UNIL | Université de Lausanne

Unicentre
CH-1015 Lausanne
http://serval.unil.ch

Year : 2016

ESTIMATING THE TIME SINCE DISCHARGE OF HANDGUN CARTRIDGES USING A NOVEL HEADSPACE SORPTIVE EXTRACTION (HSSE) APPROACH

Gallidabino Matteo

Gallidabino Matteo, 2016, ESTIMATING THE TIME SINCE DISCHARGE OF HANDGUN CARTRIDGES USING A NOVEL HEADSPACE SORPTIVE EXTRACTION (HSSE) APPROACH

Originally published at : Thesis, University of Lausanne

Posted at the University of Lausanne Open Archive http://serval.unil.ch
Document URN : urn:nbn:ch:serval-BIB_0346012579FB9

Droits d'auteur
L'Université de Lausanne attire expressément l'attention des utilisateurs sur le fait que tous les documents publiés dans l'Archive SERVAL sont protégés par le droit d'auteur, conformément à la loi fédérale sur le droit d'auteur et les droits voisins (LDA). A ce titre, il est indispensable d'obtenir le consentement préalable de l'auteur et/ou de l'éditeur avant toute utilisation d'une oeuvre ou d'une partie d'une oeuvre ne relevant pas d'une utilisation à des fins personnelles au sens de la LDA (art. 19, al. 1 lettre a). A défaut, tout contrevenant s'expose aux sanctions prévues par cette loi. Nous déclinons toute responsabilité en la matière.

Copyright
The University of Lausanne expressly draws the attention of users to the fact that all documents published in the SERVAL Archive are protected by copyright in accordance with federal law on copyright and similar rights (LDA). Accordingly it is indispensable to obtain prior consent from the author and/or publisher before any use of a work or part of a work for purposes other than personal use within the meaning of LDA (art. 19, para. 1 letter a). Failure to do so will expose offenders to the sanctions laid down by this law. We accept no liability in this respect.

UNIL | Université de Lausanne

ESTIMATING THE TIME SINCE DISCHARGE OF HANDGUN CARTRIDGES USING A NOVEL HEADSPACE SORPTIVE EXTRACTION (HSSE) APPROACH

PHD THESIS SUBMITTED TO
THE *ECOLE DES SCIENCES CRIMINELLES*
OF THE *UNIVERSITÉ DE LAUSANNE*

MATTEO GALLIDABINO

LAUSANNE
2016

ISBN 2-940098-73-5

Unil
UNIL | Université de Lausanne
Ecole des sciences criminelles
bâtiment Batochime
CH-1015 Lausanne

IMPRIMATUR

A l'issue de la soutenance de thèse, le Jury autorise l'impression de la thèse de M. Matteo Gallidabino, candidat au doctorat en science forensique, intitulée

« Estimating the time since discharge of hand-gun cartridges using a novel headspace sorptive extraction (HSSE) approach »

Le Président du Jury

Professeur Pierre Esseiva

Lausanne, le 29 février 2016

Acknowledgements

The following research was carried out at the *Ecole des Sciences Criminelles* (ESC) of the *University of Lausanne* (UNIL), under the supervision of Prof. Céline Weyermann, associate professor in forensic science. The dissertation committee was additionally composed by Prof. Pierre Esseiva, vice-director of ESC and president of the jury, Dr. Balthasard Jung, head of the *Scientific Research Service* (SRS) of the *Forensiches Institut Zürich* (FOR), Dr. Arie Zeichner, former head of the *Toolmarks and Material Lab* of the *Israel Police's Division of Identification and Forensic Science* (DIFS), and Prof. Franco Taroni, associate professor in forensic science at ESC. I am sincerely grateful to all the members of the committee for reading the manuscript, as well as for their comments and feedbacks. In particular, I would like to thank:

- My "boss", Prof. Céline Weyermann for proposing to me this fascinating research topic at the beginning of my doctorate and supporting me all throughout it. I am deeply thankful to her for the encouragement in the face of the numerous frustrating moments I faced, for putting her trust in me when I had "alternative" ideas on the path to follow, as well as for assisting me in the numerous projects we undertook together during the long time I spent at ESC.
- Prof. Franco Taroni for the fruitful discussions about interpretation and the role of the evidence we had over these years, since when I was an ignorant undergraduate student until the last days of my doctoral thesis. I am highly thankful to him for having sacrificed numerous lunch breaks to answer my questions, but also for securing my rope during the numerous free-climbing sessions we did.
- Prof. Pierre Esseiva for the numerous debates, especially in the last years of my thesis, about chemometrics and data-treatment methods. I am particularly grateful to him for suggesting to me possible statistical solutions to give reasonable meanings to my analytical results and, particularly, for showing me the potential in the application of my research in a forensic perspective when I had lost all hope. Furthermore, I thank him for allowing me to exploit the analytical instruments of the Drug Profiling's group.
- Dr. Balthasard Jung and Dr. Arie Zeichner, for their helpful suggestions, comments and ideas during this research, as well as the time dedicated to reading the final result. I consider both brilliant forensic scientists and I hope to work with them again in the future.
- The *Swiss National Science Foundation* (SNSF) and the *Fondation pour l'Université de Lausanne*, the first for granting my first three years of research, as well as most analyses of

my doctoral thesis, and the second for partially financing the presentation of the main results during the 7^{th} *European Academy of Forensic Science Conference* (EAFS) in 2015 in Prague.

- The ESC, and more generally the UNIL, for giving me the possibility to enjoy the precious resources of their laboratories.

This research would never have been possible without the support and encouragement of numerous other persons. In this regard, I am particularly grateful to:

- Prof. Francesco Saverio Romolo for introducing me to the field of gunshot residue analysis and always pushing my reflections (and research) further.
- Prof. Olivier Delémont for his fruitful advices when I needed his professional point of view, as well as for giving me the possibility to collaborate in numerous projects on gunshot residues and explosives, which furthered my interest in these topics and to gain considerable experience.
- Dr. Juan Manuel Garcia Gongora for his valuable moral and technical support during the development and optimisation of the analytical method, and more generally for teaching me that there is no problem you can't fix in analytical chemistry without a rigorous methodological approach.
- Prof. Alex Biedermann for the numerous debates on interpretation, as well as for offering me the opportunity to participate in a pedagogical project on the pre-assessment of evidence, which helped me to better understand some essential problems of our discipline.
- Prof. Pierre Margot and Prof. Olivier Ribaux for presenting to me the different possibilities in academia for my professional future and helping me to pursue my career in this field.
- Dr. Đurdica Hazard for giving me the opportunity to be more deeply involved in the daily life of the firearm investigation's group, as well as to better express my teaching potential in gunshot residue analysis and interpretation of firearms-related evidence during the bachelors' and masters' seminars.
- Dr. Alain Gallusser for allowing me to actively participate as invited lecturer in the teaching of a part of his course on the investigation of firearm-related crimes, as well as for always giving a thought on my opinions.
- My other colleagues and friends of the firearm investigation's group, namely Samuel Rodrigues, Damien Rhumorbarbe and Denis Werner, for the amazing moments we spent together in organising the practical work and discussing on the problems of teaching such a complex field that is the forensic investigation of firearms.
- Anne Marville and Eric Dürst for their administrative and logistic support during these years.

I would like to reserve a special thanks to my other colleagues, friends and fellow doctoral students for all the precious moments I shared with them. In particular:

- Rudolf Haraksim, who, even after several misunderstandings and disagreements in the first occasions we met, has repeatedly proven to be an unconditional friend. I thank him for all the help he provided me during my moving to London and all the crazy moments we spent together (and I hope we will spend again), drinking prosecco but never staying too late on our way back home.
- Emanuele Sironi, with whom I had more than one debate about every imaginable argument during the long 13 years (or maybe more) we have known each other, but who I considered one of the most competent people in his field and one of the most lovable people I have ever met in my life. I am sincerely grateful to him for the many fruitful discussions we had, which surely helped me to improve as a scientist and as a person.
- Frederic Béen, with whom I shared every type of moment, from the worst to the best, during the last 10 years. I would especially like to thank him for being such an indispensable support, for both moral and technical problems, a pleasant officemate and a key friend in the darkest, as well as the brightest, hours. I sincerely hope our professional paths will cross again in the future.
- My other recent and, even less recent, officemates, Agathe Barreau, Martin Fürbach and Lisa Benaglia, for simply being the most enjoyable colleagues I could have met in this adventure and, now, such valued friends.
- Sonja Bitzer, Danny Lambert, Patrick Juchli and Aurèle Scoundrianos, with whom I started this adventure a long time ago. I would like to thank them for being such helpful and lovely companions.
- All my friends, and especially the crews of the West Swiss (Florentin Coppey, Vincent Monnet, Benoit Pyronnet), Lugano (Ivan Sforza, Daniel Durante, Thomas Stefanelli) and Mendrisio (Thiago Solcà, Alessandro Ammann, Gion Camata) for all the (not so) relaxing moments, but also for their priceless moral support during these years.
- All the collaborators of ESC, especially Daniel Alvarez, Valentin Desarzens, Saman Rodriguez and Arnaud Ehrsam, for all the funny moments in the labs of ESC, as well as for the many nonsense discussions they often involved me in.
- Laurence Hamdan, for proof-reading the final version of this manuscript.
- My lovely girlfriend, Marie Gorka, for comprehensively demonstrating her patience during the last, very stressful months of my thesis, as well as all the perfect time shared together.

- Of course, my family for always supporting and believing in me. If I could reach this achievement in my life, it is especially thanks to them.

Finally, I would like to thank everyone who I forgot to cite but has anyway supported me and contributed to the achievement of this work, in one way or another.

Résumé

Estimer le temps depuis la décharge des douilles peut être une information utile dans l'investigation de crimes liés aux armes à feu. Au cours du siècle dernier, de nombreuses approches ont été proposées. Les plus prometteuses se basent sur le suivi de la diminution au cours du temps des résidus de tir volatils dans l'espace de tête (et plus particulièrement, des composés les plus volatiles, tels que le naphtalène). Au regard des bons résultats obtenus dans des applications analogues (par exemple l'estimation du temps depuis la dernière décharge d'armes à feu et de cartouches de fusil), la *solid phase micro-extraction* (SPME) suivie par de la chromatographie en phase gazeuse a été récemment suggérée pour l'analyse des cartouches d'armes de poings tirées. Cependant, des résultats insatisfaisants ont été reportés, principalement en raison de limitations analytiques (problèmes de reproductibilité et de sensibilité), de la faible sélection de composés ciblés et du choix des modèles interprétatifs.

Afin d'étudier davantage le potentiel de l'analyse de l'espace de tête pour estimer le temps depuis la décharge des cartouches d'armes de poing, une nouvelle approche a été développée dans le cadre de cette thèse de doctorat. Cette approche se fonde sur l'extraction des douilles par *headspace sorptive extraction* (HSSE), qui est une technique plus exhaustive (et donc, plus reproductible et sensible) que la SPME. Les composés cibles ont été choisis sur la base d'une étude préliminaire, et l'ensemble de la méthodologie analytique a ensuite été optimisée en utilisant une approche statistique multivariée basée sur la théorie des plans d'expérience. Les résultats ont montré de bonnes performances analytiques en termes de limites de détection et de répétabilité. Son application à des échantillons réels a révélé que les quantités de composés volatils diminuent rapidement dans les premières heures après la décharge (< 10 h), mais ils ne disparaissent pas totalement de l'espace de tête des douilles. Ainsi, en utilisant un modèle d'interprétation multivarié, il a été possible de fournir des estimations du temps depuis la décharge jusqu'à 48 h et de différencier des cartouches récemment tirées de celles âgées. A ce propos, les meilleures approches ont été une combinaison de normalisation par paires suivie d'une transformation logarithmique (i.e., *pairwise log-ratios*, PLR) et l'implémentation des paramètres de vieillissement ainsi obtenus dans des modèles *random forests* (RF) ou *partial least squares* (PLS). Les effets de plusieurs facteurs d'influence (par exemple la position de la cartouche, l'humidité et la température) ont été étudiés plus avant. Alors que tous affectaient d'une certaine manière la cinétique de vieillissement des composés, ils ne portaient pas atteinte à la précision de la datation des modèles multivariés, du moment qu'il n'y avait pas de différence significative entre le matériel indiciaire et celui de référence en question. Un modèle évaluatif a également été développé et a permis d'assigner un rapport de vraisemblance aux résultats analytiques considérant des hypothèses alternatives sur le temps depuis la décharge.

Ainsi, les résultats ont permis de montrer que l'analyse en espace de tête peut effectivement fournir des preuves utiles pour aider les praticiens à répondre aux questions concernant le temps depuis la décharge d'une cartouche. Des recherches complémentaires restent nécessaires, mais les résultats sont prometteurs vers le développement d'une méthodologie complète de datation.

Abstract

Estimating the time since discharge of handgun cartridges may be a useful piece of information in the forensic investigation of firearm-related crimes. Many approaches were reported throughout the last century. The most promising were generally based on analysing over time the decrease in the headspace concentration of dissipating compounds released during the discharge (especially, the most volatile ones, such as naphthalene). Following the good results obtained in analogous applications (i.e., the estimation of the time since last discharge of firearms and shotgun shells), headspace solid phase micro-extraction (SPME) followed by gas chromatography was recently suggested to analyse shot handgun cartridges. However, unsatisfactory results were reported, likely because of analytical limitations (reproducibility and sensitivity issues) and/or weak choices in target compounds and interpretative models.

In an attempt to further study the potential of headspace analysis to estimate the time since discharge of handgun cartridges, a novel approach was developed in this PhD research. This was based on the extraction of fired cases by headspace sorptive extraction (HSSE), which is more exhaustive (and thus, more reproducible and sensitive) than SPME. Target compounds were selected on the basis of a preliminary study, and the entire analytical methodology was then optimized using a multivariate statistical approach based on the design-of-experiments theory. Results showed good analytical performances in terms of detection limits and repeatability. Its application to real specimens revealed that the amounts of volatile GSR compounds rapidly decreased in the first hours after discharge (< 10 h), but they did not totally disappear from cartridge headspaces. Thus, through multivariate interpretation models, it was possible to provide time-since-discharge estimates up to 48 h and to differentiate recently fired cases from older ones. The best combinations of pre-treatments and multivariate regression models were pairwise ratios followed by logarithm transformation (i.e., PLR) and random forests (RF) and/or partial least squares (PLS). The effects of several influential factors (i.e., cartridge position, humidity and temperature) were further studied. While all affected in some way the ageing kinetics of the single GSR compounds, they did not affect the multivariate models' dating accuracy, as long as there was no significant difference between reference and examined material. An evaluative model was also developed and allowed assigning a likelihood ratio to analytical outcomes given alternative hypotheses on the time since discharge forwarded by the parties at trial.

Thus, headspace analysis of spent cartridges was found to provide helpful evidence to assist forensic practitioners in answering questions about time since discharge. Further research is needed, but results toward the development of a complete dating methodology, and its implementation in real forensic cases, are promising.

Table of contents

Abbreviations

cfSPME	Cold-fibre SPME
DOE	Design of experiment
DPA	Diphenylamine
FID	Flame ionization detector
GC	Gas chromatography
GPE	Gum-phase extraction
GSR	Gunshot residue
HMF	Heavy metal free
HSSE	Headspace sorptive extraction
ID	Internal diameter
IGSR	Inorganic GSR
IS	Internal standard
KNN	*k*-nearest neighbors
LR	Likelihood ratio
LC	Liquid chromatography
LD	Liquid desorption
LOD	Limit of detection
MARS	Multivariate adaptive regression splines
MS	Mass spectrometry
NLR	Non-linear regression
NN	Neural network
OD	Outer diameter
OGSR	Organic GSR
OVAT	One-variable-at-a-time
PA	Polyacrylate
PAH	Polycyclic aromatic hydrocarbon
PDMS	Polydimethylsiloxane
PLS	Partial least squares
pGSR	Primer GSR
pSPME	Planar SPME
RF	Random forest
RH	Relative humidity
SBSE	Stir bar sorptive extraction
SIM	Selected ion monitoring
SPME	Solid phase micro-extraction
SVM	Support vector machines
SX	Sinoxyd
TD	Thermal desorption
TEA	Thermal energy analyser
TLC	Thin layer chromatography
TFME	Thin film micro-extraction

CHAPTER 1

Foreword

1. Foreword

In the forensic investigation of firearms-related cases, law enforcement authorities often require evidence to link a firearm seized on a suspect to bullets or cartridges found at the crime scene. For this purpose, mark comparisons between reference and examined materials (i.e., bullets or spent cartridges) are generally commissioned [1-4]. Inferring the culpability of the firearm's owner is however a more complex process, which often requires complementary information. In fact, it is not uncommon in real cases that the validity of a spent cartridge collected on the scene is contested by the defence, stating for example that it had been fired long before the commission of the crime [5]. When faced with this kind of situation, estimating the time since last discharge may be particularly useful to reach a conclusion [5-7].

In the literature, some research has been devoted to the estimation of the time since discharge of handgun cartridges. The most promising ones were generally based on the quantification of the compounds released during the discharge (such as naphthalene) [8-10]. These compounds form the gunshot residue (GSR) and decrease over time in barrels and empty cartridges, making their residual quantity very interesting for dating purposes. Following the good results obtained in analogous applications (i.e., the estimation of the time since last discharge of firearms and shotgun shells) [11-13], headspace solid phase micro-extraction (SPME) followed by gas chromatography was tested to analyse these compounds in spent handgun cartridges as well [14]. However, unsatisfactory results were reported, which make most of the current studies discouraging concerning the possibility of implementing such procedures in casework practice. It should nonetheless be noted that, in addition to the natural complexity of the specimens themselves, various other factors linked to the development of the approach could be said to have contributed to this failure. Amongst them, we can find the extraction technique, its optimisation, the targeted compounds and the interpretation models.

In an attempt to more objectively evaluate the potential of headspace analysis of spent cases for providing helpful evidence in forensic frameworks, a more systematic study was thus carried out in this doctoral research. This passed though the implementation of alternative analytical and interpretative techniques, which were ideally more suitable for the purpose of dating spent cartridges. Especially, a novel analytical approach was developed, which involved the extraction of fired cases by headspace sorptive extraction (HSSE), a more exhaustive (and thus, better performing) extraction technique in comparison to SPME [15]. Alternative interpretative methods were also developed and tested. These were based, on one hand, on multivariate regression models for directly inferring the time since discharge and, on the other hand, on the assignment of likelihood ratios to deal with those situations where adversarial hypotheses on the time since discharge are forwarded by the parties at trial.

The research was organised as follows. First, a strategy to extract spent handgun cartridges by HSSE was developed and tested (Chapter 6). Then, the method was used to characterise the volatile GSR fractions of different types of cartridges, in order to identify the best target compounds (Chapter 7). This was successively optimised using a multivariate statistical approach based on the design-of-experiments theory (Chapter 8), and its potential to estimate the time since discharge of 9 mm cartridges was assessed using different multivariate regression models (Chapter 9). Finally, an evaluative model, based on the assignment of likelihood ratios, was developed and tested (Chapter 10).

CHAPTER 2

Gunshot residue

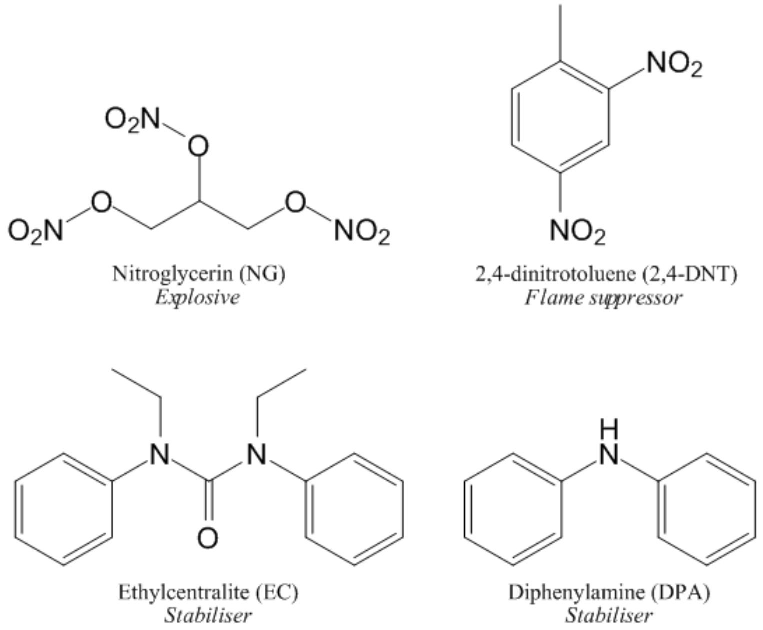
2. Gunshot residue

GSR is a very complex and heterogeneous mixture of unreacted species, explosion products and other materials [16-20]. While its formation is characteristic of every discharge event, its final composition strongly reflects the ammunition used [21]. Thus, a knowledge of the principles of the chemistry of cartridges is helpful to understand the composition of GSR, and will be presented in the next sub-chapter. An overview of the species identified in GSR and their formation mechanisms will be also proposed, highlighting those compounds that could be useful in estimating the time since discharge of firearms and spent cartridges.

2.1. Chemistry of the cartridge

A gun cartridge generally consists of four main components: the primer cup (containing the primer mixture), the propellant and the bullet, all of which are contained by a cylinder-shaped cartridge case [1, 3, 16]. Bullets are generally formed by a lead core, which can be coated or jacketed with harder metals. Several coating and jacket materials exist, but they usually are alloys of copper, zinc and/or nickel. The same metals are also used in the fabrication of the cases, although the jacket and case compositions can differ in the final cartridge [16]. Concerning the primer mixture, this is essentially a highly percussion-sensitive low-order explosive composed of initiators and oxidisers amalgamated to other supporting chemicals such as fuels and sensitizers [3, 16]. Early priming compositions mainly consisted of mercury fulminate ($Hg(CNO)_2$, initiator) and potassium chlorate ($KClO_3$, oxidiser), but they were likely to cause the rapid rusting of the barrel and were thus substituted with other less corrosive formulations [16]. Today, two main categories of primer mixtures exist: Sinoxyd-type (SX) and heavy metal free (HMF) [16, 22]. Usually, the SX primers are composed by lead styphnate (initiator), barium nitrate ($Ba(NO_3)_2$, oxidiser) and antimony sulphide (Sb_2S_3, fuel). They are slightly toxic because of the systematic presence of heavy metals. This toxic hazard is decreased in HMF primers, which usually contain diazole (initiator) and a mixture of zinc peroxide and titanium metal powder (playing the roles of oxidiser and fuel, respectively). Sensitisers typically used in both formulations are tetracene and trinitrotoluene [16, 22].

Finally, propellants are low-order explosive mixtures, which contain oxidisers along with other additives. The first propellant used in firearms was black powder, a mechanical mixture of charcoal, potassium nitrate (KNO_3, saltpetre) and sulphur [16]. Charcoal and sulphur acted as the fuel, whilst potassium nitrate supplied the oxygen necessary for combustion. However, black powder was a very inefficient explosive as it gave a heavy and hygroscopic fouling, which accelerated the rusting of the barrel and obscured the firer's view [16]). Consequently, it was gradually replaced by smokeless powders. These are extruded granular materials, which can be subdivided into single- or double-bases depending on the oxidiser they contain: pure nitrocellulose or a mixture between nitrocellulose and nitroglycerine [16, 23]. Various other chemicals are also added for specific purposes. Amongst them are stabilisers (centralites and/or diphenylamines), plasticisers (phthalates, polyester adipate and/or urethane) and flame suppressors (dinitrotoluenes) [16, 23]. Most of the chemicals which compose the smokeless powders were often reviewed in the literature (see examples in Figure 2.1) [16, 19, 24]. Nonetheless, the exact composition of smokeless powder can vary greatly from ammunition to ammunition and many different formulations have actually been commercialised. Additionally, they can also differ in the morphological characteristics of the flakes, which strongly affect their explosion kinetics [1, 16, 25].



Nitroglycerin (NG)
Explosive

2,4-dinitrotoluene (2,4-DNT)
Flame suppressor

Ethylcentralite (EC)
Stabiliser

Diphenylamine (DPA)
Stabiliser

Figure 2.1 *– Chemical structures of some compounds typically found in modern smokeless powders.*

2.2. Formation and composition of GSR

The stepwise ignition of the primer and propellant produces a large volume of incandescent gaseous materials, whose rapid expansion accelerates the bullet [1, 3]. A secondary effect of this process is the formation of GSR [16-20]. Several types of materials actually compose the GSR (Table 2.1), and these can be classified regarding their physical form/condition, and chemical nature. Thus, we can differentiate between particulate materials and free compounds, as well as between inorganic and organic species.

Physical state		***#***	***Chemical nature***		***Mechanisms of Formation***	***Mechanisms of aging***
Class	***Type***		***Organic (OGSR)***	***Inorganic (IGSR)***		
Particulate materials	Macroscopic powder flakes	I.	Powder compounds *(diphenylamine, etc.)*		Incomplete ignition	Falling off + chemical transform.
	Microscopic metallic particles	II.		Metals oxides *(oxides of Pb, etc.)*	Vaporization + condensation	Falling off
		III		Metals *(metallic Pb, etc.)*	Surfaces wrenching	Falling off
Free species	Gases	IV.		Light compounds *(CO, NO_x, etc.)*	Stoichiometric combustion reaction	Diffusion
	Condensed compounds	V.	Aromatics *(naphthalene, etc.)*		Pyrolisis and pyrosynthesis	Evaporation + diffusion
		VI.		Light compounds (H_2O)	Stoichiometric combustion reaction	Evaporation + diffusion
		VII.		Soluble ions *(nitrites, nitrates)*	Propellant degradation	Chemical transformation
		VIII.	Powder compounds *(diphenylamine, etc.)*		Powder vaporization	Evaporation + diffusion, and/or chemical transform.

***Table 2.1** – Summary of the main categories of GSR components produced by modern ammunition.*

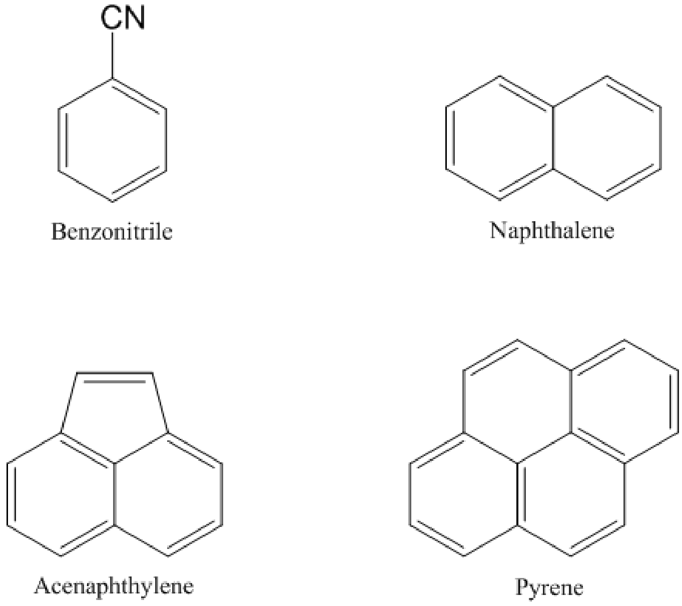
Particulate materials can be further divided into macro-particles (> 50 µm) and micro-particles (< 50 µm). As the explosion yield of the smokeless powders is never complete, unburnt and partially burnt propellant flakes are released during the discharge and compose a macro-fraction of particles found in GSR [25]. These carry with them the same organic species contained in the original smokeless powder, and their composition and aspect are thus strongly variable from case to case. Pun & Gallusser [25] exhaustively reviewed the possible morphological features of powder residues. Micro-particles, conversely, mostly come from the condensation of metals vaporised during the discharge, and, because of this formation mechanism, have a characteristic spherical form [17, 26-28]. The primary metallic source of these types of residues is the primer mixture/cup, and thus they are collectively known under the specific name of "primer GSR" (pGSR) [18, 19]. Because of the differences in the primer-mixture formulations, the actual composition of the metallic particles can greatly differ depending on the type of ammunition employed. In particular, particles formed from SX cartridges are predominantly composed of oxides of heavy metals such as lead, barium and antimony, while particles from HMF cartridges usually contain titanium and zinc as major components [23, 29, 30]. Other minor metals can also be present due to other minor compounds in the primer mixture and smokeless powder, as well as due to contaminations from the case and/or, more rarely, the firearm itself. Examples of composition have been reported in the literature [22, 29]. In addition to particles coming from condensation phenomena, some others are produced by mechanical wrenching of the cartridge and firearm surfaces due to their mutual friction, and have consequently an amorphous shape instead of a spherical form. The primary source of these sub-fraction is the bullet base, and they are thus mainly composed of metallic lead [31].

Free species can be split into gases and compounds in a condensed state (i.e., compounds which are liquid or solid at environmental temperature and pressure). The discharge of a cartridge releases very hot gases, which are produced through the stoichiometric ignition reaction of the propellant. These are mainly carbon and nitrogen oxides (CO, CO_2 and NO_x), as well as molecular hydrogen (H_2) and nitrogen (N_2) [16, 32]. Water (H_2O) is also produced, but it rapidly condenses after the discharge. Apart from these light molecules, it is well known that a significant portion of the organic molecules composing the smokeless powder does not undergo conventional stoichiometric reaction pathways, and low levels of many other heavier compounds are also found in GSR vapours. Most of them are pyrolitic byproducts, the formation of which is due to the oxygen-deficient and high-temperature conditions persisting during the discharge [33-35]. Such extreme conditions leads to the creation of radicals, which could add to other stable molecules and set in motion a waterfall mechanism resulting in the formation of larger compounds. Benzene, toluene and styrene are generally the first species to be formed by the stabilisation of unsaturated aliphatic radical species through cyclisation. Then, successive additions can lead (through further cyclisation phenomena) to naphthalene and from there to the synthesis of other heavier polycyclic aromatic hydrocarbons (PAHs). Branched or substituted derivatives of the previous compounds (e.g., methylnaphthalene) can be released as side-products; similarly, heteroatoms can take part in the reaction mechanism and be incorporated in the structure of the final products [7, 36-38]. Hundreds of PAHs from 2 up to 6 rings, substituted derivatives of benzene, and heterocyclic compounds have been identified in GSRs. Particularly, Ase *et al.* [39] identified more than 100 different traces species in the exhaust of a military rifle and an anti-tank gun. Andrasko *et al.* [11] confirmed the presence of some of them in shotgun barrels and Weyermann *et al.* [40] identified 34 compounds in two types of 9 mm Parabellum ammunition. Some of these molecules are shown in Figure 2.2.

The high-temperature conditions persisting during the cartridge discharge also leads to the rapid vaporisation of a significant amount of smokeless powder components, which deposits without having reacted on the surfaces near the firearm after the discharge [23, 41]. Finally, a fraction of the condensed GSR is composed of soluble ions. Modern ammunition mainly produces nitrates (NO_3^-) and nitrites (NO_2^-), originating from the decomposition of the powder nitro-compounds [42-44], and some traces of sulphates (SO_4^{2-}) [45-47]. On the other hand, older ammunition based on black powder left a wider variety of inorganic ions, which actually composed the larger part of the remaining fouling

[45, 47]. Most of these ions originated from potassium salts as potassium carbonate (K_2CO_3), potassium sulphide (K_2S), potassium sulphate (K_2SO_4), potassium nitrite (KNO_2) and potassium thiocyanate (KSCN) [45, 47], which were co-released with some unconsumed charcoal after the discharge.

All the inorganic compounds collectively form what is called the "inorganic GSR" (IGSR), while organic compounds compose the "organic GSR" (OGSR).



Benzonitrile

Naphthalene

Acenaphthylene

Pyrene

***Figure** 2.2 – Chemical structures of some explosion products identified in GSRs of modern ammunition.*

CHAPTER 3

Estimation of the time since discharge

3. Estimation of the time since discharge

Several approaches to estimate the time since discharge have been suggested in the literature, and their evolution has been directly correlated with the introduction of new gunpowder formulations, as well as the development of analytical techniques. Generally, they can be classified into two large categories: those based on the visual inspection of macroscopic characteristics changing over the time (e.g., rust), and those based on the quantification of the residual amount of target analytes using either wet chemistry or chromatographic methods. In this chapter, research works, both in the field of the estimation of last discharge of firearms and cartridges, are reviewed. An overview of the major approaches for interpreting analytical results is also presented in a related section.

3.1. Analytical approaches to estimate the time since discharge

3.1.1. Approaches based on visual inspection

As previously mentioned, black powder was the first propellant used in ammunition. It yielded a predominantly inorganic residue, which underwent chemical transformations (i.e., oxidation) during ageing that influenced its colour and properties [46]. Thus, all the pioneering works in the field largely exploited these physical changes to estimate the time since discharge of firearms and cartridges employing black powder. In the first investigation of its kind, published in the early 20th century, Sonnenschein [45] observed that the inner surface of a recently used barrel looked black/blue in colour, and in turn developed rusty stains and white/green crystals over time. These were initially attributed to the formation of iron oxide (Fe_2O_3) and iron sulphate ($FeSO_4$), respectively. Exploiting these transformations, the author claimed to be able to discriminate a recent use (< 24 h) from an older one (> 24 h).

A more in-depth study was later carried out by Silveira [45, 46] in 1926, in an attempt to develop a methodology for estimating the time since discharge of rifles and related cartridges. In particular, he determined that the dark colour of inner surfaces of barrels and cartridges immediately after their discharge was due to the deposition of distinct traces of carbon and iron sulphide (FeS) in the form of a black/blue crystalline mass. This turned white/green after 24 h because of the oxidation of iron sulphide to iron sulphate ($FeSO_4$), supporting the hypothesis of Sonnenschein. Being more hygroscopic, iron sulphate also absorbed moisture, which evaporated after 2 – 5 days turning the mass colour from white/green into grey. Finally, metallic iron oxidised (into Fe_2O_3) and GSR crystals further acquired a brown colour 5 days after discharge. Over time, this rusty residue continued spreading until it covered the whole metallic surface. Thus, the author explicitly suggested to check the colour of the remaining fouling, the quantity of absorbed moisture and the size of the rust spread to estimate the time since discharge. The occurrence of particular odours was also considered an important observation, given the presence of hydrogen sulphide (H_2S) in GSR during the first hours of ageing. Furthermore, Silveira claimed that all these characteristics easily allowed the discrimination of recent shots (up to a maximum of 5 – 10 days, depending on the case) from older ones, but he also pointed out that obtaining more accurate time estimates would be very difficult in casework conditions. Very similar results were obtained in more recent investigations by Sokol [45] and Lucas [47], further supporting the importance of visual inspection in estimating the time since discharge of firearms and cartridges employing black powder. Sokol, in his work on small firearms and related cartridges was, nevertheless, less optimistic than Silveira about the possible ranges of conclusions. He suggested redefining the threshold of a "recent" discharge to 3 – 6 days. Similarly, he stated that recognising shots more recent than 24 h, as done by Sonneschein, would be too hard in real casework.

With the gradual introduction of smokeless powders from the beginning of the 20th century, dating methods based on visual inspection became less important because of the significantly lower solid residue released. Moreover, smokeless powders do not form the intense residual black crystalline mass characteristic of black powder residues [47]. For these reasons, few reliable approaches based on the exploitation of physical changes were reported. In one of the rare contributions, Silveira [45, 46] noted that the residue left by smokeless powders had hygroscopic properties similarly to that of black powder. Particularly, he noticed that it rapidly adsorbed moisture in the first hours after discharge, presenting a wet aspect 12 – 24 h after discharge. The moisture successively evaporated and totally disappeared after 2 days. Thus, he proposed to date the discharge of rifles and related cartridges employing smokeless powder through the visual quantification of the humidity absorbed by the residue, in addition to the spreading of rust, which inevitably started developing 5 days after discharge.

Lucas [47] mainly investigated changes in composition and colour, and stated that no marked difference was found between fresh and older (a few days) smokeless powder residues. Thus, he implicitly concluded that dating of firearms using smokeless powders was not feasible on the basis of these features. Nonetheless, he noted that a characteristic smell was generally present after firing, which decreased in intensity over time, providing some evidence on the time since discharge. This smell could not be attributed to hydrogen sulphide (H_2S), because of the total absence of sulphides in GSR of this type of propellant. Contrary to Lucas, Sokol [45] observed that smokeless powder residues of small firearms and related cartridges, changed colour slightly from light to dark grey 3 – 5 days after discharge in barrels. However, he did not find this characteristic sufficiently reliable to provide indications of the time since discharge. He rather proposed to exploit residual humidity and rust, which he considered the only characteristics which evolved in a sufficiently rapid and reliable way.

Preliminary studies definitely showed that a change in the visual aspect of the smokeless powder GSR deposed in barrels or spent cases after firing was an unreliable criterion to estimate the time since discharge. Thus, the possibility of exploiting physical changes in characteristics other than the deposited residues was further investigated in following works. In 1968, Meier [48] presented in a conference report the general approach adopted by the *Wissenschaftliche Dienst* (WD) of Zurich in estimating the time since discharge of firearms and spent cases. He stated that a particular methodological importance was placed on checking the amount of dust deposited on external metallic surfaces (and in the barrel, if the firearm was kept vertically), given that its inspection could easily help recognising if a firearm had been recently used or not. Nonetheless, he also admitted that this type of examination was not always useful, given that no dust is deposited on firearms stored in holsters. The possible exploitation of rust was also explored, but was not considered a reliable approach because of the scarce case-to-case reproducibility and its very slow formation rate in indoor environments.

Exploiting dust deposition could be an interesting and simple method to estimate the time since discharge, but the fragility of this type of trace strongly discouraged further investigations in the specialised literature. In fact, it could be easily and involuntary removed during crime scene investigation [48]. As a consequence, many subsequent authors preferred to focus on better understanding the mechanisms underlying the corrosion and formation of rust. In 1977, in an attempt to provide evidence in a murder case, Shanahan [49] investigated the corrosion behaviour of .22 calibre brass spent cartridges buried in 50 mm garden soil during 18 weeks. He found that cases coming from the same batch showed extremely variable spread and colour of corrosion products after ageing, even if they were buried side by side. In fact, surface area coverage of rusting products ranged from 5 to 100 %. Moreover, he found that, being superficial in nature, rust traces could be easily removed by handling. Therefore, as similarly reported by Meier, the author implicitly stated that no useful information on the time since discharge could be obtained from this type of trace. This statement was largely supported by a recent work by Wogan *et al.* [50], which focused on evaluating the corrosion behaviour of handguns in aqueous environments to estimate their time since immersion. The latter authors observed that the corrosion rate and rust properties could vary greatly between different firearms, even if all were made of stainless steel. This was mainly attributed to minor differences in alloys used for their production. Furthermore, they found that the composition of the ageing medium could affect the rusting process because of the possible presence of corroding agents. Thus, they concluded that the time since immersion is not the only variable influencing the rust formation, and that other parameters should be taken into account to better model this process. Concerning specifically the ageing medium, Kerkhoff *et al.* [51] reported chloride (Cl^-) and sulphur dioxide (SO_2) as common corroding agents.

In 1986, Bridgemon [5] published a study on the formation of rust on brass cartridges stored in soil, which reported contradictory results in comparison to the aforementioned contributions. In fact, he observed that no substantial difference was noted in the rate of tarnishing between various spent cases, even belonging to different brands and calibres. Spent cartridges developed various colours after one month, but were almost totally covered by a characteristic olive-brown coloration after six months of ageing. The dissimilarity between the studies of Bridgemon and Shanahan could partly be attributed to a difference in the characteristics of the soils used for experiments: in fact, while Shanahan carried out the ageing tests in garden soil, Bridgemon set his experiments in desert sand. In any case, the latter author also studied dirt accumulation and soot disappearance as ageing phenomena for dating purposes. He thus found that cartridges deposed at ground level were totally filled with soil within a month, mainly because of air movement. Furthermore, the amount of sooting was visibly reduced within a day, and mostly disappeared at the end of the first week. Sooting never persisted more than a month. He finally concluded that an estimation of the time since deposition might be possible by exploiting all these characteristics. Nonetheless, he also admitted that all observations were qualitative by nature and other examiners would need to duplicate the study in order to provide evidence in a real case. Finally, Kerkhoff *et al.* [51] published in 2015 the results of a preliminary study on the development of corrosion after firing of cartridges and bullets made by different metals (i.e., brass, lead and steel). They observed that steel elements corroded very rapidly, while lead ones were less affected. However, no considerations were reported on variability within analogous elements, and it remains unclear if such ageing phenomena would actually be useful for dating purposes.

In addition to work directly related to the estimation of the time since discharge, a substantial number of contributions in the field of the corrosion of firearm elements was devoted to study its effect on source identification. The most recent and exhaustive work is one by Larrison [52], who focused on the investigation of corrosion of copper-jacked bullets and brass cases in open air, soil, water and a dog carcass after their firing. Results showed that corrosion in water and the dog carcass was significantly quicker and that elements may no longer be identifiable after two years of ageing in these media.

3.1.2. Wet chemistry methods

In early work on the estimation of time since discharge of firearms and cartridges employing black powder, all the analytical methodologies highlighted the importance of exploiting physical characteristics (and thus the role of the barrel/cartridge visual inspection). Wet chemistry methods were also often suggested as confirmatory methods to improve the confidence in the time-since-discharge estimates.

In his pioneering work on the estimation of the time since discharge of firearms employing black powder, Sonnenschein [45] suggested checking filtered GSR extracts (obtained by washing the barrels with distilled water after their visual inspection) for their colour, odour and presence of specific compounds. In particular, he observed that the extracts from firearms discharged within 2 h, generally presented a characteristic smell of hydrogen sulphide (H_2S) and a pale yellow colour, while older ones were darker and did not smell. The use of lead acetate, barium chloride and potassium ferricyanide were recommended for testing the presence of sulphides (S^{2-}), sulphates (SO_4^{2-}) and ferrous ions (Fe^{2+}), respectively (Table 3.1). In fact, the author noticed that sulphides totally disappeared in the first 2 h since discharge, while sulphates persisted for up to 24 h. Revelation for ferrous ions could be positive until 6 days.

Ion	***Name***	***Recommended colorimetric test***	***Temporal window***		***Authors***
			Min	***Max***	
S^{2-}	Sulphide	Lead acetate	0	2 – 5 h	Sonnenschein [45], Lucas [47]
SO_4^{2-}	Sulphate	Barium chloride	0	24 h	Sonnenschein [45], Lucas [47]
$S_2O_3^{2-}$	Thiosulphate	Silver nitrate	2 – 5 h	∞	Lucas [47]
SCN^-	Thiocyanate	Ferric chloride + mercury perchloride	No evolution		Lucas [47]
Fe^{2+}	Ferrous	Potassium ferricyanide	0	6 d	Sonnenschein [45], Lucas [47]
Fe^{3+}	Ferric	Potassium thiocyanate	28 h	∞	Lucas [47]
NO_2^-	Nitrite	Naphthylamine hydrochloride	2 – 5 h	∞	Lucas [47]
NO_3^-	Nitrate	Phenolsulphonic acid	Never detected		Lucas [47]

Table 3.1 *– Ions suggested to estimate the time since discharge of firearms and cartridges employing black powder, together with the recommended colorimetric tests to detect their presence in GSRs.*

Analogous approaches were later proposed by Lucas [47] and Sokol [45]. Lucas, in particular, reported a thorough study on the gradual appearance/disappearance of sulphides (S^{2-}), sulphates (SO_4^{2-}), thiosulphates ($S_2O_3^{2-}$), thiocyanates (SCN^-), ferrous ions (Fe^{2+}), ferric ions (Fe^{3+}), nitrites (NO_2^-) and nitrates (NO_3^-) over time. Many of his results largely agreed with those of Sonnenschein. He thus observed that sulphides normally disappeared in the first 4 – 5 h after discharge because of their gradual oxidation in sulphates (primary product) and thiosulphates (secondary product), the amount of which consequently increased over time. Furthermore, he observed that trace levels of ferrous ions were always present from the beginning, but oxidised to ferric ions during the first days after discharge. Nitrites only appeared after 4 – 5 h, as they decomposed in presence of alkaline sulphides. Nitrates were apparently never detected, contrary to thiocyanate which was initially present but did not present any reliable evolution. Tests used to semi-quantify all these ions are reported in Table 3.1. The author suggested to apply them on liquid extracts, obtained by pouring distilled water (20 mL for rifles and shotguns, and 10 mL for rifles for pistols and revolvers) down the barrel from the breech. An approach using cotton-wool swabs, which were then squeezed in order to obtain a test solution, was also proposed, but less satisfactory results were generally obtained.

The introduction of smokeless powders significantly valorised the role of wet chemistry in comparison to visual inspection in methodologies used to estimate time since discharge. This was mainly because of the minimal physical changes occurring after discharge on the inner surfaces of firearms and cartridges employing these types of propellants. Nevertheless, its use was not yet fully established at the beginning of the 20th century, and few in-depth investigations were reported.

Both Lucas [47] and Silveira [45, 46] recognised that the most important difference between smokeless powders and black powder was that the solid residue was mainly composed of nitrites and nitrates. In his investigation on rifles and related cartridges, Silveira suggested brucine and/or diphenylamine tests to semi-quantify nitrates in scratched GSR samples. The decreasing intensity of reactions over time (a consequence of the diminution in nitrates content) could provide some evidences on the time since discharge, thus allowing to discriminate recent shots from older ones. Likewise, Lucas tried to exploit changes in residue composition (i.e., nitrites, nitrates and sulphates) to date the discharge but, contrary to Silveira, he did not find any marked difference between fresh and (few days) older residues. This could be attributed to a difference in the sampling technique. In fact, while Lucas preferred performing tests on a GSR solution obtained by washing the barrel with distilled water, Silveira applied them on solid GSR samples obtained by scratching. The latter was found to be a more sensitive approach for the chemical tests.

It was not until the late 1960s, and the widespread use of smokeless powders, that more systematic work on wet chemistry methods was proposed. Meier [48] published the results of an extensive study on the quantitative evolution of nitrites in barrels and spent cartridges after shooting. His method involved the sampling of GSR with water or dry tissues, followed by the application of (unspecified) colorimetric reactions. His experiences definitely demonstrated that the composition of solid residues of smokeless powders actually changed over time, but he did not recommend exploiting them for dating purposes because of reliability issues in following their evolution.

Considering the low precision obtained when exploiting the evolution of nitrates and nitrites, Price [53] opted for a totally different (and revolutionary) approach. Thus, instead of studying the different chemical transformations which take place in the solid GSR, he proposed to follow the evolution of the residual amounts of dissipating compounds in the volatile fraction of GSR. In his investigation, he focused on carbon monoxide (CO) remaining in firearms after the discharge. Barrel vapours were aspirated in tubes containing silica gel impregnated with potassium pallado-sulphite producing a colorimetric reaction in the presence of carbon monoxide. Through this procedure, the author affirmed being able to differentiate recent discharges from less recent ones (particularly, the simple presence of carbon monoxide was found to be indicative of a recent shot). Encouraged by these promising results, Sinha [54] later proposed a method to estimate the time since discharge of shotgun shells by quantifying the dissipation rate of nitrogen oxides (NO_x). A filter paper containing a solution of L-naphthylamine and sulphonic acid was placed over the mouth of the cartridge and the time taken by nitrogen oxides to escape through the hole and develop a pink colour was measured. This was found to be correlated to the time since discharge, and could thus be used as a metric for dating estimations. The author reported that this method allowed the determination of whether a shotgun was last used within 24 h, 2 to 5 day, or 6 to 10 days before analysis.

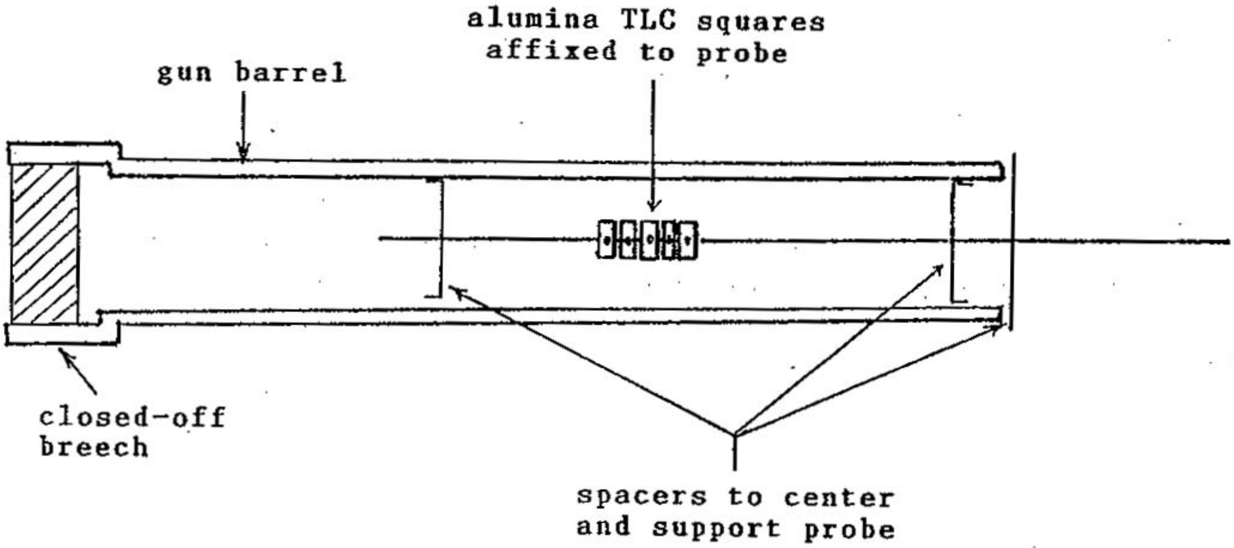


Figure 3.1 – *Schematic diagram of the analytical approach proposed by Voskertchian & Pavilova to estimate the time of last discharge of shotguns. Reproduced with permission from [55].*

The measurement of dissipating compounds was considered a very encouraging approach, but early work was based on a subjective evaluation of the intensity of colorimetric reactions. Because of this, low precision was attached to time-since-discharge estimates. Voskertchian & Pavilova suggested two analytical approaches to solve the problem. The first was based on electron spin resonance [56, 57] but did not receive much attention because of the limited availability of these devices in forensic laboratories. The second involved the use of a conventional spectrophotometer [55, 58]. The latter consisted of the application of a nitronyl nitroxide solution on small squares of plastic-backed alumina plates, which were then exposed to the inner atmosphere of the examined barrel (Fig. 3.1). In the presence of residual nitrogen monoxide (NO), a coloured product was developed and the intensity of the reaction was measured by spectrophotometry. Several plates were simultaneously inserted in the barrel and retrieved at different intervals. Thus, the reaction's kinetic rate could be measured and exploited as a metric to infer the time since discharge. The authors claimed a precision of ± 1 day on inferring times since discharge of recently (5 – 7 days) used shotguns.

3.1.3. Chromatographic methods

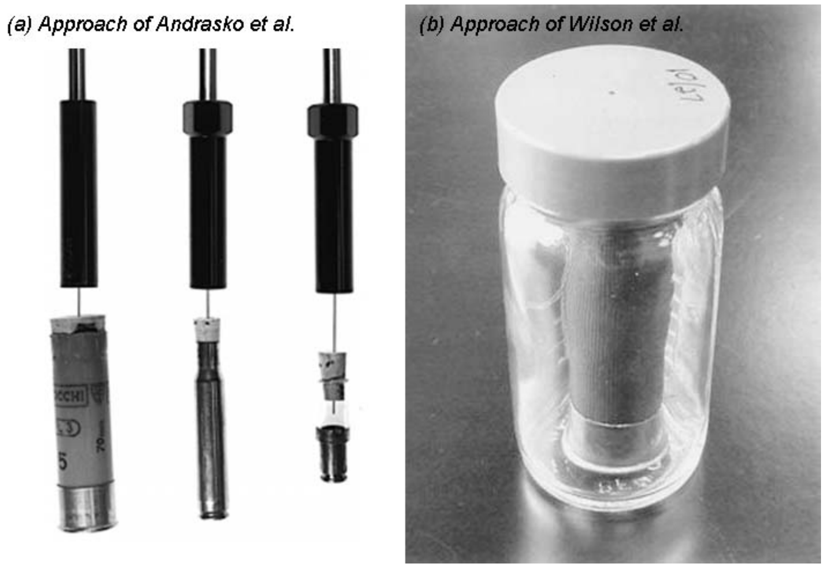
While the introduction of instrumental methods by Voskertchian & Pavilova allowed for a more objective evaluation of the analytical results, the low reliability of the sampling techniques still limited their reliability. Therefore, alternative approaches were developed using non-specific sampling methods instead of colorimetric reactions. Chromatographic steps were thus generally implemented in the analytical protocols to allow the separation and quantification of singly extracted compounds.

In a 1996 work, Aginsky *et al.* [59] first proposed to follow the nitration of the diphenylamine (DPA), deposed on inner metallic surfaces after firing, for dating the discharge of pistols and spent cartridges. According to their procedure, residues were sampled by liquid extraction with dichloromethane. Cotton clothes wetted with the solvent were used to extract DPA from pistol barrels, while cartridges were treated by immersion. Extracts were then analysed by gas chromatography (GC) coupled to mass spectrometry (MS), or by thin layer chromatography (TLC). Results showed that several mono- and polynitrated DPAs were observable in aged residues, which were usually not found immediately after the shot. However, nitration is a slow reaction and only very old residues (1 year or older) could be differentiated from "fresh" ones, limiting the practical cases where this method would be useful.

More recently, Andrasko *et al.* [11-14] further developed the idea of analysing the volatile fraction of GSR, and proposed a totally new method based on the sampling of dissipating compounds by solid phase micro-extraction (SPME). SPME is a solvent-free preparation technique which involves the use of a fused silica fibre coated with a small amount of extracting phase [60, 61], such as polydimethylsiloxane (PDMS) or polyacrylate (PA). Behaving like high viscosity rubbery liquids, these fibres can interact with different kinds of analytes and simultaneously co-extract them. In their first work in 1998, Andrasko *et al.* [11] applied the technique to estimate the time since last discharge of shotguns. The method suggested sampling volatile GSR compounds directly from the inner atmosphere of the barrels by inserting a SPME fibre at the muzzle position (Fig. 3.2). Polyacrylate was suggested as extraction coating and sampling was carried out for 30 minutes at laboratory temperature. The fibre was then analysed in GC by thermally desorbing it in the inlet. Many characteristic explosion byproducts were identified by MS, particularly a large range of PAHs. Nonetheless, the authors suggested to solely select naphthalene and an unidentified decomposition product of nitrocellulose (named “TEA2”) as target analytes, and to follow their decreases using two different GC systems coupled to a flame ionization detector (FID) and a thermal energy analyser (TEA), respectively. Partial ageing curves were thus constructed by daily analysis of the barrels, until complete disappearance of any analytical response. This allowed an estimation of the time since last discharge by comparison with a complete reference curve obtained on analogous materials. The authors admitted that no exact estimation could be provided by this methodology (mainly because of the numerous variables affecting diffusion kinetics in real cases), but claimed a good precision in discriminating recently used shotguns (2 –3 days), from those used in the last 7 – 14 days, and/or from those used beyond 21 days.

Figure 3.2 *– Sampling of a .30-06 rifle using SPME, as suggested by Andrasko et al. Reproduced with permission from [12].*

SPME had several advantages over earlier approaches. For example, being a non-specific sampling method, several volatile GSR compounds were simultaneous identified contrary to colorimetric reactions (which are usually selective for one class of compounds only). It also allowed the extraction of very unreactive molecules (such as PAHs), which represent the major class of investigated compounds amongst explosion products. These characteristics, together with instrumental detection, make it an efficient and promising technique for dating purposes. In fact, a real case application was reported for the determination of the last discharge of a shotgun [6]. Furthermore, the possibility to transfer the method to other types of firearms was investigated by the same authors in a series of successive papers. In 2000, Andrasko & Stahling [12] reported an attempt to estimate the last discharge of sporting rifles, by testing the sampling of TEA2 from both the breech and muzzle. While most of the tested rifles showed highest compound concentrations in the breech position, systematically sampling both was recommended in order to achieve a better precision. The authors thus reported the possibility of discriminating between a recent discharge (2 – 3 days) and one several weeks older. In 2003, pistols and revolvers were tested by sampling TEA2 from the muzzle position [13]. However, TEA2 could be followed only for a few days (1 – 2 weeks) and inconsistent results were obtained. Finally, the same authors reported an attempt to apply the method to spent cartridges from different types of firearms [14]. In this case, a cork was fitted at the case opening and the SPME fibre was pushed through for sampling (Fig. 3.3a). TEA2 and naphthalene were chosen as target analytes. Additionally, nitroglycerin was followed using GC-TEA. The concentration of these molecules was found to be higher in spent shotgun shells, compared to rifle and handgun cartridges. Nonetheless, as for handguns, their escape was globally very rapid and could be followed only for a few days (1 – 3 weeks, depending on the calibre) with inconsistent time-since-discharge estimates.



(a) Approach of Andrasko et al.

(b) Approach of Wilson et al.

Figure 3.3 *– Sampling of cartridges using SPME, as suggested by (a) Andrasko et al. and (b) Wilson et al. Reproduced with permission from [14] and [62].*

In order to improve the robustness of the methodology and allow its application to a larger range of cases, Andrasko *et al.*, as well as independent research groups, described further upgrades of the method. In 2003, Wilson et *al.* [62] suggested some significant modifications to Andrasko & Stahling's method for the analysis of spent shotgun shells. Having recognised that the rapid disappearance of any compound is a major problem in the analysis of spent cases, they recommended placing the latter in sealed vials, thus preventing volatile GSR compounds from escaping prior to laboratory analysis (Fig. 3.3b). The solution proved to be effective, and was generally adopted by successive studies in the field. Furthermore, they suggested to use MS instead of FID. In this way, they were able to follow naphthalene in spent 12/70 shells for 4 – 5 weeks. However, no dating approach was directly proposed, and no estimated time-since-discharge error was thus reported. Additional target analytes were studied (i.e., DPA, biphenylene and a fourth unidentified compound), but they were found to be unreliable for following shell ageing.

Successively, a research group from the *Institut de Recherche Criminelle de la Gendarmerie National* (IRCGN) published the results of their extensive study on the estimation of the time since last discharge of shotguns [7, 10]. The authors confirmed that the escape rate of volatile GSR compounds from barrels was affected by many factors. Nonetheless, they also found that more robust conclusions could be obtained by simultaneously exploiting multiple compounds in the same GC run instead of using single target analytes as done by previous works. 1-methylnaphthalene, 2-methylnaphthalene and acenaphthylene were thus exploited in addition to naphthalene, and the developed multi-residue GC-FID method allowed the recognition of recently used shotguns (2 –5 days) with enhanced confidence, from those used in the last 5 – 10 days, and/or from those used beyond 10 days. Tests on different shotgun calibres, ranging from 12/70 to .22 LR, gave similar results [7]. Furthermore, strategies to prevent the escape of volatile GSR compounds from barrels prior to laboratory analysis were tested, and the use of lead stoppers or aluminium foils proved to be effective. The suggested improvements seemed promising, and an application to a real case was reported. The same research group also attempted to transfer the method to pistols and revolvers [10]. However, similarly to previous studies, they found that the disappearance of the molecules was globally too rapid to be followed over 24 h since discharge with GC-FID. The possibility of following the decrease of stabilisers (i.e., DPA and EC) and heavy PAHs (i.e., phenanthrene, acenaphthene and fluorene) with GC-MS was investigated. Poor results were again encountered, and the authors finally concluded that the SPME method was not sufficiently reliable to estimate the time since discharge of handguns in real cases.

Major methodological changes were proposed more recently. Weyermann *et al.* [40] published the results of a study on the characterisation of the volatile GSR fraction in spent handgun cases using SPME coupled to GC-MS. Numerous new and potentially interesting analytes were identified. However, contrary to what was observed by Wilson *et al.* on shotgun shells, the authors found that a punctual SPME extraction of a spent case substantially affected the concentration of the residual volatile GSR amounts staying in the cartridge. Thus, they claimed that a sequential-sampling procedure was not applicable to estimate the time since discharge, at least not of handgun calibres. A single extraction procedure was attempted, however a poor reproducibility of measurements from cartridge to cartridge was observed, potentially affecting the reliability of the time-since-discharge estimates. Very similar conclusions were successively reported by researchers of the *Bundeskriminalamt* (BKA) in an unpublished internal study [63]. Particularly, the authors tried to upgrade the method by using a comprehensive two-dimensional GCxGC hyphenated to time-of-flight MS approach, as well as trying to optimise SPME extraction by raising the temperature. They found that an extraction temperature of 55 °C was optimal for the selected GSR compounds (i.e., 3-methyl-1-butanol, toluene, butyl acetate, hexyl acetate, cyclohexanone, benzonitrile, indene and naphthalene), but, despite these improvements, low reliable ageing curves were always obtained. More recently, Chang *et al.* [64] reported an attempt to estimate the time since discharge of spent handgun cartridges by exploiting DPA, DBP and naphthalene. SPME extraction temperature was set to 66 °C, and a multi-residue GC-FID method was used. The authors claimed to be able to recognise whether a cartridge was fired within 1 day, 5 days, 10 days, 20 days, 30 days or more. However, cartridges were aged in open vials, and this might have quenched their ageing kinetics and headspace concentrations, making the reported results not representative of a real case.

In 2012, IRCGN researchers proposed a new sampling strategy for shotguns [65]. Instead of using SPME fibres to directly sample the inner atmosphere of barrels, they suggested to swab down the barrel with a dry cotton wad, and to preferentially extract the latter after putting it in a closed vial. The method was claimed to be simpler and less time consuming, but did not allow for the application of a sequential-extraction approach, in keeping with the latest methodologies used for the analysis of spent cartridges. Nonetheless, promising preliminary results were reported. Table 3.2 shows a summary of the analytical settings of the different published works using SPME for dating purposes.

3.2. Interpretation of the analytical results

Different types of evidential information can be extracted from analytical outcomes. On one hand, the most probable time since discharge can be inferred. This approach is suited for investigative situations. On the other hand, results can be weighed against alternative hypothesis on the facts previously forwarded by the parties (i.e., the prosecution and the defence) in order to assess which of them is best supported. This corresponds to assigning an evidential value and is typical of evaluative situations.

Discharge of firearms

Authors	**Firearms**	**Coating**	**T_{ext}**	**t_{ext}**	**Detector**	**Targets**
Andrasko et al. [11]	Shotguns	PA	AT	30 min.	TEA, FID	TEA2, NPT
Andrasko et al. [6]	Shotguns	PA	AT	20 min.	TEA, FID	TEA2, NPT
LARATES [66]	Shotguns	?	AT	17 hours	FID, MS	NPT
Wohlwend [67]	Shotguns	PDMS	AT	30 min.	FID	NPT
IRCGN [7, 10]	Shotguns	PDMS	AT	30 min.	FID, MS	NPT, 2-MNP, 1-MNP, ACY
IRCGN [65]	Shotguns	PA	?	?	MS	NPT, 2-MNP, 1-MNP, ACY
Andrasko et al. [12]	Rifles	PA	AT	20 min.	TEA	TEA2
Andrasko et al. [13]	Handguns	PDMS/Carb.	AT	40 min.	TEA	TEA2

Discharge of cartridges

Authors	**Firearms**	**Coating**	**T_{ext}**	**t_{ext}**	**Detector**	**Targets**
Andrasko et al. [14]	All kinds	PA	AT	20 min.	TEA, FID	TEA2, NPT, NG
Wilson et al. [62]	Shotguns	PA	AT	20 min.	MS	NPT, BIP, DPA, Unkn
Weyermann et al. [9, 40]	Handguns	PA	AT	40 min.	MS	*(characterization study)*
Schmidt (BKA) [63]	Handguns	PDMS/DVB	55 °C	40 min.	ToF-MS	3MB, TOL, EA, CYHONE, BNZ, HA, IND, NPT
Chang et al. [64]	Handguns	PA	66 °C	21 min.	FID	DPA, DBP, NPT

Table 3.2 *– Published work using SPME-GC to estimate the date since discharge and corresponding experimental settings (T_{ext} and t_{ext} are the extraction temperature and time, respectively). AT stands for "ambient temperature". For targets compounds, the following abbreviations were used. 1MNP: 1-methylnaphthalene, 2MNP: 2-methylnaphthalene, 3MB: 3-methyl-1-butanol, ACY: acenaphtylene, BIP: biphenylene, BNZ: benzonitrile, CYHONE: cyclohexanone, DBP: dibutyl phthalate, DPA: diphenylamine, EA: ethyl acetate, HA: hexyl acetate, IND: indene, NG: nitroglycerin, NPT: naphthalene, TOL: toluene, Unkn: unidentified compound.*

3.2.1. Inferring the time since discharge (investigative models)

From a general point of view, inferring a punctual time since discharge or a temporal interval best explaining the analytical results made on examined specimens is the most classical approach adopted to interpret them. In fact, inferential models are common in the literature. Early dating approaches mainly relied on the evaluation of some nominal indicators, as the presence/absence of specific ions in the solid GSR or the characterisation of the colour of the inner metallic surfaces. Consequently, the time since discharge was estimated by comparing the qualitative assessments carried out on the examined material with those of a tabulated set of reference data. This essentially allowed discriminating a recent discharge from an older one, or eventually to classify the discharge into a given time interval (whose limits were case dependent).

