddy lump and large crystals had formed on the upper parts of the sides of the receptacle. For these reasons, the portion of sample submitted to high relative humidity was withdrawn from the experiment. At that time (t = 5), two samples were collected: one from the muddy part and one from the crystals. The chemical nature of the crystals is unknown, however given their % N (see results below), it is likely that it corresponded to re-crystallised pure ammonium nitrate. The isotope ratios of both types of samples were measured. They are plotted on the following figures with the same colour (blue) but different symbols.

1.2.1 Influence on nitrogen

The influence of the environmental conditions on the δ^{15}N value and % N of the ammonium nitrate sample is illustrated in **Figure 7.5**.

The δ^{15}N measurements under the different conditions at t = 0 reflects the intravariability of the AN fertiliser. These variations are within the range of values plotted in the Shewart chart of control sample L5 and reflects the intravariability of the sample. Under high relative humidity (blue dots), the δ^{15}N value is not significantly modified, although a change in the aspect of the sample was observed during the experiment. Even after 5 months, the physical phenomenon that split the sample into two entities of different aspect (mud and crystals) did not result in a significant fractionation. The value of the crystals are slightly depleted compared to the muddy part, but the difference is not statistically significant ($p > 0.05$). This moderate difference may come from the fact that the sample is not much enriched in ^{15}N, limiting therefore the effects of isotopic fractionation.

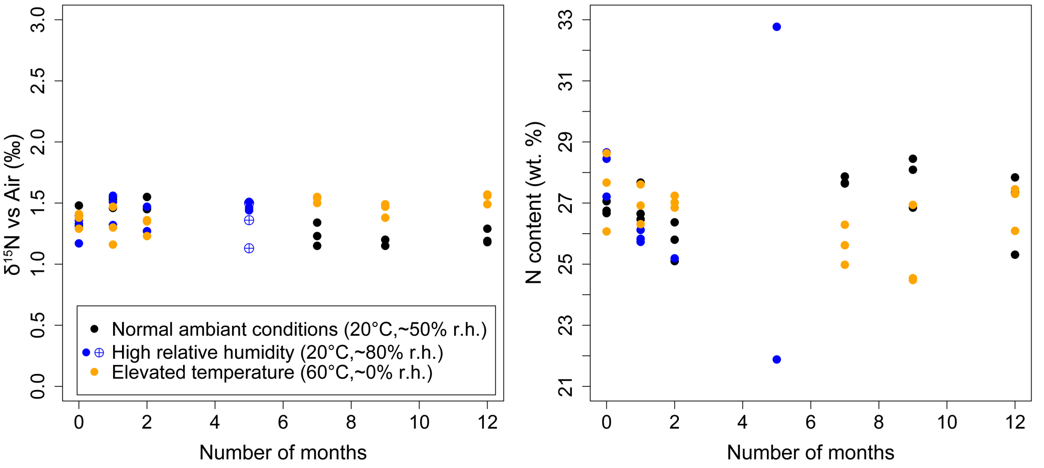

Figure 7.5. Evolution of the δ^{15}N value (left) of AN fertiliser sample L5 and its % N (wt. %) (right) under different environmental conditions over a period of 12 months. The two blue symbols at t = 5 represent one sample collected from the muddy prills (plain blue dots) and a second sample collected from the crystals that had formed on the sides of the receptacle (blue dots with a cross).

The % N of the portion stored under high relative humidity shows a slight decrease, which is also displayed by the sample stored under normal ambient conditions. A major difference in % N concerns the samples collected at $t = 5$ (not all visible on the plot). While the % N of the crystals was rather repeatable with a mean around 35.5 % (not visible on the plot), that of the muddy part of the sample was highly variable (only two points are plotted on the graph, the third is out.). Given the mean % N of the crystals, it is possible that it corresponded to pure ammonium nitrate that re-crisallised after having been dissociated by water. Concerning the muddy prills, as the samples slowly attract water, weighed amounts of the samples included moisture or water, influencing thus the effective amount of weighed fertiliser and resulting in a highly variable % N.

The δ^{15}N value of the AN fertiliser stored under elevated temperature (orange dots) is slightly more enriched than that of the portion stored under ambient conditions (for $t = 7$, 9 and 12). An isotopic difference of 0.2 ‰, which is statistically significant ($p < 0.05$), is measured between means at $t = 0$ (encompassing all 9 measurements) and $t = 12$. At the same time, the % N at $t = 7$ and 9 is also smaller. δ^{15}N measurements at $t = 7$, 9 and 12 and % N measurements at $t = 7$ and 9 might be indicative of a reaction involving nitrogen, such as for black powder. However, these differences should be interpreted with caution given the intravariability of the sample (see **Figures 6.30** and **6.34**, **pages 203** and **206**). Thus, the δ^{15}N value is not significantly influenced by elevated temperature. Nevertheless, these results need to be confirmed by additional experiments, either on a longer period or, in a simpler way, with higher temperatures experiments. Besides, further work could test the accelerated ageing of several ammonium nitrate fertilisers, in order to see whether the additives have an impact on the ageing of the fertiliser.

1.2.2 Influence on oxygen

Figure 7.6 illustrates the influence of the different environmental conditions on the δ^{18}O value and % O of the AN fertiliser. As all δ^{18}O measurements were performed one year after the end of the storage experiment, samples of the portion exposed to elevated temperature collected at t = 0 and t = 12 were analysed first. Because the δ^{18}O values measured at t = 12 were not significantly different from the ones collected at t = 0, further analysis of the samples stored under elevated temperature was not undertaken.

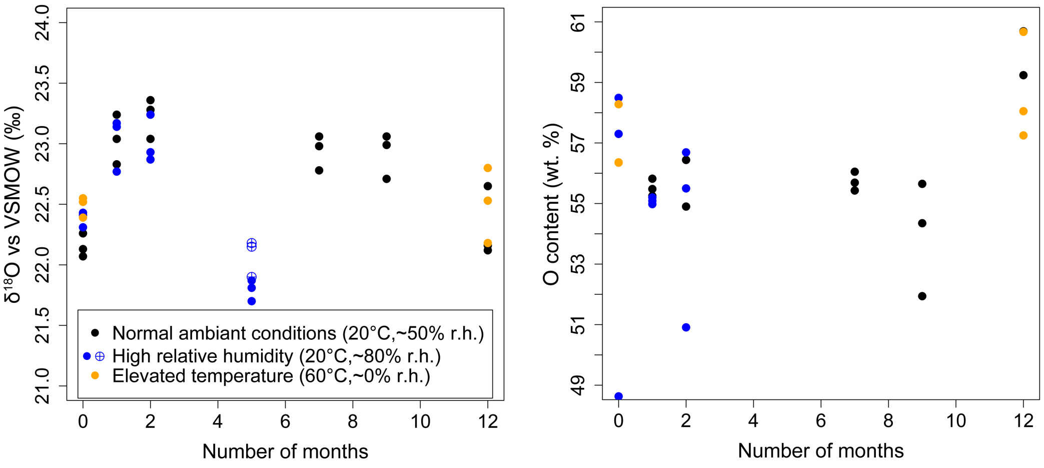

Figure 7.6. Evolution of the δ^{18}O value (left) of AN fertiliser sample L5 and its % O (wt. %) (right) under different environmental conditions over a period of 12 months.

The δ^{18}O values and % O measured at t = 0 reflect the intravariability of sample L5. However, the mean δ^{18}O value at t = 0 is curiously lower than the mean of control sample L5 (see **Table 6.10**). Except for values at t = 0 and 12 for normal ambient conditions and t = 5 for high relative humidity conditions, the evolution of the δ^{18}O value of samples stored under ambient and high relative humidity conditions (black and blue dots on the plot) is within the calculated expanded uncertainty (0.4 ‰) and intravariability of control sample L5 (range of about 1 ‰). The δ^{18}O values of both samples collected at t = 5 are depleted by approximately 1 ‰. This depletion is possibly due to atmospheric water vapour, as the δ^{18}O of water range from about -5 ‰ to -15 ‰ [Saurer & Siegwolf 2004]. It is however unclear, why this effect was not already seen after 1 or 2 months exposure. It is possible that dissociation of AN fertiliser by water and the subsequent re-crystallisation involved an exchange of oxygen between water and nitrate.

The % O of the samples did not show any significant increase or decrease. Despite a calculation of the % O relative to AN std, measurements at t = 0 and t = 12 are all too high except for one measurement (48.6 %). The measurements of the sample stored under ambient conditions are out of the plot with more than 62 % O. These high oxygen yields in conjunction with their respective lower δ^{18}O values appear to come from analytical interference. Indeed, measurements collected at t = 0 and t = 12 were analysed in the same sequence. These anomalies can originate from an incomplete conversion of standards into CO or from an air leak in the system. Similarly, the poor repeatability of % O measurements at t = 0, 2 and 9 likely comes from the problems identified in chapter 6, **Section 3.2**. The oxygen yields of samples collected at t = 5 under high relative humidity are not visible on the plot with approximately 40 % O. As the samples contained much water, the weighed samples encompassed AN fertiliser and water, leading to a reduced % O.

Thus, except for samples collected at t = 5 under high relative humidity, the variations of the δ^{18}O value and % O are within the precision of the method. There is no significant influence of ambient and elevated temperature conditions on oxygen. Only high relative humidity affected the δ^{18}O value of the sample after 5 months exposure, leading to a 1 ‰ depletion. However, as all samples were analysed about one year after the end of the experiment, they were exposed to the ambient water vapour present in the glass container and possibly to the water vapour of the surrounding atmosphere. Therefore, the time elapsed between collection and analyses renders the interpretation of the 1 ‰ difference delicate, as additional sorption or exchange mechanisms may have occurred in the meantime. Therefore, the depletion of 1 ‰ cannot be directly related to the exposure to high relative humidity conditions.

As the samples were not analysed immediately, the influence of high relative humidity on the δ^{18}O value of the samples could not be precisely evaluated. In an attempt to further investigate the influence of water on the δ^{18}O value of the samples, an additional experiment, in which samples were analysed immediately after collection, was undertaken involving the short exposure of samples to water very depleted in ^{18}O.

1.3 Discussion

The results of this experiment showed the stability of the isotopic profile of black powder and AN fertiliser under ambient storage conditions over one year. Under high relative humidity, the isotopic profile of black powder was not influenced, while the δ^{18}O value of AN fertiliser displayed a depletion of about 1 ‰ after 5 months. The δ^{15}N value of AN fertiliser was not affected though. These results confirm those obtained by Benson who studied the influence of atmospheric moisture on the δ^{15}N value of AN prills for 8 days [Benson 2009]. No significant variation was reported between samples exposed to laboratory environment (left uncovered on a laboratory bench) and those stored in a desiccator.

Concerning temperature of storage, the results of this experiment provide an indication on the irreversible effects of elevated temperature (60°C) on the δ^{15}N and δ^{18}O values of black powder, resulting in a 1.4 ‰ enrichment of the δ^{15}N value and a 3.6 ‰ depletion of the δ^{18}O value after one year. Besides, the exposure of AN fertiliser to elevated temperature induced a slight δ^{15}N enrichment of 0.2 ‰ after one year. However, as this difference is small, interpretation of results would benefit from additional accelerated ageing experiments undertaken at higher temperatures and involving supplementary AN fertilisers with different additives.

This study brings useful information on the evolution of the isotopic profile under different environmental conditions. Nevertheless, interpretation of results is limited by:

- the restricted number of samples of each type (one black powder and one ammonium nitrate). Further research involving several black powders from different manufacturers and ammonium nitrate fertilisers with distinct additives could confirm the results or produce complementary data about the stability of the isotopic profile. As the stability of isotope ratios concerns the atomic level of a substance, any additive, coating or even contaminant may influence the activation energy necessary for a reaction to take place;

- the limited accelerated ageing conditions considered in this experiment (one temperature for elevated temperature conditions and one value of relative humidity for high relative humidity conditions). Additional temperatures and relative humidity states would reveal whether reactions follow an Arrhenius behaviour and provide additional data for the calculations;

- the time elapsed between the collection of the samples and their analyses. For different reasons, the samples could not always be analysed immediately after their collection. Thus, there is an undeniable influence of the time accounting between the collection and the analysis of the sample. This time fluctuated between 0 and 12 months for carbon. For nitrogen all samples were re-analysed with the second method 4 years after the end of the experiment. For oxygen the time between collection and analysis was between 11 and 23 months. As an example, the effective storage period of a sample exposed at 60°C for 2 months, but which was analysed only 2 months later, corresponds in reality to an actual period of 2 months under elevated temperature and 2 supplementary months stored in the dark in a glass container sealed with Parafilm under ambient conditions (conditions of storage after collection).

The interaction of the Parafilm or the air in the glass container with the sample was also considered. For carbon, the δ^{13}C value of the black powder stored in a room for 12 months under ambient conditions was compared to that of a black powder stored in the dark in a glass container sealed with Parafilm for 12 months. No significant difference was observed between the two samples, indicating that the storage of the sample in the dark and in a flask sealed with Parafilm did not cause any interference with the determination of δ^{13}C.

For nitrogen, the comparison of data is even more difficult, as all samples were re-processed with the second nitrogen method about 4 years after the last sample collection. Therefore, all samples were collected after their storage time (between 0 and 12 months) and stored at ambient conditions in the dark in the glass container for 4 to 5 years. Because the second method produced different δ^{15}N values, it is not possible to compare data obtained with the first method. However, it is interesting to note that data produced with the first nitrogen method, obtained in 2008 [Gentile *et al.* 2009], show the same influence of elevated temperature on the δ^{15}N value of black powder. Results obtained with this first method were less accurate and less precise, however a similar isotopic difference (1.3 ‰) was also detected between the means measured at t = 0 and at t = 12. This seems to indicate that the δ^{15}N values of all samples collected 4 to 5 years earlier did not change during the storage in the dark in the glass container or that they were all affected in the same way. This supports the hypothesis that the exposure of black powder to elevated temperature (60°C) induces irreversible effects, which can still be measured 5 years later.

For oxygen, all δ^{18}O data on black powder and ammonium nitrate were acquired one year after the last sample collection. However, no significant difference was observed between samples stored for two years in the dark in a sealed glass container and those stored for one year in a room under ambient conditions for one additional year in the dark in a glass container.

Implications Results show that the isotopic profile can be subject to changes according to the environmental conditions. These changes are, for black powder, irreversible, which means that, in a forensic perspective, the comparison of the isotopic profile of two specimens coming from the same "source", but with different histories (which is almost unavoidable), can lead to a wrong discrimination (they are considered as specimens of different "sources", while they are not), contributing to increase the rate of false negative. Inversely, the comparison of two specimens coming from distinct "sources" and presenting by chance, a similar isotopic profile due to the ageing of one of the specimen, may lead to a wrong association or identification (the specimens are said to come from the same "source", while they are not in reality), producing thus a false positive. This situation producing a false positive seems however less likely to occur than the case resulting in a false negative. If we were to take these isotopic shifts into consideration for the evaluation of isotopic information in real cases, calculations based on Arrhenius equation offer the possibility to estimate either the time equivalence necessary to observe a given isotopic change under defined storage conditions or the expected isotopic change under given environmental conditions. In the present research, the estimation of the time equivalence to observe the measured isotopic shifts when the sample is stored under normal ambient conditions oscillates between 10 and 247 years, depending on the activation energy considered. As mentioned before, these figures are indicative and would need to be strengthened with the correct and empirical activation energy. Nevertheless, modelling the ageing of a substance requires to have knowledge or indications on the storage conditions of the substance.

2 Short term exposure to moisture

The cellulose standard, the MIX, the AN std, the black powder samples A1 and the AN fertiliser sample L5 were placed in a desiccator in which the desiccant was replaced by a container filled with highly depleted water of known δ^{18}O value (-250 ‰). The exposure to water vapour was operated at room temperature during 3 days (72 hours). Samples were collected after 6, 12, 24 and 72 hours. Standards and samples were analysed in triplicates using the Zero Blank autosampler at the beginning of the experiment before any exposure to moisture and immediately after each collection. Standards and samples exposed to highly depleted water vapour were analysed along with the traditional standard cellulose kept dry in a desiccator in order to correct raw data to the international reference scale.

2.1 Black powder

The mean δ^{18}O values and the standard deviations of cellulose, MIX and black powder exposed to depleted water are plotted as a function of time in **Figure 7.7**. The δ^{18}O value of cellulose showed to be rather constant during exposure, with a slight depletion (-0.3 ‰) after 72 hours. The difference is however not significant given the intravariability of cellulose. In comparison, the δ^{18}O value of black powder and the MIX were more variable during exposure. After 72 hours, black powder also showed a depletion, which is however not statistically significant ($p > 0.05$). While the repeatability of cellulose was not affected by water exposure, the slight depletion of black powder was associated with a poorer repeatability (see lower part of the figure).

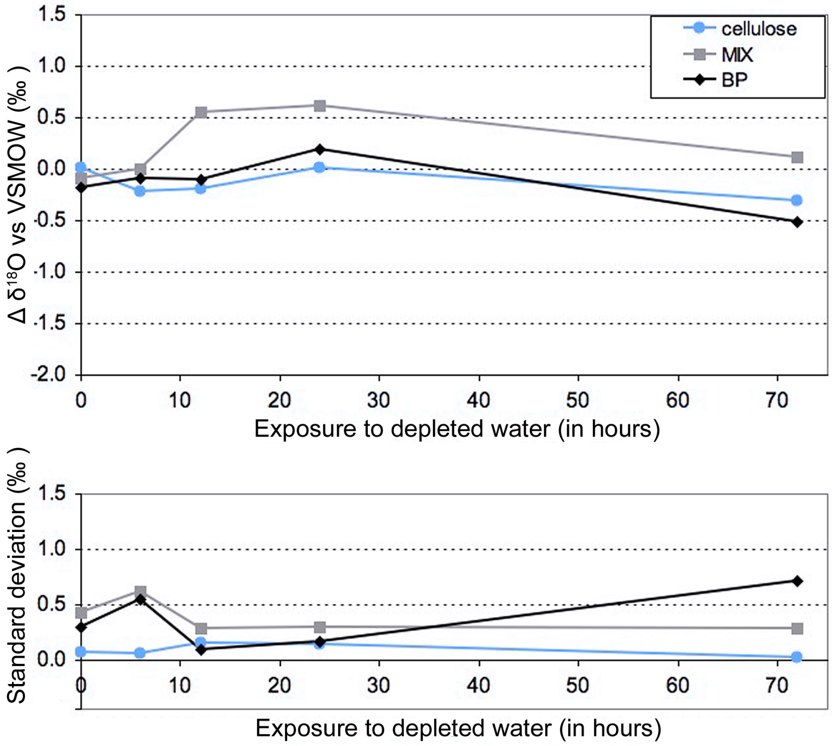

Figure 7.7. Difference of the mean δ^{18}O value with its expected value (upper part of the figure) for cellulose, MIX and the black powder sample exposed to depleted water and analysed after t = 0, 6, 12, 24 and 72 hours exposure. The lower part of the figure illustrates the evolution of the associated repeatability as a function of hours of exposure.

Figure 7.8 illustrates the evolution of the % O of cellulose, MIX and black powder under these conditions. The exposure to moisture induces a clear loss and variability in the measurement of the % O of the MIX and the black powder sample after 72 hours.

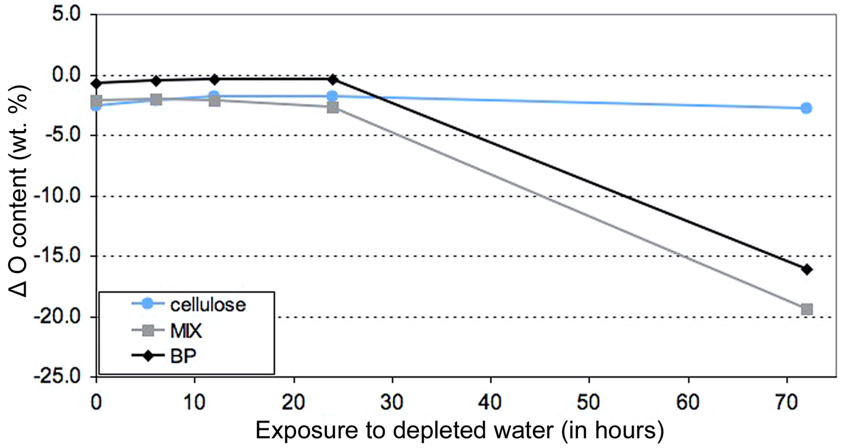

Figure 7.8. Difference of the % O (wt. %) with its expected value for the MIX, black powder and cellulose during 72 hours exposure to depleted water.

2.2 Ammonium nitrate fertiliser

Figure 7.9 displays the influence of heavily depleted water exposure on the δ^{18}O value of AN std, AN fertiliser and cellulose. Except for samples collected after 6 hours (depletion of 1.5 ‰ for the AN fertiliser), there is no significant effect on the δ^{18}O value of ammonium nitrate between the ones collected at t = 0 and t = 12 hours ($p > 0.05$). In addition, the decrease in repeatability of AN std and AN fertiliser variability at t = 72 hours is also observable (see lower part of the figure). The depletion registered for AN fertiliser and AN std only at t = 6 hours is actually due to a downward drift in the instrument response (shown by a dotted line on the plot) ; it is therefore not significant.

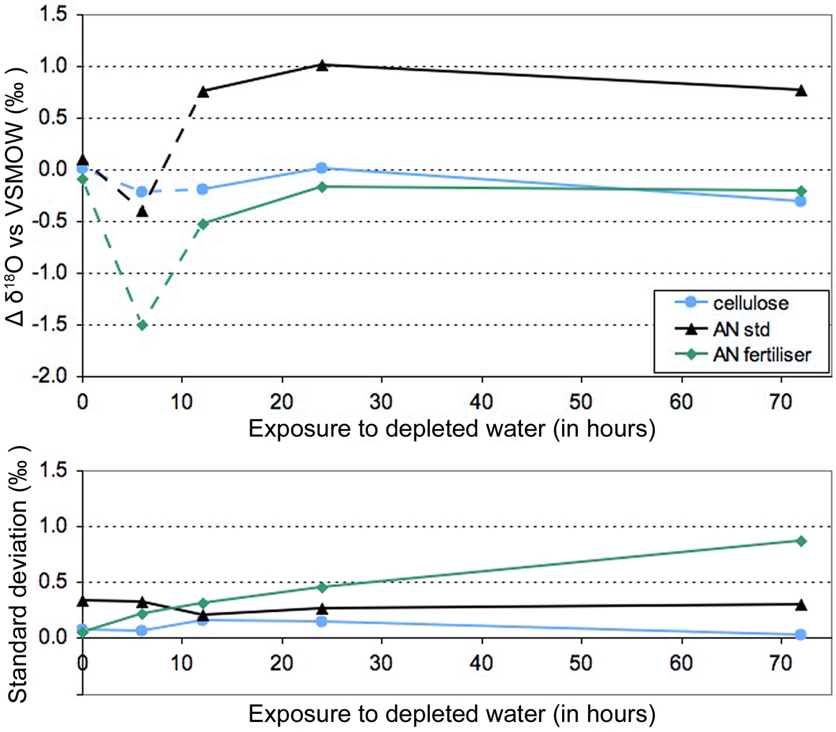

Figure 7.9. Difference of the mean δ^{18}O value (upper part of the figure) with its expected value for cellulose, AN std and AN fertiliser exposed to depleted water and analysed after t = 0, 6, 12, 24 and 72 hours exposure. The lower part of the figure illustrates the evolution of the associated repeatability as a function of hours of exposure.

Plotted as a function of time in **Figure 7.10**, the difference of % O with its expected value shows a similar trend as for black powder. The % O of AN std and AN fertiliser dramatically diminish (already after 6 to 12 hours for AN std), leading to a significant loss of oxygen after 72 hours.

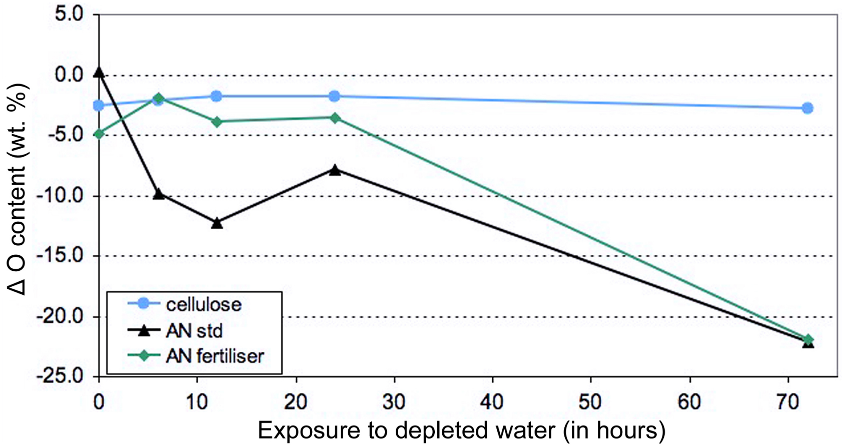

Figure 7.10. Difference of the % O (wt. %) with its expected value for the AN std, AN fertiliser and cellulose during 72 hours exposure to depleted water.

2.3 Discussion

Results show that depleted water vapour exposure does not induce fundamental change on the δ^{18}O value of black powder and ammonium nitrate in the short term. Somehow surprising for hygroscopic substances, this indicates that water is adsorbed but that there is no exchange of oxygen at the atomic level. These results confirm the conclusions of Benson, who found similar results when exposing chemical grade AN and AN prills to atmospheric moisture for 7 days [Benson 2009]. The δ^{18}O value was not significantly different between samples stored in a desiccator and those left uncovered on a laboratory bench. She also dried the samples for 3 hours in an oven heated at 60°C after moisture exposure. This drying step did not result in a significant change of the δ^{18}O value, supporting the hypothesis of water sorption rather than water exchange.

It is also interesting to compare the results of this experiment with those presented by Saurer and Siegwolf, although the nature of the samples is different [Saurer & Siegwolf 2004]. They observed a clear impact of water vapour on the δ^{18}O value of cellulose of different types of trees equilibrated with laboratory air. The difference between dried samples and those that were equilibrated could be as large as 1 ‰. However, at that period, measurements were made without a Zero Blank autosampler. In this experiment, as the δ^{18}O of cellulose was not significantly affected by the exposure to depleted water, this could indicate that the use of a Zero Blank autosampler efficiently removes the sorbed water and prevent from any adverse effect on the δ^{18}O value. With that type of autosampler, the samples are vacuum-dried for 30 minutes through the evacuation of air and moisture from the autosampler, before being refilled with helium. This step likely removes the exogenous oxygen from the depleted water vapour and atmospheric moisture. Thus, the use of a Zero Blank autosampler is recommended when processing hygroscopic substances for oxygen isotope analysis.

Although accuracy of the isotope ratio was not changed, the exposure to moisture clearly affected the repeatability of the $\delta^{18}O$ value and % O. Additional tests performed without the Zero Blank autosampler showed that the repeatability was even poorer when moisture from the sample was not eliminated. It is worth noting that Benson also experienced large imprecision in the $\delta^{18}O$ measurement of AN prills.

Finally, an unexpected decrease of the % O of the black powder and AN fertiliser could indicate that water possibly dissociated the nitrate based molecule after some time. This hypothesis assumes that, when the sample is submitted to vacuum in the autosampler, residual moisture and hydrolysed oxygen from the molecule are evacuated and result in a loss of oxygen. Yet, this loss does not affect significantly the $\delta^{18}O$ value.

CHAPTER 8

Differentiation of sources of black powders

Contents

1 Introduction

In chapter 5, hypothesis IV.1 defined the source of black powder as the manufacturer. In the following sections, we will examine the results of the experiments to evaluate the intravariability of black powder produced by a manufacturer. Several levels of information have been considered: first, the variability within a kilo (or half kilo) of black powder, secondly, the variability within production batches and finally the variability within the production of a manufacturer.

For the manufacturer of Aubonne, information on the manufacture of black powder and the constitution and definition of a batch were collected at the powdery of Aubonne [Fiaux 2006]. This knowledge allowed performing a sampling of the daily production and their associated final batch in the production line in order to evaluate the variability measured by isotope ratio analyses. For Wano and Vonges, the intravariability of a kilo and batch was evaluated on the basis of samples from half kilo obtained for each batch. Intervariability was evaluated by assessing the variability between manufacturers.

2 Carbon content and isotope ratio

2.1 Variations within a manufacturer

2.1.1 Variations within a kilo and half kilo

Figure 8.1 illustrates the δ^{13}C and % C variations measured within a kilo and half kilo of black powder, quantity which is typically representative of the amount sold separately as unit on the market [Fiaux 2006]. The δ^{13}C and % C intravariabilities in a kilo or half kilo of black powder is slightly larger for Aubonne and lower for Wano. In reality, measurements of samples of Wano and Vonges were obtained from two different, but contemporaneous sequences, while those of the sample of Aubonne came from several sequences of analysis. Indeed, the sample of Aubonne reported here corresponds to the control sample A1, inserted in each sequence of analysis. Thus, its measurements reflect the variability of isotopic measurements obtained in several sequences over one year and a half and correspond to the variability of the method for black powder material. It is comprehensively larger than that of the other two manufacturers. The variability measured in the samples of Wano and Vonges is within that of the black powder control sample, indicating therefore that they do not correspond to the isotopic variations of the samples themselves but rather to variations of isotopic measurements (variability of the method).

2.1.2 Variations within and between batches

Figures 8.2 and **8.3** present, for each manufacturer, the frequencies of the δ^{13}C and % C differences calculated between pairs of measurements on samples coming from the same batch. For the three manufacturers, the δ^{13}C variability within a batch of black powder of the three manufacturer presents an excellent and similar repeatability, with mostly a difference of 0.1 to 0.2 ‰. The δ^{13}C intravariability within batches of Aubonne reached however 0.9 ‰ on occasions[1]. Concerning the

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[1] Note however that the number of measurements for Aubonne is much larger than for the other two manufacturers.

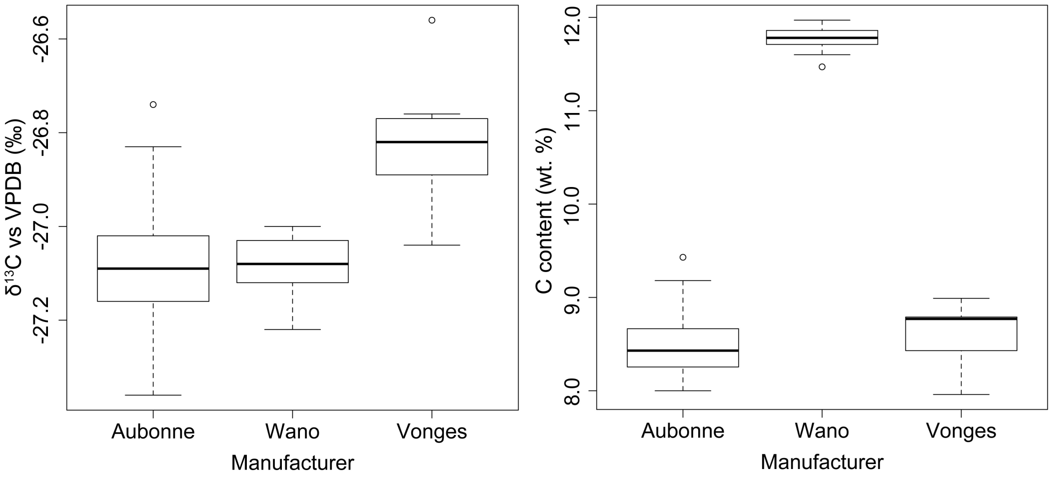

Figure 8.1. δ^{13}C and % C variations measured within a kilo (for Aubonne, n = 35) and half kilo of black powder (for Wano and Vonges, n = 9 for each).

% C, the variability within a batch of black powder is similar for each manufacturer, with most variations being smaller than 0.5 %. Overall, the δ^{13}C and % C variations measured within a batch do not exceed the variability of the control sample of Aubonne (see previous subsection) and reflect thus the variability of the method.

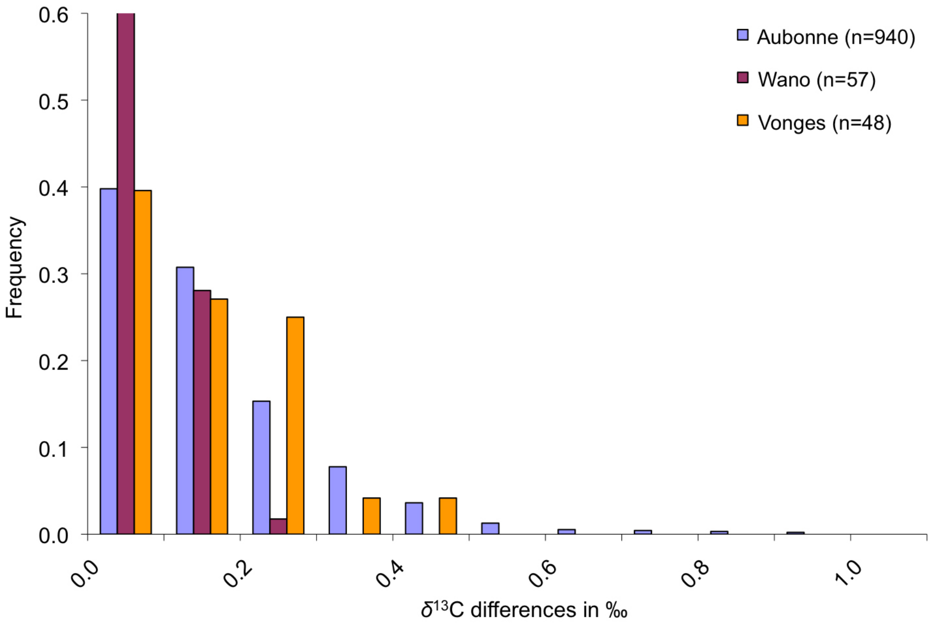

Figure 8.2. δ^{13}C differences between pairs of measurements on samples coming from the same batch, for each manufacturer.

The δ^{13}C values and % C intravariability measured in the samples of Aubonne is displayed in **Figures 8.4** and **8.5**. For the record, A1 to A5 are 5 batches of

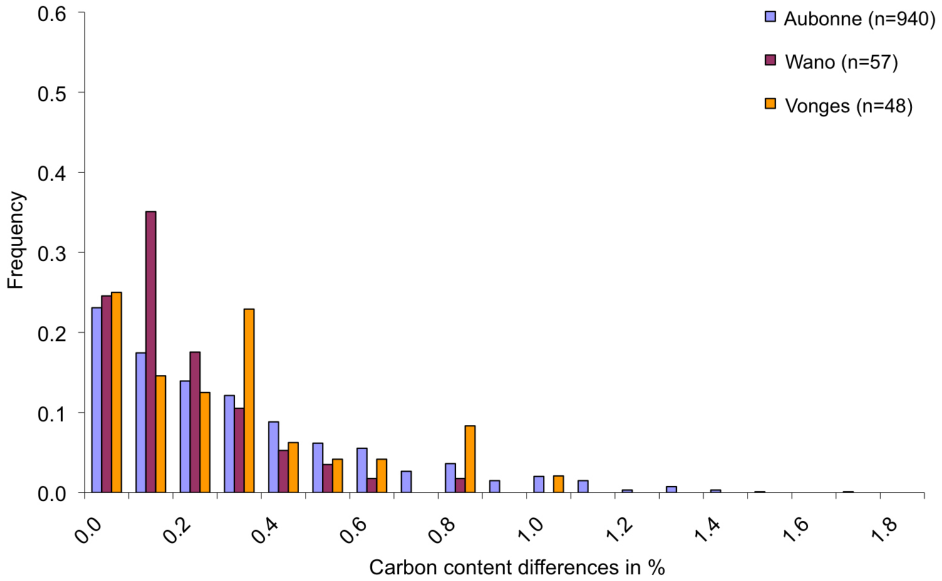

Figure 8.3. % C differences between pairs of measurements on samples coming from the same batch, for each manufacturer.

shooting black powder produced between 2006 and 2008, while A10 is a civilian mortar black powder. Samples A2.1 to A2.4, A3.1 to A3.3, A4.1 to A4.3, A5.1 to 5.4 were taken from daily productions before they were mixed together to constitute the final batch (end product). Samples A1, A2.0, A3.0, A4.0, A5.0 and A10 were taken from a bag or a unit containing the end product, that is black powder representative of the product sold on the market. For sake of clarity, **Table 8.1** presenting the samples provided by the powdery of Aubonne recalls Table 5.3 presented earlier.

A clear distinction in δ^{13}C and % C can be observed in **Figures 8.4** and **8.5** between the types of black powder produced by Aubonne: batches A1 to A5 (mean $\delta^{13}\mathrm{C} = -27.0 \pm 0.2$ ‰; mean % C $= 8.4 \pm 0.3$ %) have a different δ^{13}C value and % C from that of A10 (mean $\delta^{13}\mathrm{C} = -28.5 \pm 0.1$ ‰; mean % C $=$ 11.9 ± 0.9 %). These differences between the types of black powder produced by the same manufacturer were not so obvious in the production of Wano: W3, W4, W5 shooting powder samples have a δ^{13}C value closer to that of samples W6, W7 and W8 sporting powder than that of W1 and W2 (shooting powder). The % C of Wano shooting powder is similar between samples W1 to W5, however, for Wano sporting powder (samples W6 to W8), W6 shows a clearly different % C (for illustration, see **Figures 8.6** and **8.7** further presented). This δ^{13}C and % C variability within the type of black powder produced by one manufacturer is also observable for samples from Vonges (samples V1 to V5).

Batches A1, A2 and A5 present a similar δ^{13}C value with a comparable variability between daily productions (see **Figure 8.4**). On the contrary, batches A3 and

Type of powder	Period of production	Batch number	Reference number	Samples
Shooting powder n°2	July 2006	040.906	A1	1 x 1 kg
Shooting powder n°2	January 2007	290.107 batch	A2.0	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production A	A2.1	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production B	A2.2	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production C	A2.3	1 x 100 g
Shooting powder n°2	January 2007	290.107 day production D	A2.4	1 x 100 g
Shooting powder n°2	October 2007	151.007 batch	A3.0	1 x 100 g
Shooting powder n°2	October 2007	151.007 day production A	A3.1	1 x 100 g
Shooting powder n°2	October 2007	151.007 day production B	A3.2	1 x 100 g
Shooting powder n°2	October 2007	151.007 day production C	A3.3	1 x 100 g
Shooting powder n°2	March 2008	170.308 batch	A4.0	1 x 100 g
Shooting powder n°2	March 2008	170.308 day production A	A4.1	1 x 100 g
Shooting powder n°2	March 2008	170.308 day production B	A4.2	1 x 100 g
Shooting powder n°2	2003	170.308 day production C	A4.3	1 x 100 g
Shooting powder n°2	October 2008	071.008 batch	A5.0	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production A	A5.1	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production B	A5.2	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production C	A5.3	1 x 100 g
Shooting powder n°2	October 2008	071.008 day production D	A5.4	1 x 100 g
Mortar powder Böller n°5	unknown	-	A10	1 x 100 g

Table 8.1. Samples of black powder provided by the powdery of Aubonne.

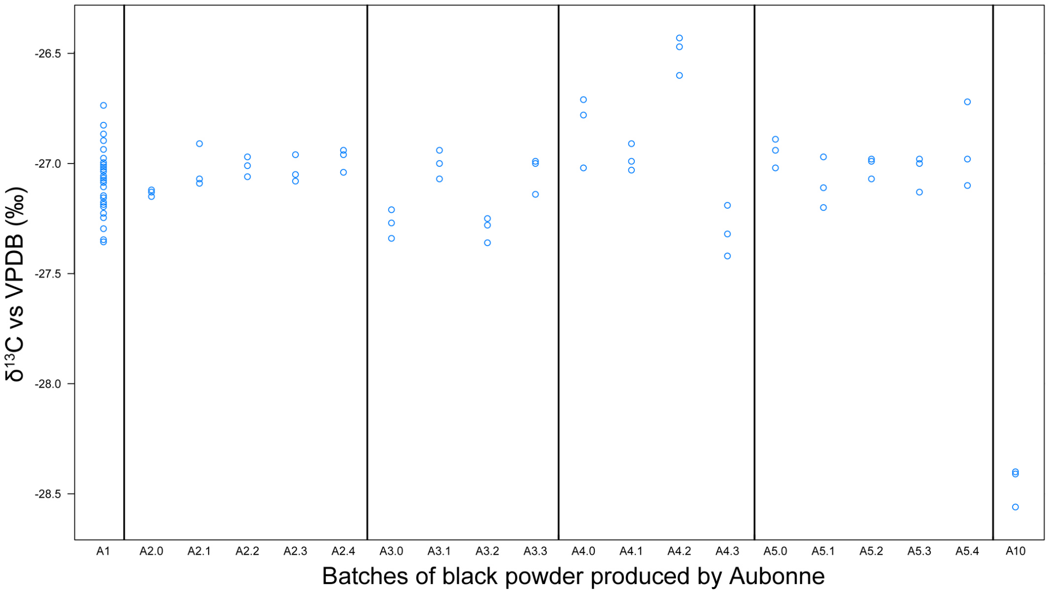

Figure 8.4. δ^{13}C values measured in the samples of Aubonne. Batches are separated by solid lines. Samples A2.1 - A2.4, A3.1 - A3.3, A4.1 - A4.3, A5.1 - 5.4 were taken from daily productions before their mixture to constitute the end product (final batch). A1, A2.0, A3.0, A4.0, A5.0, A10 were sampled from the end product.

A4 show more disparities between the daily productions and the final batch. An interesting point concerns the δ^{13}C values of the final batches A2.0, A3.0, A4.0 and A5.0. Although the final batch is made of several daily units, the δ^{13}C values of the sample does not always reflect the isotopic variability and value measured in the daily productions (for example, A2.0 and A4.0 do not cover all the values measured in the corresponding daily productions). However, the δ^{13}C value and % C of sample A1, obtained with 35 measurements, show a large dispersion of values.

Concerning the intravariability of the % C shooting powder, batches A1 to A5 exhibit slight variations (see **Figure 8.5**). Except for A4 which has a slightly lower % C than the others, the measurements of the % C of A1 cover most of the variations observed in batches A2, A3 and A5.

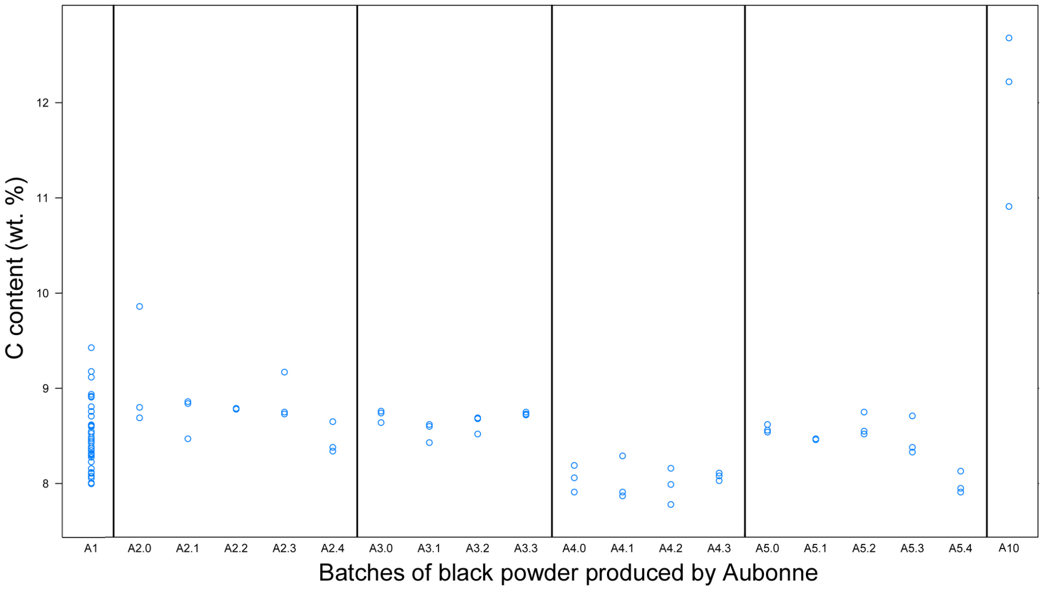

Figure 8.5. % C measured in batches and daily productions of Aubonne.

2.2 Variations between manufacturers

The δ^{13}C variability between manufacturers is illustrated in **Figure 8.6** by the different batches. For a given type of black powder, the δ^{13}C value measured in the production of one manufacturer is relatively constant, while that of another is highly variable. The black powder of Aubonne produced between July 2006 and October 2008 (A1 to A5) exhibit a constant δ^{13}C value, while the shooting powders of Wano (W1 to W5) and Vonges (V1 to V5) present more variability for the period of time monitored (2003 to 2007 for Wano and 2005 to 2007 for Vonges). Except for the batches with a significantly different δ^{13}C value, such as for batches W1 and W2, the δ^{13}C intervariability between manufacturers is rather limited.

The variability of the % C between manufacturers is represented in **Figure 8.7**. As denoted by the difference between the shooting powders of Wano and Aubonne, there is a variability in % C between manufacturers. This feature showed to vary according to the type of black powder produced by a manufacturer. Nevertheless, the % C also displays large variations within a given type of black powder, as for batches W6 to W8.

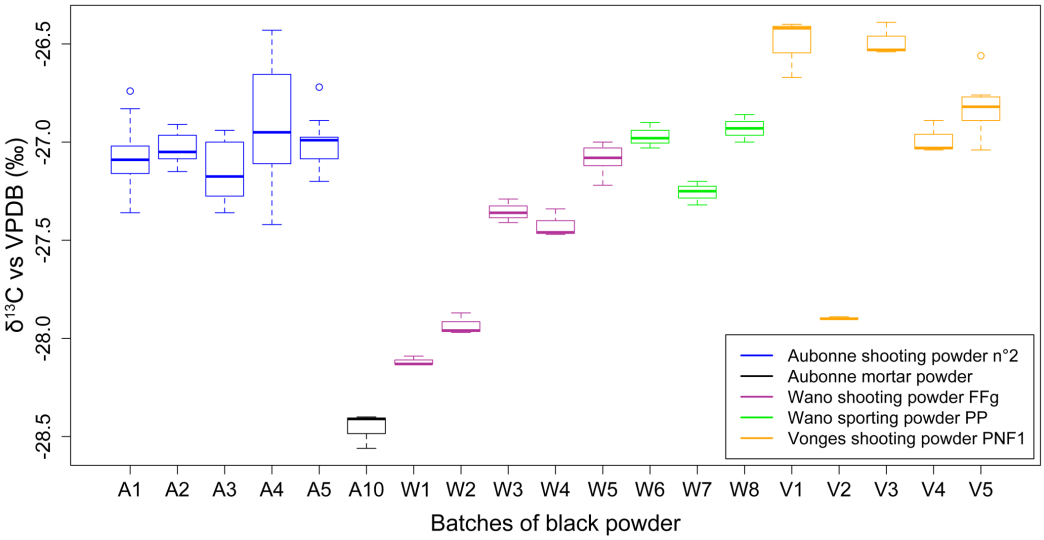

Figure 8.6. Variability of the δ^{13}C value between the production of Aubonne, Wano and Vonges. For each batch, n = 3, except for A1 (n = 35), A2 and A5 (n = 15), A3 and A4 (n = 12), W5 and V5 (n = 9).

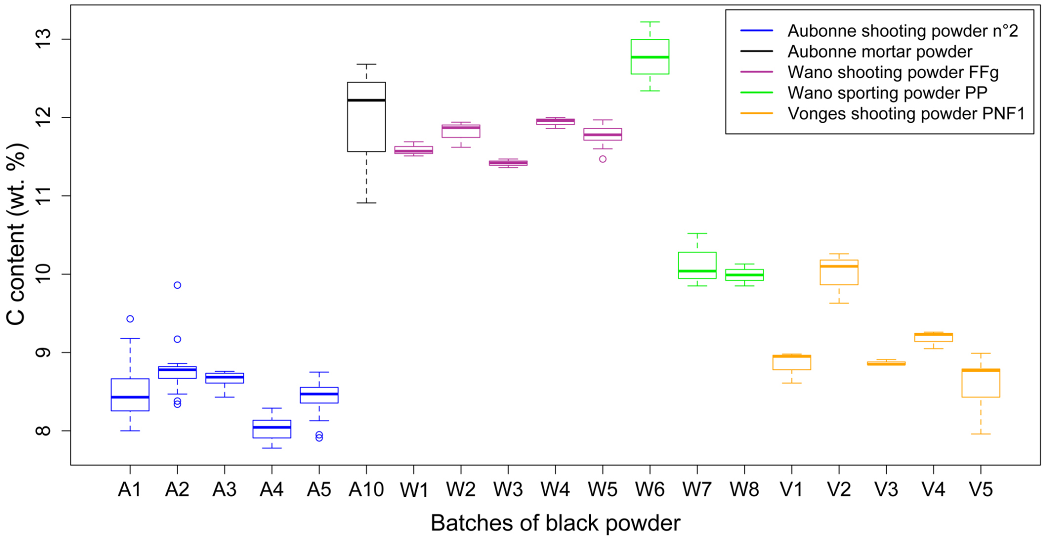

Figure 8.7. Variability of the % C between the production of Aubonne, Wano and Vonges. For each batch, n = 3, except for A1 (n = 35), A2 and A5 (n = 15), A3 and A4 (n = 12), W5 and V5 (n = 9).