The introduction of instrumental techniques allowed switching from nominal to quantitative (and thus, more accurate) indicators. Improved interpretative methods could thus be developed using more formal chemometric techniques. A first attempt to apply statistics to the estimation of the time since discharge, and especially a combined approach based on regression analysis and interpolation, was presented by Voskertchian & Pavilova [55, 58]. Particularly, they suggested to measure the daily reaction rate between nitrogen monoxide (NO) and nitronyl nitroxide using several plastic-backed alumina probes, and to plot the within-day apparent reaction constants (determined by regression analysis between the intensity of the nitronyl nitroxide colour reaction and the exposure time) as a function of the time since discharge. This allowed a partial ageing profile to be obtained, which could then be compared to a complete reference curve (i.e., an ageing profile established with the same set of firearm/ammunition as the one used in the investigated case) in order to reach a conclusion, with a normal post-assigned reproducibility of ± 1 day.

Andrasko *et al.* [11-14] adopted a very similar approach, but recommended the acquiring of partial ageing profiles by daily measurements of the residual quantity of naphthalene and/or TEA2 using SPME-GC until complete disappearance of any analytical response, and by plotting this quantity over the time since discharge. A major drawback encountered in the application of this methodology lay in the fact that the partial aging profiles were strongly dependent on the initial quantity of the measured compound. Particularly, this was considered an issues in those situations where no case-related reference cartridges were available. Thus, they suggested applying the following normalization:

$$Q_n = I_n / I_{n-1} \qquad \text{Eq. 3.1}$$

where I_n and I_{n-1} are the naphthalene peak area measured the day *n* and the day before respectively. Thanks to this solution, the normalised partial aging profiles were quantity-independent and the authors claimed that a generic reference ageing profile could be used as comparison.

In an attempt to adapt the SPME-GC method for systematic use in real cases, Persin *et al.* [7] of the IRCGN significantly modified the previous interpretation methodology by introducing a double-step and threshold-based evaluation. Following the latter, examined firearms were analysed by SPME-GC-FID at their arrival in laboratory, and, if four target compounds (i.e., naphthalene, 1-methylnaphthalene, 2-methylnaphthalene and acenaphthylene) were simultaneously detected, the last discharge was considered very recent (generally, less than 5 days). Otherwise, the decrease of naphthalene was followed daily and the partial ageing profile was compared with a reference curve, as explained above. Conclusions were given considering previously defined decision thresholds. Particularly, the comparison aging curve was divided into three intervals depending on the naphthalene decrease rate. Thus, if the first point of the partial ageing profile fell inside a specific frame, it was inferred that the last discharge was between the interval's limits (which were specific for the given ammunition/firearm system). The approach of Persin *et al.* is rather conservative and authors stated systematic use in real cases.

All the latest work in the field implemented a multi-residue chromatographic method in their protocol. This potentially allowed switching from interpretative approaches, essentially based on the consideration of a single compound at a time (i.e., univariate approaches), to the systematic exploitation of all detected analytes simultaneously (i.e., multivariate approaches). However, few works have explored this possibility. The only attempt to apply a multivariate regression method (specifically, partial least squares, or PLS) was reported by Schmidt [63] in an unpublished internal study in collaboration with the BKA using 8 target compounds (i.e., 3-methyl-1-butanol, toluene, butyl acetate, hexyl acetate, cyclohexanone, benzonitrile, indene and naphthalene). Good linearity between estimated and actual times since discharge was obtained in the first 14 days of ageing. However, the method was not validated with an independent dataset, and doubts remained about its actual efficiency in real cases.

3.2.2. Assigning an evaluative value (evaluative models)

In order to measure the evidential value of analytical outcomes and address evaluative frameworks, probabilistic approaches based on the assignment of likelihood ratios (LRs) are considered reliable methods in forensic literature [68, 69]. However, applications to shot dating were uncommon. In one of the rare attempts, researchers of the IRCGN tried to extend their interpretative methodology to evaluative frameworks, and published their model in a brief note [70]. This novel approach was always based on the split of the ageing into categorical temporal frames (< 5 days, 10 – 15 days, and 20 – 25 days), but instead of suggesting the most probable one on the basis of the analytical outcomes, probabilities were assigned under of each of them using Chebyshev's inequality theorem and a large database of previous analysis on different firearm/cartridge sets. This approach was interesting. However, many methodological and calculation errors were recognized in mathematical proofs. For example, the pertinence of Chebyshev's inequality to estimate probabilities could be contested, as it does not properly allow the determination of the probabilities of a quantitative analytical outcome itself, but rather that of obtaining values beyond its value. Moreover, results for any single GSR compound were naively multiplied together assuming independency, a premise which is surely not realistic and largely overestimates the weight of the evidence.

CHAPTER 4

Sorptive extraction

Further details on sorptive extraction theory can be found in Appendix A

4. Sorptive extraction

Analytical procedures involve several steps before obtaining a result. The main issue is that most analytical instruments have difficulties in directly handling the sample matrix. For example, a solid sample cannot be directly injected in GC. Thus, a preparation step is usually necessary to isolate the analytes of interest from the matrix [60]. Sample preparation techniques can be divided into solvent-based and solvent-free. Amongst the latter, we find the so-called sorptive extraction methods, such as SPME.

Methods for estimating the time since discharge have implemented different extraction techniques in their analytical protocols in order to study the ageing of many compounds of interest and, in this regard, sorptive extraction have been particularly exploited through the use of SPME. The fundamental principles of sorptive extraction will thus be presented in this chapter. The specifics of SPME will then be discussed, together with a critical review of its advantages and disadvantages. Alternative sorptive extraction methods will also be presented. For the readers wishing to get more details on these subjects, a more comprehensive theory on sorption extraction can be find in Appendix A.

4.1. Fundamental principles of sorptive extraction

Sorption is a physical and chemical process by which one substance takes up and holds another. It can be further split into adsorption and absorption, depending on whether the considered substance simply interacts with the coating surface or consequently migrates into its bulk [71, 72]. Given that liquid phases, such as PDMS and PA, are prevalently used as extraction coatings, extraction by absorption is more often encountered. The latter is, by the nature of the phenomena involved, a technique based on partitioning equilibrium. This is, for a given analyte, mainly controlled by the respective partitioning coefficient ($K_{p/s}$) between the coating phase and the sample phase (which is essentially the headspace of the vial in the case of the extraction of spent cartridges). Moreover, it is also dependent upon the phase ratio (i.e., the ratio between the volumes of the phase and sample, designated as β), and thus on the amount of coating phase available on the extracting support. The global relationship between these variables is shown by the following equation [73]:

$$K_{p/s} = \frac{C_p}{C_s} = \frac{m_p}{m_s} \cdot \frac{V_s}{V_p} = \frac{m_p}{m_s} \cdot \beta \qquad \text{Eq. 4.1}$$

where C_p, C_s, m_p and m_s are the target analyte concentrations and masses at equilibrium in the coating phase and the sample, respectively, and V_p and V_s are the volumes of the coating and the sample. The ratio between these volumes is the phase ratio β. The recovery at equilibrium (R), expressed as the ratio of the extracted amount of analyte (m_p) over the original amount of analyte in the sample ($m_0 = m_p + m_s$) is determined by rearranging terms in Eq. 4.1 [73]:

$$R = \frac{m_p}{m_0} = \frac{K_{p/s}/\beta}{1 + K_{p/s}/\beta} \qquad \text{Eq. 4.2}$$

From Eq. 4.2, it is then clear that the extraction efficiency increases with decreasing β, and thus with increasing V_p on extraction supports. Furthermore, it increases with increasing $K_{p/s}$. The partition coefficient $K_{p/s}$ is a thermodynamic variable which depends on the force of the chemical interaction between the analyte and the coating. Thus, it is influenced by a variety of conditions, including the chemical natures of the analyte and coating (particularly, their relative polarity), changes in temperature, derivatisation of polar functional groups, as well as the presence of organic solvents in the sample matrix. Conversely, the phase ratio β is basically a parameter which is fixed by selecting a specific vial type and coating thickness [73].

4.2. Sorptive extraction techniques

4.2.1. Solid phase micro-extraction (SPME)

By permanently applying a sorbent material to an appropriate support, sorption can be exploited to extract analytes of interest from a given matrix. The main difference amongst the different sorptive techniques mainly lies in what kind of support the sorbent material is mounted. In SPME, it is permanently attached to fused silica [60]. The latter is incorporated into a micro-syringe, and can be exposed and/or withdrawn by the movement of a plunger. When stored in the needle, the fibre is protected from physical damages and allows, for example, transportation and/or penetration of a septa. Inversely, when the fibre is exposed, the extraction of sample is allowed. As in every sorptive extraction process, SPME extraction involves two steps: partitioning of analytes between the coating and the sample matrix, followed by desorption into an analytical instrument [60]. In the first step, the coated fibre is exposed to the sample (or its headspace), which causes the target analytes to partition from the sample matrix onto the coating through the aforementioned principles. The fibre bearing concentrated analytes is then transferred to an instrument for desorption and analysis.

Given its miniaturised needle-based design, SPME is a very simple, only slightly-invasive method and, for these reasons, generated a lot of interest in analytical sciences including forensic science [74]. In fact, it allows for the storing of samples (e.g., spent cartridges) in vials, with the possibility of extracting them later by simply piercing the vial septa [40, 62]. Furthermore, it can be easily inserted into small cavities, such as firearm barrels [11-13]. Finally, it allows thermal desorption in conventional GC inlets, and, thus, the direct on-line injection/analysis in GC instruments, a characteristic which greatly decreases analysis time and complexity [60].

Despite its advantages in terms of analytical rapidity, the amount of sorptive coating on fibres is very limited, i.e. in the order of 0.5 µL for a typical 100 µm PDMS fibre. Consequently, phase ratios β are usually large, which in turn make extraction efficiency for many analytes (especially the most polar) quite low [73, 75] (Fig. 4.1). In the context of cartridge dating, this may partially explain the reproducibility issues observed in the different applications and the fact that ageing curves quickly fall below instrumental detection limits. Moreover, the development of extensive multi-residue methods composed of analytes with different physicochemical properties is particularly challenging with SPME, because of the relatively elevated selectivity of the coatings for limited groups of compounds.

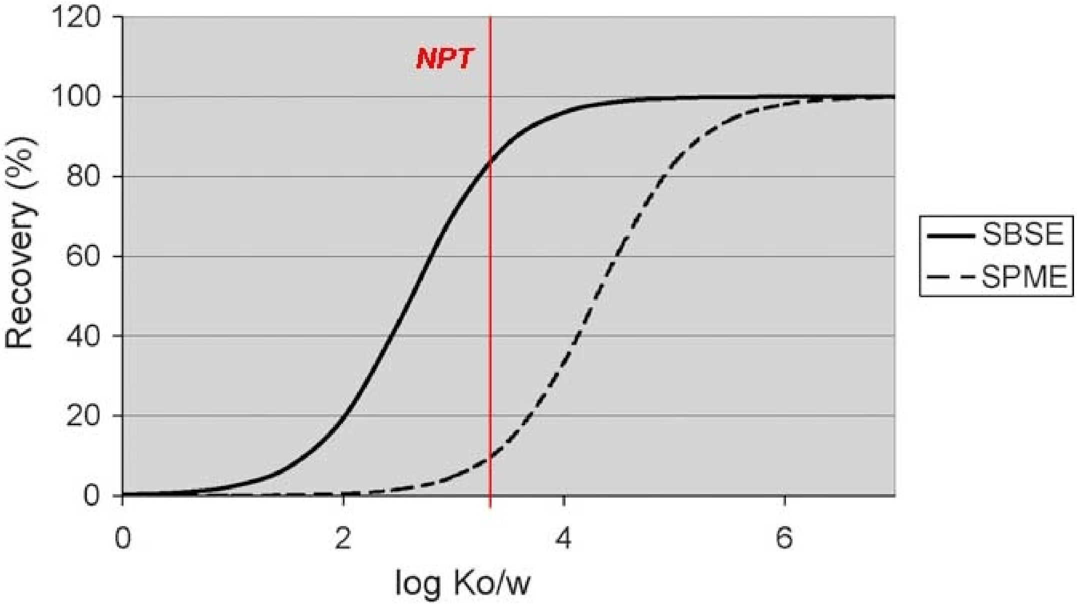


Figure 4.1 – Theoretical recovery as a function of solute polarity (here quantified with the logarithm of the octanol/water partition coefficient, log Ko/w) for SPME (PDMS, 0.5 µL) and SBSE/HSSE (PDMS, 40 µL). The red line is the naphthalene (log Ko/w = 3.3). Reproduced with permission from [73].

4.2.2. High-capacity sorptive extraction methods

In order to avoid the previously mentioned limitations, the simplest approach to increase extraction efficiency consists of improving the global capacity of the extraction technique. Referring to Eq. 4.2, a first solution to achieve this objective would be to decrease β, and thus to increase the amount of coating volume V_p on the extraction device. Unfortunately, this is not possible by keeping the same needle-based design as in SPME, since the coating volume would be too thick to be stored in such a device. Thus, alternative designs were developed, which led to the introduction of completely new sorptive extraction techniques. Some examples are thin film micro-extraction (TFME) [76], planar solid phase micro-extraction (pSPME) [77-79], gum-phase extraction (GPE) [80-82], stir bar sorptive extraction (SBSE) [83-85], and the application of the latter to headspace analysis, namely headspace sorptive extraction (HSSE) [15]. Another solution to increase capacity would be to increase $K_{p/s}$ by locally modifying the characteristics of the support. On this subject, Zhang & Pawliszyn [86] noted that the analyte absorption by a polymeric coating is an exothermic process, which is thus encouraged by low temperatures. Thus, they suggested to improve the capacity of SPME by internally cooling the coating. This idea led to the development of the cold-fibre SPME (cfSPME) [86-89].

Offering better extraction efficiency than traditional SPME, all the aforementioned improvements are very attractive for the analysis of low-concentrated samples. Nevertheless, their application is prevalently limited to very specific fields and no commercialized devices are currently available. In this regard, SBSE (and its headspace application, HSSE) represents a very interesting alternative, since it is currently the only one for which ready-to-use devices are being sold, under the commercial name “Twisters” manufactured by Gerstel.

4.2.3. Stir bar sorptive extraction (SBSE) and headspace sorptive extraction (HSSE)

SBSE was initially developed in environmental chemistry as a means to enhance the low extraction efficiencies of SPME in the analysis of aqueous samples. The original idea was, thus, to coat a glass stir bar with a very thick layer of sorbent phase (typically, PDMS) and use it to stir an aqueous solution of interest [83]. In this way, solute were able to be extracted and enriched into the coating. Good results were obtained [85, 90, 91], and the method was consequently also adapted for headspace extraction through suspending stir bars in the headspaces of the samples, a variation which is generally referred to as HSSE [15, 92].

Involving the same types of phases as SPME, the basic extraction principles of these techniques are identical. However, the coating volume on SBSE/HSSE stir bars is much larger (50 – 250 times larger than SPME) and accounts for the majority of the observed differences [73]. The total amount of extracted analytes, which is significantly higher with SBSE/HSSE compared to SPME (Fig. 4.1), is among the major advantages of the technique. Repeatability is also generally improved since SBSE/HSSE is more exhaustive than SPME.

Due to their significantly larger diameters, stir bars cannot be placed in ordinary GC inlets to recover analytes. Desorption is thus accomplished by *ad-hoc* on-line thermal devices coupled to GC instruments [83], or by liquid extraction [93, 94]. In the latter setting, analytes are back-extracted using organic solvents, a fraction of which is injected in GC or LC. Many recent publications proved the benefits of these tools for trace and ultra-trace analysis in numerous fields of analytical chemistry, such as the analysis of foods and environmental pollutants (reviewed in [73, 91, 95, 96]). Based on these considerations, the application of SBSE/HSSE to the analysis of volatile GSR fraction seems promising.

CHAPTER 5

Aims of the research and methodological approach

5. Aims of the research and methodological approach

5.1. State of the art

As reviewed in previous chapters, GSR is a complex trace, composed of hundreds of species of differing natures which, after discharge, are all simultaneously subjected to different ageing phenomena. Several approaches have been suggested in the literature to estimate the time since discharge of both firearms and cartridges. Amongst them, the most promising ones were generally based on following the decrease in headspace concentration of dissipating compounds released during the discharge (in particular, the most volatile ones, such as naphthalene) over time. Recently, headspace SPME followed by GC has been introduced as an extraction technique for these analytes. However, while promising results were obtained for estimating the time since last discharge of firearms and shotgun shells, this approach did not provide satisfying results for handgun cartridges. For example, extracted amounts of target compounds were found to rapidly fall below limits of detection (LODs) [13, 14, 40], and intra- and inter-day inconsistencies were often observed [40, 66, 67]. Thus, no reliable approach currently exists for these kinds of specimens.

In isolating the causes of this failure, the natural complexity of the specimens themselves could certainly qualify as a contributing factor. Indeed, spent handgun cases are difficult supports, which, due to their limited surfaces and small volumes, do not allow deposition of large amounts of GSR and also promote their rapid decrease. Moreover, shot-to-shot variability in released amounts of GSR compounds are surely significant, because of variations in the explosion conditions during firing and/or in the quantity of smokeless powder loads in original cartridges. Beyond these problems, however, four additional factors can be recognised as potential contributors to the scarcity of results: the extraction technique, its optimisation, the targeted compounds and the interpretation models.

Since its first implementation in the analysis of volatile GSR fraction, SPME has been the extraction technique of choice due to its simplicity, low invasiveness and non-exhaustiveness, allowing sequential sampling of the same specimen [11]. However, the low exhaustiveness of SPME is also its main drawback, as it does not allow the technique to perform well (both in terms of repeatability and sensitivity) in diluted and complex samples such as the headspaces of spent cases. To this, it should be added that comprehensive optimisation of the extraction conditions has rarely been carried out in published studies, a lack which has surely contributed to further worsening its actual extraction performances. Concerning target analytes, all works in the field essentially focused on a small set of compounds with relatively high volatility and, consequently, a low actual persistence in spent cases. Naphthalene has often been selected as a target, but was also found to disappear very rapidly from cartridges [10, 40]. Some other molecules, with higher persistence (e.g., methylnaphthalenes, biphenylene, acenaphthylene and DPA), have sporadically been tested [10, 62, 64], but no systematic investigation has ever been carried out. Finally, few studies have been devoted to the development of adequate interpretative models for analytical outcomes. Concerning the direct inference of the time since discharge, it should be noted that most published dating approaches have been essentially based on the assessment of one compound at a time through very simplistic (and often, not statistical-based) techniques; in this regard, very few attempt have been made to simultaneously consider all the available chemical information in a unique interpretative multivariate model. In particular, no chemometrical approach has ever been tried. Even worse, no convincing model to measure the evidential value of analytical outcomes and address evaluative frameworks has ever been reported. Therefore, it is difficult from the currently available knowledge to objectively evaluate the potential of headspace analysis of spent cases for providing helpful evidence in forensic frameworks.

5.2. Methodological considerations

Considering the aforementioned points, the aim of this PhD research was to further explore the possibility of extracting useful dating information from the headspace of spent cases adopting a more systematic investigative approach. The latter passed through the implementation of alternative technological innovations to both analytical and interpretative aspects. As described by Gentile *et al.* [97] and Girod *et al.* [98], the development of new technologies in forensic science must consider various aspects, which in summary pertain to:

1. the target analytes;
2. the analytical technique;
3. the optimisation and validation of the analytical procedure;
4. the ageing parameters;
5. the interpretation approaches.

As it is challenging from the currently available knowledge to identify if one (or more) of these aspects actually constitutes a weak point of the presently adopted headspace methodologies, improvements to all of them were studied and implemented. This was done in order to carry out the intended investigation in the most favourable conditions to try and reach the most objective conclusions. In the following sub-sections, the adopted methodological approach is presented.

5.2.1. Target analytes

Following the evolution of dissipating compounds has been experimentally assessed as a promising approach to follow the ageing of GSRs in many different situations [11-14]. However, until now, only compounds with low boiling points and rapid decrease rates have been tested, as they were judged adequate for estimating the time since last discharge of firearms. In this regard, it should nonetheless be remarked that GSR consists of many other volatile molecules, most of which have high boiling points, which could be more interesting to estimate the time since discharge of spent handgun cartridges [40]. A more systematic investigation of alternative target analytes in handgun GSRs has thus been carried out (Chapter 7).

5.2.2. Analytical technique

As various pertinent compounds were expected to be identified in GSRs, implementation of a method incorporating a broad range of molecules with different physico-chemical characteristics has been considered an appealing solution. The implementation of a multi-residue method has thus been deemed necessary.

Unspecific extraction approaches were evaluated in order to achieve this aim and it was decided not to discard sorptive techniques, as they were finally considered the most suitable to extract organic compounds from complex matrices [73]. In this regard, most of the previous studies in the field implemented sequential-extraction approaches using SPME. However, recent literature has proven that a significant fraction of headspace is always removed when sorptive techniques are applied to spent handgun cartridges, even SPME which is relatively lowly exhaustive [40]. Consequently, sequential-extraction methodologies were discarded, in favour of a punctual-extraction approach exploiting a more exhaustive technique. With respect to this choice, HSSE was selected as it was considered an elegant alternative to SPME to improve extraction efficiency and obtain a more comprehensive overview of ageing processes. Indeed, HSSE has been easily implemented in other analytical fields, with notable results reported [15, 99-106]. A HSSE method was thus developed in this PhD research (Chapter 6). Furthermore, the possibility of using deuterated standards spiked in vials just before extraction was evaluated to improve reproducibility (Chapter 8).

5.2.3. Optimisation and validation

A HSSE-based approach usually involves several experimental factors influencing analytical performances, especially in the extraction and desorption steps. With regard to extraction, temperature is particularly important. In fact, raising the temperature during extraction usually promotes the vaporisation of analytes and their recovery [60]. While this strategy has largely been applied in many sub-fields of analytical chemistry, and especially in the analysis of PAHs for environmental purposes [107, 108], few attempts were carried out to apply it to the forensic analysis of volatile GSR compounds. Extraction time is also usually very important [60]. Thus, the effects of these factors were investigated in this PhD research (Chapter 8). Concerning desorption, a thermal approach was adopted. Desorption temperature, time and flow, as well as cryo-focusing temperature, were consequently also investigated for their optimal conditions.

Optimisation can be undertaken according to two approaches: the traditional one-variable-at-a-time (OVAT) approach which consists of varying one factor at a time by keeping constant the remaining factors, or the multivariate approach which consists of following a precise design of experiment (DOE) [97]. As the latter involves varying multiple factors at a time, it allows for taking into account interactions between the latter and leads to a more comprehensive evaluation of the optimum conditions [109-111]. DOE was thus adopted for the optimisation of all the experimental parameters retained in this research.

5.2.4. Ageing parameters

For proper interpretation of chemical data, two factors are of extreme importance: the selection of adequate ageing parameters and the development of reliable interpretative approaches.

Ageing parameters are qualitative and/or quantitative variables extracted from analytical outcomes, which show an evolution over time and enable the extraction of useful dating evidence from spent cases. Most works in the field were based on direct use of the extracted peak areas of target compounds [7, 40, 64]. Nonetheless, data pre-processing using chemometrical approaches could provide more reliable ageing parameters and several strategies were investigated in this PhD research (Chapter 9). Particularly, as a multi-residue method was implemented, feature engineering through pairwise ratio normalisation was evaluated. Indeed, this strategy was reported to significantly correct size-effects and analytically-induced variations [112, 113] and could thus prove beneficial for cartridge-dating purposes.

5.2.5. Interpretative approaches

As previous works mainly dealt with the implementation of new analytical solutions to approach dating problems, few of them actually proposed reliable statistical models to interpret the data. Consequently, this problem was thoroughly studied in this work, where both investigative and evaluative models were approached.

Investigative models proposed in the literature to estimate the time since discharge were mainly based on univariate data (i.e., one compound at time) [7, 11, 64]. This is an inefficient method when faced with multiple analytes and, as a multi-residue was developed in this PhD research, particular focus was set on studying the potential of multivariate models (especially, non-linear ones) (Chapter 9). Thus, regression techniques such as partial least squares (PLS), multivariate adaptive regression splines (MARS), artificial neural networks (NN), random forests (RF), k-nearest neighbors (KNN) and support vector machines (SVM) were all tested.

Concerning evidence evaluation under alternative hypotheses, very few have essentially been done to date and no adequate model has currently been proposed to deal with dating problems in firearms. A preliminary, evaluative model based on LRs was thus also developed to interpret data in a legal perspective when two alternative propositions on the facts are forwarded by the prosecution and the defence (Chapter 10).

5.3. Summary of the objectives

In summary, the following objectives were completed:

- Objective 1 (Chapter 6): develop a novel HSSE extraction method for the analysis of the volatile organic GSR compounds in spent cases of handgun calibres.
- Objective 2 (Chapter 7): identify potential alternative target compounds exploitable in a high-capacity HSSE method by extensively characterising the GSRs from different types of ammunition.
- Objective 3 (Chapter 8): comprehensively optimize the method for the extraction and analysis of the most promising target analytes, and implement an internal standard approach based on deuterated standards for reducing analytical variability.
- Objective 4 (Chapter 9): study the potential of the optimised method to follow the ageing of target compounds and to estimate the time since discharge of spent 9 mm cartridges using multivariate regression models.
- Objective 5 (Chapter 10): develop a logical LR-based approach to interpret the analytical results in an evaluative framework.

CHAPTER 6

Development of a HSSE method

Mainly based on:

Gallidabino, M., Romolo, F.S., Bylenga, K., & Weyermann, C. (2014). Development of a headspace sorptive extraction method to study ageing of volatile compounds in spent handgun cartridges. *Analytical Chemistry*, 86(9), 4471-4478.

Reprinted in Appendix B

6. Development of a HSSE method

In recent studies, SPME has shown promising results for shot dating of firearms. However, reproducibility and sensitivity issues were observed for handgun cartridges [14, 40]. An alternative, more exhaustive, sorptive extraction method was thus adopted in this PhD research in order to allow a more objective characterisation of ageing processes in spent handgun cases. Headspace extraction by stir bars was selected (i.e., HSSE). In this regard, as stir bar extraction was conceptually developed as an exhaustive sorptive alternative to SPME for water analysis [83], few HSSE methods were reported in analytical chemistry, none of which focused on spent cases. Thus, the purpose of this pilot research was to develop an HSSE-based approach to extract the organic vapour fraction of GSRs in handgun cartridges after firing. The first step consisted of finding a strategy to easily sample spent cartridges and suspend stir bars in their headspace. Subsequently, a multi-residue method was developed to back-extract sampled GSR compounds and analyse them in GC-MS. Application of the method to follow the evolution of GSR in aged spent cartridges was finally carried out to evaluate the feasibility of developed approach to estimate the time since discharge.

***Figure 6.1** – Developed extraction approach.*

6.1. Development of the HSSE method

Calibres .45 ACP and .357 Magnum were selected for this investigation. .45 ACP cartridges possess large cases (an outer diameter of about 12 mm), while those of .357 Magnum cartridges are relatively long (about 32 mm). Consequently, it was assumed that an extraction strategy optimised based on these cases could be applied to most handgun cartridges.

Figure 6.1 shows the adopted approach. Cartridges were put in 20 mL HSSE-dedicated crimp glass vials. These were found to be a good solution because of the large opening (i.e., 13 mm), which fits well with most handgun calibres. Commercial stir bars produced by Gerstel (i.e., Twisters) were selected as sorptive devices. These were suspended in the case headspaces with the aid of special glass inserts. The latter prevented stir bars from falling onto the samples, and also guaranteed a good chemical stability to the PDMS coating. They were thus preferred to other published alternatives, such as magnetically attaching stir bars to metallic rods placed in the headspace of the vials. Then, vials were closed using 20 mm crimp caps.

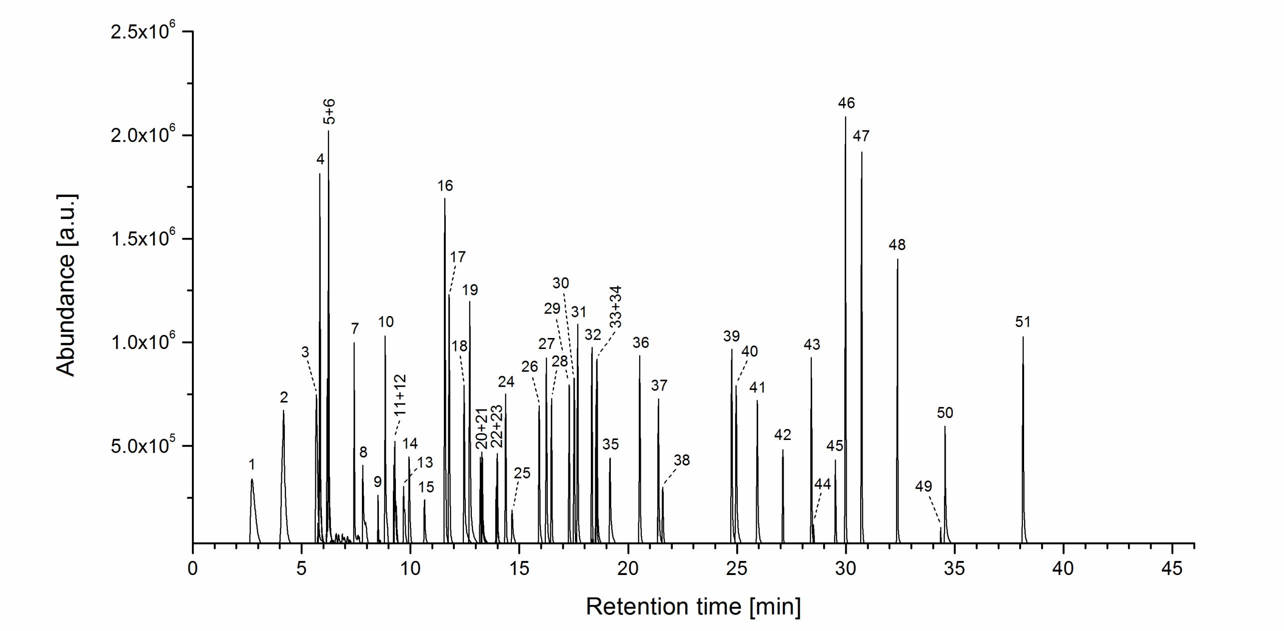


***Figure 6.2** – Superimposed extracted ion chromatograms (EICs) of the injected standards. Corresponding compounds to numbers can be found in the Appendix B.*

For back-extraction of volatile GSR compounds sampled with sorptive stir bars, thermal desorption (TD) was selected as the desorption approach because it was more rapid and exhaustive than other alternatives, such as liquid desorption (LD). TD was applied using an *ad-hoc* unit developed by Gerstel (i.e., TDU). This was connected on-line to a programmed temperature vaporisation (PTV) inlet, which was able to cryo-focus the compounds at very low temperature during desorption and perform cool split/splitless GC injection. Both apparatus were mounted on a traditional GC-MS instrument. A HP5-MS was selected as the column, given its good selectivity towards the molecules expected to be present in GSR [114-116].

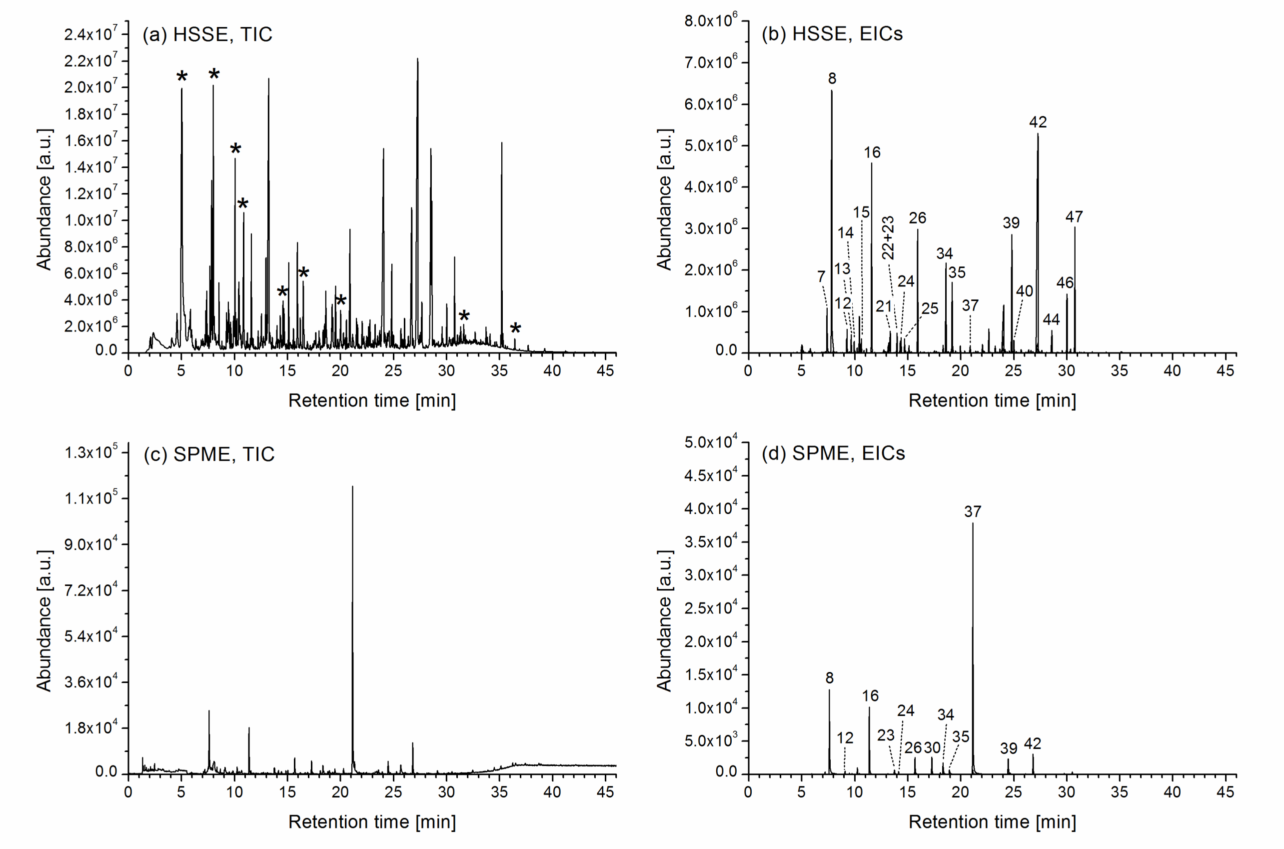


Figure 6.3 – *Total ion chromatograms (TICs) and extracted ion chromatograms (EICs) of a fired Samson .357 Magnum cartridge extracted by HSSE (TIC: a; EICs: b) and SPME (TIC: c; EICs: d), at an extraction temperature of 80 °C. EICs are given as the superimposed traces for 12 ions of particular interest. Asterisks in the HSSE TIC indicate peaks belonging to major siloxane oligomers.*

Preliminary tuning of the desorption, injection and chromatography steps was carried out using liquid standards from 55 molecules previously identified in GSR. The extracted ion GC-MS chromatograms of the liquid standards are shown in Figure 6.2. The developed method allowed the detection of 51 of the 55 injected compounds. Even if a PTV injector and a cool injection technique were used, some thermo-labile molecules could not be detected. Particularly, nitroglycerine degraded during thermo-desorption and/or in the injection port and was thus not observed in chromatograms. N-nitroso-DPA also degraded but was totally converted to DPA [117]. Co-elution of some compounds could not be avoided, but only 2 pairs of isomers (p-/o-xylene, and 1,3-/1,4-dicyanobenyene) could not be differentiated on the basis of their mass spectra. Figure 6.3a-b shows the chromatograms of a HSSE stir bar after 72 h extraction at 80 °C of a spent cartridge. Generally, HSSE chromatograms were characterised by a large number of peaks. Some of them were siloxane oligomers, which are formed during thermo-desorption by the oxidative degradation of the PDMS coating [118]. The thick coating layer also produces a slight background noise along the chromatograms. Nevertheless, given the high selective mass spectra of siloxane compounds, the additional signals did not affect the identification of target analytes.

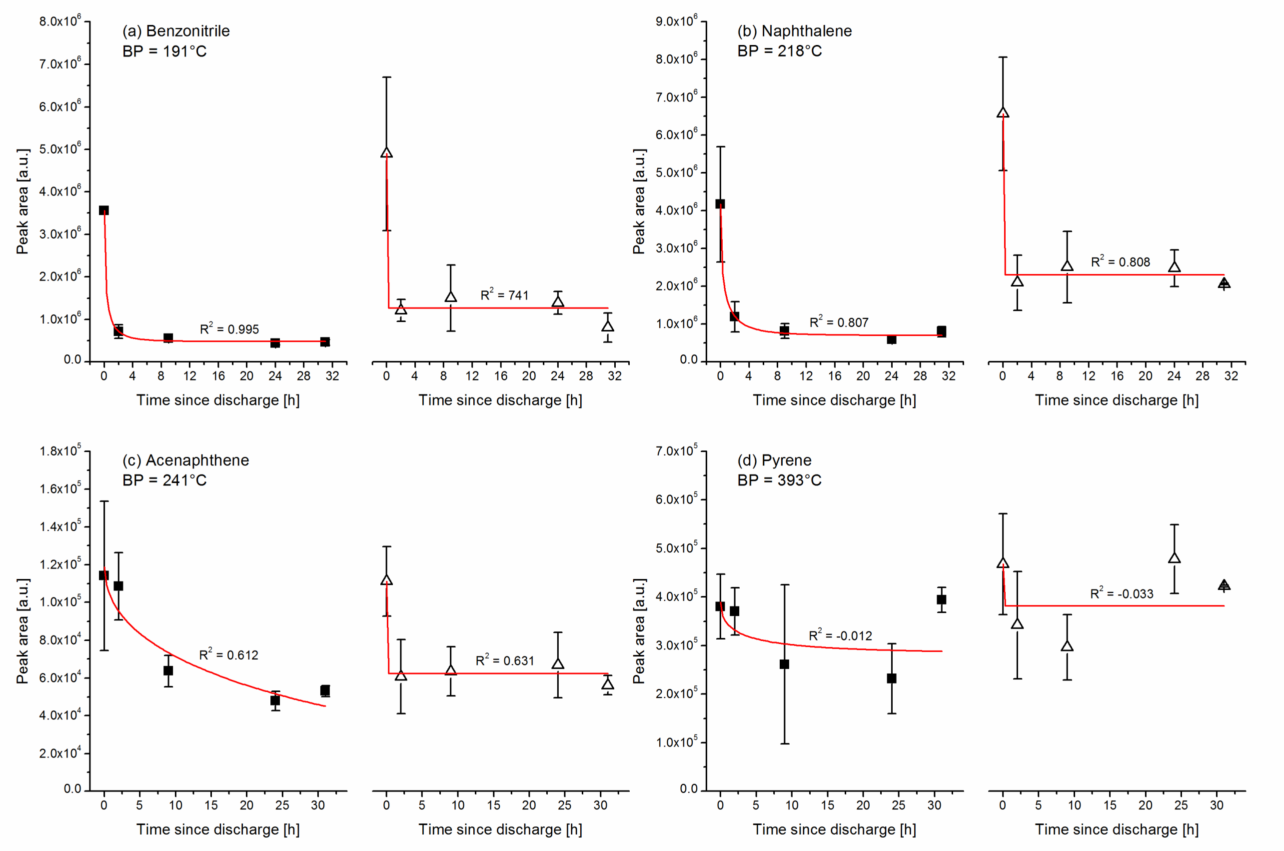


__Figure 6.4__ – Evolution of the peak areas observed by HSSE-TD-GC-MS for 4 target analytes in two types of .45 ammunition: Magtech (squares) and Geco (triangles). Measurements were fitted using non-linear regression.

6.2. Comparison to SPME

For the sake of comparison with SPME, a cartridge belonging to the same ammunition as that previously analysed by HSSE was extracted using a PA-coated SPME fibre for 40 min at 80 °C (optimized conditions) (Fig. 6.3c-d). Unlike HSSE, TICs obtained by SPME did not contain any major breakdown product. Nonetheless, significantly fewer target analytes were observed in EICs.

Inter-day RSDs were determined as a measure of intermediary precision for nine types of ammunition (always belonging to .45 ACP and .357 Magnum calibres) extracted at 80 °C for 72 h using stir bars of different batches. RSDs globally ranged from 3 to 70 %. This interval narrowed from 3 to 43 % if acenaphtylene and pyrene were excluded. RSDs for SPME previously reported in literature for the extraction of 9 mm Parabellum cartridges were 71% for naphthalene and 157 % for benzonitrile. The same compounds extracted by the developed HSSE method yielded RSDs between 5 – 34 % and 9 – 33 %, respectively, which were largely better. Thus, while SPME was less intrusive than HSSE, it was also less sensitive and reproducible. HSSE represents a promising alternative extraction method for dating purposes.

6.3. Ageing of volatile GSR compounds

The evolution of the detectable target compounds as a function of time was followed during a period of 31 h with two different .45 ACP ammunition from Magtech and Geco, loaded with a single- and a double-base smokeless powder, respectively. Most of analytes significantly diminished over time (Fig. 6.4). Geco ammunition generally gave faster and less reproducible ageing profiles compared to those obtained from Magtech for the same molecules. In fact, analytes in Geco cartridges globally levelled off 2 h after discharge, whereas ageing in Magtech ammunition appeared to be more variable. A pre-treatment approach was tested to stabilise ageing trends and reduce the problem of data variability. This was based on the pairwise ratio normalisation between extracted compounds. As it was assumed that substances simultaneously produced during discharge were affected by the same variability-introducing factors, only these were normalised to each other. Spearman rank correlation was used to recognise this dependency. Among 1327 pairs of compounds, 407 (about 31 %) were found to be strongly correlated (> 0.7) in at least one type of cartridge. In many cases, the ageing profiles of the ratios presented less variability and slower decrease rates in comparison to those of the single compounds. A representative example is shown in Figure 6.5, where the ageing profiles of acenaphthene and phenanthrene are reported for the two tested types of ammunition, along with those of their ratios. Thus, coefficients of determination (R^2) after regression analysis were clearly better for the ratios than the original compounds. Furthermore, the decrease rates for the ratios were slower and actually presented a rather linear trend. This is an obvious advantage for dating purposes given the absence of a levelling off. Generally, of the 407 ratios tested, 154 (38 %) and 264 (65 %) presented slower decrease rates than both their composing molecules in the Magtech and Geco ammunition, respectively.

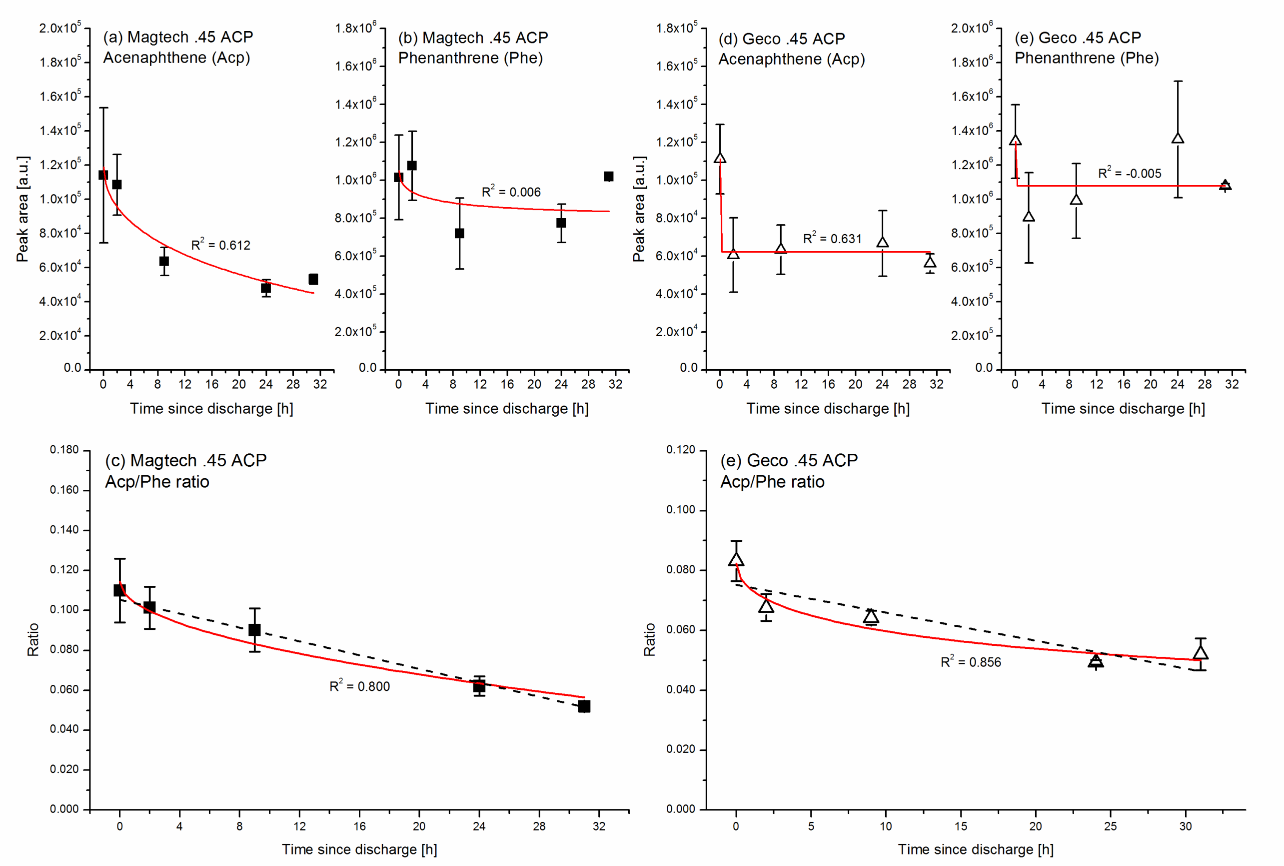


Figure 6.5 *– Evolution over time of selected compounds and their respective ratios in two .45 ACP cartridges. Solid lines are the regression curves obtained by fitting the data with non-linear regression, while dashed lines are the curves obtained by simple linear regression.*

6.4. Chapter conclusion

A novel approach based on HSSE extraction followed by TD and multi-residue analysis in GC-MS was developed to comprehensively follow the ageing of volatile GSR organic compounds in handgun cartridges. The method was tested on 55 compounds expected to be present in GSR, 51 of which could eventually be detected. Compared to SPME, HSSE was found to be more reproducible and effective, allowing simultaneous analysis of more compounds when applied to the same cartridge. The method was finally employed to follow the evolution of volatile GSR compounds in two types of ammunition. Results showed that many compounds presented noticeable ageing profiles. Pairwise ratio normalisation was also tested and proved to be beneficial both in reducing the variability of the ageing profiles and for increasing the observable ageing interval. These results were thus encouraging toward the use of HSSE-TD-GC-MS for the analysis of volatile GSR fraction and their application in shot dating. The method should now be optimised and validated based on selected compounds.

CHAPTER 7

Characterisation of volatile GSR compounds in fired cartridges and selection of targets

Mainly based on:

Gallidabino, M., Romolo, F.S., & Weyermann, C. (2015).
Characterisation of volatile gunshot residues in fired handgun cartridges by headspace sorptive extraction. *Analytical and Bioanalytical Chemistry*, 407(23), 7123-7134.

Reprinted in Appendix C

7. Characterisation of volatile GSR compounds in fired cartridges and selection of targets

Most of the previously published works on the estimation of time since discharge were based on the selection of certain target analytes. Naphthalene and the unidentified TEA2 compound were regarded as promising in dating shotguns and rifles [11, 12], but their usefulness was questionable for handguns and related cartridges [10, 13, 14, 40]. Their rapid disappearance and the low reproducibility of detected amounts between cartridges were thought to be contributing causes. Consequently, alternative target analytes must be considered.

While other volatile organic compounds have been identified in GSR [11, 39, 40], very few untargeted investigations have been performed, and in any case none using sorptive high-capacity extraction techniques. The lack of exhaustive investigations on the composition of volatile GSR opens the possibility to exploit additional target compounds pertinent to dating time since discharge using an HSSE-based approach. Thus, the composition and variability of volatile GSRs produced by different types of ammunition has been comprehensively studied in this chapter. Intra-variability (i.e., variability within the same firearm and cartridge set) and inter-variability (i.e., variability between different firearm and cartridge sets) between each detected compound were also evaluated, as well as their evolutions over time.

7.1. Identification of volatile GSR compounds

Spent cartridges from nine types of ammunition belonging to two handgun calibres (.357 Magnum and .45 ACP) were extracted at two different temperatures (20 and 80 °C). Use of different extraction temperatures allowed the study of compounds with different properties, i.e., high-volatiles (at 20 °C) and semi-volatiles (at 80 °C). The largest PDMS-coated HSSE stir bars available (2 cm length with a 1 mm thick coating, corresponding to a PDMS volume of 110 µL) were used, in order to guarantee the best recoveries even for the more polar analytes [119].

Extracted volatile GSR fractions were mainly composed of explosion products, but unaltered smokeless powder additives and other contaminations were also identified. Globally, 166 compounds were recurrently found in the different cartridges. A high variety of aromatic molecules was observed, and most of them were explosion products or smokeless powder additives. In fact, 109 of the 166 recurrently detected compounds have at least one aromatic ring in their structure. PAHs were the most diversified group. Non-branched and branched PAHs with up to 5 rings were identified (the most light was indane, while the most heavy benzo[a]pyrene). Branched PAHs were prevalently derivatives of napththalene. Derivatives of benzene were also very common between the different GSRs.

Several molecules containing hetero-atoms were observed. Amongst them, nitrogen-containing PAH homologs and aromatic nitriles were relatively diversified. This large number of nitrogenized species is compatible with the characteristics of the exploding material, as smokeless powders are rich in nitrogen-containing substances, such as nitroesters (i.e., explosives) and aromatic amines/ureas (i.e., stabilisers). Substances containing other hetero-atoms, mainly sulphur and oxygen, were nonetheless identified. Sulphur probably originates from primer mixtures rather than smokeless powders, as most producers currently use antimony sulphide to produce priming mixtures [16].

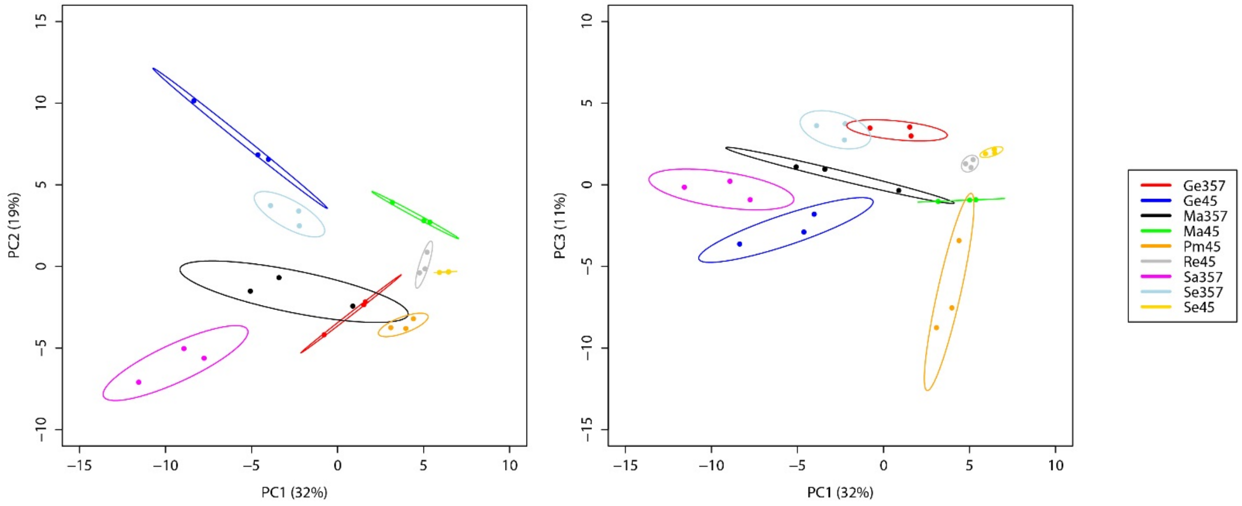


Figure 7.1 *– Plot of the first three principal components (PC) after principal components analysis (PCA) of GSR data (extracted at 80 °C). Only data concerning identified explosion products and smokeless powder additives were analysed. Explained variance of each PC is reported in brackets.*

In addition to aromatic molecules, several aliphatics were observed in GSRs. A large fraction was composed of linear alkanes and alkenes, respectively from C11 to C24, and from C10 to C17. Branched alkanes such as pristine and phytane were also detected. It is very unlikely that all these compounds were produced during the explosion. In this regard, the heptadecane/pristane and octadecane/phytane pairs may indicate the presence of heavy petroleum distillates, such as mineral oils [120]. Thus, this group of compounds might be ascribed to the contamination of spent cases by lubricants during the production process [121].

7.2. Characteristics of GSRs

Of the 166 identified compounds, 141 were present in the GSRs of every cartridges, making their qualitative compositions very similar. Nonetheless, proportions between compounds significantly varied, and the actual characteristics of each GSR were very different. This can be easily visualised using principal component analysis (PCA) (Figure 7.1). GSRs belonging to different sources could be fairly discriminated, even if some of them shared quite similar compositions (i.e., Re45 and Se45). In order to better appreciate these differences, proportions of selected compounds were plotted in Figure 7.2. No clear correlation between the characteristics of the GSRs and those of the respective smokeless powders were determined, but it was found that smokeless powders with different compositions could produce similar GSRs, and vice-versa. It can thus be deduced that other factors may play an important role in the GSR formation (e.g., the pressure and temperature conditions during the discharge) [35, 122].

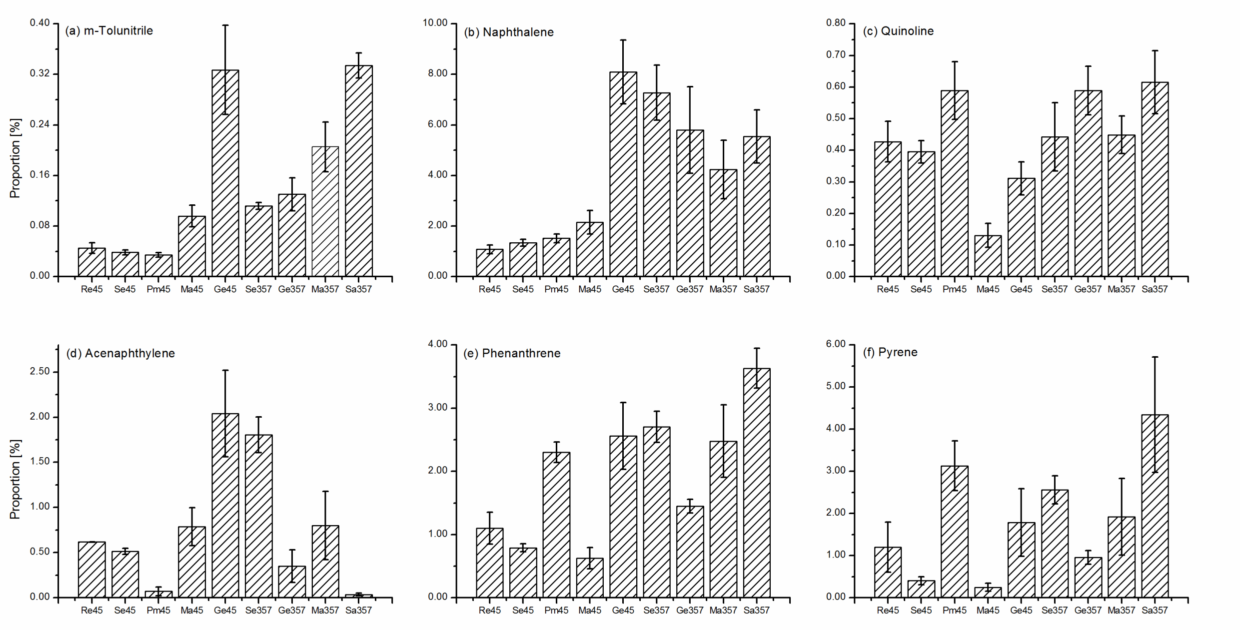


Figure 7.2 – *Proportions of certain selected compounds amongst cartridges.*

7.3. Intra- and inter-variability of single compounds (t = 0 h)

Target analytes for dating purposes should fulfil the following criteria:

1. always be present in the GSRs released from every cartridge/firearm system;
2. ideally, be present in high amounts;

3. show a high reproducibility within the discharges of the same cartridge/firearm system;
4. show a high reproducibility between the discharges of different cartridge/firearm systems;
5. have a significant evolution over a relevant range after the discharge.

These characteristics were further investigated for the group of analytes solely composed by the explosion byproducts and smokeless powder additives identified in GSRs.

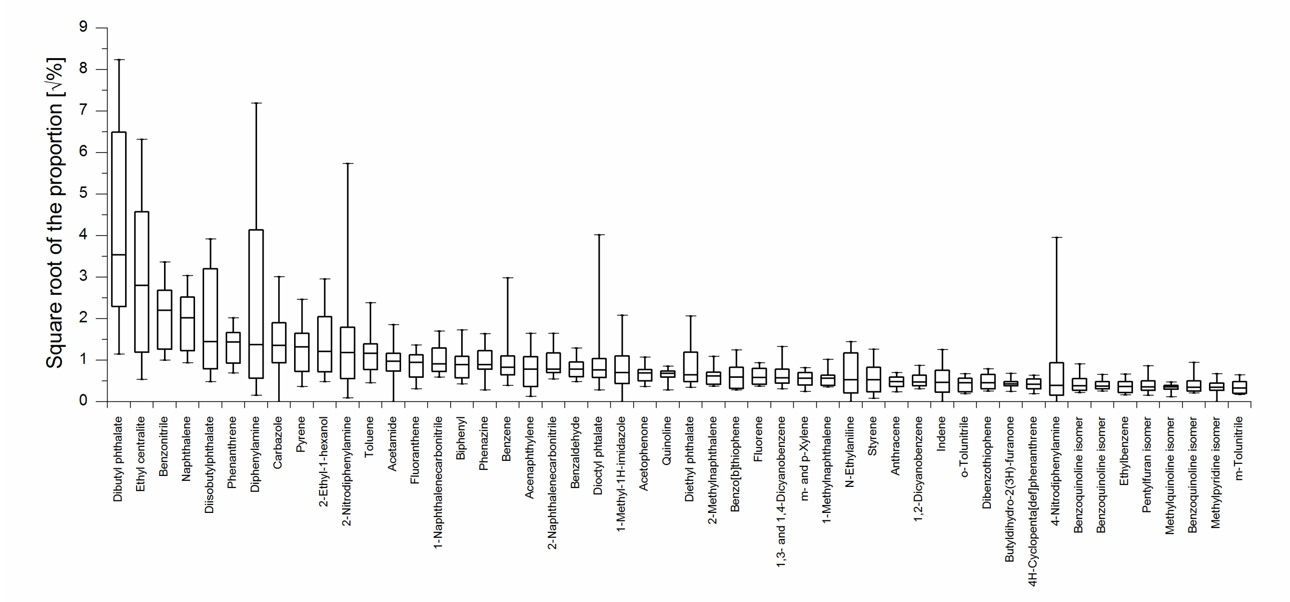


Figure 7.3 *– Distribution amongst GSRs of the 50 most abundant explosion products and gunpowder additives (after extraction at 80 °C). To improve graph legibility, the units of the y-axis are given as the square root of the proportion of the total sum of peak areas.*

Concerning variability, only a few explosion byproducts or powder additives were not observed in all GSRs, these were mainly the heaviest PAHs (chrysene and benzo[a]pyrene), 1,4:3,6-dianhydro-α-D-glucopyranose, toluene, phenyl thiocyanate, 1,2,3,4-tetrahydro-1,1,6-trimenthylnaphthalene, isoquinoline, nitrotoluene, 2,4-dinitrodiphenylamine isomers, 1-methyl-3,3-diphenylurea (akardite II) and dioctyl phthalate. Figure 7.3 shows the distribution of the remaining compounds amongst the cartridges. It is noteworthy that smokeless powder additives generally composed a large fraction of the GSRs and were the most abundant substances in chromatograms. Particularly, dibutyl phthalate, ethylcentralite, diisobutylphthalate, diphenylamine, 2-ethyl-1-hexanol and 2-nitrodiphenylamine were, on average, the 6 most abundant smokeless powder additives detected in GSRs. Proportions of explosive products were smaller, but still significant for most of them. In fact, benzonitrile and naphthalene represented a relatively large fraction of each analysed GSR, followed by phenanthrene, carbazole, pyrene, fluoranthene, biphenyl, acenaphthylene. Consequently, many aromatics with up to 4 rings could be quantitatively detected after the discharge of all types of ammunition analysed.

Intra- and inter-cartridge RSDs were investigated in more depth (Fig 7.4). Proportions of smokeless powder additives were slightly less reproducible within the different sources than those of explosion products (median intra-cartridge RSDs of 39 and 24 %, respectively). Furthermore, the proportions of smokeless powder additives also showed higher inter-variability than those of explosion products. In fact, the amount of smokeless powder additives in GSRs often ranged from trace to major-compounds levels depending on the types of ammunition (median inter-cartridge RSD of 121%). For comparison, the median inter-cartridge RSD obtained for compounds associated to explosion products was 75 %.

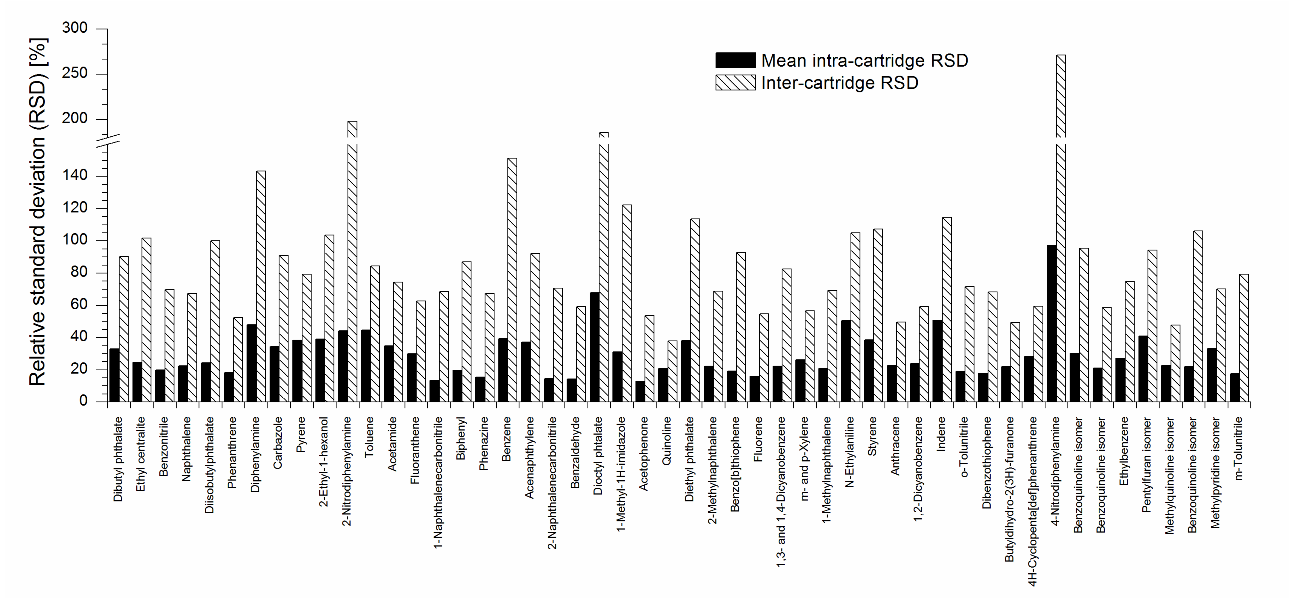


***Figure 7.4** – Comparison of mean intra-cartridge RSDs and inter-cartridge RSDs amongst the different cartridges for the proportions of the 50 most abundant explosion products and gunpowder additives identified in GSRs (after extraction at 80 °C).*

7.4. Evolution over time (t > 0 h)

The evolution of the compounds as a function of time was also investigated. Several molecules decreased and levelled-off during ageing, indicating at least two distinct phases in the diffusion of GSR compounds over time. In the first ageing stage, the decrease was generally rapid. High amount of vapours were released during discharge and the excess was rapidly lost because of the important gradient formed between the internal and external atmospheres. Then, equilibrium between compounds in the vapour phase and those deposed on surfaces was reached, making their decrease slower in this second phase. Because of this non-linear diffusion trend, most of the substances were still detected 31 h after discharge, making the difference between GSR at $t = 0$ h and $t > 0$ h essentially quantitative and not qualitative.

Specific rates of decrease were found to be dependent on the nature of the considered compound. On one hand, the different smokeless powder components seemed to remain essentially constant over time, or at least, the patterns did not follow any recognisable ageing trend. Given that these compounds come from powder flakes before firing, their high persistence can be down to a difficulty in escaping from the flake residues after discharge. On the other hand, most of the explosion products significantly diminished over time and their decrease rates were actually found to be correlated with their boiling points (BPs). For example, naphthalene (BP = 218 °C) diminished quickly from spent cartridges (70 % of its signal was lost in the first 2 h after discharge), whereas acenaphthene (BP = 279 °C) decreased more slowly and did not yet level off after 31 h. Figure 7.5 shows this dependence by plotting decrease rates versus the GC retention times for selected compounds.

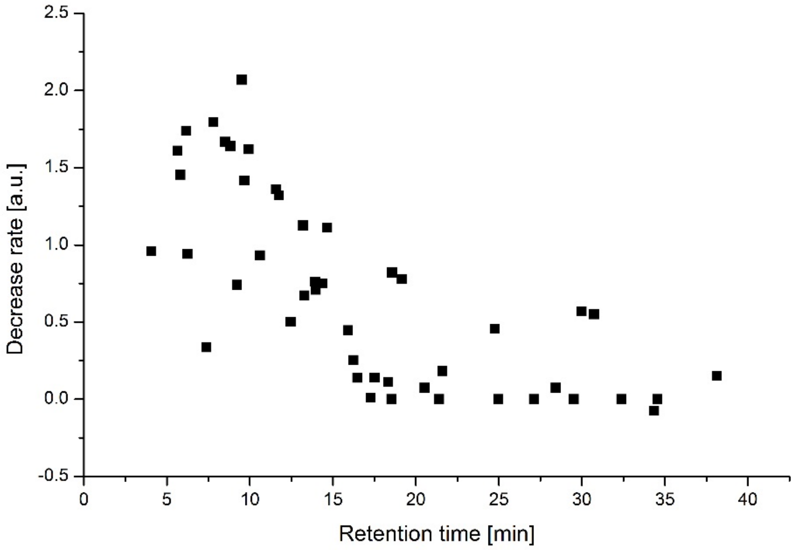


***Figure** 7.5 – Rates of decrease (determined by non-linear regression) for selected analytes detected in the Magtech .45 ACP spent cartridges versus their respective retention times (tR).*

7.5. Choice of target compounds

Target compounds finally retained and included in the final method are listed in Table 7.1. This list includes 26 explosion products, which were selected in order to cover the broadest permissible range of rates of decrease and, thus, provide complementary pieces of information about the age of cartridges. Even if not presenting noticeable ageing behaviours, 3 smokeless powder additives (diphenylamine, ethylcentralite and dibutyl phthalate) were also included for the purpose of characterising the nature of the smokeless powder used by the cartridges submitted to analysis.

#	***t_R***	***Compound***	***BP [°C]***
1	*5.899*	*Benzonitrile*	191
2	*6.647*	*Indene*	182
3	*6.951*	*o-Tolunitrile*	205
4	*7.245*	*m-Tolunitrile*	213
5	*7.444*	*p-Tolunitrile*	218
6	*7.961*	*Benzyl nitrile*	234
7	*8.763*	*Naphthalene*	218
8	*9.704*	*Quinoline*	238
9	*10.109*	*Isoquinoline*	243
10	*10.139*	*1,3-Dicyanobenzene*	?
11	*10.161*	*1,4-Dicyanobenzene*	?
12	*10.746*	*Indole*	254
13	*10.804*	*1-Methylnaphthalene*	245
14	*11.148*	*2-Mehtylnaphthalene*	241
15	*11.379*	*1,2-Dicyanobenzene*	?
16	*12.529*	*Biphenyl*	256
17	*14.026*	*Acenaphthylene*	270
18	*14.178*	*Biphenylene*	?
19	*14.791*	*Acenaphthene*	278
20	*15.102*	*1-Naphthalenecarbonitrile*	299
21	*15.580*	*2-Naphthalenecarbonitrile*	305
22	*16.836*	*Fluorene*	298
23	*17.517*	*Diphenylamine*	302
24	*19.719*	*Phenanthrene*	340
25	*19.835*	*Anthracene*	351
26	*20.977*	*Ethylcentralite*	?
27	*21.591*	*Dibutyl phthalate*	340
28	*22.561*	*Fluoranthene*	384
29	*23.020*	*Pyrene*	393

***Table 7.1** – Promising compounds for dating purposes implemented in the final method, together with their retention times (t_R) and boiling points (BP).*

7.6. Chapter conclusion

A characterisation study was carried out using the developed HSSE approach on 9 different types of ammunition, in order to identify interesting compounds for dating. Globally, 166 molecules were identified. It was observed that a significant proportion of the volatile GSR fraction was dominated by vapours of smokeless powder compounds. Nonetheless, numerous explosion byproducts were also identified. Aromatics (i.e., benzene derivatives and PAHs) were a particularly varied group, and molecules up to 5 rings were detected. The behaviours of the single compounds were further studied. Following this, smokeless powder additives were found to be unsuitable for dating because the released amounts of additives were less reproducible between and within types of ammunition. Furthermore, additives did not show any significant decrease over the first day of ageing. Explosion byproduct, conversely, are suitable target compounds for dating, as most of them were more reproducible and showed recognisable ageing behaviours. Thus, in addition to naphthalene, many other analytes with different physico-chemical characteristics were found to be interesting and could provide complementary evidence about cartridge age. 29 analytes were finally selected as targets to be included in the final HSSE method.

CHAPTER 8

Optimisation of the HSSE method and ageing study of target compounds in 9 mm cartridges

Mainly based on:

Gallidabino, M., Romolo, F.S., & Weyermann, C. (2016).
Time since discharge of 9 mm cartridges by headspace analysis, part 1: comprehensive optimisation and validation of a headspace sorptive extraction method (HSSE). *Submitted to Forensic Science International*.

Reprinted in Appendix D

8. Optimisation of the HSSE method and ageing study of target compounds in 9 mm cartridges

As a HSSE-TD-GC-MS method involves several experimental parameters which can affect final performance, detailed optimisation is generally a mandatory step in order to minimise the analytical sources of variability. This was the purpose of the following chapter. Furthermore, in an attempt to further improve reproducibility, a spiking approach was also implemented using deuterated internal standards (ISs) introduced directly in the sampling vials before extraction. The following parameters were optimised: the type of HSSE stir bar, the spiking method (solvent type and volume), the extraction conditions (temperature and time) and the thermal desorption conditions (desorption temperature, time, gas flow and cryo-focusing temperature). A DOE-based approach was applied and a surface-response methodology was exploited to select the optimal analytical conditions [109-111]. Finally, the optimised method was used to study the evolution of the target analytes over time in 9 mm Parabellum cartridges. The impact of varying the cartridge storage conditions on ageing profiles was also investigated (i.e. cartridge position, temperature and humidity), in order to preliminary identify those factors which should be taken into account for interpreting outcomes in a forensic context.

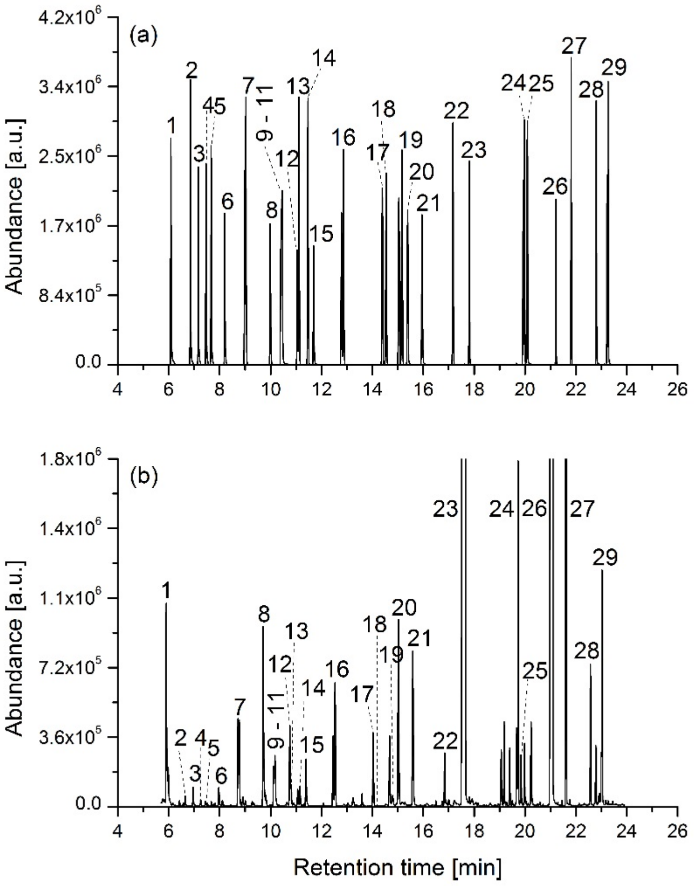


***Figure 8.1** – Examples of chromatograms for (a) the direct injection of liquid standards with the optimised separation method, and (b) a 9 mm spent cartridge from Geco, analysed after 1 h of ageing. Correspondence between numbers and compounds is reported in Table 7.1.*

8.1. Optimisation of the GC-MS method

29 analytes were selected as targets on the basis of the observations carried out in Chapter 7 (Table 7.1). The GC temperature ramp was optimised by liquid injection of a mixed solution of these compounds, deuterated analogues and common contaminants. A method with a run time of 23.9 min was developed (Fig. 8.1), which consequently brought an approximately two-fold improvement in terms of run time in comparison to the previously developed method. Furthermore, a selected ion monitoring (SIM) method was implemented as the MS mode, in place of the previous scan-based method (Chapter 6). One target ion and two qualifiers were then selected for each analyte (see Appendix D for details).

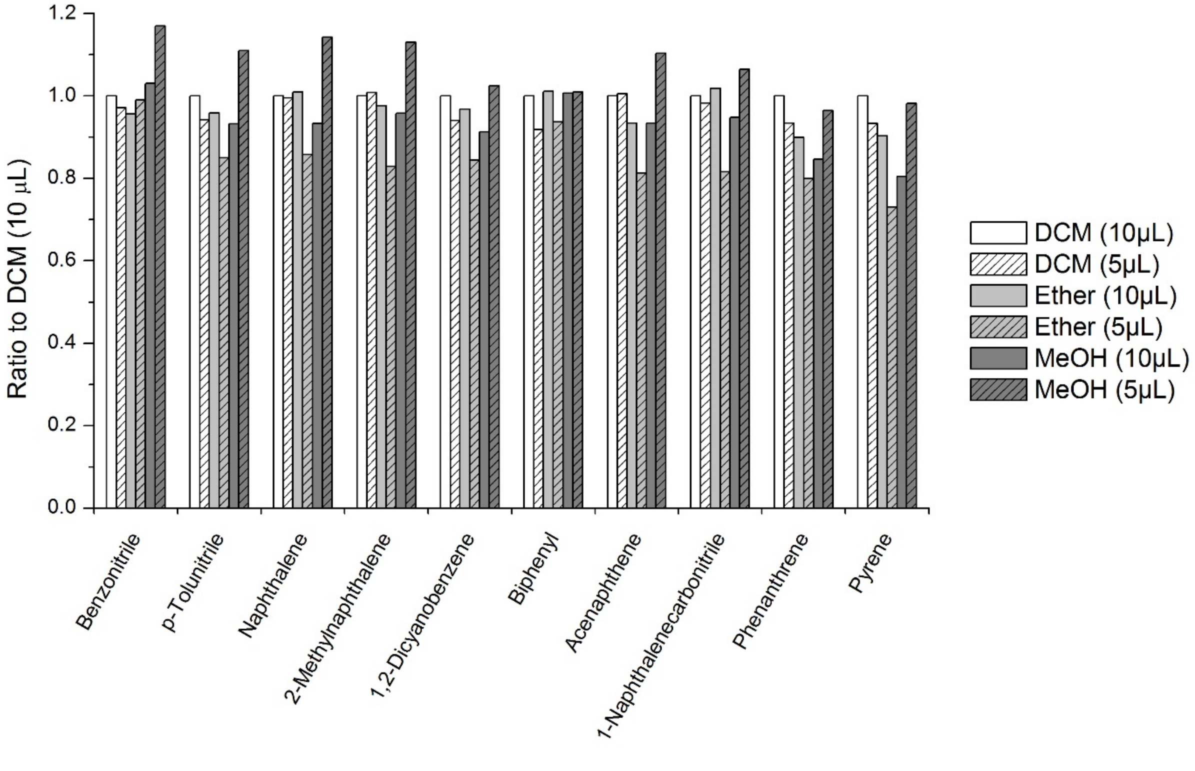


Figure 8.2 *– Observed responses for selected compounds after HSSE extraction of samples spiked using different carrier solvents and volumes. Values are normalised by those observed using 10 μL of dichloromethane (DCM).*

8.2. Optimisation of the spiking strategy

The developed IS method involved spiking a mixed solution containing 5 ng of each standards into the vials after cartridge sampling, but just before extraction. In this regard, 5 deuterated molecules were selected as ISs: naphthalene-d8, biphenyl-d10, acenaphthene-d10, phenanthrene-d10 and pyrene-d10. The solvent used for spiking was found to affect the chromatographic performances, and was hence optimised (type and spiking volume). This was particularly important as the same strategy was also adopted to spike known amounts of target compounds for the purpose of optimisation of the extraction and desorption steps. 10 µL and 5 µL spike volumes of dichloromethane, diethyl ether and methanol were investigated. Globally, peak shapes and resolutions were not affected by changing the solvent, but detected amounts significantly varied (see Figure 8.2). 10 µL spikes in diethyl ether gave similar responses to those in dichloromethane, but halving the volume also caused a significant decrease in the compound responses. Consequently, volume reduction was not possible with this solvent. Best results were obtained by using 5 µL spikes in methanol.

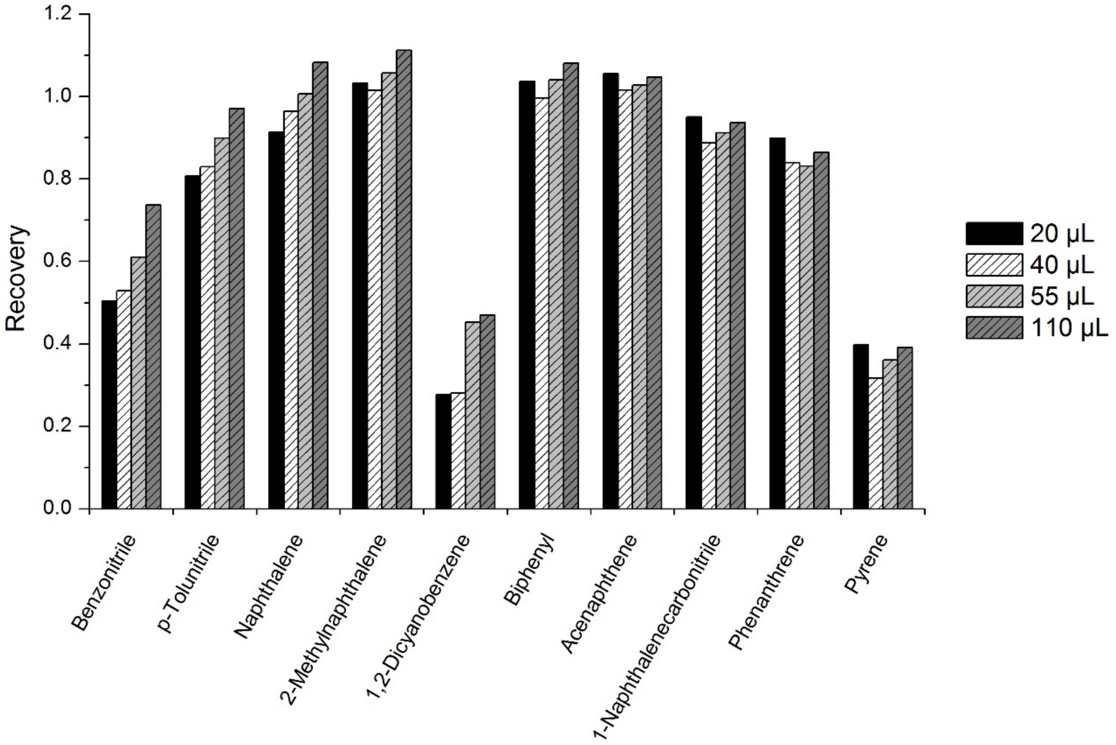


***Figure 8.3** – Observed responses for selected compounds after HSSE extraction of spiked blank cartridges with stir bars having different coating volumes. Values are normalised by those observed by direct injection.*

8.3. Optimisation of the extraction step

The optimal phase volume coated on stir bars was investigated. Generally, a significant difference in the detected amounts was detected after extraction with stir bars of different sizes (Figure 8.3), particularly for the most volatile. For instance, detected peak areas for benzonitrile after extraction with a 20 µL stir bar were approximately 1/3 smaller than those detected with a 110 µL stir bar. This difference became less significant for less volatile analytes (such as biphenyl and pyrene). Despite the lower signal intensities, a coating volume of 20 µL was selected for subsequent experiments in order to avoid excessive solvent loading onto stir bars, which could cause solvent-induced column degradation. This was deemed acceptable, considering that the most volatile compounds are more prone to rapidly disappearing from spent cartridges, and are consequently less interesting for dating purposes.

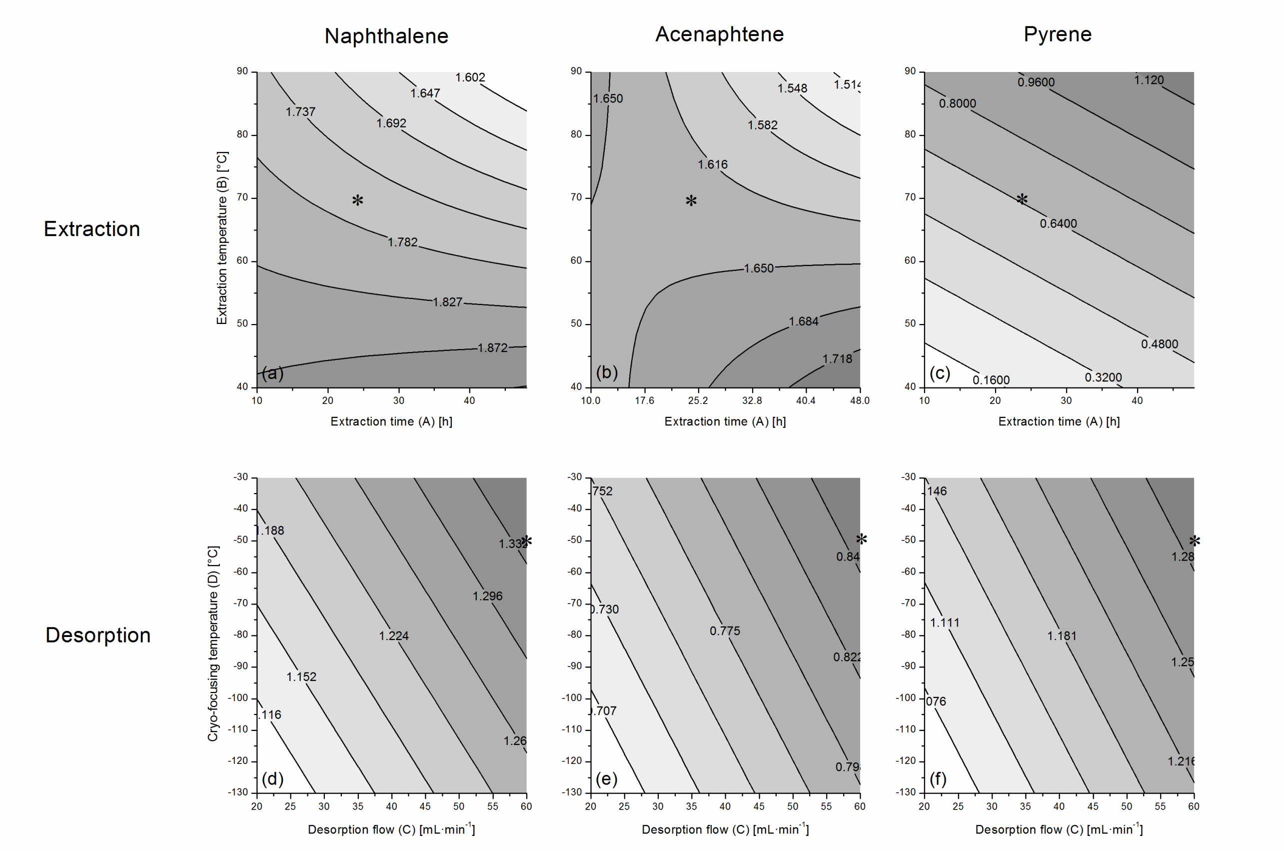


***Figure 8.4** – Evaluated surface responses for selected compounds and particularly influent parameters in the optimisation of both the extraction (a-c) and desorption conditions (d-f). The asterisks (*) indicates the chosen optimal conditions.*

A DOE approach was used to optimise the extraction temperature and time (see the obtained response surfaces in Figures 8.4a-c). From a routine work perspective, extraction time is a constraining parameter and was thus firstly tuned. It can be observed that the effect of extraction time in the studied interval (i.e., 10 – 48 h) was generally not significant for the most volatile compounds. On the contrary, the less volatile ones needed longer extraction times in order to reach satisfactory recoveries. Values between 24 and 48 h were considered as globally acceptable, and finally a value of 24 h was selected for practical reasons. Regarding extraction temperature (investigated interval: 40 – 90 °C), there was a clear conflicting trend amongst compounds (Figures 8.4a-c): raising temperature had a negative effect on the recovery of the most volatile compounds while it was positive on those of the less volatile ones (especially, phenanthrene and pyrene). Thus, an intermediary value of 70 °C was finally selected.

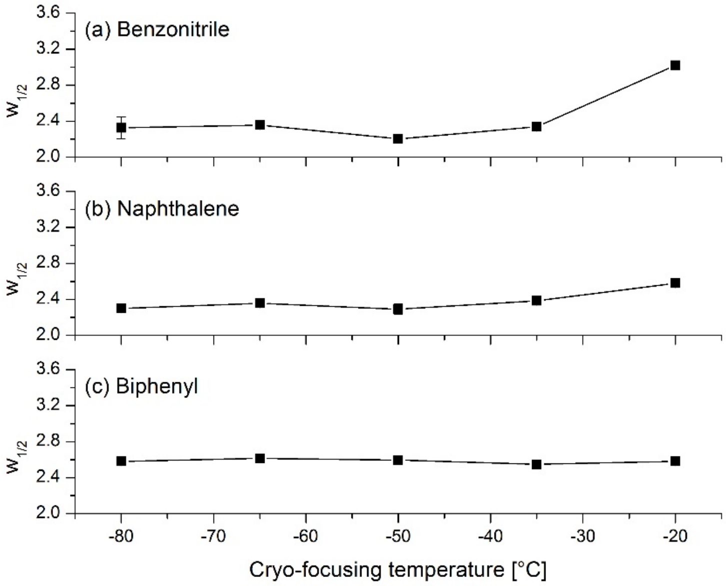


Figure 8.5 *– Plot of the peak widths at half height ($w_{1/2}$) for selected target analytes as a function of the cryo-focusing temperature.*

8.4. Optimisation of the desorption step

A DOE approach was also used to optimise the desorption conditions (i.e., the maximal desorption temperature, desorption time after reaching the final desorption temperature, desorption flow and cryo-focusing temperature). The effects of desorption time and desorption temperature (optimisation ranges: 5 – 10 min, and 250 – 300 °C, respectively) were generally not significant for any target analytes. Consequently, 300 °C was chosen as the desorption temperature to avoid the condensation of low volatility compounds in the inlet, and desorption time was set to 5 min to minimise run time. Setting the remaining variables (i.e., desorption flow and cryo-focusing temperature in the optimisation ranges of 20 – 60 mL min^{-1}, and -130 – -30 °C, respectively) to high values also allowed to reach larger signals (see response surfaces in Figures 8.4d-f). Nonetheless, even if larger peak areas were obtained at high cryo-focusing temperatures, significant peak tailing was also detected for most volatile analytes. This was likely due to non-optimal cryo-focusing effects during desorption. Thus, the effect of cryo-focusing temperature on the peak shapes was further investigated between -80 °C to -20 °C. Cryo-focusing temperatures lower than -50 °C did not allow a significant reduction of peak widths at half heights (see Figure 8.5). For this reason, a temperature of -50 °C was selected and desorption flow was set to 60 mL min^{-1}.

8.5. Analytical performance characteristics

A summary of the chosen optimal conditions is reported in Table 8.1, while Table 8.2 reports the analytical performance characteristics of the optimised method. In agreement to other stir-bars-based approaches for the analysis of PAHs [85, 90, 115], LOD values of all compounds deposed in cartridges were at picograms level. Repeatability of peak areas normalised by the corresponding deuterated standards (measured in terms of RSD) ranged from 0.3 % to 16.0 % in empty vials, and from 0.2 % to 20.0 % in blank cartridges. Thus, the additional analytical error introduced by the matrix itself was globally insignificant.

Step	***Factor***	***Type***	***Experimental values/ranges***	***Optimal conditions***
Spiking	*Spiking solvent*	D	DCM, Ether, MeOH	MeOH
	Spiked volume	D	5 µL, 10 µL	5 µL
Extraction	*PDMS volume (stir bars)*	D	20 µL, 40 µL, 55 µL, 110 µL	20 µL
	Extraction temperature	C	From 40 to 90 °C	70 °C
	Extraction time	C	From 10 to 48 h	24 h
Desorption	*Desorption temperature*	C	From 250 to 300 °C	300 °C
	Desorption time	C	From 5 to 10 min	5 min
	Desorption gas flow	C	From 20 to 60 mL min^{-1}	60 mL min^{-1}
	Cryo-focusing temperature	C	From -130 to -30 °C	-50 °C

Table 8.1 *– Optimised experimental factors, with their experimental values/ranges and chosen optimal conditions. "Type" indicates whether the corresponding factor was categorical ("D") or continuous ("C"). For solvents, DCM = dichloromethane, Ether = diethyl ether, MeOH = methanol.*

On the contrary, the repeatability of target compounds in real cartridges analysed immediately after discharge was significantly higher and ranged from 18.3 % to 66.9 %. A considerable degree of variability in real cases was consequently due to the specimen itself rather than analytical factors. This might be explained by an inhomogeneity in the composition of different cartridges or slight differences in temperature and pressure in the firearm chamber during discharge. Normalisation of peak areas to deuterated standards generally improved significantly the repeatability of the measured values, as well as their intermediary precisions (see data in Table 8.2). Comparability of ageing curves acquired at different weeks also improved by using deuterated standards, as it can be seen in Figure 8.6. Thus, the results strongly supported the benefit of using co-extracted ISs during HSSE extraction.

Analytes			**LODs [pg]**		**Recoveries**		**RSDs (absolute peak areas)**				**RSDs (normalized peak areas)**			
							Repeatability			**IP**	**Repeatability**			**IP**
#	**t_R**	**Compound**	**Empty vials**	**Blank cart.**	**Empty vials**	**Blank cart.**	**Empty vials**	**Blank cart.**	**Fired cart.**	**Fired cart.**	**Empty vials**	**Blank cart.**	**Fired cart.**	**Fired cart.**
1	*5.899*	*Benzonitrile*	50	500	54%	50%	7.9%	4.6%	43.2%	80.1%	5.1%	4.0%	36.3%	57.7%
2	*6.647*	*Indene*	50	50	80%	75%	4.8%	3.3%	35.5%	70.6%	1.1%	2.2%	30.2%	52.0%
3	*6.951*	*o-Tolunitrile*	10	10	90%	85%	4.8%	4.4%	40.7%	66.8%	0.7%	2.3%	34.3%	48.1%
4	*7.245*	*m-Tolunitrile*	50	50	95%	89%	4.1%	4.7%	34.6%	69.9%	0.7%	2.0%	28.2%	54.1%
5	*7.444*	*p-Tolunitrile*	10	10	89%	81%	6.0%	5.4%	33.4%	68.8%	2.3%	4.8%	30.3%	53.1%
6	*7.961*	*Benzyl nitrile*	10	10	101%	67%	4.4%	9.1%	54.9%	83.3%	1.6%	7.4%	53.0%	75.9%
7	*8.763*	*Naphthalene*	50	50	97%	91%	4.5%	2.9%	36.5%	68.0%	0.3%	0.4%	31.3%	54.1%
8	*9.704*	*Quinoline*	5	5	100%	76%	6.0%	7.7%	42.0%	61.5%	1.7%	2.6%	40.4%	53.6%
9	*10.109*	*Isoquinoline*	500	500	102%	59%	18.0%	14.8%	40.7%	51.7%	16.0%	10.0%	36.7%	45.4%
10	*10.139*	*1,3-Dicyanobenzene*	500	500	103%	38%	5.0%	10.5%	63.1%	88.1%	0.8%	6.9%	60.5%	77.1%
11	*10.161*	*1,4-Dicyanobenzene*	500	500	103%	38%	5.2%	10.5%	63.1%	88.1%	0.7%	6.8%	60.5%	77.1%
12	*10.746*	*Indole*	50	50	88%	47%	7.7%	8.4%	52.1%	77.4%	3.3%	6.3%	49.5%	63.3%
13	*10.804*	*1-Methylnaphthalene*	50	50	106%	99%	4.6%	3.5%	35.2%	63.3%	0.8%	0.9%	32.2%	57.7%
14	*11.148*	*2-Mehtylnaphthalene*	50	50	108%	103%	4.4%	3.2%	35.9%	63.2%	1.0%	1.2%	34.8%	58.6%
15	*11.379*	*1,2-Dicyanobenzene*	500	500	99%	28%	5.1%	11.3%	69.1%	90.6%	1.1%	8.4%	66.9%	83.6%
16	*12.529*	*Biphenyl*	1	1	109%	104%	4.5%	3.9%	54.3%	77.4%	0.3%	0.2%	50.3%	74.4%
17	*14.026*	*Acenaphthylene*	50	50	108%	103%	5.6%	3.4%	46.3%	67.7%	1.3%	1.0%	42.9%	62.8%
18	*14.178*	*Biphenylene*	50	50	105%	96%	5.2%	4.5%	42.3%	67.9%	1.0%	1.5%	39.9%	64.8%
19	*14.791*	*Acenaphthene*	5	50	111%	106%	4.8%	3.4%	43.7%	60.8%	0.7%	0.5%	38.8%	54.4%
20	*15.102*	*1-Naphthalenecarbonitrile*	50	50	104%	95%	5.1%	4.8%	48.6%	66.6%	0.7%	3.7%	46.5%	63.3%
21	*15.580*	*2-Naphthalenecarbonitrile*	10	10	101%	82%	5.6%	7.5%	50.8%	68.1%	0.9%	4.2%	49.4%	64.4%
22	*16.836*	*Fluorene*	10	10	118%	103%	4.7%	4.1%	44.9%	62.0%	1.1%	4.3%	43.3%	61.9%
23	*17.517*	*Diphenylamine*	5	5	96%	83%	4.4%	5.7%	16.1%	21.7%	1.3%	5.8%	18.3%	28.5%
24	*19.719*	*Phenanthrene*	1	5	97%	90%	5.2%	6.1%	42.8%	57.2%	0.4%	0.3%	42.5%	56.2%
25	*19.835*	*Anthracene*	5	5	102%	89%	5.7%	8.2%	40.5%	54.4%	0.5%	2.1%	40.4%	54.0%
26	*20.977*	*Ethylcentralite*	50	50	89%	64%	5.6%	13.6%	30.0%	31.8%	3.5%	10.5%	31.8%	49.7%
27	*21.591*	*Dibutyl phthalate*	>500	>500	118%	52%	9.4%	24.7%	41.8%	43.3%	9.5%	20.0%	44.2%	55.6%
28	*22.561*	*Fluoranthene*	50	100	103%	48%	6.3%	21.8%	42.4%	57.9%	1.2%	1.5%	42.0%	56.1%
29	*23.020*	*Pyrene*	50	100	96%	40%	7.1%	21.6%	43.8%	60.6%	0.4%	1.2%	43.6%	57.8%
		Min.	1	1	54%	28%	4.1%	2.9%	16.1%	80.1%	0.3%	0.2%	18.3%	57.7%
		Median	50	50	101%	82%	5.2%	5.7%	42.4%	70.6%	1.0%	2.6%	40.4%	52.0%
		Max.	500	500	118%	106%	18.0%	24.7%	69.1%	66.8%	16.0%	20.0%	66.9%	48.1%

Table 8.2 *– Analysed target compounds, together with corresponding analytical performance characteristics for the optimised method. LODs are reported as the absolute amounts deposed in cartridges. "IP" is the intermediary precision.*

8.6. Ageing of target compounds in 9 mm cartridges

Several 9 mm Parabellum cartridges from the brand Geco were analysed using the optimised method at different times since discharge. This specific calibre was chosen because it is commonly use to perpetrate firearm-related crimes in Switzerland, and more generally in Europe. Examples of ageing profiles are reported in Figure 8.7.

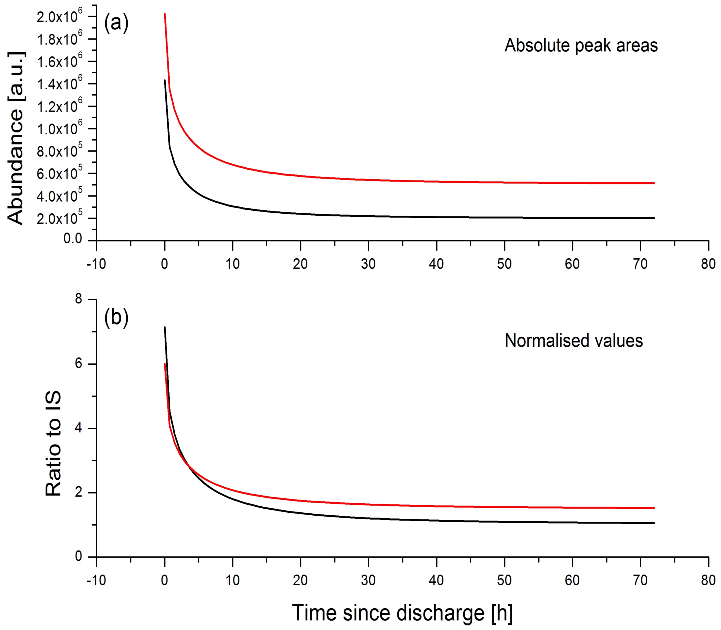


Figure 8.6 *– Comparison of two ageing curves for pyrene taken at 3 month intervals (a) without normalisation and (b) with normalisation to the corresponding deuterated standard (pyrene-d10). After IS normalisation, a better superposition between the profiles was obtained, proving a better inter-day precision and comparability.*

Compound	***Cartridge position***		***Temperature***		***Relative humidity***	
	p-values	***< α = 0.010***	***p-value***	***< α = 0.010***	***p-values***	***< α = 0.010***
Benzonitrile	0.001	X	<0.001	X	0.996	
p-Tolunitrile	<0.001	X	<0.001	X	0.480	
Naphthalene	<0.001	X	<0.001	X	0.333	
2-Methylnaphthalene	<0.001	X	<0.001	X	0.013	
1,2-Dicyanobenzene	0.005	X	<0.001	X	<0.001	X
Biphenyl	<0.001	X	<0.001	X	<0.001	X
Acenaphthene	<0.001	X	<0.001	X	<0.001	X
1-Naphthalenecarb.	<0.001	X	<0.001	X	<0.001	X
Phenanthrene	<0.001	X	<0.001	X	<0.001	X
Pyrene	0.002	X	<0.001	X	<0.001	X

Table 8.3 *– P-values after ANOVA F-tests for testing the significance of storage conditions on the compounds' rates of decrease (C coefficient). P-values close to 0 support the hypothesis that the specific condition is significant on the decrease rate of the considered compound, while p-values closed to 1 support the alternative hypothesis.*

As previously remarked in .45 ACP cartridges during the preliminary studies (Chapters 6 and 7), also in 9 mm Parabellum, the rates of decrease of each selected explosion product depended on their volatility. Thus, the detected signals for the most volatile explosion products heavily dropped in the first hours since discharge and quickly levelled-off, while those of the less volatile compounds followed a slower decrease. This further indicated that different compounds could give complementary evidence about the time since discharge, independently from the calibre.

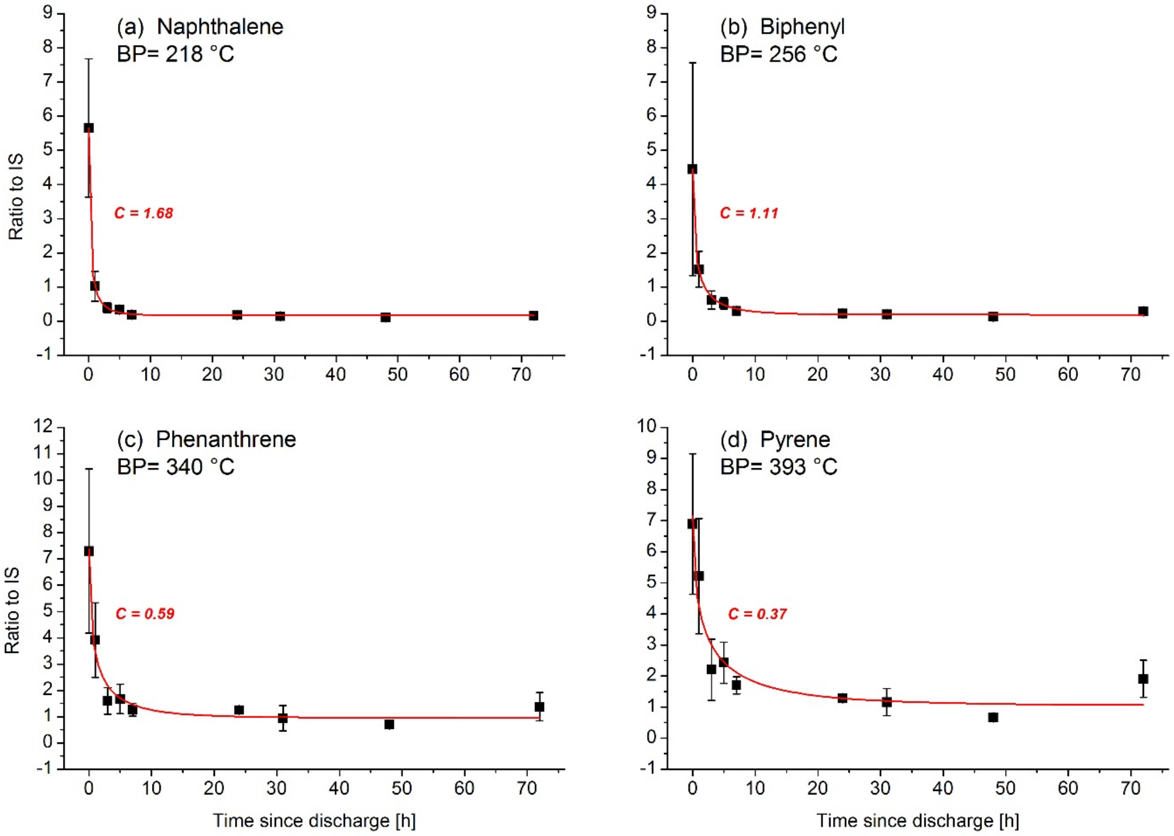


Figure 8.7 *– Examples of ageing curves for 4 selected target compounds: naphthalene, biphenyl, phenanthrene and pyrene. "BP" is the boiling point.*

The effect of different influencing factors (i.e., storage temperature, relative humidity and cartridge position) on the decrease rates was investigated through ANOVA (Table 8.3 and Fig. 8.8). Observations strongly supported the hypothesis that the temperature has an effect on the decrease of all compounds studied, and that this was generally positively correlated to the decrease rates. Indeed, at low temperatures, the disappearance of compounds was slower, and vice-versa.

Effects of relative humidity and cartridge position were never reported before, but results also supported the hypothesis that they could have significant effects on the aging of most compounds. In particular, laying the cartridge horizontally instead of placing it vertically largely seemed to accelerate the rate of decrease. This may be due to an increase in the evaporation surface when placed horizontally. Such an observation is of particular importance given that cartridges are more commonly encountered in a lying position in actual caseworks. Concerning relative humidity, results did not lend themselves to supporting the hypothesis that its effect was significant for the most volatile compounds, while the contrary was true for the least volatile ones. Furthermore, the correlation between decrease rates and relative humidity was not linear, with maximal decrease rates at around 75 % relative humidity for all the studied compounds.

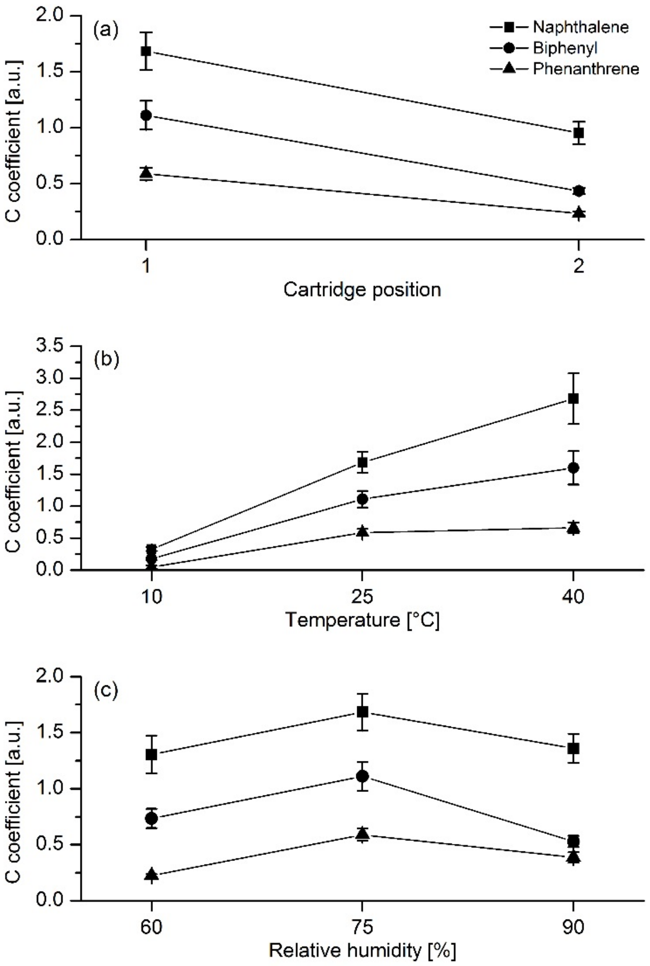


Figure 8.8 *– Evolution of the C coefficients (i.e., decrease rates) as a function of (a) cartridge position, (b) environmental temperature, and (c) relative humidity. For cartridge position, "1" indicates horizontal, while "2" vertical.*

8.7. Chapter conclusion

The proposed HSSE-TD-GC-MS method was comprehensively optimised for the analysis of 29 volatile organic GSR compounds. Several experimental parameters affecting the chromatographic, extraction and desorption steps were identified and tuned using a multivariate statistical approach based on DOE. Additionally, an IS approach based on deuterated analogues was developed and implemented to reduce analytical variability. The developed method presented excellent analytical performances. Low LOD values were obtained for the selected target analytes in relatively short run times. Application to real cartridges showed that the use of co-extracted internal standards also allowed an improvement in repeatability of the measured signals as well as comparability between ageing curves acquired at different days. Moreover, the optimised procedure allowed a substantial decrease in the total time necessary to process a single cartridge in comparison to the previously tested method (Chapter 6).

Using the optimised method, the ageing of the single target GSR compounds was thoroughly studied in spent 9 mm Parabellum cartridges. Most compounds demonstrated a significant decrease over time, with actual decrease rates dependent on the compounds boiling points. This further indicated that different compounds could provide complementary evidence about the time since discharge. The impact of varying the cartridge storage conditions on ageing profiles was also investigated, and results supported the hypothesis that all the variables investigated (i.e. cartridge position, temperature and humidity) could have an impact on GSR decrease rates. This should be accounted for when implementing reliable interpretation methodologies.

CHAPTER 9

Application of chemometric methods to estimate the time since discharge of 9 mm cartridges

Mainly based on:

Gallidabino, M., Romolo, F.S., & Weyermann, C. (2016).
Time since discharge of 9 mm cartridges using headspace analysis, part 2: ageing study and estimation of the time since discharge using multivariate regression. *Submitted to Forensic Science International*.

Reprinted in Appendix E

9. Application of chemometric methods to estimate the time since discharge of 9 mm cartridges

Previous methods for inferring the time since discharge essentially involved the independent assessment of each single co-extracted compound at a time (especially, naphthalene and TEA2) [7, 11, 64]. Indeed, very few attempts have been made to simultaneously consider all the available chemical information in a unique interpretative model, although this could significantly improve the accuracy of the estimated times. As a multi-residue method was developed and optimised in this work for the analysis of 29 volatile organic GSR compounds, investigating the implementation of chemometric multivariate methods and its improvement on the interpretation of data were nonetheless of particular interest. Consequently, their application to 9 mm Parabellum cartridges (a very common pistol calibre amongst crimes involving firearms in Europe) has been investigated in this chapter. Particularly, different pre-treatments and multivariate regression methods were tested on data for their performance [123]. Then, the best models were selected, evaluated and time-since-discharge estimates compared to univariate models. A casework perspective was generally adopted. Hence, training of models was performed using a limited number of reference cartridges and their validation was carried out on data obtained from different runs. The impact of varying the cartridge storage conditions was also investigated (i.e. cartridge position, temperature and humidity).

9.1. Selection of the best multivariate models

In an attempt to implement all sources of information about time since discharge linked to the single target molecules into a unique estimation model, the following multivariate regression techniques were tested on the data acquired at “standard” conditions (i.e., 25 °C, 75 % relative humidity, horizontal positioning) [123]: partial least squares (PLS), multivariate adaptive regression splines (MARS), artificial neural networks (NN), random forests (RF), k-nearest neighbors (KNN) and support vector machines (SVM). Improving regression performances by applying different pre-treatments was also investigated, including normalisation, transformation and scaling methods [112, 113, 124]. These included, for example, total sum normalisation (TSN), probabilistic quotient normalisation (PQN) and pairwise log-ratios normalisation (PLR) as normalisation approaches, as well as logarithm (Log), square root (Sqrt), fourth root (4thr) and inverse (Inv) as transformations (see Appendix E for details).

384 models were thus evaluated. Root mean square error (RMSE, i.e., the model mean error on inferring estimates over the entire studied time-since-discharge interval) was exploited as a criterion

for the preliminary triage of the models. Overall, RMSEs ranged from 8.8 to 27.8 h with a median value of 13.0 h. The medians for the specific regression methods did not vary significantly from this value, proving that a certain coherence was observed between the different regression techniques. Despite this, specific combinations of pre-treatments and regression methods were nonetheless found to perform much better than others. In this regard, RF and PLS preceded by PLR normalisation showed the best general performances with observed RMSEs of 10.1 and 10.6 h, respectively, and very low evidences of over-fitting (contrary, for example, to KNN and NN models). Both these models were thus chosen for further inspection, as well as for evaluating the potential of the HSSE method to infer time since discharge.

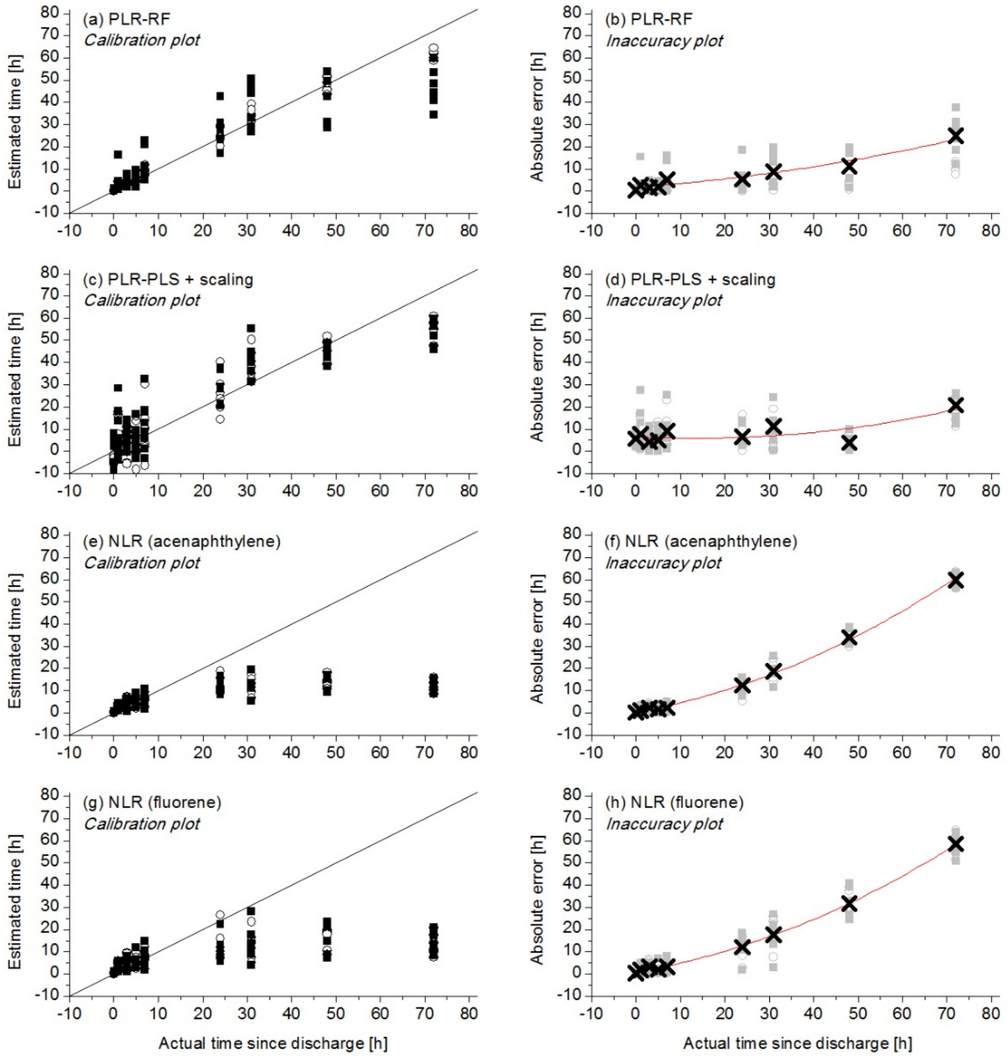


***Figure 9.1** – Calibration and inaccuracy plots for the best multivariate and univariate regression models. For inaccuracy plots, cross-points represent the local means, while overall mean tendencies (approximated by quadratic regression) are represented by solid lines.*

9.2. Accuracy in the estimation of time since discharge

Table 9.1 reports inaccuracy (i.e., the average magnitude of absolute error) obtained with the two retained multivariate models (see also Figures 9.1a-d). Using the RF model, inaccuracy values of 2.5, 6.5, 13.7 and 23.2 h were estimated for 5h-, 24h-, 48h- and 72h-old cartridges, which correspond to percentages of about 50, 27, 28 and 32 % in relation to the actual times since discharge, respectively. The PLS model was shown to perform worst at the shortest time since discharge, with an inaccuracy estimated at about 6.5 h across the entire 0 – 24 h range. For the sake of illustration, this corresponded to 126 % of relative error at 5 h since discharge.

Actual time [h]	*PLR-RF*		*PLR-PLS + scaling*		*NLR (acenaphthylene)*		*NLR (fluorene)*	
	I [h]	*%*	*I [h]*	*%*	*I [h]*	*%*	*I [h]*	*%*
1	1.8	183 %	6.7	665 %	0.8	79 %	1.3	132 %
5	2.5	50 %	6.3	126 %	2.4	47 %	2.8	56 %
24	6.5	27 %	6.4	26 %	12.8	53 %	12.7	53 %
48	13.7	28 %	10.4	22 %	32.9	69 %	31.8	66 %
72	23.2	32 %	18.9	26 %	60.8	84 %	58.3	81 %

Table 9.1 *– Estimated inaccuracy (I) and relative percentage to the actual time since discharge (%) obtained with the best multivariate and univariate models.*

The larger average inaccuracy of the PLS model in comparison to the RF one for freshly fired cartridges was surely due to a wider spread of estimation errors obtained on the cartridges actually analysed in the first 0 to 24 h since discharge. This could in turn be attributed to a statistical reason, namely the difficulty of the PLS algorithm in correctly taking into account the considerable non-linear nature of the data in this initial ageing stage [123]. Despite this, slightly lower inaccuracies were observed for the PLS model in comparison to the RF one for > 48h-old cartridges. In fact, local average inaccuracies of 10.4 h and 18.9 h were estimated for 48h- and 72h-old cartridges, which correspond to only 22 and 26 % of the actual times since discharge, respectively. In consequence, the two models appeared to be complementary to some extent. The model based on RF was anyway slightly preferred for the purpose of estimating punctual time-since-discharge estimates due to the lower average inaccuracy over the entire studied temporal range, which could overall be rated as satisfying, considering the large random variations in data which cannot be reduced by statistical means (see Chapter 8).

Notwithstanding, it is noteworthy to mention that the local inaccuracy on the time-since-discharge estimates increased as a function of the actual cartridge age independently of the model applied. This trend led to systematic negative biases in estimates for cartridges older than 48 h for all the models, which are most probably due to the level-off in the amount of every volatile GSR compounds after this threshold and the subsequent instalment of a steady state in the ageing profiles. Maximal time limits able to be estimated with acceptable accuracy were thus seen to be around 48 h, at least with the tested 9 mm Geco cartridges and the adopted experimental methodology.

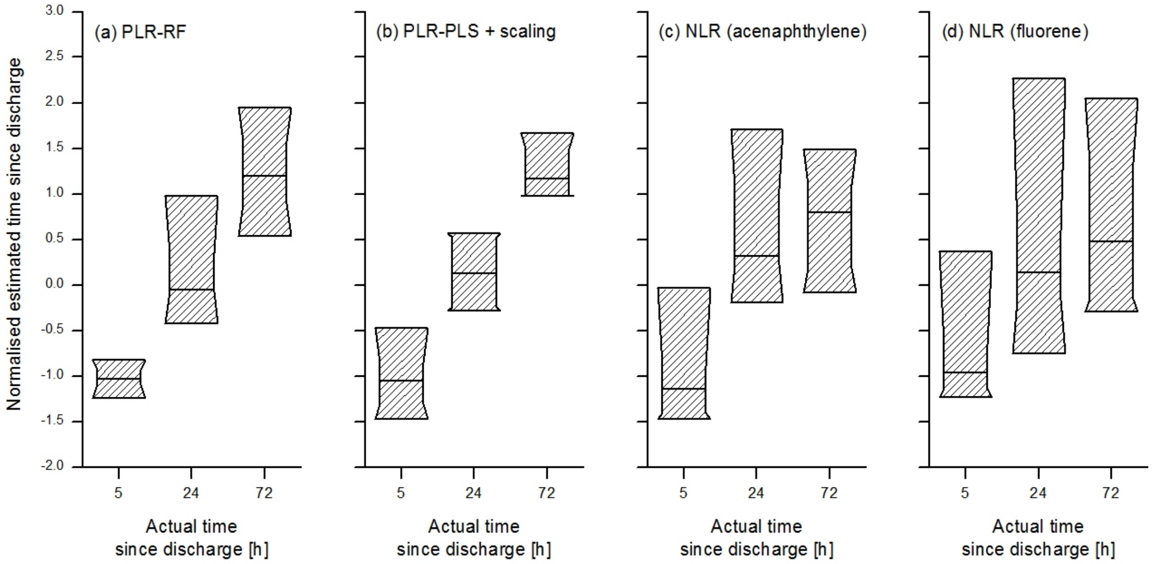


Figure 9.2 – Notched boxplots (10/25/75/90 percentiles) of the distribution of the times since discharge estimated by the best multivariate and univariate regression models, as a function of three actual cartridges ages. Estimates were centred and normalised to their standard deviation to improve visualisation.

9.3. Differentiation of cartridges at different ageing stages

Even if estimation inaccuracies beyond 48 h of ageing were found to be generally too significant to allow a sufficiently accurate inference of the time since discharge, it is noteworthy that analyses performed at distinct ageing stages seemed to present differentiable distributions of estimates and thus also the potential to be mutually discriminated. As an alternative to direct time-since-discharge inference, the potential to separate recently fired from older cartridges was then preliminary investigated.

Figures 9.2a-b show the distributions of the time-since-discharge estimates obtained by the RF and PLS models as a function of three actual cartridge ages (i.e., 5 h, 24 h and 72 h). As it can be noted, 5h-old cartridges could easily be differentiated from 24h- and 72h-old ones, independently from the multivariate model considered. A slight overlap between results obtained on cartridges aged for 24 h and 72 h was observed for the RF model, while they were clearly differentiable for the PLS model. Thus, these results strongly supported the hypothesis that analyses carried out on cartridges at different ageing stages actually convey sufficiently divergent chemical information to be, at least, discriminable from each other. More generally, they point toward the possibility of identifying recently fired from older cartridges even if an accurate estimation of the shooting time is not readily available. From a forensic perspective, this is important as it would potentially allow for differentiating between relevant and irrelevant cartridges at a crime scene and/or discriminating between alternative hypotheses brought forth by the prosecution and the defence. Future work on the use of classification methods, instead of regression ones, to correctly assess these situations may thus be promising. In this regard, it is interesting to note again the complementarity of the two tested chemometric techniques. Indeed, separation between cartridges at different ageing stages was slightly better with PLS than RF.

9.4. Comparison to univariate regression models

For the sake of validation, comparison of estimation accuracy obtained by the retained multivariate models with that achievable by traditional univariate regression approaches was attempted. In this regard, inference on time since discharge through the interpretation of ageing profiles of single compounds has often been suggested in the literature, but no statistical tool has ever specifically been proposed. Since then, heteroscedastic non-linear regression analysis (NLR) has been adopted in this study as a reference univariate approach, as it previously proved to be an adequate statistical technique to model ageing profiles of volatile compounds in spent cases (see Chapter 8.6).

NLR was individually applied to every target compound in order to obtain compound-specific estimates of time since discharge. RMSEs for all univariate NLR models ranged from 20.1 to 26.2 h (corresponding to pyrene and indene, respectively), while coefficients of determination (R^2) never surpassed 0.588 (the highest being acenaphthylene). These metrics were considerably worse than those obtained from most multivariate models, thus equally denoting worse global regression performances. Inaccuracy profiles were investigated. Values for < 10h-old cartridges attained with the two univariate models considered the best in terms of overall fitting efficiency (i.e., acenaphthylene and fluorene) were comparable to the RF one. Despite that, average inaccuracy obtained on cartridges older than 10 h were significantly higher than those of both PLS and RF (Table 9.1 and Fig. 9.1e-h). Furthermore, the distribution of time-since-discharge estimates obtained on 5h-, 24h- and 72h-old cartridges showed strong overlapping (Fig. 9.2c-d). Therefore, results support the hypothesis that univariate approaches are less appropriate to gaining helpful evidence on the ageing state of spent cases. Enhanced results could actually be obtained by simultaneously assessing all the available chemical information in a unique multivariate model instead of interpreting single compounds at a time.

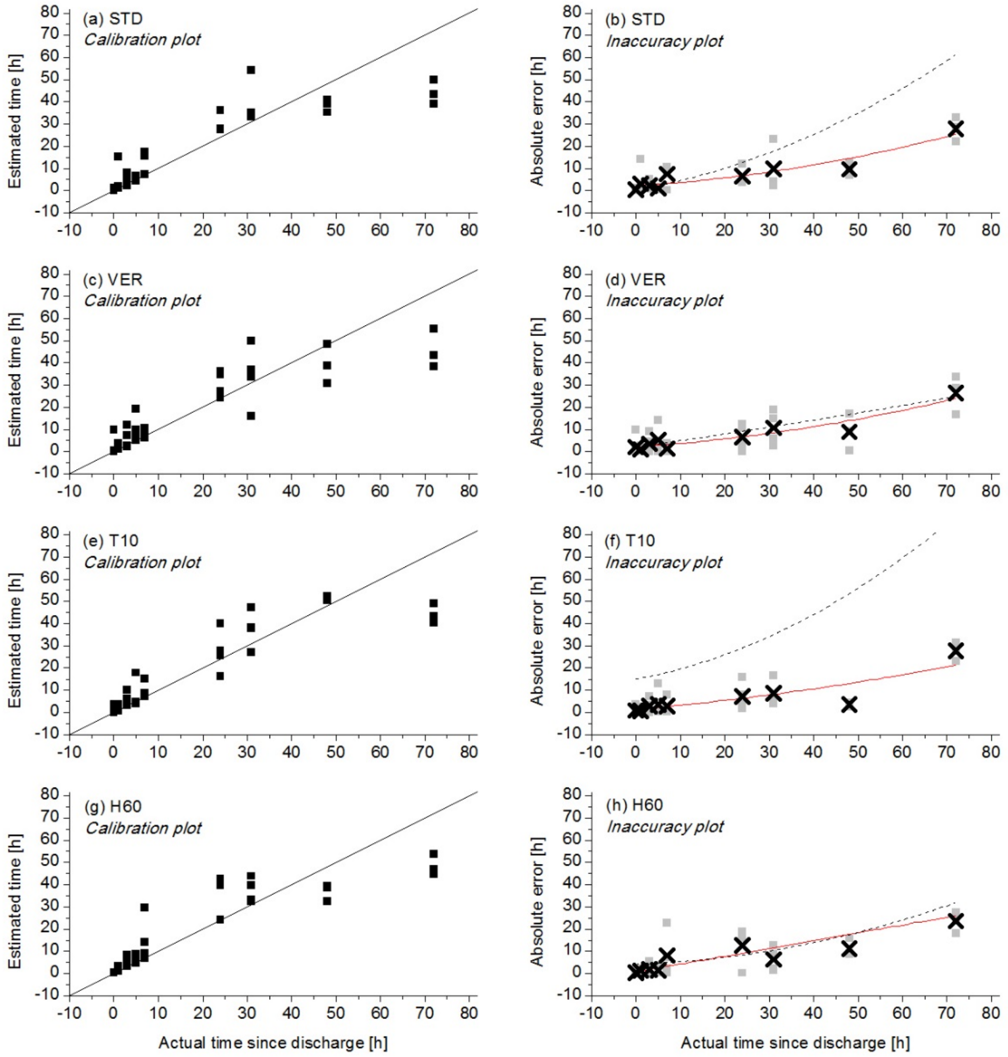


Figure 9.3 – Calibration and inaccuracy plots obtained with PLR-RF under different storage conditions, where the model was trained on reference cartridges aged at the same conditions as the cartridges in question. For inaccuracy plots, cross-points represent local means, while overall mean tendencies (approximated by quadratic regression) are represented by solid lines. Dashed lines represent overall mean inaccuracy for univariate NLR models solely trained on the acenaphthylene data.