3 Nitrogen content and isotope ratio

3.1 Variations within a manufacturer

3.1.1 Variations within a kilo and half kilo

The δ^{15}N intravariability measured within a kilo and half kilo of black powder, illustrated in **Figure 8.8**, is similar for Aubonne and Vonges, while that of Wano is very large with a maximum difference up to 3.2 ‰ (vs. 0.6 ‰ and 1.0 ‰ for Aubonne and Vonges, respectively). The % N intravariability is larger for Vonges (with a range of 1.9 %) than for Aubonne and Wano which showed a repeatable % N (with a range of 0.4 %). Measurements of Wano and Vonges samples were obtained in two different sequences, while measurements for Aubonne came from five different sequences (the measurements presented here for Aubonne correspond to those of the black powder control sample A1 inserted in each sequence of analysis). The measurements of the 3 samples are however contemporaneous and were obtained within 4 days difference. This indicates that the δ^{15}N variability of Wano and the % N of Vonges are larger than the variability of the method and are therefore attributed to the variability of the sample itself.

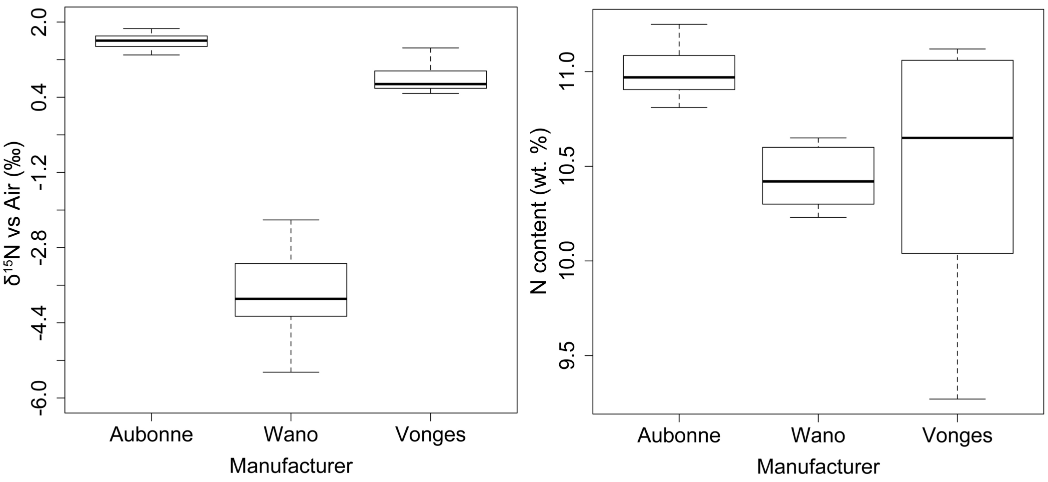

Figure 8.8. δ^{15}N and % N variations measured within a kilo (for Aubonne, n = 16) and half kilo of black powder (for Wano and Vonges, n = 9 for each).

3.1.2 Variations within and between batches

Figures 8.9 and **8.10** illustrate the δ^{15}N and % N differences calculated between pairs of measurements on samples coming from the same batch. Aubonne and Vonges exhibit a similar distribution of the δ^{15}N differences, with most of them lying below 0.6 ‰ (see **Figure 8.9**). On the contrary, Wano presents a large δ^{15}N batch intravariability with differences up to 3.6 ‰. Moreover, the distribution of the differences for Wano is relatively flat and appears particular to that manufacturer.

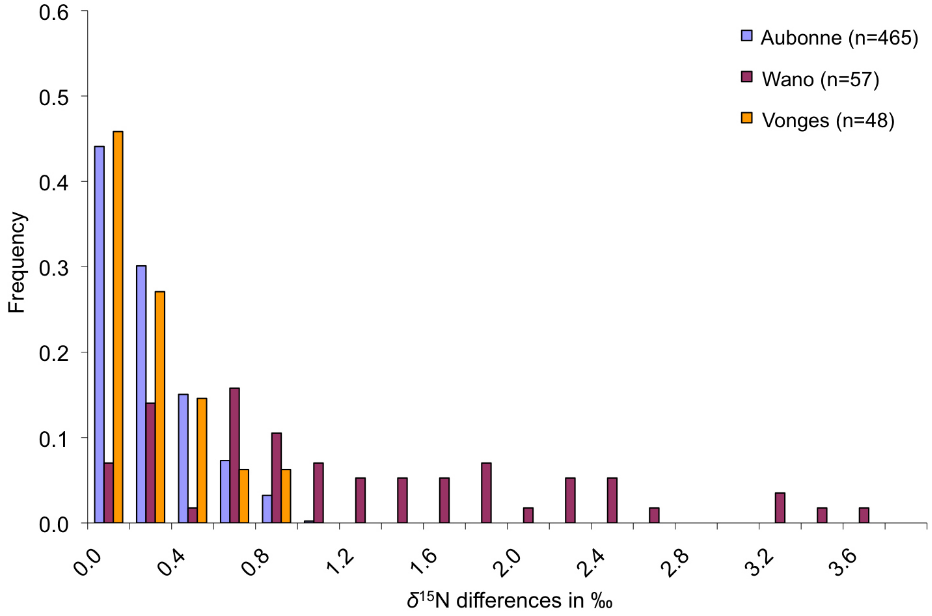

Figure 8.9. δ^{15}N differences between pairs of measurements on samples coming from the same batch, for each manufacturer .

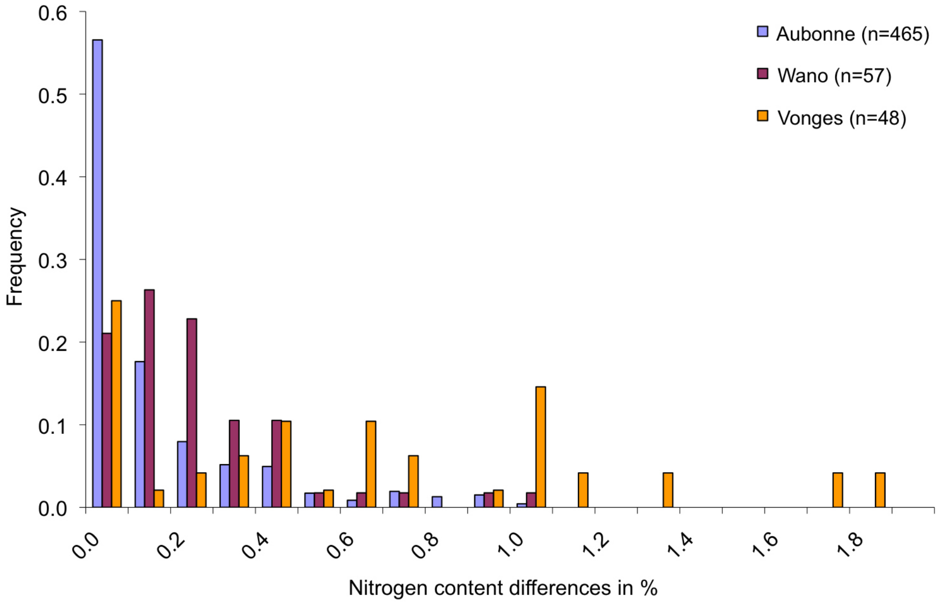

Figure 8.10. % N differences for each manufacturer between pairs of measurements on samples coming from the same batch, for each manufacturer.

Most % N differences are below 0.5 %, except for Vonges which exhibits a larger batch intravariability with differences of up to 1.8 % (see **Figure 8.10**). The examination of the batch intravariability thus confirms the observations made within a kilo and half kilo of black powder.

Figures 8.11 and **8.12** present the δ^{15}N value and % N measured in the samples of Aubonne. The δ^{15}N variability within a batch can be as large as 1.0 ‰, as for batches A2 and A4 (see **Figure 8.11**). As for carbon, a concerning point is the δ^{15}N values in the final batch (A2.0, A3.0, A4.0 A5.0) which are not always representative of the values measured in the daily productions. As an example, samples taken from the final batch A2.0 have a mean δ^{15}N value of 0.5±0.1 ‰ in contrast to the daily production A2.1 with a δ^{15}N value of 1.2±0.2 ‰.

There is however no distinct δ^{15}N value or % N regarding the type of black powder (A1 to A5 or A10).

The variability of the % N within a batch is moderate, though there are few outlying points (see **Figure 8.12**). Except for these measurements, the analysis of each sample is fairly repeatable (< 0.1 %). By contrast, the intravariability of A1 is larger (0.4 %) but results from a larger number of measurements (n=16). Contrarily to δ^{13}C and δ^{15}N, the % N measurements made in the final batches mostly reflect the values measured in the daily productions.

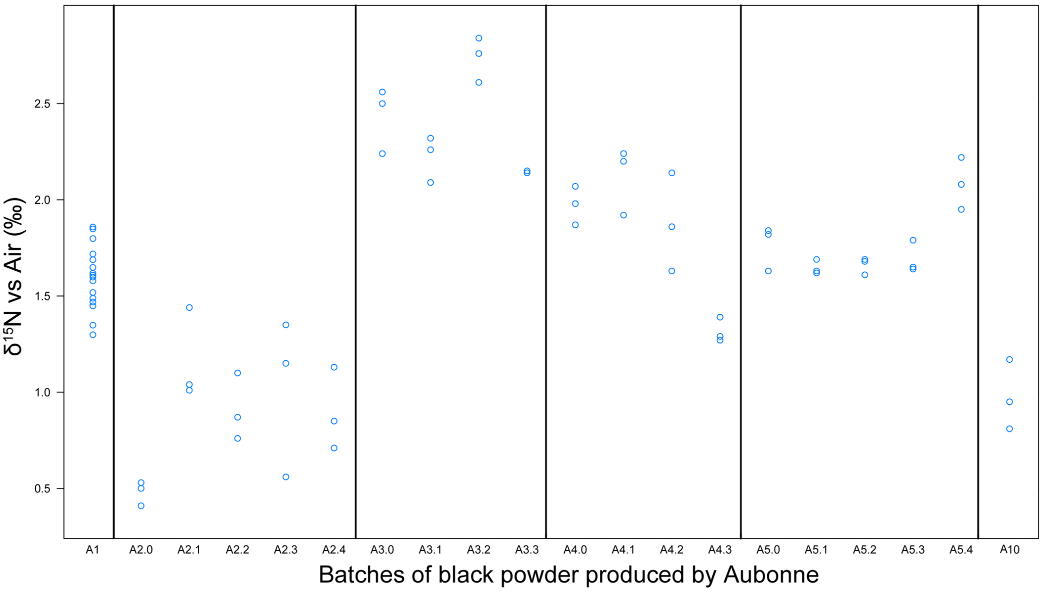

Figure 8.11. δ^{15}N measured in the samples of Aubonne. Batches are separated by solid lines.

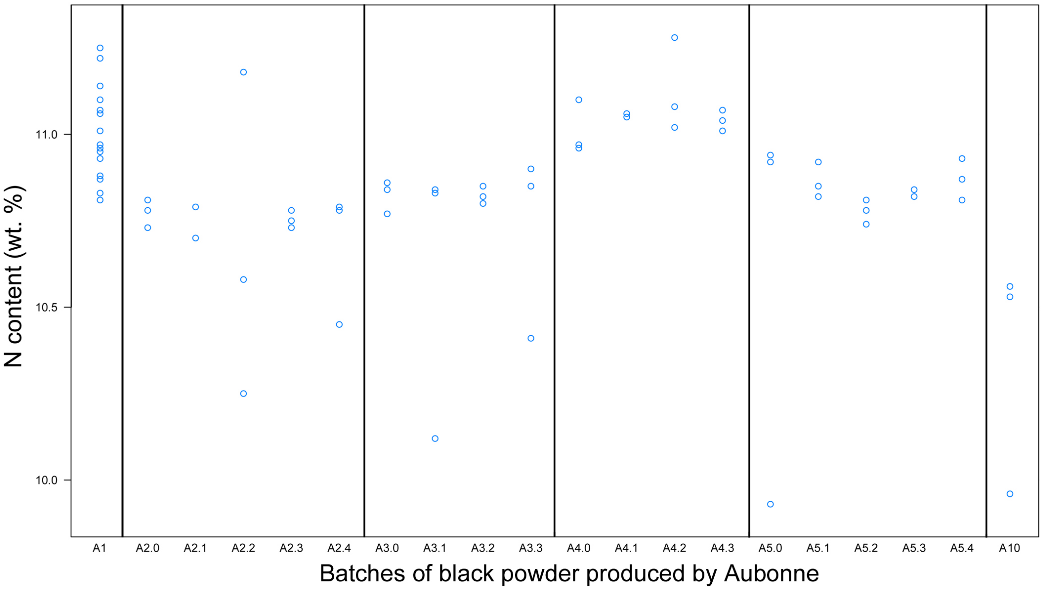

Figure 8.12. % N measured in the samples of Aubonne. Batches are separated by solid lines.

3.2 Variations between manufacturers

Figure 8.13 illustrates the variability between the production of the three manufacturers. Wano has a particular signature with batches of black powder which are very depleted in ^{15}N. In contrast, the similarity of the δ^{15}N value of batches of Aubonne and Vonges does not allow discriminating them. As for carbon, variations between batches of the same type of black powder also exist for δ^{15}N, as shown by the batches of shooting (W1 - W5) and sporting (W6 - W8) powders of Wano.

The % N variability between manufacturers appears rather limited as displayed in **Figure 8.14**. A given % N can not be attributed to a given type of black powder or a manufacturer: some batches of the same type of black powder have very different % N (for instance W6 versus W7 and W8).

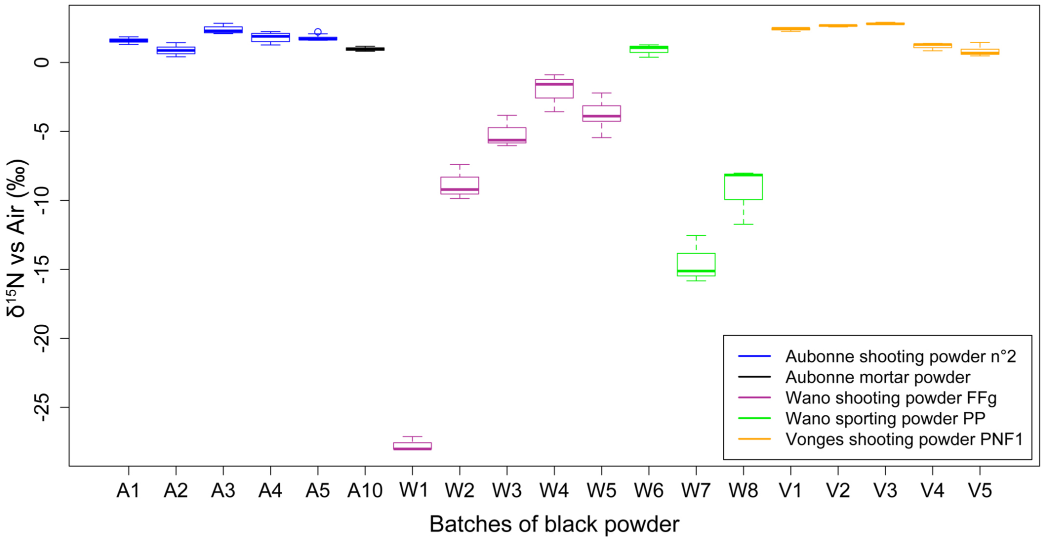

Figure 8.13. Variability of the δ^{15}N value between the production of Aubonne, Wano and Vonges. For each batch, n = 3, except for A1 (n = 16), A2 and A5 (n = 15), A3 and A4 (n = 12), W5 and V5 (n = 9).

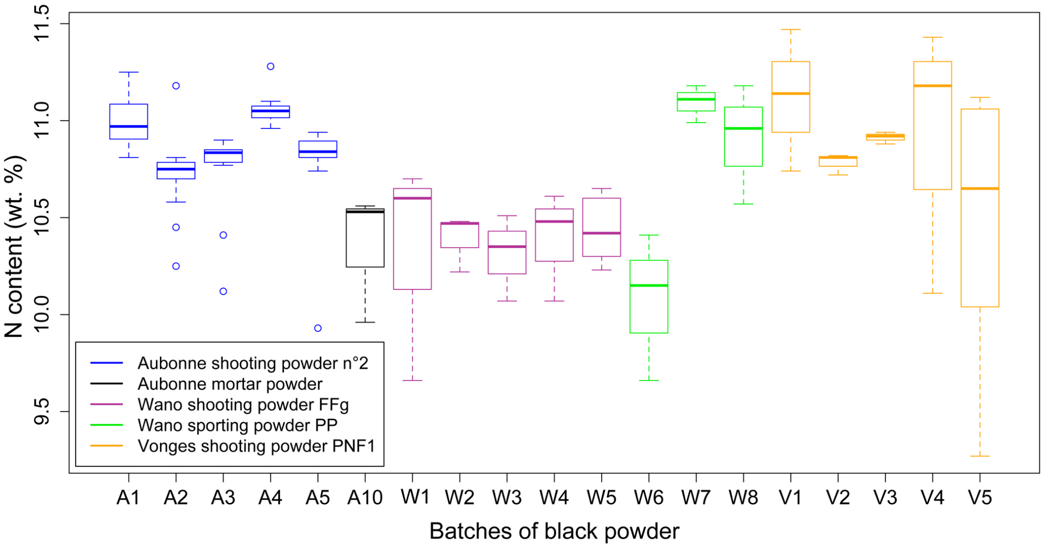

Figure 8.14. Variability of the % N between the production of Aubonne, Wano and Vonges. For each batch, n = 3, except for A1 (n = 16), A2 and A5 (n = 15), A3 and A4 (n = 12), W5 and V5 (n = 9).

4 Oxygen content and isotope ratio

4.1 Variations within a manufacturer

4.1.1 Variations within a kilo and half kilo

Figure 8.15 represents the δ^{18}O and % O intravariability measured within a kilo and half kilo of black powder. For both variables, Aubonne shows the largest variability within a kilo, with a range of 1.5 ‰ for δ^{18}O, while it is smaller for Wano and Vonges (0.5 ‰ and 0.4 ‰, respectively). As mentioned in Chapter 6, the isotopic variations of Aubonne sample likely come from the isotopic heterogeneity of the matrix itself. The measurements for Aubonne correspond to that of control sample A1, which were obtained from 12 sequences of analysis over a period of 2 months. In contrast, the samples of Wano and Vonges were measured on the same day but in two distinct sequences of analysis. This explains why, for both features, their variability is smaller than that of Aubonne. The large variability of % O in Aubonne sample also likely originates from the non optimum conversion of oxygen or the interference of N_2, as highlighted in chapter about oxygen measurement (see Chapter 6 for further details).

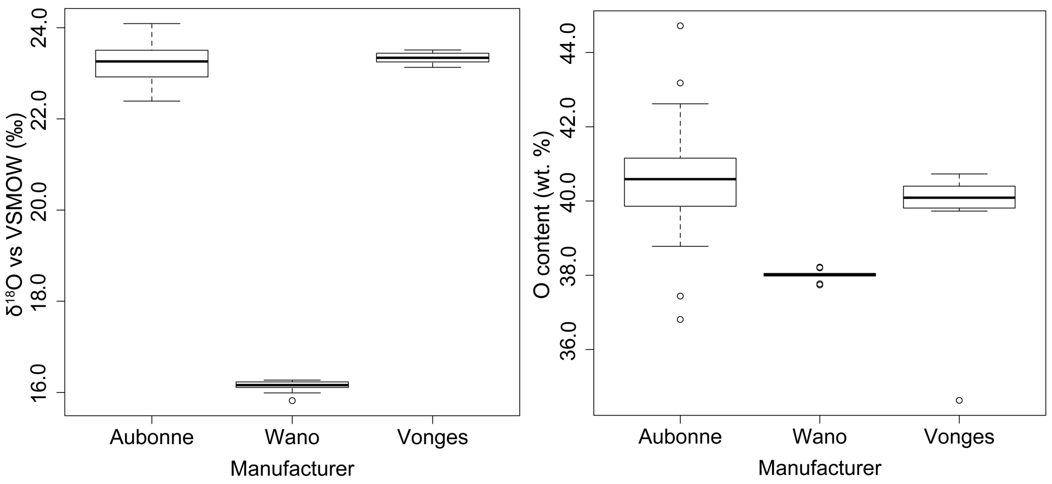

Figure 8.15. δ^{18}O and % O variations measured within a kilo (for Aubonne, n = 59) and half kilo of black powder (for Wano and Vonges, n = 9 for each).

4.1.2 Variations within and between batches

Figures 8.16 and **8.17** reveal the distribution of the δ^{18}O and % O differences between pairs of measurements made on samples coming from the same batch. As Wano and Vonges exhibit a similar distribution of the differences between pairs of measurements, Aubonne displays a widespread distribution with differences of up to 1.5 ‰ for δ^{18}O and 6 % for % O. These large differences are also observable in the batches of Vonges regarding the % O.

Most δ^{18}O variations within a batch are below 0.6 ‰ and most % O variations are below 1 %. With a very important number of analyses, Aubonne exhibits the largest variations (up to 1.7 ‰ for δ^{18}O and about 6 % for % O). These variations correspond to the range of variations reported in the previous subsection, which means that within batch variations are smaller or similar to the variability associated to the method.

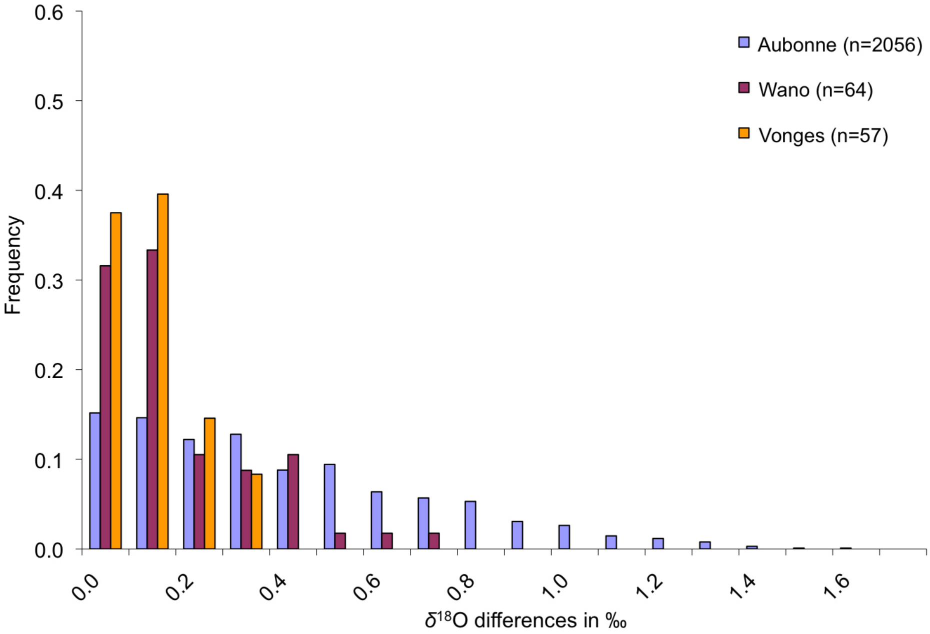

Figure 8.16. δ^{18}O differences calculated for each manufacturer between pairs of measurements on samples coming from the same batch.

Figures 8.18 and **8.19** illustrate the variability within the batches of Aubonne. The striking feature of both plots is the large variability of δ^{18}O and % O measured within the kilo of black powder A1. This wide variability precludes any interpretation of the variations observed between samples coming from daily productions and final batches (A2.0, A3.0, A4.0, A5.0, A10) or from the different batches. As a result, the variations measured in A1 is representative of the variations within the production of Aubonne.

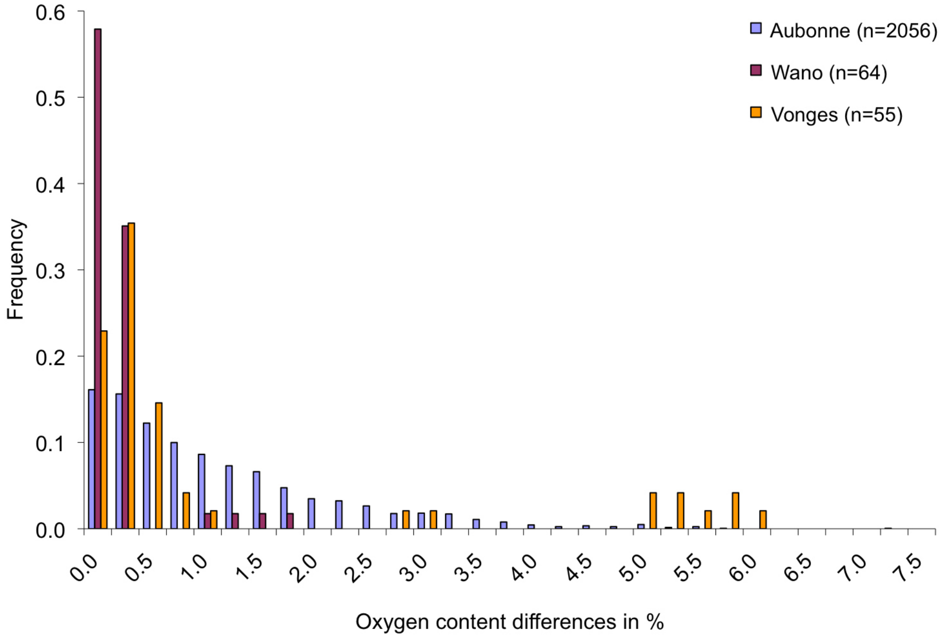

Figure 8.17. % O differences calculated for each manufacturer between pairs of measurements on samples coming from the same batch.

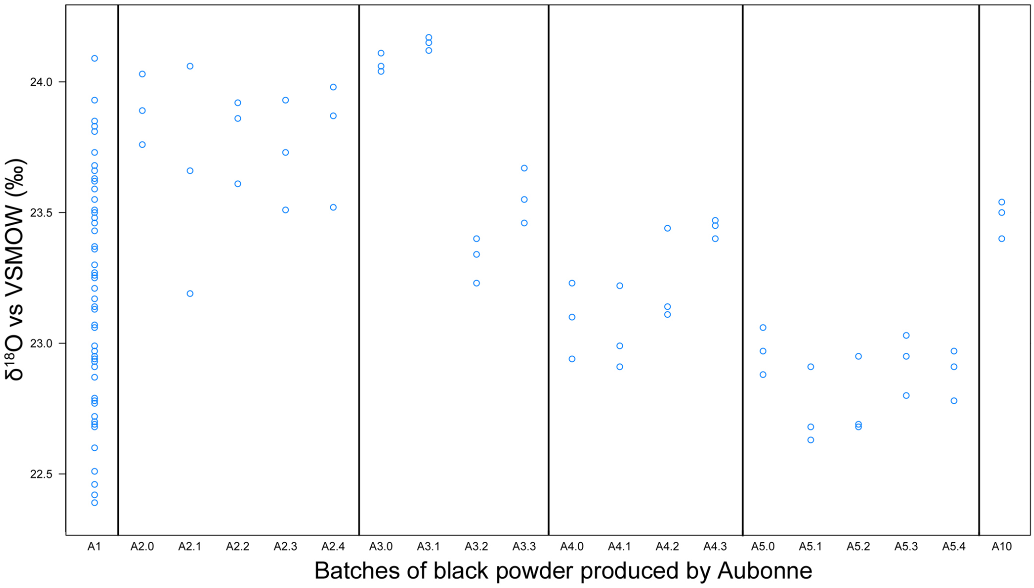

Figure 8.18. δ^{18}O measured in the samples of Aubonne. Batches are separated by solid lines.

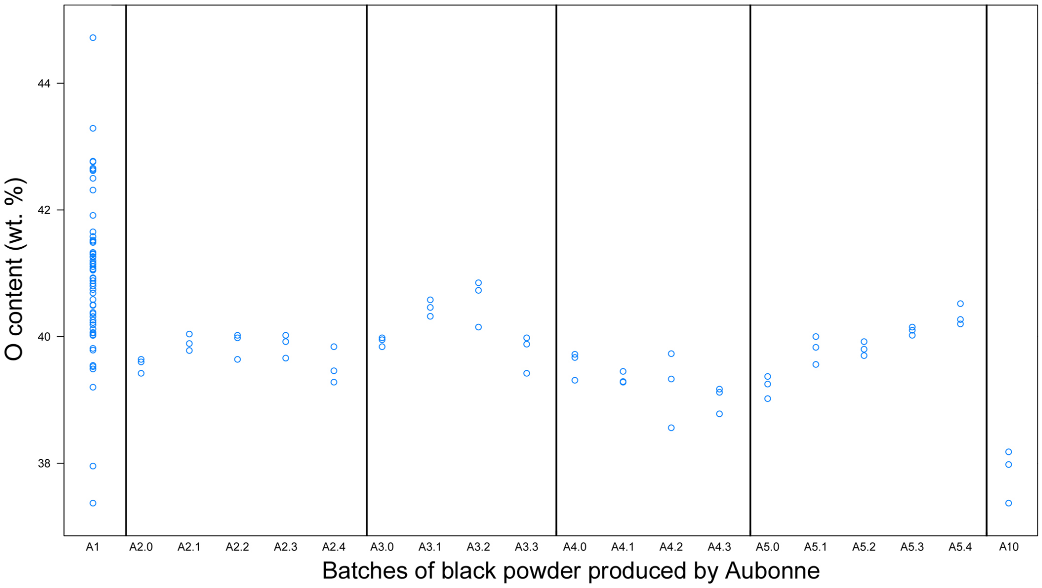

Figure 8.19. % O measured in the samples of Aubonne. Batches are separated by solid lines.

4.2 Variations between manufacturers

The variability of the δ^{18}O value and % O between the three manufacturers is presented in **Figures 8.20** and **8.21**. Despite the large intravariability of the δ^{18}O value and % O within the production line of Aubonne, a clear distinction exists between the δ^{18}O value of the samples of Wano and the ones of Aubonne and Vonges. There is however no clear difference between the types of powder.

The variability of the % O between the three manufacturers is very limited and given the large variations inherent to the method itself, the potential of discrimination of this feature is restricted within the dataset.

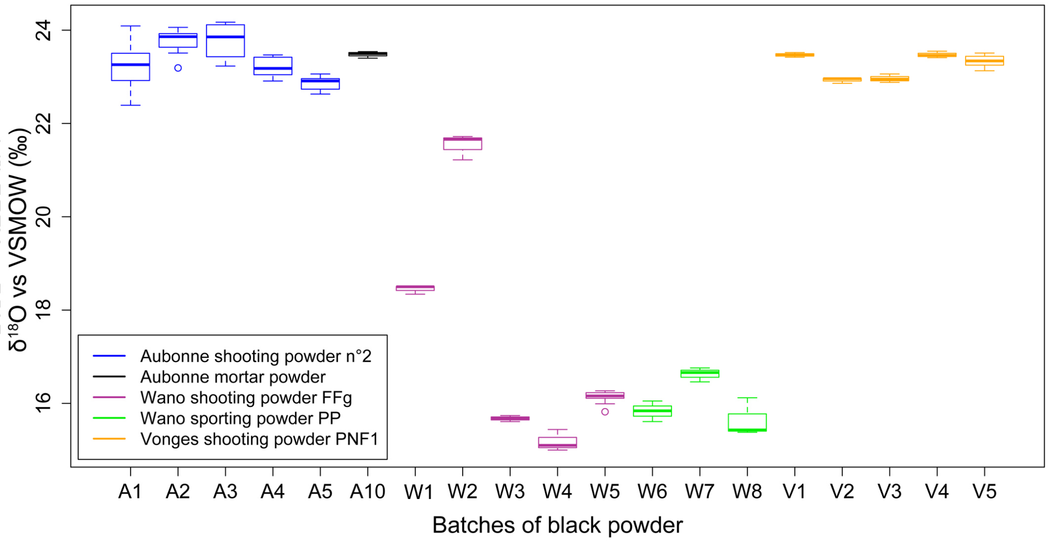

Figure 8.20. Variability of the δ^{18}O value between the production of Aubonne, Wano and Vonges. For each batch, n=3, except for A1 (n=59), A2 and A5 (n=15), A3 and A4 (n=12), W5 and V5 (n=9).

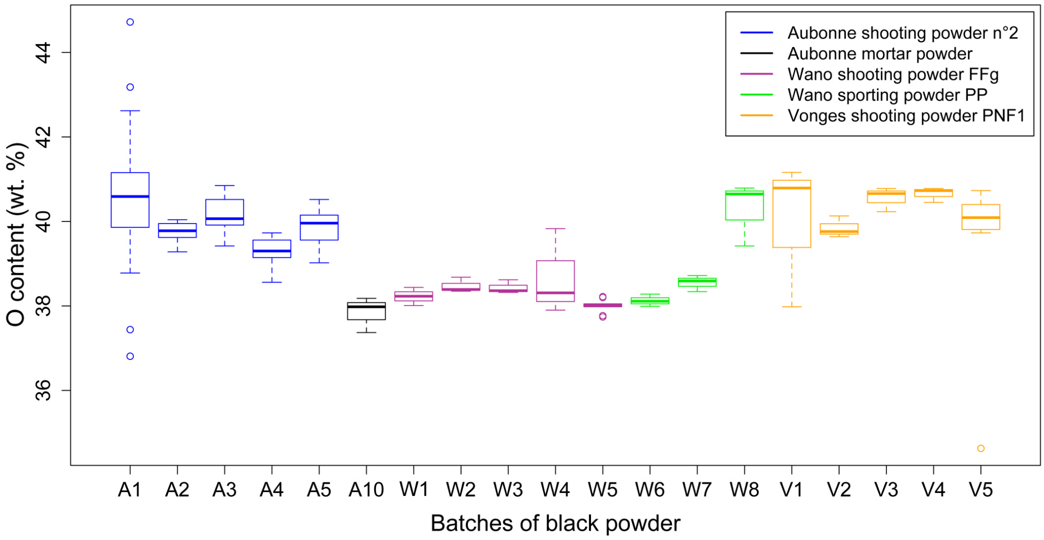

Figure 8.21. Variability of the % O between the production of Aubonne, Wano and Vonges. For each batch, n=3, except for A1 (n=59), A2 and A5 (n=15), A3 and A4 (n=12), W5 and V5 (n=9).

5 Differentiation of sources using combined features

5.1 C, N and O variations: in essence

In the previous sections, variations of isotope ratios and elemental content were observed at different production levels:

- Within the production of one manufacturer, significant isotopic and elemental variations were observed, for some manufacturers, between different types of black powder, and, for others, between batches of the same type of black powder.
- Between manufacturers of black powder, there are δ^{13}C and % C variations between productions. However, there are overlapping measurements around -27 ‰ for δ^{13}C and around 9 % for % C. While the % N and % O variations between the productions of the manufacturers are very limited, the δ^{15}N and δ^{18}O values show to be different for one of the manufacturer, suggesting possible discrimination.

Given these observations, the level of source which will allow the best discrimination needed to be further investigated. For each applied statistical method, the findings were considered given the different levels of source, that is the manufacturer, type of black powder and batch levels. Exploratory analyses were undertaken to observe the structure of the data set and whether clusters could be outlined, regarding the known classes of the samples. The distinction of the different classes was also evaluated through distance measurement. Finally, the ability of supervised classification models to predict the known classes of samples according to the three levels of source was assessed.

The different preprocessing of data (range scaling, standardisation and scaling rows to constant total) did not favourably influence the discrimination between groups. Most of the time, this step even reduced the variance within the data set or between the classes. Results presented in the following subsections were obtained on non transformed data (i.e. value obtained after normalisation to the international reference scale).

5.2 Exploratory statistical methods

5.2.1 Principal component analysis

Principal components analysis (PCA) of the data set was undertaken in order to observe the distribution of the black powder samples. The variables considered for PCA were δ^{13}C, δ^{15}N, δ^{18}O, % C, % N and % O. **Figure 8.22** displays the scores of the samples projected on the first two principal components (PC1 and

PC2). Letters a) and b) present the visualisations by batch (one colour by batch) and by type of black powder (one colour by type). The first two dimensions (PC1 and PC2) explain more than 95 % of variance, indicating that they properly model the information contained in the data set. Examination of the variable factor map against their first two component loadings highlights that δ^{15}N, δ^{18}O and the % C summarise most of the measured variance (see **Figure 8.23**).

The production of Wano appears different from that of Aubonne and Vonges. Each batch of Wano has distinct scores, while that of the other 2 manufacturers are clustered together creating an overlapping area. Although some batches and types of black powder are distinct from each other, a clear differentiation is not visible in **Figure 8.22**.

Additional PCA undertaken without the particular profile of Wano did not offer a better distinction of Aubonne and Vonges productions (see **Figure 8.24**). Only the batch A10 showed to be significantly different from the rest of the Aubonne production.

5.2.2 Hierarchical clustering analysis

Hierarchical clustering analysis (HCA), using the Euclidean distance measurement, was undertaken to see whether the samples could be grouped according to the classes suggested by the information on production (manufacturers, types of black powder or batches levels). The quality of clustering was best when the number of clusters corresponded to the number of manufacturers of BP. Nevertheless, a confusion matrix shows that only 61.5 % of the samples are correctly attributed to the clusters when considering the manufacturer level (see **Figure 8.25**), while it decreased to 7.4 % and 16.4 % when considering the type or batch level, respectively. Given the overlap of many classes, this approach is not suitable for the present research problem.

5.2.3 Distance measurement

The distance or similarity between samples belonging to the same and to different classes was measured using Pearson correlation coefficient. Whatever the level of source considered (manufacturer, type of BP and batch), intravariability and intervariability distributions displayed an important overlap, leading to a high rate of false positives (FP). This is illustrated in **Figure 8.26**, which shows the distribution of Pearson correlation coefficient for samples of the same batch (intra) and of different batches (inter) of black powder.

This large overlap, which is observable for each level of source, is due to the fact that the target variables are constant among the production line of some manufacturers,

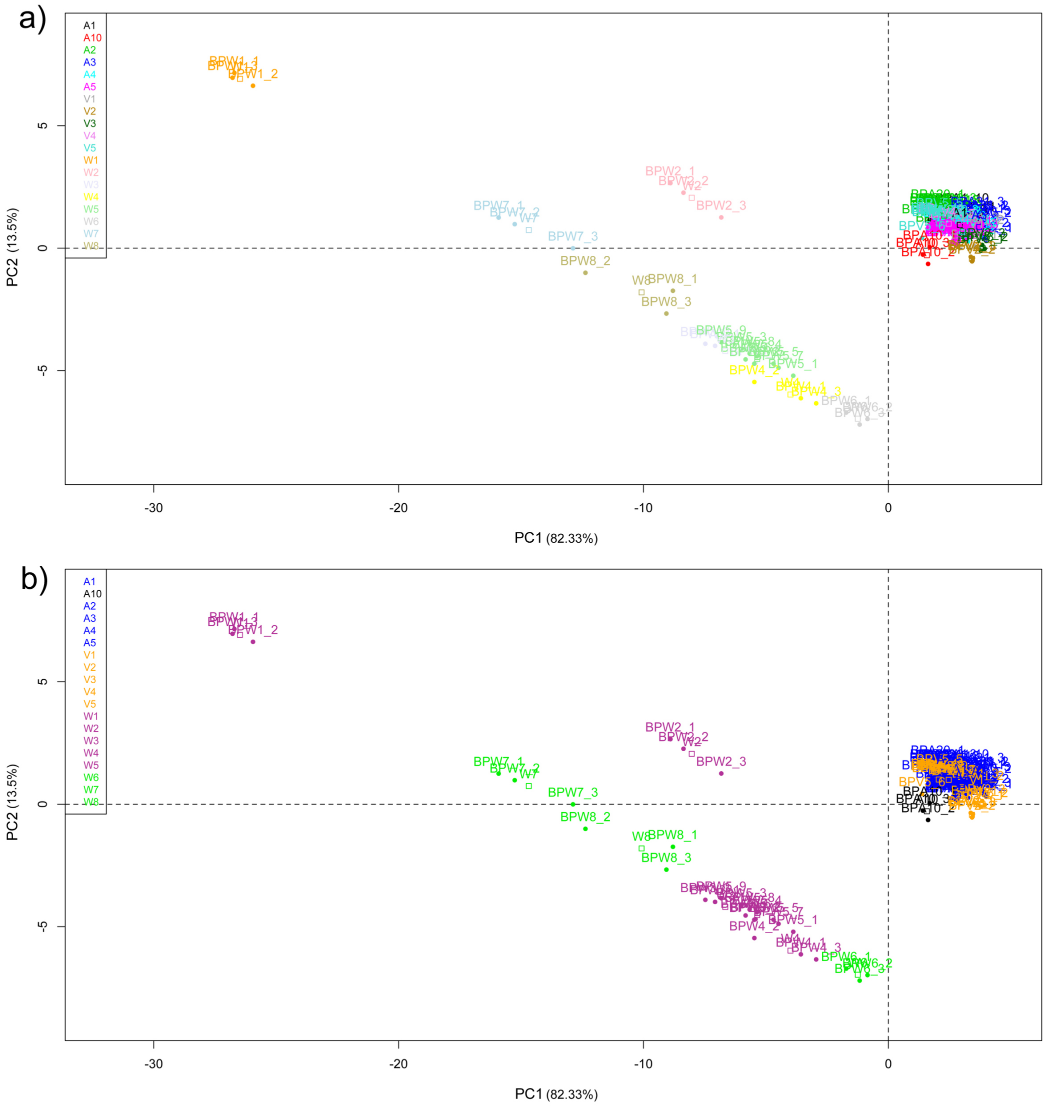

Figure 8.22. PCA performed on the data set including the productions of the 3 manufacturers. Results are illustrated with a colouring according to a) the different batches of manufacturers; b) the types of black powder.

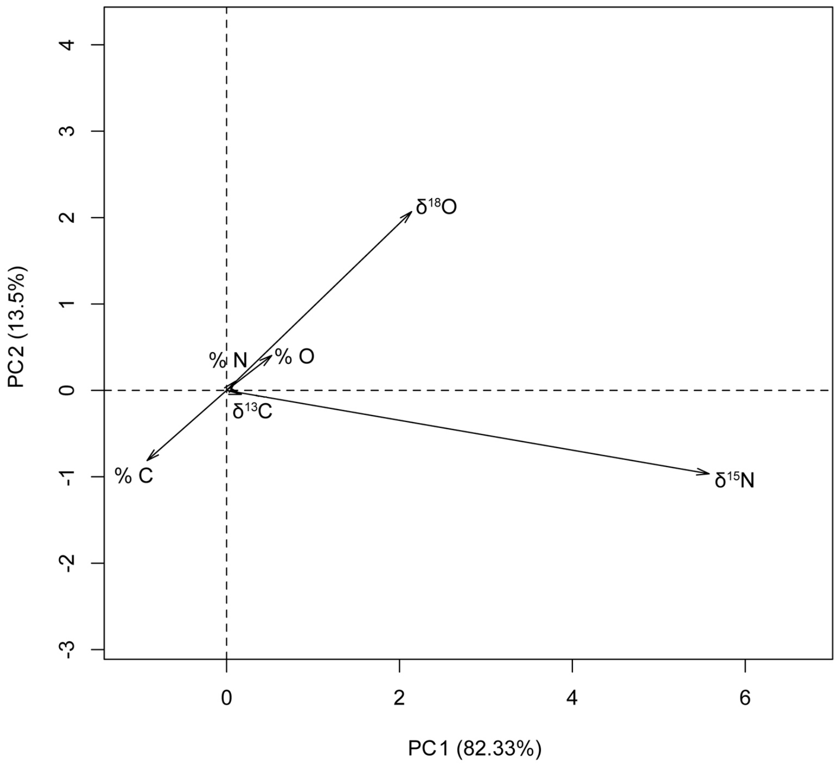

Figure 8.23. Representation of the loadings of the variables.

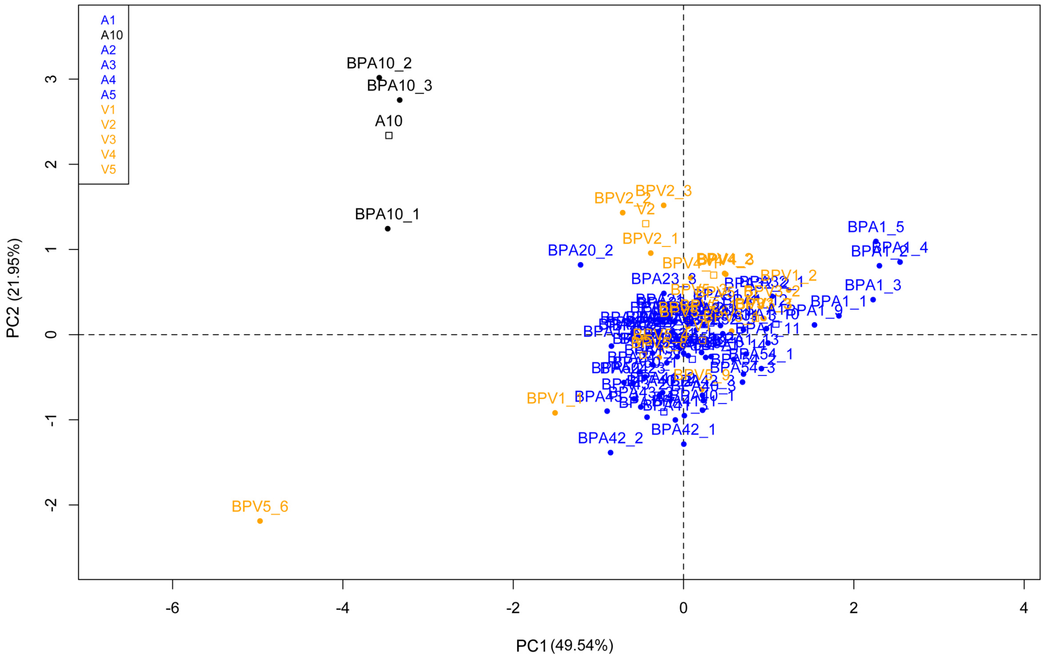

Figure 8.24. PCA performed on the data set including the productions of Aubonne and Vonges. Results are illustrated with a colouring according to the types of black powder.

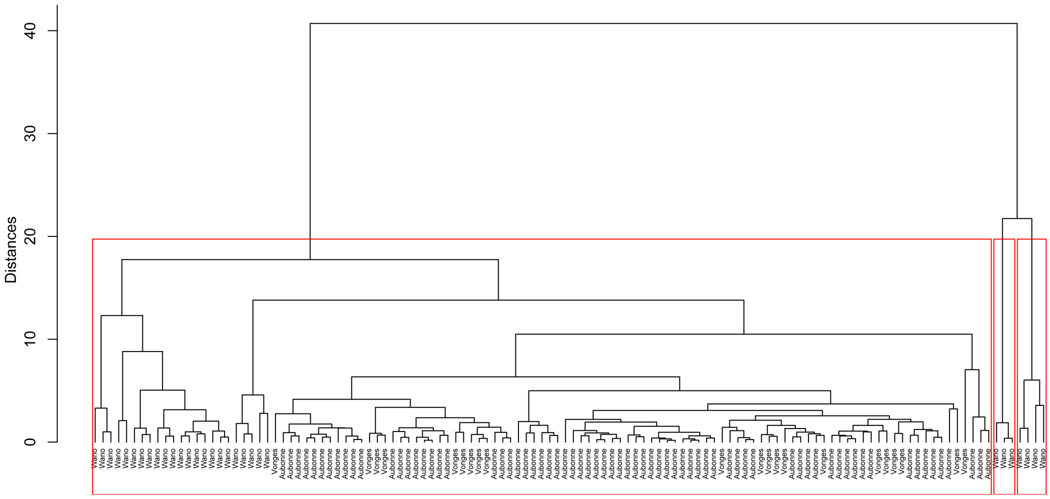

Figure 8.25. HCA performed on the whole data set using the Euclidean distance. 61.5 % of the black powder samples are correctly attributed to the corresponding cluster, when considering the manufacturer level (number of clusters = 3).