9.5. Robustness of multivariate models to changes in storage conditions

All the influential variables previously investigated in this work (i.e. cartridge position, temperature and relative humidity) were shown to affect GSR ageing rates (Chapter 8.6). Thus, a major concern was to determine if differences in estimation accuracy could equally be encountered between models which were trained using the same regression method but on cartridges aged under distinct storage conditions. This was tested by refitting the RF model on datasets from cartridges aged under distinct ageing circumstances, and by successively determining estimation accuracy on left-out observations.

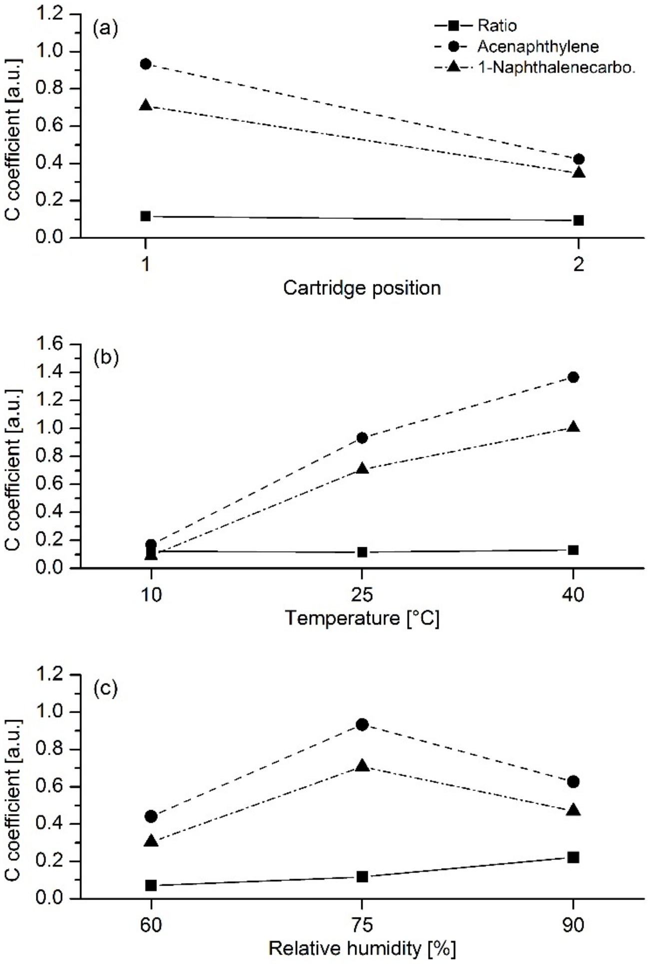


Figure 9.4 *– Evolution of the decrease rates for the acenaphthylene / 1-naphthalenecarbonitrile ratio and their composing molecules as a function of (a) cartridge position, (b) environmental temperature, and (c) relative humidity. For cartridge position, "1" indicates horizontal, while "2" vertical.*

No significant shifts in estimation accuracy were observed between all the trained models. The inaccuracy profiles were indeed very similar in all the situations (Fig. 9.3), proving a good robustness of the retained RF approach under different storage conditions. In this regard, it should be noted that PLR normalisation could have had a significant role in preventing shifts in estimation accuracy, as the decrease rates of most ratios were found to be less affected by influential factors than their respective composing molecules (Fig. 9.4).

9.6. Effect of differences between reference and examined cartridges

Even if the above results are definitely important for the development of a generalised interpretative approach, the main problem in real forensic cases still lies in the fact that the exact storage conditions encountered by a specific examined cartridge from a crime scene are rarely known. Effectively, differences between the storage conditions applied to reference materials and those actually present during the ageing of the examined cartridges are very likely, thus making any statistical model trained on reference data not suitable for time-since-discharge estimations. The consequence of these situations were primarily investigated by refitting the RF model on datasets from cartridges aged at distinct ageing circumstances, and by successively determining estimation accuracy on datasets from cartridges aged under different conditions.

Suboptimal accuracies were noted in many cases, especially when differences in storage temperature were introduced. Figure 9.5 shows some examples. Thus, when time-since-discharge estimation was attempted on cartridges aged at 10 °C using a model trained with data acquired at 25 °C, outcomes were affected by strong inaccuracies which globally under-estimated the true values. In contrast, predicted times since discharge for cartridges aged at 40 °C (15 °C above the training dataset) were mostly over-estimated (mainly within the first hours of ageing). Nonetheless, it could be noted that consequences were more limited when differences in cartridge position and relative humidity were introduced (Fig. 9.5).

ANOVA of the RMSEs estimated on all the validation datasets further confirmed these observations. Thus, very limited evidence to supporting the hypothesis that differences in cartridge position between the training and validation datasets are significant on the estimation accuracy of the models were provided by ANOVA (p-value = 0.111). On the contrary, results moderately supported the hypothesis that differences in the relative humidity can have an impact on estimation accuracy (p-value = 0.021), and strongly supported the hypothesis that differences in storage temperature have a significant effect on estimation accuracy (p-value < 0.001).

From a general point of view, it should be noted that the magnitude of differences between validation and training datasets as tested in this research was relatively large. In fact, it can be assumed that, in real cases, a smaller uncertainty would exist between experimental and actual storage conditions. Nonetheless, these results point toward the fact that a case-by-case approach should be adopted for interpreting data in caseworks. Reference materials, ideally, should be stored under similar conditions as examined cartridges (in addition to being of the same type) in order to train accurate case-related dating models. Using general reference models does not seem to be a reliable strategy. In consequence, knowledge of the environmental conditions occurring at the crime scene before cartridge sampling should be collected.

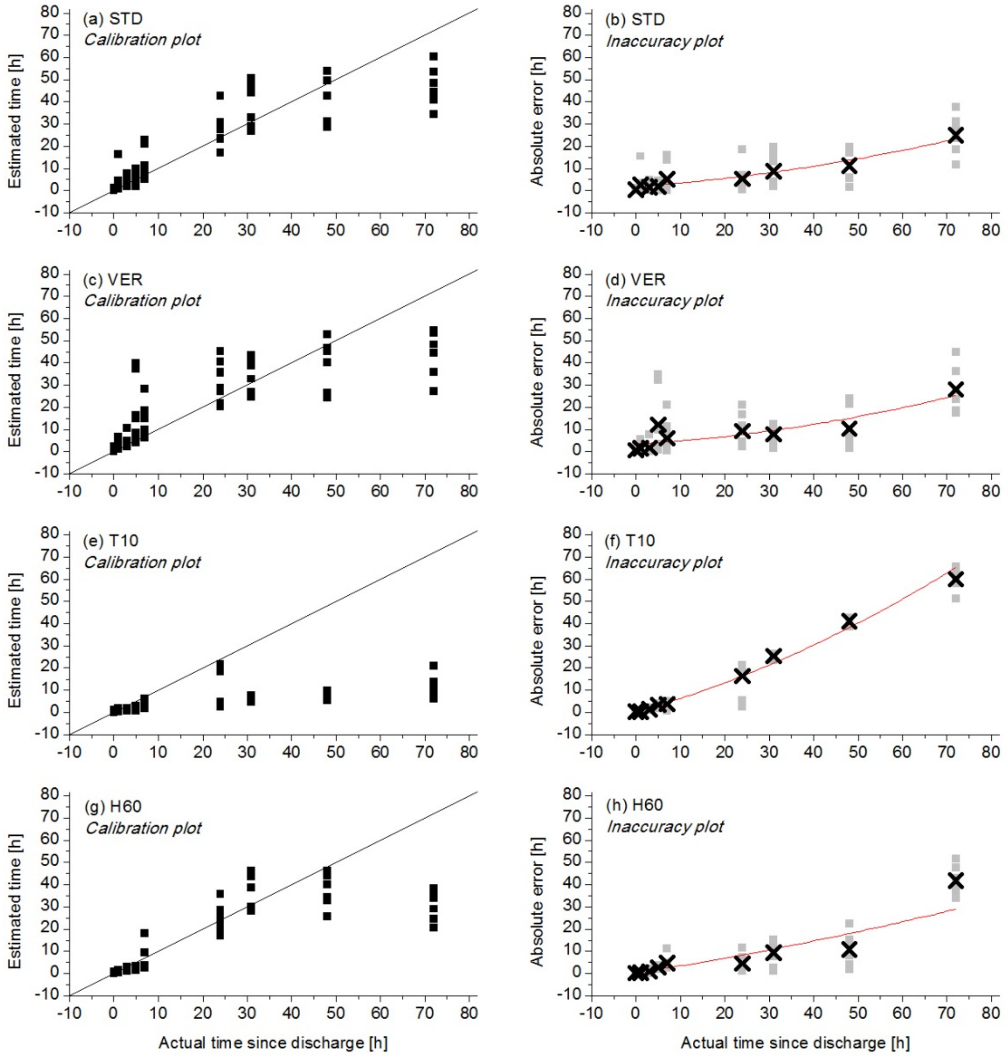


Figure 9.5 *– Calibration and inaccuracy plots obtained by PLR-RF under different storage conditions in the specific case where the model was trained on reference cartridges aged under different conditions to the ones in question. In particular, the reference model was trained on cartridges aged under "standard" conditions. For inaccuracy plots, cross-points represent local means, while overall mean tendencies (approximated by quadratic regression) are represented by solid lines.*

9.7. Compounds importance in characterising global cartridge age

As preliminarily observed in Chapter 6.3, pairwise ratios normalisation (hereafter logarithmic transformation, i.e. PLR) proved to be a valuable pre-treatment step. Nonetheless, a large number or different ageing parameters are generally created by this procedure and identifying those which have more impact on regression performances is certainly of strong interest. In this regard, the RF algorithm involves an internal scoring method which automatically ranks predictors as a function of their relative impact on the fitting procedure. Results of this method have thus been used to also assess the importance of each ratio obtained through PLR normalisation for characterising the ageing of spent cartridges. Of the 210 ratios obtained from all the possible pairwise combinations between the retained analytes, a total of 8 ratios generated from different combinations of 8 target compounds were found to be particularly significant in every trained model. These were (in order of decreasing importance):

(1) acenaphtylene / phenanthrene,
(2) acenaphtylene / anthracene,
(3) acenaphtylene / acenaphtene,
(4) fluorene / anthracene,
(5) acenaphtylene / 1-naphthalenecarbonitrile,
(6) indole / fluoranthene,
(7) fluorene / phenanthrene,
(8) acenaphtylene / fluoranthene.

Upon closer inspection of their ageing profiles, it could be remarked that all these ratios decreased over time similarly to the signals of the single composing molecules (see examples in Figure 9.6). Nonetheless, decrease rates of ratios were often slower, thus yielding more linear ageing curves. This could partially explain the better regression performances averagely obtained after PLR normalisation in comparison to other pre-treatment approaches.

Concerning the single compounds, acenaphthylene appeared in 5 of the 8 most influent ratios. Indole, phenanthrene, anthracene and fluoranthene were each involved in 2 ratios, while acenaphthene, 1-napthalenecarbonitrile and fluorene were each in only 1 ratio. All 8 substances are characterised by boiling points higher than 254 °C (the lowest being indole). Therefore, most of the pertinent information to estimate the time since discharge of 9 mm cartridges seemed to be linked to the least volatile molecules. Even if strongly exploited in the literature, naphthalene was not highlighted as particularly significant for characterising the ageing of the chosen handgun cartridges, probably because of its overly-rapid disappearance in this kind of specimens which also makes it poorly informative for dating purposes.

9.8. Chapter conclusion

The potential of various pre-treatments and multivariate regression methods was investigated to estimate the time since discharge of spent 9 mm cartridges after analysis by HSSE-TD-GC-MS. In this regard, regression methods based on RF and PLS, preceded by data pre-treatment using PLR, presented particularly good estimation performances in comparison to other multivariate models. The accuracy of the predicted outcomes showed a strong potential for estimating the time since discharge up to 48 h of ageing and/or to differentiate recently fired from old cartridges (e.g., less than 5 h compared to more than 1 – 2 days), under stable storage conditions. This was definitely better than any univariate compound-specific model trained on data, and supported the hypothesis that enhanced evidential information could actually be obtained by simultaneously assessing multiple compounds in a unique inferential model. More generally, these results were important because they showed that useful temporal information could be extracted from the analysis of the volatile GSR fraction in spent cartridges prior to forensic mark examination, contrary to what was previously reported in published work involving SPME. The least volatile molecules, such as acenaphthylene, seemed to play a more important role in characterizing the cartridge age than the most volatile ones (e.g., naphthalene).

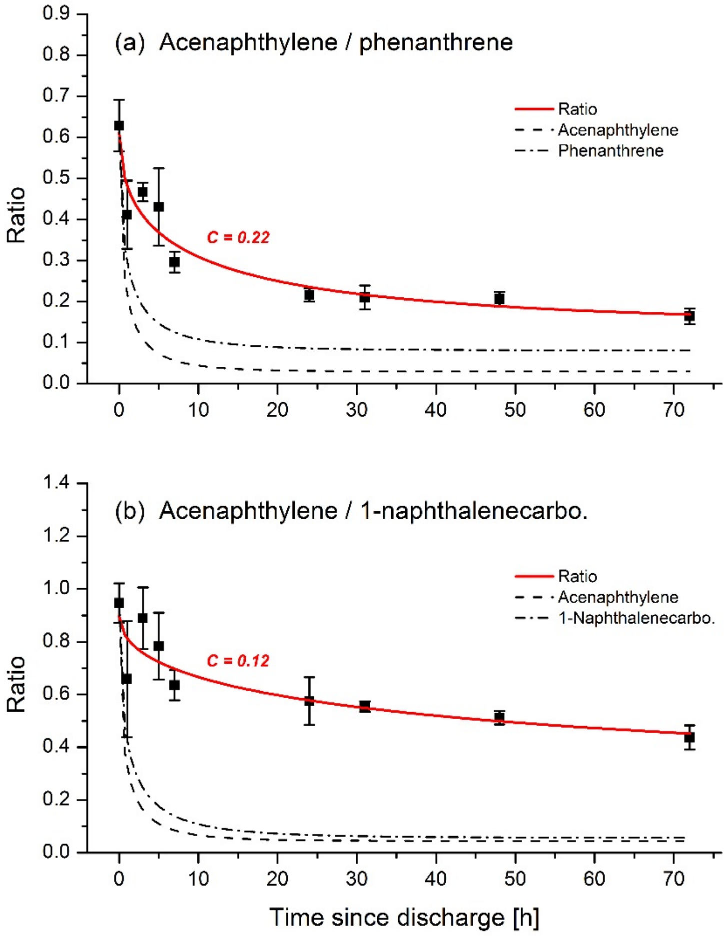


Figure 9.6 *– Examples of ageing curves for 2 particularly influent pairwise ratios. Dashed lines indicates ageing profiles for the respective composing compounds.*

Robustness to differences in the storage conditions applied to reference materials and those actually present during the ageing of the examined cartridges was also tested. While differences in cartridge position and relative humidity were found to be marginally more significant, those in temperature conditions were particularly prone to introducing significant errors to the time-since-discharge estimates. This highlighted the necessity of adopting a case-by-case interpretative approach, as well as of implementing strategies to gain some knowledge of the storage conditions occurring at the crime scene before fixation in order to minimise differences in ageing conditions between reference and examined cartridges.

CHAPTER 10

A logical approach for the evaluation of analytical results

Mainly based on:

Gallidabino, M., Weyermann, C., Romolo, F.S., & Taroni, F. (2013). Estimating the time since discharge of spent cartridges: a logical approach for interpreting the evidence. *Science & Justice*, 53(1), 41-48.

Reprinted in Appendix F

10. A logical approach for the evaluation of analytical results

Little previous work has dealt with the development of comprehensive models to interpret certain data. This is particularly true for evaluative frameworks, namely, those situations when two adversarial propositions of the time since discharge are forwarded by the parties (i.e., prosecution and defence), and it is of interest to evaluate the evidential value of the analysis carried out on an examined specimen against both of them. Indeed, no complete and reliable approach exists which can deal with this problem. Models developed in Chapter 9 are rather useful to estimate the most probable time since discharge given analytical results, and, for this reason, can be solely applied to investigative frameworks.

In order to determine the evidential value of a set of analytical observations, the use of a probabilistic approach based on the assignment of LRs was suggested in numerous forensic fields and is currently gaining a strong consensus within the scientific community [68, 69]. This is because such methodology is considered a particularly robust, transparent and impartial approach to evaluate evidences [125]. Thus, the purpose of this last research step was to develop a valid strategy to assign LRs to outcomes obtained from the analysis of volatile GSR fractions in spent cartridges in the light of competitive propositions.

10.1. General evaluative model

In an evaluative framework, the role of the scientist is to assess the probability of a given evidential element under different alternative hypotheses: the ratio between these probabilities is called the LR [68, 69]. The formulation of the hypotheses depends on the circumstances of the case. In this context, we focus on the case where a suspect admits having fired the examined cartridge but he contests the proposed discharge time [5]. The following hypotheses on the course of the events can therefore be suggested:

1. T_p: the examined cartridge was fired at the same time as the commission of the crime with the suspect's firearm and ammunition.
2. T_d: the examined cartridge was fired prior to the commission of the crime with the suspect's firearm and ammunition.

The expert's role is to assess the probability of observing q (i.e., the analytical results obtained on the examined cartridge) given respectively T_p and T_d. The LR (defined with the letter V) is given by the ratio of these two likelihoods:

$$V = \frac{p(q|T_p)}{p(q|T_d)} \qquad \text{Eq. 10.1}$$

If V is greater than 1, the analytical results support T_p, and vice-versa if V is smaller. For the sake of illustration, we consider q as the observed value in the examined spent cartridge of a given ageing parameter, let's say the residual amount of naphthalene (multivariate approaches are also possible but will not be discussed here; see [126] for further details). In this case, q is a particular observation of the random variable Q, the unknown quantity of naphthalene in the cartridge. This variable is continuous because q can ideally take any value between 0 and ∞. We assume that Q is normally distributed, so $Q \sim N(\mu;\sigma^2)$. Therefore, if the values of the distribution parameters (the mean and the variance) are known, the density for a given $Q = q$ is provided by the following density distribution:

$$f_Q(q|\mu,\sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}}\exp\left[-\frac{(q-\mu)^2}{2\sigma^2}\right] \qquad \text{Eq. 10.2}$$

From a practical point of view, it should be noted that the more recent the discharge time is, the greater the amount of organic GSR compounds remaining in the spent cartridge is (and vice-versa for longer intervals). Consequently, the distribution parameters for Q depends on the hypothesis that has been put forth, and the formula in Eq. 10.1 can be substituted by the following definition [127, 128]:

$$V = \frac{f_Q(q|\mu_p,\sigma_p^2)}{f_Q(q|\mu_d,\sigma_d^2)} \qquad \text{Eq. 10.3}$$

where μ_p, μ_d, σ_p^2 and σ_d^2 are the parameters characterising the distribution of the chosen ageing parameter under each of the two given propositions.

10.2. Estimation of the distribution parameters

In practice, the exact values of the different distribution parameters are unknown and they should be estimated. The simplest approach to achieve this objective would be to plan two replicate series of experiments with adequate reference material. The spent cartridges belonging to the two groups are then analysed after the intervals defined by the hypothesis T_p and T_d, respectively. The estimates $\hat{\mu}_p$, $\hat{\mu}_d$, $\hat{\sigma}_p^2$ and $\hat{\sigma}_d^2$ are provided by determining the sample means and variances of the two groups of measurements. However, it is possible in a real case that both parties change their explanations about the events on the basis of some new pieces of information gathered during an investigation, which would then require a further evaluation of the evidence under a new revised hypotheses. Therefore, a statistical tool to describe the true relationship between Q and the time since discharge T and estimates the probability of a given value $Q = q$ for each given $T = t$ would be desirable. A non-linear heteroscedastic regression model was proposed for this purpose. A regression model can be formally described as follows [129, 130]:

$$Q = \mu_t + \varepsilon_t \qquad \text{Eq. 10.4}$$

where μ_t is the mean of Q at t, and ε_t the measurement error. Assuming that ε_t is randomly distributed, that is $\varepsilon_t \sim N(0;\sigma_t^2)$, it is deducible that $Q \sim N(\mu_t;\sigma_t^2)$, where σ_t^2 is the variance of Q at t. Both the mean μ_t and the variance σ_t^2 could be described by functions (say f and g, respectively), and the following functions were found adequate in this context [131-136]:

$$f(t,\theta) = \theta_1 + \theta_2 \cdot e^{-\theta_3\sqrt{t}} \qquad \text{Eq. 10.5}$$

and

$$\begin{aligned} g(t,\theta,\tau) &= \tau_1^2 \cdot (\mu_t)^{\tau_2} \\ &= \tau_1^2 \cdot f(t,\theta)^{\tau_2} \end{aligned} \qquad \text{Eq. 10.6}$$

where θ_1, θ_2, θ_3, τ_1 and τ_2 are estimatable regression parameters (see Annexe F for details). Starting from a series of comparison shots carried out at different times after discharge (such as those represented in Figure 10.1), the parameter estimation can easily be performed by the maximum likelihood method and a computerised iterative resolution algorithm [129, 136, 137].

10.3. Examples of application

Table 10.1 shows distribution parameters at different times since discharge estimated by regression analysis using the suggested approach on a series of reference analyses, along with the likelihoods for different values of q. On the contrary, Table 10.2 summaries the LRs (V) obtained through the analysis of different pair of hypotheses which could have been forwarded by the parties. We observed that, for a given measurement q, the magnitude of V clearly depends on the considered pair of hypotheses. Generally, greater discrimination is obtained for the propositions in scenario I (T_p = 4 h vs. $T_d = 32$ h). This is normal considering that the estimated distributions for q given $T_p = 4$ h and $T_d = 32$ h are only slightly overlapping in comparison with other scenarios (Figure 10.2) and thus less "similar". In fact, for the scenario where the distributions are the most overlapping (i.e., scenario IV, $T_p = 9$ h vs. $T_d = 20$ h), the discrimination between hypotheses is globally weaker.

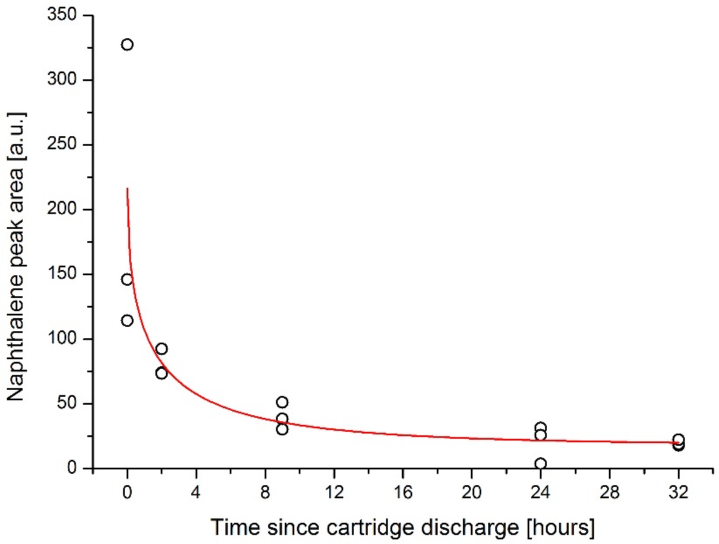


Figure 10.1 *– Decrease of the naphthalene peak area measured on reference cartridges. The scale of the vertical axis is the chromatographic peak area divided by 1000. The central line represents the mean tendency curve.*

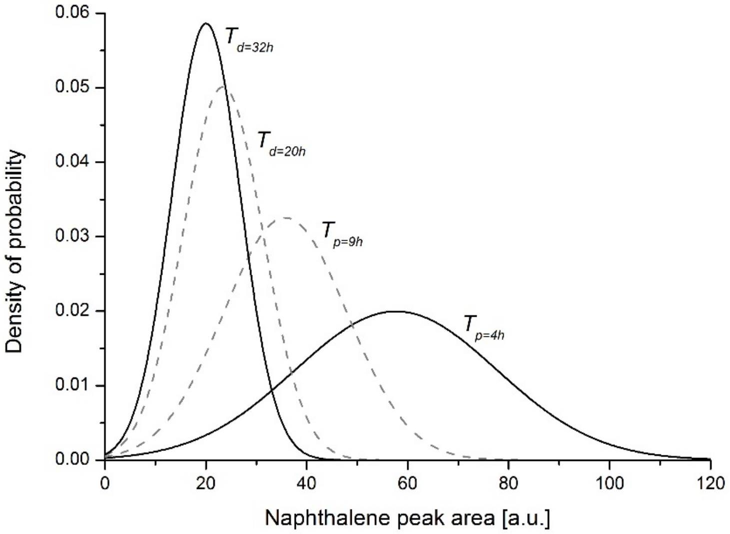


Figure 10.2 *– Examples of density distributions for Q (i.e., the chromatographic peak area of naphthalene) estimated with the regression model at four intervals after discharge. It is evident that the distributions for $T_p = 4$ h and $T_d = 32$ h are less overlapped with respect to the distributions for $T_p = 9$ h and $T_d = 20$ h.*

Figure 10.3 shows the evolution of V as a function of q. It is noteworthy that V had a minimum value, for which the evidence maximally support the defense hypothesis T_d. However, it never has a well-defined maximum, and very large values supporting the prosecutor's hypothesis T_p are theoretically possible. Furthermore, it should be noted that the increase of V over the neutral value of 1 ($logV = 0$) is more rapid than its decrease below this threshold (see also Table 10.2). These observations are coherent since they reflect the intrinsic uncertainty in small quantities of volatile GSR compounds. In fact, small *q* values have two reasonable explanations: a sufficiently long time has passed between discharge and analysis (the small extracted quantity is due to a true decrease in naphthalene) or the shot is recent but only a small quantity of compounds was produced (the small extracted quantity is due to a large deviation from the mean, which is still probable considering the large distributions of *q* for the shorter discharge times). On the contrary, large *q* values are fundamentally explained only by a short interval since discharge. This shows that a LR-based approach allows one to proportionally weigh all the possible explanations in the final result.

Time after discharge [h]	*Hypothesis*	*Estimates [a.u.]*		*Densities*			
		$\hat{\mu}$	$\hat{\sigma}^2$	*q = 17.00 a.u.*	*q = 28.00 a.u.*	*q = 39.00 a.u.*	*q = 50.00 a.u.*
4	$T_{p=4h}$	**57.69**	**398.76**	2.50×10^{-3}	6.61×10^{-3}	1.29×10^{-2}	1.85×10^{-2}
9	$T_{p=9h}$	**35.73**	**150.24**	1.01×10^{-2}	2.67×10^{-2}	3.14×10^{-2}	1.65×10^{-2}
20	$T_{d=20h}$	**23.39**	**63.38**	3.63×10^{-2}	4.24×10^{-2}	7.32×10^{-3}	1.88×10^{-4}
32	$T_{d=32h}$	**20.04**	**46.30**	5.31×10^{-2}	2.96×10^{-2}	1.21×10^{-3}	3.62×10^{-6}

Table 10.1 *– Interpolated estimates for the mean and variance of Q (i.e., the distribution of the naphthalene peak area) given different intervals after discharge. The right side of the table shows the probability densities associated with some selected measurements q at different intervals after discharge.*

Scenarios	*I*		*II*		*III*		*IV*	
	$T_{p=4h}$ *vs.* $T_{d=32h}$		$T_{p=9h}$ *vs.* $T_{d=32h}$		$T_{p=4h}$ *vs.* $T_{d=20h}$		$T_{p=9h}$ *vs.* $T_{d=20h}$	
Measurements	V	$log_{10}V$	V	$log_{10}V$	V	$log_{10}V$	V	$log_{10}V$
q = 17.00 a.u.	0.05	-1.33	0.19	-0.72	0.07	-1.16	0.28	-0.55
q = 28.00 a.u.	0.22	-0.65	0.90	-0.04	0.16	-0.81	0.63	-0.20
q = 39.00 a.u.	10.66	1.03	25.98	1.41	1.76	0.25	4.29	0.63
q = 50.00 a.u.	5124.79	3.71	4564.41	3.66	98.89	2.00	88.07	1.94

Table 10.2 *– Assigned likelihood ratios (V) and related logarithmic values associated with some selected measurements q under different scenarios.*

10.4. Chapter conclusion

A novel logical approach to interpret data in the field of shot dating using LR was developed. This suggested modeling the expected values for a given ageing parameter at any time since discharge using a normal distribution, and to estimate the distribution parameters using a non-linear regression model. The proposed approach was tested on univariate data exploiting the residual quantity of naphthalene, but is compatible with other compounds and/or ageing parameters (e.g. pairwise ratios between compounds), as long as appropriate regression equations are employed. A multivariate approach could also potentially be implemented using multivariate regressions analysis. In fact, a preliminary model to deal with these situations has already been implemented by the present author and reported in a MSc thesis; refer to [126] for details.

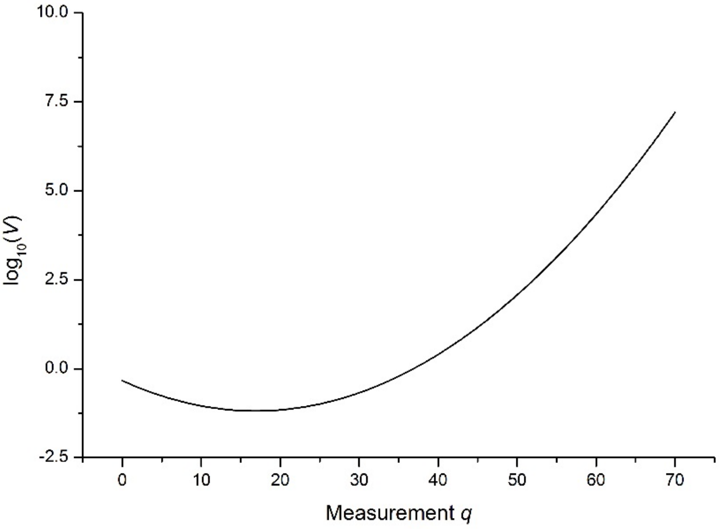


Figure 10.3 *– Estimated likelihood ratio (V, T_p = 4 h vs. T_d = 20 h) as a function of different observed measurements q (in this case, the chromatographic peak area of naphthalene). V is reported as its logarithmic value.*

CHAPTER 11

Discussion of a formal dating approach for caseworks

11. Discussion: formal dating approach for caseworks

The possibility of providing helpful ageing information from the headspace analysis of spent cases has been systematically studied in this research. It was thus observed that estimating the time since discharge of 9 mm cartridges up to 48 h of ageing with satisfying accuracy, or at least to differentiate recently fired from old cartridges (e.g., less than 5 h compared to more than 1 – 2 days), may be feasible under stable storage conditions. Furthermore, the opportunity to provide evidential values in the light of alternative propositions was also shown through the use of an evaluative model. These results should be considered in themselves a valuable achievement. In fact, they definitely prove that undertaking such examinations may be useful in some situations, contrary to what was reported in previous published studies. The interest in pursuing the research is hence also stressed.

Implementation of dating methods in casework practice is nonetheless a complex procedure, which requires the consideration of various aspects, from the initial crime scene investigation to the interpretation of evidence. Indeed, dating methods should ideally be integrated in existing forensic and investigation procedures, in such a way as to minimise the impact on routine protocols. All these aspects subject the acquisition of examined and reference materials to practical constraints. In this chapter, a general discussion about these topics is suggested, and the latter has been extended in order to include a critical insight on the solutions implemented in the present research. A formal implementation approach has finally been proposed (see Figure 11.1).

11.1. Analytical method and relative constraints

As previously reviewed, numerous analytical approaches could potentially be applied for the purpose of shot dating. However, many of them might physically damage the specimens and should thus be avoided, in order not to compromise the subsequent comparison of physical firearm marks, which remains the most important step in the investigative procedure. In this regard, the study of the evolution over time of the volatile GSR fraction through headspace extraction techniques is appealing as it allows the minimisation of such issues. Various extraction and analytical techniques could nonetheless be applied. What should be kept in mind is that every technique is different, and has distinctive advantages and disadvantage which should be thoroughly evaluated in order to be efficiently applied to real cases. In particular, sensitivity, reproducibility and selectivity, as well as invasiveness, simplicity (in terms of time and number of analytical steps) and availability (of the devices and instruments necessary to implement the method) are all important factors to consider for potential implementation in caseworks (Table 11.1).

In this research, HSSE was employed. Given its high extraction capacity, this extraction technique has several advantages over non-exhaustive sorptive methods, such as SPME (Table 11.1). It actually allows for better sensitivity and reproducibility, as well as for lower selectivity which leads to the extraction of a broader range of compounds [73, 83]. Because of this, HSSE has been an essential research instrument to comprehensively study GSR composition and allows the gaining of a better, more objective understanding of ageing processes in spent handgun cartridges. Indeed, this would not be possible with less exhaustive extraction methods. Nonetheless, it is acknowledged that it is also an invasive and relatively complex technique. Importantly, it does not allow the analysis of an enclosed specimen without re-opening its transport vessel or implementing more complex capping solutions, an important drawback in evaluating its routine application in forensic practice. In this regard, SPME would be more suitable thanks to its needle-based design, but it is also analytically less performant, as widely recognised. Some solutions to tackle these issues can be suggested. On one hand, further research could be performed in order to evaluate the possibility of adapting the multi-residue method developed in this research using a SPME-based approach. On the other hand, other extraction techniques could be considered. In this regard, an alternative which combines the advantages of both techniques is cfSPME (see Chapter 4.2). In fact, this extraction technique merges the sensitivity of a high-capacity approach with the low invasiveness of SPME [86-89], and thus combines the possibility of adopting a performant multi-residue method with the possibility of easily analysing samples in a conventional GC-MS instrument. For these reasons, it could be an interesting solution for future investigations.

Characteristics	***SPME***	***HSSE***	***cfSPME***
Sensitivity	–	+	+
Reproducibility	–	+	+
Selectivity	–	+	+
Invasiveness	+	–	+
Simplicity	+	–	+
Availability	+	+	–

Table 11.1 *– Characteristics of some sorptive methods. "+" and "–" indicate a positive or a negative point for that method, respectively.*

11.2. Interpretation of analytical outcomes in a forensic perspective

Situations where temporal information on the time elapsed since discharge of an examined cartridge proving helpful are numerous, but they could also be essentially different in relation to the kind of evidence required. As a consequence, this implies distinct ways of interpreting the analytical findings depending on the context.

If no suspect is arrested or he does not release any declaration ("missing-man" and "no-comment" situations, respectively, as described by Jackson *et al.* [138]), the inquiry should still be considered in its investigative stage. In these cases, the chemical analysis of specimens could provide evidence to reconstruct the course of the events through chronologically placing a shooting in time. Alternatively, it could also allow the identification of those specimens which are most likely to have been fired during the alleged offence (information which is useful, for example, in order to focus only on pertinent specimens for further examination and/or to recognise imported evidences). When these kind of situations are encountered, inferential models allowing the estimation of the time since discharge best explaining the analytical results are surely the most appropriate. Univariate models either based on graphical superposition of ageing profiles or regression analysis have been proposed in the literature. Nonetheless, as proved in this work, the consideration of only one ageing parameter at a time could be insufficient and/or particularly prone to errors. Simultaneously considering multiple ageing parameters allows the maximising of the reliability of the obtained time estimates, and thus adopting a multivariate interpretation model should be preferable. On this subject, this research has shown that RF and PLS could be valid regression methods, and also that a normalisation approach as PLR may be necessary to increase the accuracy of the obtained time-since-discharge estimates. Implementation of classification models in place of regression ones seemed also promising and could be addressed in future work. It should nonetheless be noted that the use of any multivariate approach (in regression or classification mode) implies the adoption of a multi-residue approach for analysis. The implementation of this kinds of approach in analytical methods thus appears essential in any future development in the field.

If a suspect is apprehended and forwards an alternative hypothesis on the course of events in contrast with that proposed by the prosecutor, the inquiry should be considered in its evaluative stage [138, 139]. For example, the suspect might admit that the specimens were fired by the seized firearm and ammunition, but contest the time since discharge, placing the firing event before the crime for legitimate reasons. From an interpretative point of view, these situations are slightly different in relation to those discussed above, and they thus also need the application of different interpretation strategies. In fact, the chemical analysis of the specimens should rather provide evidence to preferentially support one hypothesis through the assignment of an evidential value to the analytical outcomes. On this subject however, there is currently a lack of statistical methods in the literature in comparison to those devoted to the direct inference of time-since-discharge estimates. This is particularly annoying given that the estimation of time since discharge is often more pertinent in testing scenarios forwarded by the defence than for investigative purposes, as shown in many of the rare reported cases (e.g., [5, 6, 49]). Fundamentally, a logical approach based on the evaluation of LRs is suggested, considering that this methodology is mostly accepted as a particularly robust, transparent and impartial approach to evaluate evidences [125], and is widely gaining consensus amongst practitioners. Thus, the preliminary model developed in this PhD research seems valuable, also because it allows for further extension to account for multi-residue information through the use of multivariate distributions, as proven in [126]. Further research in this direction could be promising. In this regard, it should be noted that the use of multivariate distributions seems to be mandatory in order to account for correlations between compounds. Naïve approaches based on the multiplication of the evidential values obtained on the single ageing parameters presupposing their independency should be avoided, given that this premise is surely not realised and would lead to a large overestimation of the global evidential value. Furthermore, the pertinence of Chebyshev's inequality to estimate probabilities as suggested by [70] could also be contested, as it does not properly allow the determination of the probabilities of a quantitative analytical outcome itself, but rather that of obtaining values beyond its quantitative value.

11.3. Sampling of examined material

Application of best practices at the crime scene is essential to guarantee useful exploitation of the collected specimens, and this is especially true if dating will potentially become necessary. In this regard, many results obtained in this work suggest some important general guidelines to take into account for sampling both examined and reference materials (Fig. 11.1).

Usually, examined firearm-related specimens (e.g., spent cases and bullets) are put in soft paper bags for preservation and transport. While this type of packaging guarantees a protection against degradation of physical traces, it is not satisfactory for dating purposes as it does not halt ageing processes. In fact, freezing the state of the examined specimens under the conditions in which they were found at the crime scene is crucial for subsequent dating, given that target ageing parameters decrease very quickly in the first hours after discharge and transport in non-airtight conditions is likely to uncontrollably affect the ageing kinetics. In the specific case of spent cartridges, collection in sealable glass vials should be carried out systematically if dating is expected. This procedure is easily implementable in current crime-scene processing protocols, and very effective in blocking the dissipation of volatile GSR compounds [9, 62]. Caps with septa are strongly suggested if syringe-based extraction techniques (e.g., SPME and cfSPME) might be adopted to enable analysis without re-opening the vials. For invasive extraction techniques (e.g., HSSE), an extraction device should be placed inside before closing the vial or more sophisticated capping methods should be developed in the future for properly using these kinds of techniques in the laboratory.

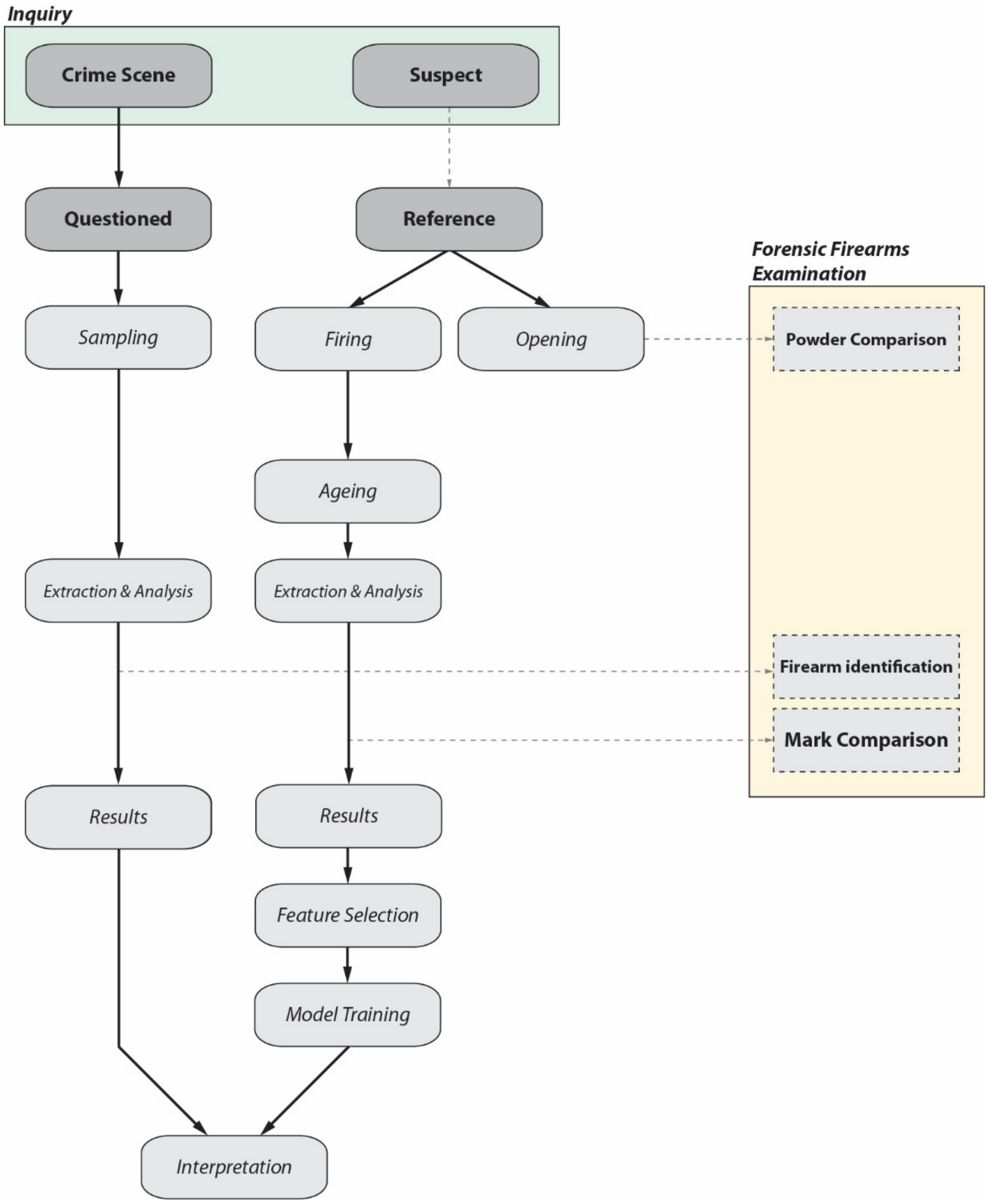


***Figure 11.1** – Suggested formal approach for dating in a casework*

A major conclusion which can be drawn from this PhD research is that interpretation of the analytical outcomes obtained on examined specimens should be a comparative procedure, in which the latter are weighted against relevant reference models (i.e., a case-by-case approach should be applied). In this regard, GSR ageing profiles are strongly dependent on numerous influencing factors, one amongst them being storage conditions. Thus, in order to allow a minimising of the differences between those factors which can influence ageing between examined and reference materials, the state of the examined specimens must be well documented upon arrival at the crime scene. Environmental conditions and specimen position should be noted, as well as any other elements which might indicate that its state was altered after firing. Temperature, relative humidity and wind conditions (airflow) are particularly important environmental parameters, as proved in this PhD research, and should imperatively be measured at the scene. If these parameters might have changed before sampling (e.g., day/night cycles), pre-existing conditions should be reconstructed by technical means. It is admitted that this purpose is not easy to reach and could constitute the main difficulty during investigation. Nonetheless, useful data could be obtained from meteorological records and/or direct post-crime in situ measurements. The experimental approach systematically applied in forensic entomology is a reliable methodology to obtain such information (see for example, Wyss & Cherix [140]), and could easily be adapted for current crime-scene protocols for the investigation of firearm-related crimes. Concerning specimen position, crucial parameters include its state (horizontal/vertical), as well as its relative location at the crime scene.

Upon arrival at the laboratory, chemical analysis should be prioritised over the examination of physical traces, because, once the vials are opened, concentrations of volatile compounds are modified and no useful analysis can be carried out. At the end of this procedure, specimens can be transferred to the forensic firearm-marks examination section.

11.4. Reference material and general analytical protocol

As reported above, interpretation of the analytical outcomes obtained on examined specimens is a comparative procedure, in which the latter are weighted against pertinent reference ageing models. Particularly, in addition to storage conditions, materials used to build reference models are equally prone to affect GSR ageing profiles and should thus be the most similar to those actually used during the alleged offence. In this regard, particularly important factors are the type of ammunition (brand and calibre), its production batch (due to the fact that smokeless powder can change between batches), and the firearm (which, for estimating the time since discharge of cartridges, could equally be important because of differences in working pressures and temperatures). Thus, in a case where a suspect is apprehended, their person should usually be searched for firearm and ammunition boxes, as they would constitute the best reference material. Otherwise, the reference firearm and ammunition should be selected on the basis of physical and chemical characteristics of the specimens. Independently from this situation, examined and reference materials should share comparable characteristics, both at the physical and chemical level. Applying case-unrelated reference materials and/or models does not seem to be a reliable strategy, considering the multitude of factors which could have a serious impact on the ageing profiles. Again, a case-by-case approach is strongly advised.

Providing evidence that supports the hypothesis of whether the seized firearm has been used or not to fire the examined specimens is a fundamental step in the investigation of firearm-related crimes. Thus, mark comparison between reference and examined materials should be a priority, and some reference cartridges should be kept apart to perform shooting tests if this is particularly urgent for the specific case. The remaining reference cartridges can then be used for dating purposes. Alternatively, the reference cartridge chemically analysed for volatile GSR compounds could successively be exploited for mark comparison purposes, as the headspace procedures developed so far (SPME and HSSE) essentially avoid physical alterations to the processed specimens. In any case, it is strongly suggested to open and analyse one supplementary reference cartridge in order to establish and compare the composition of the smokeless powder [25].

For the chemical analysis of reference material, groups of cartridges should be aged over different times after discharge. As the variability in the data is expected to be relatively high, genuine replica are very important and should be planned. Experimental plans such as those presented in Chapter 9 and 10 could be taken as examples. Independently from the type of case, it is essential that reference cartridges are submitted to the same treatment and ageing conditions as those expected to have been experienced by the examined specimens. A climatic chamber can be used to simulate specific storage conditions (e.g., temperature, relative humidity, airflow, light, position). Additionally, it is also suggested to schedule the analysis of all reference samples in the same analytical run, in order to reduce intra-day variability. The formal implementation approach showed in Figure 11.1 is proposed.

CHAPTER 12

Conclusion

12. Conclusion

Estimating the time since discharge of handgun cartridges has often been reported in literature. Nonetheless, currently available studies show discouraging results concerning the possibility of implementing such procedures in casework practice, and few applications to real cases have actually been reported. In analysing the methodologies adopted in these studies more deeply, it is apparent that they were rarely systematic due to very selective choices regarding extraction methods, target compounds and interpretative models. Hence, a tangible uncertainty currently exists on the actual helpfulness to criminal investigation of submitting cartridges to this kind of examination. In this regard, the purpose of this doctoral research was to tackle this issue through the exploitation of alternative analytical and interpretative tools. Especially, it aimed to increase the fundamental knowledge on the composition and ageing of volatile GSR, to develop a more appropriate headspace-based analytical method, as well as to suggest reliable statistical strategies to comprehensively interpret data in forensic frameworks.

From an analytical point of view, a novel punctual-sampling, semi-quantitative, HSSE approach was developed for the extraction of volatile GSR compounds from spent cartridges and their successive analysis by TD-GC-MS. This showed very satisfying analytical performances. In particular, better sensitivity and repeatability than previous methodologies (e.g., SPME) was observed. Volatile GSRs from different types of ammunition were then characterised using the developed approach, in order to identify adequate compounds for dating purposes. Explosion byproducts were found to be particularly suitable, as most of them were highly concentrated in GSRs, relatively reproducible within- and between-cartridges and significantly decreased over time (within 1 – 3 days). On the contrary, unreacted smokeless powder components, such as DPA, were significantly more variable and thus less interesting. 29 target compounds with different physico-chemical characteristics were finally selected, and the analytical approach comprehensively optimised based on these.

The optimised HSSE method was successively applied to study ageing phenomena in spent 9 mm cartridges. The main findings revealed that the amounts of volatile GSR compounds rapidly decreased in the first hours after discharge (< 10 h), but they did not totally disappear from cartridge headspaces. Thus, it was still possible to provide time-since-discharge estimates up to 48 h using multivariate interpretation models or, at least, to differentiate recently fired cases from older ones. Random forests and partial least-squares proved to be valuable regression methods to achieve these purposes, especially after pre-treatment of data based on pairwise ratios normalisation and logarithm transformation. The chosen chemometric tools presented significantly enhanced estimation performances in comparison to inferential univariate models based on the solely exploitation of single compounds at time.

The effects of several storage conditions (i.e., cartridge position, humidity and temperature) were further studied. While it was noted that all of them affected the rates of decrease of the single compounds in one way or another, cartridge position and humidity showed more limited impact. Conversely, temperature had major effects on ageing profiles, and it was thus recognised as a very important variable. Estimating time since discharge using multivariate models was thus attempted by varying storage conditions, but accuracy of conclusions was found not to be strongly dependent on the specific situation, as long as no significant difference existed between reference and examined materials. Nonetheless, a larger inaccuracy was actually observed when time since discharge was attempted on examined cartridges stored at different ageing conditions than those used for training the estimation models. Application of a dating methodology to real cases would consequently necessitate knowledge of the storage conditions before crime scene investigation. Strategies to measure them might include collection of information through meteorological data and/or post-crime measurements (as already systematically done in forensic entomology). Finally, an evaluative model was developed to interpret the data in a legal perspective. This allowed the calculation of likelihood ratios (LRs) to quantify the evidential value of analytical outcomes in the light of two adversarial hypotheses forwarded by the parties.

In conclusion, the results obtained in this PhD thesis showed that useful information could actually be extracted by headspace analysis of small calibre cartridges (contrary to previous research). HSSE combined with chemometrical tools was seen to be a very useful methodology for research purposes, as it enabled gaining considerable knowledge about ageing processes in spent cases. Further research should focus on the implementation of the proposed approach in practice. This might require adapting a less invasive headspace extraction (applicable on-site by untrained personal) as well as more in-depth studies about the effects on data interpretation that differences in storage conditions between examined and reference material have.

BIBLIOGRAPHY

Bibliography

1. Gallusser, A., M. Bonfanti, and F. Schütz, *Expertise des armes à feu et des éléments de munitions dans l'investigation criminelle*. 2002, Lausanne, CH: PPUR.
2. Warlow, T., *Firearms, the law and forensic ballistics*. 2nd ed. 2005, Boca Raton, FL: CRC Press.
3. Heard, B.J., *Handbook of firearms and ballistics*. 2nd ed. 2008, Hoboken, NJ: John Wiley & Sons.
4. Bonfanti, M., *Exploitation et interprétation des traces présentes sur les projectiles et les douilles.* Canadian Society of Forensic Science Journal, 1999. **32**(1): p. 25-37.
5. Bridgemon, R.R., *Cartridge case aging.* AFTE Journal, 1986. **18**(1): p. 49-52.
6. Andersson, C. and J. Andrasko, *A novel application of time since the latest discharge of a shotgun in a suspect murder.* Journal of Forensic Sciences, 1999. **44**(1): p. 211-213.
7. Persin, B., et al., *Évaluation de la date d'un tir.* Canadian Society of Forensic Science Journal, 2007. **40**(2): p. 65-85.
8. Wohlwend, C., *La datation des résidus de tir dans le canon*, 2000, University of Lausanne.
9. Belaud, V., *Identification par SPME-GC-MS des résidus de tirs volatils retrouvés dans les douilles: étude du vieillissement de composés cibles*, 2008, University of Lausanne.
10. Rigal, O., *Analyse des résidus de tir par arme à feu en SPME GC/MS: caractérisation d'un tir*, 2007, University of Paris-Sud.
11. Andrasko, J., T. Norberg, and S. Stahling, *Time since discharge of shotguns.* Journal of Forensic Sciences, 1998. **43**(5): p. 1005-1015.
12. Andrasko, J. and S. Stahling, *Time since discharge of rifles.* Journal of Forensic Sciences, 2000. **45**(6): p. 1250-1255.
13. Andrasko, J. and S. Stahling, *Time since discharge of pistols and revolvers.* Journal of Forensic Sciences, 2003. **48**(2): p. 307-311.
14. Andrasko, J. and S. Stahling, *Time since discharge of spent cartridges.* Journal of Forensic Sciences, 1999. **44**(3): p. 487-495.
15. Tienpont, B., et al., *High capacity headspace sorptive extraction.* Journal of Microcolumn Separations, 2000. **12**(11): p. 577-584.
16. Wallace, J.S., *Chemical analysis of firearms, ammunition, and gunshot residue*. 2008, Boca Raton, FL: CRC Press.

17. Romolo, F.S. and P. Margot, *Identification of gunshot residue: a critical review.* Forensic Science International, 2001. **119**: p. 195-211.

18. Meng, H.-H. and B. Caddy, *Gunshot residue analysis - a review.* Journal of Forensic Sciences, 1997. **42**(4): p. 553-570.

19. Dalby, O., D. Butler, and J.W. Birkett, *Analysis of gunshot residue and associated materials - a review.* Journal of Forensic Sciences, 2010.

20. Zeichner, A., *Firearm Discharge Residue: analysis of*, in *Wiley encyclopedia of forensic science*, A. Jamieson and A. Moenssens, Editors. 2009, John Wiley & Sons: Chichester, UK.

21. Rowe, W.F., *Residues*, in *Encyclopedia of forensic sciences*, J.A. Siegel, P.J. Saukko, and G.C. Knupfer, Editors. 2000, Academic Press: San Diego, CA.

22. Chang, K.H., et al., *Gunshot residue analysis and its evidential values: a review.* Australian Journal of Forensic Science, 2013. **45**(1): p. 3-23.

23. Romolo, F.S., *Organic gunshot residue from lead-free ammunition*, 2004, University of Lausanne.

24. Taudte, R.V., et al., *Detection of gunshot residues using mass spectrometry.* BioMed Research International, 2014.

25. Pun, K.-M. and A. Gallusser, *Macroscopic observation of the morphological characteristics of ammunition gunpowder.* Forensic Science International, 2008. **175**: p. 179-185.

26. Wolten, G.M. and R.S. Nesbitt, *On the mechanism of gunshot residue particle formation.* Journal of Forensic Sciences, 1980(533-545).

27. Basu, S., *Formation of gunshot residues.* Journal of Forensic Sciences, 1982. **27**(1): p. 72-91.

28. Schwoeble, A.J. and D.L. Exline, *Current methods in forensic gunshot residue analysis*. 2000, Boca Raton, FL: CRC Press.

29. Meng, H.-H. and H.-C. Lee, *Elemental analysis of primer mixtures and gunshot residues from handgun cartridges commonly encountered in Taiwan.* Forensic Science Journal, 2007. **6**(1): p. 39-54.

30. Brozek-Mucha, Z., *Distribution and properties of gunshot residue originating from a Luger 9 mm ammunition in the vicinity of the shooting gun.* Forensic Science International, 2009. **183**: p. 33-44.

31. Wolten, G.M., et al., *Particle analysis for the detection of gunshot residue, part I: scanning electron microscopy / energy dispersive X-ray characterization of hand deposits from firing.* Journal of Forensic Sciences, 1979. **24**(2): p. 409-422.

32. Moxnes, J.F., et al., *Lead free ammunition without toxic propellant gases.* Propellants, Explosives, Pyrotechnics, 2013. **38**: p. 255-260.

33. Richter, H. and J.B. Howard, *Formation of polycyclic aromatic hydrocarbons and their growth to soot: a review of chemical reaction pathways.* Progress in energy and combustion science, 2000. **26**: p. 565-608.

34. Ravindra, K., R. Sokhi, and R. Van Grieken, *Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation.* Atmospheric Environment, 2008. **42**: p. 2895-2921.

35. Mastral, A.M. and M.S. Callén, *A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation.* Environmental Science & Technology, 2000. **34**(15): p. 3051-3057.

36. Cropek, D.M., P.A. Kemme, and J.M. Day, *Pyrolytic decomposition studies of AA2, a double-base propellant*, 2001, US Army Corps of Engineers.

37. Cropek, D.M., P.A. Kemme, and J.M. Day, *Incineration by-products of AA2, NC fines, and NG slums*, 2001, US Army Corps of Engineers.

38. Cropek, D.M., et al., *Use of pyrolysis GC/MS for predicting emission byproducts from the incineration of double-base propellant.* Environmental Science & Technology, 2002. **36**: p. 4346-4351.

39. Ase, P., et al., *Propellant combustion product analyses on an M16 rifle and a 105mm caliber gun.* Journal of Environmental Science and Health A, 1985. **20**(3): p. 337-368.

40. Weyermann, C., et al., *Analysis of organic volatile residues in 9mm spent cartridges.* Forensic Science International, 2009. **186**: p. 29-35.

41. Mach, M.H., A. Pallos, and P.F. Jones, *Feasibility of gunshot residue detection via its organic constituents, part II: a gas chromatography-mass spectrometry method.* Journal of Forensic Sciences, 1978. **23**(3): p. 446-455.

42. Lichtenberg, W., *Methods for determination of shooting distance.* Forensic Science Review, 1990. **2**(1): p. 37-62.

43. Dillon, J.H., *The modified Griess test: a chemically specific chromatophoric test fo nitrite compounds in gunshot residues.* AFTE Journal, 1990. **22**(3): p. 243-250.

44. Turkel, H.W. and J. Lipman, *Unreliability of dermal nitrate test for gunpowder.* 1955. **46**: p. 281-284.

45. Sokol, J., *Die Bestimmung des Zeitpunktes, wann eine Waffe das letztemal beschossen und eine Partone verfeuert werde*, 1939, University of Bern.

46. Mezger and Heess, *Die Bestimmung des Zeitpunktes, wann eine Waffe das letztemal beschossen und eine Patrone verfeuert wurde.* Archiv für Kriminologie, 1930. **87**: p. 239-242.

47. Lucas, A., *Firearms, cartridges and projectiles*, in *Forensic Chemistry and Scientific Criminal Investigation*, A. Lucas, Editor 1935, Edward Arnold & Co.: London.

48. Meier, J., *Détermination du temps écoulé depuis le tir.* Revue Internationale de Police Criminelle, 1970. **234**: p. 22-26.

49. Shanahan, R., *Corrosion of new, fired, 0.22-Caliber, Long-Rifle brass cartridge cases buried in soil.* Journal of Forensic Sciences, 1977. **22**(2): p. 614-627.

50. Wogan, M.E., K.S. Webster-Hoffmeyer, and C.M. Grgicak, *Corrosion behaviour of four handguns in aqueous environments: corrosion product characterization and effects on estimating the time since deposition.* Science & Justice, 2013. **53**(3): p. 363-370.

51. Kerkhoff, W., D. Hazard, and M. Lopatka, *Atmospheric corrosion of bullets and cartridge cases as an indicator of time since discharge.* AFTE Journal, 2014. **46**(1): p. 51-58.

52. Larrison, R.M., *Degradation of fired bullets and cartridge cases in different environmental mediums.* AFTE Journal, 2006. **38**(3): p. 223-230.

53. Price, G., *Recent advances in ballistics laboratory methods.* Journal of Forensic Science Society, 1968. **8**(2): p. 83-90.

54. Sinha, J.K., *Time of firing of shot shells.* Journal of Forensic Sciences, 1976. **21**(1): p. 171-175.

55. Voskertchian, G.P. and G.V. Pavilova, *Spectrophotometric determination of time since discharge of firearms.* AFTE Journal, 1994. **26**(3): p. 216-222.

56. Voskertchian, G.P. and G.V. Pavilova, *The possibility of determining the time of shooting by ESR spectroscopy*, in *41st annual meeting of the american academy of forensic sciences*1989: Las Vegas, NV.

57. Voskertchian, G.P. and G.V. Pavilova, *Impact of different parameters on determination of time since discharge of firearms by ESR*, 1988, All-union institute of forensic sciences: Moskow.

58. Voskertchian, G.P. and G.V. Pavilova, *Spectrophotometric determination of time since discharge of firearms.* Crime Laboratory Digest, 1995. **22**(1): p. 4-10.

59. Aginsky, V., V. Lesnikov, and G. Sorokina, *Time of shooting - feasibility of discriminating "fresh" and "old" organic gunshot residues*, in *14th meeting of the international assocaition of forensic sciences*1996: Tokyo.

60. Pawliszyn, J., *Solid phase microextraction: theory and practice*. 1997, New York: John Wiley & Sons.

61. Pawliszyn, J., *Quantitative aspects of SPME*, in *Applications of solid phase microextraction*, J. Pawliszyn, Editor 1999, The Royal Society of Chemistry: Cambridge, UK.

62. Wilson, J.D., J.D. Tebow, and K.W. Moline, *Time since discharge of shotgun shells.* Journal of Forensic Sciences, 2003. **48**(6): p. 1298-1301.

63. Schmidt, M., *Analytik von Patronenhülsen zur genauen Bestimmung des Schussabgabe-Zeitpunkts*, 2010, TU Bergakademie Freiberg.

64. Chang, K.H., C.H. Yew, and A.F.L. Abdullah, *Study of the behaviors of gunshot residues from spent cartridges by headspace solid-phase microextraction-gas chromatographic techniques.* Journal of Forensic Sciences, 2015. **60**(4): p. 869-877.

65. Frère, B., et al. *Time since discharge of a firearm*. in *6th European Academy of Forensic Science Conference*. 2012. The Hague.

66. LARATES, *Estimation de la date de dernière utilisation d'une arme à feu*.

67. Wohlwend, C., *La datation du tir à l'aide de techniques instrumentales: étude préliminaire de la méthode de datation proposée par Andrasko*, 2001, University of Lausanne.

68. Aitken, C.G.G. and F. Taroni, *Statistics and the evaluation of evidence for forensic scientists*. 2nd ed. 2004, Hoboken, NJ: John Wiley & Sons.

69. Robertson, B. and G.A. Vignaux, *Interpreting evidence: evaluating forensic science in the courtroom*. 1995, Chichester, UK: John Wiley & Sons.

70. Sauleau, C., et al., *L'Interprétation des résultats d'analyse: une première approche sur la comparaison de caractéristiques chimiques des traces illustrant l'emploi de l'outil statistique au service des experts.* Spectra Analyse, 2014. **300**: p. 42-48.

71. Risticevic, S., et al., *Protocol for solid-phase microextraction method development.* Nature Protocols, 2010. **5**(1): p. 122-139.

72. Gorecki, T., *Solid versus liquid coatings*, in *Applications of solid phase microextraction*, J. Pawliszyn, Editor 1999, The Royal Society of Chemistry: Cambridge, UK.

73. David, F. and P. Sandra, *Stir bar sorptive extraction for trace analysis.* Journal of Chromatography A, 2007. **1152**: p. 54-69.

74. Meola, S., *Utilisation de la microextraction sur phase solide en science forensique*, 2012, University of Lausanne.

75. Dietz, C., J. Sanz, and C. Cámara, *Recent developments in solid-phase microextraction coatings and related techniques.* Journal of Chromatography A, 2006. **1103**: p. 183-192.

76. Bruheim, I., X. Liu, and J. Pawliszyn, *Thin-film microextraction.* Analytical Chemistry, 2003. **75**: p. 1002-1010.

77. Guerra, P., H. Lai, and J.R. Almirall, *Analysis of the volatile chemical markers of explosives using novel solid phase microextraction coupled to ion mobility spectrometry.* Journal of Separation Science, 2008. **31**: p. 2891-2898.

78. Guerra, P., S. Gura, and J.R. Almirall, *Dynamic planar solid phase microextraction-ion mobility spectrometry for rapid field air sampling and analysis of illicit drugs and explosives.* Analytical Chemistry, 2010. **82**: p. 2826-2835.

79. Gura, S., et al., *Enhancement in sample collection for the detection of MDMA using a novel planar SPME (PSPME) device coupled to ionmobility spectrometry (IMS).* Drug Testing and Analysis, 2009. **1**: p. 355-362.

80. Baltussen, E., et al., *Retention model for sorptive extraction-thermal desorption of aqueous samples: application to the automated analysis of pesticides and polyaromatic hydrocarbons in water samples.* Journal of Chromatography A, 1998. **805**: p. 237-247.

81. Baltussen, E., et al., *Equilibrium sorptive enrichment on poly(dimethylsiloxane) particles for trace analysis of volatile compounds in gaseous samples.* Analytical Chemistry, 1999. **71**: p. 5193-5198.

82. Baltussen, E., et al., *A new method for sorptive enrichment of gaseous samples: application in air analysis and natural gas characterization.* Journal of High Resolution Chromatography, 1997. **20**: p. 385-393.

83. Baltussen, E., et al., *Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles.* Journal of Microcolumn Separations, 1999. **11**(10): p. 737-747.

84. Baltussen, E., et al., *Automated sorptive extraction-thermal desorption-gas chromatography-mass spectrometry analysis: determination of phenols in water samples.* Journal of Microcolumn Separations, 1999. **11**(6): p. 471-474.

85. Popp, P., C. Bauer, and L. Wennrich, *Application of stir bar sorptive extraction in combination with column liquid chromatography for the determination of polycyclic aromatic hydrocarbons in water samples.* Analytica Chimica Acta, 2001. **436**: p. 1-9.

86. Zhang, Z. and J. Pawliszyn, *Quantitative extraction using an internally cooled solid phase microextraction device.* Analytical Chemistry, 1995. **67**: p. 34-43.

87. Carasek, E., E. Cudjoe, and J. Pawliszyn, *Fast and sensitive method to determine chloroanisoles in cork using an internally cooled solid-phase microextraction fiber.* Journal of Chromatography A, 2007. **1138**: p. 10-17.
88. Carasek, E. and J. Pawliszyn, *Screening of tropical fruit volatile compounds using solid-phase microextraction (SPME) fibers and internally cooled SPME fiber.* Journal of Agricultural and Food Chemistry, 2006. **54**(8688-8696).
89. Martendal, E. and E. Carasek, *A new optimization strategy for gaseous phase sampling by an internally cooled solid-phase microextraction technique.* Journal of Chromatography A, 2011. **1218**: p. 367-372.
90. Kolahgar, B., A. Hoffman, and A.C. Heiden, *Application of stir bar sorptive extraction to the determination of polycyclic aromatic hydrocarbons in aqceous samples.* Journal of Chromatography A, 2002. **963**: p. 225-230.
91. Prieto, A., et al., *Stir-bar sorptive extraction: a view on method optimisation, novel applications, limitations and potential solutions.* Journal of Chromatography A, 2010. **1217**: p. 2642-2666.
92. Bicchi, C., C. Cordero, and P. Rubiolo, *A survey on high-concentration-capability headspace sampling techniques in the analysis of flavors and fragrances.* Journal of Chromatographic Science, 2004. **42**.
93. Do Rosário, P.M.A. and J.M.F. Nogueira, *Combining stir bar sorptive extraction and MEKC for the determination of polynuclear aromatic hydrocarbons in environmental and biological matrices.* Electrophoresis, 2006. **27**: p. 4694-4702.
94. Serôdio, P., M. Salomé Cabral, and J.M.F. Nogueira, *Use of experimental design in the optimization of stir bar sorptive extraction for the determination of polybrominated diphenyl ethers in environmental matrices.* Journal of Chromatography A, 2007. **1141**: p. 259-270.
95. David, F., B. Tienpont, and P. Sandra, *Stir-bar sorptive extraction of trace organic compounds from aqueous matrices.* LC-GC North America, 2003. **21**(2): p. 108-118.
96. Lancas, F.M., et al., *Recent developments and applications of stir bar sorptive extraction.* Journal of Separation Science, 2009. **32**: p. 813-824.
97. Gentile, N., et al., *On the use of IRMS in forensic science: proposals for a methodological approach.* Forensic Science International, 2011. **212**: p. 260-271.
98. Girod, A., *Etude de la composition initiale et du vieillissement des traces digitales*, 2015, University of Lausanne.

99. Li, X.-M., et al., *Determination of polycyclic aromatic hydrocarbons in air by stir bar sorptive extraction-thermal desorption-gas chromatography tandem mass spectrometry.* Chinese Journal of Analytical Chemistry, 2011. **39**(11): p. 1641-1646.
100. Ochiai, N., et al., *Determination of trace amounts of off-flavor compounds in drinking water by stir bar sorptive extraction and thermal desorption GC-MS.* Analyst, 2001. **126**: p. 1652-1657.
101. David, F., et al., *Elucidation of the hoppy aroma in beers by stir bar and headspace sorptive extraction followed by thermal desorption - CGC - MS/PFPD*, 2001, Gerstel.
102. Bicchi, C., et al., *Headspace sorptive extraction (HSSE), stir bar sorptive extraction (SBSE), and solid phase microextraction (SPME) applied to the analysis of roasted arabica coffee and coffee brew.* 2002.
103. Demyttenaere, J.C.R., et al., *Use of headspace solid-phase microextraction and headspace sorptive extraction for the detection of the volatile metabolites produced by toxigenic fusarium species.* Journal of Chromatography A, 2004. **1027**: p. 147-154.
104. Splivallo, R., et al., *Discrimination of truffle fruiting body versus mycelial aromas by stir bar sorptive extraction.* Phytochemistry, 2007. **68**: p. 2584-2598.
105. Lorenzo, C., et al., *Non-destructive method to determine halophenols and haloanisoles in cork stoppers by headspace sorptive extraction.* Journal of Chromatography A, 2006. **1114**: p. 250-254.
106. Bicchi, C., et al., *Headspace sorptive extraction (HSSE) in the headspace analysis of aromatic and medicinal plants.* Journal of High Resolution Chromatography, 2000. **23**(9): p. 539-546.
107. Doong, R.-A., S.-M. Chang, and Y.-C. Sun, *Solid-phase microextraction and headspace solid-phase microextraction for the determination of high molecular-weight polycyclic aromatic hydrocarbons in water and soil samples.* Journal od Chromatographic Science, 2000. **38**: p. 528-534.
108. Eriksson, M., et al., *Determination of hydrocarbons in old creosote contaminated soil using headspace solid phase microextraction and GC-MS.* Chemosphere, 2001. **44**: p. 1641-1648.
109. Leardi, R., *Experimental design in chemistry: a tutorial.* Analytica chimica acta, 2009. **652**: p. 161-172.
110. Lundstedt, T., et al., *Experimental design and optimization.* Chemometrics and intelligent laboratory systems, 1998. **42**: p. 3-40.
111. Box, G.E.P., J.S. Hunter, and W.G. Hunter, *Statistics for experimenters: design, innovation and discovery*. 2nd ed. 2005, Hoboken, NJ: John Wiley & Sons.

112. Filzmoser, P. and B. Walczak, *What can go wrong at the data normalization step for identification of biomarkers?* Journal of Chromatography A, 2014. **1362**: p. 194-205.

113. Lehallier, B., et al., *Systematic ratio normalization of gas chromatography signals for biological sample discrimination and biomarker discovery.* Analytica chimica acta, 2012. **733**: p. 16-22.

114. Singh, A.K., D. Spassova, and T. White, *Quantitative analysis of polychlorinated biphenyls, organochlorine, insecticides, polycyclic aromatic hydrocarbons, polychlorinated hydrocarbons and polynitrohydrocarbons in spiked samples of soil, water and plasma by selected-ion monitoring gas chromatogrphy-mass spectrometry.* Journal of Chromatography B, 1998. **706**: p. 231-244.

115. Prieto, A., et al., *Development of a stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry method for the simultaneous determination of several persistent organic pollutants in water samples.* Journal of Chromatography A, 2007. **1174**: p. 40-49.

116. Gfrerer, M., B.M. Gawlik, and E. Lankmayr, *Validation of a fluidized-bed extraction method for solid materials for the determination of PAHs and PCBs using certified reference materials.* Analytica Chimica Acta, 2004. **527**: p. 53-60.

117. Jelisavac, L. and M. Filipovic, *Determination of diphenylamine and its mono-derivatives in single-base gun propellants during aging by high performance liquid chromatography.* Chromatographia, 2002. **55**: p. 239-241.

118. Stopforth, A., *Stir bar sorptive extraction and gas chromatography - mass spectrometry for the analysis of biological matrices*, 2007, University of Stellenbosch.

119. Bicchi, C., et al., *Impact of phase ratio, polydimethylsiloxane volume and size, and sampling temperature and time on headspace sorptive extraction recovery of some volatile compounds in the essential oil field.* Journal of Chromatography A, 2005. **1071**: p. 111-118.

120. Stauffer, E., J.A. Dolan, and R. Newman, *Fire debris analysis*. 2007, London, UK: Academic Press.

121. Arfsten, D.P., et al., *Acute and subacute dermal toxicity of Break-Free CLP: a weapons cleaning and maintenance compound.* Journal of Applied Toxicology, 2005. **25**: p. 318-327.

122. Masclet, P.A., A. Bresson, and G. Mouvier, *Polycyclic aromatic hydrocarbons emitted by power stations, and the influence of combustion conditions.* Fuel, 1987. **66**: p. 556-562.

123. Kuhn, M. and K. Johnson, *Applied predictive modeling*. 2013, New York: Springer.

124. Dieterle, F., et al., *Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures: application in ^1H NMR metabonomics.* Analytical chemistry, 2006. **78**: p. 4281-4290.

125. Jackson, G., *The scientist and the scales of justice.* Science & Justice, 2000. **40**(2): p. 81-85.

126. Gallidabino, M., *Une approche pragmatique pour l'évaluation de la preuve dans les cas impliquant l'estimation de la date du tir*, 2010, University of Lausanne.

127. Biedermann, A. and F. Taroni, *A probabilistic approach to the joint evaluation of firearm evidence and gunshot residues.* Forensic Science International, 2006. **163**: p. 18-33.

128. Royall, R., *On the probability of observing misleading statistical evidence.* Journal of the American Statistical Association, 2000. **95**(451): p. 760-768.

129. Huet, S., et al., *Statistical tools for nonlinear regression: a practical guide with S-Plus and R examples*. 2nd ed. 2004, New York: Springer.

130. Johnson, R.A. and D.W. Wichern, *Applied multivariate stastistical analysis*. 5th ed. 2002, Upper Saddle River, NJ: Prentice-Hall.

131. Lociciro, S., et al., *Dynamic of the ageing of ballpoint pen inks, quauntification of phenoxyethanol by GC-MS.* Science & Justice, 2004. **44**(3): p. 165-171.

132. Guerrier, B., et al., *Drying kinetics of polymer films.* AlChE Journal, 1998. **44**(4): p. 791-798.

133. Weyermann, C., et al., *Minimum requirements for application of ink dating methods based on solvent analysis in casework.* Forensic Science International, 2011: p. In press.

134. Weyermann, C., *Mass spectrometric investigation of the aging processes of ballpoint ink for the examination of questioned documents*, 2005, University of Giessen.

135. Weyermann, C., et al., *A GC/MS study of the drying of ballpoint pen ink on paper.* Forensic Science International, 2007. **168**: p. 119-127.

136. Ritz, C. and J.C. Streibig, *Nonlinear regression with R*. 2008, New York: Springer.

137. Draper, N.R. and H. Smith, *Applied regression analysis*. 3rd ed. 1998, New York: John Wiley & Sons.

138. Jackson, G., et al., *The nature of forensic science opinion - a possible framework to guide thinking and practice in investigations and in court proceedings.* Science & Justice, 2006. **46**(1): p. 33-44.

139. Jackson, G., C.G.G. Aitken, and P. Roberts, *Practioner guide no.4: case assessment and interpretation of expert evidence*, 2014, Royal Statistical Society's Working Group on Statistics and the Law.

140. Wyss, C. and D. Cherix, *Traité d'entomologie forensique*. 2006, Lausanne, CH: PPUR.

APPENDIX A

Theory and optimisation of sorptive extraction methods

A. Theory and optimisation of sorptive extraction methods

All the sorptive extraction techniques (e.g., SPME, SBSE, HSSE) are based on the same modes of operation. This means that construction materials, utilisation, working theory and optimisation parameters are globally very similar. In the following section, the physicochemical principles of sorptive extraction theory are presented in more detail. Refer to [1-4] for further explanations.

A.1. Coatings and sorption phenomena

In sorptive extraction, transport of analytes to the sorbent material starts immediately after exposing the latter to the sample. However, the specific phenomena which lead to this concentration are slightly different depending on the type of coating employed. Coatings can be homogeneous polymeric phases (globally known as "liquid" phases), or porous particulate solid materials imbedded in a supporting polymeric phases ("solid" phases) [5, 6]. Liquid phases, like poly(dimethylsiloxane) (PDMS) or poly(acrylate) (PA), are polymers which are over their glass transition temperature. Thus, they behave like high viscosity rubbery liquids and extract analytes via an absorption process. On the contrary, solid co-polymeric phases like poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB), poly(dimethylsiloxane)/carboxen, (PMDS/Carboxen), carbowax/divinylbenzene (CAR/DVB), and carbowax/template resin (CAR/TPR) are mixed coatings, in which the primary extracting phase is a porous solid. The latter extracts analytes via adsorption rather than absorption.

Absorption and adsorption are different phenomena. The only common feature between them is that both start with the substances getting attached to the surface of the coatings [5, 6]. Then, in absorption, they dissolve in the coating and diffuse to its bulk, while they stay on the surface of the solid phase in adsorption. Whether they migrate to the bulk of the coating or remain on its surface essentially depends on the diffusion coefficients of the analytes in the coating, which are similar to those of organic solvents for liquid phases and are thus several magnitudes higher than for solid phases. From a more practical point of view, a crucial difference between absorption and adsorption lies in the fact that, in adsorption, there are a limited number of surface sites where the interaction with analyte can take place: this means that, when all active sites are occupied, the phase is saturated and no more analyte can be trapped. Considering that liquids are miscible in any proportion, saturation in absorption does not occur. Consequently, the dynamic range for solid phases cannot be as broad as for liquid ones. Moreover, molecules with higher affinity for the surface can replace molecules with lower affinity, and the amount of the analyte extracted can be affected by the matrix composition (a phenomenon called "displacement effect"). This leads to severe problems in analyte quantification.

Advantages of solid phases are nevertheless their greater sensitivity for certain compounds [4]. Liquid phases, behaving like organic solvents, are actually limited to the extraction of non-polar and less volatile molecules which possess large partition coefficients in organic solvents (e.g., PAHs). Solid phases are more sensitive to polar and very volatiles molecules having low partition coefficients in organic solvents (e.g., VOCs).

A.2. Thermodynamic considerations

Following their physical state, samples can be solid, liquid or gaseous. This means that an analytical system can be composed from two or more phases [7]. In a 2-phases system, only the sample and the sorbent material are present: such a situation is possible when the sample is gaseous or liquid as long as no headspace is present. In a 3-phases system on the other hand, the system is composed of the sample, the sorbent material and an intermediate fluid where the latter is placed: such a situation comes for solid samples immersed in a solvent or for liquid with an overhanging headspace. Systems of higher orders are possible if, for example, a solid sample is immersed in a liquid with an overhanging headspace or some insoluble contaminants are present in a liquid extract. Extraction can be performed by posing the sorbent material directly in the sample (an extraction mode known as "direct immersion", or DI, which is normally used for liquid or gaseous samples), or by placing it in the headspace ("headspace extraction", HS, mainly used for solid and "dirty" liquid samples) [1, 4]. For liquid coatings working under absorption, extraction is driven by the partitioning of a compound between two (or more) immiscible phases. The partitioning process may be described by a dimensionless parameter called "partition coefficient" ($K_{p/s}$). Sorptive extraction is typically completed when analyte concentration has reached distribution equilibrium between sample matrix and the coating. In a 2-phases system, extracted analyte amount at this condition is done by the following expression [1, 8]:

$$m_p = C_0 \cdot \frac{K_{p/s} V_p V_s}{V_s + K_{p/s} V_p} \qquad \text{Eq. A.1}$$

where m_p is the amount extracted by the coating, C_0 is the initial concentration of the target analyte, V_s is the sample volume, V_p is the sorbent material volume and $K_{p/s}$ is the partition coefficient of the analyte between the sorbent material and the sample. Eq. A.1 reduces to Eq. 4.1 by assuming that $C_0 = m_0 / V_s = (m_s + m_p) / V_s$ and rearranging. Given that no saturation is possible in absorption, once equilibrium is reached the amount of each analyte extracted is constant; consequently, maximum sensitivity for absorptive extraction is only obtained under equilibrium conditions.

For solid phases working under adsorption, extraction is driven by the partitioning of a compound between a fluid phase and a solid surface. This partitioning is not identical to the previous one and may be described by a different dimensionless parameter called "Langmuir's distribution coefficient" (Ψ). In a 2-phases system where only an analyte is present, its amount at equilibrium conditions is given by the following expression [9]:

$$m_p = C_0 \cdot \frac{\Psi V_p V_s (C_{max} - C_{eq})}{V_s + \Psi V_p (C_{max} - C_{eq})} \qquad \text{Eq. A.2}$$

where C_{max} is the maximum concentration of active sites on sorbent material, C_{eq} is the concentration of the analyte on the sorbent material at equilibrium, and Ψ is the Langmuir's distribution coefficient of the analyte between the sorbent material and the sample. The form of this dependence is very similar to the corresponding for liquid coatings (Eq. A.1) except for the presence of the sorbent material concentration term $(C_{max} - C_{eq})$. This expression is only valid if one analyte is extracted. However, several compounds are generally present in real matrices and saturation of the solid surface can decrease the extracted amount for specific compounds [9]. Moreover, it should be noted that displacement effects lead to a decrease in the extracted amount of more volatile compounds by the less volatiles molecules when their equilibrium is reached. This implies that a choice must be effectuated and the maximal sensitivity for some compounds must be sacrificed in favour of other molecules. Pre-equilibrium extractions (i.e., retracting the fibre before reaching global equilibrium) are thus generally applied for adsorptive extractions in order to avoid displacement effects.

A.3. Kinetic considerations

Sorptive extraction can be performed by a passive or a dynamic approach [1, 10]. In passive extraction, the sorptive device is simply exposed to the sample and the transport of analytes to the coating is guaranteed only by their natural diffusion in the medium. In the dynamic approach, transport of the analytes to the sorbent material is favoured by forcing the convention of the medium with, for example, a pump or a stirrer. Adoption of this kind of extraction mode is useful mainly to increase the kinetics of the extraction, but it does not influence its thermodynamics, i.e., the equilibrium is reached more rapidly but maximal extracted quantity is the same as in infinite-time sampling. Several dynamic methods were proposed in literature; these methods can be divided into two general groups.

(1) In on-site environmental analysis, analytes are dispersed in infinite volumes (e.g., air or fluvial waters). Given that an infinite sample volume is available, sample can be continuously renewed around the extraction support by creating a continuous sample flow through it. In this situation, extracted analyte amount is very negligible in rapport to the general amount in the infinite sample volume.

(2) On the other hand, in laboratory grab analysis, analytes are normally contained in a finite volume vessel. Given that the sample volume is finite, sample properties cannot be continuously renewed and, in this case, dynamic approaches are only useful to favour analyte diffusion from the periphery of the vessel to the extraction support by increasing convention speed. In this case, extracted analyte amount is normally non-negligible.

A.4. Method optimisation

To maximise extraction efficiency, a number of experimental parameters must be carefully considered and optimised. Important experimental variables are the (1) chosen coating, (2) extraction mode, (3) separation/detection system, (4) agitation method, (5) analyte derivatisation, (6) sample volume and phase ratio, (7) sample pH, (8) ionic strength, (9) water content, (10) organic solvent content, (11) sample temperature, (12) extraction time, (13) desorption conditions [1, 4, 10].

As previously mentioned, possible extraction modes are basically two: direct immersion (DI) and headspace (HS). Each of these modes can further be implemented with a passive or a dynamic approach. From a matrix-based perspective, DI is generally used for gaseous and simple liquid samples. Whereas, HS is preferentially used for complex liquid and solid samples as it allows protecting the sorptive coating from physical damage, as well as from irreversible sorption of high molecular mass and other non-volatile contaminants in the sample. HS is also the preferred approach when sample modifications, which could be potentially dangerous for the coating (i.e., decreasing pH to very low values), should be applied. Otherwise, from an analyte-based perspective, HS is more suitable for the extraction of analytes of medium-to-high volatility and low-to-medium polarity, considering that these compounds are extracted faster from the headspace (i.e., they are more concentrated in the headspace than in the sample). By analogy, DI should be used for the extraction of compounds with low-to-medium volatility and medium-to-high polarity.

Adjusting the sample pH is sometimes useful considering that sorptive coatings extract only neutral species: extraction efficiency is therefore enhanced by fully converting charged analytes into their neutral forms. On the other hand, addition of salt (normally, sodium chloride, NaCl) allows for an increase in the ionic strength of a solution, which in turn leads to a decrease in the water solubility of many organic compounds and to an enhanced concentration in the extraction coating ("salting-out effect"). Another useful sample modification is the addition of organic solvents (generally, methanol). In fact, apolar compounds tend to be adsorbed on the extraction vessel walls and the presence of solvents can inversed this trend. However, a particular attention should be paid to the fact that organic solvents also decrease the distribution constants of the analytes between sample and coating. Their addition should thus be controlled and kept minimal. Temperature could have a significant effect on the extraction efficiency. Increasing the temperature allows for an increase in the vapour pressures of the analytes and, thus, to increase the headspace capacity. Furthermore, diffusion coefficients of the analytes are improved, with obvious enhancements to the extraction rates (kinetic positive effect). However, it also leads to a warming of the coating, which translates into a decrease in the analyte partition constant (thermodynamic negative effect). As a consequence, the optimal temperature will depend on the purpose of the analysis (speed or sensitivity), and should be determined experimentally. Finally, extraction time is also a question of compromise. Short extraction times allow fast analyses to be achieved, but also lead to a pre-equilibrium extraction with lower sensitivity and reproducibility. Long extraction times allow this problem to be prevented because of the establishment of an equilibrium between sample and coating, but analyses become potentially much longer, especially if very apolar compounds are analysed.

A.5. Quantification of analytes

Regarding sorptive extraction methods, "quantification" is an ambiguous term which can refer to 2 different concepts: (1) calibration (i.e., the determination of the analyte concentration in the sample) or (2) absolute quantification (i.e., the determination of the analyte concentration in the extraction support). Approaches for calibration can be further divided in 2 general groups: (1) traditional calibration approaches and (2) analytical calibration approaches. The first allows determining the actual analyte concentration (or total mass) in the sample directly from the detector signal, while the second allows back-inferring the actual concentration (or total mass) from the absolute analyte amount on the extraction support previously determined through absolute quantification. In the latter case, absolute quantification is thus a necessary step to calibrate. A summary of these procedures will be exposed in the following paragraphs. Refer to [1, 11, 12] for further explanations.

A.5.1. Absolute quantification

Analyte amount loaded into the extraction phase is proportional to the detector signal by a parameter which is known as the "response detector coefficient". Absolute quantification is simply the determination of this parameter for a given instrument and it is normally performed by liquid injection of standard solutions. Different solutions with increasing analyte concentrations are thus injected in the instruments, in order to determine the relation between the signal response and the injected amount: then, a new response value obtained from injection of extracted analytes can be compared to the obtained curve to determine the corresponding injected amount. This procedure relies on the assumption that the sample transfer efficiency in the injector is the same for both liquid injections and extractor desorption (i.e., the analyte masses transferred to the column are comparable for both injection types). For SPME, Ouyang et al. [13] demonstrated that this was not always true. Liner type was found to be a significant variable. In fact, sample transfer efficiencies obtained with a SPME liner without wool was different between liquid and SPME injections (i.e., poorer efficiencies are obtained for liquid injection than for SPME injection). A direct injection liner without wool (ID = 0.8 mm) was found to be the most efficient for both kinds of injections; alternatively, comparable results are obtained if SPME injections are carried out with SPME liner without wool (ID = 0.8 mm) and liquid injection are made with SPME liner fitted with wool (ID = 0.8 mm). Cross-sectional area of the space between the liner and the column, as well as the carrier gas flow rate and the length of the column inside the liner were others sensible parameters.

A.5.2. Traditional calibration

Traditional calibrations are empirical approaches that directly relates the detector response to the analyte concentration in the sample (C_0) without using any analytical relationship derived from the fundamental theory of sorptive extraction. Given that the concentration is directly related to the response, the response detector factor is an integral part of the global equation relating these two quantities, and should thus not directly be determined. Traditional calibration approaches can be divided into:

- *External calibration* – in external calibration, for each target analyte, the sample in question is extracted with the same method as a series of reference materials with increasing analyte concentration. The calibration curve is then determined by plotting analyte responses against actual concentrations: a new response value obtained from the injection of extracted analytes can be compared to this curve to determine the corresponding concentration in the sample. It is the simplest calibration approach, but it assumes that matrix effects between sample and reference materials are comparable, the extraction conditions are the same, and the efficacy of the extraction phase is constant. Unfortunately, the combination of these assumptions is rarely satisfied in reality.

- *Internal calibration* – internal calibration differs from external calibration by the introduction of internal standards (ISs) at a specific stage of the analysis in both the sample and the reference calibration standards. A single IS or multiple ISs can be used. In internal calibration with multiple ISs, an IS for each analyte of interest is introduced in the extraction procedure. These ISs are generally deuterated molecules considering that they should have the same physicochemical properties as for target compounds. In internal calibration with a single IS, one IS is globally used for all the target molecules. This IS should have comparable properties to the target compounds but, actually, it can have different physicochemical behaviours. After IS introduction, extraction is performed with the same method as the sample. The calibration curve is determined by plotting the analyte/standard response ratios against initial concentration: a new ratio value obtained from injection of extracted analytes can be compared to this curve to determine the corresponding concentration in sample.

  Using headspace sorptive extraction techniques, ISs can be introduced directly in the sample or in the extraction support (by a stepwise approach). This choice can have different effects. If multiple ISs are used and they are introduced directly in the sample, internal calibration can eliminate matrix effects (which leads to an increase in method validity) and coating usury effects (which leads to an increase in accuracy). This is because the ISs account for the variability introduced by the matrix and the coating. On the other hand, if ISs are introduced in the extraction support, only coating usury effects can be eliminated. More generally, if a single IS is used, the method cannot completely eliminate matrix and coating usury effects, but only minimise them. The extension of these benefits mainly depends on the similarity between the properties of the IS and the different target molecules. The main problem of adopting a multiple IS approach is that suitable deuterated molecules are difficult to find, they are expensive and they are generally more unstable than standard compounds. This is the reason because a single IS approach is normally preferred, although it is not a totally rigorous method. Nevertheless, for both approaches, extraction conditions must always be constant between sample and standard solutions.
- *Standard addition* – in standard addition, the sample is divided into many comparable sub-units. One sub-sample is left unaltered the others are spiked with known increasing quantities of analyte. Then, all the samples are extracted with the same method. The division of the

sample in sub-units is not mandatory: the same sample can be spiked different times with increasing quantities of the target analyte. The calibration curve is then determined by plotting analyte responses against initial concentrations. Because the same matrix is always used, standard addition method totally eliminates the problems of the matrix effects, but not the assumptions of constant extraction efficiency and conditions.

- *Multiple extraction calibration* – previous methods are difficult to apply to the quantification of analytes in solid sample due to the lack of certified reference materials used as standards for calibration. Multiple extraction calibration was developed to overcome this problem. This technique requires performing several consecutive extractions of the same sample. In this way, the total area corresponding to the complete extraction of the analyte can be calculated using the following equation:

$$A_T = \frac{A_1}{1-\beta} \qquad \text{A.3}$$

Where A_1 is the peak area observed after the first extraction and β is a coefficient calculated from the linear regression between the logarithms of the areas A_i and the corresponding index i: $\ln A_i = (i-1)\ln\beta + \ln A_1$. It should be noted that total peak area is a parameter that is not influenced by the matrix. Consequently, calibration curve (i.e., the curve obtained by plotting total peak areas against initial concentrations) can be obtained by analysing any kind of standard samples. For example, it is possible to calibrate a soil extraction method with standard solutions. Given its design, this approach permits *a priori* the elimination of all the problems recurring in traditional calibration approaches (i.e., heterogeneous matrix effects, differences in extraction efficiency and different extraction conditions). The only requirement is that the decrease in the extracted analyte between successive sorptive extractions must be considerable. Thus, it is solely applicable to SPME but not to other high-capacity sorptive extraction method, such as SBSE and HSSE.

A.5.3. Analytical calibration: equilibrium-based approaches

Equilibrium-based calibration approaches are a group of methods which are based on the determination of the initial analyte concentration (or total mass) from its absolute amount previously quantified on the sorption device. Thus, this procedure essentially employs the fundamental relationships of the thermodynamic theory of sorptive extraction (see Chapter A.2) in order to mathematically back-calculate the analyte concentration. For this reason, they start from two basic assumptions. The first is that the analyte partition coefficient $K_{p/s}$ between sample and coating is known. This further requires the extraction process to be controlled by absorption, and consequently implies that these calibration approaches are only applicable in those cases where liquid coatings are used. The second assumption is that the absolute analyte amount extracted by the absorbent material is known, and thus, that it was previously quantified with the method presented in Chapter A.5.1. Therefore, equilibrium-based calibration approaches are normally used in sequence with absolute quantification. The most appropriate equilibrium-based approach to use depends on whether the hypothesis that the extraction equilibrium has either been reached or not. In the latter case, extraction referred to is in pre-equilibrium mode.

- *At equilibrium* – when analyte concentration is effectively at equilibrium, the initial concentration of the analyte in the sample is related to its absolute amount in the sorptive phase by the following equation, which is a simple of rearrangement of Eq. A.1:

$$C_0 = m_p \cdot \frac{K_{p/s}V_p + V_s}{K_{p/s}V_pV_s} \qquad \text{A.4}$$

When the sample becomes very large (e.g., environmental analysis), this equation can be simplified to:

$$C_0 = m_p \cdot K_{p/s}V_p \qquad \text{A.5}$$

Conversely, in cases where the extraction is exhaustive, or when the sample volume is very small and $K_{p/s}$ is very large, Eq. A.4 simplified to:

$$C_0 = m_p \cdot \frac{1}{V_s} \qquad \text{A.6}$$

The latter relationship implies that all the analytes in the sample are extracted on the sorptive phase and the concentration of the target analyte can easily be calculated. Thus, it is the preferential approach for high-capacity sorptive methods, and/or when the recovery is known.

- *At pre-equilibrium* – if equilibrium is not reached, the following relation is preferred:

$$C_0 = m_p \cdot \frac{1}{1 - \exp\left(-at\right)} \cdot \frac{K_{p/s}V_p + V_s}{K_{p/s}V_pV_s} \qquad \text{A.7}$$

where *a* is a constant that depends on the extraction phase, the headspace and sample volumes, the mass transfer, the distribution coefficient, and the surface area of the extraction phase.

Equilibrium-based calibration methods are extremely precise as long as variables affecting the analyte partition are known. On this subject, it should be taken into consideration that the partition coefficient $K_{p/s}$ depends on the temperature, matrix properties and coating efficiency: consequently, some additional empirical relations must be known to adjust its value if extraction conditions are changed.

A.5.1. Analytical calibration: diffusion-based approaches

Diffusion-based calibration approaches are another group of analytical calibration methods. These were essentially developed for the calibration of the sorptive extraction of 2-phase systems under short extraction times (i.e., pre-equilibrium conditions), and allow only the determination of the analyte concentration in the environment directly surrounding the coating. Therefore, they are mainly used for on-site sampling (for example, of air or water). Several diffusion-based calibration approaches have recently been suggested. In comparison to equilibrium-based counterparts, most of these surpasses the assumption that the extraction process is driven by absorption, and they are consequently applicable also to solid coatings.

- *Time weighted average (TWA) calibration* – a TWA sampling is performed when the analyte gains access to the coating only by means of diffusion through a static air/water gap. In SPME, for example, this is true when the fibre is retracted at a known distance into its needle housing during the extraction. Thus, the amount of analyte accumulated during sampling time can be predicted by considering Fick's first law of diffusion. The following relationship is applicable:

  $$C = m_p \cdot \frac{Z}{ADt} \qquad \text{A.8}$$

  where *C* is, here, the average concentration of the target analyte in air/water during the time *t*, *Z* is the diffusion path length, *A* is the cross-sectional area of the needle and *D* is the diffusion coefficient of the target analytes in the air/water.
- *Interface model* – the interface model is a method which can be used for calibration of rapid extraction techniques using solid coatings. It is based on the assumption that molecules must pass through a static boundary layer surrounding the coating to be extracted:

$$C = m_p \cdot \frac{\ln\left((b+\delta)/b\right)}{2\pi D_g L t} \qquad \text{A.9}$$

where C is the concentration of the analyte in the bulk air, L is the length of the coated rod, D_g is the gas-phase molecular diffusion coefficient, b the outer radius of the coating and δ is the thickness of the boundary layer surrounding the coating.

- *Kinetic calibration (or, in-fibre standardisation technique)* – kinetic calibration is a method which can be applied only in the cases where a liquid coating is used. In fact, the theory behind this calibration method lies in the assumption that absorption of analytes can be described by $m_t/m_p = 1 - \exp(-at)$, where m_t is the amount of analyte extracted at the time t, m_p is the amount of analyte extracted at equilibrium ($t = \infty$), and a is a measure of how fast equilibrium can be reached. This is essentially realised only with liquid coatings. The calibration is accomplished by a 2-step procedure: the extraction device is firstly exposed to a standard material containing a reliable deuterated IS in order to pre-load it onto the coating, then the extraction is performed by exposing the same device again to the sample. Form the previous relation, it follows that, if a deuterated IS is pre-loaded onto the coating, desorption of the IS during the analyte extraction can be used to calibrate the extracted concentration of the corresponding analyte:

$$C_0 = m_t \cdot \frac{q_0}{K_{p/s} V_p (q_0 - Q)} \qquad \text{A.10}$$

where q_0 is the amount of pre-loaded IS in the extraction phase and Q is the amount of the standard remaining in the phase after its exposure to the sample for the sampling time.

Analogously to equilibrium based-approaches, these kinds of calibration methods are extremely precise as long as variables affecting the different coefficients are known. In particular, diffusion coefficient D depends on the temperature and medium, while the thickness of the boundary layer δ is affected by the stirring conditions of the sample.

Bibliography

1. Pawliszyn, J., *Solid phase microextraction: theory and practice*. 1997, New York: John Wiley & Sons.

2. Pawliszyn, J., ed. *Applications of solid phase microextraction*. 1999, The Royal Society of Chemistry: Cambridge, UK.
3. Lord, H. and J. Pawliszyn, *Evolution of solid-phase microextraction technology.* Journal of Chromatography A, 2000. **885**: p. 153-193.
4. Risticevic, S., et al., *Protocol for solid-phase microextraction method development.* Nature Protocols, 2010. **5**(1): p. 122-139.
5. Gorecki, T., *Solid versus liquid coatings*, in *Applications of solid phase microextraction*, J. Pawliszyn, Editor 1999, The Royal Society of Chemistry: Cambridge, UK.
6. Mani, V., *Properties of commercial SPME coatings*, in *Applications of solid phase microextraction*, J. Pawliszyn, Editor 1999, The Royal Society of Chemistry: Cambridge, UK.
7. Górecki, T. and J. Pawliszyn, *Effect of sample volume on quantitative analysis by solid-phase microextraction, part 1: theoretical considerations.* Analyst, 1997. **122**: p. 1079-1086.
8. Arthur, C.L., et al., *Automation and optimization of solid-phase microextraction.* Analytical Chemistry, 1992. **64**: p. 1960-1966.
9. Górecki, T., X. Yu, and J. Pawliszyn, *Theory of analyte extraction by selected porous polymer SPME fibres.* Analyst, 1999. **124**: p. 643-649.
10. Pawliszyn, J., *Quantitative aspects of SPME*, in *Applications of solid phase microextraction*, J. Pawliszyn, Editor 1999, The Royal Society of Chemistry: Cambridge, UK.
11. Ouyang, G. and J. Pawliszyn, *SPME in environmental analysis.* Analytical and Bioanalytical Chemistry, 2006. **386**: p. 1059-1073.
12. Ouyang, G. and J. Pawliszyn, *Recent developments in SPME for on-site analysis and monitoring.* Trends in Analytical Chemistry, 2006. **25**(7): p. 692-703.
13. Ouyang, G., et al., *Calibration of solid-phase microextraction for quantitative analysis by gas chromatography.* Journal of Chromatography A, 2005. **1097**: p. 9-16.

APPENDIX B

Development of a novel headspace sorptive extraction method to study the aging of volatile compounds in spent handgun cartridges

Published in:

Analytical Chemistry, **2014**, 86(9), 4471-4478.

Authors: Gallidabino, M., Romolo, F. S., Bylenga, K., & Weyermann, C.

B. Development of a novel headspace sorptive extraction method to study the aging of volatile compounds in spent handgun cartridges

Abstract

Estimating the time since the last discharge of firearms and/or spent cartridges may be a useful piece of information in forensic firearm-related cases. The current approach consists of studying the diffusion of selected volatile organic compounds (such as naphthalene) released during the shooting using solid phase micro-extraction (SPME). However, this technique works poorly on handgun cartridges because the extracted quantities quickly fall below the limit of detection.

In order to find more effective solutions and further investigate the aging of organic gunshot residue after the discharge of handgun cartridges, an extensive study was carried out in this work using a novel approach based on high capacity headspace sorptive extraction (HSSE). By adopting this technique, for the first time 51 gunshot residue (GSR) volatile organic compounds could be simultaneously detected from fired handgun cartridge cases. Application to aged specimens showed that many of those compounds presented significant and complementary aging profiles. Compound-to-compound ratios were also tested and proved to be beneficial both in reducing the variability of the aging curves and in enlarging the time window useful in a forensic casework perspective. The obtained results were thus particularly promising for the development of a new complete forensic dating methodology.

B.1. Introduction

Estimating the time since the last discharge of firearms and/or spent cartridges may be a useful piece of information in firearm-related cases [1, 2]. In fact, the validity of the collected evidence may sometimes be contested by stating that a seized gun was not recently fired, or alternatively, that a questioned empty cartridge found at the crime scene had been fired long before the commission of the crime. In such cases, estimating the time since discharge would help in reaching a conclusion.

Several approaches were previously suggested to address this issue, and most exploited the measurement of changes in the gunshot residue (GSR) after discharge [3-8]. The term “GSR” refers to the different materials released as a secondary result during the discharge of a firearm [9, 10]. These mainly include unburned and partially burned flakes of smokeless powder [11, 12], condensed metallic particles formed after the explosion of the primer [13, 14], and many explosion products and by-products coming from the deflagration of the propellant and the primer mixture [15, 16]. GSR is thus a complex and heterogeneous mixture composed of both organic and inorganic species, possibly undergoing some aging mechanisms after discharge. In this regard, many organic GSR substances have significant vapour pressures and are thus particularly suitable for estimating the time since the last discharge [15]. Amongst them, we can find smokeless powder components such as nitroglycerin, diphenylamine, ethylcentralite, dibutylphthalate and 2-ethyl-1-hexanol [17, 18]. Many organic reaction by-products were also identified, and these are mainly derivatives of benzene (e.g., benzonitrile and tolunitrile) and polycyclic aromatic hydrocarbons (PAHs) (e.g., naphthalene, acenapthene and pyrene) [19].

Solid phase micro-extraction (SPME) proved to be a promising solution for sampling such compounds in a GSR dating method [15]. SPME is a solvent-free extraction technique based on the partition equilibrium of analytes between a matrix and a small amount of sorbent phase coated on a fused silica fibre [20]. GSR dating approaches using this technique were generally based on measuring the evolution of selected target compounds by repeated sampling from the inner space of the questioned objects (such as barrels or spent cartridges). In this way, a partial aging curve was constructed and could be compared to a complete reference profile to infer the time since discharge [15]. Naphthalene and some unidentified decomposition products of nitrocellulose were suggested as target compounds [15]. Particular attention was devoted to the choice of the optimal coating material. Polydimethylsiloxane (PDMS), polyacrylate (PA), and mixed carboxen/polydimethylsiloxane (CAR/PDMS) were tested, and it was found that PA offered better sensitivity towards the selected target analytes [16]. Gas chromatography (GC) was generally recommended for analysis, coupled to a thermal energy analyser (TEA) for the nitrocellulose decomposition products [15, 21], or to a flame ionization detector (FID) [15] or mass spectrometer (MS) [22] for naphthalene.

SPME proved to be promising for dating the last discharge of shotguns, rifles, and related shells/cartridges [15, 21, 23]. However, when the method was applied to handguns and their cartridges, repeatability issues [16] and aging curves quickly falling below the limits of detection for the considered target compounds [16, 23, 24] were observed. No comprehensive studies were carried out using more effective extraction techniques and/or targeting less volatile compounds than naphthalene and nitrocellulose decomposition products. Therefore, the possibility of dating the discharge of handgun firearms and cartridges by sampling their GSR volatile organic compounds is still largely unexplored.

The purpose of this research was to study the aging of several GSR volatile organic compounds using a novel high capacity extraction technique, the so-called headspace sorptive extraction (HSSE). While HSSE and SPME are based on the same extraction principles, the extracting support of the former is a magnetic stir bar coated with a larger volume of sorbent phase in comparison to SPME fibres (up to 110 μL vs. a maximum of 0.5 μL, respectively) [25]. This feature generally guarantees better recovery yields with benefits for sensitivity and repeatability [25, 26], making HSSE particularly promising for studying more comprehensively the GSR aging processes in spent cartridges. Thermal desorption coupled to gas chromatography/mass spectrometry (TD/GC/MS) was used to recover and analyse the volatile compounds absorbed by HSSE stir bars. A data post-treatment approach involving compound-to-compound ratios was also applied in order to reduce variability of the aging curves and increase the measurable time ranges. These implementations resulted in an innovative analytical procedure expected to bring a significant contribution in a key forensic field.

B.2. Experimental section

B.2.1. Reference substances and solvents

Pure standards of 55 compounds previously identified in GSRs were purchased from various chemical companies (see details in Electronic Supporting Material, ESM): (#1) benzene, (#2) toluene, (#3) ethylbenzene, (#4a) p-xylene, (#4b) m-xylene, (#5) styrene, (#6) o-xylene, (#7) benzaldehyde, (#7) benzonitrile, (#9) 2-ethyl-1-hexanol, (#10) indene, (#11) acetophenone, (#12) o-tolunitrile, (#13) m-tolunitrile, (#14) p-tolunitrile, (#15) benzyl nitrile, (#16) naphthalene, (#17) benzo[b]thiophene, (#18) benzothiazole, (#19) quinoline, (#20) isoquinoline, (#21a) 1,4-dicyanobenzene, (#21b) 1,3-dicyanobenzene, (#22) indole, (#23) 2-methylnaphthalene, (#24) 1-methylnaphthalene, (#25) 1,2-dicyanobenzene, (#26) biphenyl, (#27) 2-ethylnaphthalene, (#28) 2,6-dimethylnaphthalene, (#29) 1,4-dimethylnaphthalene, (#30) acenaphthylene, (#31) biphenylene, (#32) acenaphthene, (#33) 4-methylbiphenyl, (#34) 1-naphthalenecarbonitrile, (#35) 2-naphthalenecarbonitrile, (#36) fluorene, (#37) diphenylamine, (#38) benzophenone, (#39) phenanthrene, (#40) anthracene, (#41) carbazole, (#42) ethylcentralite, (#43) dibutylphthalate, (#44) 2-nitrodiphenylamine, (#45) 1-methyl-3,3-diphenylurea, (#46) fluoranthene, (#47) pyrene, (#48) 4-nitrodiphenylamine, (#49) 2,4-dinitrodiphenylamine, (#50) chrysene, (#51) benzo[a]pyrene, (#52) nitroglycerin, (#53) N-nitrosodiphenylamine (compounds were numbered according to their elution order, while co-eluting and undistinguishable compounds were annotated with letters). For each substance, a working standard solution was prepared at a concentration of 1 mg·mL^{-1} in methanol (puriss. grade) purchased from Sigma/Aldrich (Buchs, Switzerland), except for anthracene, chrysene and benzo[a]pyrene which were dissolved in chloroform (purum grade) also purchased from Sigma/Aldrich. Nitroglycerin was already dissolved in a mixture of ethanol/methanol (97:3).

B.2.2. Ammunition and test shootings

Nine types of ammunition were purchased in 2011 from various sellers in Switzerland: five .45 ACP cartridges (produced by Geco, PMC, UMC, Sellier & Bellot and Magtech) and four .357 Magnum cartridges (produced by Geco, Sellier & Bellot, Samson and Magtech). All ammunition contained double-base smokeless powders (i.e., they contained both nitrocellulose and nitroglycerin as main explosives) except for the .45 ACP Magtech cartridges which contained a single-base smokeless powder (i.e., it contained only nitrocellulose).

The test shootings were carried out using a Colt 1911 semi-automatic pistol (.45 ACP) and a Colt Python revolver (.357 Magnum). Development of the method was carried out using all types of ammunition, while the aging profiles were solely acquired on the Geco and Magtech .45 ACP cartridges. In fact, spent cartridges from semi-automatic pistols are actually found more often at a crime scene given that they are ejected from the firearm after discharge, compared to revolver cartridges which stay in the revolving cylinder. Before each shooting event, firearms were carefully cleaned with dry cleaning patches. Five consecutive shots with a generic type of ammunition were additionally performed to remove any excess oils and lubricants. Then, test cartridges were fired by singly loading them in the magazine/barrel.

Spent cartridges analysed at time $t = 0$ h were immediately recovered after discharge and put in 20 mL HSSE-dedicated crimp glass vials (Gerstel, Sursee, Switzerland). 20 mL crimp vials were found to be a good solution because their large opening (i.e., 13 mm) fits well with most handgun calibres. Vials were closed with 20 mm crimp caps equipped with 3.0 mm PTFE/silicon septa (Gerstel). Spent cartridges analysed at time $t > 0$ h were aged with openings facing upward in an air conditioned laboratory kept at a temperature of about 20 °C. For each type of test, cartridges were fired in three replicates.

B.2.3. HSSE extraction of spent cartridges

HSSE stir bars are commercialized under the name of "Twister$^{©}$" by Gerstel. The largest available PDMS-coated HSSE stir bars (2 cm length with a 1 mm coating thickness, corresponding to a PDMS volume of 110 µL) were purchased. These were chosen to provide the maximum extraction capability. Stir bars were always thermo-conditioned before use. To do this, they were put in ad-hoc glass desorption tubes (Gerstel) and placed in a Gerstel TC tube conditioner. The conditioning procedure suggested by the producer (30 min at laboratory temperature, followed by 90 min at 300 °C and about 60 min for cooling down) was used.

For the extraction, one stir bar was suspended with the aid of a special glass insert (Gerstel) in the headspace of each vial containing one fired cartridge. Cartridges were extracted in a laboratory oven at 80 °C for 72 h. Before analysis, vials were reopened. Then, the stir bars were retrieved and placed in reconditioned desorption tubes. Finally, the tubes were capped with special transportation adapters (Gerstel) and placed on the GC tray for TD/GC/MS analysis.

B.2.4. TD/GC/MS analysis of stir bars

Stir bars were thermally desorbed using a Gerstel TDU thermal desorption unit connected to a Gerstel CIS-4 programmed temperature vaporizing injector. These devices were mounted on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (Agilent Technologies, Basel, Switzerland). The system was also equipped with a Gerstel MPS multi-purpose sampler which was used to automatically load tubes containing stir bars into the TDU.

Desorption mode was splitless, desorption flow 40 mL·min^{-1}, and desorption pressure 9.07 psi. The desorption ramp was programmed as follows: 20 °C for 0.5 min, ramped to 250 °C at 60 °C·min^{-1} and held at this temperature for 10 min (total desorption time of 14.3 min). The transfer line temperature between TDU and CIS-4 was 280 °C. Liners for CIS-4 were obtained from Gerstel and packed with quartz-wool. The cryo-focusing temperature was -80 °C. The PTV injection ramp was programmed as follows: -80 °C for 0 min, ramped to 300 °C at 600 °C·min^{-1} and held at this temperature for 1.37 min (total injection time of 2 min). Splitless mode was used during this time. After injection, the injector was additionally programmed to decrease at a rate of 720 °C·min^{-1} down to 270 °C and held at this temperature for 10 min in split mode to condition the liner for the next injection.

GC separation was performed on a HP-5MS (30 m x 0.25 mm x 0.25 μm) column from Agilent. The carrier gas was helium, and column flow was maintained at 1.2 mL·min^{-1}. The oven ramp was programmed as follows: 40 °C for 2 min, ramped to 100 °C at 10 °C·min^{-1}, ramped to 200 °C at 5 °C·min^{-1}, ramped to 280 °C at 5 °C·min^{-1} and held at this temperature for 10 min (total chromatographic time of 46 min). The transfer line between the column and the MS was at 280 °C. Ionisation was carried out through electron impact (EI). Masses were scanned from m/z 40 to 500 without solvent delay. MS source and quadrupole temperatures were 230 °C and 150 °C respectively.

B.2.5. TD/GC/MS analysis of reference standards

Liquid standards were injected into the instrument and analysed to obtain their MS spectra and retention times. The injection was performed by equipping a desorption tube with a special glass insert and a special transportation adapter for liquid injection, both furnished by Gerstel. Before analysis, the tube was automatically inserted into the TDU, and 1 µL of solution was injected into the insert. TD/GC/MS parameters were the same as written above, except for the CIS-4 cryo-focusing temperature which was set to 0 °C in order to vent solvents during desorption. Moreover, a solvent delay of 4 min and the split mode were adopted.

B.2.6. SPME extraction of spent cartridges

Automated SPME extractions were performed as previously described in Weyermann et al. [16], apart from extraction temperature which was set to 80 °C (instead of 20°C). A PA-coated fibre (85 µm layer) purchased from Supelco was used. Analyses were performed on an Agilent 6890N gas chromatograph equipped with a normal split/splitless injector and coupled to an Agilent 5973 inert mass selective detector. Separation and detection parameters were identical to those adopted for the TD/GC/MS method.

B.2.7. Data treatment

If not specified otherwise, aging curves were fitted with the following equation previously derived from the diffusion theory [27]:

$$S = A + B \cdot \exp\left(-C \cdot \sqrt{t}\right) \qquad \text{Eq. B.1}$$

where *S* is the expected peak area mean, *t* is the time since discharge, *A* and *B* are two size constants (for t = ∞, *A* represent the minimal value of the signal, while for t = 0, *A* + *B* is its maximal value), and *C* is a characteristic curve constant which is related to the decrease rate.

To test correlation between compounds, the Spearman’s rank correlation coefficient (r_s) was estimated for each tested type of ammunition between all the couples formed by the combination of the selected target compounds.

B.3. Results and discussion

B.3.1. Analysis of liquid standards

Analysis of liquid standards from 55 molecules previously identified in GSR was carried out in order to evaluate the injection and separation parameters of the TD/GC/MS method. The method allowed detecting 51 of the 55 injected compounds (Figure B.1 and ESM). Even if a PTV injector and a cold injection technique were used, some thermo-labile molecules could not be detected. Particularly, nitroglycerin (#52) degraded during thermo-desorption and/or in the injection port and was thus not observed in chromatograms. Lower temperatures should be adopted in order to avoid this phenomenon [16]. N-nitrosodiphenylamine (#53) also degraded but was totally converted into diphenylamine [28]. Some compounds co-eluted. Amongst them, the co-eluting couples p-xylene/m-xylene (#4a/#4b) and 1,4-dicyanobenzene/1,3-dicyanobenzene (#21a/#21b) actually showed undifferentiable mass spectra and could not be differentiated. On the contrary, styrene/o-xylene (#5/#6), acetophenone/o-tolunitrile (#11/#12), indole/2-methylnaphthalene (#22/#23), and 4-methylbiphenyl/1-naphthalenecarbonitrile (#33/#34) could be differentiated. The same was possible for isoquinoline (#20) which co-eluted with both 1,4-dicyanobenzene and 1,3-dicyanobenzene (#21a/#21b). Good chromatographic efficiency and resolutions were reached, as Figure B.1 illustrates. The only exceptions were the first two eluting compounds (i.e., benzene and toluene) which had relatively large peak widths. This is due to the cold injection method, which causes the slow release of the most volatile molecules into the GC column. Substances eluting after 5 min did not present this problem.

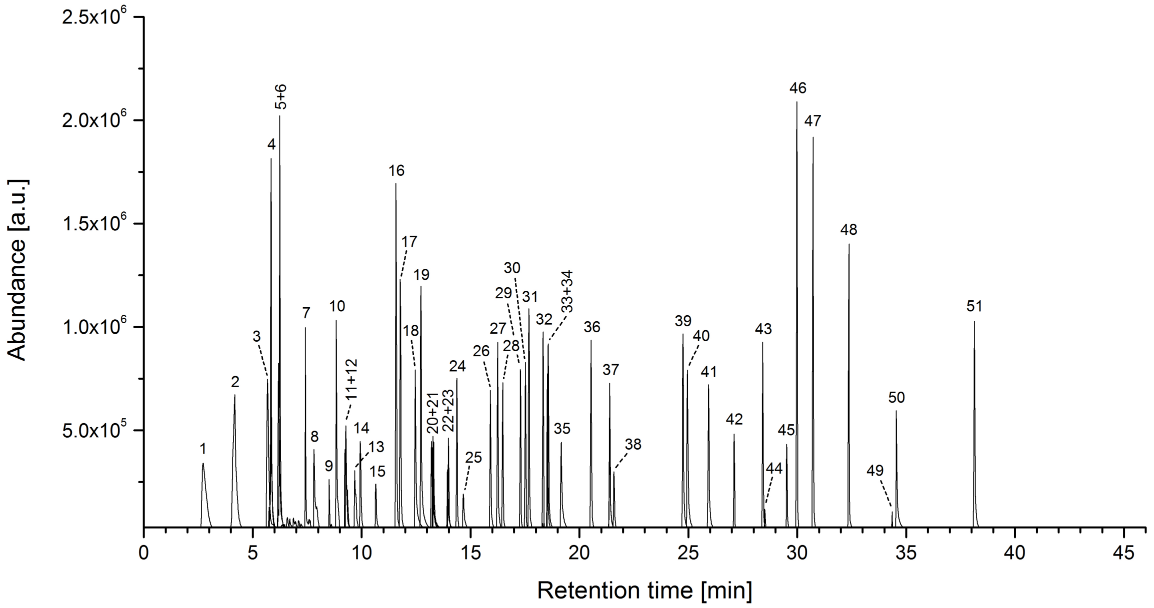


Figure B.1 – Superimposed extracted ion chromatograms (EICs) of the injected standards. Correspondence between numbers and peaks is explained in ESM.

B.3.2. HSSE analysis of spent cartridges

Three cartridges belonging to each of the nine types of ammunition were fired and extracted at 80 °C by HSSE stir bars and analysed using TD/GC/MS. Generally, HSSE total ion chromatograms (TICs) were characterised by a large number of peaks. Some of them were siloxane oligomers, which are formed during thermo-desorption by the oxidative degradation of the polydimethylsiloxane coating

[29]. As a representative example, Figure B.2a shows the TIC of a Samson .357 Magnum cartridge. The thick coating layer also produces a slight background noise along the chromatograms. Nevertheless, given the high selectivity of the mass spectral ions belonging to siloxane compounds, the additional signals did not affect the identification of target analytes. This is well illustrated in Figure B.2b where the superimposed extracted ion chromatograms (EICs) belonging to 12 ions of particular interest are shown. Apart from four analytes, all target compounds were detected in the GSR of all ammunition types. Differences between ammunitions were mainly quantitative (i.e., difference in their relative peak areas). The four compounds detected in only some types of cartridges were 1-methyl-3,3-diphenylurea (4 occurrences), 2,4-dinitrodiphenylamine (3 occurrences), chrysene (8 occurrences) and benzo[a]pyrene (1 occurrence).

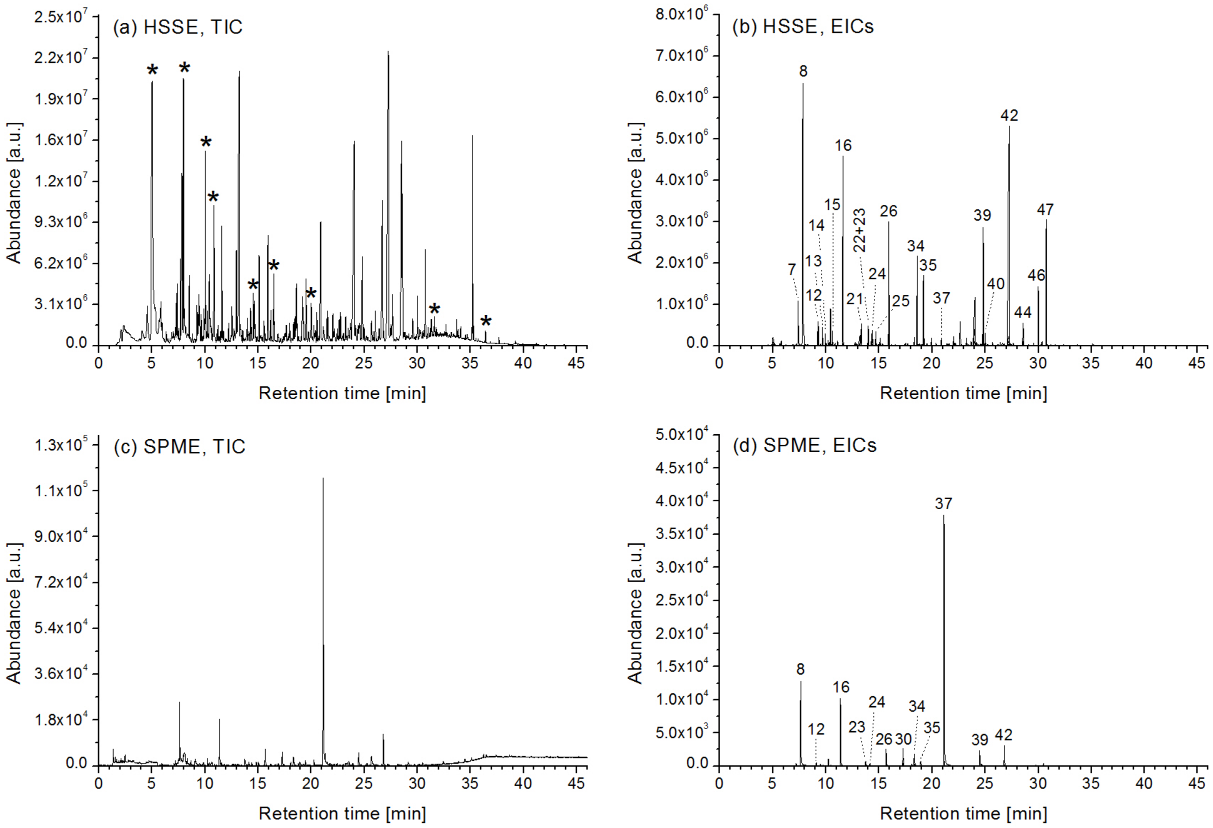


Figure B.2 *– Total ion chromatograms (TICs) and extracted ion chromatograms (EICs) of a fired Samson .357 Magnum cartridge extracted by HSSE (TIC: a; EICs: b) and SPME (TIC: c; EICs: d), at an extraction temperature of 80 °C. EICs are given as the superimposed traces for 12 ions of particular interest. Asterisks in the HSSE TIC indicate peaks belonging to major siloxane oligomers.*

For comparison, Samson .357 Magnum cartridges were also extracted by a PA-coated SPME fibre. Unlike HSSE, TICs obtained by SPME did not contain any major breakdown products (Figure 2c). This is both due to the difference in coatings and to the smaller volume of phase on fibres in comparison to stir bars. Despite this advantage, significantly fewer compounds were observed in SPME chromatograms as opposed to the HSSE ones. This was evident by comparing HSSE and SPME EICs for the 12 previously discussed ions in Figure B.2b and B.2d, respectively.

For comparison, Samson .357 Magnum cartridges were also extracted by a PA-coated SPME fibre. Unlike HSSE, TICs obtained by SPME did not contain any major breakdown products (Figure B.2c). This is both due to the difference in coatings and to the smaller volume of phase on fibres in comparison to stir bars. Despite this advantage, significantly fewer compounds were observed in SPME chromatograms as opposed to the HSSE ones. This was evident by comparing HSSE and SPME EICs for the 12 previously discussed

B.3.3. Measurement precision

Relative standard deviations (RSDs) of the peak areas for representative compounds were calculated in order to measure the precision of the analytical method (see details in ESM). Many sources of variability can affect precision during HSSE extraction of spent cartridges. Thus, measurement errors may be due to variations in the analysis, the HSSE extraction, the sample preparation, the discharge, and/or the powder composition [16]. In order to take into account all these factors, RSDs were calculated for cartridges analysed on different days (i.e., between-day precision) and using stir bars from different batches. Concerning explosion products, RSD values ranged from 3% to 70% in all the nine types of ammunition. This interval narrowed down to 3% - 43% without considering acenaphthylene and pyrene, which were the least reproducible compounds. RSD values for SPME are rarely mentioned in literature, but a recent study reported 71% for naphthalene and 157% for benzonitrile in spent 9mm Parabellum cartridges [16]. The same compounds extracted by HSSE yielded better RSD values between 5% - 34% and 9% - 33%, respectively. For gunpowder compounds, the RSD values were generally higher than for explosion products, ranging from 5% to 110%. This lower reproducibility may indicate that residual gunpowder in the cartridge is very variable from shot to shot. For SPME extraction of spent cartridges, RSD values of 96% and 153% were reported for diphenylamine and 2-ethyl-1-hexanol respectively [16]. Using HSSE to extract the same compounds, RSD values between 7% - 110% and 8% - 73% were respectively obtained.

B.3.4. GSR aging

The evolution of the detectable target compounds as a function of time was followed using the developed HSSE method. This study was carried out during a period of 31 h with two different types of .45 ACP ammunition: Magtech (single-base) and Geco (double-base). Generally, the GSR chromatograms strongly evolved during the cartridges' aging but not all the target substances had the same behaviour over time. Aging rates were actually found to be dependent on the nature of the considered compound and slightly influenced by the type of cartridge.

On the one hand, the different gunpowder components seemed to remain essentially constant over time, or at least did not follow any recognizable aging trend in both types of ammunition during the studied period. Considering that these compounds were mainly trapped in unburned powder flakes, their high persistence can be ascribable to a difficulty in escaping from this matrix, which in turn leads to an important slowing-down of their loss from the cartridges. Thus, they seem of little use for dating purposes, considering also their weak reproducibility (see previous section).