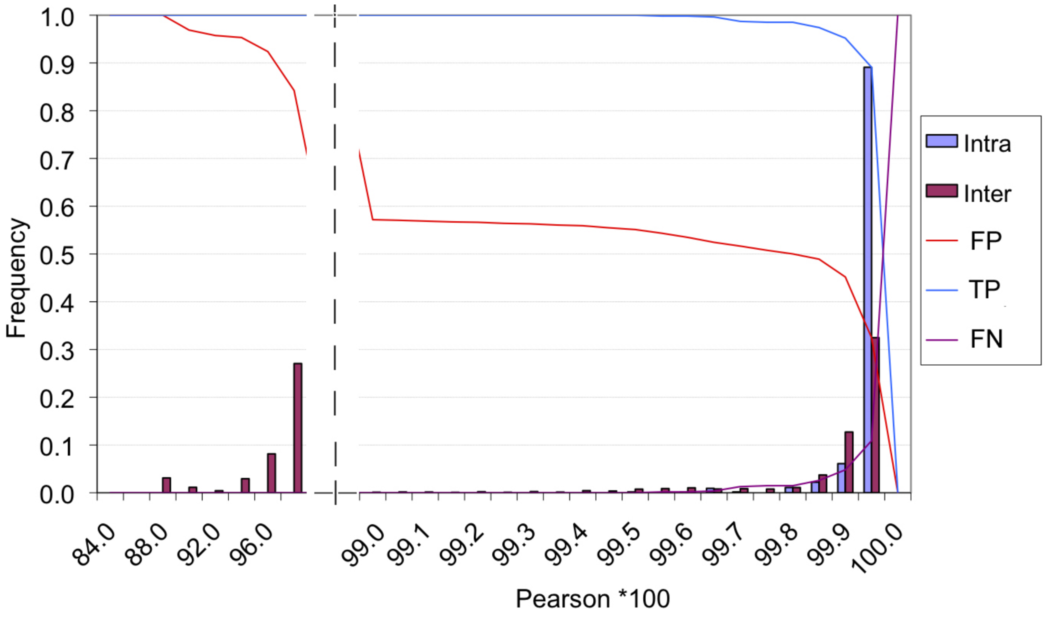

Figure 8.26. Distribution of Pearson correlation coefficient for samples coming from the same batch of black powder (Intra) and samples coming from different batches of black powder (Inter).

while they are highly variable within the production of others. This leads to important values of FP, i.e. high rates of samples predicted as linked to the source, while they are in fact not. Conversely, setting a higher threshold to decrease the FP rate results in an increasing number of false negatives (FN) (samples predicted to come from different sources, while in reality they come from the same one). **Table 8.2** reports the rates of FP and FN according to a Pearson threshold corresponding to about 5 % of false negatives. With such distributions, even when considering higher thresholds (99.9) for the type of BP for example, the minimum of FP is 17 % with a corresponding rate of FN of 30 %, meaning that in 1/5 of the cases, the link between samples is not correct and in 1/3 of the cases, the information that samples are linked is missed.

Level of source	**Threshold**	**FP (%)**	**FN (%)**	**Specificity**	**Sensitivity**
Manufacturer	99.0	35	6	0.65	0.94
Type of BP	99.7	32	5	0.68	0.95
Batch	99.9	45	5	0.55	0.95

Table 8.2. Respective percentages of false positives (FP), false negatives (FN), specificity and sensitivity, according to the threshold of Pearson correlation coefficient.

ROC (Receiver Operating Characteristics) curves allow monitoring the sensitivity and specificity of a two-class test and provides a response on its performance to predict the actual class of a sample. ROC curves were used to visualise the efficiency of the Pearson correlation coefficient method to separate the two populations of samples (samples coming from the same source vs. samples coming from different sources) when considering the manufacturer, the type and the batch as the source level. The advantage of ROC curves is that they provide the sensitivity and specificity of a two-class test or of a statistical method considering the different test thresholds. **Figure 8.27** plots the ROC curves considering the manufacturer, type and batch level as the source. The use of the Pearson distance to discriminate samples based on the three considered levels of source provides similar performance, as shown by the similar curves and area under the curve, although the performance is slightly better for the batch level. Ideally, a perfect method would have given a 0 false positive rate and a 1 true positive rate, with a curve showing a right angle in the upper left corner of the graph and an area under the curve (AUC) of 1. None of the three curves demonstrated a perfect performance of the test. They all show a non negligible proportion of false positives but their performance is better than a random classifier[2] with an AUC varying between 0.82 and 0.89. This indicates that the three systems are not perfect but are able to determine whether two samples are coming from the same source or not with a certain number of false positives.

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[2] a random classifier produces half positives and half negatives and yields an AUC of 0.5.

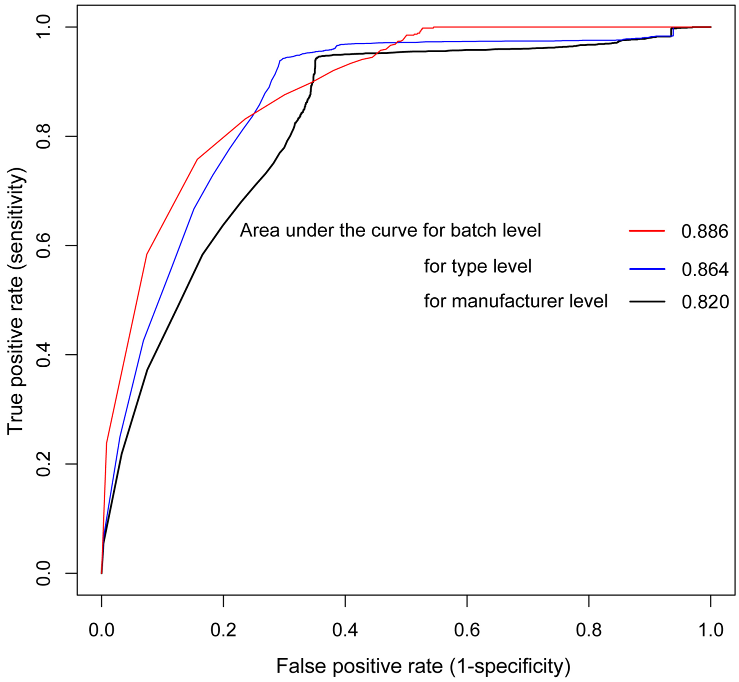

Figure 8.27. ROC curves of Pearson correlation coefficient when considering the manufacturer, type and batch of BP as the level of source.

5.3 Supervised classification methods (LDA, RF, SVMs)

Linear discriminant analysis (LDA), Random Forest (RF) and Support Vector Machines (SVMs) were applied to the data set in order to evaluate their performance to predict the source of a sample. The levels of source considered were the manufacturer, type and batch of black powder. The classification models were built using 2/3 of the data set (training set, n = 82). Their capacity to predict correctly the source of a sample was validated using the remaining 1/3 of the data set (test set, n = 41). The training and test steps were repeated 100 times with samples randomly chosen for every iteration. The performance of the classifiers was assessed through the mean percentage of correctly classified samples (% CR - correct rate) as well as wrongly classified samples (% ER - error rate). In addition, the models were also tested by integrating the classification of 5 blind BP samples analysed in triplicate (n= 15). The classification of these 15 measurements was undertaken with each iteration. The classification performance of the blind samples was compared on the basis of the mean % CR and % ER, as well as the median.

Application of the classifiers to raw and preprocessed data sets revealed that raw data, as well as range scaling and standardisation produced better results than scaling rows to constant total. As raw data and the other 2 data preprocessing gave similar performance, only results obtained with the raw data set are presented in the following subsections.

5.3.1 LDA model optimisation

Optimisation of linear discriminant analysis (LDA) model was performed through variables selection. The less discriminant variables were removed from the model when their coefficient of linear discriminants was close to zero and the model was re-evaluated without these variables. The classification model by type of BP exhibited two superfluous variables: % O and and % N. Excluding these variables from the model slightly increased the classification ability of the classifier. For each source level, the variability in the optimised models accounted for by linear discriminant functions 1 and 2 (LD1 and LD2) was higher than 95 %, with LD1 explaining most of the difference between groups.

5.3.2 RF model optimisation

Two parameters can be modified in order to optimise the performance of the model: $\mathrm{m_{try}}$, the number of subset variables randomly chosen at each node, and $\mathrm{n_{tree}}$ corresponding to the number of trees in the forest.

For classification, the default value of m_{try} is $\sqrt{p}$, where p is the number of descriptors. Although this parameter is not reported to significantly affect RF performance [Breiman 2001, Svetnik *et al.* 2003], it is advised to investigate its effect on the model. For each source level to predict (manufacturer, type and batch), the value of m_{try} was set from 1 to 6, 6 being the maximum number of variables in the data set. The quality of classification of the model was assessed through the Out-of-Bag (OOB) estimate error rate. m_{try} set at 2 gave the best performances for the 3 source levels.

The number of trees has to be sufficiently large in order to allow the OOB error rate to stabilise. A number of 500 trees is generally adequate for small data sets [Svetnik *et al.* 2003]. The evolution of the OOB error rate as the number of trees increased was plotted for the prediction of each level of source. The overall OOB error rate showed to stabilise already after 100 to 150 trees. **Figure 8.28** illustrate the overall OOB error rate as well as that of each the class (the manufacturers) as the number of tree increases. The fact that the overall OOB error rate does not increase along with the increasing number of trees indicates that the model does not overfit. In supervised learning, complex models can overfit, meaning that they overinterpret data, which in turn produce poor predictive performance [Brereton 2009]. The tests performed with 1000 and 2000 trees did not improve the performances of the model.

Whatever the level of source considered, variable importance ranking showed that % N and % O were the less important in the model. However, removing these variables from the data set gave lower classification performance.

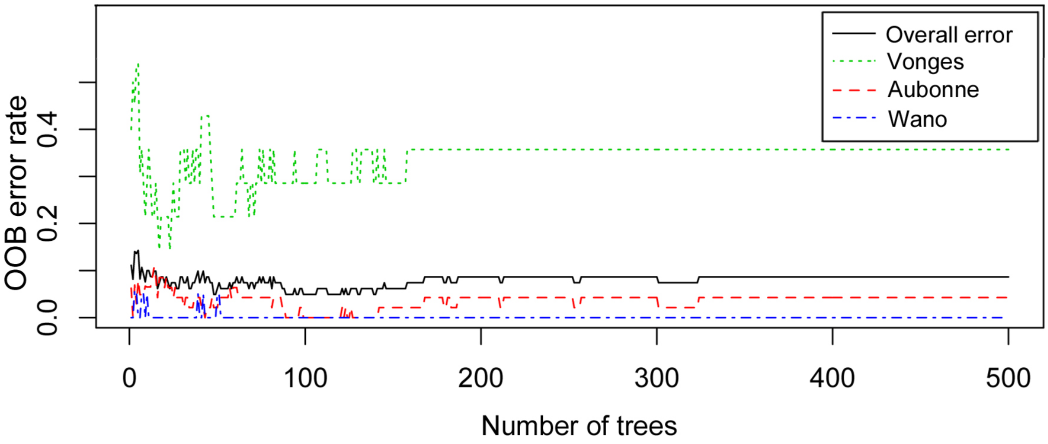

Figure 8.28. Overall OOB error rate and OOB error rate for each manufacturer as the number of trees in the forest increases.

5.3.3 SVMs model optimisation

When applying support vector machines with a radial basis function kernel (SVMs RBF), the RBF radius γ and the penalty parameter C are the two tuneable parameters. As C defines the accepted deviation of the model from the optimal solution, a large C value makes the model more tolerant to misclassification, while a low C forces the model to adopt a more complex solution to reduce the misclassification rate. The adequate value of these parameters are strongly dependent on the data structure and it is advised to optimise them to obtain the best performance [Dixon & Brereton 2009]. By performing a grid search, the values of C were tested between 0.1 and 10000 and the values of γ between 0.0001 and 100 for each training set, using a 10-fold cross validation. The higher % CR defined the best combination of the parameters. For the manufacturer level, the model used 31 support vectors, with cost set at 100 and γ at 0.1. For the type of black powder level, a number of 41 support vectors were used with cost and γ set at 10 and 0.1, respectively. For the batch level, the best cost and γ were 1000 and 0.01, respectively, defining a total number of 75 support vectors. These optimised parameters were then used to create the model on the training set. The model was then applied to the test set.

5.3.4 LDA, RF and SVMs models validation

Table 8.3 presents the performance of the optimised LDA, RF and SVMs models through the mean values obtained on 100 iterations. According to literature, a % CR higher than 50 % suggests that the classifier is able to discriminate between the different groups [Brereton 2009]. The higher the % CR is, the better the classifier. LDA achieved good results when classifying the sample according to the manufacturer (with about 90 % of CR) or according to the type and batch of BP (about 80% of CR). However, non linear based classifiers outperformed LDA. Considering both the means reported in **Table 8.3** and the medians illustrated in **Figure 8.29**, RF and SVMs demonstrate similar performance to classify sample according to the manufacturer and type of BP, yet classification per batch is better performed by RF.

Level of source	Classifiers	mean % CR	mean % ER
Manufacturer	LDA	89.0 ± 3.4	11.0 ± 3.4
	RF	**93.3 ± 3.8**	**6.9 ± 3.8**
	SVMs	92.0 ± 4.2	8.0 ± 4.2
Type of BP	LDA	85.6 ± 4.2	15.0 ± 4.4
	RF	**92.7 ± 3.5**	**7.3 ± 3.5**
	SVMs	91.8 ± 4.4	8.2 ± 4.4
Batch	LDA	78.4 ± 5.1	21.6 ± 5.1
	RF	**86.5 ± 4.7**	**13.5 ± 4.7**
	SVMs	79.5 ± 5.9	20.5 ± 5.9

Table 8.3. Performance of the classifiers determined on 100 iterations to predict the manufacturer, type and batch of the test set samples. The best performance is highlighted in bold for each source level.

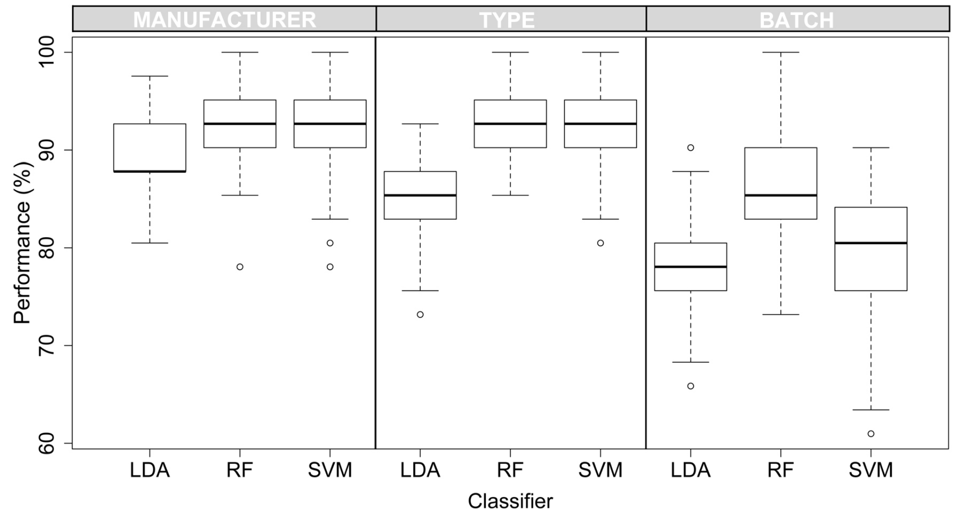

Figure 8.29. Comparison of the performance of the classifiers (in % CR) on 100 measurements to predict the source of the test set samples, according to the manufacturer, type of BP and batch.

The large range of performance of the classifiers for batch prediction, illustrated on **Figure 8.29**, likely comes from the small number of samples (n=3) describing some classes. Finally, the comparison of the medians with the mean values shows little difference, indicating that any of the two can be used as a reasonable estimate of % CR and % ER for performance comparison.

Given the overlap of several classes highlighted by the scores plots of PCA (see earlier subsection on PCA), the best performances of non linear models over LDA are understandable, as linear models cope less easily with such overlap. Numerous applications of non-linear SVM and RF reported their excellent classification ability to deal with complex data structure [Broséus *et al.* 2011, Qi 2012, Liu *et al.* 2013]. However, the best performances of RF over SVMs to classify samples according to the batch arise from its ability to deal with small data sets [Qi 2012, Liu *et al.* 2013]. Indeed, some batch were only represented by 3 measurements. SVMs models are only based on the samples on the margins of each class which define the support vectors (SVs). The optimal hyperplane separates two classes by maximising the margin between them. As 2 measurements out of 3 are selected in the training phase, these 2 measurements are defined as the margin of the class and constitute the SVs. The small number of measurements thus reduces the definition of the margins of the class and may lead the model to misclassify the third measurement.

Classification of blind samples The blind tests consisted in BP samples selected by an external operator. These chosen samples corresponded to A1, A3, W5, A4 and V2 and were each analysed in triplicate. The results of classification of the 5 blind samples yielded by LDA, RF and SVMs models are reported in **Table 8.4** and illustrated in **Figure 8.30**. Prediction of the manufacturer and type of blind samples was better achieved by RF with about 95 % CR. In contrast, when predicting the batch of blind samples, the performance of the 3 classifiers drastically decreased. While the classification of the test set according to batch was performed with a reasonable % CR (about % 80 CR for the 3 classifiers), these results question the validity of the classification models for batch prediction. These lower performances could reflect an overfitting phenomenon. Although overfitting is known to occur in SVMs models, RF does not overfit [Svetnik *et al.* 2003, Breiman 2001, Qi 2012, Liu *et al.* 2013]. As the performances of the 3 classifiers show the same trend, it is very likely that the representativeness of the blind data set all alone is responsible for the poor % CR.

Indeed, looking closer at the classification results, batches of Aubonne (A1, A3 and A4) are overall classified in wrong classes, most of the time in another batch of Aubonne, sometimes in batches of Vonges. This is not surprising given the consistency of Aubonne's production and the similarity of profile between Aubonne and Vonges batches. Finally, the small number of samples in the blind set plays a major role in the low performance of the models. One misclassified blind sample (representing 3 measurements, n=3) equals to 20 % ER (1/5 batch), while the wrong classification of a test set sample corresponds to an % ER of 5 % (1/19 batch). This also explains the higher % CR of RF for manufacturer and type classification. Thus, these results on the blind set samples cannot be totally compared with those of the validation set.

SVMs and RF showed the best performance when predicting the manufac-

Level of source	Classifier	mean % CR	mean % ER
Manufacturer	LDA	63.6 ± 13.2	36.4 ± 13.2
	RF	**94.5 ± 8.6**	**5.5 ± 8.6**
	SVMs	78.0 ± 8.5	22.0 ± 8.5
Type of BP	LDA	84.5 ± 11.6	17.9 ± 11.6
	RF	**96.5 ± 5.4**	**3.5 ± 5.4**
	SVMs	69.9 ± 10.2	30.1 ± 10.2
Batch	LDA	38.2 ± 8.8	62.7 ± 9.0
	RF	**40.7 ± 8.0**	**59.3 ± 8.0**
	SVMs	33.7 ± 7.8	66.3 ± 7.8

Table 8.4. Performance of the classifiers calculated on 100 iterations to predict the manufacturer, type and batch of 5 blind samples analysed in triplicate (n=15). The best performance is highlighted in bold for each source level.

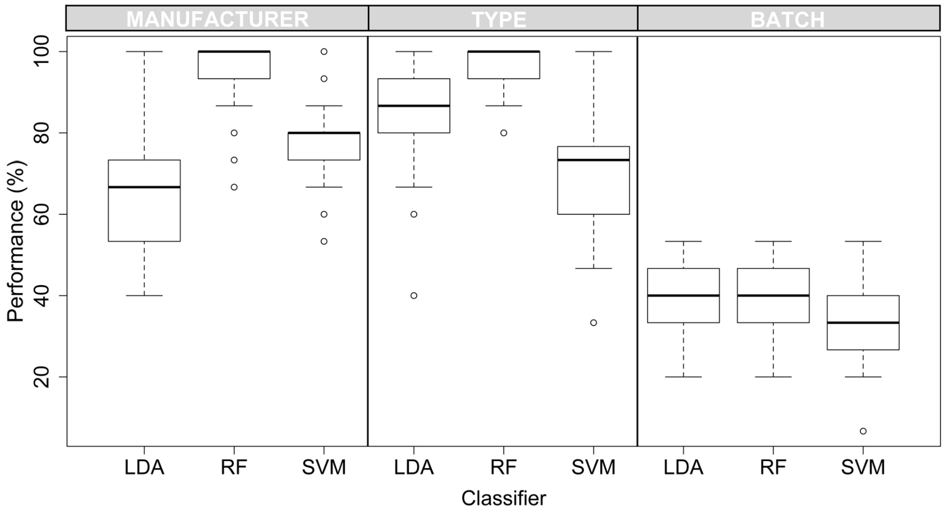

Figure 8.30. Comparison of the performance of the classifiers (in % CR) on 100 measurements to predict the source of the 5 blind BP samples according to the manufacturer, the type of BP and the batch.

turer and the type of BP of the test set samples with about 7 % ER. RF was the most efficient to predict the batch of the test set samples with about 13 % ER. The efficiency of RF was confirmed with the classification of blind BP samples according to the manufacturer and the type of BP. The classification of the blind samples according to their batch cannot be compared to that of the test set.

6 Discussion

6.1 Sampling representativeness

Sampling from the production chain of Aubonne included daily productions, as well as end products (final batches) resulting from the mix of the daily productions. The results show that the δ^{13}C, δ^{15}N and δ^{18}O of the final batch were not always representative of the daily productions. As no chemical process is involved in the constitution of the final batch, these results either indicate that the sampling was not representative of the total mass of the final batch (not all the daily productions constituting the final batch were included in the sampling) or that the small analysed amounts offered a limited isotopic representativeness of the final batch. Depending on the target element, the necessary amount for one analysis ranged from 1 to 2 mg of black powder. This amount, representing in fact about 2 to 3 grains of black powder, appears incredibly insignificant compared to the 1.8 tons of the final batch. This comparison highlights the small amounts analysed compared to the produced quantities. In addition, the restricted number of analyses (n=3) per batch or daily batch was probably insufficient to obtain a representative δ value. This is corroborated by the difference of dispersion of the δ^{13}C, δ^{15}N and δ^{18}O values resulting from 3 measurements and those of the kilo of black powder A1 resulting from a larger number of measurements. However, this imperfection of the sampling representativeness does not invalidate the results of this research but increases the associated uncertainty measurement. In an ideal case, this imperfect sampling could be enlarged with additional samples to improve sampling representativeness and to reduce the associated uncertainty.

These elements highlight the difficulty to ensure a representative sampling for mass-produced substances, even when considering the characteristics of production (information on the production chain and processes, raw materials, produced quantities).

6.2 Considerations on intravariability

The variability existing within the production of a manufacturer was evaluated at different levels (variations within a kilo or half kilo of BP, within and between batches of a manufacturer, within and between manufacturers). Even when considering the batch level of manufacturers, large variations were measured as summarised in **Table 8.5**

Results presented in this chapter demonstrate that intravariability can be particularly important. This large intravariability, for example, 1 ‰ for the δ^{13}C value in Aubonne's production is significant as the BP sold on the market is directly derived from the batch. This obviously impacts on the interpretation

Manufacturer	% C [%]	% N [%]	% O [%]	$\delta^{13}C$ [‰]	$\delta^{15}N$ [‰]	$\delta^{18}O$ [‰]
Aubonne	1.8	1.0	7.3	1.0	1.0	1.6
Wano	0.9	1.0	1.9	0.2	3.7	0.7
Vonges	1.0	1.9	6.1	0.5	1.0	0.4

Table 8.5. Largest differences measured within batches of the manufacturers.

of results. As highlighted in chapter 4, published studies often neglect to report on intravariability. It is interesting to note that, each time the authors reported on it, the measured intravariability was generally larger than expected [Benson *et al.* 2009a, Schneiders *et al.* 2009]. The notion of intravariability should therefore not be underestimated. As fully part of the methodological process [Gentile *et al.* 2011], intravariability should be thoroughly evaluated, although preconceived ideas may suggest that little variations will be observed.

6.3 Observing the variability, reflecting on the notion of source

6.3.1 Isotopic and elemental variations

Not only was variability observed at the isotopic level but also in the elemental composition of black powders. In chapter 5, the source was defined at the level of the manufacturer based on the available information on the production of black powder. However, the variability of the target variables and the structure of the data set revealed that this preliminary definition of the source could possibly be refined. While some manufacturers produced a black powder with a constant profile[3] between batches, others delivered different batches of the same product with a very variable profile. This means that each manufacturer produces black powder in his own way. The manufacture of black powder does not involve any chemical process, therefore the isotopic profile of black powder directly reflects that of the raw materials used. Each manufacturer freely chooses the use of the raw materials. He can either:

- always use the same raw material for all his production, irrespective of the type of black powder he produces;
- use different raw materials for different types of powder;
- use different raw materials (different providers) for the batches of a same type of black powder.

Finally, even if the manufacturer always buys its raw materials from the same suppliers, the procurement sources of the suppliers can vary, such as for KNO_3

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[3] the profile here is defined by the studied target variables: $\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, % C, % N, % O

which can come from different geologic sources or even be synthesised.

These elements demonstrate that the variability of the profile of black powder is partly dependent on the manufacturing process (for the % C, % N and % O variables), but is also mainly dictated by the isotope signature of the raw materials used by the manufacturer.

The United States geological survey (USGS) provides minimum and maximum isotope ratios in naturally occurring materials and reagents [Coplen *et al.* 2002b]. It reports an overall range of 170 ‰ for $\delta^{15}N$ in nitrates. The range measured in this research corresponds to that of desert salt deposits (-5 to +15 ‰), soil extracts (-23 ‰ to +46 ‰) and synthetic reagents and fertilisers (-23 ‰ to +15 ‰). The negative inhomogeneous $\delta^{15}N$ values measured within samples of Wano could reflect a natural origin such as desert salt deposits. The oxygen isotopic composition of nitrate chemical reagent is reported to be close to that of atmospheric oxygen (about +24 ‰), as it was measured in the batches of Aubonne and Vonges. The $\delta^{18}O$ values of atmospheric nitrate (atmospheric N_2 which is converted to nitrate, range from +14 ‰ to +76 ‰, while that of dissolved nitrate in soils and ground waters is encompassed between -2 ‰ to +22 ‰. For carbon, $\delta^{13}C$ values vary by more than 140 ‰ in nature, although the measured range for BP in this study does not exceed 2 ‰.

6.3.2 Discrimination of sources

The three considered classifiers–LDA, RF and SVMs–are suitable for the classification of BP samples according to the 3 levels of source. Nevertheless, non linear classifiers outperformed LDA. Among the three, RF exhibited the best performance and was able to predict the manufacturer, type and batch of BP of a sample, with about 7 % ER for the first two and 13 % ER for the latter. These results not only confirm the hypothesis enunciated in chapter 5, but also refines the considered level of source. Hypothesis IV.1 is confirmed and, based on the presented results, can be modified as follows:

> Hypothesis IV.1
>
> The information extracted from the isotopic analysis of black powders allow categorising distinct manufacturers, types and batches of black powder.

These results are very promising. However, there are several points to bear in mind:

1. In light of the production market of black powder, the number of samples analysed in this research is relatively small: it represents 3 manufacturers, 5 types of BP and 19 batches of BP. Although the European production does

not count many manufacturers (see chapter 2), with import and export possibilities, the worldwide production from the U.S.A, Brazil and especially China cannot be ignored.

2. An interesting feature of isotopic values is that they are reported on an international reference scale. A possible complement to this research would be the integration of additional BP data collected by other studies involving BP. Wakelin and Lock reported on the isotopic analyses of BP specimens coming from police seizures [Wakelin 2001, Lock 2009]. Despite the fact that they analysed BP specimens coming mainly from caseworks, additional indications on year and country of origin is provided for some BP. By nourishing the model with new data, this would allow re-evaluating it with a growing data set and adapting the % CR and ER.

   The USGS report compiles large ranges of isotopic values for the elements and substances analysed in this research [Coplen *et al.* 2002b]. However, comparing the results obtained in this research with those of other studies involving black powders [Wakelin 2001, Lock 2009], it can be seen that the ranges of isotopic values reported in **Table 8.6** are very similar. Thus, with an increasing number of BP samples coming from different sources (manufacturers, types of BP or batches), it is likely that the % ER of any classification model will increase.

Research	**δ^{13}C [‰] range**	**δ^{15}N [‰] range**	**δ^{18}O [‰] range**
[Wakelin 2001]*	-28.5 to -26.0	-27.0 to +5.0	+12.0 to +24.0
[Lock 2009]	-27.5 to -25.6	-16.9 to +8.3	+14.4 to +28.9
this research	-28.6 to -26.4	-28.0 to +2.9	+15.0 to +24.2

Table 8.6. Comparison of the range of isotopic value of black powder obtained in the different studies. *: exact δ values were not available, figures were deducted from plots.

   Furthermore, the % CR and ER were obtained using supervised models. Thus, these models would be inadequate to detect new classes of samples which have a different profile from that of the training set. However, an exciting approach to deal with new classes is the use of unsupervised RF [Shi & Horvath 2006]. RF encloses a measure of "similarity", which is called intrinsic proximity measure [Breiman 2001]. This proximity, measured between pairs of samples, can be used for clustering [Svetnik *et al.* 2003]. Shi and Horvath reports on the possibility of setting threshold rules to define cluster membership. This solution, which combines the advantages of RF, appears as a very attractive feature to detect new classes or to compare distance between populations and deserves further investigations.

3. The statement of Hypothesis IV.1 is confirmed, *as long as the data structure*

is known (preliminary knowledge of classes). This is especially highlighted by the difference of performance between unsupervised and supervised approaches. Supervised methods give excellent results with low % ER, while unsupervised methods provide higher % FP rates or % ER.

In the framework of forensic investigations, one of the questions interesting the police and the criminal judicial system is whether two samples of BP could have come from the same source or are from different sources. This enquiry can occur in two situations. The first one involves a knowledge of the structure of the market or data at disposal. The profiles of manufacturers and their production are known and stored for comparison. This situation involves the use of supervised methods, which provide excellent classification results but require a complete knowledge of the market and, actually, rarely exists. However, this approach is conceivable, if the question to answer is for example, whether the incriminated black powder comes from a black powder produced by Aubonne or not. It is worth noting though that machine learning RF and SVMs act as a black box [Svetnik *et al.* 2003]. The inner relation of the models between the variables and the results are not available for examination. In a prosecutorial approach, it is difficult to conceive that the comparative evaluation of a piece of evidence may rely on such a process, which may then have heavy consequences on the life of a person. By contrast, the use of such models to produce intelligence is less disturbing, as information is used to support investigations or assist decision makers in crime reduction or prevention.

The second situation reflects a more common reality of investigations: data structure is not known. In that case, unsupervised statistical approaches provide an interesting way to compare samples and determine if they have a similar or very different profiles. These methods generate more false positives but the information they produce can be used in an intelligence-led approach. One of the essence of this approach is to gather and exploit the maximum of information to generate intelligence for decision making. In that perspective, minimising the rate of false negatives limits the loss of information.

CHAPTER 9

Differentiation of sources of AN fertilisers

Contents

1 Introduction

In **Chapter 5**, hypothesis IV.2 defined the source of AN fertiliser as the manufacturer. The different information obtained on the samples allow exploring the variability at several levels. In the following sections, the concept of intravariability of AN fertiliser is studied at the following levels: first, the variability measured within a 100 g sample and a 25 kg bag of AN fertiliser was examined, then the variability within a batch and between batches of the same type of AN fertiliser and from the same manufacturer was considered. Finally, different batches of the same manufacturer (involving all the sampled types and batches of AN fertiliser he produced were also compared. Intervariability was evaluated between the production of the manufacturers.

Table 9.1 presenting the AN fertiliser samples analysed in this research is recalled here in order to ease the reading and comprehension of the chapter.

Fertiliser type **Composition**	**Period of production**	**Manufacturer** **Country**	**Reference number**	**Samples**
AN Ca, Mg	before 2003	Unknown	L1.1-1.3	3 x 100 g in a 50 kg bag
AN	March 2007	BASF Belgium	L2	1 x 500 g
AN	March 2007	Pec Rhin France	L3	1 x 500 g
AN	March 2007	DSM Stein Netherland	L4	1 x 500 g
AN	April 2007	Kemira GrowHow Belgium	L5	1 x 500 g
AN Mg	March 2007	Yara Sluiskil Netherland	L6	1 x 500 g
AN Mg	April 2007	Yara Sluiskil Netherland	L7	1 x 500 g
AN Borax	March 2007	Lonza Switzerland	O1	1 x 100 g
AN, Mg Dolomite	April 2007	Lonza Switzerland	O2	1 x 100 g
AN $CaCO_3$	March 2007	Lonza Switzerland	O3	1 x 100 g
AN $MgSO_4$	April 2007	Lonza Switzerland	O4.1-4.3	3 x 100 g in the batch
AN schiste	spring 2006	Lonza Switzerland	O5.1-5.3	3 x 100 g in the batch
AN schiste	spring 2007	Lonza Switzerland	O6.1-6.3	3 x 100 g in the batch

Table 9.1. Samples of AN fertilisers obtained from Landor and Lonza.

2 Nitrogen content and isotope ratio

2.1 Variations within a manufacturer

2.1.1 Variations within a 25 kg bag and a 100 g sample

The δ^{15}N and % N variability measured within a 100 g sample (L5, n = 14 analyses) was compared to that of 3 samples taken in a 25 kg bag (L1.1 to L.1.3, n = 3 analyses for each sample, making a total of 9 analyses for L1) (**Figure 9.1**). L1 comes from an unknown origin and was produced before 2003 and L5 was produced in Belgium in 2007. In addition, it is worth noting that L5 was used as the control sample in every analytical sequences. The measurements of L5 reported in this section actually reflect the variability of the method, that is the variability associated with the measurement of AN fertiliser. Samples L1 and L5 show a similar δ^{15}N variability (a range of 0.2 ‰ for L1 and 0.3 ‰ for L5). This shows that the variability is comparable for samples coming from different places and produced at different periods. Thus, these variations likely reflect the variability associated with the isotopic measurement of AN fertiliser material.

The % N measured in samples L1 is larger than the % N of L5 (4.5 % versus 2.9 % N). Looking closer at the data, the 14 measurements of sample L5, the control sample for AN analyses, were obtained from 7 analytical sequences spread over 2 months. In contrast, the 9 values for L1.1 to L1.3 were measured in 2 analytical sequences run on a shorter period of time (3 days difference between the 2 sequences). Thus, L1 variations can be attributed to the intravariability of the sample itself. It is also possible that this variation comes from the sample preparation related to the method, as mentioned in **Chapter 6**. Indeed, sample preparation of the second nitrogen method involved removing the extra tin foil from the capsule. This delicate step could provoke the loss of small particles of the sample, resulting in a lower % N.

Overall, the δ^{15}N variations measured in the 25 kg bag (L1) do not exceed the variability of the 100 g sample (L5). By contrast, larger % N variations were measured in the 25 kg bag than in the 100 g sample (control sample L5).

2.1.2 Variations within and between batches

Variations within a batch The δ^{15}N and % N variability within a batch was evaluated through the analyses of fertilisers O4 (O4.1 to O4.3), O5 (O5.1 to O5.3) and O6 (O6.1 to O6.3). The three samples, taken in the huge mass provides a rough evaluation of the possible variability expected within a batch. Fertiliser O4 was produced at the same period of the year as fertiliser O6 (April 2007 for O4 and spring 2007 for O6), but both are two different types of AN fertiliser. In contrast, O5 and O6, produced with a year difference (respectively, spring 2006 and 2007),

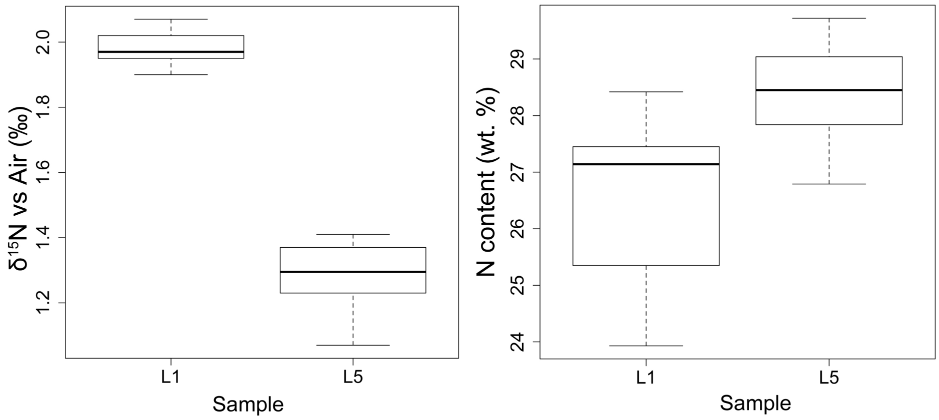

Figure 9.1. Variability of the δ^{15}N value and % N in a 25 kg bag (sample L1, n = 9) and in a 100 g sample of AN fertiliser (sample L5, n = 14).

correspond to the same type of fertiliser.

As illustrated in **Figure 9.2**, the δ^{15}N variations measured within a batch are limited. Except for O4, which has an almost uniform distribution of variations of up to 0.6 ‰, most of the δ^{15}N differences of O5 and O6 are smaller than 0.3 ‰, with variations of up to 0.4 ‰. This difference in δ^{15}N variations between, on one hand, O4 and, on the other hand, O5 and O6 shows that one type of AN fertiliser (O5 and O6) has a smaller δ^{15}N variability than the other type (O4). The range of 0.4 ‰ measured in samples O5 and O6 is similar to the δ^{15}N variability reported in the previous subsection for a 25 kg bag and a 100 g sample, and thus corresponds to the variability of the method. The variability measured in these batches is therefore similar to that measured in the 100 g sample[1]. The larger variability of O4 exceeds that of control sample L5, indicating that these variations are most likely inherent to the sample itself. Although larger, they are however in the same range of values.

Figure 9.3 represents the % N variations measured within a batch. Most variations are below 1.2 %, except for sample O5 which shows larger variations. Nevertheless, such differences of up to 4.4 %, are larger than those measured in the 100 g sample (2.9 %) but similar to those observed in the 25 kg bag (4.5 %) (see previous subsection).

Overall, the δ^{15}N and % N variations measured within a batch are similar to the variability of the method when analysing AN fertilisers. Some batches showed however larger variations: up to 0.6 ‰ and 4.4 % N difference were measured within a batch.

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[1] Note, though, that the samples to evaluate batch intravariability are limited

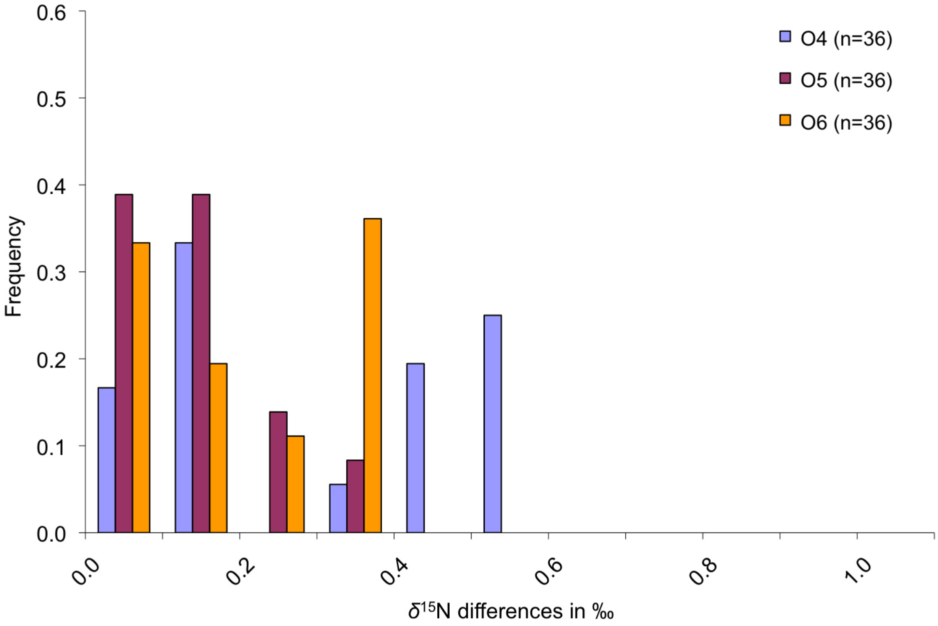

Figure 9.2. δ^{15}N differences between pairs of measurements for batches O4, O5 and O6.

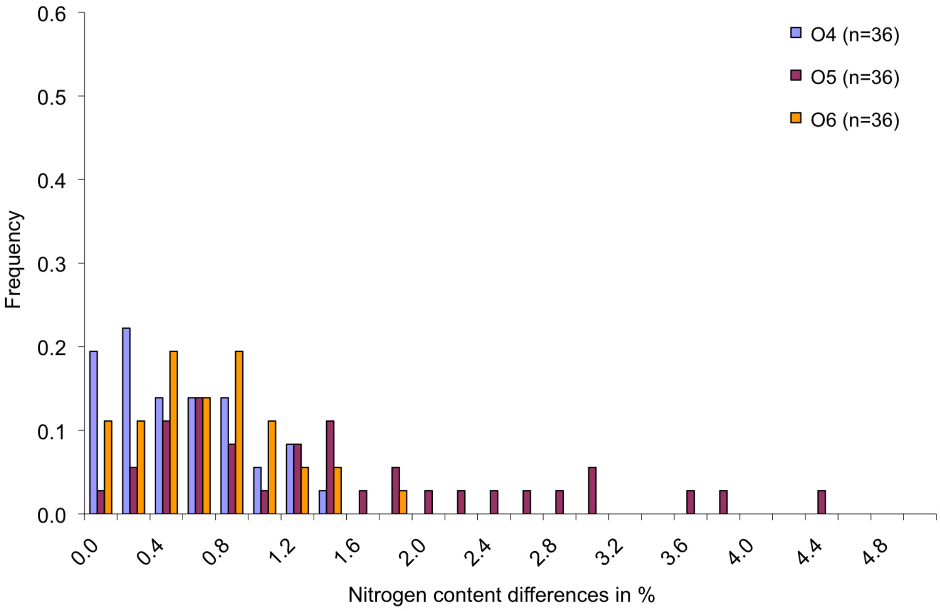

Figure 9.3. % N differences between pairs of measurements for batches O4, O5 and O6.

Variations within the same type of fertiliser The aim was to examine the variations within a type of AN fertilisers (same manufacturer, different batches of the same type). For this purpose, the variability of two types of AN fertilisers were examined: one type produced by Lonza (represented by batches O5 and O6) and one type produced by Yara (represented by batches L6 and L7). Samples O5 and O6 were produced with a year difference by Lonza. Samples L6 and L7 were produced in March and April 2007 by Yara. **Figure 9.4** illustrates the δ^{15}N and % N variations of the two different types of AN fertiliser. The mean and standard deviation of the batches are also presented in **Table 9.2**.

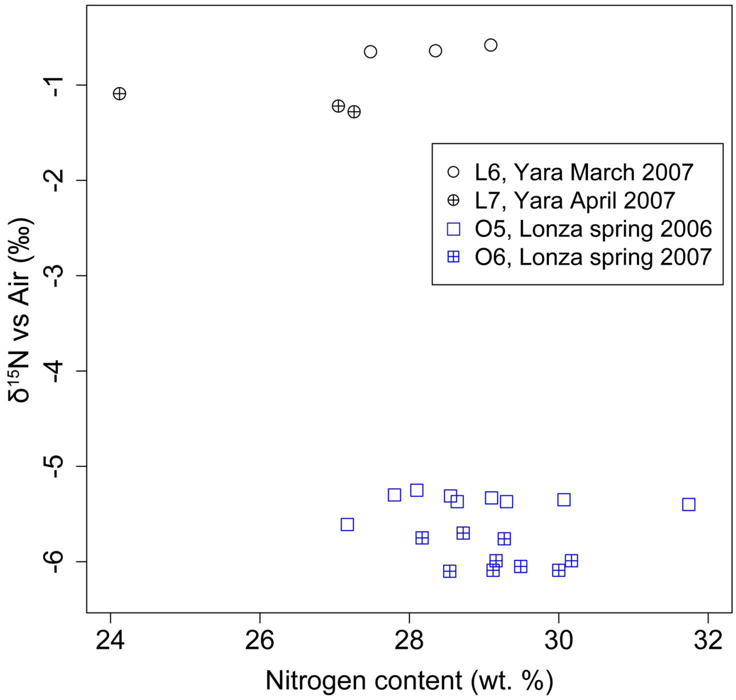

Figure 9.4. δ^{15}N value and % N of batches of the same type of AN fertiliser.

Batch	Manufacturer Fertiliser type	% N ± SD [%]	δ^{15}N [‰ vs Air]
L6	Yara	28.3 ± 0.8	-0.6 ± 0.1
L7	AN, Mg	26.1 ± 1.8	-1.2 ± 0.1
O5	Lonza	28.9 ± 1.4	-5.4 ± 0.1
O6	AN, schiste	29.2 ± 0.7	-5.9 ± 0.2

Table 9.2. Mean and standard deviation (SD) of δ^{15}N values and % N of batches L6 and L7 (n = 3 for each), and batches O5 and O6 (n = 6 for each).

As illustrated in **Figure 9.4**, there is no significant differences between the % N of the batches of one type of AN fertiliser, except for one measurement of sample L7 which exhibits a lower % N. This lower value most likely comes from the sample preparation for nitrogen analysis, as explained in **Chapter 6**.

In contrast, the batches of the same type of AN fertiliser exhibit different δ^{15}N values, as shown by the means reported in **Table 9.2**. Considering the the variability of the method and variations measured within a batch (see previous subsection), this δ^{15}N difference between batches of the same type of AN fertiliser appears significant. These elements suggest that batches of the same type of AN fertiliser are not distinguishable based on their % N, but may be differentiated based on their δ^{15}N value.

Variations between batches of the same manufacturer The variability within the production line of a manufacturer was evaluated through samples O1 to O6 produced by Lonza (all types considered). One of the particularity of the production line of Lonza is the synthesis of one batch of pure ammonium nitrate, which is subsequently mixed with different additives to form the various types of AN fertilisers. The δ^{15}N value and % N of the samples of the batches are plotted in **Figure 9.5**.

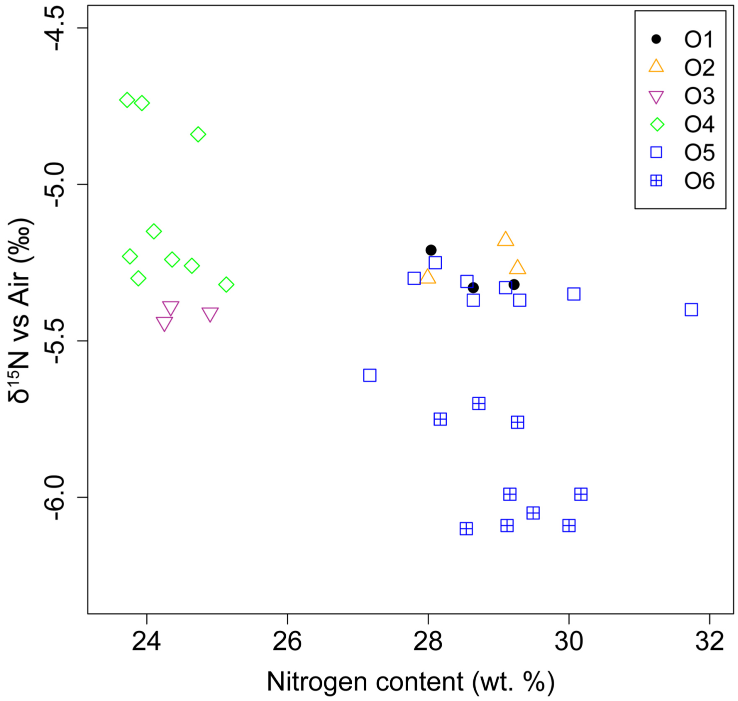

Figure 9.5. δ^{15}N value and % N of batches produced by the manufacturer Lonza.

Although the δ^{15}N value of the batches oscillates between -6.1 ‰ and -4.7 ‰, is it is fairly similar within the production line of the manufacturer. As there is no additional information on whether some fertiliser batches were manufactured with the same batch of pure AN, it is difficult to interpret any δ^{15}N variations with this lack of information. On the contrary, the % N is less homogenous between the different types of AN fertilisers. Samples O3 and O4 present a lower % N (between 23.7 and 25.1 %) compared to the rest of the batches (between 27.2 and 31.7 %). This difference in % N comes from the type of AN fertiliser, which contains less nitrogen. The AN fertiliser with $CaCO_3$ as additive (sample O3) and the one with $MgSO_4$ (sample O4) contain in reality 23 and 25 % N, respectively [Kummer 2007]. The rest of the AN fertilisers from Lonza contain either 27 or 27.5 % N. Note, though, that the measured % N is greater than the theoretical value for all samples. This indicates that, although measured with the optimized nitrogen method, the conversion of bulk material into N_2 is still not completely quantitative.

2.2 Variations between manufacturers

The δ^{15}N and % N variability between manufacturers are illustrated in **Figures 9.6** and **9.7**. The large δ^{15}N variability (between - 6.1 ‰ and + 2.1 ‰) and the small intravariability per manufacturer[2] suggest this variable as an interesting discriminant feature (**Figure 9.6**). The production of Lonza has a completely different δ^{15}N value from that of other manufacturers. In contrast, the % N of each manufacturer shows more distribution and overlap (**Figure 9.7**). Except for samples O3 and O4, with a lower % N, the productions of the other manufacturers are within the same range (about 27 % N), rendering % N less interesting for sample discrimination.

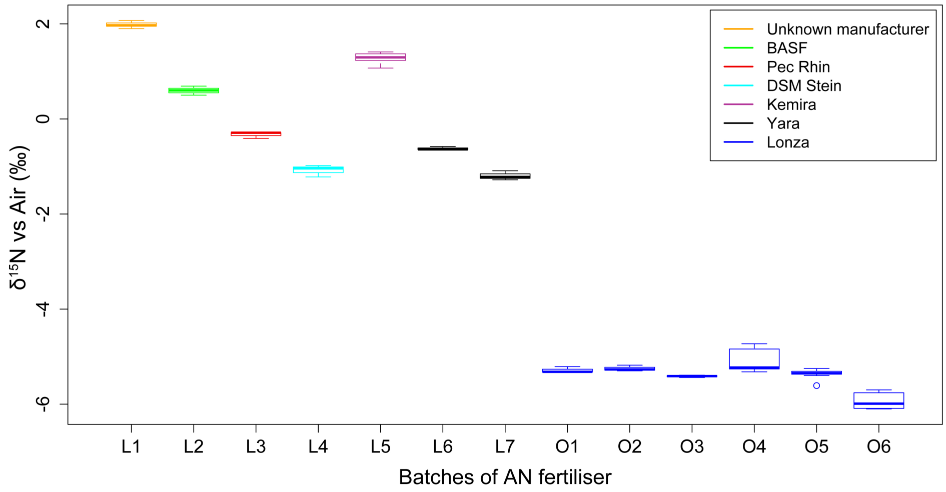

Figure 9.6. Variability of the δ^{15}N value of batches of AN fertilisers between manufacturers. For L1, O4 to O6, n = 9; for L5, n = 14. For the rest of the samples, n = 3.