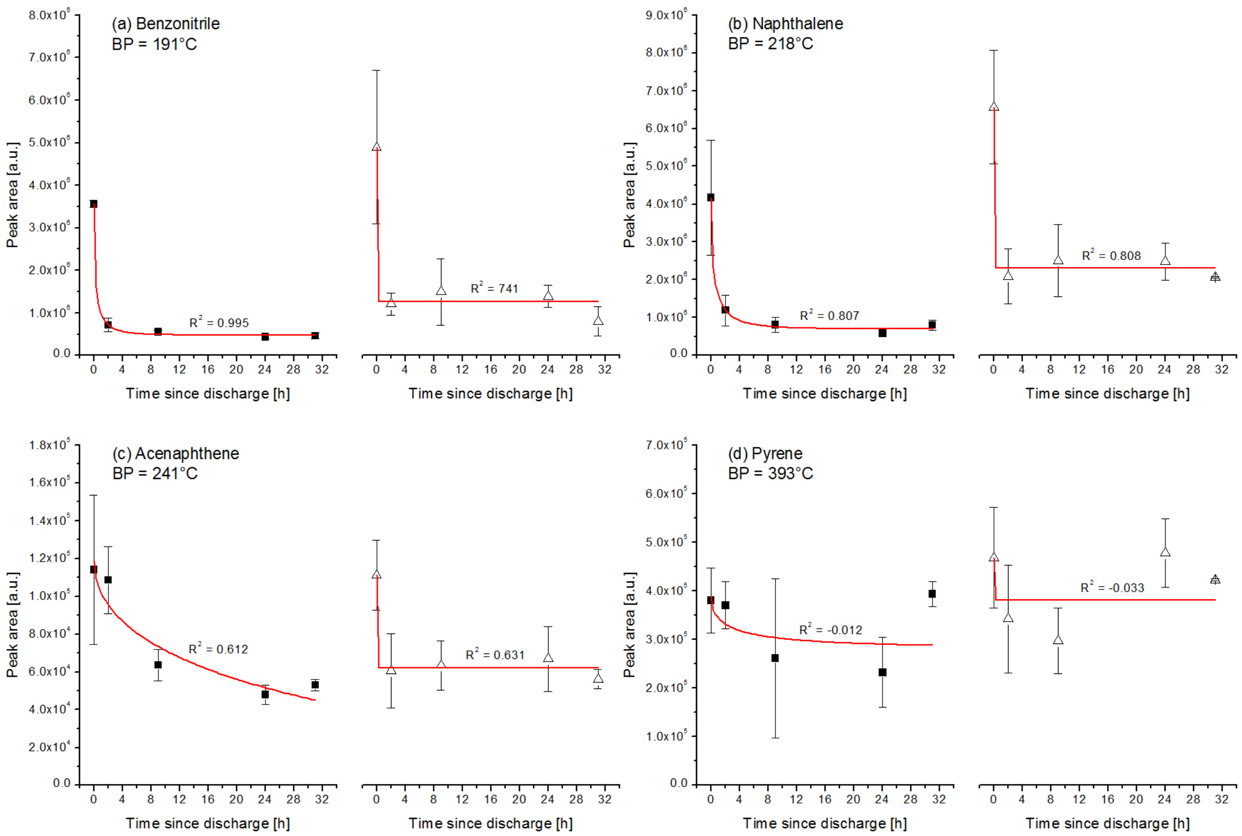


Figure B.3 *– Evolution of the peak areas observed by HSSE/TD/GC/MS for 4 target analytes in two types of .45 ammunition: Magtech (squares) and Geco (triangles). Measurements were fitted with Eq. B.1.*

On the contrary, most of the explosion products significantly diminished over time (Figure B.3). Geco ammunition generally gave faster and less reproducible aging profiles compared to those obtained from Magtech for the same molecules. In fact, analytes in Geco cartridges globally levelled off 2 h after discharge, while aging in Magtech ammunition appeared to be more variable. In the latter case, the substances' decrease rates were found to be directly correlated with their boiling points (BPs).

For example, naphthalene (BP = 218 °C [30]) diminished quickly from spent cartridges (70% of its signal was lost in the first 2 h after discharge), while acenaphthene (BP = 279 °C [30]) decreased more slowly and did not yet level off after 31 h (Figure B.3). As a general representation of the relationship between the BPs and decrease rates, Figure B.4 shows the plot of the estimated *C* parameters of the compounds' aging curves versus their GC retention times in Magtech cartridges (according to equation B.1, *C* is related to the decrease rates). It can thus be noticed that the higher the retention time of the compound is, the slower its decrease.

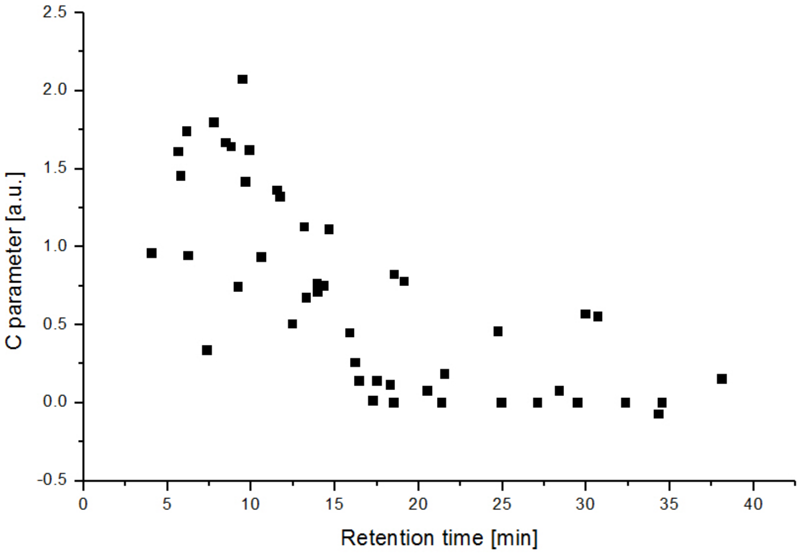


Figure B.4 *– Characteristic C constant for the aging curves (Eq. B.1) of all the target analytes detected in the Magtech .45 ACP spent cartridges versus their respective retention time (tR). C is related to the decrease rate.*

Levelling-off of the aging curves seemed to indicate that the diffusion of GSR compounds was actually characterised by two distinct phases. In the first aging stage, the decrease was generally rapid. As a high amount of vapours was released during discharge, the excess was probably lost rapidly because of the important gradient formed between the internal and external atmospheres. Then, equilibrium between compounds in the vapour phase and those adsorbed on surfaces such as walls and particles was probably established, making their decrease slower in this second phase. If we considered the aging curve of naphthalene (Figure B.3a), while its decrease was very rapid and essentially levelled off 2 h after discharge, the residual signal was still relatively high after 31 h. The same observation was previously reported for the escape of naphthalene and nitrocellulose decomposition products from barrels [15]. Because of this non-linear diffusion trend, most of the selected target substances were still detected in both cartridges 31 h after discharge, making the difference between GSR chromatograms at t = 0 h and t > 0 h essentially quantitative and not qualitative.

Reliability of aging profiles was also correlated to the compounds' BPs. Aging curves for low-BP compounds generally gave higher coefficients of determination (R^2) after regression analysis in comparison to high-BP compounds. For example, R^2 values for benzonitrile (BP = 191 °C [30]) reached 0.995 and 0.741 in the two types of ammunition, while values for pyrene (BP = 393 °C [30]) were quite low: -0.012 and -0.033 (Figure B.3). As a result, measurements for high-BP compounds were often not sufficiently reproducible in comparison to their decrease rates to significantly discriminate shots of different ages.

B.3.5. Ratios between compounds as aging indicators

In order to stabilize trends and solve the problem of data variability (particularly for high-BP compounds), normalization to other compounds or internal standards is a common stratagem in analytical chemistry [31, 32]. From a general point of view, an adequate normalization strategy would be to determine ratios between compounds for which the measured signal vary similarly. This is particularly important because it is essential that the reference and normalized compounds are submitted to the same variability-introducing factors. Internal standards added to the vial before the HSSE extraction would not be sufficient since they would account only for the variability introduced by the extraction procedure and the analytical method, and not for the heterogeneity of the powder composition and the shooting conditions (e.g., the temperature and pressure in the cartridge during the gunpowder explosion). Unfortunately, these latter factors are likely responsible for most of the variability affecting the results. In this regard, substances which are simultaneously produced during discharge are subjected to the same variability-introducing factors and are expected to present closer mutual fluctuations. Normalizing the response of a target compound with those of another correlated substance thus seemed a promising solution.

To test the correlation between compounds, Spearman's rank correlation (r_s) was calculated between all the couples formed by the combination of the 51 observable target analytes. Amongst the 1327 formed couples, only 407 (about 31%) were found to be strongly correlated (i.e., $r_s > 0.7$) in at least one type of cartridge. The relatively small percentage of correlated couples is not surprising. In fact, it should be noted that not all the explosion products are simultaneously produced during discharge, but their formation can be explained by multiple step reaction mechanisms in which larger products (e.g., pyrene and benzo[a]pyrene) are formed by the addition of radicals to smaller molecules (e.g., benzene and naphthalene) through different chemical pathways [33, 34]. This means that similar molecules probably come from a similar reaction pathway, resulting in high correlation. Indeed, in our research, isomers gave the best values. The correlation patterns also slightly depended on the type of ammunition. This can be explained by changes in the explosion temperature and pressure from cartridge to cartridge. However, some groups of compounds remained strongly correlated amongst the two tested types of ammunition. These are, for example, the groups formed by:

- naphthalene, 1-methylnaphthalene, 2-methylnaphthalene, biphenyl, 4-methylbiphenyl, fluorene and acenaphthene,
- phenanthrene, fluoranthene and pyrene
- benzonitrile, benzyl nitrile and the three tolunitrile isomers.

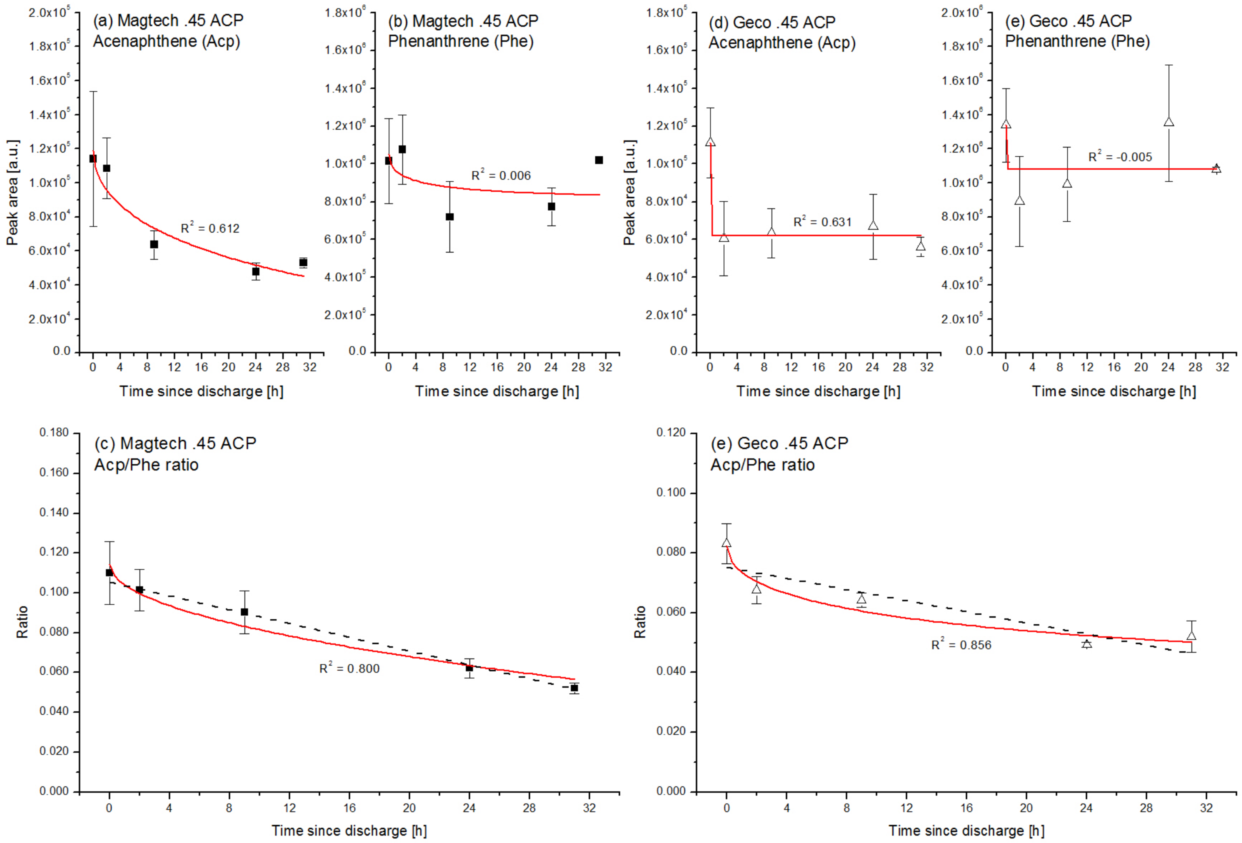


Figure B.5 – Evolution over time of selected compounds and their respective ratios in two .45 ACP cartridges. Solid lines are the regression curves obtained by fitting the data with the non-linear regression model reported in Equation (1), while dashed lines are the curves obtained by simple linear regression.

Ratios between signals belonging to the different couples of correlated molecules were determined in order to evaluate their usefulness for dating purposes. In many cases, the aging curves of the new indicators presented more reproducible values and slower decrease rates in comparison to those of the single molecules. A representative example is shown in Figure B.5, where the aging curves of acenaphthene (Acp) and phenanthrene (Phe) are reported for the two tested types of ammunition, along with those of their ratios (Acp/Phe). For single molecules, dispersion of peak areas was relatively high at the different tested ages yielding low R^2 after regression analysis (i.e., 0.612 and 0.631 for Acp, and 0.006 and -0.005 for Phe in Magtech and Geco cartridges, respectively). On the contrary, dispersion of the ratios' values was smaller and resulted in improved R^2 values for the Acp/Phe aging curves (0.800 and 0.856, respectively). In addition to this, it is also evident that the decrease rates for the Acp/Phe aging curves were slower than those of the single components. Indeed, they actually presented a quite linear trend. This slow-down effect was particularly significant for low-BP correlated molecules such as benzonitrile and o-tolunitrile. In fact, while these analytes showed a very quick decrease in their signals after discharge and reached a steady state after a couple of hours, the aging curve of their ratio did not to seem to level off after 31 h of aging. 407 ratios were tested in this work, and amongst them, 154 (38%) presented slower decrease rates than both their composing molecules in the Magtech ammunition, and 264 (65%) in the Geco ammunition.

Generally, the decrease rates of the ratios obtained by inter-normalizing compounds depended on the difference between the decrease rates of the respective components. For example, ratios obtained from substances showing comparable decrease rates tended to be constant over time (i.e., they did not show any appreciable decrease), while for compounds showing very different rates, the decrease rate of the obtained ratio was closer to that of the fastest decreasing substance. Intermediate situations were the most interesting because they allowed effectively slowing down the decrease rates and conserving a noticeable monotonic decrease. Apart from acenaphthene/phenanthrene and benzonitrile/o-tolunitrile, particularly interesting combinations were benzyl nitrile/1-naphthalenecarbonitrile, benzyl nitrile/2-naphthalenecarbonitrile, 1-methylnaphthalene/phenanthrene, 2-methylnaphthalene/phenanthrene, biphenyl/phenanthrene, and acenaphthene/fluorene.

B.3.6. Implementation in forensic casework

As demonstrated above, the suggested HSSE approach offers several advantages over existing extraction procedures (e.g., SPME) for analysing GSR volatile organic compounds in handgun cartridges (i.e., sensitivity and reproducibility). However, further research will be necessary in order to apply the approach in real forensic cases. Particularly, GSR aging kinetics in daily casework will be affected by the ammunition and firearm used, as well as other influence factors, such as the storage conditions. For example, Andrasko et al. [23] demonstrated that GSR compounds' diffusion was influenced by the environmental temperature and found that cold weather did quench the aging. They also showed that calibre and ammunition type were important parameters affecting the aging of the selected target compounds. Other factors such as humidity, wind speed, or cartridge position have not been addressed yet, but would probably affect GSR aging as well. Future works should thus consider these parameters in order to implement HSSE/TD/GC/MS for dating in casework [32].

B.4. Conclusions

A novel approach for studying the aging of gunshot residue (GSR) volatile organic compounds in handgun cartridges was developed in this work. This approach was based on the headspace sorptive extraction (HSSE) of the fired cartridges followed by thermal desorption and analysis using gas chromatography/mass spectrometry. The developed analytical method allowed, for the first time, the detection of 51 GSR compounds from fired cartridge cases. Moreover, compared to solid phase micro-extraction (SPME), HSSE was found to be more reproducible and effective, allowing simultaneous analysis of more compounds when applied to the same spent cartridge.

The method was applied to follow the evolution of GSR volatile organic compounds in two types of .45 ACP ammunition. Results showed that many compounds presented noticeable aging profiles and could thus be exploited for the purpose of estimating the time since discharge. Compound-to-compound ratios were tested and proved to be beneficial both in reducing the variability of the aging curves and in enlarging the time window useful for producing data in a forensic context. The obtained results are particularly promising regarding the full development of a forensic dating methodology for handgun and related cartridges. The analytical method should now be validated for practical forensic implementation, taking into account the factors influencing aging, in particular, the circumstances surrounding the discharge.

Acknowledgements

This work has been kindly supported by the Swiss National Foundation (Grant no. PP00P1_123358). The authors would like to thank Dr. Juan Manuel Garcia Gongora and Thibault Genessay from the Institut de Police Scientifique (Lausanne, Switzerland), as well as Dr. Arie Zeichner, former member of the Israeli Police (Jerusalem, Israel), for their precious advice.

Bibliography

1. Andersson, C. and J. Andrasko, *A novel application of time since the latest discharge of a shotgun in a suspect murder.* Journal of Forensic Sciences, 1999. **44**(1): p. 211-213.
2. Bridgemon, R.R., *Cartridge case aging.* AFTE Journal, 1986. **18**(1): p. 49-52.
3. Lucas, A., *Firearms, cartridges and projectiles*, in *Forensic chemistry and scientific criminal investigation*, A. Lucas, Editor 1935, Edward Arnold & Co.: London.

4. Sokol, J., *Die Bestimmung des Zeitpunktes, wann eine Waffe das letztemal beschossen und eine Partone verfeuert werde*, 1939, University of Bern.
5. Price, G., *Recent advances in ballistics laboratory methods.* Journal of Forensic Science Society, 1968. **8**(2): p. 83-90.
6. Sinha, J.K., *Time of firing of shot shells.* Journal of Forensic Sciences, 1976. **21**(1): p. 171-175.
7. Voskertchian, G.P. and G.V. Pavilova, *Spectrophotometric determination of time since discharge of firearms.* AFTE Journal, 1994. **26**(3): p. 216-222.
8. Aginsky, V.N., V. Lesnikov, and G. Sorokina, *Time of shooting: feasibility of discriminating "fresh" and "old" organic gunshot residues*, in *Proceedings of the 14th meeting of the International Association of Forensic Sciences*1996.
9. Romolo, F.S. and P. Margot, *Identification of gunshot residue: a critical review.* Forensic Science International, 2001. **119**: p. 195-211.
10. Dalby, O., D. Butler, and J.W. Birkett, *Analysis of gunshot residue and associated materials - a review.* Journal of Forensic Sciences, 2010. **55**(4): p. 924-943.
11. Pun, K.-M. and A. Gallusser, *Macroscopic observation of the morphological characteristics of ammunition gunpowder.* Forensic Science International, 2008. **175**: p. 179-185.
12. Andrasko, J., *Characterization of smokeless powder flakes from fired cartridge cases and from discharge patterns on clothing.* Journal of Forensic Sciences, 1992. **37**(4): p. 1030-1047.
13. Basu, S., *Formation of gunshot residues.* Journal of Forensic Sciences, 1982. **27**(1): p. 72-91.
14. Wolten, G.M. and R.S. Nesbitt, *On the mechanism of gunshot residue particle formation.* Journal of Forensic Sciences, 1980. **25**(3): p. 533-545.
15. Andrasko, J., T. Norberg, and S. Stahling, *Time since discharge of shotguns.* Journal of Forensic Sciences, 1998. **43**(5): p. 1005-1015.
16. Weyermann, C., et al., *Analysis of organic volatile residues in 9mm spent cartridges.* Forensic Science International, 2009. **186**: p. 29-35.
17. Laza, D., et al., *Development of a quantitative LC-MS/MS method for the analysis of common propellant powder stabilizers in gunshot residue.* Journal of Forensic Sciences, 2007. **52**(4): p. 842-850.
18. Mach, M.H., A. Pallos, and P.F. Jones, *Feasibility of gunshot residue detection via its organic constituents, part I: analysis of smokeless powders by combined gas chromatography-chemical ionization mass spectrometry.* Journal of Forensic Sciences, 1978. **23**(3): p. 433-455.

19. Cropek, D.M., et al., *Use of pyrolysis GC/MS for predicting emission byproducts from the incineration of double-base propellant.* Environmental Science & Technology, 2002. **36**: p. 4346-4351.
20. Pawliszyn, J., *Solid phase microextraction: theory and practice*. 1997, New York: John Wiley & Sons.
21. Andrasko, J. and S. Stahling, *Time since discharge of rifles.* Journal of Forensic Sciences, 2000. **45**(6): p. 1250-1255.
22. Wilson, J.D., J.D. Tebow, and K.W. Moline, *Time since discharge of shotgun shells.* Journal of Forensic Sciences, 2003. **48**(6): p. 1298-1301.
23. Andrasko, J. and S. Stahling, *Time since discharge of spent cartridges.* Journal of Forensic Sciences, 1999. **44**(3): p. 487-495.
24. Andrasko, J. and S. Stahling, *Time since discharge of pistols and revolvers.* Journal of Forensic Sciences, 2003. **48**(2): p. 307-311.
25. Tienpont, B., et al., *High capacity headspace sorptive extraction.* Journal of Microcolumn Separations, 2000. **12**(11): p. 577-584.
26. Baltussen, E., et al., *Stir bar sorptive extraction (SBSE), a novel extraction technique for aqueous samples: theory and principles.* Journal of Microcolumn Separations, 1999. **11**(10): p. 737-747.
27. Gallidabino, M., et al., *Estimating the time since discharge of spent cartridges: a logical approach for interpreting the evidence.* Science & Justice, 2013. **53**(1): p. 41-48.
28. Jelisavac, L. and M. Filipovic, *Determination of diphenylamine and its mono-derivatives in single-base gun propellants during aging by high performance liquid chromatography.* Chromatographia, 2002. **55**: p. 239-241.
29. Stopforth, A., *Stir bar sorptive extraction and gas chromatography - mass spectrometry for the analysis of biological matrices*, 2007, University of Stellenbosch.
30. Lide, D.R., *CRC Handbook of chemistry and physics*. 74th ed. 1994, Boca Raton, FL: CRC Press.
31. EPA, *Method 8000C, rev. 3: determinative chromatographic separations*. 2003.
32. Weyermann, C., et al., *Minimum requirements for application of ink dating methods based on solvent analysis in casework.* Forensic Science International, 2011. **210**(1-3): p. 52-62.
33. Richter, H. and J.B. Howard, *Formation of polycyclic aromatic hydrocarbons and their growth to soot: a review of chemical reaction pathways.* Progress in Energy and Combustion Science, 2000. **26**: p. 565-608.

34. Ravindra, K., R. Sokhi, and R. Van Grieken, *Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation.* Atmospheric Environment, 2008. **42**: p. 2895-2921.

APPENDIX C

Characterization of volatile organic gunshot residues in fired handgun cartridges by headspace sorptive extraction

Published in:

Analytical and Bioanalytical Chemistry, **2015**, 407(23), 7123-7134.

Authors: Gallidabino, M., Romolo, F. S., & Weyermann, C.

C. Characterization of volatile organic gunshot residues in fired handgun cartridges by headspace sorptive extraction

Abstract

In forensic investigation of firearm-related cases, determination of the residual amount of volatile compounds remaining inside a cartridge could be useful in estimating the time since its discharge. Published approaches are based on following the decrease of selected target compounds as a function of time by using solid phase micro-extraction (SPME). Naphthalene, as well as an unidentified decomposition product of nitrocellulose (referred to as "TEA2"), are usually employed for this purpose. However, reliability can be brought into question given their high volatility and the low reproducibility of the extracted procedure.

In order to identify alternatives and therefore develop improved dating methods, an extensive study on the composition and variability of volatile residues in nine different types of cartridges was carried out. Analysis was performed using headspace sorptive extraction (HSSE), which is a more exhaustive technique compared to SPME. 166 compounds were identified (several of which for the first time), and it was observed that the final compositional characteristics of each residue were strongly dependent on its source. Variability of single identified compounds within and between different types of cartridge, as well as their evolution over time, were also studied. Many explosion products containing up to 4 aromatic rings were found to be globally present in high proportions. 27 of them (excluding naphthalene) also presented detectable decreases during the first 24 h. Therefore, they could be used as complementary target analytes in future dating methods.

C.1. Introduction

In the forensic investigation of criminal cases, residual materials released during firearm discharges can be exploited in order to identify a person involved in a gunfire event, estimate a shooting distance, determine the ammunition type, or to estimate the time elapsed since discharge [1-3]. These residual materials are collectively known as gunshot residue (GSR), and mainly consist of flakes from partially reacted smokeless powder, vaporized species from all the cartridge elements, microscopic metallic particles and explosion products from gunpowder and primer mixtures [1-3]. Given their importance in forensic science, the specific composition and variability of some of them were thoroughly studied. This is the case for solid particulate residues (i.e., smokeless powder flakes and microscopic metallic particles), which are generally used to identify a shooter and/or estimate a shooting distance [4-7].

Early researches in the field of volatile GSR compounds mainly referred to environmental and health issues [8, 9], neglecting the areas of applications in forensic science. More recently, it was found that their diffusion kinetics could be useful to estimate the time since a cartridge or firearm was last discharged [10-13]. Since then, choosing the most accurate target compounds has been a central concern, and questions concerning composition and variability of volatile GSR compounds has become again of great interest. It is well known that the main compounds released during the cartridge discharge are oxidation products. Sequential explosion of the various organic species composing the gunpowder and primer mixtures produces very hot gases, essentially consisting of H_2O, CO, CO_2, H_2 and N_2 [9, 14]. However, low levels of many other heavier compounds were also found in GSR vapours. Most of them are pyrolitic byproducts, the formation of which is due to the oxygen-deficient and high-temperature conditions persisting during the discharge. Such extreme conditions lead to the formation of radicals, which could add to other stable molecules and set in motion a waterfall mechanism resulting in the formation of larger compounds [15, 16]. Benzene, toluene and styrene are generally the first species to be formed by the stabilisation of unsaturated aliphatic radical species through cyclization. Then, successive additions can lead (through further cyclization phenomena) to naphthalene, and from there to the synthesis of other heavier polycyclic aromatic hydrocarbons (PAHs). Branched or substituted derivatives of the previous compounds (e.g., methylnaphthalenes) can be released as side-products; similarly, hetero-atoms can take part in the reaction mechanism and be incorporated in the structures of the final products [17, 18]. Hundreds of PAHs from 2 up to 6 rings, substituted derivatives of benzene and heterocyclic compounds have been identified in GSRs. Particularly, Ase et al. [8] identified more than 100 different trace species in the exhaust of a military rifle and an anti-tank gun using trapping methods followed by gas chromatography coupled to mass spectrometry (GC/MS) and high performance liquid chromatography with ultraviolet detection (HPLC/UV). Andrasko et al. [10] confirmed the presence of some of these compounds in shotgun barrels by in-situ sampling of vapours with headspace solid phase micro-extraction (SPME), followed by analysis with GC coupled to thermal electron analyser (TEA), flame ionization detector (FID) or mass spectrometry (MS). Using SPME-GC/MS, Weyermann et al. [19] characterized the residues of two types of 9 mm Parabellum ammunition and reported the identification of 34 compounds (confirmed with reference standards). Recently, Gallidabino et al. [20] looked for specific compounds in GSRs and identified 51 molecules in the headspace of fired cartridges using headspace sorptive extraction (HSSE) followed by GC/MS.

Most of the published approaches for estimating the time since discharge were based on the selection of target analytes amongst the previously identified molecules, and the comparison of their residual quantity against aging profiles obtained from pertinent reference material (i.e., the firearm/cartridges set presumably used for committing the crime). Thus, naphthalene and an unidentified decomposition product of nitrocellulose (referred to as “TEA2”) were regarded as promising in order to estimate the time elapsed since a shotgun, rifle or related ammunition were last fired [10-12, 21]. However, their usefulness for dating the discharge of handguns and smaller cartridges was questioned because they disappear too rapidly (few hours) and their amounts are not reproducible enough [13, 19]. Until now, no comprehensive qualitative and quantitative studies on GSR compositions released by handgun cartridges have ever been carried out to the best of our knowledge, leaving a gap on possible useful target compounds.

Thus, this research aimed at comprehensively evaluating the composition and variability of volatile GSRs produced by nine different types of ammunition belonging to two handgun calibres (.357 Magnum and .45 ACP). At first, compounds found in cartridges after discharge were extracted and identified. As sampling method, HSSE was selected. HSSE is a recent extraction technique, which was introduced for trace and ultra-trace analysis [22]. While it is based on the same principle of headspace SPME, the extraction support in HSSE is not a thin fibre but a magnetic stir bar coated with a larger volume of polydimethylsiloxane (PDMS) phase (up to 110 µL vs 0.5 µL for SPME) [22]. For this reason, limits of detection are improved and the discrimination toward more polar compounds is generally reduced, making HSSE a very valuable technique for extensive characterization of various sample types, as recently proved in many analytical domains [22-24]. The method was recently applied to volatile GSR compounds, leading to a good extraction recovery for some selected compounds [20]. Following the identification step, intra-variability (i.e., variability within the same firearm and cartridge set), inter-variability (i.e., variability between different firearm and cartridge sets) and evolution over time of the identified compounds were studied in order to select promising target molecules for dating purposes.

C.2. Materials and methods

C.2.1. Reference substances, solvents and ammunition

Pure standards of 55 compounds were purchased (see Electronic Supplementary Material, ESM, for details). For each substance, a working standard solution was prepared at a concentration of 1 $mg \cdot mL^{-1}$ in methanol (puriss. grade) purchased from Sigma/Aldrich (Buchs, Switzerland), except for anthracene, chrysene and benzo[a]pyrene which were dissolved in chloroform (purum grade) also purchased from Sigma/Aldrich. Nitroglycerin was already dissolved at a concentration of 1 $mg \cdot mL^{-1}$ in a mixture of ethanol/methanol (97:3).

Nine different types of ammunition were purchased during 2011 from sellers in Switzerland (see Table C.1). Five of them belonged to .45 ACP calibre (producers: Geco, PMC, UMC, Sellier & Bellot and Magtech) and the remaining four to .357 Magnum (producers: Geco, Sellier & Bellot, Samson and Magtech). From each ammunition box, two cartridges were opened using a kinetic hammer, and the smokeless powder analysed by GC/MS using a method adapted from Romolo [25] (see results in Table C.1).

Abbreviation	***Characteristics***					***Smokeless powder composition***						
	Calibre	***Brand***	***Bullet mass [gr]***	***Bullet Type***	***Powder weight [gr]***	***NG***	***DPA***	***EC***	***DBP***	***2NDPA***	***AKII***	***DF***
Ge45	*.45 ACP*	*Geco*	230	FMJ	6.0	M	m	M	-	-	-	-
Pm45	*.45 ACP*	*PMC*	230	FMJ	5.1	M	M	-	-	-	-	-
Re45	*.45 ACP*	*Remington UMC*	230	FMJ	4.8	M	M	-	M	-	M	-
Se45	*.45 ACP*	*Sellier & Bellot*	230	FMJ	5.0	M	M	-	M	m	-	-
Ma45	*.45 ACP*	*Magtech*	230	FMJ	5.5	-	-	M	-	-	-	-
Ge357	*.357 Mag.*	*Geco*	158	FMJ	6.3	M	M	M	-	-	M	-
Se357	*.357 Mag.*	*Sellier & Bellot*	158	FMJ	6.2	M	M	-	M	-	-	-
Sa357	*.357 Mag.*	*Samson*	125	JSP	17.2	M	M	M	M	m	-	m
Ma357	*.357 Mag.*	*Magtech*	158	FMJ	14.6	M	M	M	M	-	-	-

Table C.1 *– Cartridges selected for the study, their characteristics and the composition of their smokeless powders determined by GC/MS (NG = nitroglycerine, DPA = diphenylamine, EC = ethylcentralite, DBP = dibutylphthalate, 2NDPA = 2-nitrodiphenylamine, AKII = 1-methyl-3,3-diphenylurea (akardite II), DF = dioctyl fumerate). "M" indicates a major compound (>1% of the total area count) and "m" a minor compound (0.2 - 1% of the total area count); "FMJ" indicates full metal jacket bullets and "JSP" jacket soft point bullets. Masses are given in grains (1 grain = 64.80 mg).*

C.2.2. Test shootings

Test shootings were carried out using a Colt 1911 semi-automatic pistol (.45 ACP) and a Colt Python revolver (.357 Magnum). Before starting the test shootings, firearms were carefully cleaned with dry cleaning patches. Then, test cartridges were fired by singly loading them in the magazine/cylinder. Cartridges were handled while wearing latex gloves in order to minimize contaminations from hands. Latex gloves were preferred over nitrile ones since preliminary tests showed that the latter introduced several unwanted impurities in samples, especially long-chain aliphatic compounds. Weapons were never greased between the different test shootings.

Spent cartridges analysed at time t = 0 h were immediately recovered after discharge and put in 20 mL HSSE-dedicated crimp glass vials (Gerstel, Sursee, Switzerland). These vials were found to be suitable because their large opening (i.e., 13 mm) fits well with most handgun calibres. A stir bar was suspended with the aid of a special glass insert (Gerstel) to the headspace of each vial containing a fired cartridge. Then, vials were rapidly closed with 20 mm crimp caps equipped with 3.0 mm polytetrafluoroethylene/silicon septa (Gerstel). Glassware was previously conditioned in a ventilated laboratory oven at 80 °C for 8 h. Some analyses were also performed on fired cartridges aged for 24 h (t = 24 h) in an air-conditioned laboratory kept at a temperature of about 20 °C.

C.2.3. HSSE extraction of spent cartridges

HSSE stir bars are commercialized under the name of "Twister©" by Gerstel. The largest PDMS-coated HSSE stir bars available (2 cm length with a 1 mm coating thickness, corresponding to a PDMS volume of 110 μL) were purchased. Stir bars were always thermo-conditioned before use. To do this, they were put in *ad-hoc* glass desorption tubes (Gerstel) and placed in a Gerstel TC tube conditioner. The conditioning procedure suggested by the producer (30 min at laboratory temperature, followed by 90 min at 300 °C and about 60 min to cool down) was used.

Preliminary tests indicated that a sampling temperature of 80 °C promotes extraction of less volatile compounds as opposed to a temperature of 20°C, whereby the recovery of the most volatile compounds decreases in some cases. Different specimens of the same type of ammunition (same box) were thus analysed at the two temperatures in order to guarantee the detection and identification of a larger range of compounds. For extraction at 20 °C, closed vials were put in an air-conditioned laboratory with controlled temperature, while extractions at 80 °C were performed in a ventilated laboratory oven. Extraction time was set at 72 h. Before analysis, the sample vials were reopened, stir bars were removed and placed in reconditioned desorption tubes. Finally, the tubes were capped with special transportation adapters (Gerstel) and placed on the GC tray for GC/MS analysis. For each type of ammunition, three different cartridges from the same box were analysed per temperature. Procedural blanks were also systematically carried out. These consisted of empty vials, which were handled in the same way as those used for sampling the cartridges, and had been in contact with the same environments. Three blanks per extraction condition were analysed.

C.2.4. Analysis of stir bars

Stir bars were thermally desorbed using a Gerstel TDU thermal desorption unit, connected to a Gerstel CIS-4 programmed temperature vaporizing injector. These devices were mounted on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (Agilent Technologies, Basel, Switzerland). The system was also equipped with a Gerstel MPS multi-purpose sampler, which was used to automatically load tubes containing stir bars into the TDU.

Desorption mode was splitless, desorption flow 40 mL·min^{-1}, and desorption pressure 9.07 psi. Desorption ramp was programmed as follows: 20 °C for 0.5 min, increased to 250 °C at 60 °C·min^{-1} and held at this temperature for 10 min (total desorption time of 14.3 min). The transfer line temperature between TDU and CIS-4 was 280 °C. Liners for CIS-4 were obtained from Gerstel and packed with quartz-wool. The cryo-focusing temperature was set at -80 °C. The PTV injection ramp programming was as follows: -80 °C for 0 min, increased to 300 °C at 600 °C·min^{-1} and held at this temperature for 1.37 min (total injection time: 2 min). Splitless mode was used during this time. After injection, the injector was additionally programmed to decrease at a rate of 720 °C·min^{-1} to 270 °C and held at this temperature for 10 min in split mode in order to condition the liner for the next injection.

GC separation was performed on a HP-5MS (30 m x 0.25 mm x 0.25 μm) column from Agilent. The carrier gas was helium, and column flow was maintained at 1.2 mL·min^{-1}. The oven ramp was programmed as follows: 40 °C for 2 min, increased to 100 °C at 10 °C·min^{-1}, increased to 200 °C at 5 °C·min^{-1}, increased to 280 °C at 5 °C·min^{-1} and held at this temperature for 10 min (total chromatographic time: 46 min). The transfer line between the column and the MS was set at 280 °C. Ionisation was carried out through electron impact (EI). Masses were scanned from m/z 40 to 500 without solvent delay. MS source and quadrupole temperatures were 230 °C and 150 °C, respectively.

C.2.5. Injection of standard solutions

In order to inject the standard solutions, a desorption tube was equipped with special glass insert and transportation adapter for liquid injection (both furnished by Gerstel). Before analysis, the tube was automatically inserted into the TDU, and 1 μL of solution was injected into the insert. GC/MS parameters were the same as mentioned above, except for the CIS-4 cryo-focusing temperature, which was set to 0 °C in order to vent solvents during desorption. Moreover, a solvent delay of 4 min and split mode were adopted.

C.2.6. Data processing

Chromatograms acquired at time t = 0 h were used for peak identification. These were analysed using the Enhanced Data Analysis software provided by Agilent. Preliminary identification of the mass spectra of the eluted peaks was attempted with the NIST08 library. Hits were evaluated by visual inspection of the corresponding spectra. Afterward, liquid standards were injected to confirm the identity of compounds of particular interest. Detection of a compound was accepted if its mass fragmentation pattern was coherent with the results of the library search (additionally, retention time had to match for the molecules with available standards).

After identification, target ions were selected for all the compounds and the corresponding peak areas were extracted. The presence of a compound in a particular type of ammunition was accepted if the intensity of its target ion was greater than the average signals in the procedural blanks plus three times the standard deviation [26] in at least one of the analysed cartridges.

For further statistical analyses, the observed peak areas were normalised to the respective cartridge total sum (unless otherwise indicated in-text). In this way, proportions were calculated. Principal component analysis (PCA) was applied on data in order to study the composition variability. For this purpose, variables were further mean-centred and scaled with their standard deviations. In order to find molecules with significant decreases over time, specimens belonging to two types of ammunition (Ma45 and Ge45) were aged for 24 h and a Student t-test was performed on the slope passing through the values measured at $t = 0$ h and 24 h. If the slope was negative and significantly different from zero ($\alpha = 0.1$), the hypothesis of decrease was accepted.

C.3. Results and discussion

C.3.1. Identification of the volatile compounds in GSR

Spent cartridges from nine types of ammunition were extracted using HSSE at two different temperatures (20 °C and 80 °C). The obtained chromatograms were generally characterised by a large number of peaks. This is well illustrated in Figure C.1, where an example of total ion chromatogram (TIC) is shown.

166 peaks were found to be recurrent between TICs from different cartridges. Their identities are reported in ESM. It is noteworthy that the analysed GSRs presented a high variety of aromatic molecules. In fact, 109 of the 166 recurrently detected compounds have at least one aromatic ring in their structure. As already reported in previous works, PAHs were the most diversified group. Non-branched PAHs with up to 5 rings were identified. Most of them were already reported in the literature as being released after the discharge of handgun cartridges (e.g., naphthalene, biphenyl, acenaphthene, phenanthrene, pyrene, benzo[a]pyrene) [10, 19, 20]. Indane was previously identified solely in the exhaust of military rifle [8], while azulene, diphenylmethane, phenylnaphthalene and 4H-cyclopenta[def]phenanthrene were found for the first time in this study. Many branched and substituted derivatives of some PAHs (especially naphthalene) were also observed. 1- and 2-methylnaphthalene, 1- and 2-naphthalenecarbonitrile, as well as some isomers of dimethylnaphthalene were already reported in the literature [18-20] and found in the GSRs of all the cartridges. Additionally, the improved sensitivity of the HSSE method allowed the detection of diisopropylnaphthalene, methylindene, 1,2,3,4-tetrahydro-1,1,6-trimenthylnaphthalene and some

isomers of the ethenylnaphthalene. Benzene and its derivatives were also very common between the different residues. In fact, benzene, ethylbenzene, styrene and xylene isomers were always identified, while toluene was found in the exhausts of eight out of nine types of cartridges.

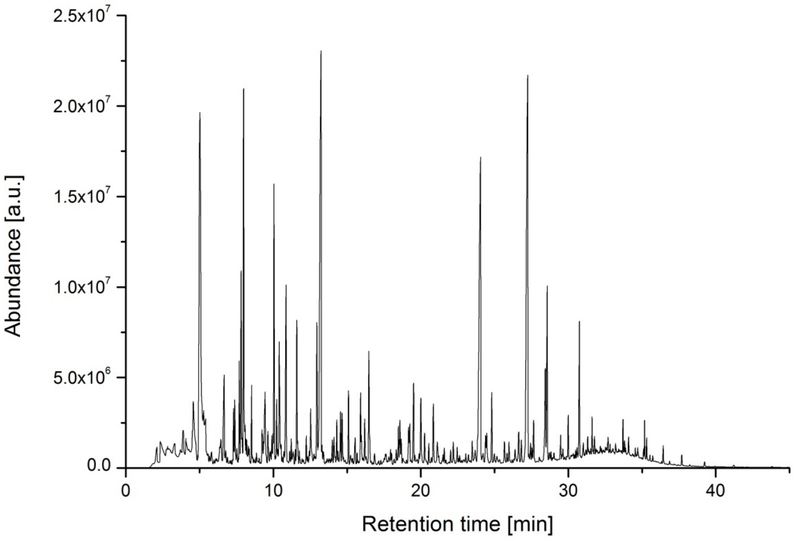


Figure C.1 *– Example of HSSE chromatogram of a fired cartridge (Samson .357 Magnum, extracted at 80 °C).*

Several molecules containing hetero-atoms were observed. As previously reported in the literature, nitrogen-containing PAH homologues were a relatively diversified group. Quinoline and carbazole were observed in the residues of all types of cartridges, while isoquinoline was only detected in three of them. Phenazine was previously observed in the exhaust of smokeless powder after pyrolysis and incineration [17], but never found in GSRs prior to our study. Methylpyridine was already reported to be present in the exhausts of military rifles [8], but never in those of spent cartridges. Methylimidazole and benzoquinoline isomers were never reported before. Molecules with nitrogen-containing functional groups were also frequent. Aromatic nitriles were a particularly represented class. Amongst them, benzonitrile and benzyl nitrile, as well as the isomers of tolunitrile, dicyanobenzene and naphthalenecarbonitrile were common to all residues. This is in agreement with previous studies [19, 20]. Ethenylbenzonitrile was never reported before, but two of its isomers were found in all residues. The large number of nitrogenized species is compatible with the characteristics of the exploding materials. In fact, smokeless powders are rich in nitrogen-containing substances, such as nitroesters (i.e., explosives) and aromatic amines/ureas (i.e., stabilizers). Nitrogen atoms from all these compounds can thus participate in the reaction mechanism and therefore be found in the structures of the final products.

Many amines, ureas and nitro-compounds were identified. Amongst amines and ureas, the three main stabilizers used in smokeless powders were detected, i.e., diphenylamine, ethylcentralite and 1-

methyl-3,3-diphenylurea (akardite II). The presence of the first two molecules in GSRs was already reported before [10, 19-21], and they were detected in all the analysed residues even if they were not detected in the respective smokeless powders. In addition, akardite II (which was detected in gunshot exhausts for the first time in this work) was observed in four out of nine residues, but only in two smokeless powders. These observations are consistent with the nature of smokeless powder manufacturing. Indeed, it is not rare that manufacturers blend powder lots in order to achieve optimal ballistic performances [27]. Traces of minor compounds can thus be detected in GSRs, especially with a high sensitive extraction technique such as HSSE. Some nitro-derivatives of diphenylamine (mainly, the isomers of the nitrodiphenylamine and the 2,4-dinitrodiphenylamine) were also identified in the GSRs of some cartridges. These are degradation products of smokeless powders, which are normally produced during cartridge aging. However, previous researches reported that their formation could be significantly accelerated at high temperatures [28]. Thus, in-vial formation of these compounds during extraction at 80 °C seems probable, considering that few of them were observed by extraction at 20 °C of the same types of cartridges. Other identified aromatic nitro-compounds were nitrobenzene, nitrophenol and nitrotoluene. The last two molecules were already identified in the exhaust of military rifles [8], and detected in all the types of cartridges in our study. Considering their high thermolability, also these compounds were probably produced from the degradation of the different gunpowder additives rather than from explosion reactions. Dinitrotoluenes were never detected in GSRs (and neither in the smokeless powders), even after ion extraction.

Several substances containing hetero-atoms other than nitrogen were identified. Particularly, many compounds of sulphur (i.e., benzo[b]thiophene and dibenzothiophene) and oxygen (i.e., dibenzofuran) were observed in the residues of all the cartridges. Amongst these substances, only benzo[b]thiophene was previously reported in the literature [20]. Benzothiazole, an aromatic molecule containing both nitrogen and sulphur, was previously detected only in military rifle exhausts [8]. Sulphur probably originates from primer mixtures rather than smokeless powders. In fact, most producers currently use antimony sulphide to produce priming mixtures [29]. In addition, some aromatic aldehydes and alcohols, such as benzaldehyde, benzophenone and acetophenone, were also detected. Methylphenol was identified for the first time in this study. 4-phenylazophenol, a yellow dye (Solvent Yellow 7), was found in the residue of two cartridges. As it is generally used as a celluloid colorant and lacquer component, it may be employed as an additive in smokeless powders [30]. Chlorinated aromatics (i.e., isomers of dichlorobenzene, dichlorophenol, chlorobenzaldehyde, dichlorobenzaldehyde and dichlorobenzamide) were found in the residues of all cartridges, but remain unknown in the literature. Sources of chlorine in GSRs are difficult to track down. Modern primers are normally free of chlorates because of their corrosive properties [29]. It is supposed that chlorine originates from the degradation of plastic materials based on poly(vinyl chloride) [31]. Presence of the aforementioned compounds could thus be ascribable to a contamination due to the production and packing of the cartridges and/or specimen handling.

Phthalates are the esters of benzene-1,2-dicarboxylic acid. These compounds are commonly employed in the production of smokeless powders as plasticizers. Dibutyl phthalate is the most widely used and was already found in residues [19, 20]. On the contrary, dimethyl, diethyl and dioctyl phthalates were previously identified only in smokeless powder formulations [29, 32]. Particularly, dioctyl phthalate (also known as bis(2-ethylhexyl) phthalate) is a very common phthalate-based plasticizer, produced from the reaction with 2-ethyl-1-hexanol, which was also detected in the analysed GSRs. Salicylates (i.e., the esters of 2-hydroxybenzoic acid) and benzoates (i.e., esters of benzoic acid) share structural similarity with phthalates and were identified in residues. Salicylates are normally used as fragrances, while benzoates as carriers. Thus, they might be contaminations adsorbed on the cartridge surface.

Even though few aliphatic substances were previously identified in GSRs, several were observed in the analysed cartridges during this research. A large fraction was composed of linear alkanes and alkenes. In fact, linear alkanes from C11 to C24 and linear mono-alkenes from C10 to C17 were present in the exhausts of all the cartridges. Branched alkanes such as pristane and phytane were also detected. Even if these compounds were not found in the smokeless powders, it is very unlikely that they were produced during the explosion. In this regard, the couples heptadecane/pristane and octadecane/phytane may indicate the presence of heavy petroleum distillates, such as mineral oils [33]. Thus, these groups of compounds might be ascribable to a contamination of spent cartridges by lubricants during the production process or cleaning of the weapon [34]. A similar mechanism could also explain the presence of methyl dehydroabietate (a marker of wood tar, generally used for waterproofing [35, 36]) in the residues. Saturated fatty acids and related esters were another important class of aliphatic molecules found in GSRs. Lauric (C12:0), mystic (C14:0), palmitic (C16:0) and stearic acid (C18:0) were detected, along with some of their isopropyl and butyl ester derivatives. Given the fact that they were not found in the blanks, their presence might be the result of a contamination of the cartridge surface or vials by fingerprint residues [37, 38].

Amongst the other aliphatics, 2(5H)-furanone was already identified in the literature as an important pyrolytic byproduct of smokeless powder [17, 18] and was observed in all residues. Butyldihydro-2(3H)-furanone was firstly reported in this work and found to be very common amongst cartridges.

Similarly, 1,4:3,6-dianhydro-α-d-glucopyranose was previously identified as a main pyrolitic byproduct of cellulose [39]. Considering that the main explosive in smokeless powders is nitrocellulose, this could explain its presence in seven out of nine residues.

C.3.2. Characteristics of GSRs from the different types of ammunition

While an extraction temperature of 20 °C proved to be useful to identify some of the most volatile compounds, 80 °C generally allowed reaching higher recoveries. Consequently, only data acquired at this condition were considered hereafter.

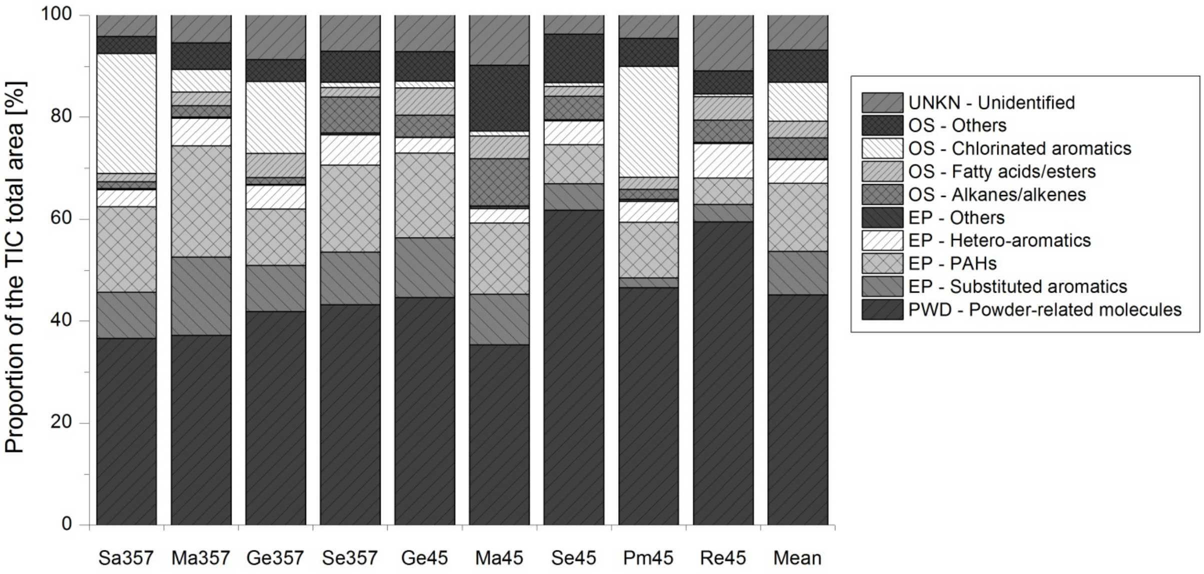


Figure C.2 *– Proportions in TICs of the different compound classes amongst all the analysed GSRs (after extraction at 80 °C). "PWD" = smokeless powder additives, "EP" = explosion products, "OS" = other sources, and "UNKN" = unidentified compounds. Classes coincide with those reported in Table S2 in Electronic Supplementary Material.*

Figure C.2 shows the percentages of each class of compounds on the total sum of peak areas in TICs. As can be seen from the figure, molecules coming from smokeless powder were always the dominant class, with proportions ranging from 36.6% to 61.8% (average: 45.0%). This suggests that a significant amount of smokeless powder compounds were adsorbed on the inner cartridge walls and/or did not undergo combustion during explosion. Concerning the explosion products, branched/substituted aromatics and PAHs were normally the second most abundant classes in GSRs. Proportions for branched/substituted aromatics went from 5.2% to 15.4% (average: 8.5%). Internal composition of this class was strongly variable from one cartridge to another, but nitrile- and alkyl-substituted benzene derivatives always constituted the major sub-classes. PAHs proportion ranged from 5.2% to 17.0% (average: 13.4%). Except for one type of ammunition (Pm45), PAHs mainly consists of 2-rings compounds and the proportions for heavier PAHs decreased as a function of the number of rings. Prevalence of small aromatics is consistent with the discharge process. Indeed, the bullet departure is normally very rapid, and conditions promoting the addition of radicals onto smaller molecules are probably not held for a sufficient time to produce heavier molecules. However, it should also be noted that sampling was done through headspace extraction, which is less sensitive towards heavy molecules with low volatility. The other classes of explosion products were generally below 10%. Particularly, the proportions of hetero-aromatics were comprised between 2.8% and 6.8% (average: 4.5%). This shows that, although heteroatoms are partly involved in the process of pyrosynthesis of byproducts, their inclusion in molecular rings was very limited. The only exception was Re45, where the proportion of hetero-aromatics was slightly larger than that of the other explosion products. Proportions of alkanes/alkenes and fatty acids/esters ranged from 1.3% to 9.3% (average: 4.1%), and from 1.6% to 5.4% (average: 3.3%), respectively. Therefore, these compounds were not very abundant in the GSR profiles. On the contrary, the proportion of chlorinated aromatics ranged from 0.7% to 23.5% (average: 7.6%), and consequently represented a significant percentage of the chromatograms of some cartridges (i.e., Sa357, Ge357 and Pm45).

Focusing only on smokeless powder additives and explosion products, results showed that the sum of their peak areas was dependent on the type of ammunition. This means that different sources also yielded different amounts of GSR by the employed method (see ESM). However, no significant correlation between the signals and the initial quantity of smokeless powder in the cartridge was observed. In fact, many cartridges with very different initial quantities of powder gave similar peak area sums. For instance, Pm45 (5.1 gr of smokeless powder) showed comparable signals as Sa357 (17.2 gr). On the other hand, cartridges filled with similar powder weights could release different amounts of GSR. This is, for example, the case of Re45 (4.8 gr), which gave significantly lower signals compared to Se45 (5.0 gr). The diversity of smokeless powder ballistic properties (mainly, powder vivacity) is likely to be the cause of these observations. In fact, some powders undergo a more or less exhaustive explosion reaction than others and, thus, release different amounts of HSSE-detectable compounds.

Of the 166 identified compounds, 141 were found to be present in the GSRs of every cartridge, making their qualitative compositions very similar. Nonetheless, proportions between compounds were significantly different. These can be easily visualised using principal component analysis (PCA). Figure C.3 shows the plot of the first three principal components after applying PCA solely on explosion products and gunpowder additives. GSRs belonging to different sources could be fairly discriminated, even if some of them shared quite similar compositions (i.e., Re45 and Se45). In order to better appreciate these differences, proportions for some selected compounds were plotted in Figure C.4.

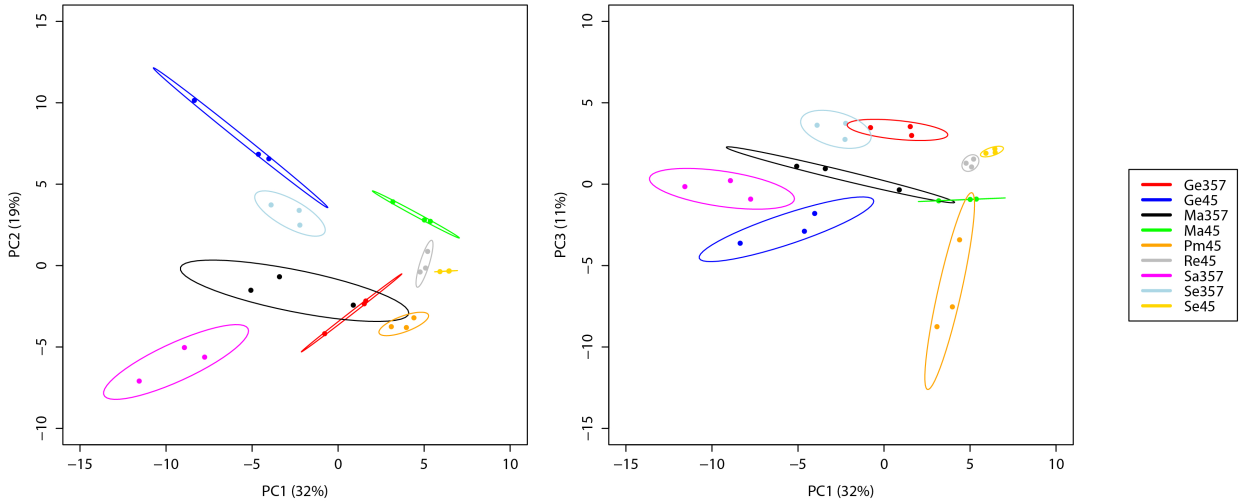


***Figure C.3** – Plot of the first 3 principal components (PC) after principal components analysis (PCA) of GSR data (extracted at 80 °C). Only data concerning identified explosion products and smokeless powder additives were analysed. Explained variance of each PC is reported in brackets.*

Correlations between compounds were calculated, and it was found that molecules presenting similar structures were also strongly correlated. This was, for example, the case of phenanthrene and pyrene ($r = 0.92$), benzaldehyde and benzonitrile ($r = 0.95$), benzaldehyde and biphenyl ($r = 0.94$), m-tolunitrile and 1-naphthalenecarbonitrile ($r = 0.97$) as well as naphthalene and 2-methylnaphthalene ($r = 0.80$). This result is consistent with their formation process, which involves the sequential addition of radicals in order to form larger and larger molecules. Consequently, even if different proportions of the various explosion products could be released between different cartridge types, those sharing similar structures are more prone to be generated at the same time and to be correlated within the same ammunition. Moreover, the compounds in the previous couples also shared similar chemical properties (i.e., boiling point, vapour pressure and polarity), which makes their extraction behaviours very similar and contribute to the high observed correlations.

Smokeless powder formulation certainly affects post-discharge GSR composition. However, it is also true that cartridges using very similar powder formulations (i.e., Se45 and Se357, as well as Ma357 and Sa357) showed differentiable GSRs. In the same manner, the similitude between the residues released by Re45 and Se45 was very interesting because their pre-discharge smokeless powder formulations were found to be significantly different (Table C.1). It can thus be deduced that other factors (e.g., pressure and temperature conditions during the discharge) may play an important role and therefore influence the composition of the GSRs. This was also supported by the fact that no significant correlation between the composition of the smokeless powders and the respective GSRs were observed (i.e., there was no evidence that a powder containing specific additives preferentially generated given compounds). Instead, it was already proved that parameters such as burning conditions during the combustion of organic matter are particularly relevant in controlling the pyrosynthesis of new species [40, 41].

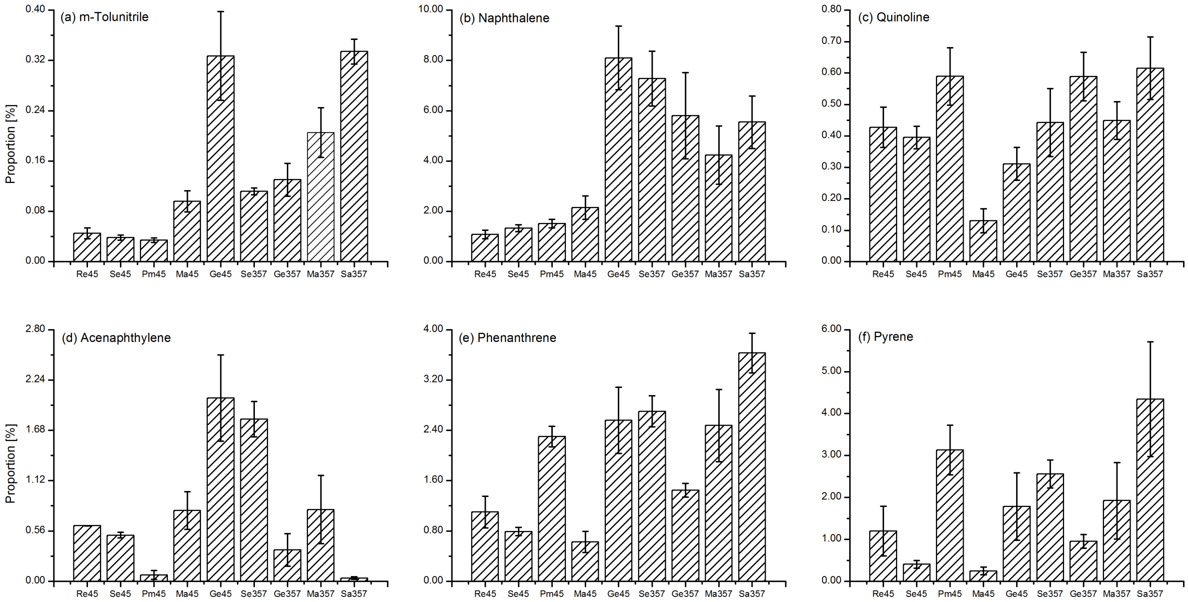


Figure C.4 – *Proportions for some selected compounds amongst cartridges.*

C.3.3. Identification of potential target compounds for time since discharge estimation

Target analytes for dating purposes should fulfil the following criteria:

1. always be released during the discharge (i.e., they should be present in the GSRs released by every type of cartridge);
2. ideally be present in high amounts;
3. their signals should show low intra-variability between the discharge of the same cartridge and firearm set;
4. their signals should show low inter-variability between different cartridge/firearm sets;
5. have a significant evolution during a relevant time range after discharge.

These characteristics were investigated for each explosion product and smokeless powder additive previously identified in GSRs.

Firstly, variability of compounds at t = 0 h in all the analysed types of ammunition was studied. Amongst the identified explosion products and smokeless powder additives, only a few molecules were not observed in all the residues (see ESM). These are mainly the heaviest PAHs (chrysene and benzo[a]pyrene), 1,4:3,6-dianhydro-α-d-glucopyranose, toluene, phenyl thiocyanate, 1,2,3,4-tetrahydro-1,1,6-trimenthylnaphthalene, isoquinoline, nitrotoluene, 2,4-dinitrodiphenylamine isomers, 1-methyl-3,3-diphenylurea (akardite II) and dioctyl phthalate.