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[2] This limited intravariability may also be due to the small number of measurements for some samples

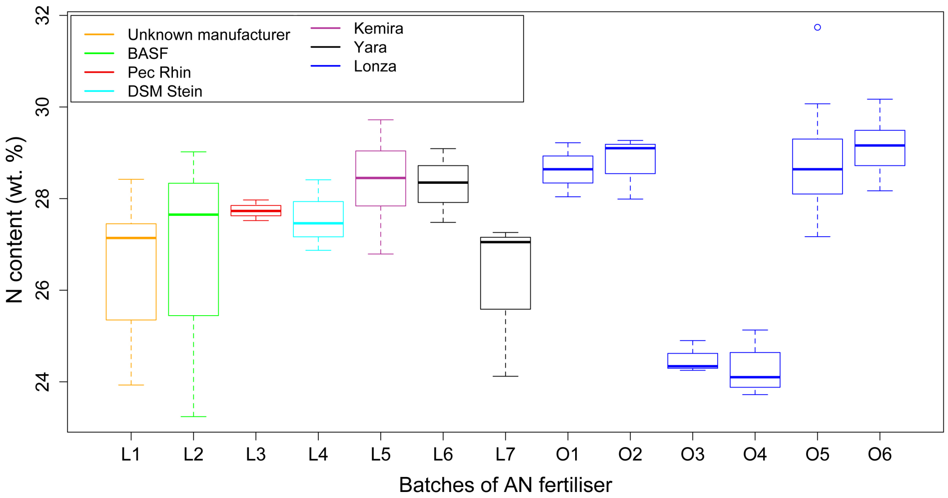

Figure 9.7. Variability of the % N of batches of AN fertilisers between manufacturers. For L1, O4 to O6, n = 9; for L5, n = 14. For the rest of the samples, n = 3.

3 Oxygen content and isotope ratio

3.1 Variations within a manufacturer

3.1.1 Variations within a 25 kg bag and a 100 g sample

The variability of δ^{18}O and % O was evaluated in a 100 g sample (L5) (n = 34) and a 25 kg bag (L1), through the analysis of 3 samples taken in the bag (L1.1 to L.1.3) (n = 9) (**Figure 9.8**). While the δ^{18}O value of sample L5 lies between 22.0 ‰ and 23.7 ‰, that of sample L1 is much more restrained (from 24.9 ‰ to 25.4 ‰). Excluding the outliers, the range of δ^{18}O variations in sample L5 is more reasonable (1.0 ‰). The difference in δ^{18}O variability is explained by the fact that the 34 measurements of the control sample L5 were obtained from 13 analytical sequences run over 2 months, while the 9 measurements of sample L1 were analysed on 2 different days in 2 analytical sequences. Thus, the variability of sample L1 is within the variability of the method, which is reflected by the δ^{18}O measurements of control sample L5.

The % O variability expands over a similar, though large, range (8.6 % and 5.8 % for L1 and L5, respectively). Without outliers, the % O covers a moderate range of 2.7 % for both samples. The % O variability measured in the 25 kg bag (sample L1) is within the variability of the control sample L5 (100 g sample), indicating that the intravariability of AN contained in the 25 kg bag is smaller or equal to the variability of the method.

Overall, the δ^{18}O and % O variations measured in the 25 kg bag (L1) were not larger than the variations of the method.

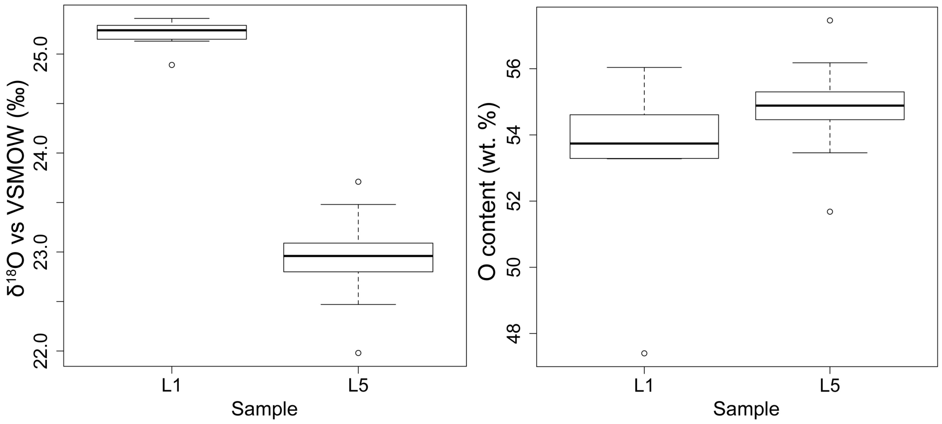

Figure 9.8. Variability of the δ^{18}O value and % O in a 25 kg bag (sample L1, n = 9) and in a 100 g sample of AN fertiliser (sample L5, n = 34).

3.1.2 Variations within and between batches

Variations within a batch The variability of δ^{18}O and % O within a batch was evaluated on the basis of the three samples taken for AN fertilisers O4 (O4.1 to O4.3), O5 (O5.1 to O5.3) and O6 (O6.1 to O6.3). **Figures 9.9** and **9.10** illustrate the differences in δ^{18}O and % O calculated between pairs of measurements. Most δ^{18}O variations measured within a batch are below 0.7 ‰. δ^{18}O differences can however reach 1.4 ‰. It is interesting to note that, as for nitrogen, sample O4 exhibits the largest δ^{18}O variability. This variability is larger than that associated to the method and appears therefore inherent to the sample itself.

Variations of % O within a batch primarily lie below 3 %, although differences of up to 6 % were also measured. The δ^{18}O and % O variability measured within batches O5 and O6 correspond to that observed in the previous subsection for a 25 kg bag and a 100 g sample and therefore are within the variability of the method.

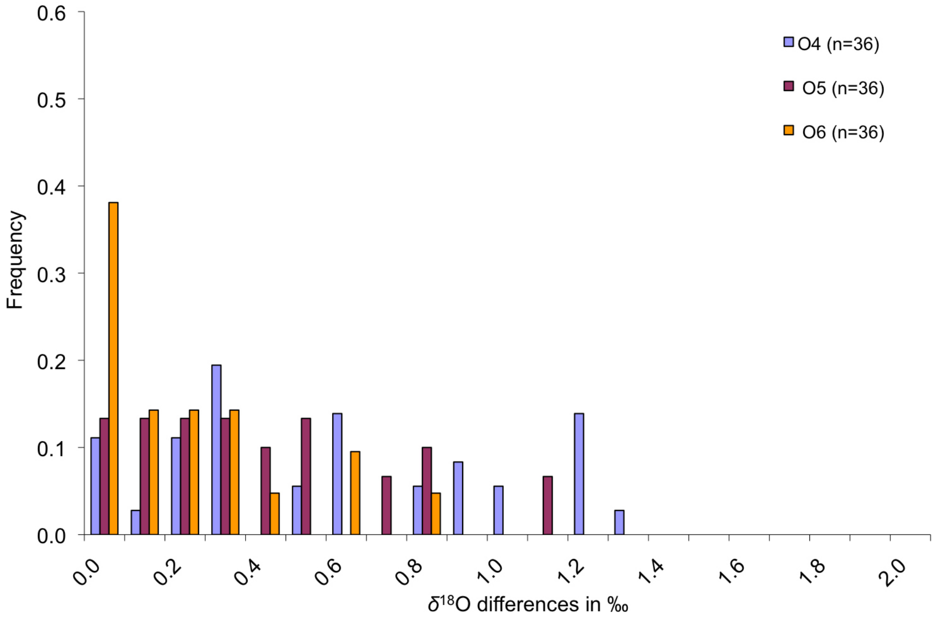

Figure 9.9. δ^{18}O differences between pairs of measurements for batches O4, O5 and O6.

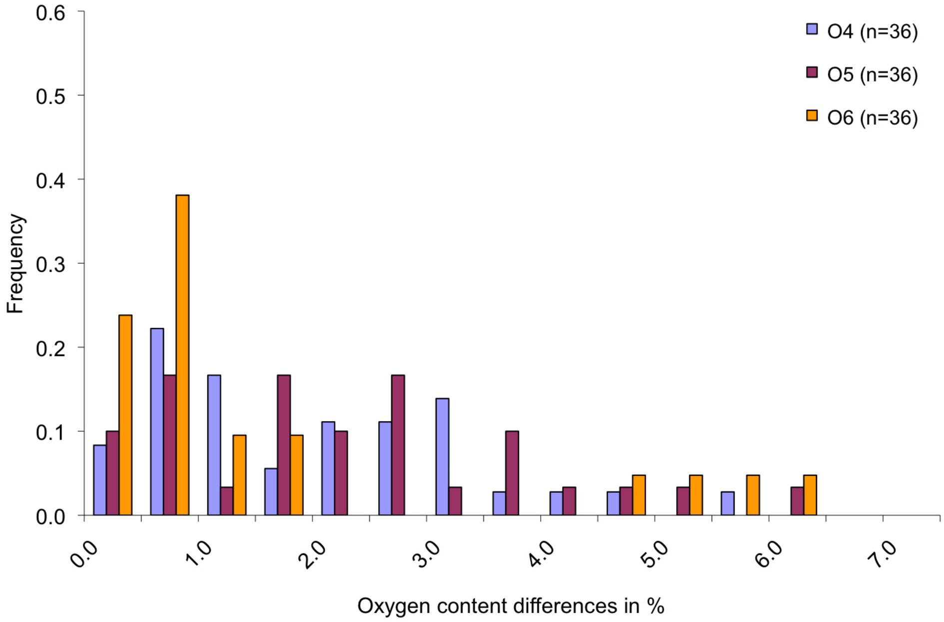

Figure 9.10. % O between pairs of measurements for batches O4, O5 and O6.

Variations within the same type of fertiliser The δ^{18}O and % O variations between batches of the same type of AN fertiliser were evaluated by comparing AN fertilisers L6 and L7 and AN fertilisers O5 and O6. Their δ^{18}O value and % O are presented in **Figure 9.11**. **Table 9.3** presents the mean and standard deviation of the batches.

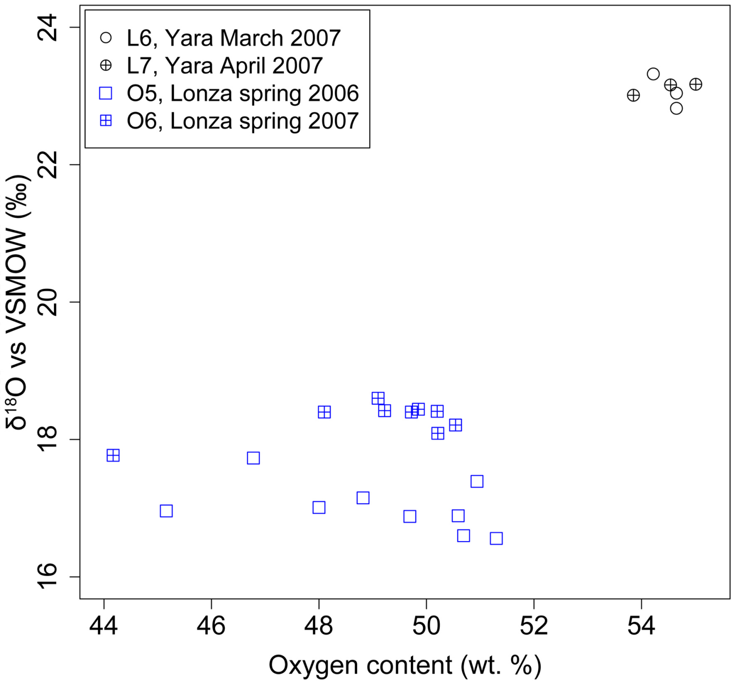

Figure 9.11. δ^{18}O value and % O of batches of the same type of AN fertiliser.

Batch	Manufacturer Fertiliser type	% O ± SD [%]	δ^{18}O±SD [‰ vs VSMOW]
L6	Yara	54.5 ± 0.2	23.1 ± 0.3
L7	AN, Mg	54.5 ± 0.6	23.1 ± 0.1
O5	Lonza	49.1 ± 2.1	17.0 ± 0.4
O6	AN, schiste	49.0 ± 2.0	18.3 ± 0.2

Table 9.3. Mean and standard deviation (SD) of δ^{18}O values and % O of batches L6 and L7 (n = 3 for each), and batches O5 and O6 (n = 6 for each).

Batches of the same type have similar % O, both for batches produced with a month difference (L6 and L7) and with a year difference (O5 and O6). The δ^{18}O values of batches of a same type of AN fertiliser are in a similar range. The means of batches L6 and L7 produced by Yara are not significantly different, however there is a difference of 1.3 ‰ between the mean δ^{18}O values of batches O5 and O6 produced by Lonza with a year difference. This difference is however similar to the variability of the method reflected by measurements of sample L5 (see **Subsection 3.1.1**). These elements suggest that the δ^{18}O value and % O of a type of AN fertiliser produced by a manufacturer are in similar range of values. Thus, batches of the same type of AN produced at different periods of time may be difficult to distinguish.

Variations between batches of the same manufacturer The δ^{18}O and % O of the different AN fertilisers (O1 to O6) produced by Lonza were examined to evaluate the variability within the production of the manufacturer. Samples of Lonza cover a large range of % O (from 44.2 to 57.7 %) and have a variable δ^{18}O value (from 16.5 to 20.1 ‰) (**Figure 9.12**). Although some samples have a similar δ^{18}O and % O (samples O1, O5, O6), others have different means % O (sample O4) or δ^{18}O values (samples O2 and O3). The additives used for the different types of AN fertiliser contribute to the bulk % O and δ^{18}O value and may be at the origin of the variability of these features within the production of Lonza. However, it does not explain the large dispersion of values.

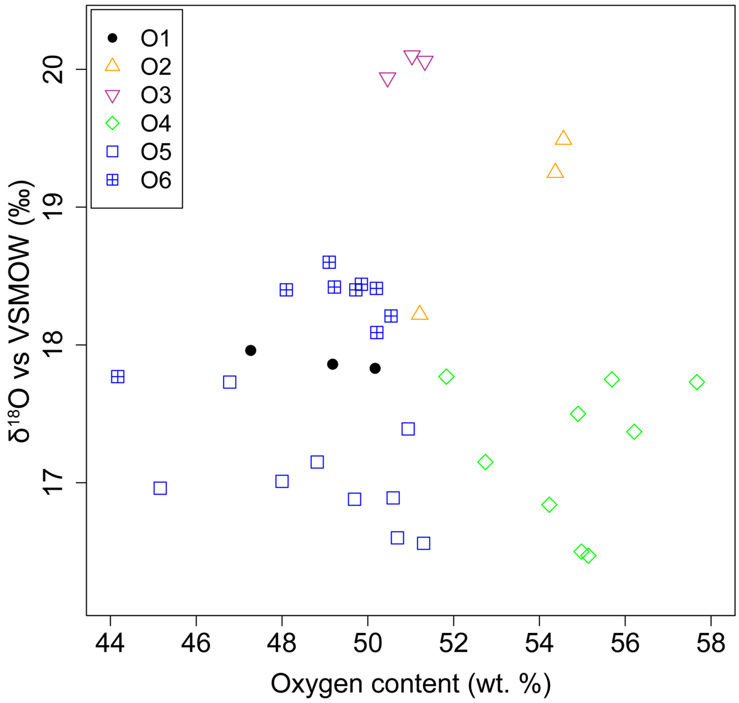

Figure 9.12. δ^{18}O value and % O of batches of AN fertiliser produced by the manufacturer Lonza.

3.2 Variations between manufacturers

Figures 9.13 and **9.14** present the δ^{18}O and % O variability between manufacturers. Despite its large intravariability, Lonza (O1 to O6) has a lower distinct δ^{18}O value than the other manufacturers. The unknown manufacturer (sample L1) and BASF (sample L2) have also a different δ^{18}O value. These δ^{18}O variations may assist in discriminating AN samples produced by different manufacturers.

Despite limited variability between L1 to L7, differences in % O may be detected between various AN fertilisers, such as between samples of Lonza (O1 to O6) and Yara (L6 and L7). Indeed, according to the additives mixed with AN, different % O may be measured and may assist in differentiating some AN fertilisers.

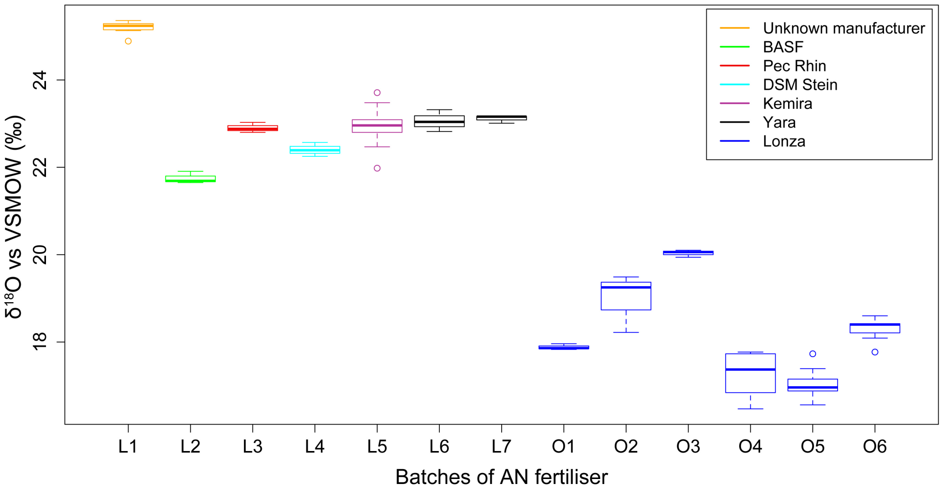

Figure 9.13. Variability of the δ^{18}O value between manufacturers of AN fertilisers. For L1, O4 to O6, n = 9; for L5, n = 14. For the rest of the samples, n = 3.

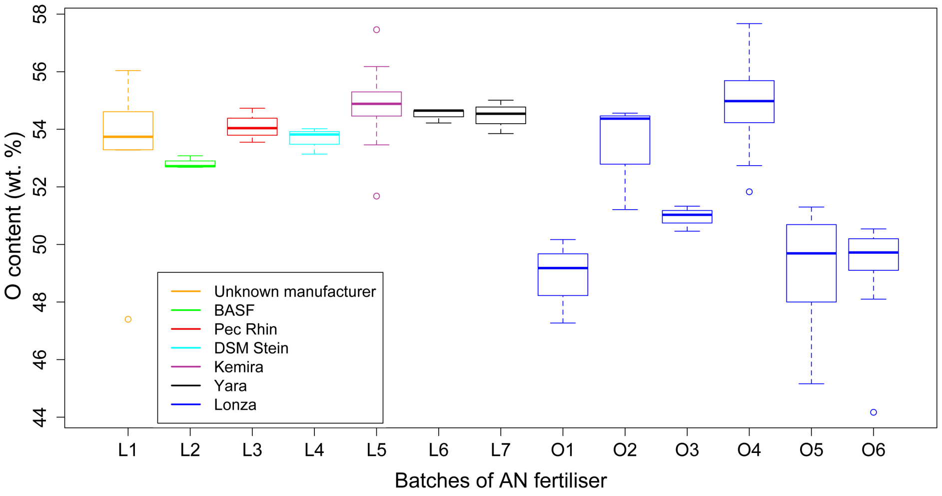

Figure 9.14. Variability of the % O between manufacturers of AN fertilisers. For L1, O4 to O6, n = 9; for L5, n = 14. For the rest of the samples, n = 3.

4 Differentiation of sources using combined features

4.1 N and O variations: in essence

The previous sections showed that variations in nitrogen and oxygen contents and isotope ratios could be observed at different levels of production. These findings are summarised hereafter:

- Between batches of the same type of AN fertiliser, there is no significant difference in % N and % O. The δ^{18}O value does vary between some batches of the same type, but are not significant in comparison to the variability of the method. There are however slight, but significant, differences in δ^{15}N values which may assist in differentiating some batches.

- Between batches of the same manufacturer, the % N and % O is generally similar. However, several types of fertilisers produced by the same manufacturer may display significantly different % N and % O, resulting from different compositions. Differences in δ^{15}N and δ^{18}O values also exists within the production line of a manufacturer.

- Between manufacturers of AN fertilisers, most productions display a similar % N and % O. Nevertheless, few have a slightly different elemental N and O composition. Regarding the nitrogen and oxygen isotopic composition, different δ^{15}N values are observed between the productions of some manufacturers. However, the isotopic value of most productions are within the same range (between -2 to 2 ‰ for δ^{15}N values and about 23 ‰ for δ^{18}O values).

The level of source defined by hypothesis IV.2 in **Chapter 5** considered the manufacturer level of AN fertiliser. However, as described above, the examination of the variability within and between the productions of manufacturers showed the existence of elemental and isotopic variations not only between manufacturers but also, for some samples, between types and batches. This suggests that all the possible levels of source should be considered instead of focusing only on the manufacturer level.

Unsupervised statistical methods were applied to the data set in order to uncover its structure and to observe if clusters were agglomerated following one of the source levels. Distance measurement was also used to evaluate the separation between intra and intervariability considering the 3 source levels. In the end, the performance of supervised classification models to predict class samples according to a given source level was assessed.

These statistical treatments were applied to raw and preprocessed data. Overall, data preprocessing (range scaling, standardisation, scaling rows to constant total) gave better results for the application of unsupervised methods, while raw data (i.e. normalised to the international reference scale only) yielded similar or even better results than preprocessed data for supervised methods.

4.2 Exploratory statistical methods

4.2.1 Principal component analysis

Considering the δ^{15}N, δ^{18}O values, the % N and O, PCA applied to row scaled data allowed explaining over 96 % of the variance of the data set with two principal components. The information extracted from the samples can therefore be summarised almost completely by two underlying dimensions. As illustrated by the plot of the variables (**Figure 9.15**), the first principal component (PC1) is mainly negatively correlated with δ^{15}N and δ^{18}O, and positively to % O. The variables δ^{15}N and δ^{18}O are highly correlated (0.74), indicating that part of the information of these variables is redundant. The second principal component (PC2) is mainly associated with % N and negatively with % O. **Figure 9.16** illustrates the results of the scores of samples against the first two principal components. The upper part illustrates the samples coloured according to their manufacturer, the lower part shows the distribution of the samples according to the type of AN fertiliser. According to the isotope ratio values, the production of Lonza is well separated from the samples imported by Landor.

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4.2.2 Hierarchical clustering analysis

The quality of clustering produced by hierarchical clustering analysis (HCA) was evaluated comparing the obtained clusters with the possible levels of source (the manufacturer, type of AN fertiliser and batch). Using standardised data, 24.3 % of the samples were in the correct cluster when considering the manufacturer level (k = 7)[3]. For type (k = 11) and batch levels (k = 13), these percentages were 13.5 % and 48.6 %, respectively on row scaled data. A visualisation of the results obtained for the batch level is presented in **Figure 9.17**. This method does not yield optimum results to separate the data set according the source levels of interest.

4.2.3 Distance measurement

Distance measurements were calculated using the Pearson correlation coefficient between samples coming from the same source (intravariability) and from different sources (intervariability). The levels of source considered were the manufacturer, type of AN fertiliser and batch. When considering the manufacturer as the level

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[3] k being the number of clusters

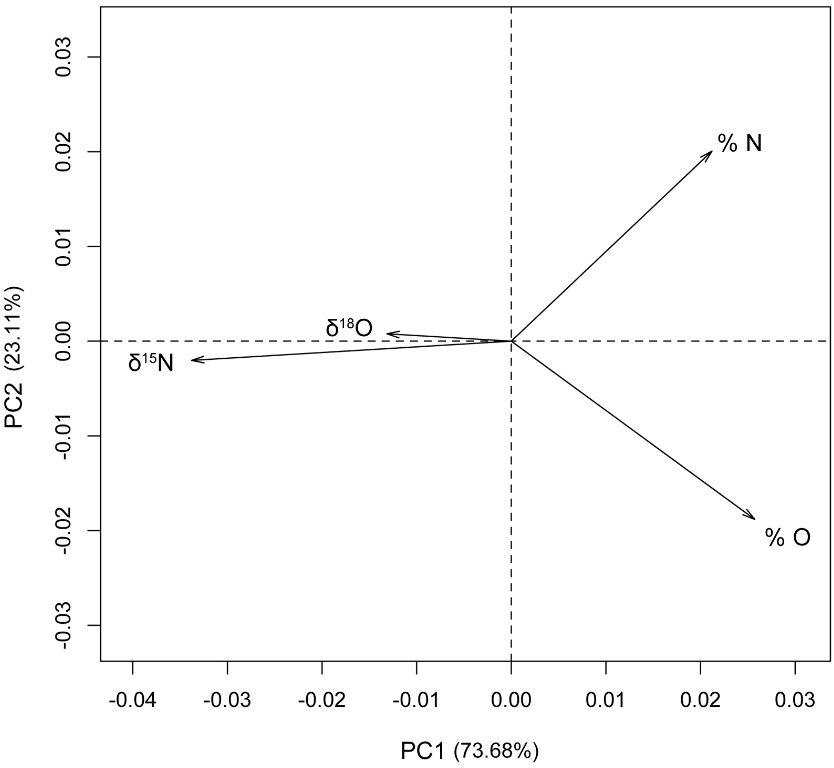

Figure 9.15. Representation of the loadings of the variables for row scaled data.

of source, the distribution of the intra and intervariability were similar, resulting in a large number of false positives. A better separation between the intra and intervariablity was obtained when the level of source considered was the type or batch of AN (**Figure 9.18**). Both gave similar plot. However, whatever the level of source considered, the distribution of both populations (intravariability and intervariability) overlapped and led to high FP and FN rates, as reported in **Table 9.4**. The threshold indicated in the table was chosen in order to balance FP and FN. Increased to 99.9, this threshold would have reduced the FP rate to 17 % and led to 40 % FN, meaning that in 1/5 of the cases, the link established between two samples is not true and in 2/5 of the cases, the information that samples are linked is not detected.

Level of source	Threshold	FP (%)	FN (%)	Specificity	Sensitivity
Manufacturer	98.0	91	6	0.07	0.94
Type of BP	99.5	46	5	0.54	0.95
Batch	99.5	48	5	0.52	0.95

Table 9.4. Respective percentages of false positives (FP), false negatives (FN), specificity and sensitivity according to the threshold of Pearson correlation.

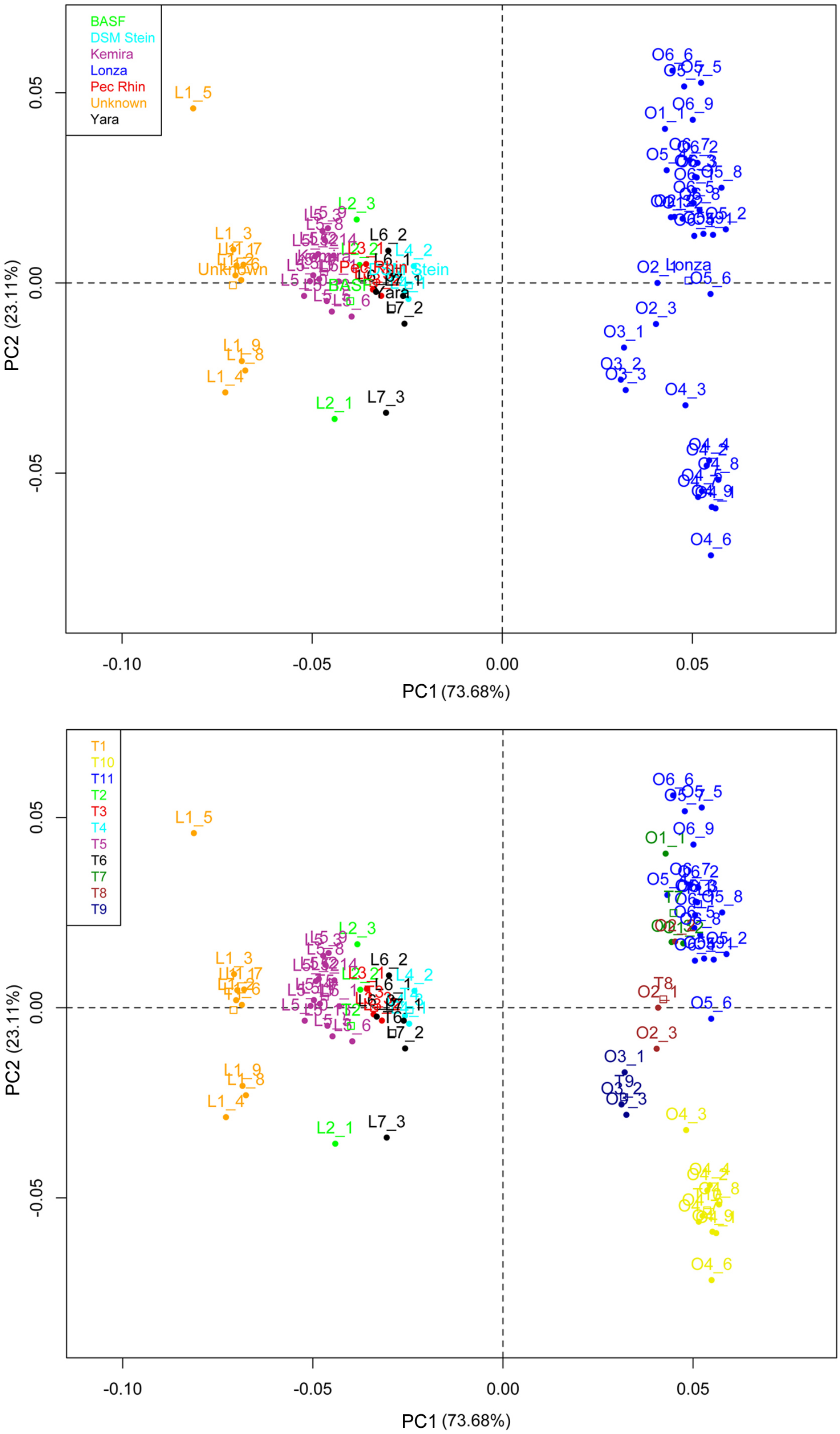

Figure 9.16. PCA performed on row scaled data. On the upper part of the figure, each manufacturer is represented by one colour. On the lower part, each type is coloured.

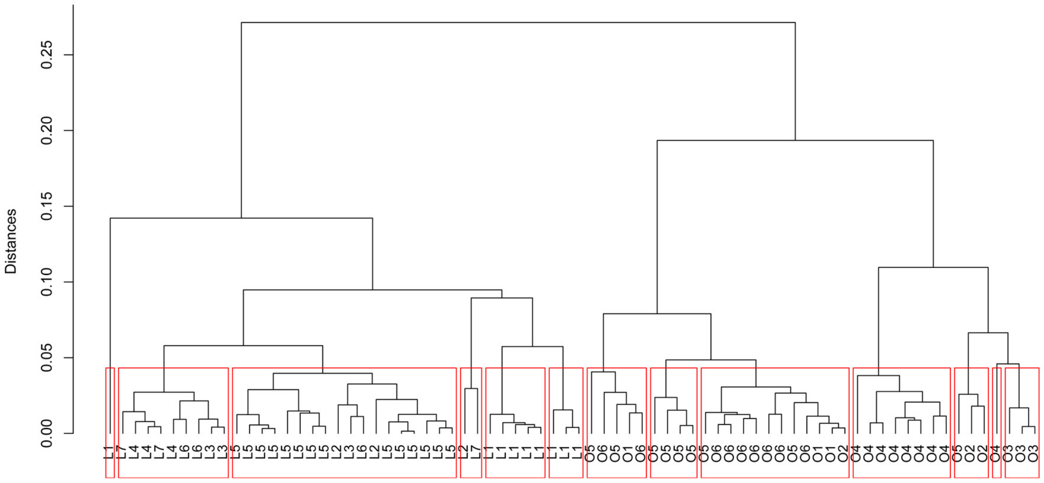

Figure 9.17. HCA using the Euclidean distance operated on scale to row data. When considering the batch level, 48.6 % of the samples are correctly separated between clusters (k = 13).

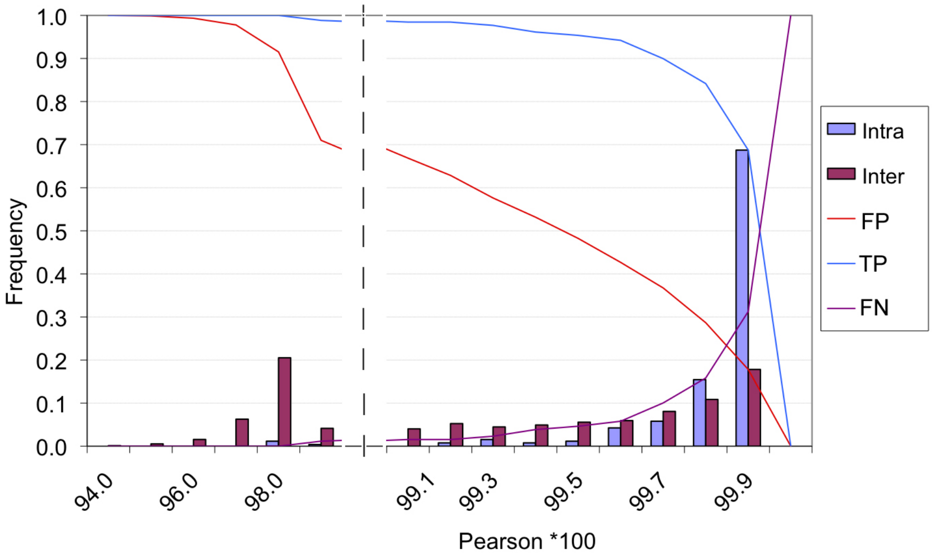

Figure 9.18. Distribution of Pearson correlation coefficient calculated on raw data for samples coming from the same batch (Intra) and from different batches of AN fertilisers (Inter).

The ROC curves presented in **Figure 9.19** show the performance of the model using the Pearson correlation coefficient to discriminate samples when considering the manufacturer, type and batch of AN fertiliser as the level of source. The ROC curve of the manufacturer level results in an AUC slightly larger than 0.5, indicating that the manufacturer model barely perform better than random. By contrast, the ROC curves of the batch and type levels perform better with an AUC of about 0.84. These performance are not perfect–the ideal model producing 0 % of false positives and 0 % of false negatives–however, they are able to discriminate samples coming from the same source and from different sources with a better efficiency than a model guessing at random.

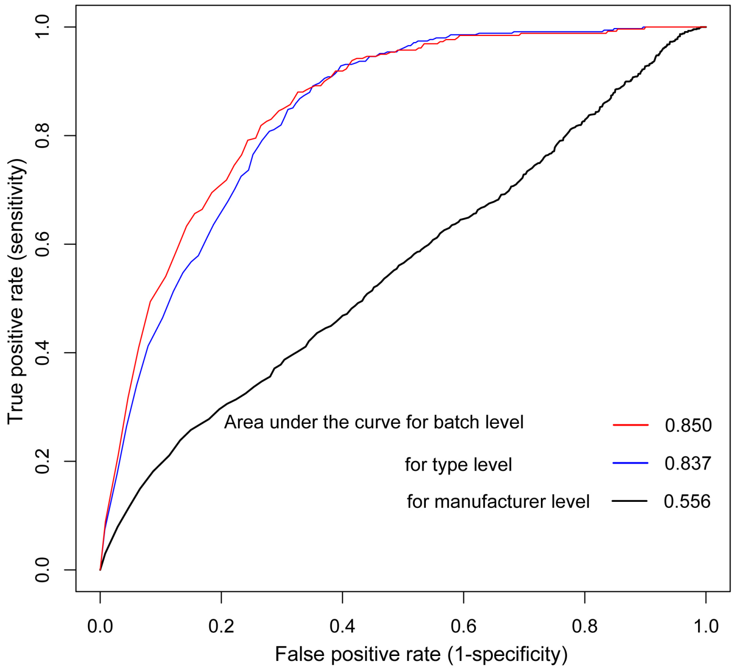

Figure 9.19. ROC curves of Pearson correlation coefficient when considering the manufacturer, type and batch of AN fertiliser as the level of source.

4.3 Supervised classification methods (LDA, RF, SVMs)

Linear discriminant analysis (LDA), random forest (RF) and support vector machines (SVMs) were applied to the data set in order to evaluate their performance to predict the manufacturer, type and batch of an AN fertiliser sample. The models were built using 2/3 of the data set (training set). The remaining 1/3 (test set) was used to validate the models. The split between the training and test sets was performed 100 times with samples chosen randomly at each split. On each iteration, the classification models were also evaluated with the prediction of the manufacturer, type and batch of 4 blind AN fertiliser samples, each analysed in triplicate (n = 12). The performance of the models were assessed through the mean percentage of correctly classified samples, corresponding to the correct rate (% CR) and that of wrongly classified samples, reported as the error rate (% ER).

The classifiers were applied to the raw data set as well as preprocessed data. The different data preprocessing did not improve the performance of the classifiers. Thus, the results presented in the following subsections were obtained with the raw data set, i.e. isotope ratio were only normalised against the international reference scale.

4.3.1 LDA model optimisation

Various tests were undertaken to remove the superfluous variables. For the classification by manufacturer and type, % O had a coefficient close to zero for the first linear discriminant, thus meaning that the variable had a limited effect on the scores of the samples. The performance of the model did not decrease without % O, indicating that the variable was not necessary to the discrimination of samples according to the different levels of source. The suppression of δ^{18}O was also tested as δ^{15}N and δ^{18}O values were highly correlated. However, this reduced the performance of the model. The optimised LDA model, which accounted for 100 % of the variability of the data set with two discriminant functions, was then constructed with the training set and evaluated with the test set.

4.3.2 RF model optimisation

Optimisation of the RF model was achieved through testing a series of value for $\mathrm{m_{try}}$, the number of randomly variables chosen at each node and $\mathrm{n_{tree}}$, the number of trees in the forest. Although the default value of m_{try} for classification is $\sqrt{p}$, with p the number of descriptors or variables, the effect of $\mathrm{m_{try}}$ value ranging between 1 to 4 was evaluated on the model performance through the overall Out-of-Bag (OOB) estimate error rate [Breiman 2001, Svetnik *et al.* 2003]. The lowest overall OOB error rate was obtained with $\mathrm{m_{try}}$ set at 2 for the 3 levels of source considered.

A sizeable number of trees in the forest ensures the stabilisation of the OOB error rate. Although 500 trees are reported as sufficient for small data sets, it is advised to test the effect of this parameter on the classification results of the model [Svetnik *et al.* 2003]. A $\mathrm{n_{tree}}$ corresponding to 500, 1000 and 2000 trees was tested. As illustrated on **Figure 9.20** which plots the overall OOB error rate against the increasing number of trees for a classification by manufacturer, the overall OOB error rate already stabilises after 100 to 150 trees for the 3 levels of source.

Variables importance ranking highlighted that % O and % N were the less influent variables in the classification model. While the model constructed without % N

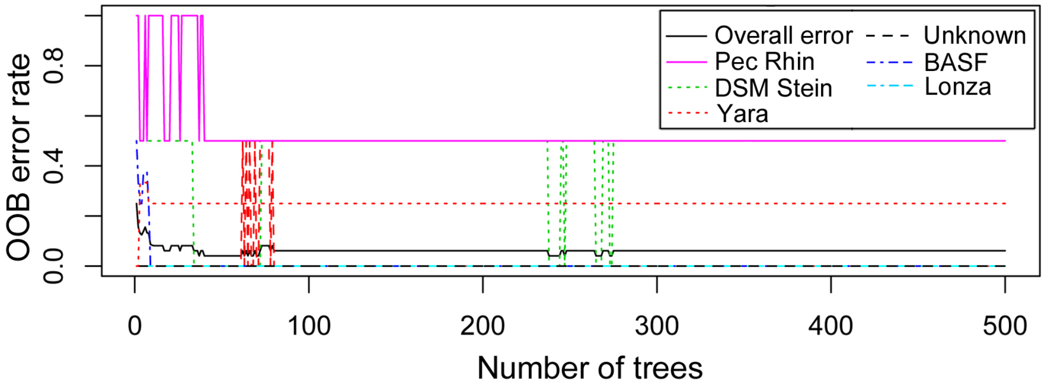

Figure 9.20. Overall OOB error rate and OOB error rate for each manufacturer of AN fertiliser as the number of trees in the forest increases.

variable led to lower percentage of correct rate (% CR), removing % O from the model gave better performances. The % O was therefore discarded from the final model.

4.3.3 SVMs model optimisation

The support vector machines with a radial basis function kernel (SVMs RBF) model was optimised by searching for the best values of the RBF radius γ and the penalty parameter C, which is a trade-off parameter which defines the complexity of the solution. Using a 10-fold cross validation, this optimisation was achieved for each training set using a grid search testing C values set between 0.1 and 10000 and γ values between 0.0001 and 100. The higher % CR defined the best combination of parameters. The model was then built with the optimised parameters using the training set and evaluated using the test set. In the final model, the cost value was set at 1000 for the manufacturer, 10 for the type and 100 for the batch prediction. For the 3 levels of source, the best γ value was 0.1.

4.3.4 LDA, RF, SVMs models validation

The 3 classifiers were able to discriminate between the different groups whatever the level of source considered with a % CR (correct rate) ranging between about 77% and 98%. **Table 9.5** presents the mean % CR and % ER (error rate) of LDA, RF and SVMs when classifying the test set samples according to the 3 levels of source considered. The distribution of the % CR obtained on 100 iterations with the 3 classifiers is illustrated in **Figure 9.21**. The adequacy between means and medians indicates that any of the two can be used as an estimate of correctly classified samples.

Among the 3 classifiers, RF outperformed LDA and SVMs for manufacturer, type

Level of source	**Classifiers**	**mean % CR**	**mean % ER**
Manufacturer	LDA	90.0 ± 3.6	10.0 ± 3.6
	RF	**97.8 ± 3.5**	**2.2 ± 3.5**
	SVMs	89.1 ± 4.6	10.9 ± 4.6
Type of AN	LDA	77.0 ± 5.1	23.0 ± 5.1
	RF	**90.5 ± 5.8**	**9.5 ± 5.8**
	SVMs	84.3 ± 6.1	15.7 ± 6.1
Batch	LDA	84.3 ± 4.1	15.7 ± 4.1
	RF	**93.7 ± 4.6**	**6.3 ± 4.6**
	SVMs	80.3 ± 7.0	19.7 ± 7.0

Table 9.5. Performance of the classifiers determined on 100 iterations to predict the manufacturer, type and batch of the test set samples. The best performance is highlighted in bold for each source level.

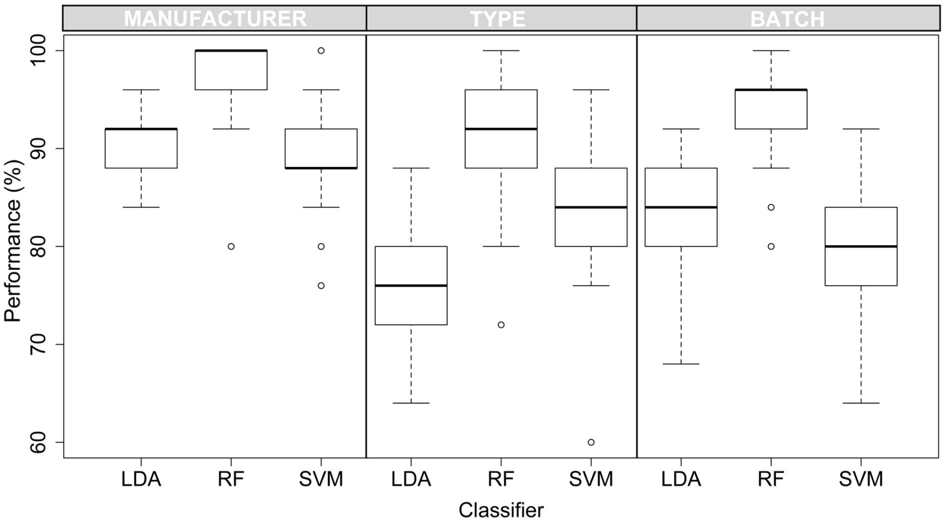

Figure 9.21. Comparison of the performance of the classifiers (in % CR) on 100 measurements to predict the source of the test set samples, according to the manufacturer, type and batch of AN fertiliser.

and batch prediction of the test set with less than 10 % ER. Except for type prediction, SVMs yielded similar results to LDA, despite its ability to define complex boundaries. It is likely that the size of the classes is partly responsible for it. At the manufacturer level, 3/7 classes (40 %) are composed of 3 measurements, while at the type and batch level this figure raises to 6/11 (50 %) and 8/13 (60 %), respectively. Although the performance for manufacturer prediction is good, the % CR decreases as the proportion of classes with 3 measurements increases. One characteristic of SVMs is that support vectors are defined as being on the margins of the class. With a small class of 3 measurements, 2 measurements are randomly chosen to construct the model during the training phase. These 2 points necessarily become the support vectors describing the margins of the class. As the number of data is limited, the borders of the class are less well defined and a classification error can occur more easily.

Classification of blind samples Four blind AN fertilisers samples were selected by an external operator. They were analysed in triplicate (n = 12) and were incorporated to the evaluation of the classification models in order to predict the manufacturer, type and batch of AN fertiliser. Blind test corresponded to samples L4, L6, O4.3 and O6.2. The performance of each classification model measured on 100 iterations is reported as mean % CR and % ER in **Table 9.6** and illustrated in **Figure 9.22**. These results correspond to those obtained with the test set and confirm the ability of RF to deal with small data set, especially with classes grouping few observations.

The largest % ER of batch prediction comes from the misclassification of blind sample L6 in class L7 (which occurred in 36 % of the cases). Interestingly, both batches were produced by Yara in March and April 2007, respectively. This indicates that batches of the same type of AN fertiliser may less easily be distinguished, although the model succeeded in 64 % of the cases. The results obtained with the blind samples are excellent (100 % CR for manufacturer and type prediction). However, the fact that all classes are not represented in the blind set must be kept in mind. These results confirm that the classification models are able to discriminate between samples according to the 3 levels of source.

Level of source	**Classifiers**	**mean % CR**	**mean % ER**
Manufacturer	LDA	68.0 ± 8.8	32.0 ± 8.8
	RF	**99.8 ± 1.2**	**0.2 ± 1.2**
	SVMs	72.2 ± 9.0	27.8 ± 9.0
Type of AN	LDA	67.3 ± 10.4	32.7 ± 10.4
	RF	**100.0 ± 0.1**	**0.0 ± 0.1**
	SVMs	71.0 ± 8.9	29.0 ± 8.9
Batch	LDA	69.3 ± 6.5	30.7 ± 6.5
	RF	**89.8 ± 6.9**	**10.2 ± 6.9**
	SVMs	60.0 ± 9.6	40.0 ± 9.6

Table 9.6. Results of classification of the classifiers determined on 100 iterations to predict the manufacturer, type and batch of the 4 blind AN fertiliser samples analysed in triplicate. The best performance is highlighted in bold for each source level.

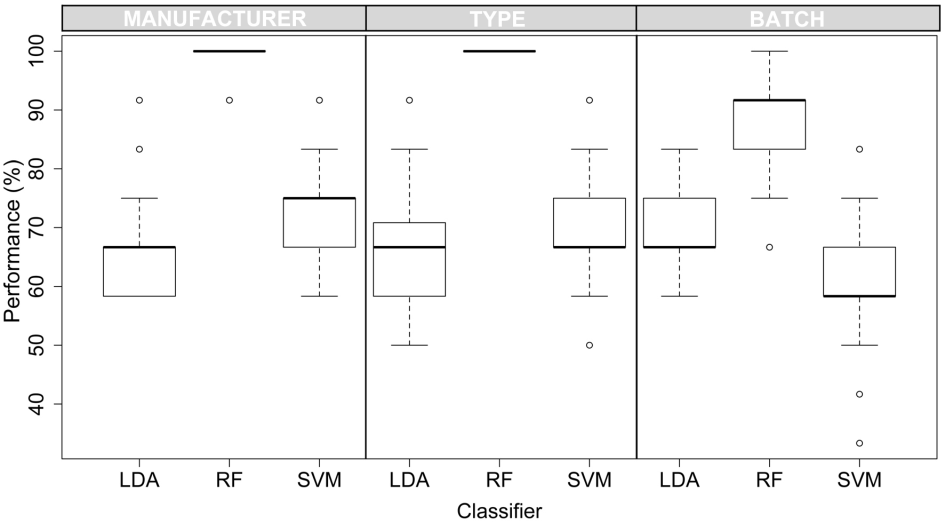

Figure 9.22. Comparison of the performance of the classifiers (in % CR) on 100 measurements to predict the source of the blind samples (n = 12), according to the manufacturer, type of AN fertiliser and batch.

5 Discussion

5.1 Sampling and measurement representativeness

Part of the AN fertilisers sold on the Swiss market is produced in Switzerland, the rest is imported mostly by the company LANDOR. Although the sampling analysed in this research does not cover all types of AN fertilizers which can be bought on the Swiss market, the sampling of this research was obtained from Lonza, the Swiss manufacturer and LANDOR, the importer. These samples selected for this research are therefore representative of AN fertilisers sold on the Swiss market. Despite the collaboration of Lonza, obtaining such sampling has shown to be difficult. Given the structure of the distribution market, obtaining a larger number of samples involving more types and batches of AN fertilisers sold in Switzerland without the collaboration of the different manufacturers and importers is a tricky task.

Besides, the sampling and the analyses undertaken in this research involved a notion of reduction factor coming from:

- masses sampled over the whole production of a batch: one to three samples of 100 g or 500 g sampled in a batch of several tons represent a limited portion;
- masses sampled for analysis (for example, for δ^{15}N, only about 0.35 mg of AN fertiliser is analysed).

This reduction factor raises the question of measurements representativeness. In particular, the number of analyses performed have a direct impact on the estimation of the isotopic homogeneity. The larger the analysed quantity and the number of analyses, the smaller the uncertainty associated with the measurement (the estimation). In this research, a given number of samples could only be analysed in triplicates, which means that the uncertainty associated with such measurement is larger. It is not possible to know whether the obtained measurements are really representative of the produced batches. Nevertheless, these measurements reflect an estimation of the "true" value of the produced batched associated with a part of uncertainty.

5.2 Considerations on intravariability

As demonstrated by the results of this research, up to 0.6 ‰ δ^{15}N variations were measured within a batch of AN fertiliser and up to 0.8 ‰ δ^{18}O variations in most cases, with however exceptions up to 1.3 ‰. Concerning elemental variability, up to 4.5 % and 8.6 % were measured for % N and O within a batch, respectively. These large elemental variations also encompass the variability from sample preparation for nitrogen and from the method itself for oxygen. **Table 9.7** summarises the largest variations measured within batches of the manufacturers.

Target variable	**Unknown**	**BASF**	**Pec Rhin**	**DSM Stein**	**Kemira**	**Yara**	**Lonza**
% N [%]	4.5	5.8	0.4	1.5	2.9	3.1	4.6
% O [%]	8.6	0.4	1.2	0.9	4.0	1.2	6.4
δ^{15}N [‰]	0.2	0.2	0.1	0.2	0.3	0.2	0.6
δ^{18}O [‰]	0.5	0.3	0.2	0.3	1.3	0.5	1.3

Table 9.7. Largest differences measured within batches of AN fertilisers of the manufacturers.

Among the few studies undertaken on AN fertilisers, only Benson provides some data about the intravariability of manufacturers. She measured the intravariability of 3 types of AN fertiliser, each produced by different manufacturers, over given periods of time [Benson 2009]. Differences in δ^{15}N value of up to 4.1 ‰ and in δ^{18}O value of up to 3.0 ‰ were measured within one manufacturer over 64 days (see **Table 9.8**).

Target variable	**Manufacturer 1 (d = 64)**	**Manufacturer 2 (d = 231)**	**Manufacturer 3 (d = 359)**
δ^{15}N [‰]	4.1	2.6	0.8
δ^{18}O [‰]	3.0	2.5	2.0

Table 9.8. Range of variations of 3 types of AN fertiliser, each produced by one manufacturer, measured by Benson, with d being the number of days [Benson 2009].

The results of Benson indicate that the isotopic profile of a manufacturer's production can vary much, rendering the identification of a particular manufacturer even more difficult as the range of isotopic variations of AN fertilisers is restricted (see further discussion). However, although she collected samples on different periods of time, she never refers to batch production or to the structure of production. It is therefore not possible to know if these variations are attributed to different batches or correspond to production inhomogeneity. This highlights the significance of collecting information on the source of the samples in order to be able to better interpret results. In addition to her results, there is a lack of data on the intravariability of a type of AN fertiliser in literature. In order to provide a robust interpretation of isotopic variations measured in AN fertilisers, it is necessary to collect these fundamental data in conjunction with information on the source of the samples.

5.3 Discrimination of sources

Random forests model was able to classify test set samples according to the manufacturer, type and batch of AN fertilisers with excellent % CR (between 90 % and 98 %). However, regarding batch classification, it is important to recognise that data on batches of the same type of AN fertiliser were rather limited in the sampling (2 types of AN with each one represented by 2 batches). These results should therefore be considered with caution. More data on batches of the same type of AN are needed for an adequate evaluation of supervised models abilities to differentiate samples at this level of source.

Concerning samples classification according to manufacturers and types of AN fertiliser, the ability of RF to predict the class of a sample within the given sampling is excellent. This confirms hypothesis IV.2 stated in **Chapter 5** and shows that even the type of AN fertiliser can be inferred from such information.

The results prove that hypothesis IV.2 is confirmed and can be specified by the following statement:

> Hypothesis IV.2
>
> The information extracted from the isotopic analysis of ammonium nitrate fertilisers allow categorising distinct manufacturers and types of AN fertilisers.

Several elements should however be considered:

1. The sampling involved a limited number of classes for each source level (7 manufacturers and 11 types). This research would certainly benefit from including more classes in the model, which would provide a more representative estimate of the performance of the classification model.

Indeed, published data attest to the similarity of the ranges of measured values. **Table 9.9** presents the range of δ values measured on AN fertiliser samples by different authors. Although it is difficult to draw relevant conclusions on samples discrimination without considering intra and intervariability, the table informs on the similar and, for nitrogen, limited, ranges of δ values measured up to now.

Research	δ^{15}**N [‰] range**	δ^{18}**O [‰] range**
[Vitòria *et al.* 2004]	-0.7 to +2.5	+25.1
[Widory *et al.* 2009]*	-2.4 to +0.8	+21.6 to +23.3
[Lock 2009]	-1.8 to +2.2	+13.2 to +26.2
[Benson 2009]*	-4.0 to +2.0	+13.0 to +21.5
this research	-6.1 to +2.1	+16.5 to +24.4

Table 9.9. Comparison of the range of isotopic values of AN fertilisers from the different studies. *: exact δ values were not available, figures were deducted from plots.

Bateman and Kelly reported on the narrow range of δ^{15}N values for synthetic fertilisers (all types considered) centred around 0 ‰. Most of fertilisers they analysed had δ^{15}N values between -2 ‰ and +2 ‰, very few had a nitrogen isotopic value above 4 ‰ [Bateman & Kelly 2007]. These results correspond to that obtained in this research and are coherent as nitrogen of both nitric acid and ammonia, the two raw materials necessary to the manufacture of AN, is derived from air, which has a value around 0 ‰. Coplen *et al.* also mentioned that nitrogen in both synthetic ammonium reagents and nitrate fertlisers have δ^{15}N value between -6 ‰ and +6 ‰, with an average of 0 ‰ [Coplen *et al.* 2002b]. Theses references confirm that the ranges of δ values appears limited. Therefore, increasing the data set on which classification models are evaluated would certainly yield larger % ER than that found in this research. Nevertheless, the results of this chapter show that elemental and isotopic dimensions bring useful information to categorize samples, using random forests, a simple and efficient classification model.

2. As for black powders, hypothesis IV.2 is true as long as the categories or the classes of the model are known. The implementation of such system in a real forensic environment would require to have a memory of all the possible productions of AN fertilisers and to update this knowledge regularly (which also means with the collaboration of the manufacturers) in order to provide a reliable answer. This way of doing is difficult to implement. Most situations which occur in a forensic framework involve specimens of unknown sources. As the structure of data is not known, unsupervised methods are more appropriate to the framework of forensic investigations. Although their performance is

lower than that of supervised models (producing more false positives), they showed to perform better than a random classifier using distance measurement and to provide useful information which can be used for operational purposes.

Chapter 10

General discussion

Contents

The objective of this research was to evaluate the contribution of the information provided by EA-IRMS technique applied to black powder (BP) and ammonium nitrate (AN) fertiliser samples in a forensic context. This consisted of determining whether the profile of measured characteristics was stable and whether the extracted information allowed us to discriminate between samples at different levels of source. The methodology of the research, as well as the usefulness of this additional information in the investigation of explosives, are discussed below.

1 Methodology of the research

The research evaluated the transversal and longitudinal variability of the samples profile. The study of the influence of environmental conditions on the profile of the samples was important to gain knowledge about the stability of the target variables. The measurement of intravariability and intervariability of sources was essential to assess the discrimination potential of the information extracted by isotope analysis. Furthermore, the unexpected behaviour of standards during analytical sequences led to further investigations.

1.1 Analytical part

The methods for measuring carbon, nitrogen and oxygen were evaluated using organic laboratory standards routinely employed in the analytical sequences of the laboratory of Paul Scherrer Institut, as well as working standards with a similar chemical composition to the samples of this research. This methodology of analysis revealed that the nitrogen method commonly used to determine the δ^{15}N value in the community of the isotope scientists was not ideal for inorganic substances such as nitrates. Both the nitrogen yield (% N) and the precision of the δ^{15}N value were affected. Various modifications to the parameters of the elemental analyser were tested, such as the analytical cycle length, the timing between sample drop and oxygen injection, and the suppression of oxygen injection. The repeatability of the δ^{15}N values of nitrates drastically improved when oxygen was suppressed from the analytical cycle. In contrast, the injection of oxygen was necessary for the analysis of organic working standards in order to obtain reliable and repeatable results.

These findings highlight that organic and inorganic substances cannot be processed with the same method in EA-IRMS, and underlines the need to consider the chemical nature of the substance to analyse when developing the method. The conversion of the bulk material into a simple gas (through a flash combustion followed by a reduction step for carbon and nitrogen, and through pyrolysis for oxygen) is dictated by high temperature reactions which are dependant on the speciations at stake. When measuring oxygen, the cellulose and nitrate standards also displayed differences in oxygen yields (% O), which denotes different thermochemical behaviours. As samples are analysed along with standards in the analytical sequence in order to report results on the international scale, the similarity of chemical nature and matrix between the analyte and the standard should not be seen as a recommendation as reported in literature, but definitely as a requirement.

1.2 Stability study

Heat and humidity are the most frequent parameters tested in stability studies. The influence of heat and humidity on the profile of a sample was evaluated over 12 months with the same BP and AN fertiliser samples. The storage of the AN fertiliser sample was not influenced by elevated temperature conditions, but under high relative humidity, its δ^{18}O value decreased by 1 ‰. In contrast, the profile of BP was not affected by high relative humidity conditions. However, its exposure to elevated temperature resulted in a δ^{15}N enrichment of 1.4 ‰ and a δ^{18}O depletion of 3.6 ‰. In addition to this long term exposure experiment, the same samples were briefly exposed to highly depleted water vapour in order to see the influence on their δ^{18}O value and % O. Water vapour did not significantly modify the δ^{18}O and % O. The use of a Zero blank autosampler proved to be efficient in removing the moisture sorbed by the samples.

This stability study helped gain an important insight on the influence of temperature and high relative humidity on the profile of AN fertiliser and BP.

Depending on the conditions under which the specimens are stored or on their history, false negatives can be obtained in a framework of source inference. Furthermore, the observations on ageing are useful when giving precautions on how a specimen should be stored. Similarly, the use of a Zero blank auto sampler for oxygen analysis is needed for hygroscopic substances, such as AN.

1.3 Source discrimination

One major advantage of the sampling collected in this research is the known source of the samples. Three levels of source (manufacturer, type and batch levels) were represented with the AN fertiliser and BP samples. The variability within and between sources was evaluated considering each level of source and showed that intravariability could be larger than expected. Some sources had a very variable profile, while others presented a very constant one. In some cases, the very large variability of the feature was particular to one manufacturer, as observed for the δ^{15}N value of Wano's black powders. This demonstrates that the measurement of intravariability differs from one source to another, and is crucial in a source inference approach.

The elemental composition (% C, % N, % O) was also showed to vary between some samples. This aspect is significant, as it may enable further discrimination between samples and, moreover, is easily calculated without additional effort from the data recorded by the software Isodat. It is interesting to note that, in literature, this feature is rarely reported with isotope ratios.

Unsupervised and supervised data analysis were undertaken on raw and preprocessed data. Data preprocessing proved to be unnecessary for the multivariate statistical analyses of the data set. Data visualisation with unsupervised methods revealed overlapping groups whatever the chosen level of source. This means that in a source inference perspective, a non negligible number of false positives (samples which appear to be linked, while in fact they are not) can be produced with unsupervised statistical tools. Far from meaning that information is non exploitable, this overlap between samples coming from different sources indicate that the information produced with such approaches is accompanied with a larger uncertainty. Applied supervised classification methods included linear (linear discriminant analysis (LDA)) and non linear models (random forests (RF) and support vector machines (SVMs)). Among the 3 classifiers, RF gave the best performance to classify AN samples according to the manufacturer and type. For the prediction of BP samples according to the 3 levels of source, RF model also achieved the best performances, although SVMs showed similar results as RF for manufacturer and type prediction. While SVMs suffered from classes with very few observations, RF handled this characteristic of the sampling well. RF thus appears as an interesting classification model for small data sets, as also mentioned in literature [Qi 2012, Liu *et al.* 2013]. It can be applied to the data set without data preprocessing and feature selection. In addition, it is robust against overfitting, impervious to outliers and has only two parameters to optimise [Liu *et al.* 2013]. Nevertheless, although supervised methods provide better results than unsupervised ones (smaller error rate), unsupervised treatments actually reflect the situation encountered in real forensic caseworks with specimens of unknown classes being compared together. The comparison of specimens using exploratory methods is tainted with a larger uncertainty which cannot be accurately quantitated. However, this does not preclude the use of the information in the enquiry.

The number of represented sources and the number of samples per source in the sampling were limited. In particular, the number of different batches of the same type of AN fertiliser was too small. Without the close collaboration of manufacturers, it is difficult to obtain a large and representative sampling of their production. Although the sampling of this research does not present an image of the whole market, but only a part of it, the samples are representative of the products typically purchasable in armouries for BP or shops for AN fertilisers. Besides, the samples come from a known source, an important feature ensuring a reliable interpretation of results and rarely present in publications.

2 Exploitation in a forensic context

2.1 In favour of an intelligence-led approach

The results demonstrate that the information extracted from the isotopic analysis of BP and AN fertiliser can be used in a forensic context. As developed in **Chapter 3**, this information can be used in different ways.

In an investigative perspective or a preventive approach, the information is exploited for its ability to provide intelligence. This intelligence can assist in taking decisions and actions. It can either be used to provide leads and orientate the inquiry, to detect or strengthen the connection between linked events or to highlight new tendencies in bombings (new manufacture mode, new substances, etc.). This approach is based on the exploitation of all dimensions of information, whatever the quality and quantity, as long as it is useful for investigations or law enforcement. This means that even if the information conveyed by traces is imperfect, present in minute amounts or whose origin or source is not necessarily certain, it has the potential to produce meaningful elements useful in an intelligence approach.

In a prosecutorial approach, the information is used as evidence in court. Contrarily to an intelligence-led process whose aim is to find patterns, trends, series, suspects, the crime execution, etc. or to reconstruct the sequence of events, the prosecutorial approach occurs in a framework with less degrees of freedom, with a defined set of alternative hypotheses, generally to convict. This narrow exploitation of information, which often comes down to a one-to-one comparison, requires the forensic scientist to know and be able to quantify the uncertainties associated with it. Because of the outcome of the trial, this process tolerates less uncertainty than the provision of intelligence.

Currently, routine methods of analysis of inorganic explosives are unable to differentiate substances coming from distinct sources. The isotopic analysis of black powder and ammonium nitrates has shown that IRMS is capable of differentiating samples coming from distinct manufacturers, batches and daily productions. Thus, *IRMS definitely elicits a new dimension of information which could not be obtained with previous methods.*

However, the results of this research also showed that, for nitrogen and oxygen, fundamental issues pertaining to the conversion of the substance into simple gas during the analysis do exist. This highlights the limits of current knowledge and the need for further research regarding the technique used to extract this information. Some environmental factors (moisture, heat) also proved to be able to influence the elemental and isotopic profile of these substances. Although evaluated in one year, the small number of tested samples and environmental conditions delimits the restricted extent of knowledge. All the parameters which may influence the profile are not known. These elements highlight the uncertainty currently associated with such information.

In addition, several unsupervised and supervised statistical methods applied to infer the source of the samples were evaluated. According to the context of the question, for different reasons, the uncertainty related to both supervised and unsupervised approaches is difficult to quantify accurately for an evidential exploitation of the information. However, in a criminal intelligence based approach, even with such clouds of uncertainty, the information extracted from the isotope analysis of BP and AN may be useful to generate intelligence.

This intelligence can assist investigators in the context of the specific case, such as in the first phase of the inquiry, by providing new leads to investigators. For example, the profile of the explosive charge of an unexploded IED corresponds to that of grains of substance found in the back of a car in the vicinity of the scene. This element provides the police officers with information on the potential means of transport of the bomb and can be the starting point of further investigations. In a more advanced phase of the inquiry, the similarity or dissimilarity of the profile between the incriminated substance and an explosive substance found at the home of a person may either help exclude potential suspects or confirm the elements gathered during investigations.

On a larger scale, the information extracted from traces, a pattern or series can assist in predicting and preventing crime. For instance, in a series of bombings attributed to a criminal group, the similarity of the profile between the explosive charges enables investigators to establish a link between the different events. This information provides intelligence about the size, extent and level of activity of the criminal group. In addition, the particularities of the profile could, for example, give indications about the geographic origin of the substance. Depending on the lawfulness of the substance, security actions could be the enactment of stricter regulations on its marketing or importation for specific countries, or the increase of controls at boarders. This form of intelligence based on the chemical profile of the substance is however currently not used.

For these reasons, the use of the information provided by the isotopic analysis of BP and AN in an intelligence perspective is much more appropriate than the evidentiary approach given the state of current fundamental scientific knowledge.

2.2 Using the information: scenarios of comparison

In an intelligence led-approach, two scenarios of comparison are possible:

- the one-to-one or one-to-many comparisons (a substance is contained in an intact improvised explosive device and is compared to substances, for example found at the home of a suspect or in similar devices). The results of the comparison provides intelligence, which confirms or infirms the hypothesis made during the inquiry, as well as corroboratory or contradictory elements of investigation.
- the one-to-all comparison (the investigators do not have a substance to compare to). The substance is compared to a database assembling the characteristics of several–in the ideal case, all–possible manufacturers' productions or to a database containing the profile of casework samples. The candidates with similar characteristics to that of the substance can be used to provide new leads to the police or orientate investigations.

The second case of comparison involves the use of a database containing the productions of the manufacturers or the characteristics of casework samples. In that sense, the IRMS technique offers large possibilities for collaboration on a national level or at international level as the isotopic results are reported on an international reference scale. Thus, samples analysed on different EA-IRMS and with different methods may be compared against each other. Different laboratories could therefore contribute to the construction and updating of the database. This would allow forensic scientists to search for a particular profile of a substance or observe the evolution of the profile of the manufacturers' productions and, at the same time, accumulate data on intra and intervariability. In the case of a database storing the profile of casework samples, this could provide information on potentially linked cases. The development of databases is part of the ongoing discussions in the FIRMS since the creation of the network.

However, using such a database shared between several laboratories necessitates above all fundamental issues related to the conversion of the bulk substance into the target analyte to be solved or at least understood. The results of this research showed that incomplete conversion of the bulk substance into simple gas (N_2, CO_2 or CO) can occur, mainly due to the method. In order to provide robust and comparable data, it is necessary to first identify the influencing factors in the analytical method.

The creation and activities of the FIRMS network have much improved the knowledge on the research performed by the different institutes and has published a good practice guide to assist in high quality isotope ratio measurements [Carter, J. F. and Barwick, V. J. (Eds) 2011]. The FIRMS network offers a solid ground of connections between specialists and provides an excellent structure for the exchange of knowledge and data to progress in the collection of fundamental data.

3 Perspectives

The results obtained in this research show that the isotopic analyses of AN and BP provide relevant information exploitable in explosives investigations. Further research is however needed in order to gain a comprehensive knowledge of the technique and its application to BP and AN. The following issues could be the object of further investigations:

1. The stability of the elemental and isotopic profiles of a substance is influenced by its composition. Even small variations, such as different additives, the presence of impurities or the origin of a raw material (for example, synthetic or natural KNO_3) can modify the stability of a substance, as it modifies the necessary activation energy. Further research involving supplementary BP and AN samples could confirm these results or bring additional elements.
2. A more detailed study on the variability within and between batches of types of AN fertiliser would extend the existing knowledge and allow an assessment on whether discrimination of AN batches is possible.
3. As discussed in **Chapters 8** and **9**, the published studies report similar isotopic ranges for both BP and AN fertilisers. Thus, the rate of correctly classified samples may decrease with the addition of new samples to the model. A point that has not been achieved yet in the forensic applications of IRMS is the exchange of data or samples from other laboratories to perform

a larger scale comparison for a given substance. Taking advantage of the international isotope ratio reference scale, an interesting project would be to feed the models with data collected by other researchers, when enough information on the source is available. The assessment of the models performance would thus take into account larger populations, more representative of what is on the market.

Additional data incorporated to the models may encompass new classes or new sources. As SVMs and RF models are supervised methods, they are only able to classify samples in classes on which the model was trained. However, RF includes an intrinsic proximity measure [Breiman 2001]. Unsupervised RF is able to highlight new classes if threshold rules are set on the proximity measure [Shi & Horvath 2006]. This application appears promising and is definitely a perspective to investigate.

4. The description of the samples already showed that the physical characteristics of BP and AN could allow distinction between some samples. These characteristics could be combined to the information produced by isotopic analyses and the ability of such combination to discriminate between sources assessed.

5. The application of IRMS to other explosives could be studied. However, it appears important to undertake fundamental research and favour the collection of data on intravaraibility and intervariability.

6. The compound specific isotope analysis of AN fertiliser using IC or CE coupled to IRMS, could bring some additional discrimination between samples on the δ^{15}N value. However, this technique does not exist yet.

7. The rare publications on the isotopic analysis of postblast residues reported the non conservation of the isotopic profile. However, available data for AN are limited and such research has not been undertaken with black powder yet. Further research investigating the effects of explosions on the profile of black powders and AN fertilisers could provide a deeper view of the exploitability of this piece of information in post-blast investigations, in particular to which extent the physical and chemical phenomena of explosions alter the informative potential of the trace.

8. The construction of a database to collect the characteristics of the manufacturers' productions would assist in using this information in an intelligence-led approach. However, in order to be useful, an up-to-date database must follow the evolution of the market and must be regularly fed with samples in order to have a representative image of the market. This will require a tremendous monitoring of the market which would be easier to implement with the collaboration of the manufacturers. Constructing a database storing the characteristics of casework samples appears less constraining though. It is interesting to note that the FEL planned to develop a database, with the support

of the FIRMS network, grouping a large range of explosives from different "locations and batches to assist with future identifications". Nevertheless , the purpose of the database and the exploitation of the information remained unclear [Lock *et al.* 2005].

CHAPTER 11

Conclusion

The routine analytical techniques used in explosives investigations allow the determination of the chemical nature of the explosive charge. However, further discrimination between samples is limited. For several years now, IRMS is the object of numerous publications in forensic science, which report on its ability to distinguish between samples of the same chemical nature. The review of literature reported in this work has highlighted the large contribution of these studies, the diverse fields of applications and the dimensions of the extracted information. Despite this prolific literature, the results of the studies raised questions, as a lot of research suffers from methodological weaknesses. Moreover, there is a lack of fundamental data, which represents a severe limitation to a reliable interpretation of the significance of the results and their contribution to forensic issues.

The aim of this research was to evaluate the forensic utility of the isotopic analyses of BP and AN fertilisers in investigations of explosives, through studying the variability of the profile over time (stability) and the variability of samples within and between sources. For this purpose, the developed methodology involved using samples of known sources in order to be able to interpret results with confidence. Great importance was given to intra and intervariability measurement and examination, as well as the use of unsupervised and supervised statistical methods to extract the relevant information from the data set. This methodological approach has not been deployed–or only partially–in forensic applications of IRMS so far.

During the analysis, some problems related to the unexpected behaviour of the organic and inorganic standards were observed for nitrogen. Investigations revealed a fundamental issue of isotopic analysis: organic and inorganic substances can not follow the same analytical procedure. The first nitrogen method being inadequate for nitrates, the development of a new nitrogen method solved the problem and yielded excellent results in terms of precision and accuracy. Similarly, the oxygen yields of organic and inorganic standards displayed anomalies, indicating that the conversion of the bulk material into CO was not quantitative. Again, this appears related to the different thermochemistry of the substances. This point was also noted by Benson [Benson 2009] and deserves further attention. These elements demonstrate that fundamental knowledge at the method level are still required, despite the extended use of the technique.

The stability study showed that the sensitiveness of black powders to temperature and that of AN fertilisers to relative humidity could, in the long term, modify the profile of the samples. This does not call into question the utility of the profile in forensic science. Simply, depending on the storage conditions of the sample, false negatives or false positives can be obtained when comparing two samples coming from the same source.

Samples of BP could successfully be differentiated according to their manufacturer, type and batch. Samples of AN fertilisers were successfully discriminated according to the manufacturer and type. Additional data on the variability between batches of a type of AN fertiliser are needed to evaluate batch discrimination. These positive results demonstrate the added value of the information brought by the isotopic analysis of BP and AN and indicate that the technique could be inserted in the sequence of analysis of an explosive charge. The discussion about the exploitation of the information provided by this technique showed an intelligence-led approach was more suitable than a prosecutorial exploitation given the current state of knowledge.

Using this information to orientate inquiry at present is feasible for any laboratory, for instance, when comparing one substance to another or to several others (one-to-one or one-to-many comparisons). In contrast, the use of a database containing the profile of the manufacturers' productions and the profile of casework samples and fed by several laboratories or countries (one-to-all comparison) to generate intelligence in a tactical, operational or strategical perspective is less easy to achieve. The implementation and exploitation of such a database involves many points to consider, such as the supply of the database with data, keeping it up to date and sharing it between several entities, as well as the transmission and use of the information by law enforcement forces. A huge advantage of IRMS over other techniques is the international reference scale against which the results are reported. This characteristic makes IRMS ideal for data exchange and collaboration between laboratories. While databases containing isotope data exist in climatology and hydrology, such databases in forensic science are rare. In food adulteration, two official databases implemented in the 1990s are dedicated to light stable isotope of wines and fruit juices [Rossmann 2007]. Data from authentic samples of known geographical origin are collected by several countries every year. The interactive databases are accessible to all member laboratories and allow the comparison of some profiles with those stored in the database. This illustrates that sharing comparable data at a national or international level is not a problem and has already been done at the European level. However, the aforementioned issues concerning the methods remind us that some questions need to be addressed first.

The positive results of this research should stimulate further research. The knowledge on the stability of the substances could be deepened. The evaluation of the classification models on a larger data set involving additional samples or data from other researchers appears very interesting. Discrimination could be improved by combining the information of the physical characteristics and that provided by isotope analysis. The isotopic analysis of other explosives, its application to postblast residues and the implementation of a database are all fields which deserve to be explored.

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Appendix 1

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1 IC method details for anionic analyses

Anionic analyses were performed at the Scientific Research Service (Wissenschaftlicher Forschungs Dienst) in Zurich, using a Metrohm 761 compact ion chromatography instrument equipped with a Methrohm 750 autosampler and coupled to a conductivity detector. One μL of solution sample was injected on a Metrosep Anion Supp.5 column (4.0x 250mm), using an eluent made of 2mMol Na_2CO_3/ 3 mMol LiOH/ 1 mMol cyanophenol/ 4%acetonitrile with a flux of 0.80mL/min and a 11.8 MPa pressure.

All samples were prepared with deionised water. Solutions containing 40mg/L of black powder were prepared by successive dilutions in deionised water, after 5 minutes ultrasonic bath and filtering with 2mL syringe with a polytetrafluoroethylene (PTFE) microfilter (diameter 0.2um). Solutions containing 30 mg/L of ammonium nitrate fertiliser were prepared by successive dilutions in deionised water, following the same procedure as mentioned above.

A blank of deionised water was analysed between each sample. At the beginning of the sequence, and every ten analyses, a mixture of standards encompassing 9 target anions usually detected in explosives, was analysed.

Appendix 2

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2 Publications

- N. Gentile, R. T. Siegwolf, and O. Delémont. Study of isotopic variations in black powder: reflections on the use of stable isotopes in forensic science for source inference. *Rapid Communications in Mass Spectrometry*, 23(16):2559–2567, 2009
- N. Gentile, L. Besson, D. Pazos, O. Delémont, and P. Esseiva. On the use of IRMS in forensic science: proposals for a methodological approach. *Forensic Science International*, 212:260–271, 2011.
- N. Gentile, M. J. Rossi, O. Delémont, and R. T. W. Siegwolf. δ^{15}N measurement of organic and inorganic substances by EA-IRMS: a speciation dependent procedure. *Analytical and Bioanalytical Chemistry*, 405(1):159–176, 2013.

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RCM

Study of isotopic variations in black powder: reflections on the use of stable isotopes in forensic science for source inference[†]

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Isotope ratio mass spectrometry (IRMS) has recently made its appearance in the forensic community. This high-precision technology has already been applied to a broad range of forensic fields such as illicit drugs, explosives and flammable liquids, where current, routinely used techniques have limited powers of discrimination. The conclusions drawn from the majority of these IRMS studies appear to be very promising. Used in a comparative process, as in food or drug authentication, the measurement of stable isotope ratios is a new and remarkable analytical tool for the discrimination or the identification of a substance with a definite source or origin. However, the research consists mostly of preliminary studies. The significance of this 'new' piece of information needs to be evaluated in light of a forensic framework to assess the actual potential and validity of IRMS, considering the characteristics of each field. Through the isotopic study of black powder, this paper aims at illustrating the potential of the method and the limitations of current knowledge in stable isotopes when facing forensic problems. Copyright © 2009 John Wiley & Sons, Ltd.

A major field of application in forensic science concerns the establishment of the identity of source and, more particularly, the determination of the unique source of a trace.[1–3] The following scenario illustrates this aspect in the field of explosive investigation. An individual installs an improvised explosive device (IED) in a building. For some reason, the IED does not explode and the bomb is discovered intact the following day. A suspect is arrested within a few days. The police make a search of the suspect's house and find an explosive substance, which is seized and analysed. The case is brought to court and the expert is asked to express their opinion on the value of the link between the pieces of evidence found at the crime scene and at the suspect's house. The expert expresses the following conclusions: both samples are identified as black powder. However, one of the main questions raised by the prosecution concerns the source of the samples: does the black powder collected at the crime scene come from the same source as the black powder seized at the suspect's house?

The techniques widely used in routine forensic analyses, in this case the analysis of inorganic explosives, allow only conclusions on the type of explosive used.[4–8] Whether it concerns intact material or post-blast residues of explosives,[9,10] the use and interpretation of semi-quantitative data obtained by ion chromatography, gas chromatography, liquid chromatography or capillary electrophoresis is not possible within the framework of current knowledge: we can neither differentiate, nor infer a common source between samples of the same chemical nature. This situation not only reflects the state of the art in this field of investigation, but also in other areas applying the same analytical methodologies, such as in the analysis of some illicit drugs, of accelerants in fire debris or of microtraces.

Isotope ratio mass spectrometry (IRMS), whose applications are numerous and diverse, has lately emerged in forensic science. Used in a comparative approach, such as in food or drug authentication, this high-precision technology has theoretically the potential to differentiate samples of the same chemical composition. A wide variety of forensic materials has been analysed with this analytical tool and the conclusions of the studies appear very promising.

Forensic applications of IRMS

The main applications of IRMS in forensic science[11] are in the analysis of illicit drugs,[12–15] explosives,[16–20] flammable liquids,[21,22] and soil and microtraces.[23,24]

Samples of cocaine and heroin from four geographical regions were analysed using an elemental analyser coupled to isotope ratio mass spectrometry (EA-IRMS) by Ehleringer *et al.*[12] The geographical origin of the samples could be inferred on the basis of their carbon and nitrogen isotope ratios. Besacier *et al.* used the $\delta^{13}C$ analysis of major constituents in heroin samples as a complementary method

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[†]Presented at the 2nd Joint European Stable Isotope User Meeting (JESIUM), Presqu'île de Giens, France, 31 August–5 September, 2008.
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to the traditional comparative chemical analyses in order to confirm common batch samples.[13] Palhol *et al*. analysed the $^{15}N/^{14}N$ isotope ratio of 3,4-methylenedioxyphenylmethamphetamine (MDMA) in seized ecstasy tablets by gas chromatography coupled to combustion/isotope ratio mass spectrometry (GC/C/IRMS).[14] The large variation of the nitrogen isotope ratio (between −18 and 19‰) observed among the 106 samples of ecstasy tablets allowed the differentiation of samples and the determination of five major groups of samples probably having a similar synthetic pathway. The first isotopic study on explosives was reported by Nissenbaum[16] where seven samples of TNT with known countries of origin were differentiated on the basis of their carbon isotope ratio. Wakelin measured the isotope ratios of C, N, O, S and Cl by EA-IRMS for a wide range of bulk explosives.[17] For each type of explosive, the author reported the existence of isotopic variations between explosives from different sources. Ehleringer (see Phillips *et al*.[18]) analysed samples of PETN from different manufacturers. He reported that samples of explosive produced by the same manufacturer had similar isotopic values, yet were distinguishable from those from other manufacturers. Pierrini *et al*.[19] evaluated the $\delta^{13}C$ and $\delta^{15}N$ variations of 26 semtex samples under a likelihood ratio approach. The samples, analysed by EA-IRMS and assumed to come from different sources, showed wide $\delta^{13}C$ and $\delta^{15}N$ variations (ranging from −24 to −36‰ for carbon and from −3 to −25‰ for nitrogen). Smallwood *et al*. measured the $^{13}C/^{12}C$ isotope ratio of 16 compounds in 19 gasoline samples collected in different areas by GC/C/IRMS.[22] Although the isotope ratio of each compound varied in a narrow range (approximately 2 to 4‰), the majority of the samples could be distinguished from each other with a chemometric post-treatment of the results.

Integration of the isotopic information in a forensic framework

The results of the performed studies converge to the following conclusions: isotopic variations exist among substances having the same chemical composition; isotopic compositions vary between different manufacturers, production locations or production batches; and it is easier to differentiate samples when two or more isotope ratios are measured. The next natural step would be to take advantage of the convincing discrimination power of IRMS to establish and assess links between caseworks or between a trace recovered at the scene of a crime and a material seized at a suspect's house or on their person. However, the exploitation of the isotopic information as evidence requires further considerations in a judiciary system. Because the consequences of a verdict can be serious, the value of this new type of evidence has to be fully assessed whether it is presented in court or used in an operational perspective. Much research has been undertaken in a wide range of forensic applications, but several studies are still mainly at a preliminary stage. Bearing in mind its final exploitation in the service of justice, the central question remains with the evidentiary value of this isotopic information under two competitive hypotheses (H_1: the trace comes from the same source; H_2: the trace comes from a different source).

As an example, regardless of the isotope concerned, the isotopic comparison of the sample collected at the crime scene and the material of the suspect produces a 2‰ difference. In court, such an isotopic difference will without any hesitation lead the defence attorney to allege that the samples are from two different sources (H_2). On the contrary, the prosecution will be interested in the probability of observing such a difference if both samples are from the same source (H_1). Therefore, in order to evaluate this difference, it is crucial to have knowledge of the possible variations between different sources, but also within the same source. The definitions of intra-variability and inter-variability are strongly dependent on the hypothesis of interest on the source level and on the production process of the substance. In this case, the intra-variability of the source is related to the units of black powder sold on the market and indirectly to the intra-variability of a batch. The variability of the characteristics within a source can be either spatial in terms of homogeneity, or it can be time-related to the evolution of a production line or to the storage conditions for example.

EXPERIMENTAL

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios of samples of black powder were studied within and between batches of a Swiss manufacturer to evaluate the intra-variability of batches and of a production line. In addition, an experiment relating to storage conditions was undertaken to observe whether storage conditions could significantly affect the isotopic profile of the substance.

Sampling and sample preparation

Several samples of black powder grade FFg ('F' is an international coding in black powder manufacture related to the size of the grains) from different production batches were collected at the Aubonne powder factory in Switzerland. Each batch of black powder is composed by mixing several day-productions. Each day-production of black powder is stored in several bags of 50 kg. When the total amount necessary for the constitution of one batch has been produced, generally around 1.8 tons, the contents of the bags are dumped and physically mixed in a huge funnel.[25,26] The end product resulting from this mix constitutes one batch and it is finally packaged into appropriate containers for sale and export. The analysed samples come from four batches produced between July 2006 and March 2008 (Table 1). One sample of 100 g representative of the end product of the batch was collected after the mix. In addition to this, three to five other samples of 100 g each were collected in different bags, before the mixing step. Since the final batch production process consists of a mechanical mixing of the grains of black powder without any addition of other substances, it is reasonable to assume that the samples collected in different bags prior to the mixing process provide an indication of the isotopic intra-variability of a batch production. For each sample, three parts, each being the object of one analysis, were prepared and analysed to evaluate the homogeneity of the sample. Moreover, the intra-variability of 1 kg of black powder produced in July 2006 was investigated and used for the experiment about storage

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Table 1. Details on the sampling and the batch of production of the analysed samples of black powder

Designation	Sampling	Batch
CH01	1 kg of the end product	July 2006
CH02.0	100 g from the end product	January 2007
CH02.1	100 g from bag A	January 2007
CH02.2	100 g from bag B	January 2007
CH02.3	100 g from bag C	January 2007
CH02.4	100 g from bag D	January 2007
CH03.0	100 g from the end product	October 2007
CH03.1	100 g from bag A	October 2007
CH03.2	100 g from bag B	October 2007
CH03.3	100 g from bag C	October 2007
CH04.0	100 g from the end product	March 2008
CH04.1	100 g from bag A	March 2008
CH04.3	100 g from bag C	March 2008

conditions (see below). All samples were conditioned in glass vial sealed with Parafilm® and stored in the dark under ambient conditions.

Any handling of black powder was undertaken carefully, using an anti-electrostatic security system (ELME 157 kit 6090 wrist strap and benchmat, Distrelec, Nänikon, Switzerland) when possible. The effect of grinding samples on the isotope ratio results was investigated. No significant difference was noted between the isotope ratios of ground and unground black powder. On this basis and for safety reasons, black powder was analysed without being ground. The carbon and nitrogen isotope ratios were analysed separately. The amount of samples was adjusted to the carbon and nitrogen content of the sample. A typical amount of 3.50 mg for carbon analysis and 1.40 mg for nitrogen analysis was weighed and sealed in tin capsules.

^{13}C and ^{15}N analyses

Part of the isotope ratio measurements were performed using a continuous flow system consisting of an elemental analyser (EA 1108, Carlo Erba, Milano, Italy) coupled with a Conflo II Interface (Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta S, Finnigan MAT). For reasons of laboratory organisation, the rest of the analyses were then performed using the same elemental analyser coupled to a DeltaPlus XL isotope ratio mass spectrometer (Finnigan MAT). The EA comprised two quartz glass tubes reactors; one filled with chromium 3-oxide and cobalt oxide for combustion and the other filled with reduced copper for reduction. The combustion and reduction ovens were set to 1000°C and 650°C, respectively. For carbon analysis, the samples were dropped into the EA through an autosampler (AS 128, Carlo Erba). For nitrogen analysis, a Zero-Blank autosampler (Costech Analytical Technologies, Valencia, CA, USA) was used to avoid possible interferences of atmospheric N_2. Raw data were processed with Isodat 2.0 software (Thermo Fisher Corporation, Bremen, Germany). By convention, the isotopic value, noted as δ-values, expresses in per mill (‰) the deviation of the isotope ratio of the sample from the international standard (VPDB for carbon and atmospheric N_2 for nitrogen): $\delta = [(R_s - R_{ref})/R_{ref}]^{*}1000‰$, where R_s and R_{ref} are, respectively, the isotopic ratios of the sample and of the reference. Calibration was carried out using the international standards IAEA-USGS24, IAEA-CH-6, IAEA-CH7 for carbon and IAEA-N1, IAEA-N2 and IAEA-N3 for nitrogen.

Black powder, a very sensitive explosive, results from the mix of potassium nitrate (saltpetre), carbon and sulphur in approximate proportions of 75:15:10. The unusual nature and reactivity of black powder required, in the opinion of the authors and according the principle of identical treatment,[27] the use of a working standard with a similar matrix and chemical composition. An in-house working standard (MIX) made of potassium nitrate (purum p.a. >99.0%, Fluka, Buchs, Switzerland) and carbon (99.95% purity, Sigma-Aldrich, Buchs, Switzerland) was therefore constituted in proportions of 75:25. Sulphur was not added to the mix for safety reasons and to avoid its deposition in the mass spectrometer. The isotopic values for carbon and nitrogen were determined on both instruments against two certified laboratory standards with a repeatability of 0.10‰ for carbon ($n = 40$) and less than 0.15‰ for nitrogen ($n = 40$).

The laboratory standards, carefully calibrated against the international standards, have shown an overall precision through everyday use over several years of less than 0.1‰ for carbon and 0.2‰ for nitrogen. The samples of black powder were bracketed at the beginning and at the end of the sequence with three consecutive laboratory standards and three consecutive in-house standards. After every nine samples, one laboratory standard and one in-house standard (MIX) were inserted throughout the sequence. The in-house standard (MIX) acted as an isotopic reference and, if necessary, an appropriate drift-correction was applied to data by means of linear shift or regression. The laboratory standard was used as a control tool to monitor the quality of the sequence of analyses. The carbon and nitrogen concentrations were calculated on the basis of the peak areas (relative precision ±3%).

Storage experiment

The ageing of a material, mainly due to oxidation and hydrolysis, occurs naturally through the interaction of environmental factors, such as ultraviolet and visible light, heat, aggressive pollutants, moisture, etc. Accelerated ageing is implemented by exposing the material to severe conditions, generally heat or moisture, for a definite period of time chosen by the researcher.[28,29] In order to observe the ageing effect on isotopic ratios, three portions, taken from 1 kg of black powder produced in July 2006, were stored under different conditions. After an initial sampling of each portion, the first was stored under normal conditions (20°C, ~50% relative humidity) and the second under high moisture conditions (20°C, ~80% relative humidity). Electronic probes (ELPRO Ecolog, Buchs, Switzerland) were used to monitor the temperature and the relative humidity under normal and high moisture conditions. The third portion was stored under elevated temperature in an oven (60°C, ≈0% relative humidity), whose temperature was controlled by a thermostat. These samples were stored for 1 year under these conditions and sampling was carried out after a definite period of time t = 0, 1, 2, 3, 7, 9, 12 (t corresponding to the number of months). Each collected

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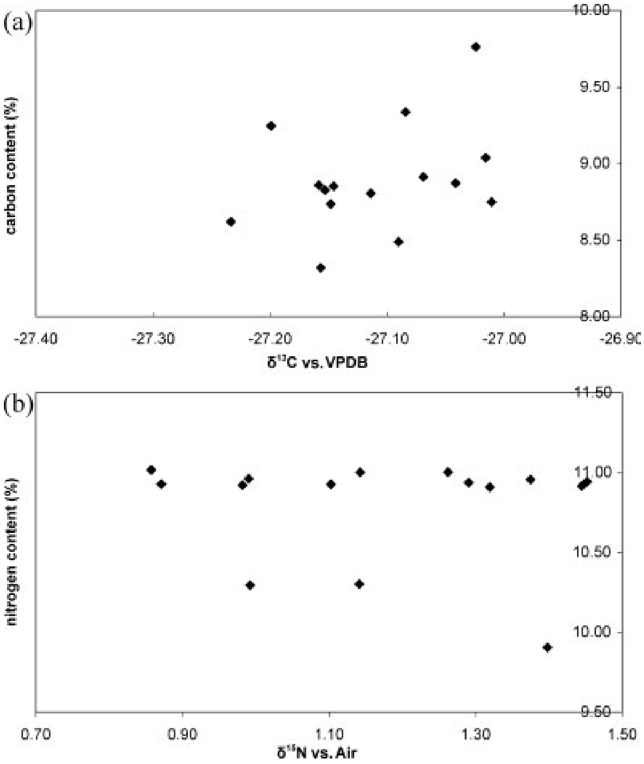

Figure 1. Isotopic δ^{13}C variations (a) and δ^{15}N variations (b) measured in 1 kg of black powder produced by the Aubonne powder factory in July 2006.

sample was conditioned in a glass vial sealed with Parafilm® and stored at ambient temperature in the dark to minimise possible interactions with light, heat and moisture in the air.

RESULTS AND DISCUSSION

Intra-variability within 1 kg of black powder

Figure 1 illustrates the isotope ratios of ^{13}C and ^{15}N measured in 1 kg packages of black powder produced in July 2006. While the measured variability of δ^{13}C was as small as 0.22‰, wider isotopic variations up to 0.60‰ were observed for nitrogen in the same 1 kg of black powder.

An interesting difference between the carbon and nitrogen concentrations (valid for measurements in Fig. 1, but also in Figs. 2 and 3) is the distribution of values. The measured nitrogen concentration within 1 kg or a batch exhibit less variation than the carbon content. The plotted measurements were obtained in different runs at different times. Thus the higher repeatability of the nitrogen concentration cannot be attributed to consecutive analyses in a sequence. A possible explanation is that all grains are glazed with graphite to retard moisture absorption and to enhance conduction. Small particles of graphite can come off with friction or small shocks, giving rise to slight variations in the carbon content.

Intra-variability within a batch

Figures 2 and 3 show, respectively, the δ^{13}C and δ^{15}N variations measured within each batch and between batches of black powder. For the δ^{13}C values, regardless of the batch, the intra-variability of the 100 g samples presents similar ranges of variations (between 0.04‰ and 0.31‰). Interestingly, there are no significant differences between the δ^{13}C values of the end product samples (CH02.0, CH03.0 and CH04.0) and the other samples collected before the mixing step occurred. This observation corroborates the hypothesis that the final mixing process does not induce carbon isotopic fractionation.

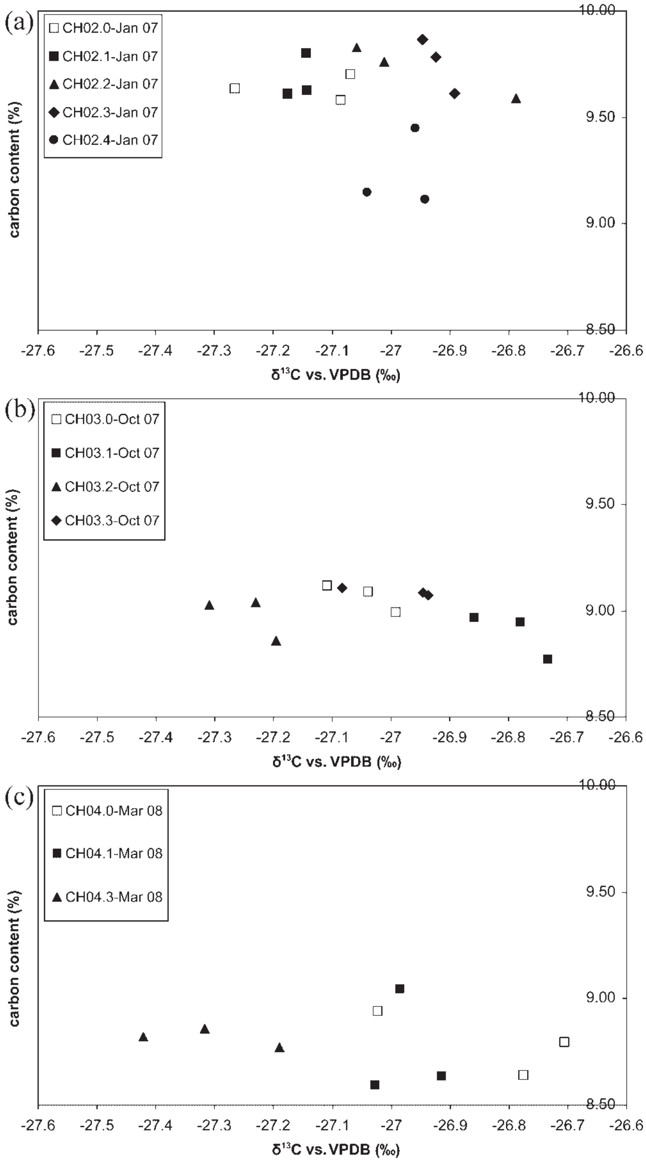

Figure 2. δ^{13}C values measured in three batches of black powder produced by the Aubonne powder factory in (a) January 2007, (b) October 2007, and (c) March 2008.