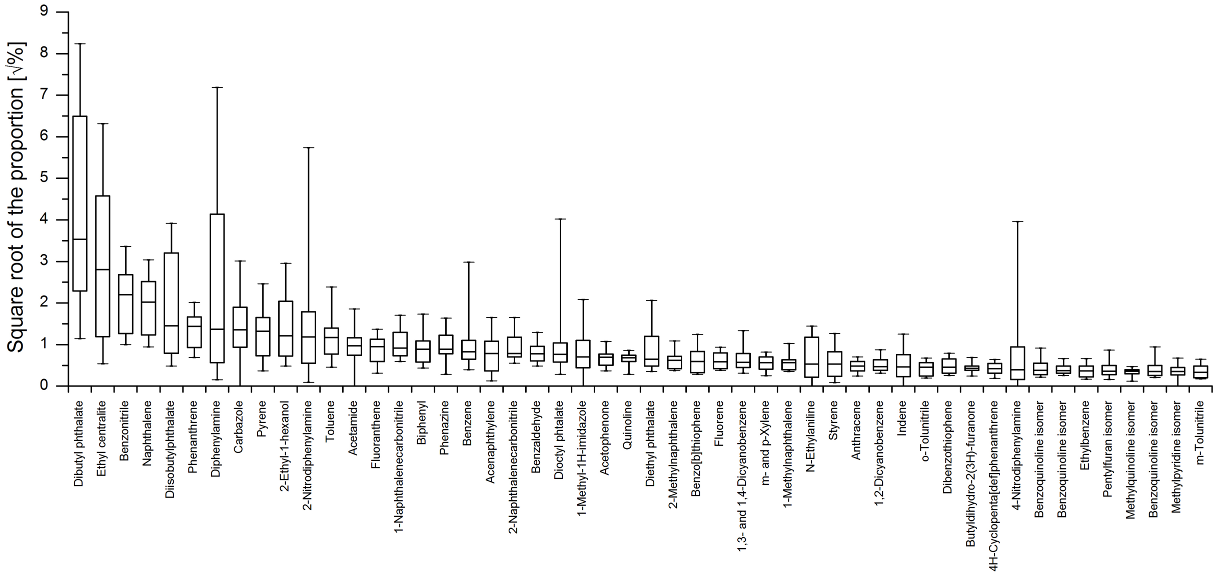


Figure C.5 *– Distribution amongst GSRs of the 50 most abundant explosion products and gunpowder additives (after extraction at 80 °C). To improve graph readability, unit of y-axis is given as the square root of the proportion of the total sum of peak areas.*

Figure C.5 shows the distribution of the remaining compounds amongst the cartridges. It is noteworthy that smokeless powder additives generally composed a large fraction of the GSRs and were the most abundant substances detected in chromatograms (Fig. C.5). This is especially true for dibutylphthalate, ethylcentralite, diisobutylphthalate, diphenylamine, 2-ethyl-1-hexanol and 2-nitrodiphenylamine, which were, on average, the six most abundant smokeless powder additives detected in GSRs. Concerning explosion products, their proportions were usually smaller but still significant in many cases. In fact, benzonitrile (aromatic nitrile) and naphthalene (2-rings PAH) represented a relatively large fraction of each analysed GSR, similarly to phenanthrene (3-rings PAH), carbazole (3-rings hetero-PAH), pyrene (4-rings PAH), fluoranthene (4-rings PAH), biphenyl (2-rings PAH) and acenaphthylene (3-rings PAH), which were less abundant. Consequently, many aromatics with up to 4 rings could be quantitatively detected after the discharge of all the analysed types of ammunition. Intra-variability was investigated (Fig. C.6). It was found that the proportions of smokeless powder additives were slightly less reproducible within the different sources than those of explosion products. In fact, the mean intra-cartridge RSD values for dibutyl phthalate, ethyl centralite and diphenylamine were of the order of 33%, 25% and 48%, while those of benzonitrile, naphthalene and phenanthrene were 20%, 22% and 18%, respectively. Globally, medians between mean intra-cartridge RSD values for compounds associated to smokeless powder and those associated to explosion products were 39% and 24%, respectively.

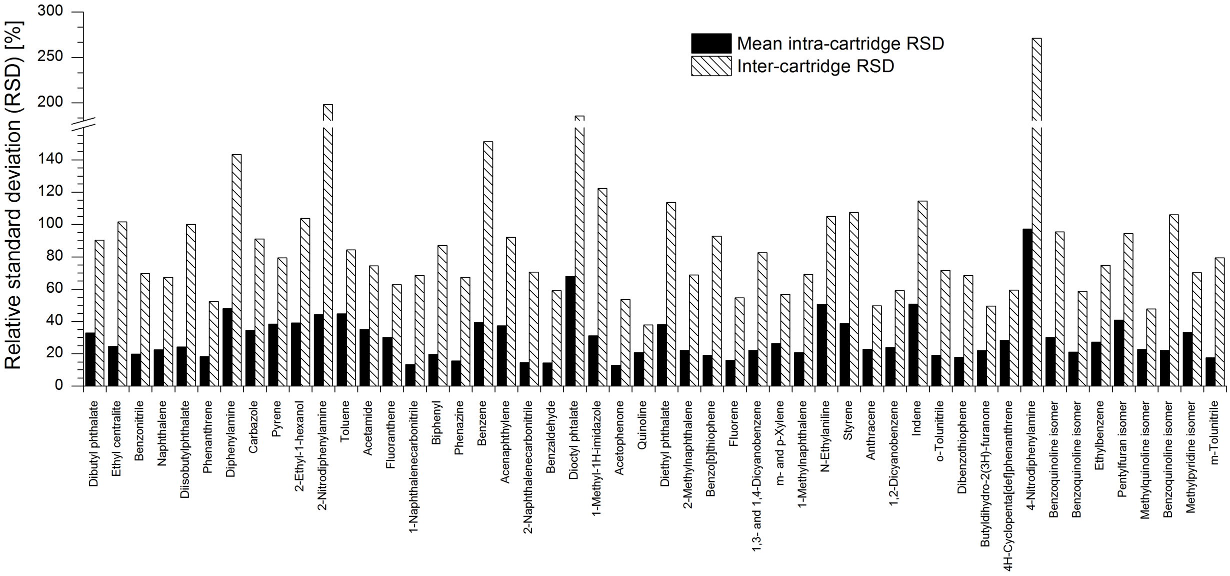


Figure C.6 *– Comparison of mean intra-cartridge RSDs and inter-cartridge RSDs amongst the different cartridges for the proportions of the 50 most abundant explosion products and gunpowder additives identified in GSRs (after extraction at 80 °C).*

Furthermore, the proportions of smokeless powder additives showed higher inter-variability than those of explosion products. In fact, the amount of smokeless powder additives in GSRs often ranged from trace to major-compound levels depending on the types of ammunition (Fig. C.5 and C.6). Inter-cartridge RSD values for dibutyl phthalate, ethyl centralite and diphenylamine were in the order of 90%, 102% and 143%, respectively. 2-nitrodiphenylamine reached 198%. For the sake of comparison, values for benzonitrile, naphthalene and phenanthrene were considerably lower: 70%, 67% and 52%, respectively. Globally, medians between inter-cartridge RSD values obtained for the compounds associated to smokeless powder and those associated to explosion products were 121% and 75%, respectively.

Aging of compounds over time (t > 0h) was investigated for two specific types of ammunition, i.e. Ma45 and Ge45. Table C.2 reports the list of compounds for which aging was proved significant in at least one of the two tested cartridges. Excluding 1,4:3,6-dianhydro-α-d-glucopyranose (which was not observed in all the residues), 27 compounds other than naphthalene were found to significantly decrease over the considered time. Amongst them, no other compound associated to smokeless powders was observed. Consequently, although smokeless powder additives were amongst the most abundant compounds detected in GSRs, they are poorly suitable as target compounds for dating purposes considering their large intra- and inter-variability, as well as the absence of significant aging in the first 24 hours after the discharge. On the contrary, many explosion products containing up to 3 aromatic rings showed a significant decrease after 24 h. Substituted benzenes (e.g., xylenes, benzonitrile, tolunitriles) were particularly represented. This is surely due to their low boiling points that render them very volatile. Additionally, naphthalene, methylnaphthalenes, biphenyl, diphenylmethane, biphenylene and acenaphthylene also showed considerable decrease. This does not apply to heavier PAHs such as phenanthrene and pyrene, at least during the considered time interval.

C.4. Conclusions

Headspace sorptive extraction (HSSE) followed by GC/MS analysis was employed to study the characteristics of volatile organic gunshot residues (GSRs) extracted from nine different types of ammunition. 166 compounds were identified. It was observed that a significant proportion of the total ion chromatograms was always dominated by smokeless powder additives which do not undergo explosion process during discharge. These were mainly amine-based and/or urea-based stabilizers such as diphenylamine and ethyl centralite, as well as their degradation products. Pyrolitic byproducts coming from the different explosion pathways were the most variegated group regarding the number of different species identified. Most of them were polycyclic aromatic hydrocarbons (PAHs) containing 2- and 3-rings (such as naphthalene, fluorene and acenaphthene) and alkyl substituted benzenes. PAHs with up to 5 rings (i.e., chrysene) were nevertheless detected in the residues of some cartridges. Several molecules presenting hetero-atoms in their structures were also observed. These were mostly nitrogen-containing hetero-PAHs (e.g., quinoline and benzoquinoline) and aromatic nitriles (e.g., benzonitrile and naphthalenecarbonitrile). Compounds of sulphur and oxygen were also identified, but they were less common.

Of the 166 identified compounds, 141 were found to be present in the GSRs of every cartridge, making the respective qualitative compositions very similar. Despite that, it was observed that the final quantitative compositional characteristics of each residue (i.e., proportions between the compounds) strongly depend on its source and could thus be very variable amongst different types of cartridges. Behaviour of the single compounds was studied more in-depth in order to identify adequate target analytes for dating purposes. Smokeless powder additives were found to be unsuitable, because their relative quantities were less reproducible between and within sources, and also because they did not show any significant decrease during the first 24 h after the discharge. On the other hand, many substituted aromatics and PAHs showed acceptable reproducibility and aging behaviours. Besides naphthalene, 27 compounds proved to be interesting analytes for forensic dating purposes.

Ref.	***Compound***	t_R	***Ge45***	***Ma45***
7	*Ethylbenzene*	5.655	x	x
8	*m- and p-Xylene*	5.795		x
10	*Styrene*	6.160		x
9	*o-Xylene*	6.241	x	x
28	*1-Methyl-1H-imidazole*	7.106	x	x
15	*Benzonitrile*	7.797	x	x
31	*Indane*	8.673	x	x
32	*Indene*	8.830		x
16	*o-Tolunitrile*	9.518	x	x
17	*m-Tolunitrile*	9.661	x	x
18	*p-Tolunitrile*	9.933	x	x
20	*Benzyl nitrile*	10.611	x	x
33	*Methylindene isomer*	10.955		x
34	*Azulene*	10.990		x
35	*Naphthalene*	11.579	x	x
64	*Benzo[b]thiophene*	11.754	x	x
22	*Ethenylbenzonitrile isomer*	11.885		x
3	*1,4:3,6-Dianhydro-α-d-glucopyranose*	12.064		x
23	*Ethenylbenzonitrile isomer*	12.212		x
68	*Indole*	13.943		x
36	*2-Methylnaphthalene*	13.983	x	x
37	*1-Methylnaphthalene*	14.367	x	x
39	*Biphenyl*	15.908	x	x
40	*2-Ethylnaphthalene*	16.241	x	
42	*Ethenylnaphthalene isomer*	16.651		x
45	*Ethenylnaphthalene isomer*	17.045		x
46	*Diphenylmethane*	17.139	x	x
48	*Acenaphthylene*	17.515		x
49	*Biphenylene*	17.677		x

Table C.2 *– List of compounds showing significant decrease after 24 h since discharge in at least one of the two tested types of ammunition (the "Ge45" and "Ma45" columns indicate for which cartridge the decrease was significant).*

This is the first time HSSE was used for characterising volatile organic compounds found in cartridges after their discharge, and also that GSRs from so many sources were compared in the same study.

The improved extraction sensitivity of HSSE in comparison to SPME allowed new compounds to be identified and, thus, to gain a better understanding of the composition, reproducibility and variability of the GSR volatile fraction. Many new dating-interesting compounds were found. This could be particularly useful for the development of novel approaches to estimate the time since discharge of spent handgun cartridges based on multiple analytes. Future works will need to focus on the further optimization of the analytical procedure and the development of robust multivariate regression models.

Acknowledgements

This work was kindly supported by the Swiss National Foundation (grant n° PP00P1_150742). The authors would like to thank Dr. Manuel Garcia Gongora, Thibault Genessay, Sarah Hochholdinger and Sonja Bitzer from the Institut de Police Scientifique (Lausanne, Switzerland) for their precious advice and for proofreading early drafts of this paper.

Bibliography

1. Chang, K.H., et al., *Gunshot residue analysis and its evidential values: a review.* Australian Journal of Forensic Sciences, 2013. **45**(1): p. 3-23.
2. Meng, H.-H. and B. Caddy, *Gunshot residue analysis - a review.* Journal of Forensic Sciences, 1997. **42**(4): p. 553-570.
3. Dalby, O., D. Butler, and J.W. Birkett, *Analysis of gunshot residue and associated materials - a review.* Journal of Forensic Sciences, 2010. **55**(4): p. 924-943.
4. Mach, M.H., A. Pallos, and P.F. Jones, *Feasibility of gunshot residue detection via its organic constituents, part I: analysis of smokeless powders by combined gas chromatography-chemical ionization mass spectrometry.* Journal of Forensic Sciences, 1978. **23**(3): p. 433-455.
5. Mach, M.H., A. Pallos, and P.F. Jones, *Feasibility of gunshot residue detection via its organic constituents, part II: a gas chromatography-mass spectrometry method.* Journal of Forensic Sciences, 1978. **23**(3): p. 446-455.
6. Basu, S., *Formation of gunshot residues.* Journal of Forensic Sciences, 1982. **27**(1): p. 72-91.
7. Wolten, G.M. and R.S. Nesbitt, *On the mechanism of gunshot residue particle formation.* Journal of Forensic Sciences, 1980(533-545).
8. Ase, P., et al., *Propellant combustion product analyses on an M16 rifle and a 105mm caliber gun.* Journal of Environmental Science and Health, 1985. **20**(3): p. 337-368.

9. Moxnes, J.F., et al., *Lead free ammunition without toxic propellant gases.* Propellants, Explosives, Pyrotechnics, 2013. **38**: p. 255-260.

10. Andrasko, J., T. Norberg, and S. Stahling, *Time since discharge of shotguns.* Journal of Forensic Sciences, 1998. **43**(5): p. 1005-1015.

11. Andrasko, J. and S. Stahling, *Time since discharge of spent cartridges.* Journal of Forensic Sciences, 1999. **44**(3): p. 487-495.

12. Andrasko, J. and S. Stahling, *Time since discharge of rifles.* Journal of Forensic Sciences, 2000. **45**(6): p. 1250-1255.

13. Andrasko, J. and S. Stahling, *Time since discharge of pistols and revolvers.* Journal of Forensic Sciences, 2003. **48**(2): p. 307-311.

14. Wallace, J.S., *Chemical analysis of firearms, ammunition, and gunshot residue*. 2008, Boca Raton, FL: CRC Press.

15. Richter, H. and J.B. Howard, *Formation of polycyclic aromatic hydrocarbons and their growth to soot: a review of chemical reaction pathways.* Progress in Energy and Combustion Science, 2000. **26**: p. 565-608.

16. Ravindra, K., R. Sokhi, and R. Van Grieken, *Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation.* Atmospheric Environment, 2008. **42**: p. 2895-2921.

17. Cropek, D.M., et al., *Use of pyrolysis GC/MS for predicting emission byproducts from the incineration of double-base propellant.* Environmental Science & Technology, 2002. **36**: p. 4346-4351.

18. Persin, B., et al., *Évaluation de la date d'un tir.* Canadian Society of Forensic Science Journal, 2007. **40**(2): p. 65-85.

19. Weyermann, C., et al., *Analysis of organic volatile residues in 9mm spent cartridges.* Forensic Science International, 2009. **186**: p. 29-35.

20. Gallidabino, M., et al., *Development of a novel headspace sorptive extraction method to study the aging of volatile compounds in spent handgun cartridges.* Analytical Chemistry, 2014. **86**: p. 4471-4478.

21. Wilson, J.D., J.D. Tebow, and K.W. Moline, *Time since discharge of shotgun shells.* Journal of Forensic Sciences, 2003. **48**(6): p. 1298-1301.

22. Tienpont, B., et al., *High capacity headspace sorptive extraction.* Journal of Microcolumn Separations, 2000. **12**(11): p. 577-584.

23. Splivallo, R., et al., *Discrimination of truffle fruiting body versus mycelial aromas by stir bar sorptive extraction.* Phytochemistry, 2007. **68**: p. 2584-2598.
24. Demyttenaere, J.C.R., R.M. Morina, and P. Sandra, *Monitoring and fast detection of mycotoxin-producing fungi based on headspace solid-phase microextraction and headspace sorptive extraction of the volatile metabolites.* Journal of Chromatography A, 2003. **985**: p. 127-135.
25. Romolo, F.S., *Organic gunshot residue from lead-free ammunition*, 2004, University of Lausanne.
26. Miller, J.N. and J.C. Miller, *Statistics and chemometrics for analytical chemistry*. 5th ed. 2005, Harlow, UK: Pearson Education Limited.
27. MacCrehan, W.A. and M. Bedner, *Development of a smokeless powder reference material for propellant and explosives analysis.* Forensic Science International, 2006. **163**: p. 119-124.
28. Bergens, A. and R. Danielsson, *Decomposition of diphenylamine in nitrocellulose based propellants, part I: optimization of a numerical model to concentration-time data for diphenylamine and its primary degradation products determined by liquid chromatography with dual-amperometric detection.* Talanta, 1995. **42**(2): p. 171-183.
29. Wallace, J.S., *Chemical aspects of firearms ammunition.* AFTE Journal, 1990. **22**(4): p. 364-389.
30. Allen, R.L.M., *Colour chemistry*. 1971, New York: Appleton-Century-Crofts.
31. Jirackova-Audoin, L., V. Bellenger, and J. Verdu, *Influence of thermal degradation on the photooxidation of PVC.* Polymer Photochemistry, 1984. **5**: p. 283-293.
32. Keto, R.O., *Comparison of smokeless powders by pyrolysis capillary gas chromatography and pattern recognition.* Journal of Forensic Sciences, 1989. **34**(1): p. 74-82.
33. Stauffer, E., J.A. Dolan, and R. Newman, *Fire debris analysis*. 2007, London, UK: Academic Press.
34. Arfsten, D.P., et al., *Acute and subacute dermal toxicity of Break-Free CLP: a weapons cleaning and maintenance compound.* Journal of Applied Toxicology, 2005. **25**: p. 318-327.
35. Font, J., et al., *Fourier transform infrared spectroscopy as a suitable technique in the study of the materials used in waterproofing of archaeological amphorae.* Analytica Chimica Acta, 2007. **598**: p. 119-127.
36. Jerkovic, I., et al., *Chemical profile of the organic residue from ancient amphora found in the Adriatic Sea determined by direct GC and GC-MS analysis.* Molecules, 2011. **16**: p. 7936-7948.

37. Girod, A., R. Ramotowski, and C. Weyermann, *Composition of fingermark residue: a qualitative and quantitative review.* Forensic Science International, 2012.

38. Girod, A. and C. Weyermann, *Lipid composition of fingermark residue and donor classification using GC/MS.* Forensic Science International, 2014. **238**: p. 68-82.

39. Aho, A., et al., *Pyrolysis of softwood carbohydrates in a fluidized bed reactor.* International Journal of Molecular Sciences, 2008. **9**: p. 1665-1675.

40. Masclet, P.A., A. Bresson, and G. Mouvier, *Polycyclic aromatic hydrocarbons emitted by power stations, and the influence of combustion conditions.* Fuel, 1987. **66**: p. 556-562.

41. Mastral, A.M. and M.S. Callén, *A review on polycyclic aromatic hydrocarbon (PAH) emissions from energy generation.* Environmental Science & Technology, 2000. **34**(15): p. 3051-3057.

APPENDIX D

Time since discharge of 9 mm cartridges by headspace analysis, part 1: comprehensive optimisation and validation of a headspace sorptive extraction (HSSE) method

Submitted to *Forensic Science International*

Authors: Gallidabino, M., Romolo, F. S., & Weyermann, C.

D. Time since discharge of 9 mm cartridges by headspace analysis, part 1: comprehensive optimisation and validation of a headspace sorptive extraction (HSSE) method

Abstract

Estimating the time since discharge of spent cartridges can be a valuable tool in the forensic investigation of firearm-related crimes. To reach this aim, it was previously proposed that the decrease of volatile organic compounds released during discharge is monitored over time using non-destructive headspace extraction techniques. While promising results were obtained for large-calibre cartridges (e.g., shotgun shells), handgun calibres yielded unsatisfying results. In addition to the natural complexity of the specimen itself, these can also be attributed to some selective choices in the methods development. Thus, the present series of papers aimed to systematically evaluate the potential of headspace analysis to estimate the time since discharge of cartridges through the use of more comprehensive analytical and interpretative techniques.

Specifically, in this first part, a method based on headspace sorptive extraction (HSSE) was comprehensively optimised and validated, as the latter recently proved to be a more efficient alternative than previous approaches. For this purpose, 29 volatile organic compounds were preliminary selected on the basis of previous works. A multivariate statistical approach based on design of experiments (DOE) was used to optimise variables potentially involved in interaction effects. Introduction of deuterated analogues in sampling vials was also investigated as strategy to account for analytical variations. Analysis was carried out by selected ion mode, gas chromatography coupled to mass spectrometry (GC-MS). Results showed good chromatographic resolution as well as detection limits and peak area repeatability. Application to 9 mm spent cartridges confirmed that the use of co-extracted internal standards allowed for improved reproducibility of the measured signals. The validated method will be applied in the second part of this work to estimate the time since discharge of 9 mm spent cartridges using multivariate models.

D.1. Introduction

In the forensic investigation of crimes involving firearms, the relevance of spent cartridges discovered at a crime scene may be contested during examination. Thus, a suspect may state, for example, that a cartridges was fired long before the commission of the alleged offense [1, 2]. In such cases, estimating the time since discharge could be a valuable piece of evidence toward reaching a conclusion, For this purpose, changes occurring in the gunshot residue (GSR) after the cartridge discharge are usually exploited [3-7]. An especially heterogeneous mixture, GSR is composed of metallic micro-particles, unburnt or partially burnt smokeless powder flakes and explosion products [8-11]. Explosion products include light di- and tri-atomic molecules (e.g., H_2O, CO, CO_2, H_2 and N_2), derivatives of benzene (e.g., benzonitrile and tolunitrile) and polycyclic aromatic hydrocarbons (PAHs) (e.g., naphthalene, acenaphthene and pyrene) [12-16]. Given their significant vapour pressures, these are volatile and disappear over time after the cartridge is fired. Therefore, most of the published dating approaches specifically focus on the study of these volatile organics.

Application of solid phase micro-extraction (SPME) as a sampling technique was first suggested by Andrasko et al. [17] in 1999, following the encouraging results obtained on shotguns [14]. In the original protocol, naphthalene and an unidentified decomposition product of nitrocellulose (referred as “TEA2”) were designed as target analytes. The residual amounts were extracted daily from the internal atmosphere of the questioned spent cartridge until the total disappearance of any response [17]. However, while partial ageing curves could be obtained using this multiple-sampling procedure, the underlying premise of this approach relied on the fact that SPME did not significantly modify the cartridge’s internal atmosphere [18]. Subsequent studies proved otherwise for small calibres [15], making it impossible to compare the obtained partial ageing profiles with reference curves acquired from analogue cartridges sampled immediately after discharge. In order to solve this problem, a single-extraction approach was evaluated [15], which avoided interferences between samples. Nonetheless, it also showed that the amounts detected in different cartridges were seldom reproducible and that largely imprecise time-since-discharge estimates were obtained.

In tracking down the causes of these issues, the natural complexity of the specimens themselves can certainly be a contributing factor. Indeed, spent handgun cases are difficult supports which, due to their limited surfaces and small volumes, do not allow deposition of large amounts of GSR. Moreover, variations in the explosion conditions during firing are surely prone to introduce shot-to-shot variability in released amounts of GSR. Beyond these typically forensic problems, however, three additional factors can also explain current limitations: the extraction technique, the targeted compounds and the interpretation models. Since its first implementation in the analysis of volatile GSR fraction, SPME has been the extraction technique of choice due to its low invasiveness and exhaustiveness, which allowed sequential sampling of the same specimen [14]. However, these characteristics are also its main drawback, as they could make the technique scarcely efficient (both in terms of repeatability and limits of detection) on diluted samples, such as headspaces of spent cases. Furthermore, most of the works in the field essentially focused on a small set of compounds with relatively high volatility (e.g., naphthalene) and few attempts have been made to simultaneously consider the whole available chemical information in a unique interpretative model. In this regard, it should actually be noted that most published dating approaches were essentially based on the assessment of one compound at the time through very simplistic (and often, not statistically-based) techniques. Consequently, the present series of paper aimed to more systematically evaluate the potential of headspace analysis to provide helpful dating evidence through the use of alternative and more efficient analytical and interpretative tools.

The main purpose of this first part was to address analytical questions and, specifically, to optimise an enhanced multi-residue method to obtain a more comprehensive overview of the ageing processes in spent cartridges. In this regard, headspace sorptive extraction (HSSE) recently proved to be a more efficient in comparison to SPME for the analysis of volatile GSR in spent cases [19], and was thus adopted. Indeed, HSSE involves the use of a stir bar coated with a layer of polydimethylsiloxane (PDMS), which is significantly thicker in comparison to SPME fibres. As a result, better recovery yields are generally achieved, benefitting detection limits and repeatability; a higher number of compounds are also generally co-extracted, providing an opportunity to follow the evolution of a greater number of molecules with a more diverse volatility range [13, 19]. Thus, 29 compounds known to be released during a cartridge discharge were selected as targets on the basis of previous studies [13, 19] and their chromatographic separation was optimised by liquid injection of a mixture of standards. A selected ion mode approach was implemented, which involved a short run time while maintaining good chromatographic resolution. Then, HSSE extraction and desorption parameters were tuned by extracting blank cartridges spiked with known amounts of analytes. Introduction to the sampling vials of deuterated analogues before extraction was also investigated to account for analytical variations and to allow for a semi-quantitative approach. The following parameters were optimised: HSSE stir bar type, spiking method (solvent and volume), extraction conditions (temperature and time) and thermal desorption conditions (desorption temperature, time, gas flow and cryo-focusing temperature). The effect of each parameter on the chromatographic step was studied and a multivariate statistical approach was adopted for their optimisation. Experiments were thus carried out following precise designs of experiments (DOE) [20-23], which involved the simultaneous variation of all the parameters over their experimental ranges. The advantage of DOE over the traditional one-variable-at-time optimisation method resides in its ability to account for interactions between the different variables and to construct response surfaces, which are helpful tools for selecting optimal analytical conditions. The optimised method was finally validated and applied to the analysis of real 9 mm spent cartridges.

#	*Analyte specifications*					*Analytical performances*												
						Selectivity	*LODs [pg]*		*Recoveries*		*RSDs (absolute peak areas)*				*RSDs (normalized peak areas)*			
											Repeatability			*IP*	*Repeatability*			*IP*
	t_R	*Compound*	*Target ion*	*Qualifiers*	*Deuterated standard*		*Empty Vials*	*Blank cart.*	*Empty vials*	*Blank cart.*	*Empty vials*	*Blank cart.*	*Fired cart.*	*Fired cart.*	*Empty vials*	*Blank cart.*	*Fired cart.*	*Fired cart.*
A	*8.716*	*Naphthalene-d8 (NPT-d8)*	136	137, 134	-	ok	-	-	-	-	-	-	-	-	-	-	-	-
B	*12.451*	*Biphenyl-d10 (BIP-d10)*	164	162, 160	-	ok	-	-	-	-	-	-	-	-	-	-	-	-
C	*14.670*	*Acenaphthene-d10 (ACE-d10)*	164	162, 160	-	ok	-	-	-	-	-	-	-	-	-	-	-	-
D	*19.664*	*Phenanthrene-d10 (PHE-d10)*	188	189, 184	-	ok	-	-	-	-	-	-	-	-	-	-	-	-
E	*22.986*	*Pyrene-d10 (PYR-d10)*	212	213, 208	-	ok	-	-	-	-	-	-	-	-	-	-	-	-
1	*5.899*	*Benzonitrile*	103	104, 76	*NPT-d8*	ok	50	500	54%	50%	7.9%	4.6%	43.2%	80.1%	5.1%	4.0%	36.3%	57.7%
2	*6.647*	*Indene*	116	117. 115	*NPT-d8*	ok	50	50	80%	75%	4.8%	3.3%	35.5%	70.6%	1.1%	2.2%	30.2%	52.0%
3	*6.951*	*o-Tolunitrile*	117	116, 90	*NPT-d8*	X	10	10	90%	85%	4.8%	4.4%	40.7%	66.8%	0.7%	2.3%	34.3%	48.1%
4	*7.245*	*m-Tolunitrile*	117	116, 90	*NPT-d8*	X	50	50	95%	89%	4.1%	4.7%	34.6%	69.9%	0.7%	2.0%	28.2%	54.1%
5	*7.444*	*p-Tolunitrile*	117	116, 90	*NPT-d8*	ok	10	10	89%	81%	6.0%	5.4%	33.4%	68.8%	2.3%	4.8%	30.3%	53.1%
6	*7.961*	*Benzyl nitrile*	117	116, 90	*PHE-d10*	ok	10	10	101%	67%	4.4%	9.1%	54.9%	83.3%	1.6%	7.4%	53.0%	75.9%
7	*8.763*	*Naphthalene*	128	129, 127	*NPT-d8*	ok	50	50	97%	91%	4.5%	2.9%	36.5%	68.0%	0.3%	0.4%	31.3%	54.1%
8	*9.704*	*Quinoline*	129	128, 130	*PHE-d10*	ok	5	5	100%	76%	6.0%	7.7%	42.0%	61.5%	1.7%	2.6%	40.4%	53.6%
9	*10.109*	*Isoquinoline*	129	128, 130	*PHE-d10*	X	500	500	102%	59%	18.0%	14.8%	40.7%	51.7%	16.0%	10.0%	36.7%	45.4%
10	*10.139*	*1,3-Dicyanobenzene*	128	129, 101	*PHE-d10*	X	500	500	103%	38%	5.0%	10.5%	63.1%	88.1%	0.8%	6.9%	60.5%	77.1%
11	*10.161*	*1,4-Dicyanobenzene*	128	129, 101	*PHE-d10*	X	500	500	103%	38%	5.2%	10.5%	63.1%	88.1%	0.7%	6.8%	60.5%	77.1%
12	*10.746*	*Indole*	117	116, 118	*PHE-d10*	ok	50	50	88%	47%	7.7%	8.4%	52.1%	77.4%	3.3%	6.3%	49.5%	63.3%
13	*10.804*	*1-Methylnaphthalene*	142	141, 115	*NPT-d8*	ok	50	50	106%	99%	4.6%	3.5%	35.2%	63.3%	0.8%	0.9%	32.2%	57.7%
14	*11.148*	*2-Mehtylnaphthalene*	142	141, 115	*NPT-d8*	ok	50	50	108%	103%	4.4%	3.2%	35.9%	63.2%	1.0%	1.2%	34.8%	58.6%
15	*11.379*	*1,2-Dicyanobenzene*	128	129, 101	*PHE-d10*	ok	500	500	99%	28%	5.1%	11.3%	69.1%	90.6%	1.1%	8.4%	66.9%	83.6%
16	*12.529*	*Biphenyl*	154	153, 152	*BIP-d10*	ok	1	**1**	109%	104%	4.5%	3.9%	54.3%	77.4%	0.3%	0.2%	50.3%	74.4%
17	*14.026*	*Acenaphthylene*	152	153, 151	*ACE-d10*	ok	50	50	108%	103%	5.6%	3.4%	46.3%	67.7%	1.3%	1.0%	42.9%	62.8%
18	*14.178*	*Biphenylene*	152	153, 151	*BIP-d10*	ok	50	50	105%	96%	5.2%	4.5%	42.3%	67.9%	1.0%	1.5%	39.9%	64.8%
19	*14.791*	*Acenaphthene*	154	153, 152	*ACE-d10*	ok	5	50	111%	106%	4.8%	3.4%	43.7%	60.8%	0.7%	0.5%	38.8%	54.4%
20	*15.102*	*1-Naphthalenecarbonitrile*	153	154, 126	*PHE-d10*	ok	50	50	104%	95%	5.1%	4.8%	48.6%	66.6%	0.7%	3.7%	46.5%	63.3%
21	*15.580*	*2-Naphthalenecarbonitrile*	153	154, 126	*PHE-d10*	ok	10	10	101%	82%	5.6%	7.5%	50.8%	68.1%	0.9%	4.2%	49.4%	64.4%
22	*16.836*	*Fluorene*	166	167, 165	*PHE-d10*	ok	10	10	118%	103%	4.7%	4.1%	44.9%	62.0%	1.1%	4.3%	43.3%	61.9%
23	*17.517*	*Diphenylamine*	169	168, 167	*PHE-d10*	ok	5	5	96%	83%	4.4%	5.7%	16.1%	21.7%	1.3%	5.8%	18.3%	28.5%
24	*19.719*	*Phenanthrene*	178	179, 176	*PHE-d10*	ok	1	5	97%	90%	5.2%	6.1%	42.8%	57.2%	0.4%	0.3%	42.5%	56.2%
25	*19.835*	*Anthracene*	178	179, 176	*PHE-d10*	ok	5	5	102%	89%	5.7%	8.2%	40.5%	54.4%	0.5%	2.1%	40.4%	54.0%
26	*20.977*	*Ethylcentralite*	120	268, 148	*PHE-d10*	ok	50	50	89%	64%	5.6%	13.6%	30.0%	31.8%	3.5%	10.5%	31.8%	49.7%
27	*21.591*	*Dibutyl phthalate*	149	150, 223	*PHE-d10*	ok	>500	>500	118%	52%	9.4%	24.7%	41.8%	43.3%	9.5%	20.0%	44.2%	55.6%
28	*22.561*	*Fluoranthene*	202	203, 200	*PYR-d10*	ok	50	100	103%	48%	6.3%	21.8%	42.4%	57.9%	1.2%	1.5%	42.0%	56.1%
29	*23.020*	*Pyrene*	202	203, 200	*PYR-d10*	ok	50	100	96%	40%	7.1%	21.6%	43.8%	60.6%	0.4%	1.2%	43.6%	57.8%
		Min.					1	**1**	54%	28%	4.1%	2.9%	16.1%	80.1%	0.3%	0.2%	18.3%	57.7%
		Median					50	50	101%	82%	5.2%	5.7%	42.4%	70.6%	1.0%	2.6%	40.4%	52.0%
		Max.					500	500	118%	106%	18.0%	24.7%	69.1%	66.8%	16.0%	20.0%	66.9%	48.1%

Table D.1 *– Analysed target and deuterated compounds, together with monitored ions and corresponding analytical performance characteristics for the optimized method. For target analytes, the column "deuterated standard" indicates the standard used for normalisation. LODs are reported as the absolute amounts deposed in cartridges. For selectivity, "ok" means that no interfering compounds significantly affected selectivity, while "X" indicates that the selectivity was compromised (see the text for further explications). "IP" is the intermediary precision.*

D.2. Material and methods

D.2.1. Materials

Based on previous research [13, 19], 29 target compounds known to be interesting target analytes for dating were selected (Table D.1). Of the selected compounds, 26 were explosion products frequently found after the discharge of handgun cartridges and the remaining 3 were additives of smokeless powders. Additionally, 5 deuterated molecules were added as internal standards and 24 molecules normally co-extracted with target analytes from spent cartridges were used in some experiments for the optimisation of the chromatographic method and the estimation of the global selectivity. Solvents used include dichloromethane (Sigma-Aldrich), acetone (Sigma-Aldrich), diethyl ether (Fluka) and methanol (Sigma-Aldrich), all of analytical grade. For each substance, a standard stock solution was prepared at a concentration of 1 mg mL^{-1} in dichloromethane. Working solutions for the various experiments were prepared from successive mixtures and dilutions of these stock solutions. Ammunition used was 9 mm Parabellum from Geco (RUAG Ammotec, Thun, Switzerland).

D.2.2. Preparation of blank cartridges

Spiking blank cartridges (i.e., blank matrices) with known amounts of target compounds was necessary for the purpose of optimisation. Blank cartridges were obtained by extracting previously discharged 9 mm cartridges in an ultrasonic bath using the following solvents: acetone, methanol and dichloromethane. For each solvent, two successive 15 min extractions were carried out. The cartridges were then allowed to dry overnight in a laboratory oven maintained at 140 °C.

D.2.3. HSSE extraction

For extraction, samples (real and spiked blank cartridges) were transferred to 20 mL HSSE-dedicated crimp glass vials (Gerstel, Sursee, Switzerland). A stir bar was suspended in the headspace with the aid of a special glass insert (Gerstel) and the vial was rapidly closed with a 20 mm crimp cap equipped with a 3.0 mm PTFE/silicon septum (Gerstel). Stir bars were always thermo-conditioned before use, i.e., they were first put into specially designed glass conditioning tubes (Gerstel) and then placed in a Gerstel tube conditioner (TC). The conditioning procedure suggested by the manufacturer was used: 30 min at room temperature followed by 90 min at 300 °C and finally 60 min (approximately) for cooling down. Prior to analysis, vials were re-opened and the stir bars were retrieved for placement into the pre-conditioned desorption tubes (Gerstel). These were then capped with special transportation adapters (Gerstel) and placed on the GC sampling tray for analysis. The type of stir bar, as well as the extraction temperature and time, were optimised (as described in the corresponding section below).

D.2.4. TD-GC-MS analysis of stir bars

Stir bars were thermally desorbed using a Gerstel thermal desorption unit (TDU) connected to a Gerstel CIS-4 programmed temperature vaporizing (PTV) injector. These devices were mounted on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (Agilent Technologies, Basel, Switzerland). The system was also equipped with a Gerstel MPS multi-purpose sampler which was used to automatically load tubes containing stir bars into the TDU.

Maximal desorption temperature, desorption time, desorption flow and cryo-focusing temperature were optimised (see corresponding section below). The other parameters were consistently set as follows. Thermal desorption was carried out in splitless mode. Initial desorption temperature was set at 25 °C for 0.5 min, raised to 720 °C min^{-1} until the final desorption temperature was reached. The transfer line temperature between TDU and CIS-4 was 300 °C. Liners for CIS-4 were obtained from Gerstel and packed with quartz-wool. The PTV injection ramp was initially set at the cryo-focusing temperature for 0.50 min, ramped to 300 °C at 720 °C min^{-1} and held at this temperature until 2 min of the total injection time. Splitless mode was used during injection after which the injection mode was switched to split in order to condition the liner for the next injection.

GC separation was performed on a HP-5MS (30 m x 0.25 mm x 0.25 µm) column from Agilent. The carrier gas was helium and column flow was maintained at 1.2 mL min^{-1}. The oven ramp was optimised by injection (1 µL) of a mixed solution of target, deuterated and contaminant compounds at a concentration of 4 mg L^{-1} (see Results for further details). Final chosen ramp was programmed as follows: 40 °C for 2 min, ramped to 100 °C at 20 °C min^{-1}, ramped to 155 °C at 5 °C min^{-1} and finally ramped to 250 °C at 12 °C min^{-1} (total chromatographic separation time of 23.9 min). Post-run temperature was set to 320 °C and held for 5 min with a column flow of 3 mL min^{-1}. The transfer line between the column and the MS was set at 280 °C. Ionisation was carried out by electron impact (EI). Selected ion monitoring (SIM) was used. The ions monitored for each target analyte are summarised in Table 1, together with the deuterated standard used for normalisation. The latter were chosen based on preliminary tests. Solvent delay was set to 5.60 min and MS source and quadrupole temperatures were 230 °C and 150 °C, respectively.

D.2.5. TD-GC-MS analysis of liquid standards

For experiments and analyses involving direct injection in TDU of standard solutions, desorption tubes were equipped with special glass inserts and transportation adapters for liquid injection (Gerstel). Before analysis, each tube was automatically inserted into the TDU, and 1 µL of the solution to be analysed was injected into the insert using the Gerstel MPS multi-purpose sampler. Analytical parameters remained unchanged.

D.2.6. Optimisation of spiking strategy

Three solvents were investigated to serve as spiking solvents: dichloromethane, diethyl ether and methanol. Furthermore, two spiking volumes were tested: 5 µL and 10 µL. In order to find the optimal combinations between these two factors, 5 ng of each target analyte and deuterated analogue were spiked into blank cartridges. Thus, concentrations of the working mix solutions were adapted in order to depose the same amounts independently of the spiking volume (1 and 0.5 mg L^{-1}, respectively). Samples were then extracted at 70 °C for 24 h.

D.2.7. Optimisation of extraction step

The extraction stage was optimised in a two-step approach. The first step was to select the most suitable stir bar. Four types of PDMS-coated stir bars are currently available from Gerstel, differing in their coating thickness and length: 0.5 mm x 1 cm (20 µL), 1 mm x 1 cm (40 µL), 0.5 mm x 2 cm (55 µL) and 1 mm x 2 cm (110 µL). These were all tested on blank cartridges spiked with both target and deuterated compounds. The extraction time and temperature were then optimised through a DOE-based approach. Experimental ranges went from 10 to 48 h for extraction time and from 40 to 90 °C for the extraction temperature. Each experimental point was repeated three times. Experiments were carried out by spiking blank cartridges with target compounds and by using the optimal stir bars for extraction. Due to variable extraction conditions, deuterated standards were not introduced before extraction but, instead, directly injected above stir bars in TDU just before desorption (1 µL of a 3 mg L^{-1}solution) using the Gerstel MPS multi-purpose sampler. This was performed in order to account, at least, for instrumental variations.

D.2.8. Optimisation of thermal desorption step

Maximal desorption temperature, desorption time after reaching the final desorption temperature, desorption flow and cryo-focusing temperature were optimised using DOE. Experimental conditions ranged from 5 to 10 min for desorption time, 250 to 300 °C for the extraction temperature, 20 to 60 mL min^{-1} for desorption flow, and -130 to -30 °C for cryo-focusing temperature. Each experimental point was repeated twice. Experiments were carried out by loading target compounds into stir bars by extraction from spiked empty vials at 70 °C for 24 h. Normalisation with deuterated analogues could not be applied due to continuous variability between injection conditions and subsequent influence on all the signals.

D.2.9. Application and evaluation of experimental designs

DOE plans were obtained and analysed using Unscrambler 10.1 software (CAMO Software AS., Oslo, Norway). Peak areas of target analytes served as monitored response variables. A major concern in the adopted optimisation approach was to minimize the number of analyses required. Hence, a sequential assembly strategy involving the fractionation of necessary experiments in different independent series was preferred [20]. Thus, for the optimisation of both extraction and desorption conditions, a full factorial design (FFD) was initially run. This was eventually extended to a central composite design (CCD) by adding the remaining axial points if the obtained models did not satisfyingly capture the true relationship between factors and responses. In assessing this, effects significance, lack-of-fit, regression significance and curvature were evaluated.

The inspection of surface responses was applied as optimisation strategy. For this, several regression models of increasing complexity (linear to quadratic) were fitted onto data. Afterwards, the model that better described the true relationship between factors and responses was selected. This corresponded, for each target compound, to the model with the highest lack-of-fit p-value and the lowest regression-significance p-value [23].

Lack-of-fit and regression significance were evaluated through Snedecor's F-test as described by Ferreira et al. [23]; curvature was tested through Student's t-test as suggested by Box et al. [20]. Amongst the 29 compounds included in the method, 10 were selected for the optimisation of the extraction and desorption steps: benzonitrile, p-tolunitrile, naphthalene, 2-methylnaphthalene, 1,2-dicyanobenzene, biphenyl, acenaphthene, 1-naphthalenecarbonitrile, phenanthrene and pyrene. These were selected in order to cover the variability in physico-chemical characteristics of the analytes extensively.

D.2.10. Estimation of analytical performance

Since the developed method was semi-quantitative, the following analytical performance characteristics could be determined: selectivity, limits of detection (LODs), repeatability, intermediary precision and recoveries [24]. Selectivity was evaluated by injecting 24 molecules known to be commonly co-released with target compounds during the discharge of handgun cartridges [24], and also by in-depth analysis of chromatograms of real spent cartridges and blank cartridges spiked with internal standards. LODs were determined in both blank cartridges and empty vials by spiking samples with diluted solutions of target compounds [25]. They were defined as the lowest amounts able to generate an S/N ratio ≥ 3 after background correction [26]. Repeatability (intra-day variability) and intermediary precision (inter-day variability) were measured by the relative standard deviations (RSDs) of the normalised peak areas after HSSE extraction. Both were determined in real fired cartridges; repeatability was additionally measured in blank cartridges and empty vials spiked with 1 ng of target compounds using the previously optimised strategy. Recoveries were determined by normalising the peak areas obtained after HSSE extraction of samples spiked with 5 ng of target compounds by those measured after direct liquid injection.

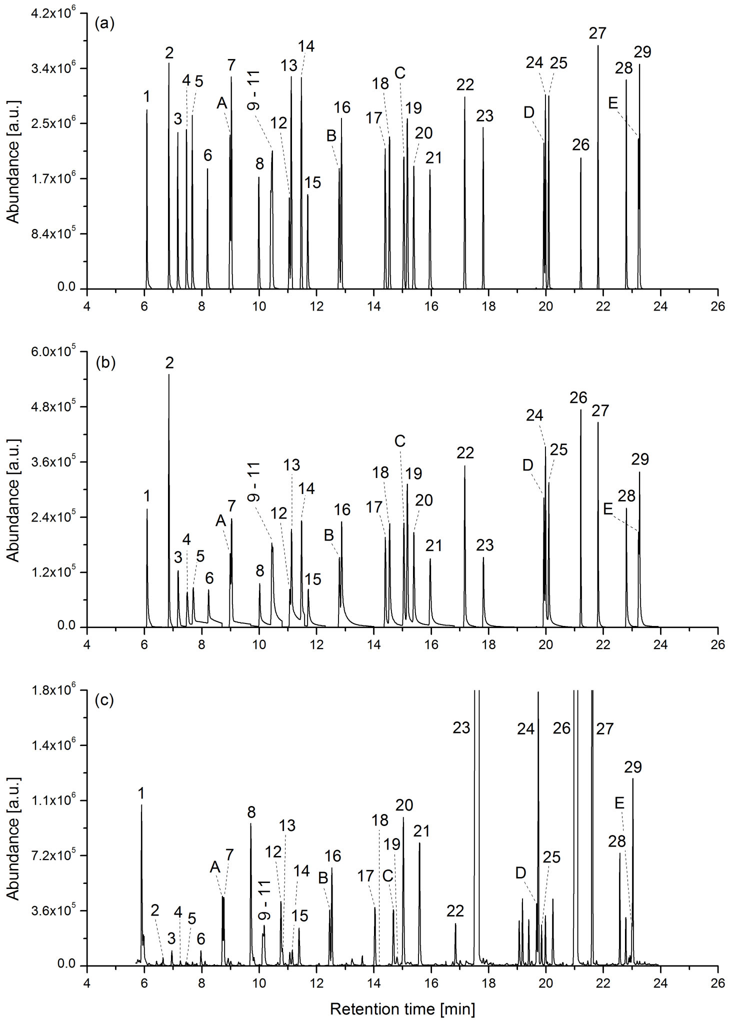


Figure D.1 *– Examples of chromatograms for (a) the direct injection of liquid standards with the optimised separation method, (b) the impact on the GC performances after analysis of 37 real samples spiked with 10 µL of dichloromethane, and (c) a 9 mm spent cartridge from Geco, analysed after 1 h of ageing. Correspondence between numbers and peaks is explained in Table D.1.*

D.2.11. Tests on real fired cartridges

Test shootings were carried out using a SIG P220 semi-automatic pistol in an internal air-conditioned shooting range. All cartridges belonged to the same production batch and were fired by singly loading them in the pistol magazine. Spent cartridges analysed at time t = 0 h were immediately sampled after discharge. Spent cartridges analysed at time t > 0 h were aged in a conditioned laboratory oven kept at constant temperature and humidity conditions (25 °C, 75 % relative humidity). Different specimens were aged for 1, 3, 5, 7, 24, 31, 48 and 72 h after which were extracted according to the proposed optimised procedure.

D.3. Results and discussion

D.3.1. Optimisation of the GC-MS method

The purpose of the present work was to optimise a fast and reliable, semi-quantitative HSSE-TD-GC-MS method capable of extraction and analysis of 29 target volatile organic GSR compounds from 9 mm Parabellum cartridges. The 29 monitored compounds and 5 deuterated analogues are reported in Table D.1. These were selected in order to cover the main classes of compounds often present in volatile GSR [13, 19].

The GC temperature ramp was optimised first by liquid injection of a mixed solution composed of target compounds and their deuterated analogues. Common contaminants were also included to guarantee the highest selectivity. A method with a run time of 23.9 min was developed (Fig. D.1a), demonstrating an improvement by a factor of 2 (approximately) in comparison to the previous published approach [13, 19]. Some compounds were hardly separable at the baseline on the chosen column (HP-5MS) without excessive loss in run time, peak resolution and peak shape. Nonetheless, all target compounds could be differentiated by their mass spectra, with the exception of isoquinoline, 1,3-dicyanobenzene and 1,4-dicyanobenzene, which co-eluted in the developed method (peak #9-11 in Figure D.1a), and were characterized by very similar mass spectra. Their signals were consequently summed in the final temperature program. A selected ion monitoring (SIM) method was implemented as the MS scan mode, in place of the previous scan-based method [13, 19]. One target ion and two qualifiers were thus selected for each analyte (Table D.1). Target compounds were extracted with HSSE stir bars and desorbed in the instruments as controls for the developed GC-MS method: no significant drifting in retention times and/or modification in fragmentation patterns were observed.

D.3.2. Optimisation of spiking strategy

The final approach involved the introduction of deuterated internal standards into extraction vials through the deposition of aliquots of a mixed solution. A similar strategy was also adopted for spiking known amounts of target compounds into blank cartridges for the optimisation of the extraction and desorption conditions. Thus, a spiking method needed to be developed.

Initially, standards were introduced into vials after cartridge sampling using 10 µL of dichloromethane. This strategy, however, caused severe peak tailing (especially for nitrogen-containing analytes) after injection of 30 to 40 real specimens (Fig. D.1b), and was thus unreliable for processing long analytical sequences. The problem was attributed to the degradation of the column head caused by the injection of an excessive volume of dichloromethane (which was co-extracted by the stir bars), as proved by the fact that chromatographic performance could be completely restored by cutting the first few centimetres off the column. Thus, while dichloromethane was found to be optimal for direct injection of small liquid volumes, the same was not true for spiking and the entire strategy was re-optimised. Particularly, the effect of different spiking solvents and volumes was investigated.

Appropriate spiking solvents for HSSE should have a sufficiently low boiling point in order to be quickly vented during the thermal desorption step and they should also have the ability to completely dissolve all target and deuterated analytes. In addition to dichloromethane, two other solvents which fulfilled these criteria were investigated: diethyl ether and methanol. Both 10 µL and 5 µL spike volumes were tested, while the spiked amounts (5 ng per compound) were kept constant by varying the solution concentration (0.5 and 1 mg L^{-1}, respectively).

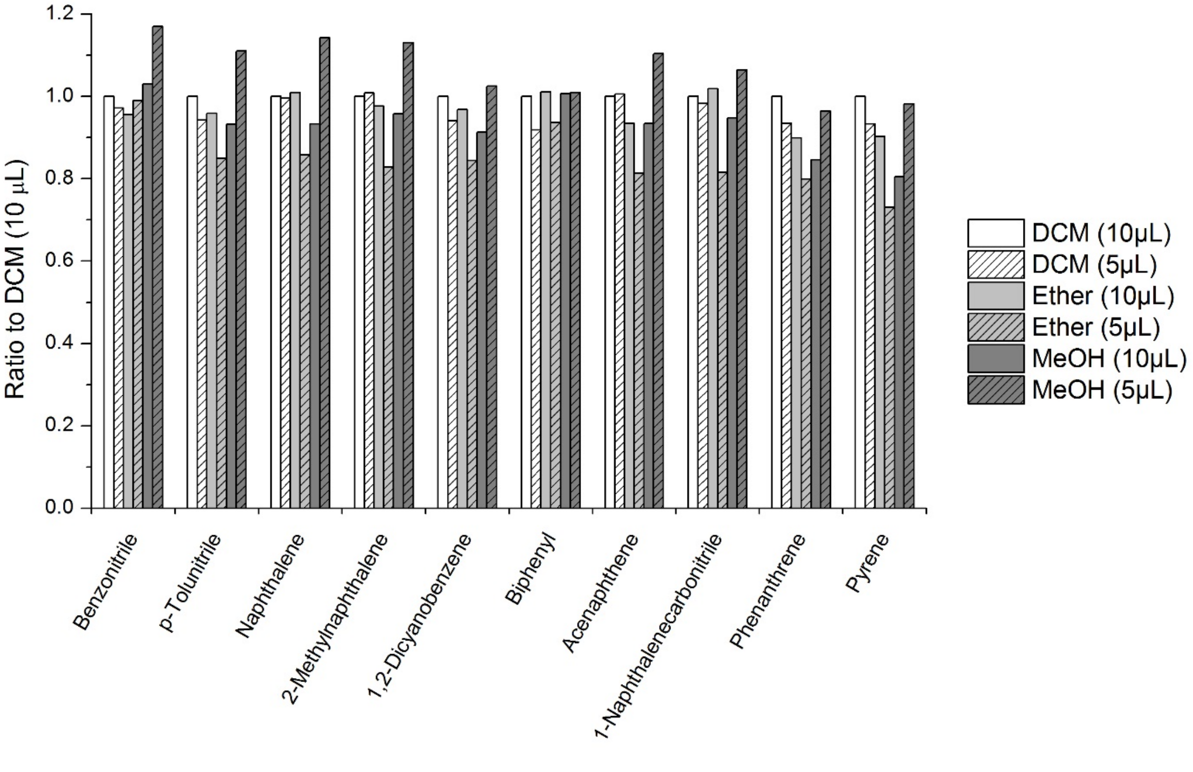


Figure D.2 *– Observed response for select compounds after HSSE extraction of samples spiked with different carrier solvents and volumes. For the sake of comparison, values are normalised by those observed using 10 µL of dichloromethane (DCM). Values for deuterated compounds were very similar to their non-deuterated analogues (not shown here).*

Globally, peak shapes and resolution were not affected by changing the solvents, but there was significant variation in the amounts detected (signal intensity). Figure D.2 shows the peak areas after HSSE extraction of some selected analytes normalised by those obtained by using 10 µL of dichloromethane (note that results for the 5 deuterated standards were very similar to their respective non-deuterated molecules). Though 10 µL spikes in diethyl ether gave similar responses to those in dichloromethane, halving the volume also caused a significant decrease in the compound responses. Consequently, volume reduction was not possible with this solvent. The best results were obtained by using 5 µL spikes in methanol. Indeed, successive tests confirmed that this method was sufficiently robust, effectively avoiding a rapid degradation of the column. The possibility of adding a time delay between the spike deposition and sealing the vial (to allow for solvent evaporation) was also investigated. However, this approach led to a systematic decrease in the signals and was thus rejected.

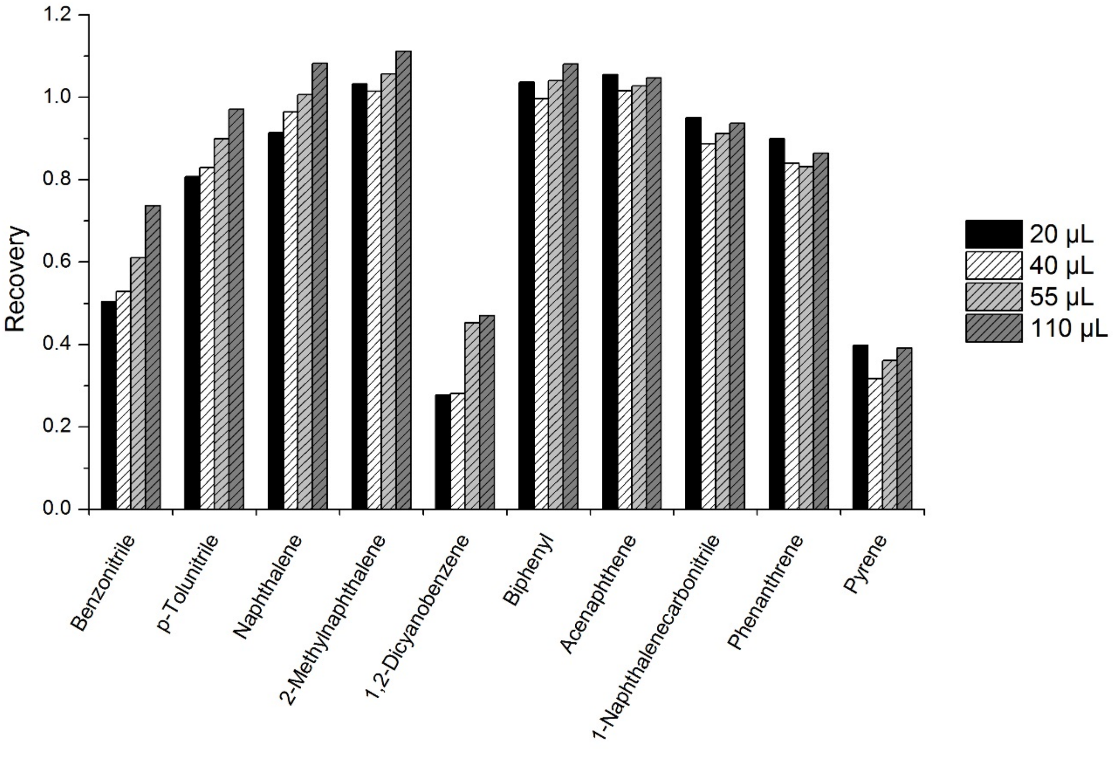


Figure D.3 *– Observed recovery for select compounds after HSSE extraction of spiked blank cartridges with stir bars of different coating volumes.*

D.3.3. Optimisation of extraction step

As previously mentioned, the HSSE extraction stage was optimised using a two-step approach. Firstly, the optimal type of stir bars was chosen. Polydimethylsiloxane (PDMS) was initially selected as the stir-bar coating because of its recognized affinity to the selected target analytes [27-29].

Contrariwise, the best coating volume was empirically investigated. Figure D.3 shows the recoveries for selected target analytes after HSSE extraction of spiked blank cartridges obtained by different coating volumes. Generally, for the most volatile compounds (those from benzonitrile to 1,2-dicyanobenzene), a significant difference in the detected amounts could be observed. For instance, detected peak areas for benzonitrile after extraction with a 20-μL stir bar were approximately 1/3 smaller than those detected with a 110-μL stir bar. This difference became less significant for the less volatile analytes (from biphenyl to pyrene, Fig. D.3). Despite the lower signal intensities, a coating volume of 20 μL was selected for subsequent experiments in order to avoid excessive solvent loading onto stir bars, which could cause solvent-induced column degradation as observed in the aforementioned preliminary tests. This was judged acceptable, considering that the most volatile compounds are more prone to rapidly disappearing from spent cartridges, and are consequently less interesting for dating purposes.

Compounds	***Effects***					***Chosen model***
	A	***B***	***AB***	***AA***	***BB***	
Benzonitrile	NS	- - -	NS	NS	NS	Interactions
p-Tolunitrile	-	- - -	-	NS	-	Quadratic
Naphthalene	NS	- - -	NS	NS	NS	Interactions
2-Methylnaphthalene	NS	- - -	-	NS	NS	Interactions
1,2-Dicyanobenzene	NS	- - -	NS	NS	-	Quadratic
Biphenyl	NS	- - -	- - -	NS	NS	Interactions
Acenaphthene	NS	-	-	NS	NS	Interactions
1-Naphthalenecarbonitrile	NS	NS	NS	NS	NS	-
Phenanthrene	+ + +	+ + +	NS	NS	NS	-
Pyrene	+ + +	+ + +	NS	NS	NS	Linear

Table D.2 *– Analysis of the effects on compound response after running a CCD on the factors retained during the optimisation of extraction step. "A" is the extraction time and "B" is the extraction temperature; "NS" indicates a not significant effect; "chosen model" indicates (for each compound) the model chosen for the inspection of the surface response (linear model w/o interactions, linear model w/ interactions or quadratic model).*

DOE was used to optimise the remaining factors, namely extraction temperature and time. A full factorial design (FFD) was initially applied. Statistical analysis of the results revealed significant main effects and interactions for most compounds (see Electronic Supporting Material, ESM). Furthermore, lack-of-fit was detected for most of the models as well as significant curvature within the studied experimental range. Important quadratic effects were consequently suspected, and it was decided to complete FFD with axial points in order to build a central composite design (CCD) and improve reliability of the inferred models. Statistical analysis of the new results indeed revealed a significant quadratic effect of the extraction temperature on the observed peak areas of p-tolunitrile and 1,2-dicyanobenzene (Table D.2). After the data was re-fit with new models, the lack-of-fit issues were solved for most compounds, with the exception of 1-naphthalenecarbonitrile and phenanthrene. These two analytes were not further considered for the optimisation of the extraction step (see ESM). The simplest acceptable models were chosen for the remaining compounds and corresponding response surfaces were inspected.

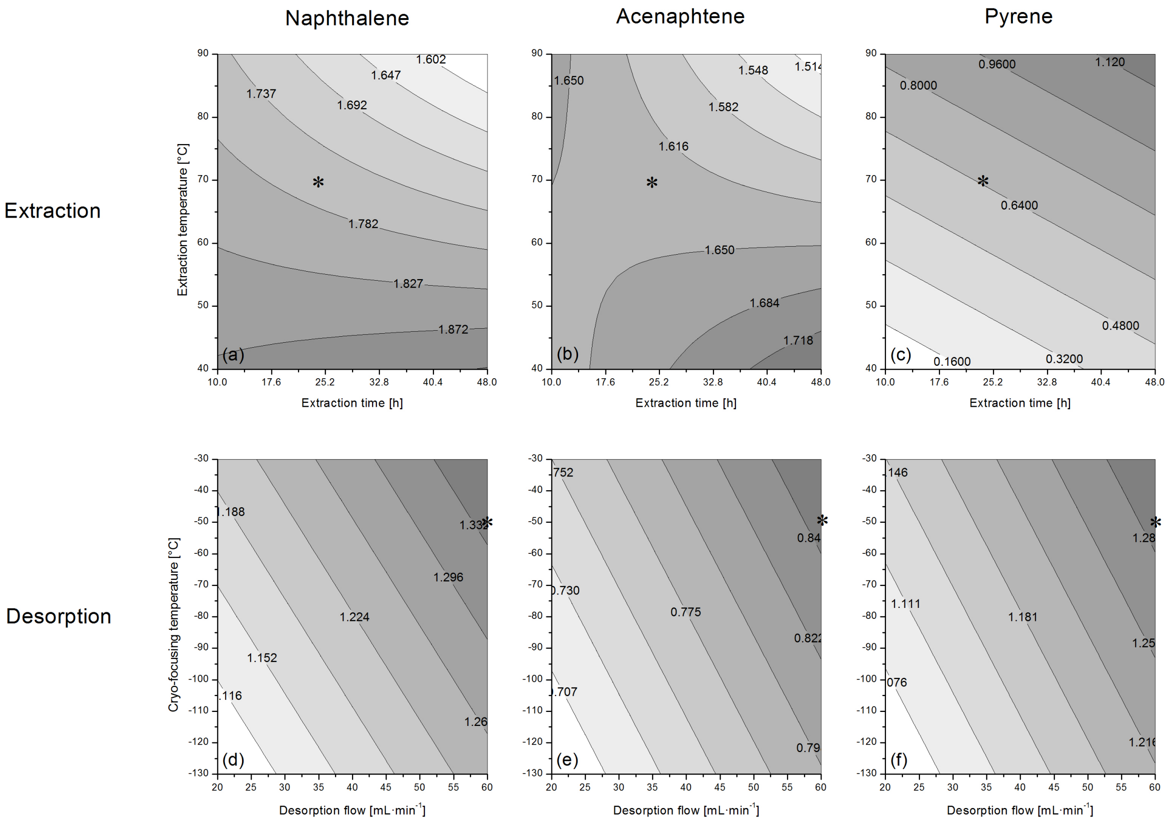


Figure D.4 – Evaluated surface response for select compounds and particularly influent parameters in the optimisation of both, the extraction (a-c) and desorption conditions (d-f). An asterisk (*) indicates the chosen optimal conditions. Complete illustrations for all the compounds involved in optimisation are reported in Electronic Supporting Material.