With regard to the overall intra-variability within each batch, the δ^{13}C isotope ratio varies by as much as 0.48‰ in the batch of January 2007, 0.58‰ in the batch of October 2007 and 0.71‰ in the batch of March 2008. As the batch results from a physical mix of all the grains of black powder produced on different days, one could reasonably expect to observe such variations in the end product samples. However, the values for the end product samples vary only by as much as 0.20‰ for sample CH2.0, 0.14‰ for CH03.0 and 0.31‰ for CH04.0. These smaller variations do, however, correspond to the

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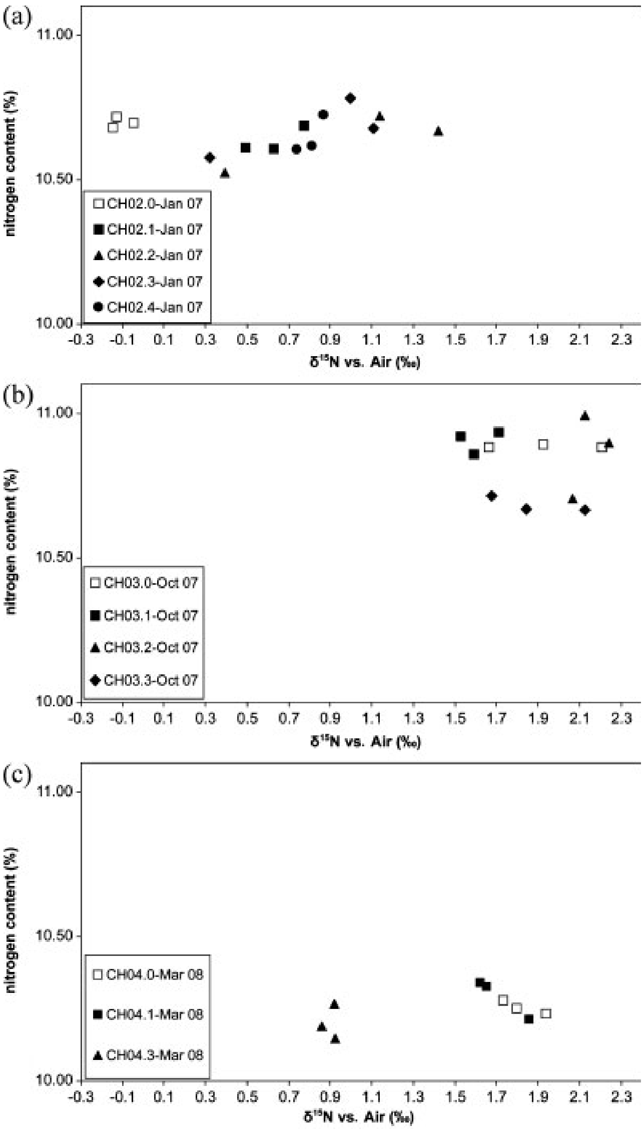

Figure 3. δ^{15}N values measured in three batches of black powder produced by the Aubonne powder factory in (a) January 2007, (b) October 2007, and (c) March 2008.

range of isotopic variations measured in the 1 kg of black powder. The three samples of a few milligrams each are probably not representative of the isotopic heterogeneity of the whole batch.

Oscillating over a wider range than the δ^{13}C values, the δ^{15}N values measured within each batch show variations of up to 1.57‰ for the batch produced in January 2007, 0.78‰ for the one manufactured in October 2007 and 1.08‰ for the one produced in March 2008. These large variations were, however, not encountered in the end product samples: their isotopic values varied by up to 0.10‰ for CH02.0, 0.54‰ for CH03.0 and 0.21‰ for CH04.0. As for carbon, these variations in the end products correspond to those encountered in the 1 kg of black powder CH01. This suggests that the batch was not sufficiently homogenous from an isotopic point of view. On the other hand, the few milligrams sampled in the 1 kg and in the 100 g samples of end product were not sufficient to obtain samples representative of the isotopic heterogeneity of the 1.8 tons batch.

Surprisingly, the δ^{15}N values of the end product CH02.0 and the other samples of the batch (CH2.1 to CH2.4) exhibit an isotopic difference (Fig. 3(a)). This difference can probably not have been induced by the simple mechanical process of mixing. However, this is probably due to the relatively small amounts analysed compared with the size of the batch.

The δ^{15}N values of the batch produced in March 2008 shows a 1‰ difference separating sample CH04.3 and samples CH04.0 and CH04.1 (Fig. 3(c)). This batch was made from the mix of daily productions during March 2008. A remaining bag produced in the year 2003 was found more recently and incorporated in the mix. The sample CH04.3 was collected in this remaining bag. Despite a clear isotopic difference, the addition of this black powder from 2003 to the rest of the batch was not reflected in the measurements; values of the end product are close to those of contemporary production.

Inter-variability between batches

Figure 4 shows a compilation of the δ^{13}C and δ^{15}N variations measured in the black powder produced by Aubonne from July 2006 to March 2008. The bivariate plot represented in Fig. 4 illustrates the existing inter-variability in the production line of the manufacturer between samples produced at different time. Except for sample CH02.0, which exhibits a distinct and unexplainable nitrogen isotope ratio, the samples are arranged in a large cluster encompassing values for carbon from −27.42‰ to −26.71‰ and for nitrogen from 0.32‰ to 2.24‰. There are no distinctive features for carbon. Interestingly, all the samples produced in October 2007 and in March 2008 have a higher δ^{15}N value than the other samples, except for the one produced in 2003. The values of sample CH04.3 are closer to the black powder produced in July 2006 and January 2007. This shift of the isotope ratio with time may indicate a change of grade, of batch or of supplier of the potassium nitrate.

Effect of storage conditions

The effect of storage conditions on the δ^{13}C values of black powder is illustrated in Fig. 5 as a function of time. The δ^{13}C values are reported in Table 2. The measurements at $t = 0$ reflect the intra-variability in the 1 kg of black powder (as reported above in Fig. 1(a)). The slight δ^{13}C variations measured after 1 and 2 months under the different conditions are within the range of the initial measured variations and can therefore be attributed to the intra-variability of the powder. As the time of exposure to heat and humidity increases, no significant δ^{13}C variations were measured. The carbon isotope ratio does not appear to be affected by environmental conditions, either natural or induced.

The δ^{15}N values of the same black powder stored under different conditions show a different pattern from that shown by carbon (Fig. 6). The δ^{15}N values are reported in Table 3. The set of values measured at the beginning of the experiment ($t = 0$) reflects the δ^{15}N intra-variability within the 1 kg of black powder (as plotted above in Fig. 1(b)). The isotopic variations observed after 1 month and 2 months exposure under different conditions oscillate within the same range as the initial measurements and can be attributed to the nitrogen isotopic intra-variability. Thus, the first 2 months of

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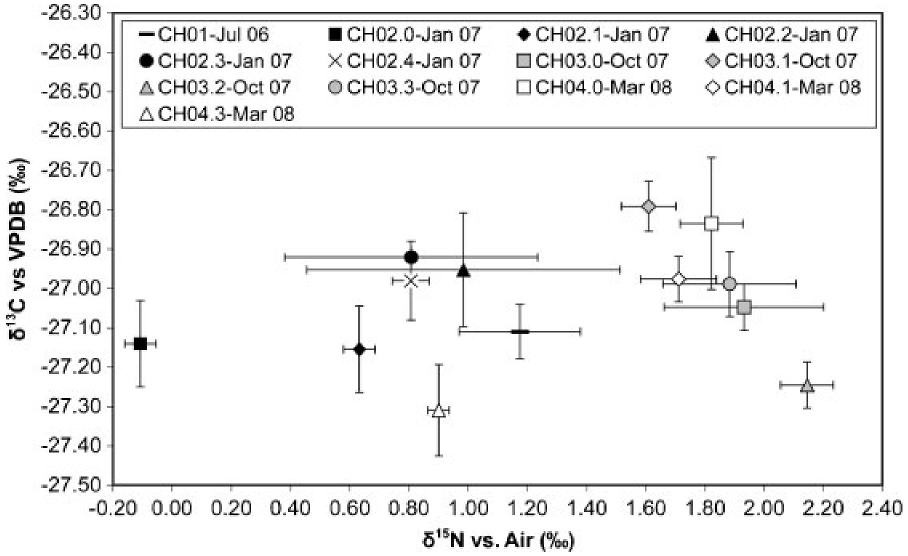

Figure 4. $\delta^{13}C$ and $\delta^{15}N$ variations between samples of black powder from four different periods of production: July 2006 (CH01), January 2007 (CH02), October 2007 (CH03), and March 2008 (CH04). The mean and the standard deviation of each sample (sd = ± 1σ) result from the analysis of at least three parts of it.

severe conditions exposure did not influence the nitrogen isotopic ratios. However, the measurements made after 7, 9 and 12 months show more disparities. The $\delta^{15}N$ values of black powder under normal ambient conditions and high relative humidity follow a very similar trend and vary within the range of the intra-variability of the sample. However, the isotopic values of the black powder exposed to high relative humidity are less repeatable and therefore produce larger standard deviations. This influenced mostly the average at t = 7, while the $\delta^{15}N$ averages for 9 and 12 months remained comparable with those of the beginning (t = 0–2). Finally, the isotopic ratios of black powder stored at elevated temperature display a significant increase (based on average) of approximately 0.5‰ after 7 months, 0.6‰ after 9 months and 1.1‰ after 12 months.

Figure 7 shows the nitrogen content of the black powder plotted versus the $\delta^{15}N$ values, after the material was stored at elevated temperature. Although the nitrogen content does not vary much, the $\delta^{15}N$ values appear to be negatively correlated to this variation. This supports the hypothesis of the evaporation of a molecule containing nitrogen under the influence of heat. The lighter, nitrogen-14-based molecules

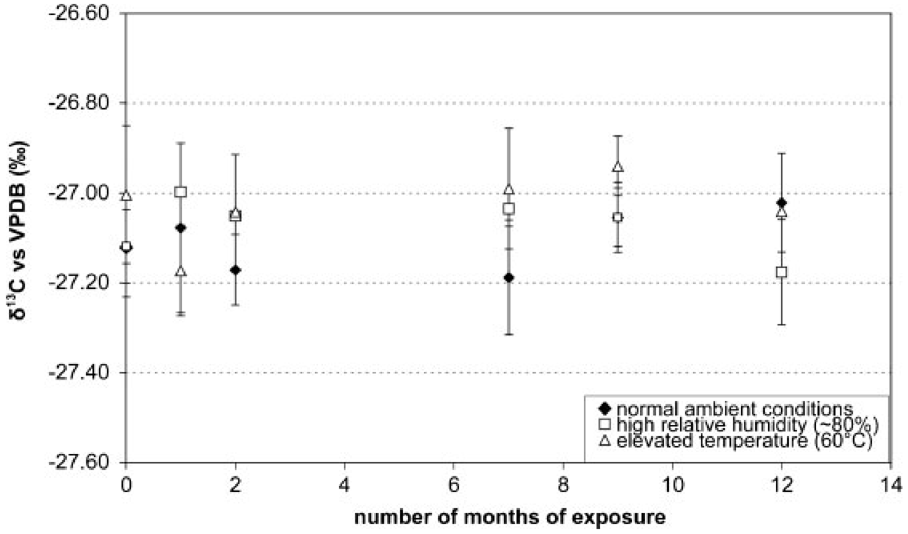

Figure 5. Evolution of the $\delta^{13}C$ value of a sample of black powder separated in three parts and stored for a period of 1 year under different conditions. One part was stored under normal ambient conditions (~20°C, ~50% relative humidity), the second under moist conditions (80–90% relative humidity) and the last one under elevated temperature (~60°C, no relative humidity). Each point results from the average of three measurements, with its respective standard deviation (sd = ± 1σ).

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Table 2. $\delta^{13}C$ of the black powder stored under different conditions. The mean and the standard deviation are based on three measurements

	$\delta^{13}C$ of black powder (‰)		
Number of months	Under normal conditions	Under elevated moisture	Under elevated temperature
0	−27.12 ± 0.11	−27.12 ± 0.08	−27.00 ± 0.15
1	−27.08 ± 0.08	27.00 ± 0.07	−27.17 ± 0.10
2	−27.17 ± 0.08	−27.05 ± 0.01	−27.04 ± 0.13
7	−27.19 ± 0.13	−27.03 ± 0.04	−26.99 ± 0.13
9	−27.05 ± 0.07	−27.05 ± 0.08	−26.94 ± 0.07
12	−27.02 ± 0.11	−27.18 ± 0.12	−27.04 ± 0.03

evaporate more easily than the heavier molecules, resulting in an isotopic enrichment of the black powder.

For unpredicted reasons, the samples could not always be analysed immediately after their collection. The results should really have been plotted taking account of the time between the sample collection and the analysis. This time fluctuated between 0.5 months and 9 months. As an example, the effective storage period of a sample exposed at 60°C for 2 months, but which was analysed only 2 months later, corresponds in reality to an actual period of 2 months under severe conditions with 2 supplementary months under ambient conditions. However, the black powder subject to normal ambient conditions did not exhibit any significant isotopic variations of carbon or nitrogen after an actual storage time of 12.5 months. Thus, the storage of the sample at ambient temperature until its analysis should not significantly affect the isotope ratios. Moreover, the impact of severe environmental conditions is logically more likely to induce isotopic variations than subsequent storage under ambient conditions. Although the ageing effects are generally irreversible, the exact nature of the physical and chemical mechanisms caused by exposure to these 'extreme' conditions on black powder is unknown and further investigations are needed to study the degree of reversibility of these processes.

The interaction of the Parafilm or the air in the glass container with the sample was also considered. $\delta^{13}C$ and $\delta^{15}N$ data from the black powder stored in a room for 12 months under normal conditions were compared with the isotope ratios of a black powder stored in the dark in a glass container sealed with Parafilm for 12 months. No significant difference was observed between the two samples, indicating that the storage of the sample in the dark and in a flask sealed with Parafilm did not cause any interference.

In addition to environmental factors that might affect the isotope ratio, aspects specific to the field of investigation have to be considered in the evaluation of evidence. Referring to the question of the prosecutor in an explosives investigation, the ultimate scope of the isotopic analysis consists indubitably of inferring a common source between the residues of explosive recovered on the site after the explosion and an intact bulk explosive found at a suspect's house. The possible interferences with the matrix of the site, a probable isotopic fractionation during the explosion or the sampling of residues are all specific features of post-blast investigation, which give rise to new questions.

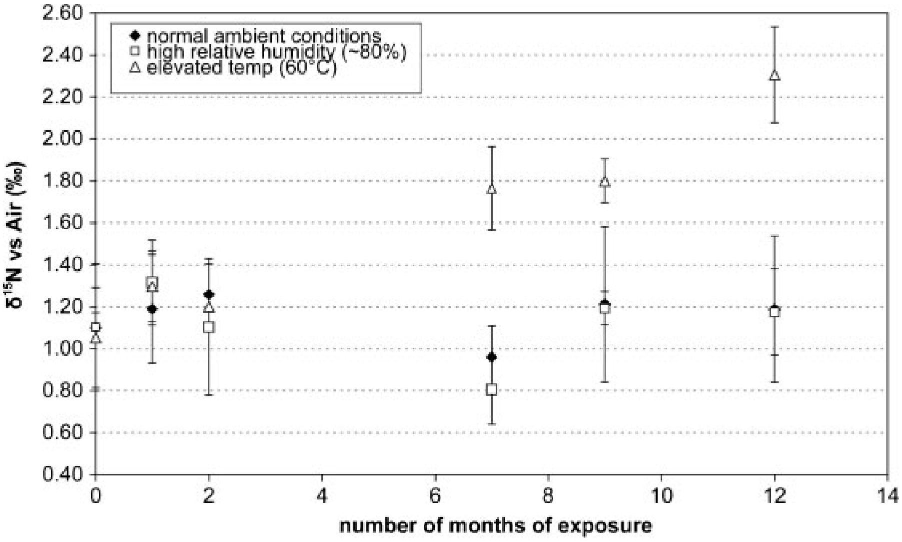

Figure 6. Evolution of the $\delta^{15}N$ value of a sample of black powder separated in three parts and stored for a period of 1 year under normal ambient conditions (~20°C, ~50% relative humidity), under moist conditions (80-90% relative humidity) and under elevated temperature (~60°C, no relative humidity). Each point results from the average of three measurements, with its respective standard deviation (sd = ±1σ).

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Table 3. $\delta^{15}N$ of the black powder stored under different conditions. The mean and the standard deviation are based on three measurements

	$\delta^{15}N$ of black powder (‰)		
Number of months	Under normal conditions	Under elevated moisture	Under elevated temperature
0	1.10 ± 0.30	1.10 ± 0.07	1.05 ± 0.24
1	1.19 ± 0.26	1.32 ± 0.20	1.30 ± 0.17
2	1.26 ± 0.14	1.10 ± 0.33	1.20 ± 0.14
7	0.96 ± 0.15	0.81 ± 0.16	1.76 ± 0.20
9	1.21 ± 0.37	1.19 ± 0.08	1.80 ± 0.11
12	1.19 ± 0.35	1.18 ± 0.20	2.31 ± 0.23

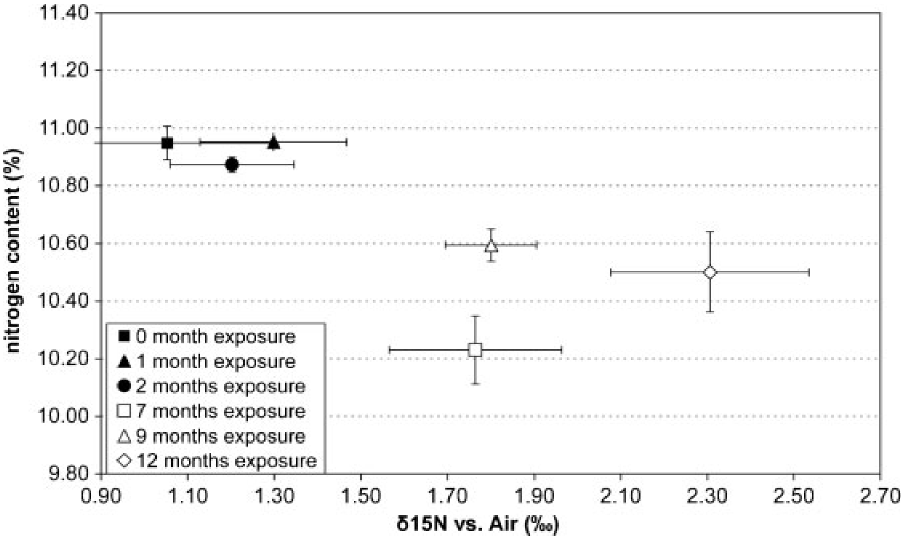

Figure 7. Plot of $\delta^{15}N$ average ($n = 3$; sd $= \pm\ 1\sigma$) versus the nitrogen content of samples stored for a period of 1 year under elevated temperature (60°C).

CONCLUSIONS

Most studies of the forensic applications of IRMS have shown that this high-precision technology could overcome limitations related to current analytical techniques in certain fields. The results presented in this study highlight on one hand the complexity of assessing the intra-variability of a mass-produced substance. On the other hand, they demonstrate the existence of significant variations, especially for nitrogen isotope ratio, within the production line of a manufacturer.

The study of the influence of storage conditions on the isotopic profile showed that the isotopic characteristics are not equally affected. While carbon is stable under the different storage conditions, nitrogen is more sensitive to environmental factors. The isotope ratio of nitrogen did not show any significant changes for black powder stored under normal conditions for a period of 12 months. However, after 7 months exposure to high relative humidity, the moisture induced poorer repeatability of the $\delta^{15}N$ values. Furthermore, the exposure of black powder at 60°C revealed a significant variation in the $\delta^{15}N$ value after 7 months exposure.

These preliminary results emphasise the need to gain comprehensive knowledge of the intra and inter-variability, as well as factors that might affect the isotope ratio, in order to assess the value of the isotopic evidence in source discrimination or source inference.

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On the use of IRMS in forensic science: Proposals for a methodological approach☆

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ABSTRACT

The flourishing number of publications on the use of isotope ratio mass spectrometry (IRMS) in forensic science denotes the enthusiasm and the attraction generated by this technology. IRMS has demonstrated its potential to distinguish chemically identical compounds coming from different sources. Despite the numerous applications of IRMS to a wide range of forensic materials, its implementation in a forensic framework is less straightforward than it appears. In addition, each laboratory has developed its own strategy of analysis on calibration, sequence design, standards utilisation and data treatment without a clear consensus.

Through the experience acquired from research undertaken in different forensic fields, we propose a methodological framework of the whole process using IRMS methods. We emphasize the importance of considering isotopic results as part of a whole approach, when applying this technology to a particular forensic issue. The process is divided into six different steps, which should be considered for a thoughtful and relevant application. The dissection of this process into fundamental steps, further detailed, enables a better understanding of the essential, though not exhaustive, factors that have to be considered in order to obtain results of quality and sufficiently robust to proceed to retrospective analyses or interlaboratory comparisons.

1. Introduction

The number of publications on the use of stable isotopes in a source inference perspective attests to the increasing popularity of isotope ratio mass spectrometry (IRMS) in forensic science. Exploratory studies on TNT and cannabis were among the very first forensic applications [1,2]. Its subsequent applications to natural and synthetic illicit drugs [3–20], organic and inorganic explosives [21–24], petroleum hydrocarbons [25–37] and to various other forensic materials [38,39] have confirmed its potential of discrimination. Benson et al. proposed an extensive review of its forensic applications [40]. More recently, a new axis of IRMS studies has focused on source inference between a manufactured substance and a starting material or precursor, as well as on the discrimination of batches of synthesised drugs according to their synthetic route [41–50].

Although research in many fields is moving forward, very few publications draw attention to the entirety of the process underlying the use of isotopic analysis in forensic science. Many studies using IRMS have been undertaken in a top-down approach, applying this technique experimentally on a random sampling. Very few are based on a comprehensive reflection of the whole process. While reference literature on important aspects of IRMS [51–58] are fundamentals of any scientific research, the specific application of this technology to forensic issues needs also to be undertaken in a forensic framework for a correct and relevant interpretation of results. Since a trace can have various and indefinite features, the forensic scientist can be faced with unpredictable matrices, as well as imperfect specimens in terms of quantities (minute amounts of analyte) or integrity (degraded or partial specimens). In addition to the quality of a trace, the final result and its interpretation should take into account the potential isotopic fractionation occurring during analyte preparation or analysis as well as source variability, while being fully conscious of the impact of sampling, calibration with standards and data treatment.

The aim of this paper is to advise a methodological framework of the whole process using IRMS methods and to highlight the significance of each step and its associated parameters on the final isotopic result. While this contribution reviews the essential elements of a sound forensic application of IRMS, it does not intend to compile an exhaustive list of all factors intervening in the IRMS process, since each application and research problem holds its own characteristics and constraints. With a selection of publications, each exemplifying specific aspects of the process,

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this methodological approach proposes anchoring elements for a comprehensive and relevant application of IRMS to forensic specimens. Overall, we emphasise the importance of considering isotopic results as part of a whole process, when applying this technology to a particular forensic issue.

Still, it should be stressed that the decision to use IRMS must be justified. Given the countless results of source discrimination obtained with IRMS, it may be tempting to overlook the possibilities of traditional analytical techniques (GC–MS, LC–MS, etc.). However, given its cost and complexity, IRMS should be considered only when other routine techniques have shown their limitations in source inference.

2. The process

The analytical process oriented towards solving a definite problem is divided into six fundamental steps (Fig. 1). Each of them, detailed below, illustrates the significance of decisive factors that have to be considered for an appropriate approach. From the delineation of the research problem to the results interpretation, the measurement of stable isotope ratios and its forensic significance result from a series of different steps, which individually requires a thoughtful consideration and a careful implementation. More than a set of guidelines, this six-step approach enables a better understanding of the essential, though not exhaustive, factors that have to be assessed in order to guarantee results of quality and robust enough to proceed to retrospective analysis (query into databases) or interlaboratory comparisons.

This methodological approach is valid for both bulk and compound specific isotope analysis (CSIA); it incorporates essential aspects common to elemental analysis (EA) and gas chromatography (GC) interfaced with IRMS.

3. Defining the research problem

3.1. Problem description

Defining the research problem through the formulation of a set of specific hypotheses helps to clarify the purpose of the research and conditions the approach to adopt. In a source discrimination or source identity inference process, the set of questions is directly related to the definition of the source in a hierarchy of propositions and, therefore, to the level of source considered, i.e. geographical origin, synthesis pathway, manufacturers level, etc. The complexity of the definition of the identity of source, brilliantly disserted by Kwan [59], stems from the multiple parameters involved in the origin of the source.

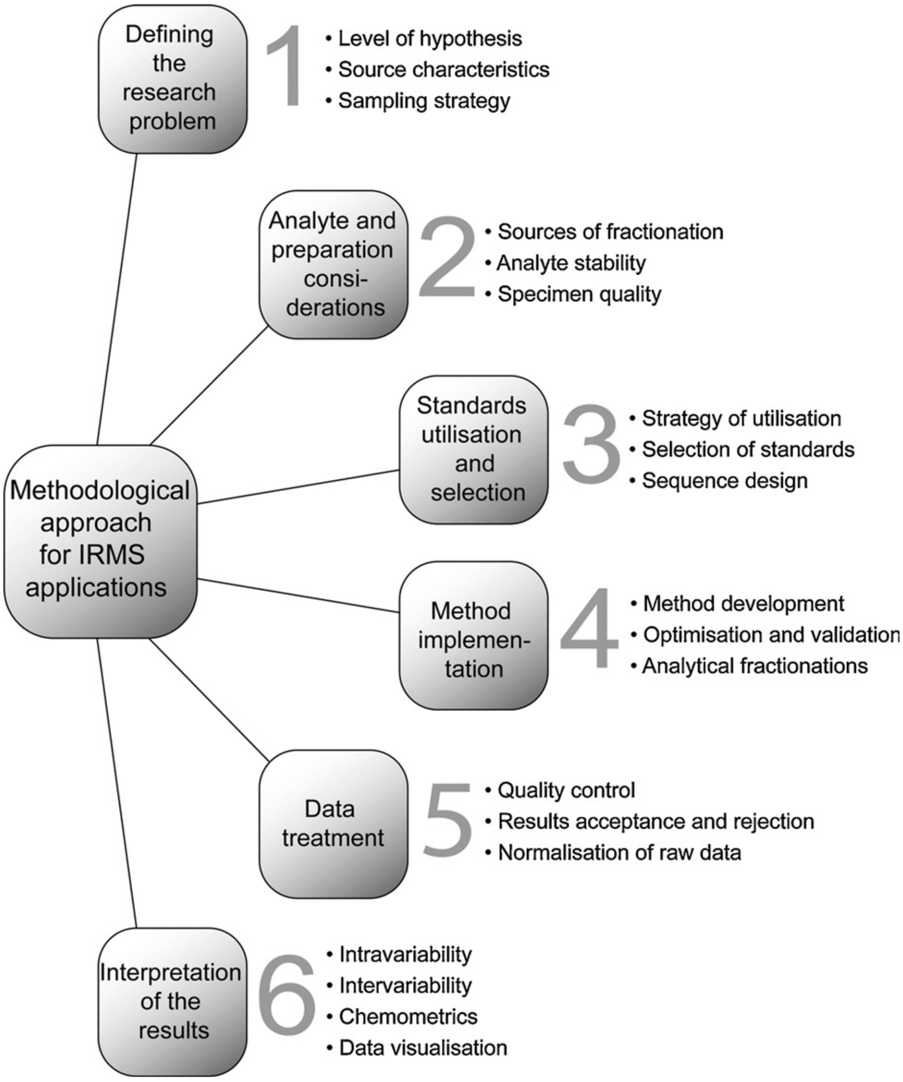

Fig. 1. The proposed methodological process decomposed into six steps and their essential parameters.

For each research problem, the source is characterised by features of the production system, distribution network and characteristics related to the consumption or use of the substance. Production features are related to the manufacturing process, including the number of batches produced per day, week, month or year, the quantities manufactured, the raw materials, the physical and chemical processes involved. The market structure of a substance is defined by the distribution networks, constituted by retailers, intermediates, dealers or sale on black market, as well as the dimensions (local or international) and quantities involved (sales unit, whole batches, etc.). The conditioning and cutting phases of illicit drugs or every new filling of the tank at a service station contributes to create a new mixture of adulterated drugs or of gasoline [60]. These are supplementary elements characterising the source. This may therefore offer additional characteristics of source identity. Yet, the complexity and limitations to define the notion of source not only arise from the intricate production and distribution features, but also from the availability of the information.

The access to such information is often uneasy, due to trade secret for mass produced substances or unreliable information as it is for illicit drugs manufacture and distribution. However, even incomplete, knowledge of these features allows to understand and make assumptions about the level at which variations between entities may be observed (geographical locations, manufacturers, retailers, batches, seizures, sales unit, etc.). It assists the forensic scientist in defining the source level (level of hypothesis) and therefore, the research problem. At the same time, when available, information on the quantities produced and distributed at a time are useful for the subsequent interpretation of results, providing indication on the frequency (rarity) of the characteristics measured.

The risks of overlooking the research problem definition can result in the following consequences: (1) an inappropriate sampling producing irrelevant data, (2) a waste of time and money, if the discrimination could possibly have been performed by routine techniques, for example such as illicit drug profiling by GC–MS [61] and (3) hampering results interpretation because the meaning of the observed variations cannot be attributed to a source level (different manufacturers, batches, seizures or sales unit, etc.). The definition of the research problem and the characterisation of the source are therefore decisive in order to define the suitable sampling for a relevant isotopic study as well as for a correct interpretation of isotopic variations.

3.2. Sampling

While analysed quantities are becoming ever smaller, the notion of sampling representativeness is more than ever paramount. A correct mass reduction is necessary for a representative and relevant sampling. The quantitative aspect [62], as well as the sampling strategy and procedure are crucial in that step [63]. Based on Gy's theory of sampling [64–66], Petersen et al. provide practical guidelines to establish a representative sampling such as considering the dimensionality of the material to sample, homogenising and avoiding grab sampling. Sampling is directly related to the definition of the research problem and to the definition of the source. In an isotopic study, this step is essential to evaluate the intravariability associated to the source, and hence, to the research problem. For example, the sampling will be totally different if we want to differentiate specimens of illicit drugs at the seizure level or their synthetic pathway. The representativeness of the sampling has a significant impact on results interpretation, as a biased sampling leads to erroneous data and can in no way be further corrected in the analytical chain.

3.3. In essence

The definition of the research problem and the notion of source highly rely on the features of production, market structure and characteristics related to the use of the substance. This knowledge is essential for a representative sampling and a reliable interpretation. Besides, the representativeness of the sampling in an isotopic study should not be ignored as it determines the quality and reliability of data.

4. Considerations on the analyte and its preparation

Any stage of the specimen history and of the analytical procedure may introduce isotopic fractionation effects. Analyte consideration and its preparation is essentially the last reflection step before starting the analyses, in which all factors that may influence the quality of the specimen and alter its isotopic integrity must be considered before analysis. Adsorption, dilution, dissolution, volatilisation, weathering or matrix interaction induced by the physical and chemical properties of the analyte or by the analyte collection, storage or preparation may cause isotopic fractionation, thereby affecting the accuracy and reproducibility of results.

4.1. Analyte considerations

Fundamental questions pertaining to the nature of the specimen (the physical and chemical state, solid/liquid, organic/inorganic, etc.), the instrument at disposal (EA-IRMS, GC/C/IRMS, LC/IRMS, etc.) and the target elements (C, N, O, H, etc.) are essential to select an appropriate method and determine the whole analytical strategy. Besides, analyte considerations such as its collection and storage conditions, as well as the eventual weathering of the specimen should raise interrogations about their potential mass discriminatory effects on the isotope ratio.

Concern about collection refers to the way analytes are sampled or collected on site. The use of specific devices or material to collect or accumulate analytes in determined environment should be evaluated under the different conditions in which it is intended to be used (concentrations, temperatures, flow, etc.) [67].

The stability of the isotopic profile, and implicitly, the way it is influenced by storage and weathering, are part of the feature of constancy enunciated by Kwan in the selection of valuable characteristics for source inference [59]. Note that, although storage and weathering notions both pertain to the exposure of the specimen under specific conditions, storage refers here to typical storage conditions for a type of specimen, while weathering stands for the alteration due to environmental conditions *other* than storage conditions. Stability, though mainly employed for storage conditions, is used here for both storage and weathering.

Storage conditions include mainly temperature, relative humidity and light, as well as the packaging and the storage time. The collection conditions and the packaging should preserve the integrity of the specimen. If not appropriate, they may alter the isotopic composition of the specimen or contaminate it, due to the presence of additives or to unsuitable permeability (sorption or transfer through the packaging walls) [68,69]. More often than not, this issue is not assessed in studies.

The weathering of a substance, like evaporation, oxidation, hydrolysis or biodegradation, either natural or accelerated, occurs by the interaction of the analyte with external factors, such as ultraviolet and visible wavelength, heat, moisture, aggressive pollutants, bacterial activities, etc. The lost of the more volatile compounds due to evaporation, the chemical transformation due to air oxidation, the modification of the chemical and isotopic composition caused by biodegradation are all physical and chemical phenomena which may affect partially or drastically the quality of the specimen. These phenomena can induce mass discrimination and prevent the evidentiary trace to be correlated with its source. The effect of weathering processes on the isotope ratio has been widely investigated in contaminants attenuation research [70–73] and in source tracing studies of hydrocarbons and oil spills [27,37]. Two comprehensive reviews of environmental forensic studies plainly expose the ins and outs of weathering effects [35,74].

The sensitivity of the isotopic profile to weathering processes is however not exclusive to environmental materials. Nevertheless, few studies have addressed the issue of integrity of the isotopic profile of specimen. Smallwood et al. exposed gasoline samples to evaporation and water-washing for one week. Despite the carbon isotopic alteration of certain compounds, most compounds were isotopically similar to neat samples [30]. The weathering of white paints under controlled conditions was investigated. After 3 months, the authors noticed a δ^{13}C depletion of 0.7‰ possibly associated with solvent evaporation [75]. Gentile et al. exposed samples of black powder under different environmental conditions. A δ^{15}N isotopic fractionation was observed for samples exposed to elevated temperature, while samples under normal ambient conditions and high relative humidity were isotopically stable [24]. By exposing human hair to artificially isotopic light and heavy water, Sharp et al. noted the re-equilibration of a reproducible portion of exchangeable hydrogen with ambient atmospheric moisture, allowing the use of δ^2H hair samples for investigation of food and water intake [38]. Despite being time consuming and not easy to implement, controlled experiments are however essential to collect fundamental data on the stability of the measured characteristics, i.e. isotope ratios, not only during the analytical process of the specimen but also prior to analysis [76]. Matrix features (i.e. pH) could influence the chemical structure of a substance causing a reaction of interconversion and affecting the isotopic profile of this substance. The evaluation of the stability of analytes in a given matrix and under specific conditions is part of a full method validation [76]. Thus, the constancy or predictability of the evolution of analytes within a matrix under standard storage [20,24] and weathering conditions is necessary for a correct and reliable interpretation of isotopic variations.

All analytes, however, do not behave identically. The risk of fractionation is smaller with some analytes such as inert and non-volatile ones. Isotopic fractionations may be moderate depending on the element and the size of the molecule involved. In theory, large molecules are less subject to fractionation. However, this issue is not always straightforward. For hydrocarbons compounds for example, isotopic fractionation is controversial. While some studies described a significant enrichment of δ^{13}C of the remaining liquid of toluene and benzene after evaporation [73], others reported a slight depletion of the non distilled fractions of aromatic compounds [77].

Beside weathering, the integrity of the specimen can also be affected by phenomena particular to certain fields, such as explosion or fire investigation. Benson et al. evaluated the effect of explosion on the isotopic composition of ammonium nitrate prills. They observed that such phenomena altered the isotopic profile [22].

4.2. Analyte preparation considerations

Analyte preparation may involve several steps from clean-up procedures, extraction processes to pre-concentration and derivatisation techniques. As every phase of the analyte preparation may induce isotopic fractionation, the effect of each step on the isotope ratio should be carefully investigated. Furthermore, interferences of the matrix with the analytes (matrix effects) or the chemical interconversion between two substances can induce mass discrimination, and therefore affect the accuracy and reproducibility of results.

Homogenisation treatments can influence the isotopic exchange properties of the specimen. Bowen et al. illustrated this phenomena and highlighted the significance of an adequate analyte preparation to countervail the effect of atmospheric water adsorption on δ^2H and δ^{18}O [78]. In the same way, derivatisation is a sensitive point in CSIA, as an extraneous element is incorporated into the molecule. This step inducing kinetic isotopic effects is delicate, as the reaction with the analyte might not be quantitative, lead to incomplete combustion or produce chromatographic effects [5,54,57,79]. In addition, as emphasised by Meier-Augenstein in [56], some derivatising agents involving silicon or halogen moieties are not suitable for CSIA.

The potential fractionation effect of the numerous extractions, pre-concentration, purification or transformation steps and their parameters should be cautiously investigated. The excellent papers of Berg et al. and Zwank et al. demonstrate the systematic evaluation of solid phase micro extraction (SPME) and purge and trap (P&T) interfaced to GC/IRMS for the determination of the isotopic signatures of contaminants in aqueous matrix [71,80]. The reported isotopic fractionations were systematic, allowing therefore the isotopic values to be corrected.

4.3. In essence

Through selected studies, this section has highlighted the importance of considering all factors that could influence the isotopic profile during the whole process before starting the analyses. Factors such as the collection, storage, weathering of a specimen, as well as the analyte preparation, matrix effect or chemical interconversion may alter the isotopic integrity of the specimen. The fundamental study and control of these parameters are essential to prevent or minimise isotopic fractionation. The knowledge or the predictability of their influence on the isotope ratio are crucial to obtain reproducible results and to properly interpret isotopic variations.

5. Standards utilisation and selection

More than in other fields, the selection of standards and their utilisation is primordial in order to obtain precise and accurate data. Critical parameters to consider are, in particular, the strategy of analysis, the choice of appropriate (certified) reference materials and laboratory standards, as well as the design of the sequence.

As pointed out by Gröning [81], there is palpable divergence between the ISO norm on international vocabulary of metrology and the common terminology referring to standards and reference material used in the stable isotope community, in addition to terms adapted from one author to another. The terms used in this section are based on the nomenclature given by Gröning; the broad term "standard" encompasses reference material, certified reference material, internal and laboratory standard as well as reference gas.

By convention, the isotopic value, noted as δ-value, expresses in per mill (‰) the deviation of the isotope ratio of the sample from the primary reference material (or international standard): $\delta = [(Rs - Rref)/Rref] \times 1000‰$, where Rs and Rref are, respectively, the isotope ratios of the sample and of the reference. As attested by the formula, the δ-value is already expressed on a relative scale. Because isotope ratio values rely on a conventional scale defined by primary reference materials, the use of reliable and traceable reference materials and standards is decisive to ensure the comparability of results. Mainly distributed by the International Atomic Energy Agency (IAEA), the National Institute of Standards and Technology (NIST) and the Institute for Reference Materials and Measurements (IRMM), stable isotope reference materials are used to calibrate laboratory equipment and laboratory standards used in routine analysis. In order to preserve their availability, laboratories are impelled to develop their own laboratory standards or find other certified reference materials for routine analysis [81,82].

5.1. Strategy of standards utilisation

The strategy of standards measurement influences the type of selected standards. In continuous-flow techniques, reference materials or standards can be introduced into the system by [51,83]: (a) reference gas pulses into the helium stream, (b) reference gas pulses directly into the ion source or (c) passing through the analytical train of the elemental analyser or gas chromatograph. In the latter case, depending on the instrumentation, the analytical strategy can involve parallel analysis (or injection for GC) of standards or co-injection of the standards (reference materials). The co-injection technique, only possible in CSIA, is undertaken by adding reference compounds to the sample injected into the GC [54,57]. In contrast to EA-IRMS where the use of internal standard is not achievable, the spike of reference compounds into the specimen and their co-injection within the same matrix guarantee, in GC/C/IRMS, that standard and analytes undergo the same conditions of analysis and ensure reproducible results [54,57,82]. This technique, recommended whenever possible, can only be undertaken if the internal standards elute in an analyte free region of the specimen chromatogram [57]. The spike-in of an internal reference material of known isotopic composition should be introduced as soon as possible in the preparatory steps, depending on the application, to monitor any isotope fractionation.

Whatever the instrumentation, the fundamental principle underlying reliable stable isotope measurements is the principle of identical treatment (IT) of sample and reference material [55]. By submitting the reference material and the sample to the same analytical pathway and same reaction conditions, this procedure allows to take into account any potential isotope fractionation occurring in the analytical train of the peripheral device (due to non quantitative injection, incomplete conversion, gas transfer, etc.). Nevertheless, the implementation of the IT principle to "real" forensic specimens is not straightforward: by definition, it requires the use of a similar and representative analyte-free matrix. Applying IRMS to authentic forensic specimens, which involve unknown and complex matrix, makes the IT principle, in the strict sense of the term, difficult to apply in practice.

Beside calibration of the equipment and laboratory standards with reference materials, standards are also sometimes used to normalise raw data to the international reference scale. Although the number and type of chosen standards definitely impact on normalisation, and therefore on the final result [84], the issue on normalisation will be addressed in the Data treatment section (see Section 7.2).

In addition, standards can be used to monitor the quality of the IRMS and of the analysis method. At this stage, a reflexion on the use of standards as quality controls should be undertaken. The evaluation of the results of such controls are discussed in Section 7.1. Through the observation of standards of known isotopic composition, quality control can be performed at different times in the analytical process. Before any analytical session, the repeated analyses of reference gas pulses allow to check the stability of the IRMS system. During chromatographic runs, the introduction of reference gas pulses with known isotopic composition, bracketing the retention time of the analytes as closely as possible, allows to control the quality of the analyte response [57].

Similarly, the daily analysis of certified reference materials or standards allows to verify the analytical response of the machine throughout the sequences of analysis, as well as to monitor the accuracy and precision in a quality control perspective. In order to evaluate the quality of the response of the whole method, the standards should go through the full analyte preparation, peripheral device and IRMS. As mentioned earlier, in CSIA, the quality of the analysis and any isotopic fractionation can be checked through the use of an internal standard of known isotopic composition and of similar chemical structure to that of the analyte [54]. If the use of an internal standard is not possible, the use of an external standard at least, with a chemical composition similar to that of the specimen should be undertaken.

The strategy of standards utilisation for calibration, normalisation and quality control often varies from one laboratory to another. The number, type and frequency of standards analysed, the way samples, standards and blanks are positioned, the number of specimen replicates between standards and within each run all design the analytical sequence [55,82]. While the frequency of the standards analysed is governed by the required precision, the number of standards and their position can influence the results. Despite an example of loading list (sequence design) suggested by Werner and Brand [55], each laboratory uses its own strategy of analysis. Nonetheless, any forensic application should raise a reflexion on the minimum requirements of the sequence design.

5.2. Selection of standards

During standards selection, elemental factors should be considered. Laboratory standards and laboratory reference materials should meet the following criteria [55,82]:

- The material should be easy to handle during preparatory steps and replaceable if exhausted.
- It must be preferably a chemical compound available at high purity, non-hygroscopic and stable over a long period of time.
- It should have a low vapour pressure at room temperature and atmospheric pressure and a good solubility in high purity solvents (in CSIA).
- The material should have a δ value within the expected range of variations of the analytes.
- The elemental mole ratio and the chemical composition of the standard should be identical or similar to the material to be analysed for an accurate and precise measurement.

In isotope ratio mass spectrometry, as in other fields [76], the notion of matrix similarity between the standards and the material to be analysed should not be overlooked. As underlined by De Bièvre et al.: "Other things being equal, the more similar are the two items to be compared, the smaller becomes the uncertainty due to the measurement itself" [85]. This sentence summarises the significance to consider the chemical and physical properties of the unknown material during their passage through the peripheral device (EA, GC/C). Matrix of forensic specimens can be particularly unpredictable and varied, making the step of standards selection a particular challenge. For some applications [24,86], the lack of appropriate certified reference materials fulfilling all quality criteria may require the laboratory to develop their own local or laboratory standards and evaluate their homogeneity and stability properties. Laboratory standards are calibrated by using reference materials with a wide range of isotopic composition, ideally bracketing the expected range of isotopic values of the specimens.

Moreover, as further highlighted for normalisation (see Section 7), the use of at least two standards is preferred [84].

When choosing the co-injection technique, additional properties of the internal standard should be considered. Its chemical structure should be similar to that of the analytes [54], the range of concentration of its compounds should be representative of that of the specimen [87] and it should not interact with the analyte. Desired properties are reviewed by Caimi et al. [88]. Blank matrix should also be spiked with the internal standard to check for any matrix effects, whenever possible.

Overall, the use of standards taking account of the properties of the forensic specimens and bracketing their unknown isotope value is definitely a challenge. The lack of appropriate reference materials fulfilling the IT principle for each application is a reality and is especially true for GC/C/IRMS applications. Additional organic and inorganic reference materials are definitely needed to comply with the increasing number of forensic applications.

5.3. *In essence*

The standards utilisation and selection step is paramount as it has a fundamental influence on the accuracy and precision of data. The selection of standards is often a difficult task, due to the lack of appropriate certified reference material for forensic specimens. Two challenging selection criteria are the consideration of the properties, as well as the unknown δ value of the specimens. The quantities of the specimen can be as little that only one analysis might be possible. The choice and use of standards, and consequently the normalisation strategy have an undeniable effect on the final result. This has to be considered in order to obtain reproducible and comparable results between retrospective analyses on the same or on different equipment, as well as between laboratories.

6. Method implementation

The implementation of a new method requires a careful development and optimisation as well as a thorough validation of the analytical method in order to obtain high quality data necessary for a correct interpretation of results.

6.1. *Method development*

Method development is an essential step that should be systematic. It requires the selection of method specifications and instrumentation to be used based on preliminary knowledge or literature for each specific application [89]. Such specifications are for example the phase system, column diameter, column length, temperature program, type of combustion and reduction reactors, combustion and reduction temperatures, carrier gas flow, as well as parameters related to the analyte preparation procedure (SPME fibre stationary phase, extraction time, purge and desorption time, etc.) and the use of the standards (co-injection, parallel analysis, physical and chemical characteristics, isotopic values, etc.). The method development stage thus encompasses the fundamental steps previously discussed.

6.2. *Method optimisation*

Method optimisation should identify the different levels for various factors that could affect the results of analysis or induce an isotopic fractionation. It should subsequently determine the optimal level for each of these factors. In addition to their evaluation and optimisation for common validation parameters, all fractionation processes possibly occurring during the analysis must be appraised, investigated and controlled. The optimisation of these factors aims at obtaining the best performances [90,91].

This optimisation can be undertaken according to two approaches:

- the traditional univariate approach consists in varying one factor at a time by maintaining constant all other factors and to evaluate its influence on the result;
- the multivariate approach makes it possible to define through experimental design the experiments to be carried out after having defined the factors to optimise and the responses to observe. It is then possible to evaluate the combined influence of the factors on the responses.

Experimental design is a powerful tool to assist method optimisation and presents many advantages compared to the traditional univariate approach [91,92]. In particular, it takes into account interactions which can exist between the factors, whereas an optimisation of one factor at a time does not. Beside providing total knowledge, experimental design requires a smaller number of experiments to be realised.

As for other analytical methods, signal independence of response of the instrument must be checked for each compound of interest [51,76]. The amount of element to be measured should be comprised within the dynamic linear range of the instrument. Ideally, the sample and standard weights should produce a signal of similar amplitude as that of the reference gas pulses. While it is much easier to deal with this issue in EA/IRMS or CSIA of a single target compound, specimens analysed by GC/C/IRMS often display multiple compounds of varying concentrations. For the best precision, isotopic measurements of compounds of such specimens require more than a single analysis, with adjustment for peaks with a particular range of concentration. An alternative of such procedure is to run standards at different concentrations to be able to correct samples with varying concentrations [82]. Back flush of components in higher concentration appears also as an alternative to prevent the saturation of the ion source [87].

A major parameter to optimise is the chromatographic resolution of individual compounds in GC/C/IRMS or of simple gas elution in EA/IRMS. Insufficient peak resolution, resulting in peak overlap of adjacent compounds, may affect their δ value. In addition, each peak must be entirely integrated. Partial integration of co-eluting compounds is known to possibly lead to erroneous isotopic values [54,87].

Isobaric interferences, due to equal mass isotopes of different elements, can originate from different sources. In carbon isotope analysis, water trap inefficiency can create interfering $^{12}C^{16}O_2H^+$ with $^{13}C^{16}O_2^+$ detection at m/z 45 [54,57]. In nitrogen analysis, a chemical trap (Ascarite) is usually placed before or after the GC column of the EA to trap CO_2 produced by the combustion of the specimen, in order to exclude CO_2 from the source. CO^+ fragment ions associated with the ionisation of CO_2^+ in the ion source, can interfere at m/z 28 and 29 and influence the $^{15}N/^{14}N$ ratio [51]. Another possibility is to extend the acquisition time to allow for the complete elution of CO_2 peak, preventing thus from any CO_2 tailing onto the subsequent specimen measurement [93]. In $\delta^{18}O$ analysis, ion current intensities produced by the $[^{12}C^{18}O]^+$ and $[^{12}C^{16}O]^+$ ions are monitored at m/z 30 and 28 for the determination of $^{18}O/^{16}O$ ratio. When analysing nitrogen-containing material by EA-IRMS, the chromatographic baseline separation of N_2 and CO is compulsory in order to avoid isobaric effect from N_2. Besides, the reaction of N_2 with traces of oxygen produces NO in the ion source interfering with m/z 30 signal [94]. Accoe et al. reviewed a series of solutions published to avoid this effect and propose the use of He dilution of N_2 peak as an effective alternative [95].

The existence of method dependant isotopic fractionation should be evaluated by means of standards with known isotopic composition following the same analytical train than specimens, including the analyte preparation phase [54,87]. In addition to potential mass discrimination occurring during analyte preparation (see Section 4.2), isotopic fractionation may arise in the peripheral device (EA or GC/C) or in the IRMS. In GC/C/IRMS, injection parameters, especially split injection, can cause isotope effect because of analytes volatilisation or amount dependant fractionation within the injector [96]. This isotope effect should be carefully investigated for each target analyte at different concentrations. Each substance is differently affected by fractionation, more particularly in the injector, a matrix sensitive part of the GC. Other method dependant isotopic fractionation can occur due to non quantitative analyte conversion into simple gas in the combustion and reduction reactors [71,97,98], chromatographic isotopic effects, unsymmetrical peaks or varying flow conditions at the open split. Ionisation quenching has also been reported by Meier-Augenstein et al. as an interference in the bulk analysis of δ^2H in nitrogen rich compounds [99]. Furthermore, in order to obtain consistent data, sources of fractionation between specimens and standards must be alike. This highlights the need for careful fundamental testing and optimisation to produce precise and accurate data [98].

The run of blanks and the evaluation of contamination or carry over in the system are entirely part of method optimisation. Caps run without content (in EA-IRMS) or run of solvent blank (in GCCIRMS) should serve to evaluate any carry over in the system originating from analyte preparation or analysis.

Data processing by the instrument software may be occulted during method optimisation, as this process is automatic. Automatic data processing include corrections for H_3^+ in H_2 analyses, $^{13}C^{17}O$ in CO analyses and $^{12}C^{17}O^{16}O$ in CO_2 analyses [100], as well as peak integration defined by integration intervals, background corrections and isotope ratios calculations. Interested readers are referred to the detailed paper of Sessions for extended description of automatic data processing [57]. Although default parameters are often used as advised by the manufacturer, the user can modify some parameters according to its requirements. To our knowledge, the effect on the final isotopic results of various data processing parameters, such as the use of different background corrections, has not been evaluated and published yet. If significant differences were to be observed by such processes, the data processing parameters would therefore have to be taken into account in longitudinal and transversal comparisons.

6.3. Method validation

Except for anti-doping applications [79,101–105], method validation of forensic IRMS applications has hardly been reported. Benson et al. described the full method validation of bulk nitrogen analysis of ammonium nitrate [93]. Validation is necessary to demonstrate that the performances of the optimised method fulfil the application requirements. Validation is carried out by evaluating various performances of the method: confirmation of identity of the analyte, selectivity, specificity, limit of detection and quantification, working and linear range, trueness (bias), precision, accuracy, sensitivity, ruggedness (robustness), recovery and measurement uncertainties [76,106,107]. The performance parameters to validate are not straightforward: it may vary depending on its application and its use as a routine or non routine analysis method. Extensive validation is required for routine analytical methods, while in single case study or imperfect specimens (degraded, decomposed, etc.), the validation experiments may be reduced, although a minimum of experiments is necessary to ensure the quality of data [76]. An unavoidable aspect of method validation is the evaluation of the expanded uncertainty on the measurement. Benson et al. reported the calculated measurement uncertainty associated with the δ^{15}N method of ammonium nitrate analysis [93].

6.4. In essence

An optimised, robust and validated analytical method provides high quality results necessary for the interpretation step. The development and optimisation of the analytical method are essential to identify the different levels of various factors and to optimise them in order to obtain the best performances of the method. The possible causes of fractionation in the analytical method must be recognised and controlled. A thorough validation demonstrates that the method is fit for purpose and will produce reliable data. In forensic science, this step is paramount as non validated methods may be contested in court, but also unreliable data may lead to erroneous conclusions.

7. Data treatment

Data treatment is more often than not a hidden part of the process, having an indisputable impact on the final results. Encompassing any raw data manipulation undertaken by the user, this step preceding interpretation involves quality control processes, the strategy of acceptance or rejection of results (specimen re-analysis) and the normalisation of raw data to the international reference scale (also known as correction or translation).

7.1. Quality control and strategy of acceptance or rejection of results

As part of a quality assurance strategy, quality control involves all procedures undertaken to ensure the reliability of analytical results. Various types of internal quality controls serve to monitor different aspects of the process (blanks, certified reference materials, spiked samples, quality control samples, blind samples). As mentioned in Section 5.1, before any sequence or at the beginning of any day measurements, the monitoring of δ values of consecutive gas pulses or of standard material allows controlling the stability and the performance of the system. Within the run and throughout the sequence, δ values of gas pulses and standards should be monitored alongside whenever possible. Adopting a threshold for maximum admissible standard deviation of repeated measurements and maximum admissible difference between the measured and true values of a standard can hold as run acceptance criteria to start the sequence of analysis. A typical example of quality control strategy is provided by Marclay et al. In addition to the normalising substance, they used a minimum admissible precision of CO_2 gas pulses to consider their instrument suitable for analysis, jointly with a 95% confidence interval of δ^{13}C value of their internal standard, ε-caprolactone, as a run acceptance criterion [15,20].

A helpful tool of internal quality check is the use of control charts to keep a record of all data and monitor if the analytical process was under control when the specimens were analysed [108]. By monitoring the central location (often the mean) and the spread associated to its measurement, performance charts allow checking if the material yields the same response over time, from run to run, day to day or month to month. This observation of the long term behaviour of the system, essential to prevent the production of misleading results, allows the detection of bias, increasing or decreasing trend or periodical changes. Mean charts, the most common type of control charts, present data plotted graphically around a centre line corresponding to the known value (either the mean of several measurements or the known isotopic value of a certified reference material) with upper and lower warning and action limits defined by the user [109]. Often, warning limits and action lines are set at 2 s and 3 s (with s being the standard deviation from repeated measurements or the combined uncertainty of the validated method) covering a respective 95.5%

and 99.7% confidence interval of all values in mean charts. When one point is outside the action limits or two consecutive points are outside the warning limits, actions should be taken by the laboratory to get the process back under control. Additional rules may be applicable as exposed by Massart et al. [109]. Other valuable charts in quality control are range charts, that allow directly visualising and controlling the process spread, cumulative sum chart (CUSUM) to rapidly detect small biases or drifts and blank charts to monitor blank values.

An additional feature of a quality assurance strategy is the evaluation of the laboratory performances through proficiency tests as an external quality control or through the analysis of blind specimens in collaborative studies.

The strategy of acceptance and rejection of specimen's results should be consistent with a quality perspective. In that sense, logic and transparency underlie this step. The observation of uncommon or abnormal results generally raises the question of the validity of the analysis. It might be tempting to re-analyse a specimen, because sufficient material is available. However, all specimens should be treated equally. Specimens should be re-analysed only when quality controls demonstrate the occurrence of an analytical problem or when the circumstances justify a second analysis. The decision to re-analyse a specimen should be legitimate. Beside acceptance criteria provided by quality controls, abnormal results can be tested for outliers with statistical tests, using for example Grubb's test in case of normal distribution or with descriptive statistics by using boxplots for non-Gaussian distribution [90,91]. In no way should data be discarded on the basis of visual appreciation.

7.2. Normalisation of raw data to the international reference scale

Because each laboratory employs different certified or calibrated reference gas and standard materials, raw δ values need to be normalised to true δ values on the international reference scale. This "conversion" step is used to counter the effect of "scale compression" in IRMS instruments and ensures that data from different laboratories are comparable. Normalisation requires the analysis of standards of known isotopic composition covering a wide range of δ values in order to draw the normalisation line [57]. Normalisation of raw data to the international reference scale can be undertaken: (1) relative to a reference gas with known isotope ratio (this procedure is however not consistent with the IT principle), (2) relative to a single reference material, (3) relative to two or more reference materials. Definitely, the normalisation strategy influences the final δ value. The contribution of Paul et al. [84] offers an excellent review of the different normalisation strategies and their pitfalls. The two-point and multiple-point linear normalisation are preferred to single-point anchoring using the reference gas or a reference material. Indeed, large normalisation errors can occur if the true δ value of the reference gas or reference material is significantly different from that of the specimens. While scale compression is more manifest for H and O isotope analysis, the IAEA recently also recommended the calibration of laboratory standards using two anchoring points for $\delta^{13}C$ scale [110]. Skrzypek et al. investigated error propagation during the normalisation step, using different numbers of standards and different numbers of repetitions of these standards [111]. The normalisation error decreases with an increasing number of standards and repetitions. To reduce significantly the error due to normalisation, they propose to use two or more standards covering the whole stable isotope scale. Through a Monte Carlo simulation, they highlighted significant differences when different sets of standards were used for the normalisation of $\delta^{13}C$ raw data. Therefore, it seems that significant discrepancies may arise from the use of different couple of standards for normalisation between different sequences within one laboratory as well as between different laboratories. The authors recommend the use of the same set of standards and the same normalisation strategy for the analysis of a specific element within an application. Clearly, this point requires further investigations for the diverse elements and the implementation of a set of guidelines to harmonise the different practices.

7.3. In essence

This section has emphasized the tremendous influence of the data treatment step. As the strategy used influences the results, discarding any data should be a transparent process, whether it results from the use of a control chart, a statistical test or from other reflections. Even elementary data treatment should be transparent to allow for comparison and reproduction of results. The quality of the results clearly depends on the application of a consistent statistical approach to the data sets.

In addition, the normalisation strategy should be clearly mentioned, as this step has also an impact on the final results and can induce large differences. This implies that interlaboratory comparisons should be undertaken with similar or identical strategies.

8. Interpretation of the results

Interpretation is the essential final step to decipher the meaning of the results. However, the part left to the interpretation of isotopic results is often neglected. More than a compilation of results, interpretation of the results is the crucial final step of the whole methodological process, where the meaning and significance of each step has to be considered.

8.1. Intravariability and intervariability

In a source inference perspective, the evaluation of intravariability and intervariability is essential to extract the relevant information from the results and perform a robust and valuable interpretation [59]. The evaluation of variations between sources is of no significance and no validity without high quality data on the intravariability of an analyte. Unfortunately, such efforts are seldom undertaken. Schneiders et al., evaluating the isotopic variations within and between batches of a manufacturer of 1-phenyl-2-propanone (P2P), an amphetamine-type stimulant precursor, produced over 5 years [46] and Gentile et al., investigating the intravariability within and between batches of a black powder manufacturer [24], demonstrate that the isotopic homogeneity of a batch or a source is not necessarily implicit. They also illustrate the difficulty of evaluating intravariability for mass produced substances, whereas the quantities analysed by IRMS are infinitely small.

Benson et al. investigated the intravariability of the production line of three Australian ammonium nitrate manufacturers over a 12 month period [22]. While some manufacturers produced ammonium nitrate prills with a fairly stable isotopic profile, other showed wide variability throughout their production line. Due to overlap and wide intravariability, the comparison of ammonium nitrate prills from Australia and other countries allowed limited discrimination between sources, but could however be used for intelligence purposes. Sewenig et al. undertook a large scale study on the isotopic analysis of 132 specimens sampled from a 1.2 ton seizure of cocaine [16]. This allowed for the first time to observe the isotopic profile of cocaine bricks in a big seizure and to confront the isotopic profile of cocaine bricks to physical characteristics such as the imprinted logo. Despite the absence of link between the logo and the isotopic profile, this study exhibited the limited variations in $\delta^{13}C$ and the wide span of $\delta^{15}N$ values. Marclay et al. demonstrated the potential for isotopic tracing of gamma-butyrolactone (GBL) through the high repeatability of $\delta^{13}C$ measurements of GBL batches and the wide intervariability observed between 19 different sources [20]. A joint evaluation of both intravariability and intervariability is a necessary basis for a robust results interpretation.

8.2. Chemometric tools

In a source inference perspective, most questions relate to the evaluation of similarities or dissimilarities between groups of specimens, or between a specimen and a source. The relationships between the specimens, embedded in a complex and multi dimensional data set, can be revealed through chemometric analysis. Chemometric tools can assist the scientist in exploiting isotopic data by providing a rapid extraction of valuable information from the data set [90,91,112]. Furthermore, they provide extensive possibilities to visualise the information contained in the data set in a relevant way.

The selection of variables to answer the formulated questions can be performed either with boxplot representations or PCA of the data set for example. By plotting the repeated δ values of measured compounds, boxplots allow to rapidly and visually select compounds with a good reproducibility (within source) or a wide variability (between sources). Complementarily, the application of principal component analysis (PCA) allows to highlight variables responsible for most of the observed variance in the data set, as well as potential correlations between the variables.

On one hand, multivariate data analysis can help to uncover the structure and characteristics in a data set, such as the existence of class or groups. These issues are addressed by using unsupervised methods, which encompass PCA and cluster analysis (hierarchical clustering, k-means, etc.). On the other hand, multivariate analysis can also aid in the classification of a specimen based on specific properties. This category of analysis appeals to supervised methods or pattern recognition methods (neural networks, k-nearest neighbour, soft independent modelling by class analogy, etc.). These methods, based on a training set defined by the specific properties, develop a classification rule to predict the belonging of new samples to the existing groups. The following studies illustrate the application of such techniques to isotopic data. Combining different types of information, Palhol et al. applied PCA on 106 seized tablets of 3,4-methylenedioxymethamphetamine (MDMA). This exploratory analysis allowed them to highlight that, the MDMA content of the tablet and the $\delta^{15}N$ value were the most important parameters in discriminating the specimens. The subsequent use of hierarchical clustering grouped specimens according their similarity, allowing the establishment of links between seized tablets [13]. The application of hierarchical clustering on $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ data of MDMA confirmed the isotopic similarity of the synthesised samples, clustered according their synthetic route [42]. The k-means cluster technique was applied to $\delta^{13}C$ and $\delta^{15}N$ values of marijuana specimens in an attempt to trace the geographical provenance of specimens seized in Brazil [14]. Boyd et al. performed sample-wise PCA, followed by a multivariate analysis of variance (MANOVA) on the principal components variables to test for differences between fuel spill specimens and potential sources of spill. A hierarchical clustering was subsequently applied to display the relationships of the data set and infer the putative source of spill specimens [34].

Statistical evaluation of data ensures an objective analysis of the data set. However, the application of chemometric tools is valuable, only when the set of formulated questions has been clearly defined and the validity of the analytical method assessed. In the absence of such approach, a chemometric analysis may lead to irrelevant, incomprehensible and wrong data interpretation. Beside the frequentist approach, a Bayesian approach can also be used to evaluate isotopic variations [21].

Interpretation of the isotopic similarity or dissimilarity between two specimens often relies on the assumption that isotopic variations result only from intra and inter source variations. However, the isotopic profile reflects a complex history. Other sub-processes or factors that could impact the observed results are generally not regarded in the evaluation of isotopic differences. Thus, interpretation should also integrate the contribution of all factors involved in each step of the process. In a forensic framework, the quality of the specimen, combined to the complexity and interferences of the matrix are, for instance, other parameters that should be considered in this final stage.

8.3. Investigative and evidentiary value

The forensic casework reported by Meier-Augenstein et al. demonstrates the possibility of using the isotopic information in a intelligence perspective [39]. Unlike in anti-doping where isotope ratios are routinely employed for prosecutorial reasons, isotope ratios have predominantly been implemented in an investigative phase, i.e. to gather intelligence, up to now. At present, interpretation strongly suffers from a lack of reference data. Research efforts jointly oriented towards the production of crucial data, such as intravariability and intervariability studies, methods validation, metrology and harmonisation of analytical procedures would assist and support its routine use as intelligence and prosecutorial information.

Still, the evaluation of isotopic results from single or particular cases (analyte or matrix rarely encountered, limited specimen amounts, degraded or altered specimen, non-routine specimens) should be undertaken carefully. Their interpretation in a prosecutorial perspective also requires validation to ensure the quality of the results [76]. Although this is highly recommended, it is not respected. The evaluation of isotopic data should only be undertaken when sufficient information on intravariability, intervariability and the influence of other parameters, such as the quality of the specimen, are available. Without such an approach, isotopic data presented as evidence in court are “weak” and may be easily disputed. Finally, isotopic data only do not provide the solution of a casework. Isotopic information should not be interpreted alone, but in conjunction with other evidence.

8.4. In essence

Interpretation of results is the essential final step of the whole methodological process where the meaning and significance of each step has to be considered. The use of helpful chemometric tools can assist the scientist to extract the relevant information from the data set. Interpretation should not only rely on the isotopic variations between sources, but also consider intravariability of the different sources. Indeed, the study of intrinsic properties such as the isotopic profile requires continuous research to collect fundamental data on intravariability for a robust and valuable interpretation. Besides, the quality of the sample (weathered, degraded specimen or in minute amounts) and the complexity of the matrix should be thoroughly considered in this final stage, as they may limit the significance of the analytical results.

9. Conclusions

The whole process underlying the forensic application of IRMS has been dissected into its fundamental steps and their associated parameters. The lack of specific steps, such as the definition of the research problem, and hence the significance of the results, as well as interpretation, weakens the meaning and the relevance of the results. Such lacks characterise forensic studies. The consideration of the entirety of the process will assist one to produce comprehensive, reliable and relevant data, and as such, will contribute to forensic literature with isotopic studies of quality.

In order to improve isotopic data exploitation and interpretation in forensic IRMS applications, the authors believe that efforts should be made towards:

- the collection of additional information on aspects characterising the source of a substance;
- the evaluation of the influence of sampling, storage and pre-analytical preparation procedures on fractionation;
- the collection of data on intervariability, simultaneously with fundamental data on intravariability;
- a transparent method validation and data treatment, including normalisation;
- interpretation of isotopic data in light of the level of hypothesis considered.

Finally, the development of guidelines to harmonise analytical strategies and procedures, especially in terms of standards utilisation, normalisation and quality control, is necessary for a coherent procedure. The adoption of unified approaches will definitely improve the quality and comparability of data on laboratory and international scales and, complementarily, will assist its gradual acceptance as prosecutorial information.

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ORIGINAL PAPER

δ^{15}N measurement of organic and inorganic substances by EA-IRMS: a speciation-dependent procedure

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Abstract Little attention has been paid so far to the influence of the chemical nature of the substance when measuring δ^{15}N by elemental analysis (EA)–isotope ratio mass spectrometry (IRMS). Although the bulk nitrogen isotope analysis of organic material is not to be questioned, literature from different disciplines using IRMS provides hints that the quantitative conversion of nitrate into nitrogen presents difficulties. We observed abnormal series of δ^{15}N values of laboratory standards and nitrates. These unexpected results were shown to be related to the tailing of the nitrogen peak of nitrate-containing compounds. A series of experiments were set up to investigate the cause of this phenomenon, using ammonium nitrate (NH_4NO_3) and potassium nitrate (KNO_3) samples, two organic laboratory standards as well as the international secondary reference materials IAEA-N1, IAEA-N2—two ammonium sulphates [$(NH_4)_2SO_4$]—and IAEA-NO-3, a potassium nitrate. In experiment 1, we used graphite and vanadium pentoxide (V_2O_5) as additives to observe if they could enhance the decomposition (combustion) of nitrates. In experiment 2, we tested another elemental analyser configuration including an additional section of reduced copper in order to see whether or not the tailing could originate from an incomplete reduction process. Finally, we modified several parameters of the method and observed their influence on the peak shape, δ^{15}N value and nitrogen content in weight percent of nitrogen of the target substances. We found the best results using mere thermal decomposition in helium, under exclusion of any oxygen. We show that the analytical procedure used for organic samples should not be used for nitrates because of their different chemical nature. We present the best performance given one set of sample introduction parameters for the analysis of nitrates, as well as for the ammonium sulphate IAEA-N1 and IAEA-N2 reference materials. We discuss these results considering the thermochemistry of the substances and the analytical technique itself. The results emphasise the difference in chemical nature of inorganic and organic samples, which necessarily involves distinct thermochemistry when analysed by EA-IRMS. Therefore, they should not be processed using the same analytical procedure. This clearly impacts on the way international secondary reference materials should be used for the calibration of organic laboratory standards.

Keywords Nitrate · Nitrogen · Isotope ratio mass spectrometry · Ammonium · Elemental analysis–isotope ratio mass spectrometry · Inorganic

Introduction

Little attention has been paid so far to the influence of the chemical nature of the substance analysed when measuring δ^{15}N by elemental analysis (EA)–isotope ratio mass spectrometry (IRMS). Although the bulk nitrogen isotope analysis of organic material appears straightforward, literature from different disciplines using IRMS provides hints that the quantitative conversion of nitrate-containing compounds to nitrogen presents difficulties. In forensic science, Aranda et al. [1] needed to add activated charcoal to their nitrate

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samples and standards to support combustion by trapping evolving O_2. Benson et al. [2, 3] noticed significantly more depleted values for the first ammonium nitrate (NH_4NO_3) standard run in a sequence. Results from interlaboratory trials also showed that the analysis of nitrates led to unusually large variations [4–6]. In addition, such features have also been observed in the field of identification of sources of nitrate in water and soils. Several studies reported that the analysis of such analytes may produce scattered results. Silva et al. [7], followed by Spoelstra et al. [8], overcame inconsistent $\delta^{15}N$ values by adding 2 mg of sucrose to nitrate salt samples on the advice of Micromass Elemental. This sugar addition method was initially described by Noguchi [9] as an improvement of the micro Dumas method for nitrogen quantitative analysis of nitro and oxidation-resistant stable species. Noguchi observed that glucose had a beneficial effect on sample combustion. Yet, Borda and Hayward [10] reported scattered nitrogen content (%N) values of nitrate esters, despite the addition of glucose to their samples. Schindler and Knighton [11] also reported the incomplete recovery of nitrogen from nitrate when KNO_3 was analysed with the Dumas method. Although these earlier applications were related to the determination of nitrogen content using the micro Dumas method in its original version, they support the fact that the conversion of solid nitrates into pure N_2 may produce inconsistent results owing to an incomplete decomposition or reduction process. This may therefore affect $\delta^{15}N$ values when EA is coupled with IRMS. Numerous discussions among the community of isotope specialists have highlighted difficulties related to the isotope analysis of nitrates [12].

The present study originated from the observation of abnormal $\delta^{15}N$ values and nitrogen content of laboratory standards and nitrate-containing samples. This difference in behaviour indicated that the conversion of nitrates into N_2 was not complete and seemed to be related to N_2 peak tailing. These first observations initiated a series of experiments set up to determine the origin of this phenomenon.

In the first part, we report the abnormal behaviour of laboratory standards following the analysis of nitrates using the EA configuration usually employed in our laboratory. In the second part, a series of experiments are reported which involved the analysis of pure NH_4NO_3 and potassium nitrate (KNO_3) samples, two organic laboratory standards and the secondary reference materials IAEA-N1, IAEA-N2 and IAEA-NO-3 (see Table 1). Experiment 1 evaluated the addition of graphite and vanadium pentoxide (V_2O_5) in different proportions to nitrates as a possible solution to eliminate peak tailing [1, 12]. In experiment 2, another EA configuration involving an additional section of copper was used in order to see whether the tailing may originate from an incomplete reduction process. The results were compared with those obtained using the EA configuration routinely employed. Finally, in experiment 3, several method parameters (helium flow, sample delay, oxygen pressure and oxygen exclusion) were modified and their influence on the peak shape, $\delta^{15}N$ value and nitrogen content of the different substances was observed.

We demonstrate the good analytical performance of EA-IRMS when nitrates and the international secondary reference materials are analysed without O_2. The results are discussed considering the chemical nature of the substances and the thermodynamic conditions imposed by the analytical technique. We conclude that these inorganic substances should not be processed using the same analytical procedure as for organic material. This necessarily influences the way international reference material should be used for the calibration of organic laboratory standards.

Materials and method

Chemicals

Nitrogen (purity greater than 99.999 %), helium (purity greater than 99.9999 %) and oxygen gas (purity greater than

Table 1 Characteristics of the international reference materials, laboratory standards and nitrates used in experiments 1–3

Substance	Origin	Use as	Composition	Theoretical N content (wt%)	Known $\delta^{15}N$ value ± SD (‰ vs. air)
IAEA-N1	International Atomic Energy Agency	International reference material	Inorganic $(NH_4)_2SO_4$	21.2	0.4±0.2 [6]
IAEA-N2	International Atomic Energy Agency	International reference material	Inorganic $(NH_4)_2SO_4$	21.2	20.3±0.2 [6]
IAEA-NO-3	International Atomic Energy Agency	International reference material	Inorganic KNO_3	13.9	4.7±0.4 [6]
Standard 1	In-house	Laboratory standard	Organic $C_xH_yN_z$	2.1	0.4±0.2
Standard2	In-house	Laboratory standard	Organic $C_xH_yN_z$	2.4	1.0±0.2
Ammonium nitrate	Fluka	Sample	Inorganic NH_4NO_3	35.0	-
Potassium nitrate	Fluka	Sample	Inorganic KNO_3	13.9	-

SD standard deviation

99.995 %) were supplied by Messer (Lenzburg, Switzerland). Chromium(III) oxide (Cr_2O_3), silvered cobaltous–cobaltic oxide ($CoO–Co_2O_3$), 15-mm-diameter quartz wool discs, reduced copper wire (0.5 mm), quartz chips, quartz glass tubes (18.5 mm×450 mm) and tin capsules for solids (3.3 mm× 5 mm) were purchased from Säntis Analytical (Teufen, Switzerland). A prepacked CHNS reactor (no. 99.0733.10) was purchased from Lüdi (Flawil, Switzerland). Magnesium perchlorate (purity 98 % or greater), KNO_3 (purity greater than 99.0 %) and NH_4NO_3 (purity 99 % or greater) were obtained from Fluka (Buchs, Switzerland). NH_4NO_3 fertiliser was supplied by Landor (Birsfelden, Switzerland). Graphite powder (1–2 μm) and vanadium(V) pentoxide (purity 99.99 %) were supplied by Sigma-Aldrich Chemie (Buchs, Switzerland). IAEA-N1 [$(NH_4)_2SO_4$], IAEA-N2 [$(NH_4)_2SO_4$] and IAEA-NO-3 (KNO_3) were obtained from the International Atomic Energy Agency (Vienna).

Standards: selection and analysis

In the first part of the study, KNO_3 and NH_4NO_3 samples were measured between laboratory standards. Laboratory standards were plant-based materials routinely employed in the analytical sequences of bulk analyses conducted by the laboratory. Although they have a chemical composition different from that of the target samples (nitrates), they have well-known δ^{15}N values, intermediate precision and long-term stability. Standard 1 (Std1) originated from the mixture of plant materials. Standard 2 (Std2) was made from catalpa leaves. The standards were scheduled at the beginning and the end of each sequence (maximum 49 positions), as well as throughout the sequence. Blanks were inserted at different positions in the sequence to monitor any carryover effects. The laboratory standards, calibrated against international reference materials, have shown an overall intermediate precision in everyday use over several years of less than 0.2 ‰ for nitrogen on both IRMS apparatus. The calculated expanded uncertainty associated with δ^{15}N measurement of plant material is 0.2 ‰ (k=2).

In the second part, NH_4NO_3 and KNO_3 samples were analysed along with international secondary reference materials (IAEA-N1, IAEA-N2, IAEA-NO-3) inserted throughout the sample sequence. Samples run with a minimum of three replicates were bracketed at the beginning and at the end of the sequences with triplicates of international reference materials, in addition to replicates inserted throughout the sequence after a maximum of 12 samples. An empty tin capsule was run as a blank at the beginning and the end of the sequence to check for any carryover effects. Laboratory standards Std1 and Std2 were also run in the sequence to check the consistency, precision and accuracy of δ^{15}N values. Besides, the sequence design was modified to investigate particular effects. The number of replicates of samples and international reference materials was adapted from three up to ten successive replicates in order to observe the evolution of the m/z 29/28 ion current ratio baseline and the δ^{15}N value. By convention, the isotope value, denoted as the δ value, expresses the deviation of the isotope ratio of the sample in per mil (‰) compared with the international standard (atmospheric N_2 for nitrogen) [13]: $\delta=[(R_s-R_{ref})/R_{ref}]$, where R_s and R_{ref} are the isotope ratios of the sample and the reference, respectively. Table 1 summarises the characteristics of the substances used in the second part of this study, with their expected δ^{15}N value and standard deviation (SD).

Sample preparation

Samples and standards were weighed in 3.3 mm×5 mm tin capsules for solids. Weighed masses were adjusted to produce a peak amplitude similar to that of the reference gas (about 2,000 mV for m/z 28) and contained the equivalent of approximately 100 μg nitrogen. Typically, about 300 μg was used for IAEA-N1 and IAEA-N2 and 720 μg for IAEA-NO-3. For laboratory standards, about 3,200 μg and 3,500 μg bulk organic material was used for Std1 and Std2, respectively. For samples, about 300 μg NH_4NO_3 and 720 μg KNO_3 were used. Reference materials, laboratory standards and samples were stored in glass desiccators. Laboratory standards and samples were enclosed in sealed glass containers, whereas international reference materials were kept in their original container.

Additives to improve combustion

In experiment 1, samples were mixed with graphite or V_2O_5 in different proportions to assist their decomposition/combustion. The preliminary EA of graphite and V_2O_5 did not show any trace of nitrogen. Weighed masses of mixtures with graphite and V_2O_5 were likewise adjusted to contain the equivalent of approximately 100 μg nitrogen. Graphite and V_2O_5 were also added to reference materials (about 20 wt%) to observe any effect on δ^{15}N values.

Instrumentation and method

A Micro MC5 analytical microbalance (Sartorius, Göttingen, Germany) was used to weigh samples and standards for isotope analysis.

A NewClassic MF ML204 analytical balance (Mettler Toledo, Greifensee, Switzerland) was used to weigh the different quantities of chemicals necessary to produce the mixtures with graphite and V_2O_5. Analyses were performed with a 1110 elemental analyser (Carlo Erba, Milan, Italy) coupled with a Conflo II interface to a Delta S isotope ratio mass spectrometer (both from Finnigan MAT, Bremen, Germany). Owing to instrument availability, some of the analyses were also performed using the same elemental analyser coupled to a DeltaPlus XP isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). The interface parameters were 0.8-bar helium and 0.8-bar reference gas. The pressure of helium and O_2 at the regulator of the elemental analyser was 150 kPa and 50 kPa, respectively. A Zero Blank autosampler (Costech Analytical Technologies, Valencia, CA, USA) was used in order to avoid possible interferences with atmospheric N_2 and moisture. Before the measurements were started, the autosampler was evacuated for 30 min and subsequently refilled with helium. The sample was dropped from the autosampler into a combustion reactor, consisting of a heated quartz tube filled with Cr_2O_3 and silvered CoO–Co_2O_3, held at 1,020 °C. By means of flash combustion, also known as Dumas combustion, the sample is processed in the presence of excess oxygen. The analytical cycle, lasting 70 s, was as follows. Oxygen was injected into the combustion reactor for 60 s from the beginning of the analytical cycle. The sample was dropped 18 s after the beginning of the cycle (analytical cycle set-up: cycle lasting 70 s, oxygen injection for 60s, sample start and stop at 18 and 20 s, respectively). The gaseous products are carried in a helium flow (80 ml/min) to a reduction reactor packed with reduced copper wire, held at 650 °C. This reduction step converts nitrogen oxides into N_2 and traps the excessive oxygen. Water is captured by a magnesium perchlorate trap. The resulting gases are separated by a PoropaQ gas chromatography column (4 m, 50 °C) before entering the ion source via the Conflo II interface. The molecules are then ionised and accelerated at approximately 3,040 V. A magnetic field deflects the ions and separates the molecules according to their mass-to-charge ratio (m/z) before they reach an array of Faraday cups positioned such that ions with m/z 28, 29 and 30 are collected separately. The time for the procedure was extended to 600 s to ensure the total elution of CO for samples containing carbon. Raw data were processed with Isodat 2.0 (Thermo Fisher, Bremen, Germany).

Elemental analyser configurations

In the first part of the study, a combustion reactor, held at 1,020 °C, consisting of a quartz tube filled with Cr_2O_3 and silvered CoO–Co_2O_3 (combustion reactor 1 in Fig. 1) was used with a reduction reactor filled with reduced copper wire. In the second part, another elemental analyser configuration using a different combustion reactor was used in experiment 2. It consisted of a pre-packed tube for CHNS analyses, maintained at 1,020 °C, containing tungsten(VI) oxide (WO_3) on alumina (Al_2O_3) and pure electrolytic copper wire (combustion reactor 2 in Fig. 1). A reduction quartz reactor, held at 650 °C and packed with reduced copper, was combined with each combustion system, forming elemental analyser configuration systems 1 and 2 (referred to further as systems 1 and 2 for the sake of simplicity) (Fig. 1).

Variations of the method parameters

Low (60 ml/min) and high (95 ml/min) helium flows were tested. The O_2 pressure at the regulator of the elemental analyser was modified to 100 kPa and 150 kPa. The effect of suppressing the O_2 injection was also investigated. Sample

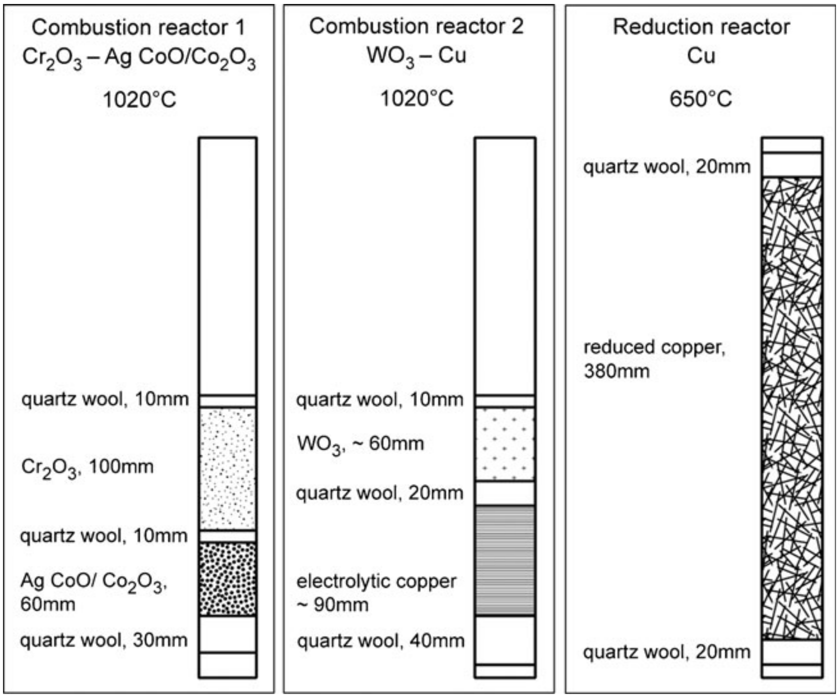


Fig. 1 Composition of the combustion and reduction reactors

delay (sample start and stop) was modified to introduce the sample at 0, 5, 10, 55 and 62 s within the analytical cycle.

Data correction (normalisation)

In the first part of the study, laboratory standards were used to correct raw data to the international scale, whereas in the second part, international secondary reference materials were used, following a two-point linear normalisation whenever possible [14]. IAEA-N2 was occasionally used alone to correct data when the peak shape, δ^{15}N value and nitrogen content of the other reference materials indicated the results were inadequate for correction. The nitrogen content (in weight per cent) was calculated on the basis of integrated peak areas of the major isotopes and determined against the theoretical nitrogen content of IAEA-N2 (21.2 wt%).

Statistical tests, quality control and uncertainty measurement

The Grubbs test was used within each sequence to determine and exclude outliers in the raw data set. Control charts were used to check the quality of the analytical response. Upper and lower warning lines were set at $\mu \pm 2$SD and upper and lower action lines were set at $\mu \pm 3$SD [15]. Differences between means were evaluated using analysis of variance [16]. The expanded uncertainty U was calculated according to the literature and using type A evaluation [17]. The expanded uncertainty was estimated at a coverage factor of 2 (k=2).

Results and discussion

Part I: unexpected behaviour of laboratory standards

Both laboratory standards Std1 and Std2 showed an intermediate precision smaller than 0.2 ‰ over a 24-month period, as illustrated by the control chart of Std1 in Fig. 2. After exclusion of the enriched δ^{15}N values corresponding to the phenomenon described in the next paragraph, the calculated intermediate precision of Std1 is 0.1 ‰ (n=217).

The unusual behaviour of Std1 (illustrated by the outlying values between measurements 127 to 222 in Fig. 2) was also observed for Std2.

Figure 3 shows the abnormal behaviour of laboratory standards, run alternatively with blanks and NH_4NO_3 samples in an analytical sequence. The δ^{15}N value of the first replicate of the laboratory standard in the sequence (in positions 7, 12, 25 and 40) following a nitrate sample was always more enriched than the other replicates and increased along the sequence (Std2 in Fig. 3a). δ^{15}N values of other replicates were not affected by this phenomenon. As the same effect was noticed for Std1 and Std2 analysed after NH_4NO_3 and KNO_3 samples, this phenomenon appears to be related to the type of substance analysed. The intensity of the phenomenon did not decrease when analysing samples containing less nitrogen [NH_4NO_3 fertiliser (27 %N) or KNO_3 (13.9 %N)] than pure NH_4NO_3 samples (35 %N). Conversely, whereas the first δ^{15}N value of a series of NH_4NO_3 replicates was more depleted than the following values, the δ^{15}N value of NH_4NO_3 samples tended to stabilise after a few samples. However, differences of up to 2 ‰ between nitrate replicates could be observed. One NH_4NO_3 measurement stands apart from the other replicates, with a δ^{15}N value of 4.1 ‰.

In addition, the variations of the δ^{15}N values of laboratory standards (Fig. 3a) seemed to be correlated with the deviations of their nitrogen contents (Fig. 3b). Whereas NH_4NO_3 samples and fertiliser exhibited a negative difference in the nitrogen content of around 2–3 % below the expected value, the first laboratory standard analysed immediately after a nitrate-containing compound displayed a slight positive deviation (Fig. 3b). A zoom-in on the y scale shows more clearly this subtle positive deviation and even reveals a slight deviation in the nitrogen content of the second or third replicate scheduled later in the time sequence (Fig. 3c).

Curiously, blanks did not allow the detection of this phenomenon. Indeed, blanks run in positions 4, 6, 10, 14 and 24 of the sequence (indicated by black arrows in Fig. 3a) did not produce nitrogen peaks. This supports the hypothesis that some nitrogen is retained in the elemental analyser and influences the δ^{15}N value of other measured

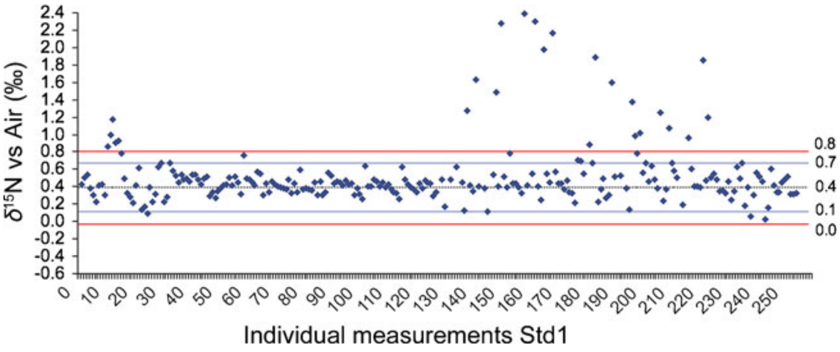


Fig. 2 Control chart of the δ^{15}N value of standard 1 (*Std1*) (N=253). The enriched δ^{15}N values (between measurements 127 and 222) correspond to the phenomenon observed and illustrated in Fig. 3. The intermediate precision calculated after exclusion of values lying outside the action limits is 0.1 ‰ (n=217)

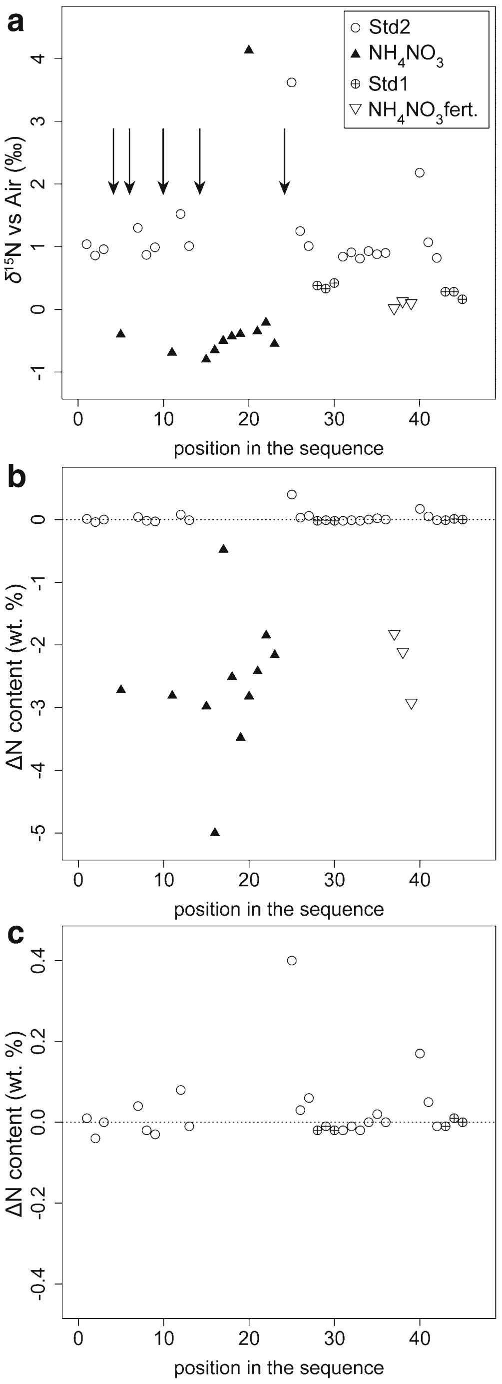


Fig. 3 **a** δ^{15}N value of NH_4NO_3 samples run alternatively in a sequence with laboratory standards Std1 and Std2, as well as blanks (their position is highlighted by *black arrows*). Note the odd value of the first replicate of a series of standards, as well as the increasing δ^{15}N values of NH_4NO_3 samples. **b** Deviation of the nitrogen content (wt%) of the same NH_4NO_3 samples and standards from their known value. NH_4NO_3 samples and fertiliser display a difference of 2–3 %N from their expected value. **c** Zoom-in of the difference in the nitrogen content (%N) in the same sequence. Laboratory standards with an odd δ^{15}N value also displayed a slight offset in their difference in nitrogen content (%N) from the expected value

samples (memory effect). Finally, the repeatability and intensity of the phenomenon were not constant from one period of time to another.

Since there is a correlation between the type of material analysed just before the standard and its δ^{15}N value and nitrogen content, it appears that the IRMS system is not the cause of these odd measurements. We rather believe that this phenomenon is caused by nitrogen remaining in the EA system, either in the combustion reactor or in the reduction reactor, resulting from non-quantitative conversion of nitrate into N_2, which is then carried over to the next sample and released in subsequent analyses.

Peak shape A close examination of the chromatograms showed that the analysis of the laboratory standards produced a peak corresponding to well-eluted N_2. Even data displaying a disparate δ^{15}N value did not present any particularities. In contrast, the N_2 peak of KNO_3 and NH_4NO_3 exhibited tail (Fig. 4). At the same time, the *m*/*z* 29/28 ion current ratio was sluggish in its return to the baseline. The peak tailing indicates that either combustion or reduction (or both) is not occurring optimally and lingers. As peak tailing was observed for nitrates, but not for the organic laboratory standards, we assumed that this phenomenon specifically pertained to the conversion of nitrate into N_2.

Owing to tailing, all N_2 was not completely integrated as illustrated by the *m*/*z* 29/28 ratio in the upper part of Fig. 4. This explains the lower conversion of nitrates in terms of nitrogen content. Moreover, tailing led to variations in peak integration, which affected the repeatability of the δ^{15}N value.

Cleaning of the combustion tube or regeneration of copper in the reduction oven as well as replacement of the chemicals in both reactors neither eliminate the problem nor did it help to determine which reactor was the source of this behaviour. We performed a series of experiments in order to determine the cause of this phenomenon.

Part II: Investigations

Experiment 1: use of additives to improve combustion

The use of graphite and V_2O_5 as additives was investigated to assess if they could assist in the combustion. At first sight, the addition of graphite to nitrate-based samples improved the N_2 peak shape. Tailing was not observed any more and the precision of the δ^{15}N value was improved when graphite was added at 20 wt%. At higher mass of added graphite, the δ^{15}N value showed a larger variability. Yet, the δ^{15}N value of nitrate-containing compounds was clearly affected (Fig. 5, plot a). This was confirmed by the biased δ^{15}N value of

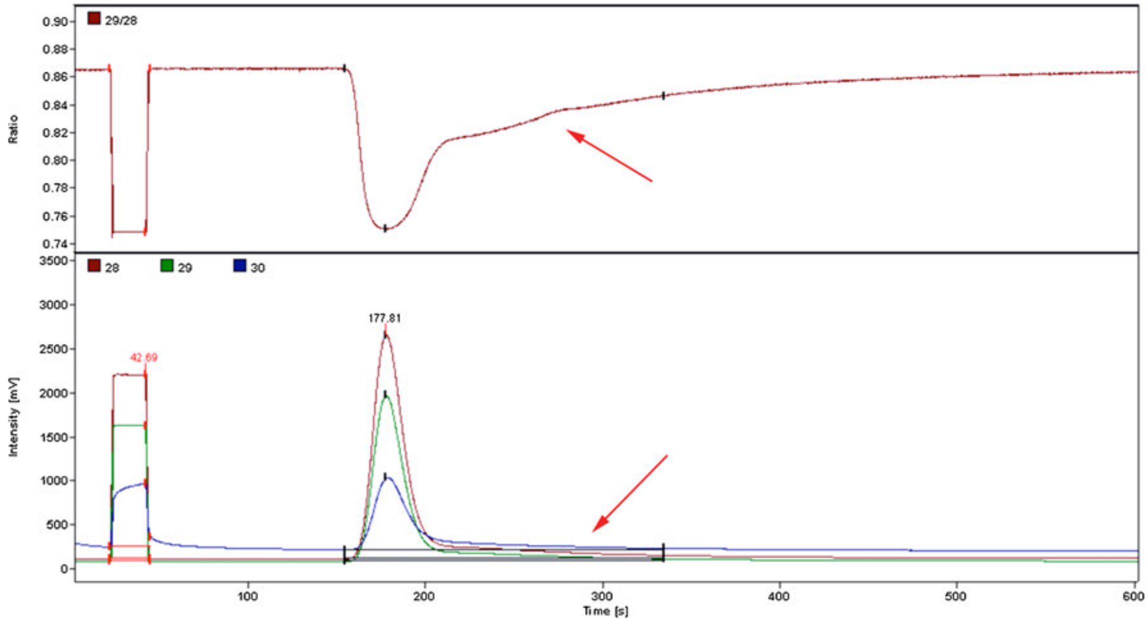


Fig. 4 δ^{15}N measurement of a KNO_3 sample. The N_2 peak of the sample exhbits tailing (*lower part*). This phenomenon is also visible from the *m*/*z* 29/28 ion current ratio (*upper part*), as highlighted by the *arrows*

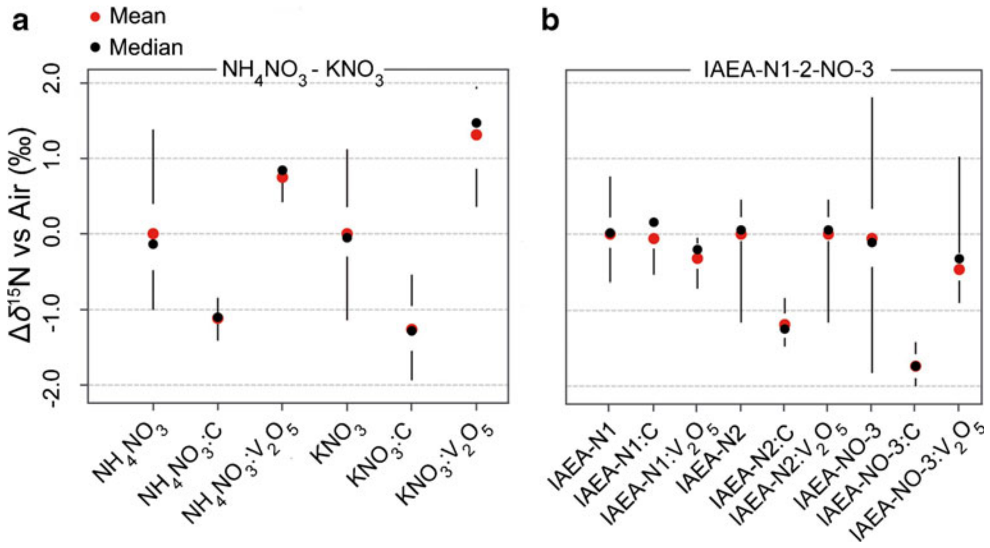
IAEA-N1, IAEA-N2 and IAEA-NO-3 mixed with the additives (Fig. 5, plot b). The literature reveals that carbon residues resulting from incomplete combustion may retain nitrogen [18]. An alternative explanation is that incomplete combustion of carbon produces CO, which may be coeluted with N_2 and alter its accuracy because the molecules CO and N_2 have the same *m*/*z* (28 for $^{12}C^{16}O$ and $^{14}N^{14}N$ and 29 for $^{13}C^{16}O$ and $^{15}N^{14}N$). A closer look at the chromatograms revealed that the ion current ratio 29/28 did not completely return to the baseline before the CO. This incomplete return to the baseline of the ratio 29/28 is probably the reason for the difference between the measured and the expected δ^{15}N value.

The addition of V_2O_5 did not eliminate peak tailing and gave inaccurate results. In proportions greater than 20 wt% it led to tailing and affected peak resolution.

Experiment 2: use of an elemental analyser configuration with an additional copper section

Another elemental analyser configuration including an additional section of reduced copper (system 2) was used in order to see whether an incomplete reduction process was the cause of the observed tailing. Results were compared with those obtained with the usual elemental analyser configuration (system 1).

Fig. 5 The positions of the mean and median indicate the deviation the δ^{15}N value of the substance from its expected value. **a** Deviation of the δ^{15}N value of NH_4NO_3 and KNO_3 mixed with 20 wt% graphite and V_2O_5 from its original value (unmixed). **b** Deviation of the δ^{15}N values of international reference materials mixed with 20 wt% graphite and V_2O_5 from their known values



Peak shape The first replicates of IAEA-N1 and IAEA-N2 in the sequence showed a peak corresponding to well-eluted N_2 in systems 1 and 2. However, after a few replicates, the *m*/*z* 29/28 ratio of IAEA-N1 and IAEA-N2 either did not always perfectly return to the baseline or showed a baseline anomaly despite a peak corresponding to well-eluted N_2. These effects were observed in systems 1 and 2 (Figs. S1–S5).

Whereas the N_2 peak of IAEA-NO-3 exhibited tailing in system 1, N_2 appeared well eluted in system 2. The absence of tailing in system 2 was also observed for KNO_3 and NH_4NO_3 samples. In system 2, the upper layer of WO_3 is heated to 1,020 °C, whereas the lower electrolytic copper layer reaches a temperature of 850 °C. Nitrogen oxides are therefore reduced by two sections of metallic copper, one at 850 °C and another at 650 °C. These results support the hypothesis that tailing in system 1 is induced by incomplete reduction. This is also corroborated by the increased tailing when a compound rich in oxygen, such as V_2O_5, is added to a sample. However, as for IAEA-N1 and IAEA-N2, nitrates also exhibited a sluggish return to the baseline in terms of the *m*/*z* 29/28 ratio after the analysis of several replicates. In contrast, the organic laboratory standards showed a peak corresponding to well-eluted N_2 in both systems.

δ^{15}N values and nitrogen content The mean δ^{15}N values of IAEA-N1, IAEA-N2 and IAEA-NO-3 determined using both systems did not show any bias compared with the expected δ^{15}N value. Systems 1 and 2 produced a similar SD (0.3 ‰ for IAEA-N1 and IAEA-N2, 0.6 ‰ for IAEA-NO-3); however, it was larger than that reported in the literature (0.2 ‰ for IAEA-N1 and IAEA-N2 and 0.4 ‰ for IAEA-NO-3) [6]. Despite the absence of tailing in system 2, the repeatability of the δ^{15}N value was not improved.

Experiment 3: variations of the method parameters and exclusion of O_2

The modification of the O_2 pressure and helium flow did not improve the analytical results for nitrates. The sample delay (sample start and stop) was adapted in order to introduce the sample at different points in the analytical cycle (sample start at 0, 5, 10, 18, 55 and 62 s), whereas oxygen was injected for 60 s from the beginning of the analytical cycle. Finally, the exclusion of oxygen from the analytical cycle was also tested.

For IAEA-N1 and IAEA-N2, the best results were obtained without injection of O_2 or when the O_2 concentration was at its lowest level (sample introduced at 0 or 5 s) (Table 2, Fig. 6). Under these conditions, N_2 was well eluted and the *m*/*z* 29/28 ratio did not show any anomaly throughout repeated analyses. The intermediate repeatability of the δ^{15}N value for these compounds is excellent (0.1 ‰) and corresponds to the estimated expanded uncertainty (less than 0.1 ‰, $k=2$).

For IAEA-NO-3 and nitrate-containing samples, the exclusion of O_2 from the analytical cycle gave, on the contrary, a peak corresponding to well-eluted N_2 with an *m*/*z* 29/28 ratio returning perfectly to the baseline, even after ten replicates (Fig. 7), whereas all conditions involving O_2 injection did not produce satisfactory results. These analytical conditions produced excellent repeatability of the δ^{15}N value of 0.1 ‰, after exclusion of outliers when present. The estimated expanded uncertainty ($k=2$) associated with the δ^{15}N measurement of nitrates (IAEA-NO-3, KNO_3 and NH_4NO_3 samples) is 0.1 ‰.

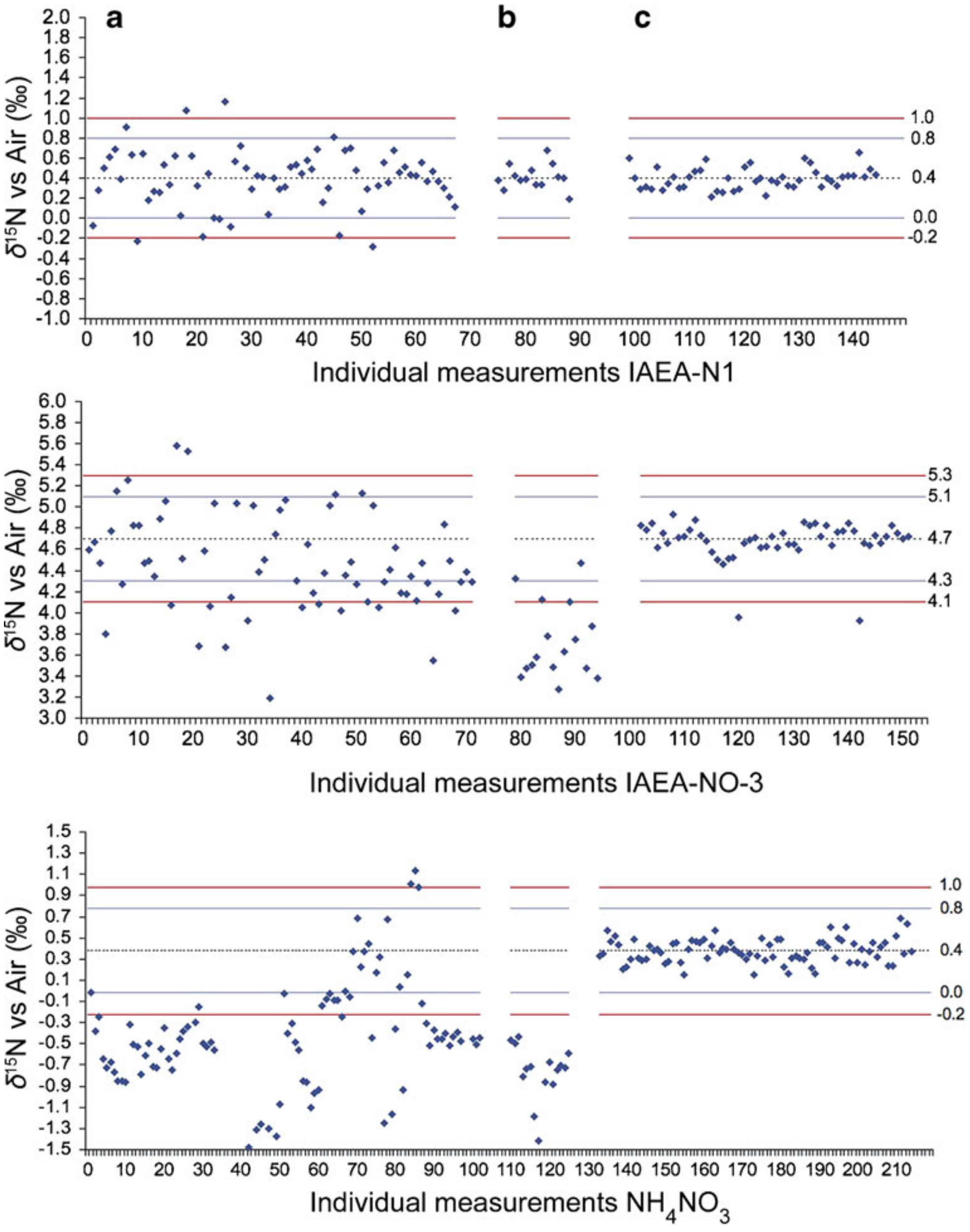
Only the organic laboratory standards showed inconsistent results when analysed without oxygen, as expected. The incomplete combustion of organic material produces CO, which may interfere with N_2 if it is not eliminated by an adequate trap ($^{12}C^{16}O$ with $m/z=28$ and $^{13}C^{16}O$ with $m/z=29$). The best results were obtained using the usual settings of the analytical cycle (oxygen for 60 s, sample start/stop at 18 s/ 20s). These conditions favour the introduction of the organic sample in an oxygen-rich environment and ensure its complete combustion.

The δ^{15}N results are supported by the nitrogen content of the inorganic substances (Fig. 8). The values for the nitrogen content of IAEA-N1 and IAEA-N2 improved when the reference material was introduced at 0 or 5 s from the beginning of the analytical cycle (with O_2 injection). The results significantly improved when the substances were analysed without O_2, with a repeatability of 0.1–0.2 %. Whereas the analysis of nitrates (IAEA-NO-3, KNO_3 and NH_4NO_3) with O_2 always produced a lower nitrogen

Table 2 δ^{15}N values and standard deviation (*SD*) of international reference materials, analysed with system 1 with and without O_2 injection. All measurements (*N*) are included in the calculated means and standard deviations

International reference material	Mean δ^{15}N vs. air (‰) ± SD				
	With O_2	*N*	Without O_2	*N*	Known δ^{15}N values
IAEA-N1	0.4±0.3	67	0.4±0.1	46	0.4±0.2 [6]
IAEA-N2	20.3±0.3	53	20.3±0.1	50	20.3±0.2 [6]
IAEA-NO-3	4.5±0.6	71	4.7±0.2	50	4.7±0.4 [6]

Fig. 6 Control charts of the δ^{15}N measurements of IAEA-N1 [$(NH_4)_2SO_4$], IAEA-NO-3 (KNO_3) and NH_4NO_3 samples analysed with system 1. **a** with oxygen injection (analytical cycle 70 s, oxygen for 60 s., sample start/stop at 18 s/20 s), **b** with oxygen injection (analytical cycle 70 s, oxygen for 60 s, sample start/stop at 0 s/2 s and 5 s/7 s), **c** without oxygen injection (analytical cycle 70 s, sample start/stop at 18 s/20 s). The expected δ^{15}N value is represented by a *central dotted black line*. For NH_4NO_3, the *dotted line* represents the mean value of measurements performed without oxygen. Upper and lower warning lines (*blue*) and action lines (*red*) are defined as two standard deviations and three standard deviations, respectively



content than expected, their analysis without O_2 yielded the expected nitrogen content with satisfactory repeatability (0.3–0.6 % for nitrates after exclusion of outliers).

Experiment 2 showed that the tailing of the N_2 peak of nitrates resulted from incomplete reduction. In 1973, Pella and Colombo [18] stated that the combustion of nitro compounds generated large quantities of nitrogen oxides which might not be quantitatively reduced. They also highlighted the influence of O_2 concentration on the determination of the nitrogen content of nitro compounds when performing EA. Nitro compounds dropped under maximum O_2 concentration resulted in inaccurate values for the nitrogen content. However, lower O_2 concentration favoured the reduction of nitrogen oxides and resulted in more accurate values for the nitrogen content.

The results of experiment 3 demonstrate the importance of the influence of oxygen on the δ^{15}N measurements of nitrate (KNO_3 and NH_4NO_3), but also of other inorganic compounds such as $(NH_4)_2SO_4$. In contrast to the study of Pella and Colombo, the introduction of nitrates at lower O_2 concentration (sample start at 0 or 5 s) did not produce satisfactory values for δ^{15}N and nitrogen content. Only the complete exclusion of oxygen from the analytical cycle led to excellent and repeatable results for nitrates.

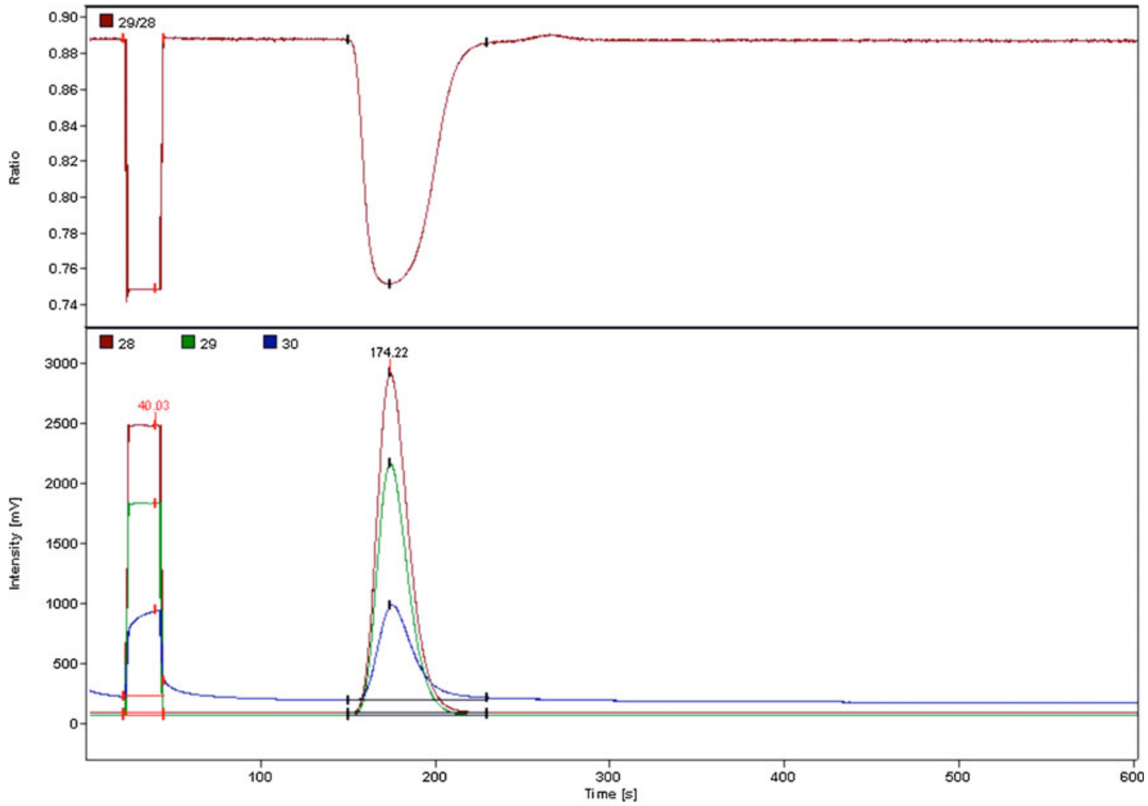


Fig. 7 δ^{15}N measurement of IAEA-NO-3 (KNO_3) obtained without oxygen injection (analytical cycle 70 s, sample start/stop at 18 s/20 s). The N_2 is well eluted and the peak does not show any tailing. In addition, the *m*/*z* 29/28 ion current ratio perfectly returns to the baseline even after ten replicates

The principle of the analytical technique is based on flash combustion, which is obtained through the sudden oxidation of tin in an oxygen-rich environment. This exothermic reaction produces a bright flash and instantly transforms the sample into gaseous combustion products at around 1,700 °C. Gaseous products are then carried through the reduction reactor for the oxides to be reduced. The requirement of injecting oxygen in excess to create an oxygen-rich environment is necessary for organic material, i.e. carbon-rich material, in order to ensure the complete oxidation of the sample. However, for highly oxidised inorganic substances such as nitrates where nitrogen assumes its highest possible oxidation number of+V, the injection of oxygen is not necessary when the sample is introduced and, even more so, is not advised. The following thermochemical considerations explain the reasons, taking into consideration the chemical nature of the substances as well as the physical and chemical conditions related to the method.

Thermochemical considerations

Using thermochemical methods [19, 20], the thermal decomposition and the equilibrium predictions of the compounds of interest explain our observations and provide guidance for performing proper laboratory procedures for reference materials. We will consider the processing of KNO_3, NH_4NO_3 and $(NH_4)_2SO_4$ as illustrative examples. Typically, a mass corresponding to roughly 2 mbar or approximately 2×10^{-3} atm of the nitrogen-containing compound is evaporated, filling a volume of approximately 50 cm^3. Runs with O_2 were performed at a partial pressure of 360 mbar, corresponding to 0.348 atm, in comparison with runs without added O_2. The present strategy consists of comparing the equilibria with and without added O_2 at temperatures of interest for each compound, considering the tables in the Appendix.

KNO_3 Crystalline KNO_3 is a stable salt whose high-temperature equilibrium is given by Eq. 1:

$$2KNO_3 \leftrightarrows K_2O + 2NO + 3/2O_2 \qquad (K_1) \qquad (1)$$

The forward direction of the equilibrium in Eq. 1 describes the high-temperature thermal decomposition starting at 1,070 K [21]. KNO_3 has been reported to decompose to potassium nitrite (KNO_2) according to Eq. 2. However, this decomposition path will not be considered in detail here because KNO_2 is less stable than the original material. In

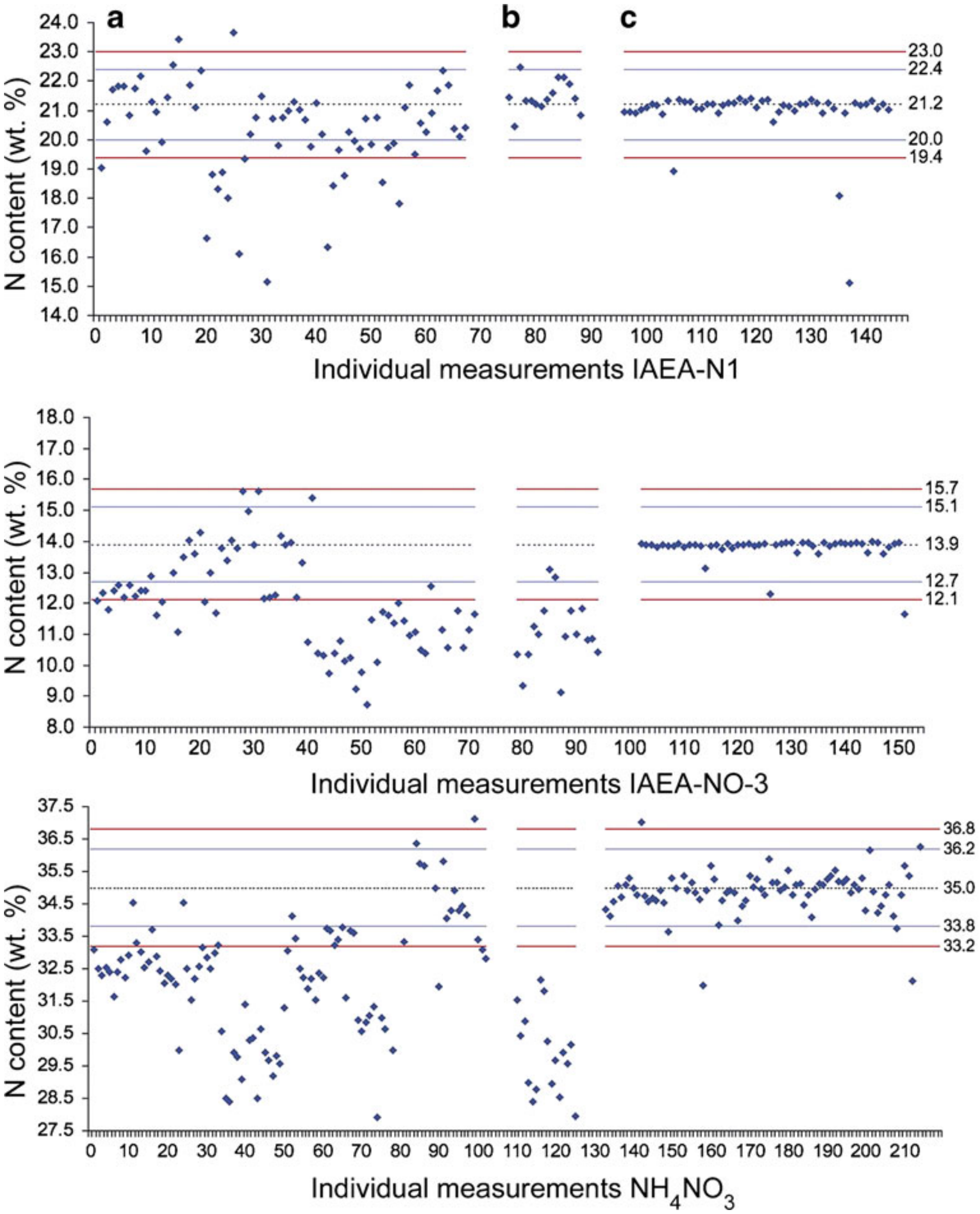


Fig. 8 Control charts of the nitrogen content of IAEA-N1 [$(NH_4)_2SO_4$], IAEA-NO-3 (KNO_3) and NH_4NO_3 samples obtained with system 1. **a** with oxygen injection (analytical cycle 70 s, oxygen for 60 s, sample start/stop at 18 s/20 s), **b** with oxygen injection (analytical cycle 70 s, oxygen for 60 s, sample start/stop at 0 s/2 s and 5 s/7 s), **c** without oxygen injection (analytical cycle 70 s, sample start/stop at 18 s/20 s). The expected nitrogen content is represented by a *central dotted black line*. Upper and lower warning lines (*blue*) and action lines (*red*) are defined as two standard deviations and three standard deviations, respectively

contrast to KNO_3, whose decomposition starts at 923 K when it is heated in air [21], the decomposition of KNO_2 starts at 700 K:

$$KNO_3 \leftrightarrows KNO_2 + 1/2O_2 \tag{2}$$

NO is the only oxide of nitrogen that is stable at temperatures in excess of 500–600 K because all other oxides of nitrogen, such as NO_2, N_2O_5, N_2O_4, N_2O_3 and N_2O_2, and oxyacids of nitrogen, such as HNO_3, HNO_4, HONO and $H_2N_2O_2$, convert to NO and O_2 at higher temperatures of interest. It therefore behoves us to consider the equilibrium in Eq. 1 as the relevant reaction sequence for the thermal decomposition of KNO_3. Of course, the equilibrium in Eq. 1 may be understood as the sum of the equilibria in Eqs. 3a and 3b, which underlines the thermal instability of N_2O_5:

$$2KNO_3 \leftrightarrows K_2O + N_2O_5 \tag{3a}$$

$$N_2O_5 \leftrightarrows 2NO + 3/2O_2 \tag{3b}$$

The relevant thermochemistry of the equilibrium in Eq. 1 is given in Table 3. Table 4 gives the ratio of the equilibrium pressures of NO and O_2 in the absence of added O_2 (second column from the left) and in the presence of O_2 (third column) as a function of temperature. The ratio of NO or nitrogen in oxidation state+II to KNO_3 with nitrogen in oxidation state+V is given in columns 4 and 5 in the absence and presence of added O_2, respectively. Adding 360 mbar of O_2 to the carrier gas for thermal processing of KNO_3 shifts the equilibrium to the left, favouring nitrogen in its high oxidation state (+V) at the expense of NO [N(+II)]. This is just a consequence of Le Chatelier's principle, of which columns 4 and 5 are a clear manifestation. Finally, the last column of Table 4 displays the factor by which the equilibrium is shifted to the left by the presence of O_2. At temperatures exceeding 1,300 K, this factor ranges from 30 to 80. The corollary, therefore, is that the presence of O_2 shifts nitrogen closer to its oxidised form, whereas we require the inverse as we are ultimately interested in the reduction of NO to N_2 in the reducing part of the elemental analyser.

Of note is the position of the equilibrium in Eq. 4 with and without added O_2, the details being given in Table 5 (thermochemistry) and Table 6 (equilibrium pressures):

$$2NO_2 \leftrightarrows 2NO + O_2 \qquad (K_2) \qquad (4)$$

The equilibrium in Eq. 4 is important in combustion/exhaust systems and shows qualitatively the same behaviour as the equilibrium in Eq. 1 as a function of temperature: added O_2 shifts the equilibrium towards NO_2 [N(+IV)] at the expense of the less oxidised form of nitrogen, namely NO [N(+II)]. However, the equilibrium lies far to the right at temperatures exceeding 500 K without the addition of O_2. The third column of Table 6 shows a rapidly decreasing ratio $r=P_{NO2}/P_{NO}$ with and without the addition of O_2 for temperatures exceeding 500 K. The addition of a large amount of O_2 increases the relative NO_2 concentration by a factor of 18.68 at 1,000 K (fourth column of Table 6) over the values without addition of O_2 (third column of Table 6). The fifth column of Table 6 displays an r/r^{O2} ratio of roughly 20 at 1,000 K, which means that P_{NO2}/P_{NO} is 20 times larger in the presence of added O_2 than in its absence. The equilibria in Eqs. 1 and 4 are sufficiently decoupled as far as the temperature is concerned, and so one does not have to take into account the equilibrium in Eq. 4 when considering the decomposition of KNO_3 (Eq. 1). That is to say that the equilibrium in Eq. 4 will not change the speciation of nitrogen between NO and NO_2 at temperatures exceeding 1,000 K because the equilibrium in Eq. 1 specifies NO_x occurs in the form of NO, with or without the occurrence of the equilibrium in Eq. 4 owing to the high-temperature stability of NO compared with NO_2.

NH_4NO_3 NH_4NO_3 decomposes according to two principal channels displayed in Eqs. 5 and 6:

$$NH_4NO_3 \leftrightarrows NH_3 + HNO_3 \qquad (5)$$

$$\begin{aligned} NH_4NO_3 &\leftrightarrows H_2O + NH_2NO_2 \leftrightarrows H_2O + N_2O \\ &\leftrightarrows N_2 + 1/2O_2 \end{aligned} \qquad (6)$$

For the sake of simplicity, we will only consider the reaction in Eq. 5, which is faster than the nitramide branch (reaction in Eq. 6) under most experimental conditions. Tables 7 and 8 give the decomposition temperatures of NH_3 and HNO_3, respectively, these being two prototypical products often encountered in thermal processing. Table 7 reveals the inherent thermodynamic instability of NH_3 towards thermal decomposition, which is independent of added O_2:

$$2NH_3 \leftrightarrows N_2 + 3H_2 \qquad (K_3) \qquad (7)$$

However, the decomposition of HNO_3, which follows a mechanism proposed by Johnston et al. [22], is governed by the same principle as the decomposition of KNO_3 (Eq. 1) and NO_2 (Eq. 4) discussed above. HNO_3 is a fairly unstable species in the gas phase and releases O_2, which makes it sensitive to the presence of added O_2 according to the reaction in Eq. 8:

$$2HNO_3 \leftrightarrows H_2O + NO_2 + NO + O_2 \qquad (K_4) \qquad (8)$$

Table 9 reveals an r/r^{O2} ratio of approximately 20 in the 300–700 K temperature range: added O_2 shifts the equilibrium from NO and NO_2 towards HNO_3 i.e., back to the higher oxidation state, i.e. N(+II) and N(+IV) ➔ N(+V), as may be seen in Eq. 8. The fact that gas-phase HNO_3 decomposes at a relatively low temperature may perhaps come as a surprise.

$(NH_4)_2SO_4$ The pattern of thermal decomposition is similar to that for NH_4NO_3, namely

$$(NH_4)_2SO_4 \leftrightarrows 2NH_3 + H_2SO_4 \qquad (9)$$

$$H_2SO_4 \leftrightarrows SO_2 + H_2O + 1/2O_2 \qquad (K_5) \qquad (10)$$

H_2SO_4 is significantly stabler than HNO_3 as revealed in Table 10 (thermochemistry) and Table 11 (equilibrium pressures). H_2SO_4 is essentially quantitatively decomposed at 700 K and releases O_2, which makes it sensitive to added O_2 according to Eq. 10. In the present case, equilibrium constant K_5 shifts SO_2 [S(+IV)] to H_2SO_4 [S(+VI)] in agreement with $r/r^{O2}=10$ in the temperature range from 500 to 900 K (Table 11). However, the thermal stability of NH_3 (K_3) is independent of H_2SO_4 as far as the decomposition reaction (Eq. 7) in a non-oxidising atmosphere is concerned because NH_3 and H_2SO_4 do not have any decomposition products in common (reactions in Eqs. 7 and 10). Therefore, both decomposition products from the reaction in Eq. 9, namely NH_3 and H_2SO_4, behave independently of each other.

However, ammonia oxidation according to the reaction in Eq. 11 has to be considered in the case when O_2 is deliberately added to the carrier gas:

$$NH_3 + 5/4O_2 \leftrightarrows NO + 3/2H_2O \qquad (K_6) \qquad (11)$$

Equation 11 is valid for catalytic oxidation, which is of relevance in the present context, whereas direct (non-catalytic) air oxidation (combustion) leads to N_2 and H_2O vapour. Table 12 displays the thermochemical data for the range from 300 to 1,800 K, including the equilibrium constant K_6. Its values throughout the given temperature range are mainly given by the stability of product H_2O vapour, which controls the position of the equilibrium in Eq. 11 Any O_2 generated as a reaction product such as in the reaction in Eq. 10 will immediately undergo reaction with NH_3 to generate NO following Eq. 11. The primary reaction (Eq. 9) may be combined with the reactions in Eqs. 10 and 11 in the following way:

$$(NH_4)_2SO_4 + 5/2O_2 \leftrightarrows SO_2 + 2NO + 4H_2O + 1/2O_2 \qquad (12)$$

The decomposition of sulphuric acid (Eq. 10), only generates 0.5 mol O_2 of the 2.5 mol O_2 necessary for complete oxidation of NH_3 according to Eq. 11. This means that roughly 10 % or less of the evolving NH_3 from the reaction in Eq. 8 will be converted to NO according to Eq. 11 if equilibrium is established because roughly half of the oxygen is consumed to oxidise nitrogen to NO and the other half of the oxygen is used to oxidise the hydrogen of NH_3 to H_2O following Eq. 11. However, in the case of added oxygen, 100 % of ammonia will be catalytically oxidised to NO owing to the strong exothermicity of the reaction in Eq. 11, leading to the enormous values of K_6 in Table 12. NO, once generated, is difficult to reduce to N_2 [23], the species of interest in the present context. It is therefore concluded, as in the cases discussed above, that the addition of O_2 in reactions of thermal decomposition of species that are already in their highest oxidation state leads to an increased extent of oxidation of reaction products that will have to be reversed in the reduction stages of the elemental analyser (Fig. 1) in order to monitor nitrogen as N_2. It is possible that this re-reduction of oxidised forms of nitrogen will lead to losses or incomplete conversion in the present experimental protocol.

In conclusion, equilibria are shifted towards species with a higher oxidation state in the presence of added O_2 whenever the equilibrium decomposition involves the formation of O_2. This is a consequence of Le Chatelier's principle and may be quantitatively assessed using high-temperature thermochemistry. The shift towards species of higher oxidation states upon addition of O_2 amounts to factors of 10–80 of the equilibrium pressures depending on the specific thermochemistry. In the present case, one wants to preserve the low oxidation states in order to support quantitative conversion of oxidised nitrogen to N_2 for analytical purposes. Using a similar strategy, Révész et al. [24] used catalysed graphite combustion of nitrates at 800 K to trap O_2 as stable carbonates. In this way, O_2 was prevented from reoxidising nitrogen to a higher oxidation state.

Concluding remarks From a chemical point of view, "oxidation" of nitrates and sulphates, including HNO_3 and H_2SO_4, should be replaced by the term "thermal decomposition" because these compounds already exist in their highest possible oxidation state. Therefore, any further attempt at oxidation is chemically impossible and thus essentially counterproductive. Instead, decomposition changes the state of oxidation of nitrogen. Taking HNO_3 as an example (Eq. (8)), oxide ions [O (−II)] are oxidised to elementary O_2 by simultaneously reducing N(+V) to N(+IV) or N(+II) depending on the temperature. This internal change of oxidation state is thermodynamically driven by the stability of nitrogen oxides that increasingly favour the lower oxidation state of N(+II), i.e. NO in Eq. 8, over the higher oxidation states corresponding to NO_2 [N(+IV)] or HNO_3 [N(+V)] with increasing temperature. In addition, it is recognised that the reduction of NO to N_2 is difficult owing to kinetic control, especially at lower temperatures, a fact that may lead to losses of nitrogen and/or to unwanted side reactions not resulting in N_2, the product of interest. This view of high-temperature reactivity in itself, supported by the aforementioned thermochemical considerations, suggests the processing of oxidised nitrogen in the absence of added O_2. This shows that it is inadequate to analyse inorganic and organic substances in the same sequence using the same method, as they undergo different thermal decomposition or combustion reactions.

The addition of sucrose and/or other oxidisable material (graphite) in nitrate decomposition experiments serves to remove (i.e. oxidise) the product O_2 but does not affect the decomposition reaction itself [1, 7–9].

Implications for the calibration of organic laboratory standards with inorganic International Atomic Energy Agency reference materials

Secondary international reference materials IAEA-N1, IAEA-N2 and IAEA-NO-3 are used to calibrate the IRMS system and the organic laboratory standards. A common procedure to calibrate a material (the future laboratory standard) is to analyse replicates of the substance with the international reference materials IAEA-N1, IAEA-N2 and IAEA-NO-3 in the same analytical sequence. This procedure, however, requires the same analytical procedure to be used for the material and the secondary international reference material. As demonstrated by our results, organic material and inorganic substances, such as $(NH_4)_2SO_4$ (IAEA-N1 and IAEA-N2) and KNO_3 (IAEA-NO-3), should not be analysed using the same analytical procedure because of their different chemical nature. The limited accessibility of organic international reference materials constrains the isotope community to use the inorganic IAEA-N1, IAEA-N2 and IAEA-NO-3 for the calibration of organic laboratory standards to the international scale.

Thus, on the basis of our results, when an organic material has to be calibrated against IAEA-N1, IAEA-N2 and IAEA-NO-3, we suggest calibrating the IRMS system by analysing IAEA-N1, IAEA-N2 and IAEA-NO-3 without oxygen first. Then, the $\delta^{15}N$ value of the future organic standard may be obtained by analysing replicates of organic material in the presence of oxygen with the calibrated IRMS system, and ideally using an organic certified reference material.

Conclusion

An unexpected series of $\delta^{15}N$ values in our laboratory standards were observed when analysing nitrates and organic laboratory standards in the same analytical sequence. A closer examination of the chromatograms showed that the N_2 peaks of nitrates exhibited tails, thus affecting the repeatability of the $\delta^{15}N$ values of nitrate and laboratory standards. A series of experiments were conducted to understand the cause of this phenomenon. In experiment 1, the use of additives to sustain combustion did not produce conclusive results. The addition of graphite to samples produced biased $\delta^{15}N$ values, whereas the addition of V_2O_5 increased the tailing of the N_2 peaks. In experiment 2, additional tests were undertaken with another elemental analyser configuration (system 2) involving a supplementary copper section. The results revealed that the tailing of nitrates originated from an incomplete reduction process. Nevertheless, despite the absence of tailing in system 2, the repeatability of the $\delta^{15}N$ values was not improved much.

Finally, modifications of the method parameters were undertaken in experiment 3. For IAEA-N1 and IAEA-N2, the introduction of the sample at 0 or 5 s after the beginning of the analytical cycle (with O_2 injection), or when oxygen was excluded, provided excellent repeatability of $\delta^{15}N$ values (0.1 ‰) without outliers. Although the exclusion of oxygen from the analytical procedure was not adequate for organic standards, the analytical procedure performed excellently and produced repeatable $\delta^{15}N$ values with only rare outliers for KNO_3 and NH_4NO_3. Under these conditions, the estimated expanded uncertainty (k=2) associated with the $\delta^{15}N$ measurement of nitrates and of ammonium sulphate compounds is 0.1 ‰. These settings also allowed a much better recovery of nitrogen, especially for KNO_3, than the analysis with O_2 injection.

We showed that organic compounds, on the one hand, and nitrates and ammonium sulphate (IAEA-N1 and IAEA-N2), on the other hand, should not be analysed using the same analytical procedure, given their different chemical nature. Although the instrumental technique was developed to transform the sample into simple gases by means of flash combustion, the thermodynamic considerations demonstrate that not all materials behave in the same way because of their different chemical nature. This aspect needs to be taken into consideration when using EA-IRMS and upholds the principle to use laboratory standards with a chemical structure as similar as possible to that of the samples. This necessarily impacts on the way inorganic secondary reference materials should be used for the calibration of organic laboratory standards.

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Appendix

Tables 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 provide supplementary material for thermochemical evaluation of standard reactions (standard state: 1 atm).

Table 3 Thermochemical data for the equilibrium in Eq. 1

T/K	ΔH^0_r/kcal/mol	ΔS^0_r/cal/mol K	ΔG^0_r/kcal/mol	K_1/(atm)$^{5/2}$
300	176.33	93.87	148.17	1.00 (−108)
800	175.80	93.53	100.97	2.51 (−28)
1,000	174.85	92.48	82.36	9.74 (−19)
1,100	174.32	91.93	73.19	2.82 (−15)
1,200	173.50	91.30	63.95	2.22 (−12)
1,300	172.46	90.46	54.87	5.90 (−10)
1,400	170.83	89.25	45.88	6.82 (−8)
1,500	168.27	87.46	37.08	3.93 (−6)
1,600	160.39	84.91	24.53	4.43 (−4)
1,700	158.46	81.32	20.21	2.51 (−3)
1,800	149.70	76.40	12.18	3.31 (−2)

Thermochemical data for KNO_3 and K_2O for the hypothetical gas phase were taken from [20]; data for NO and O_2 were taken from [19]. The thermochemistry as a function of temperature for KNO_3 and K_2O was evaluated using the seven-coefficient NASA polynomial fitting for the range from 300 to 1,000 K given in [20]. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 5 Thermodynamic data for the equilibrium in Eq. 4

T/K	ΔH^0_r/kcal/mol	ΔS^0_r/cal/mol K	ΔG^0_r/kcal/mol	K_2/atm
300	27.34	35.03	16.83	5.46 (−13)
400	27.64	35.87	13.26	5.67 (−8)
500	27.82	36.28	9.68	5.87 (−5)
1,000	27.94	36.51	−8.57	74.67
1,100	27.90	36.49	−12.24	270.31
1,200	27.84	36.45	−15.90	7.87.1
1,300	27.80	36.40	−19.52	1.913 (3)
1,400	27.74	36.36	−23.16	4.13 (3)
1,800	27.52	36.23	−37.69	3.77 (4)

Thermochemical information for NO_2, NO and O_2 was taken from [19]. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 4 Equilibrium pressures of NO and O_2 for the equilibrium in Eq. 1 at an initial pressure of KNO_3 of 2.0×10^{-3} atm (*r*) and at a prescribed O_2 partial pressure of 0.348 atm (r^{O2})

T/K	*x*	*x* (for O_2 partial pressure of 0.348 atm)	$2x/(2\times10^{-3} - 2x)$ (*r*)	$2x/(2\times10^{-3} - 2x)$ (r^{O2})	r/r^{O2}
800	3.7575 (−8)	1.2348 (−11)	3.76 (−5)	1.24 (−8)	3,042.9
1,000	5.0675 (−6)	1.9405 (−8)	5.09 (−3)	1.94 (−5)	262.5
1,100	2.9462 (−5)	2.7652 (−7)	3.04 (−2)	2.77 (−4)	109.8
1,200	1.2390 (−4)	2.5494 (−6)	1.41 (−1)	2.56 (−3)	55.3
1,300	3.7000 (−4)	1.6240 (−5)	5.87 (−1)	1.65 (−2)	35.6
1,400	7.2983 (−4)	7.5882 (−5)	2.701	8.21 (−2)	32.9
1,500	9.3748 (−4)	2.5409 (−4)	14.99	3.41 (−1)	44.02
1,600	9.93293 (−4)	6.8738 (−4)	148.10	2.199	67.35
1,700	9.97158 (−4)	8.2676 (−4)	350.86	4.772	73.52
1,800	9.99214 (−4)	9.4198 (−4)	1271.3	16.236	78.30

$P_{NO} = 2x$, $P_{O2} = 0.75P_{NO}$. *x* satisfies the governing equation $(3/4)^{3/2}x^{9/2} - K_1(10^{-3} - x)^2 = 0$ for 2.0×10^{-3} atm and $x^3\,[(3/4)0.348]^{3/2} - K_1(10^{-3} - x)^2 = 0$ for the prescribed O_2 partial pressure. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 6 Equilibrium pressures of O_2 for the equilibrium in Eq. 4 at an initial pressure of NO_x of 2.0×10^{-3} atm (r) and at a prescribed O_2 partial pressure of 0.348 atm (r^{O2})

T/K	P_{O2}/atm	$P_{NO2}/P_{NO} = r$	$(P_{NO2}/P_{NO})^{O2}$	r^{O2}/r
300	1.63 (−6)	1,226	8.00 (5)	657.5
400	7.49 (−5)	25.7	2.48 (3)	96.4
500	6.10 (−4)	4.38 (−1)	67.99	175.8
1,000	1.99274 (−3)	3.64 (−3)	6.80 (−2)	18.68
1,100	1.99616 (−3)	1.92 (−3)	3.60 (−2)	18.75
1,200	1.99775 (−3)	1.13 (−3)	2.10 (−2)	18.58
1,300	1.99855 (−3)	7.25 (−4)	1.40 (−2)	19.31
1,400	1.99901 (−3)	4.95 (−4)	9.20 (−3)	18.58
1,800	1.99968 (−3)	1.60 (−4)	3.00 (−3)	18.75

P_{NO} = x. x satisfies the governing equation $0.5x^3 - K_2(2.0\times10^{-3} - x)^2 = 0$ for 2.0×10^{-3} atm NO_x and $P_{NO2}/P_{NO} = (y/K_2)^{1/2}$, with y being the prescribed O_2 partial pressure of 0.348 atm. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 8 Thermodynamic data for decomposition of HNO_3 (Eq. 8)

T/K	ΔH^0_r/kcal/mol	ΔS^0_r/cal/molK	ΔG^0_r/kcal/mol	K_4/(atm)^2
300	35.91	74.38	13.60	1.23 (−10)
400	36.26	75.32	6.13	4.44 (−4)
500	36.31	75.37	−1.38	4.00 (0)
600	36.22	75.04	−8.80	1.62 (3)
700	35.97	74.55	−16.22	1.16 (5)
800	35.63	74.00	−23.57	2.77 (6)
900	35.24	73.46	−30.87	3.17 (7)
1,000	34.82	72.94	−38.12	2.17 (8)
1,100	34.36	72.42	−45.30	1.01 (9)
1,200	33.88	71.89	−52.39	3.52 (9)
1,300	33.39	71.28	−59.27	9.35 (9)
1,400	32.88	70.54	−65.88	1.95 (10)

Thermochemical data for HNO_3 were obtained from [20]; data for H_2O, O_2, NO_2 and NO were taken from [19]. The thermochemistry as a function of temperature for HNO_3 was evaluated using the seven-coefficient NASA polynomial fitting for the range from 300 to 1,000 K given in [20]. A number within parentheses is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 7 Thermodynamic data for decomposition of NH_3 (Eq. 7) and partial pressure of N_2 with an initial pressure of NH_3 of 2.0×10^{-3} atm

T/K	ΔH^0_r/kcal/mol	ΔS^0_r/cal/molK	ΔG^0_r/kcal/mol	K_3/(atm)^2	P_{N2}(atm)	P_{N2}/P_{NH3}
300	21.96	47.39	7.74	2.28 (−6)	5.25 (−4)	0.553
400	22.96	46.63	4.30	4.43 (−3)	9.63745 (−4)	13.3
500	23.84	52.26	−2.29	10.02	9,99741 (−4)	1,930
600	24.56	53.56	−7.57	5.75 (2)	9.99892 (−4)	4.63 (3)
700	25.16	54.49	−12.98	1.13 (4)	9.99976 (−4)	2.08 (4)
800	25.64	55.15	−18.48	1.12 (5)	9.99992 (−4)	6.25 (4)
900	26.04	55.62	−24.02	6.81 (5)	9.99997 (−4)	1.66 (5)
1,100	26.54	56.11	−35.18	9.78 (6)	9.99999 (−4)	>5.0 (5)
1,300	26.78	56.31	−46.42	6.38 (7)	1.0 (−3)	–
1,800	40.08	56.21	−61.10	2.62 (7)	1.0 (−3)	–

$P_{N2} = 1/3P_{H2} = x$. x satisfies the governing equation $27x^4 - K_3(2.0\times10^{-3} - 2x)^2 = 0$. All thermochemical data were taken from [19]. A number within *parentheses* is the exponent (base 10). For example 2.45 (−5) is 2.45×10^{-5}.

Table 9 Partial pressure of O_2 at equilibrium (Eq. 8) for an initial pressure of HNO_3 of 2.0×10^{-3} atm and a prescribed O_2 pressure of 0.348 atm

T/K	$P_{H2O} = P_{O2}$	$P_{O2}/P_{HNO3} = r$	$P_{NO} = P_{NO2}$	$P_{O2}/P_{HNO3} = \mathrm{r}^{O2}$	r/r^{O2}
300	1.38255 (−4)	8.02 (−2)	1.114 (−5)	5.633 (−3)	14.24
400	9.77335 (−4)	2.15 (1)	7.26112 (−4)	1.326	16.21
500	9.9975 (−4)	1.999 (3)	9.95369 (−4)	1.0747 (2)	18.60
600	9.99988 (−4)	4.166(4)	9.99768 (−4)	2.155 (3)	19.33
700	9.99999 (−4)	5.00 (5)	9.99973 (−4)	1.852 (4)	26.99
800	1.00 (−3)	–	9.99994 (−4)	8.333 (4)	
900	1.00 (−3)	–	9.99998 (−4)	2.500 (5)	
1,000	1.00 (−3)	–			

$P_{H2O} = P_{NO} = P_{NO2} = P_{O2} = x$. x satisfies the governing equation $x^4 - 4K_4(10^{-3} - x)^2 = 0$ for an initial pressure of HNO_3 of 2.0×10^{-3} atm and $0.348x^3 - 4K_4(10^{-3} - x)^2 = 0$ for a prescribed O_2 pressure of 0.348 atm. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

[a] Extrapolated value based on assumed (average) value $r/r^{O2} = 20.0$ and r^{O2} at 600 K.

Table 10 Thermodynamic data for decomposition of H_2SO_4 (Eq. 10)

T/K	ΔH^0_r/kcal/mol	ΔS^0_r/cal/mol K	ΔG^0_r/kcal/mol	$K_5/(\mathrm{atm})^{3/2}$
300	48.26	59.80	30.32	7.90 (−23)
500	48.15	59.58	18.36	9.33 (−9)
700	47.52	58.55	6.54	9.09 (−3)
800	47.12	58.01	0.71	0.639
900	46.70	57.51	−5.06	16.95
1,000	46.24	57.04	−10.80	230.06
1,100	45.78	56.60	−16.48	1889.6
1,200	45.30	56.19	−22.13	1.08 (4)

All thermochemical data were taken from [19]. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 11 Partial pressure of O_2 at equilibrium (Eq. 10) for an initial H_2SO_4 pressure of 2.0×10^{-3} atm and a prescribed O_2 pressure of 0.348 atm

T/K	P_{SO2}	$P_{SO2}/P_{H2SO4} = r$	P_{SO2}	$P_{SO2}/P_{H2SO4} = r^{O2}$	r/r^{O2}
300	1.37933 (−10)	6.897 (−8)	6.73812 (−13)	3.369 (−10)	204.7
500	5.8004 (−5)	2.990 (−2)	7.30922 (−6)	3.668 (−3)	8.15
700	1.98632 (−3)	145.2	1.86661 (−3)	13.994	10.38
800	1.99980 (−3)	9.999 (3)	1.99783 (−3)	9.206 (2)	10.86
900	1.99999 (−3)	1.999 (5)	1.99992 (−3)	2.499 (4)	7.99
1,000	2.0 (−3)	–	1.99999 (−3)	1.999 (5)	–
1,100	2.0 (−3)	–	2.0 (−3)	–	–

$P_{SO2} = P_{H2O} = 2P_{O2} = x$. x satisfies the governing equation $x^{5/2} - (2)^{1/2}K_5(2.0\times10^{-3} - x) = 0$ for an initial pressure of H_2SO_4 of 2.0×10^{-3} atm and $0.348x^2 - K_5(2.0\times10^{-3} - x) = 0$ for a prescribed O_2 pressure of 0.348 atm. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

Table 12 Thermodynamic data for catalytic oxidation of NH_3 (Eq. 11)

T/K	$\Delta H^0{}_r$/kcal/mol	$\Delta S^0{}_r$/cal/mol K	$\Delta G^0{}_r$/kcal/mol	$K_6/(\text{atm})^{1/4}$
300	−54.14	10.74	−57.36	6.50 (41)
500	−53.91	11.37	−59.60	1.16 (26)
700	−53.89	11.41	−61.89	2.14 (19)
800	−53,95	11.35	−63.03	1.69 (17)
900	−53.99	11.28	−64.14	3.85 (15)
1,000	−54.09	11.18	−65.27	1.88 (14)
1100	−54.19	11.09	−66.27	1.58 (13)
1,200	−54.32	10.98	−67.50	2.00 (12)
1,300	−54.43	10.87	−68.56	3.41 (11)
1,800	−55.13	10.43	−73.90	9.51 (8)

All thermochemical data were taken from [19]. A number within *parentheses* is the exponent (base 10). For example, 2.45 (−5) is 2.45×10^{-5}.

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