For the purpose of illustration, Figures D.4a-c show the surface responses of naphthalene, acenaphthene and pyrene. From a routine work perspective, extraction time is the most constraining parameter and was thus firstly tuned. It can be observed that the effect of extraction time in the studied interval (i.e., 10 – 48 h) was generally not significant for the most volatile compounds (e.g., naphthalene). On the contrary, the less volatile ones needed long extraction times in order to reach satisfactory recoveries (e.g., pyrene). Values between 24 h and 48 h were considered as globally acceptable and 24 h was selected for practical reasons. This represents a time decrease of a factor 3 in comparison to previous works [13, 19]. Regarding extraction temperature (investigated interval: 40 – 90 °C), there was a clear conflicting trend amongst analytes: raising temperature had a negative effect on the recovery of the most volatile compounds while it was positive on those of the less volatile ones (especially, phenanthrene and pyrene) (Fig. D.4a-c). Consequently, an intermediary value had to be chosen, and temperatures between 70 and 80 °C seemed adequate. In this work, 70 °C was finally selected.

Compounds	***Effects***					***Chosen model***
	A	***B***	***C***	***D***	***Inter.***	
Benzonitrile	NS	NS	+ + +	+ + +	NS	Linear
p-Tolunitrile	NS	NS	+ + +	+ + +	NS	Linear
Naphthalene	NS	NS	+ + +	+ + +	NS	Linear
2-Methylnaphthalene	NS	NS	+ + +	+ + +	NS	Linear
1,2-Dicyanobenzene	+	+ +	+ + +	+ + +	NS	Linear
Biphenyl	NS	NS	+ + +	+ + +	NS	Linear
Acenaphthene	NS	NS	+ + +	+ + +	NS	Linear
1-Naphthalenecarbonitrile	NS	NS	+ + +	+ + +	NS	Linear
Phenanthrene	NS	NS	+ + +	+ + +	NS	Linear
Pyrene	NS	NS	+ + +	+ + +	NS	Linear

Table D.3 *– Analysis of the effects on compound response after running a FFD on the factors retained during the optimisation of desorption step. "A" is the desorption time, "B" is the desorption temperature, "C" is the desorption flow, "D" is the cryo-focusing temperature and "Inter." is the combination of all the different interactions effects together; "NS" indicates a not significant effect; "chosen model" indicates (for each compound) the model chosen for the inspection of the surface response (linear model w/o interactions or linear model w/ interactions).*

D.3.4. Optimisation of thermal desorption step

An analogue DOE approach was used to optimise the factors affecting the desorption step, namely the maximal desorption temperature, desorption time after reaching the final desorption temperature, desorption flow and cryo-focusing temperature. A FFD was initially run. Factors showed very simple effect patterns on the responses of all target compounds (Table D.3). In fact, no significant interactions were observed, while main effects, when statistically significant, always had a positive influence. Statistical tests on curvatures detected significant values for some compounds (see ESM). However, these were visually examined and did not strongly deviate from linearity. Furthermore, all regression models inferred on data did not demonstrate lack-of-fit and were shown to be statistically significant. Consequently, adding axial points in order to pass from a FFD to a CCD was not judged necessary for optimisation purposes. Linear models without interactions, being globally the simplest acceptable models, were chosen for inspection of the response surfaces.

The effects of desorption time and desorption temperature (optimisation ranges: 5 – 10 min, and 250 – 300 °C, respectively) were generally not significant for all target analytes, except for 1,2-dicyanobenzene on which they had a positive effect. Hence, 300 °C was chosen as desorption temperature, mainly to avoid condensation in the liner of particularly low volatility compounds during long analytical sequences. Furthermore, desorption time was set to 5 min to minimise run time, despite the slight reduction in the recovery of 1,2-dicyanobenzene.

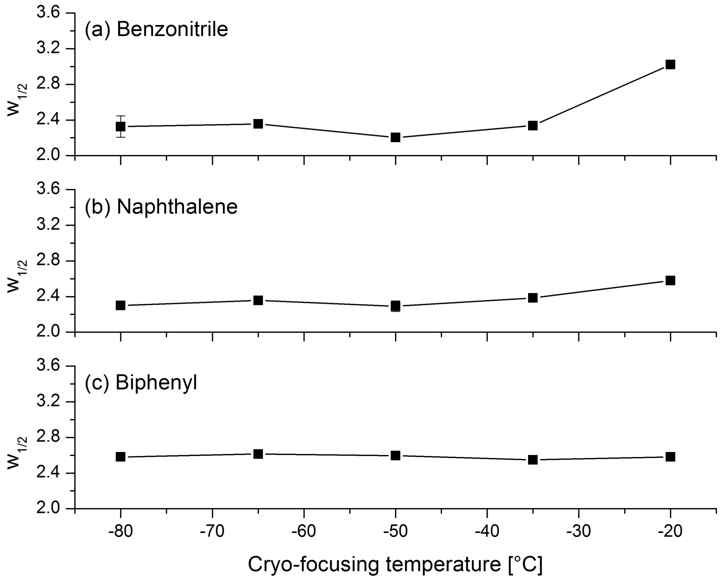


Figure D.5 – *Plot of peak widths at half height (w1/2) for select 3 target analytes as a function of cryo-focusing temperature.*

The main effects of the two remaining variables (i.e., desorption flow and cryo-focusing temperature in the ranges of 20 – 60 mL min^{-1} and -130 – -30 °C, respectively) were observed to be strongly significant and positive for all selected compounds (Table D.3). Regardless, their interaction effect was statistically irrelevant, indicating that setting both experimental parameters to high values also permitted the achievement of larger signals. This was confirmed by the inspection of the respective responses surfaces (Fig. D.4d-f). Even if larger peak areas were obtained at high cryo-focusing temperatures, it was successively noted that they also led to significant peak tailing for all of the most volatile analytes. This was likely due to a non-optimal cryo-focusing effect during desorption. Thus, the effect of cryo-focusing temperature on the peak shapes was studied in greater detail between the ranges of -80 to -20 °C on a series of new, independent experiments. Figure D.5 reports the plots of peak widths at half height for selected volatile target analytes, showing that no further worsening in peak shape was observed for cryo-focusing temperatures lower than -50 °C. For this reason, a temperature of -50 °C was selected. Desorption flow was set to 60 mL min^{-1}.

D.3.5. Analytical performance characteristics

A summary of the chosen optimal conditions is reported in Table D.4. Table D.1 reports the analytical performance characteristics of the optimised method.

Step	***Factor***	***Type***	***Experimental values/ranges***	***Optimal conditions***
Spiking	*Spiking solvent*	D	DCM, Ether, MeOH	MeOH
	Spiked volume	D	5 µL, 10 µL	5 µL
Extraction	*PDMS volume (stir bars)*	D	20 µL, 40 µL, 55 µL, 110 µL	20 µL
	Extraction temperature	C	From 40 to 90 °C	70 °C
	Extraction time	C	From 10 to 48 h	24 h
Desorption	*Desorption temperature*	C	From 250 to 300 °C	300 °C
	Desorption time	C	From 5 to 10 min	5 min
	Desorption gas flow	C	From 20 to 60 mL min^{-1}	60 mL min^{-1}
	Cryo-focusing temperature	C	From -130 to -30 °C	-50 °C

Table D.4 *– Optimised experimental factors, with their experimental values/ranges and chosen optimal conditions. "Type" indicate whether the corresponding factor was categorical ("D") or continuous ("C"). For solvents, DCM = dichloromethane, Ether = diethyl ether, MeOH = methanol.*

Selectivity was thoroughly evaluated by injection of common known contaminants and analysis of real samples. As previously mentioned, absolute discrimination of isoquinoline, 1,3-dicyanobenzene and 1,4-dicyanobenzene was not possible using the optimised GC-MS method, resulting in a mutual interference between these compounds and their sum on the chromatograms. Furthermore, both 1- and 2-naphthalenecarbonitrile partially co-eluted with 3- and 4-methylbiphenyl (potential discharge interfering compounds), respectively, which presented several common ions. Regardless, this fact has not been estimated as compromising for their actual analytical performance in practical applications because of the very low concentrations of these interfering compounds released during the discharge of handgun cartridges [13]. Both, o- and m-tolunitrile also partially co-eluted with some unidentified molecules having common ions which were detected during the analysis of real samples. This, however, was considered more problematic given the significant intensity of these interfering compounds.

LODs of all compounds deposed in cartridges were at picograms level. These are in agreement with other stir-bars-based approaches for the analysis of PAHs published in the literature [28-30]. For dibutyl phthalate, relatively important contaminations were generally detected in procedural blanks and it has been established that LOD for this compounds was > 500 pg in the vial. Despite this, its identification and monitoring over time in real GSRs should not be an issue because of the excessive amounts usually detected in spent cartridges [13]. The effect on LODs caused by the presence of a cartridge in the extraction vessel was nearly insignificant except for benzonitrile, acenaphthene, phenanthrene and pyrene, for which LODs were slightly higher.

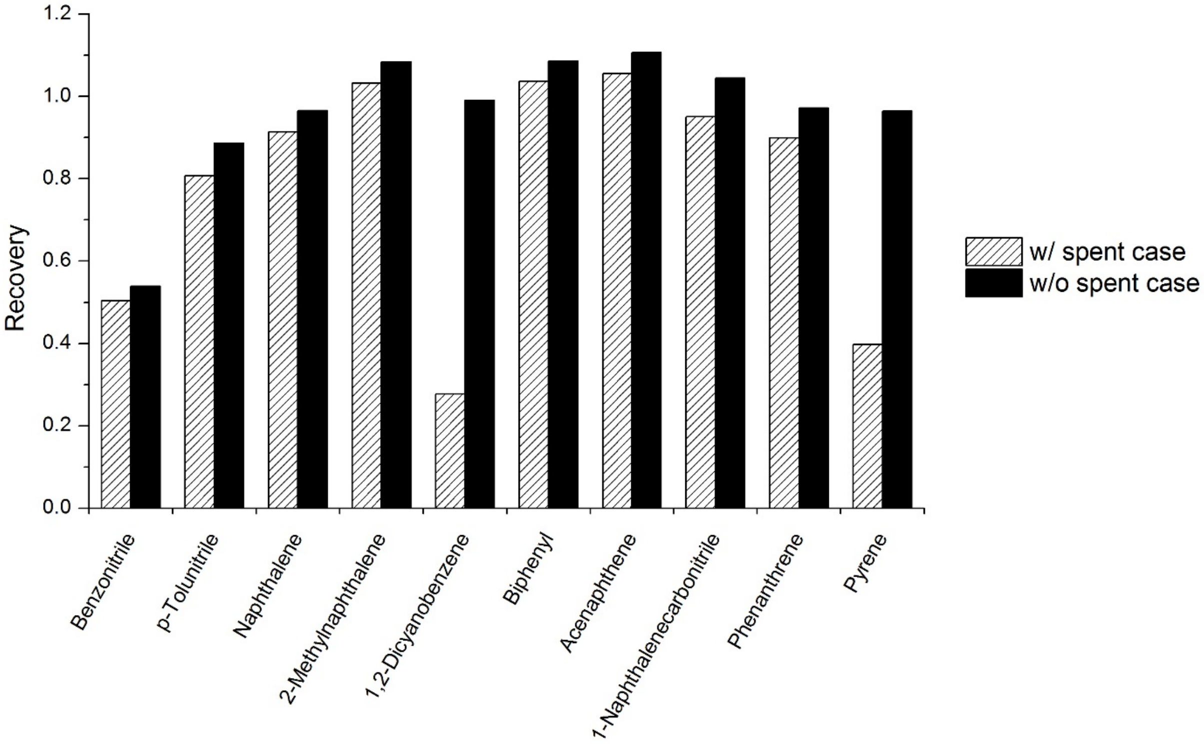


Figure D.6 *– Difference in recovery of target analytes in the presence ("w/") and absence ("w/o") of blank cartridges.*

Repeatability of compounds' peak areas normalised by the corresponding deuterated standards (measured in terms of RSD) ranged from 0.3% to 16.0% in empty vials and from 0.2% to 20.0% in the presence of cartridges. Thus, any additional analytical error introduced by the sole blank matrix, itself, was globally insignificant. On the contrary, the repeatability of target compounds in real cartridges analysed immediately after discharge was significantly higher and ranged from 18.3% to 66.9%. This indicated that a considerable degree of variability was accounted for by external non-analytical factors such as the particular cartridge used (compositional inhomogeneity between different cartridges) as well as the discharge process (differences in temperature and pressure in the firearm chamber during discharge). Normalisation of peak areas to deuterated standards generally significantly improved the repeatability of measured values along with their intermediary precisions (see data in Table D.1).

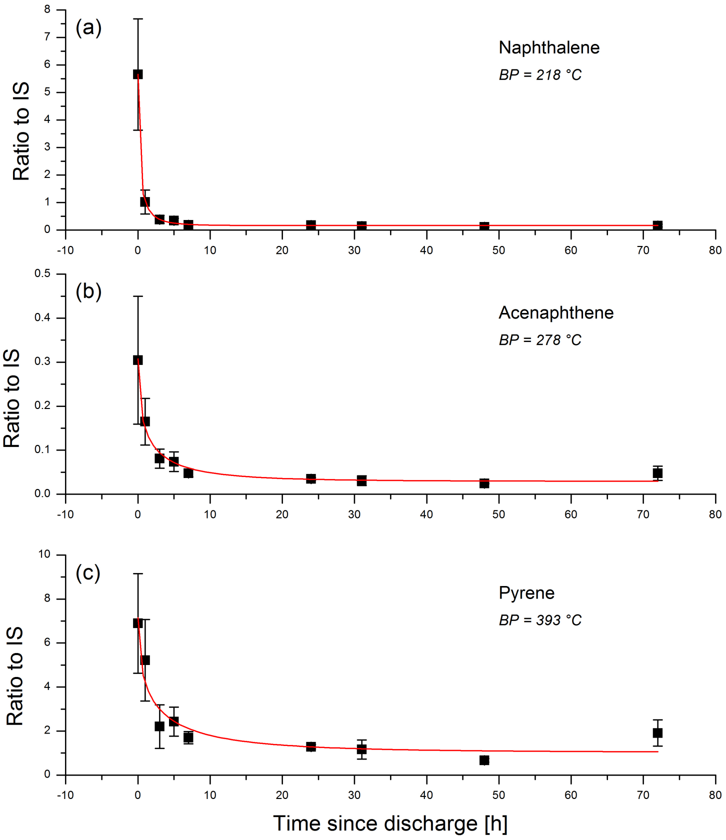


Figure D.7 *– Ageing curves for select compounds (temperature: 25 °C; relative humidity: 75%).*

Recoveries in the presence of cartridges ranged from 28% to 106%, meaning that the recovery for some target compounds was close to 100%, while for others, it was considerably lower. By comparing these results with those obtained in empty vials (recoveries from 54% to 118%), it was obvious that the presence of a cartridge in the extraction vial caused important matrix effects (see also Figure D.6). This was especially true for nitrogen-containing and lipophilic compounds. For example, recoveries for 1,2-dicyanobenzene and pyrene decreased from about 100% in empty vials to less than 40% in vials containing blank cartridges. Reasons for this may be due to adsorption phenomena on the metallic surface of the cartridges. These results stressed the importance of using blank cartridges to account for matrix effects during optimisation of the extraction conditions.

D.3.6. Application to real samples

The optimised method was finally applied to real samples. Figure D.1c shows an example of a chromatogram obtained from a 9 mm Parabellum spent cartridge after HSSE extraction. All target analytes were identified in the residue and detected across the entire tested ageing interval. Figure D.7 shows the ageing curves for some selected compounds. The decrease of most of the target analytes could be followed over the course of different days with acceptable precision in detected signals.

The use of deuterated analogues as co-extracted internal standards significantly improved the comparability of the obtained ageing curves. For example, Figure D.8 compares two ageing curves for pyrene taken at a 3 month interval, with and without normalisation to the corresponding deuterated standard (pyrene-d10). It can be observed that normalisation significantly reduced the bias between the two curves. Thus, the results strongly support the usefulness of using co-extracted internal standards to reliably study compounds' ageing kinetics and the developed approach seems to be a valuable tool for this kind of application.

rtridges stored at different ageing conditions will be performed.

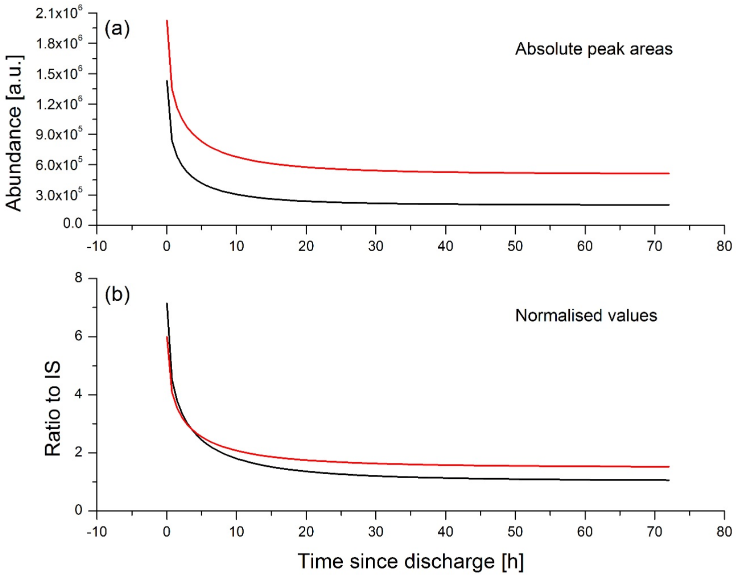


Figure D.8 *– Comparison of two ageing curves for pyrene taken at 3 months interval (a) without normalisation and (b) with normalisation to the corresponding deuterated standard (pyrene-d10).*

D.4. Conclusions

In the present work, a HSSE extraction method combined with TD-GC-MS was comprehensively optimised for the analysis of 29 volatile organic GSR compounds in spent handgun cartridges. Several experimental parameters affecting the chromatographic, extraction and desorption steps were identified and tuned. A multivariate statistical approach based on DOE was used for factors potentially involved in interaction effects. Additionally, an extracted standard approach based on deuterated standards (naphthalene-d8, biphenyl-d10, acenaphthene-d10, phenanthrene-d10 and pyrene-d10) was developed and implemented for the first time in order to reduce analytical variability.

The developed method presented notable analytical performance and particularly, low LODs for the selected target analytes in relatively short run times. Indeed, the applied optimisation procedure allowed for greatly decreasing the total time required to process a single cartridge in comparison to previously published approaches [13, 19]. Application of the novel method to real 9 mm spent cartridges showed that the use of co-extracted internal standards successfully allowed for improved repeatability of measured signals and, especially, for improving the comparability between ageing curves acquired at different times. The second part of this series will focus on testing combinations of multivariate regression models and pre-treatment strategies in order to comprehensively exploit the chemical information which can be acquired by the developed approaches. Furthermore, an evaluation of the potentials and limitations of headspace analysis for estimating the time since discharge of 9 mm ca

Acknowledgements

This work has been generously supported by the Swiss National Foundation (Grant no. PP00P1_123358). The authors would like to thank Hélène Choo, Laurence Hamdan and Thomas Miller (King’s College London, UK), as well as Natalia Pawlowska (University of Lausanne, CH) for proofreading early drafts of the article.

Bibliography

1. Andersson, C. and J. Andrasko, *A novel application of time since the latest discharge of a shotgun in a suspect murder.* Journal of Forensic Sciences, 1999. **44**(1): p. 211-213.
2. Bridgemon, R.R., *Cartridge case aging.* AFTE Journal, 1986. **18**(1): p. 49-52.
3. Sokol, J., *Die Bestimmung des Zeitpunktes, wann eine Waffe das letztemal beschossen und eine Partone verfeuert werde*, 1939, University of Bern.
4. Price, G., *Recent advances in ballistics laboratory methods.* Journal of Forensic Sciences, 1968. **8**(2): p. 83-90.
5. Sinha, J.K., *Time of firing of shot shells.* Journal of Forensic Sciences, 1976. **21**(1): p. 171-175.
6. Voskertchian, G.P. and G.V. Pavilova, *Spectrophotometric determination of time since discharge of firearms.* AFTE Journal, 1994. **26**(3): p. 216-222.
7. Aginsky, V.N., V. Lesnikov, and G. Sorokina, *Time of shooting: feasibility of discriminating "fresh" and "old" organic gunshot residues*, in *Proceedings of the 14th meeting of the International Association of Forensic Sciences*1996.

8. Romolo, F.S. and P. Margot, *Identification of gunshot residue: a critical review.* Forensic Science International, 2001. **119**: p. 195-211.

9. Dalby, O., D. Butler, and J.W. Birkett, *Analysis of gunshot residue and associated materials - a review.* Journal of Forensic Sciences, 2010. **55**(4): p. 924-943.

10. Chang, K.H., et al., *Gunshot residue analysis and its evidential values: a review.* Australian Journal of Forensic Sciences, 2013. **45**(1): p. 3-23.

11. Meng, H.-H. and B. Caddy, *Gunshot residue analysis - a review.* Journal of Forensic Sciences, 1997. **42**(4): p. 553-570.

12. Wallace, J.S., *Chemical analysis of firearms, ammunition, and gunshot residue*. 2008, Boca Raton, FL: CRC Press.

13. Gallidabino, M., F.S. Romolo, and C. Weyermann, *Characterization of volatile organic gunshot residues in fired handgun cartridges by headspace sorptive extraction.* Analytical and Bioanalytical Chemimstry, 2015. **407**(23): p. 7123-7134.

14. Andrasko, J., T. Norberg, and S. Stahling, *Time since discharge of shotguns.* Journal of Forensic Sciences, 1998. **43**(5): p. 1005-1015.

15. Weyermann, C., et al., *Analysis of organic volatile residues in 9mm spent cartridges.* Forensic Science International, 2009. **186**: p. 29-35.

16. Moxnes, J.F., et al., *Lead free ammunition without toxic propellant gases.* Propellants, Explosives, Pyrotechniques, 2013. **38**: p. 255-260.

17. Andrasko, J. and S. Stahling, *Time since discharge of spent cartridges.* Journal of Forensic Sciences, 1999. **44**(3): p. 487-495.

18. Wilson, J.D., J.D. Tebow, and K.W. Moline, *Time since discharge of shotgun shells.* Journal of Forensic Sciences, 2003. **48**(6): p. 1298-1301.

19. Gallidabino, M., et al., *Development of a novel headspace sorptive extraction method to study the aging of volatile compounds in spent handgun cartridges.* Analytical Chemistry, 2014. **86**: p. 4471-4478.

20. Box, G.E.P., J.S. Hunter, and W.G. Hunter, *Statistics for experimenters: design, innovation and discovery*. 2nd ed. 2005, Hoboken, NJ: John Wiley & Sons.

21. Leardi, R., *Experimental design in chemistry: a tutorial.* Analytica Chimica Acta, 2009. **652**: p. 161-172.

22. Lundstedt, T., et al., *Experimental design and optimization.* Chemometrics and Intelligent Laboratory Systems, 1998. **42**: p. 3-40.

23. Ferreira, S.L.C., et al., *Statistical designs and response surface techniques for the optimization of chromatographic systems.* Journal of Chromatography A, 2007. **1158**: p. 2-14.

24. Peters, F.T., O.H. Drummer, and F. Musshoff, *Validation of new methods.* Forensic Science International, 2007. **165**: p. 216-224.

25. Armbruster, D.A., M.D. Tillman, and L.M. Hubbs, *Limit of detection (LOD)/limit of quantitation (LOQ): comparison of the empirical and the statistical methods exemplified with GC-MS assays of abused drugs.* Clinical Chemistry, 1994. **40**(7): p. 1233-1238.

26. Miller, J.N. and J.C. Miller, *Statistics and chemometrics for analytical chemistry*. 5th ed. 2005, Harlow, UK: Pearson Education Limited.

27. Doong, R.-A., S.-M. Chang, and Y.-C. Sun, *Solid-phase microextraction for determining the distribution of sixteen US environmental protection agency polycyclic aromatic hydrocarbons in water samples.* Journal of Chromatography A, 2000. **879**: p. 177-188.

28. Kolahger, B., A. Hoffmann, and A.C. Heiden, *Application of stir bar sorptive extraction to the determination of polycyclic aromatic hydrocarbons in aqueous samples.* Journal of Chromatography A, 2002. **963**: p. 225-230.

29. Prieto, A., et al., *Development of a stir bar sorptive extraction and thermal desorption-gas chromatography-mass spectrometry method for the simultaneous determination of several persistent organic pollutants in water samples.* Journal of Chromatography A, 2007. **1174**: p. 40-49.

30. Popp, P., C. Bauer, and L. Wennrich, *Application of stir bar sorptive extraction in combination with column liquid chromatography for the determination of polycyclic aromatic hydrocarbons in water samples.* Analytica Chimica Acta, 2001. **436**: p. 1-9.

APPENDIX E

Time since discharge of 9 mm cartridges by headspace analysis, part 2: ageing study and estimation of the time since discharge using multivariate regression

Submitted to *Forensic Science International*

Authors: Gallidabino, M., Romolo, F. S., & Weyermann, C.

E. Time since discharge of 9 mm cartridges by headspace analysis, part 2: ageing study and estimation of the time since discharge using multivariate regression

Abstract

Estimating the time since discharge of spent cartridges can be a valuable tool in the forensic investigation of firearm-related crimes. To reach this aim, it was previously proposed that the decrease of volatile organic compounds released during discharge is monitored over time using non-destructive headspace extraction techniques. While promising results were obtained for large-calibre cartridges (e.g., shotgun shells), handgun calibres yielded unsatisfying results. In addition to the natural complexity of the specimen itself, these can also be attributed to some selective choices in the methods development. Thus, the present series of papers aimed to systematically evaluate the potential of headspace analysis to estimate the time since discharge of cartridges through the use of more comprehensive analytical and interpretative techniques.

Following the comprehensive optimisation and validation of an exhaustive headspace sorptive extraction (HSSE) method in the first part of this work, the present paper addresses the application of chemometric tools in order to systematically evaluate the potential of applying headspace analysis to estimate the time since discharge of 9 mm cartridges. Several multivariate regression and pre-treatment methods were tested and compared to univariate models based on non-linear regression. Random forests (RF) and partial least squares (PLS) proceeded by pairwise log-ratios normalisation (PLR) showed the best results, and allowed to estimate time since discharge up to 48 h of ageing and to differentiate recently fired from older cartridges (e.g., less than 5 h compared to more than 1 – 2 days). The proposed multivariate approaches showed significant improvement compared to univariate models. The effects of storage conditions were also tested and results demonstrated that temperature, humidity and cartridge position should be taken into account when estimating the time since discharge.

E.1. Introduction

In the forensic investigation of firearms-related cases, law enforcement authorities often require evidence to link a firearm seized on a suspect to spent cartridges found at the crime scene. This generally involves mark comparisons between reference and indicial material in order to reveal common patterns between physical characteristics [1]. In some cases, however, the defence does not directly contest the source of the questioned spent cartridge, but rather its relevance, by arguing that it had been fired for legitimate reasons prior to the occurrence of the alleged crime [2, 3]. If such allegations are forwarded, estimating the time since discharge can be particularly useful in helping the justice with the decision-making process [4]. A promising approach towards achieving this is to monitor (over time) the decrease of selected volatile organic substances formed during the discharge, i.e. gunshot residue (GSR) [4-10]. GSR is a chemical trace that is very heterogeneous in composition, consisting mainly of metallic micro-particles, unburnt or partially burnt flakes of smokeless powder, and explosion products [11-14]. The latter are partly composed by volatile organic compounds (such as benzonitrile, naphthalene, and pyrene), which condense on the cartridge walls and disappear over time through evaporation and diffusion phenomena [15].

Solid phase micro-extraction (SPME) coupled to gas chromatography was previously proposed as a sampling method to analyse these residual products, while naphthalene and an unidentified decomposition product of nitrocellulose (referred as "TEA2") were suggested as main target analytes [5-8]. However, unsatisfactory results were obtained in the analysis of small-calibre firearms and cartridges. As reported in the first part of this article, in addition to the natural complexity of the specimens themselves, this problem could also be attributed to three additional factors: the extraction technique, the targeted compounds and the interpretation models. Thus, the present series of paper aimed to more systematically evaluate the potential of headspace analysis to provide helpful dating evidence through the use of alternative analytical and interpretative tools.

In the previous part, analytical questions were mainly addressed. In this regard, an alternative multi-residue approach based on headspace sorptive extraction (HSSE) was optimised, in order to get a more comprehensive overview of the ageing processes in spent cartridges. The specific objective of this second part was, therefore, to use this approach in order to extensively study the ageing of spent 9 mm cartridges, as well as to develop novel, more efficient solutions to comprehensively interpret the data in a dating perspective. In this regard, the implementation of multivariate statistical methods has been investigated instead of current one-compound-at-time approaches, in an attempt to implement all sources of information about time since discharge linked to the single compounds into a unique estimation model. Specifically, numerous 9 mm Parabellum cartridges were analysed at distinct times since discharge and ageing of the single GSR compounds was first studied using heteroscedastic non-linear regression (NLR) analysis. The impact of varying the cartridge storage conditions (i.e. cartridge position, temperature and humidity) was also investigated. Then, different pre-treatments and multivariate regression methods were evaluated and compared in their ability of estimating time since discharge from analytical data. Regression methods included partial least squares (PLS), multivariate adaptive regression splines (MARS), artificial neural networks (NN), random forests (RF), *k*-nearest neighbors (KNN) and support vector machines (SVM). Concerning pre-treatments, different strategies of normalisation, transformation and scaling methods were applied. These included, for example, total sum normalisation (TSN), probabilistic quotient normalisation (PQN) and pairwise log-ratios normalisation (PLR) as normalisation approaches, as well as logarithm (Log), square root (Sqrt), fourth root (4thr) and inverse (Inv) as transformations. The best multivariate models were selected and compared to compound-specific estimates obtained by NLR. Robustness of retained models to storage conditions was finally tested. As it was considered important to evaluate dating possibilities in conditions close to real cases, a casework perspective was generally adopted. Hence, training of models was performed using a limited number of reference cartridges and their validation was carried out on data obtained from different runs.

E.2. Material and methods

E.2.1. Chemicals

Five deuterated substances were used as internal standards, i.e. naphthalene-d8 (99%), biphenyl-d10 (98%), acenaphthene-d10 (99%), phenanthrene-d10 (98%) and pyrene-d10 (98%). All deuterated standards were purchased from Cambridge Isotope Laboratories (Tewksbury, USA). Stock solutions were prepared at a concentration of 1 mg mL^{-1} in dichloromethane (Sigma-Aldrich, Buchs, Switzerland). Finally, a mixed solution of 0.6 mg L^{-1} (per analyte) was prepared by successive dilution in methanol (Sigma-Aldrich) to serve as working solution.

Dataset name	***Cartridge position***	***Temperature [°C]***	***Relative humidity [%]***
STD1	Horizontal	25	75
STD2	Horizontal	25	75
VER	Vertical	25	75
T10	Horizontal	10	75
T40	Horizontal	40	75
H60	Horizontal	25	60
H90	Horizontal	25	90

Table E.1 *– Ageing conditions for the different experimental series.*

E.2.2 Ammunition and test shooting

All experiments were carried out on Geco 9 mm Parabellum ammunition (RUAG Ammotec, Thun, Switzerland) purchased in 2014. All cartridges belonged to the same production batch. Test shootings were carried out using a SIG Sauer P220 semi-automatic pistol in an indoor shooting range. Cartridges were fired by loading them into the magazine individually. The weapon was never greased during the experimental period.

After discharge, the cartridges were transported to the laboratory and aged in a conditioned oven maintained at pre-set temperature and humidity conditions (see Table E.1). For ageing in the vertical position, the cartridge were placed in a vial rack inside the oven, while ageing in the horizontal position was carried out by laying the cartridges on aluminium rails (purchased from a local hardware store). Ageing at 25 °C temperature, 75 % relative humidity (RH) and horizontal position were defined as “standard” conditions.

E.2.3. Sampling and extraction protocol

Cartridges were sampled, extracted and analysed using the analytical method optimised in the previous part of this work. Different specimens were aged for 1, 3, 5, 7, 24, 31, 48 and 72 h. After ageing, the cartridges were collected and placed into 20 mL HSSE-dedicated crimp glass vials. An extracted internal standard approach was adopted. 5 µL of the internal standard solutions at 0.6 mg L^{-1} were added to the bottom of the vials corresponding to a spiking amount of 3 ng per deuterated molecule. Then, special glass inserts were introduced to the headspace in order to accommodate the stir bars. Stir bars used for extraction were 1 cm in length with a 0.5 mm coating. These were thermo-conditioned before use in a Gerstel tube conditioner (TC) based on the protocol suggested by the manufacturer (30 min at room temperature followed by 90 min at 300 °C and approximately 60 min for cooling down). The vials were finally sealed with 20 mm crimp caps equipped with 3.0 mm PTFE/silicon septa. Cartridges were extracted in a laboratory oven at 70 °C for 24 h. For analysis, vials were re-opened and stir bars were transferred into pre-conditioned desorption tubes. The tubes were capped with special transportation adapters and placed on the GC tray for TD-GC-MS analysis. All materials were supplied by Gerstel (Sursee, Switzerland).

E.2.4. Thermal desorption – gas chromatography – mass spectrometry (TD-GC-MS) analysis

Stir bars were thermally desorbed in a Gerstel thermal desorption unit (TDU) on-line connected to a Gerstel CIS-4 programmed temperature vaporizing (PTV) injector. These devices were mounted on an Agilent 7890A gas chromatograph coupled to an Agilent 5975C mass selective detector (Agilent Technologies, Basel, Switzerland).

Desorption of stir bars were carried out in splitless mode with a flow of 60 mL min^{-1}. The desorption ramp was programmed as follows: 25°C for 0.5 min, ramped to 300 °C at 720 °C min^{-1} and held at this temperature for 5 min. The transfer line temperature between TDU and CIS-4 was set to 300 °C. Liners for CIS-4 were packed with quartz wool, provided by Gerstel. The cryo-focusing temperature was set to -50 °C. The PTV injection ramp was programmed as follows: -50 °C for 0.1 min, ramped to 300 °C at 720 °C min^{-1}, and held at this temperature for 1.37 min (total injection time: 2 min). Splitless mode was used during injection. The injector was then switch to split mode and a supplementary conditioning time of 7 min at 300 °C was programmed.

GC separation was performed on a HP-5MS column (30 m x 0.25 mm x 0.25 µm) from Agilent. The carrier gas was helium, and column flow was maintained at 1.2 mL min^{-1}. The oven ramp was programmed as follows: 40 °C for 2 min, ramped to 100 °C at 20 °C min^{-1}, ramped to 155 °C at 5 °C min^{-1}, and finally ramped to 250 °C at 12 °C min^{-1} (total chromatographic separation time of 23.9 min). Post-run temperature was set to 320 °C and held for 5 min with a column flow of 3 mL min^{-1}. The transfer line between the column and the MS was set to 280 °C. Ionisation was carried out through electron impact (EI). Selected ion monitoring (SIM) mode was employed for MS analysis. Solvent delay was set to 5.60 min. MS source and quadrupole temperatures were 230 °C and 150 °C, respectively. Detected analytes and respective monitored ions are reported in Electronic Supporting Material (ESM).

E.2.5. Tested regression approaches

Univariate regression analysis was used to study the ageing behaviours of single extracted analytes as well as to obtain compound-based estimates of the time since discharge. In this regard, inference on time since discharge through the interpretation of ageing profiles of single compounds has been suggested in the literature, but no statistical tool has ever specifically been proposed for (definitive) decision making. Since this, heteroscedastic non-linear regression analysis (NLR) has been adopted in this study as reference univariate approach as it previously proved to be an adequate statistical technique to model ageing profiles of volatile compounds in spent cases [4, 16]. For each analyte, the peak area corresponding to the target ion was extracted. Values were then subject to a two-step normalisation: first, their peak areas were divided by a corresponding deuterated standard (see ESM); second, they were divided by the mean of the values obtained at t = 0 h in order to globally scale down the start of the ageing of all analytes to a nominal value of 1. For fitting, the following equation was adopted:

$$S = \exp\left(- C \cdot \sqrt{t} \right) \qquad \text{Eq. E.1}$$

where S is the expected peak area mean, t is the time since discharge and C is the characteristic constant of the curve, which is related to the decrease rate. Heteroscedasticity was accounted for using a power-of-the-mean function. Other pre-treatments and fitting procedures were initially tested on data. Particularly, the fitting equation suggested in [16] (which includes two supplementary size constants) was applied on log-transformed data. However, this approach was ruled out because of fitting problems encountered for most of the resampled datasets. Moreover, estimates were missing for values falling beyond the minimal value of the regression curve. Scaling the data using the mean peak area of the respective compounds at t = 0 h (which in turn allowed for eliminating size constants) was found to be a reliable strategy in solving these problems (applicable to real cases).

Concerning multivariate modelling, six regression methods were tested on data, i.e., partial least squares (PLS), multivariate adaptive regression splines (MARS), artificial neural networks (NN), random forests (RF), *k*-nearest neighbors (KNN) and support vector machines (SVM). These were applied after data pre-treatment (see following section) in reverse regression mode [17]. Therefore, the outcome variable was the time since discharge and the predictors were the various volatile organic GSR compounds. Theoretical details of the assessed methods can be found in ESM.

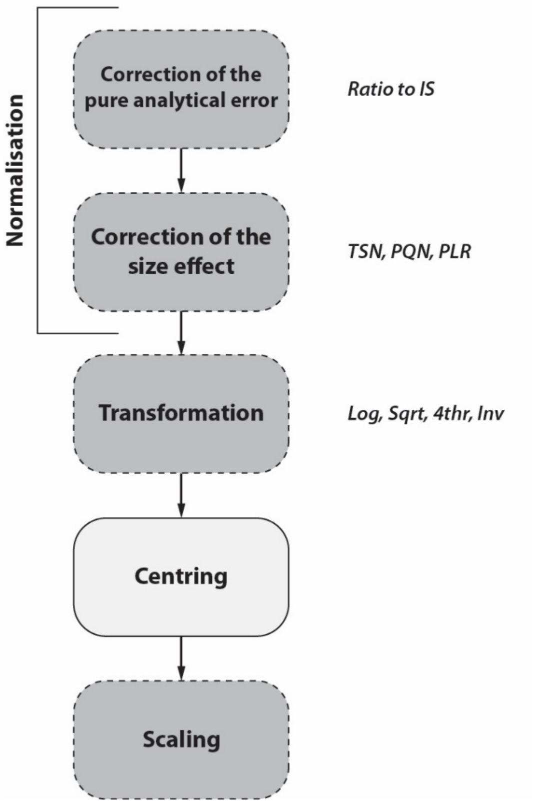


Figure E.1 *– Flowchart of adopted pre-treatment strategy for multivariate regression models.*

E.2.6. Pre-treatments before multivariate fitting

The impact of several combinations of pre-treatment methods were tested on the regression performances of multivariate models. These methods included different data normalisation, transformation and scaling approaches. For normalisation, methods for the correction of both, the pure analytical error and the size effect, were tested [18]. The application sequence of the various pre-treatment steps was kept constant in accordance with the following order: pure analytical variation correction, size effect correction, transformation, centring and scaling (Fig. E.1).

Dividing the peak areas of the target compounds by those of the corresponding co-extracted deuterated standards was investigated as a method for the correction of pure analytical error. Approaches tested for the correction of size effects included total sum normalisation (TSN), probabilistic quotient normalisation (PQN) and pairwise log-ratios normalisation (PLR). For TSN, the extracted peak areas of each target analyte were divided by their total sum within the same chromatogram, while for PQN, they were divided by a dilution quotient. This quotient was equal to the median value of ratios obtained by dividing the peak areas of single analytes in a selected chromatogram by their respective peak areas in a reference chromatogram acquired at t = 1 h (from the training dataset) [18, 19]. For PLR, all possible pairwise ratios between the peak areas of the different target analytes were calculated and then, their logarithm was determined in order to remove any variance asymmetry caused by inversion of the ratio order [1, 18]. This allowed for the creation of 210 log-ratios per chromatogram which led to excessively long training times for some regression methods, especially RF and NN. Thus, the number of log-ratios was reduced by selecting only those which presented an estimated Spearman correlation coefficient greater than 0.7 with the outcome variable (i.e., the time since discharge) across all the analyses of the training dataset. This allowed for reducing the number of log-ratios to about 60, depending on the dataset used for estimating correlation coefficients.

Applied transformations included logarithm (Log), square root (Sqrt), fourth root (4thr) and inverse transformation (Inv) [20, 21]. After PLR normalisation, only the logarithm transformation was used, for the reason explained above. Finally, scaling was performed by dividing the peak areas of each target analyte by their respective standard deviation (as estimated by the training dataset). Mean-centring of the variables was always performed before training, independently from scaling.

E.2.7. Model training and validation

Out of the 29 substances targeted with the developed method, 21 were chosen as predictors for training (see ESM). The three unaltered smokeless powder compounds (i.e., ethylcentralite, dibutyl phthalate and diphenylamine) were discarded because of low repeatability in their detected amounts [15]. Moreover, five explosion products (i.e., o- and m-tolunitrile, 1,3- and 1,4-dicyanobenzene, and isoquinoline) were also discarded due to co-elution issues compromising their detection and relative quantification (see part 1).

For all the different regression models applied in this work (univariate and multivariate), regression parameters were determined based on a training dataset by resampling using bootstrap (100 iterations) [22]. The datasets used for training (Table E.1) was dependent on the experiment. Particularly, the two datasets acquired at "standard" conditions (i.e., STD1 and STD2) were used for the initial selection of the best models and the successive study on datable ranges. The remainders were used to test the effect of storage conditions on both, compound ageing and regression performance. All multivariate regression methods used involved at least one tuning parameter (see ESM), which were optimised during the resampling procedure.

Estimation of the predictive performance of fitted models (i.e., validation) was carried out using different approaches depending on the experiment:

- Between-runs validation: in this approach, validation of a model was carried out mainly by predicting analyses from an alternative, but equivalent, dataset. Since only the ageing experiment at "standard" conditions was replicated, this approach was essentially used on STD datasets. Thus, models were trained on one of the two dataset and tested using the other. As STD datasets were composed of analyses acquired several months apart, between-runs validation also simulated the worst case scenario for which reference and questioned cartridges were analysed during different GC-MS runs.
- Within-run validation: for comparison purposes and validation of models acquired on non-replicated datasets (i.e., ageing experiments under different conditions), validation was carried out using a mixed leave-one-out cross-validation (LOOCV) and bootstrap approach. Thus, for a given dataset, each observation was recursively left out and a model was trained on the remainders using bootstrap. The excluded observations were then used for validation. This also represented the best case scenario where the reference and questioned cartridges were analysed during the same GC-MS run.

Initial model selection was carried out on the basis of the global estimation performance on the studied ageing interval. As a metric for global estimation performance, root-mean-square error (RMSE) was exploited [22]. An average RMSE was thus calculated as a result of fitting two equivalent models using between-runs validation on STD datasets (one per STD dataset). RMSE is a measure of the mean estimation error on the experimental interval. However, it does not form a specific idea about the actual estimation performance at the different times since discharge. Thus, further evaluation of the local performance was carried out by inspecting the calibration plots of the models (the plots of the estimated versus the actual times since discharge) and their inaccuracy plots (the plots of the inaccuracy versus the actual times since discharge). Particularly, inaccuracy was defined as the average of the absolute errors at a specific time since discharge [23]:

$$I_t = \frac{\sum|\hat{t} - t|}{n_t} \quad \text{Eq. E.2}$$

where I_t is the inaccuracy at the time t, $\hat{t}$ and t the estimated and actual times since discharge respectively, and n_t the number of observations. Given that inaccuracy was found to follow trends over time for most evaluated models, quadratic linear regression was applied to extrapolate mean values over all the studied ageing interval.^

E.2.8. Software and computing

Chromatograms were processed using the Enhanced Data Analysis software provided by Agilent. Regression methods were fitted using R statistical computing software and available packages (see ESM for details on used packages).

E.3. Results and discussion

E.3.1. Ageing of gunshot residue

Several 9 mm Parabellum cartridges from the brand Geco were analysed at various times since discharge using the HSSE-based method developed in part 1, after ageing at different storage conditions. From the analysis of chromatograms obtained at different times, it is already evident that most of the detected compounds decreased noticeably over time independently from the conditions applied (see examples in ESM). NLR was used in order to model the ageing of the various target analytes (see examples in Figure E.2a-d). It could thus be noticed that, while the decrease of each compound was generally rapid in the first hours since discharge, the actual rates seemed to be largely dependent on the compound’s volatility. In this regard, a negative correlation was usually observed between the *C* coefficients of the inferred ageing curves (which are strictly correlated to the compounds decrease rates) and the compounds boiling points (Fig. E.2a-d, as well as ESM). Consequently, amounts of the most volatile compounds dropped significantly within the first hour and quickly levelled-off, unlike those of the less volatile ones when the levelling-off occurred significantly later.

The different storage conditions were obtained by varying three factors: storage temperature, relative humidity and cartridge position. Their relative effects on decrease rates were investigated for each compound through ANOVA (Table E.2). It was thus noticed that the temperature had a very significant effect on the decrease of all studied compounds, and also that this was generally positively correlated to the decrease rates. Indeed, at low temperatures, disappearance of compounds was slower and vice-versa (Fig. E.3). Previous works reported similar trends for the decrease of volatile organic compounds found in barrels and other types of cartridges [5, 6].

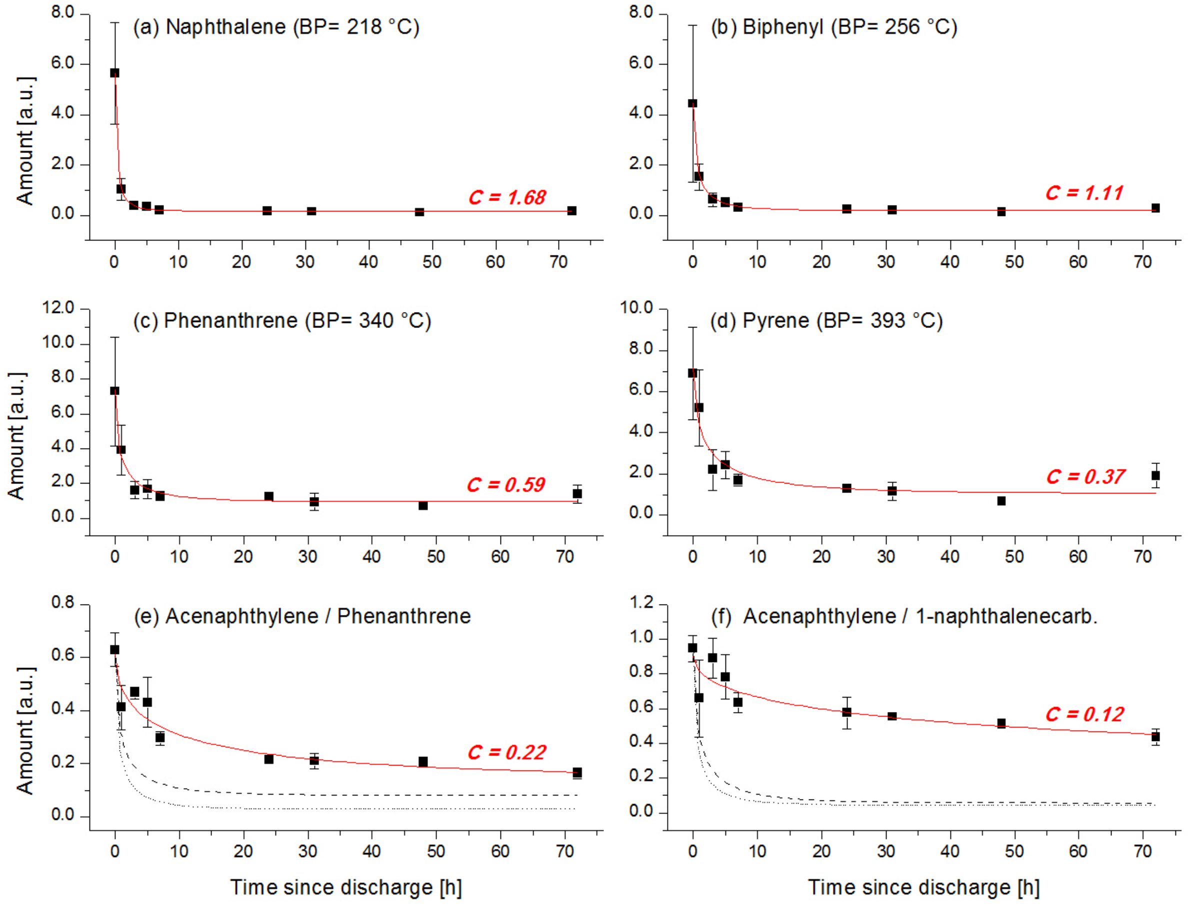


__Figure E.2__ – Examples of ageing curves for 4 selected target compounds and 2 compound-to-compound ratios: (a) naphthalene, (b) biphenyl, (c) phenanthrene, (d) pyrene, (e) acenaphthylene / phenanthrene ratio and (f) acenapthtylene / 1-naphthalenecarbonitrile ratio. "BP" is the boiling point and "C" is the decrease-rate coefficient. For the compound-to-compound ratios, dashed lines indicate ageing profiles for the respective composing molecules.

Though the effects of relative humidity and cartridge position were never previously reported, they were also found to have significant effects on the ageing of most compounds under investigation (Table E.2). Particularly, laying the cartridge horizontally rather than placing it vertically, globally, accelerated the decrease rates (Fig. E.3a-c). This may be due to an increase of the evaporation surface when cartridges were placed horizontally. Such an observation is of particular importance given that cartridges are more commonly encountered in a lying position throughout actual caseworks, and to find them standing is rare. Concerning relative humidity, its effect seemed significant only for the least volatile compounds, but not for the most volatile ones. Furthermore, the correlation between decrease rates and relative humidity in these cases was non-linear, where maximal decrease rates reached approximately 75 % RH for all the influenced compounds (Fig. E.3a-c).

Compound	***Cartridge position***		***Temperature***		***Relative humidity***	
	p-values	***Significative ($\alpha = 0.010$)***	***p-value***	***Significative ($\alpha = 0.010$)***	***p-values***	***Significative ($\alpha = 0.010$)***
Benzonitrile	0.001	X	<0.001	X	0.996	
p-Tolunitrile	<0.001	X	<0.001	X	0.480	
Naphthalene	<0.001	X	<0.001	X	0.333	
2-Methylnaphthalene	<0.001	X	<0.001	X	0.013	
1,2-Dicyanobenzene	0.005	X	<0.001	X	<0.001	X
Biphenyl	<0.001	X	<0.001	X	<0.001	X
Acenaphthene	<0.001	X	<0.001	X	<0.001	X
1-Naphthalenecarb.	<0.001	X	<0.001	X	<0.001	X
Phenanthrene	<0.001	X	<0.001	X	<0.001	X
Pyrene	0.002	X	<0.001	X	<0.001	X

Table E.2 *– P-values for ANOVA F-tests concerning the effect of varying storage conditions on compounds decrease rates (i.e., C coefficients). P-values near to 0 support the hypothesis that variations in the considered factor are significant on the decrease rate of the compound, while p-values close to 1 support the hypothesis of no significant effects.*

E.3.2. Selection of best multivariate models

Observation of the ageing tests showed different behaviours over time for the selected analytes, thus also supporting the hypothesis that different compounds could provide complementary evidences about the time since discharge. In order to exploit this situation, multivariate methods were applied. In this regard, different regression and pre-treatment strategies were tested. Particularly, six multivariate regression methods were applied (i.e., PLS, MARS, NN, RF, KNN and SVM) after pre-treatment of data using different combinations of normalisation, transformation and scaling approaches (Fig. E.1). This led to the fitting and evaluation of 384 different models (refer to ESM for details).

Screening of the multivariate models was carried out on cartridges aged at “standard” conditions on the basis of their root-mean-square error (RMSEs, i.e., the respective mean errors on the estimated times since discharge over the global experimental interval). Overall, between-runs RMSEs for all models ranged from 8.8 to 27.8 h with a median value of 13.0 h (see ESM). The medians for the specific regression methods did not significantly vary from this value, proving a certain coherence between the different regression techniques.

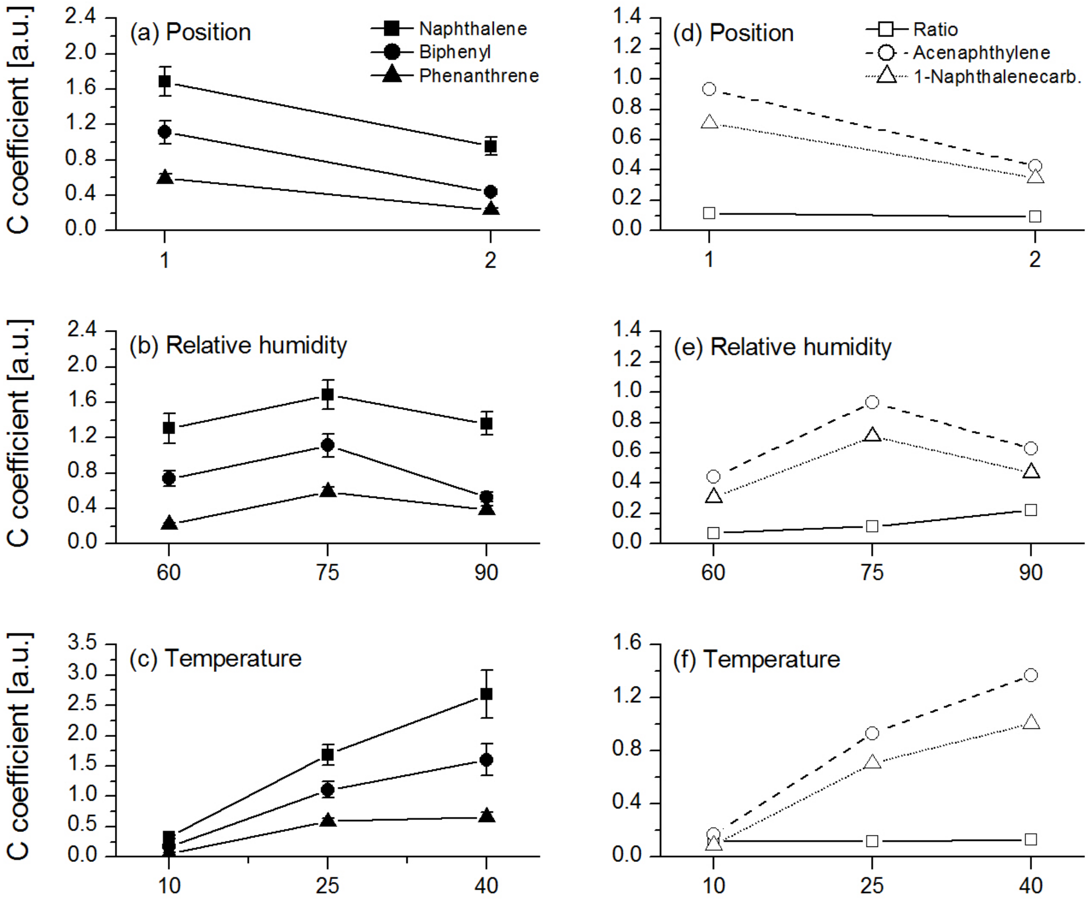


Figure E.3 *– Evolution of decrease rates (i.e., the C coefficients) of three selected molecules and the acenaphthylene / 1-naphthalenecarbonitrile ratio as a function of cartridge position, temperature, and relative humidity. For cartridge position, “1” and “2” indicate horizontal and vertical placement, respectively. For the ratio, the decrease rates of the composing molecules are equally shown for the sake of comparison.*

Despite that, specific combinations of pre-treatments and regression methods were nonetheless found to perform significantly better than others. Calibration plots for the models with lower RMSEs were inspected. Many of them (especially those based on KNN and NN) seemed presenting moderate symptoms of overfitting, which led to highly variable estimation accuracies over the experimental time range. That is, some data points were perfectly estimated, while others were affected by large estimation errors. In this regard, RF and PLS preceded by pairwise log-ratio normalisation (PLR) proved to be the most consistent (and efficient) combinations, as time-since-discharge estimates were more condensed around their local means in comparison to the other models (Fig. E.4a-d). Observed between-runs RMSEs were 10.1 and 10.6 h, respectively. Within-run RMSEs for these models, 9.8 and 12.6 h respectively, were not significantly different from between-runs RMSE values, indeed proving a good robustness with respect to data obtained from same or different runs.

E.3.3. Accuracy in the estimation

The RF and PLS models using PLR-normalised data were selected as optimal and exploited for evaluating the accuracy in time-since-discharge estimation. Table E.3 reports inaccuracy (i.e., the average magnitude of absolute error) obtained with these models over the experimental range (see also Figures E.4a-d). Using the RF model, inaccuracy values of 2.5, 6.5, 13.7 and 23.2 h were estimated for 5h-, 24h-, 48h- and 72h-old cartridges, which correspond to percentages of about 50, 27, 28 and 32 % in relation to the actual times since discharge, respectively. The PLS model performed worst at the shortest times since discharge, with an inaccuracy estimated to about 6.5 h across the entire 0 – 24 h range. For the sake of illustration, this corresponded to 126 % of relative error at 5 h since discharge.

Actual time [h]	***PLR-RF***		***PLR-PLS + scaling***		***NLR (acenaphthylene)***		***NLR (fluorene)***	
	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***
1	1.8	183 %	6.7	665 %	0.8	79 %	1.3	132 %
5	2.5	50 %	6.3	126 %	2.4	47 %	2.8	56 %
24	6.5	27 %	6.4	26 %	12.8	53 %	12.7	53 %
48	13.7	28 %	10.4	22 %	32.9	69 %	31.8	66 %
72	23.2	32 %	18.9	26 %	60.8	84 %	58.3	81 %

Table E.3 *– Estimated inaccuracy (It) and relative percentage to the actual time since discharge (%) for the best multivariate and univariate models over the experimental range. Inaccuracy values were approximated using a quadratic model.*

The larger average inaccuracy of the PLS model in comparison to the RF one for freshly fired cartridges was surely due to a wider spread of estimation errors obtained on the cartridges actually analysed in the first 0 to 24 h since discharge. This could in turn be attributed to statistical reasons, namely the difficulty of the PLS algorithm in correctly taking into account the considerable non-linear nature of the data in this initial ageing stage [22]. Despite that, a slightly lower average inaccuracy was observed for the PLS model in comparison to the RF one for > 48h-old cartridges.

In fact, inaccuracy values of 10.4 h and 18.9 h were estimated for 48h- and 72h-old cartridges, which correspond to 22 and 26 % of the actual times since discharge, respectively. In consequence, the two models appeared to be complementary to some extent, even if that based on RF was still slightly preferred because of its better average accuracy over the studied temporal range. In this regard, the obtained average accuracy was considered acceptable in view of the large random variations in such forensic data, which cannot be reduced by statistical means.

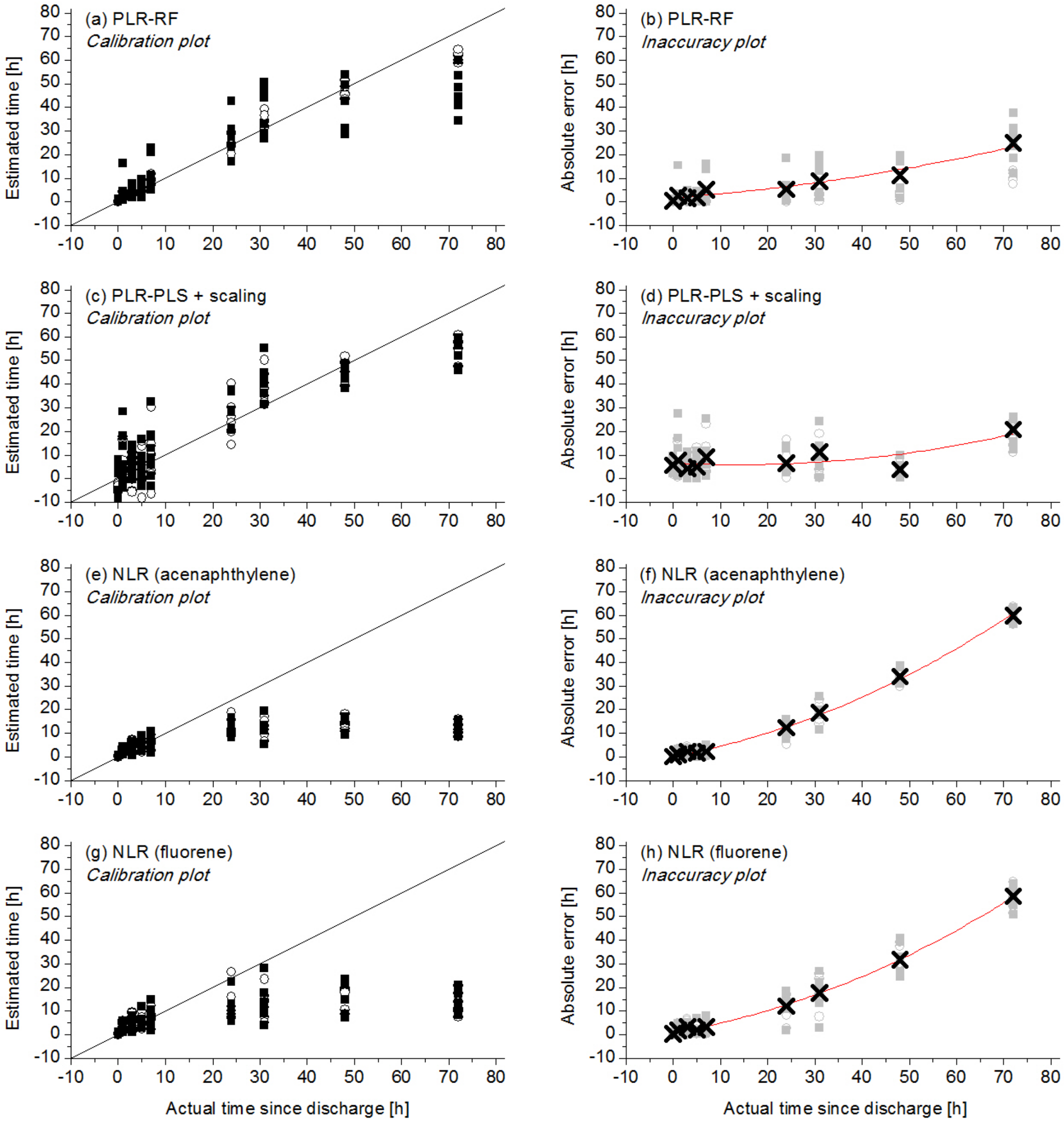


Figure E.4 *–Calibration and inaccuracy plots for the best multivariate and univariate regression models. For inaccuracy plots, cross-points represent the local means, while overall mean tendencies (approximated by quadratic regression) are represented by solid lines.*

It is noteworthy to mention that the local inaccuracy on the time-since-discharge estimates increased as a function of the actual cartridge age independently of the model applied. This trend led to systematic negative biases in estimates for cartridges older than 48 h for all the models, which are most probably due to the level-off in the amount of every volatile GSR compounds after this threshold and the subsequent instalment of a steady state in ageing profiles (Fig. E.2a-d). Improving estimation accuracy for > 24h-old cartridges was attempted by iteratively omitting data from 0h- to 7h-old cartridges during model training but, interestingly, yielded no significant improvements in observations. Maximal time limits estimable with acceptable accuracy were thus determined to be around 48 h, at least for the tested 9 mm Geco cartridges and adopted experimental methodology.

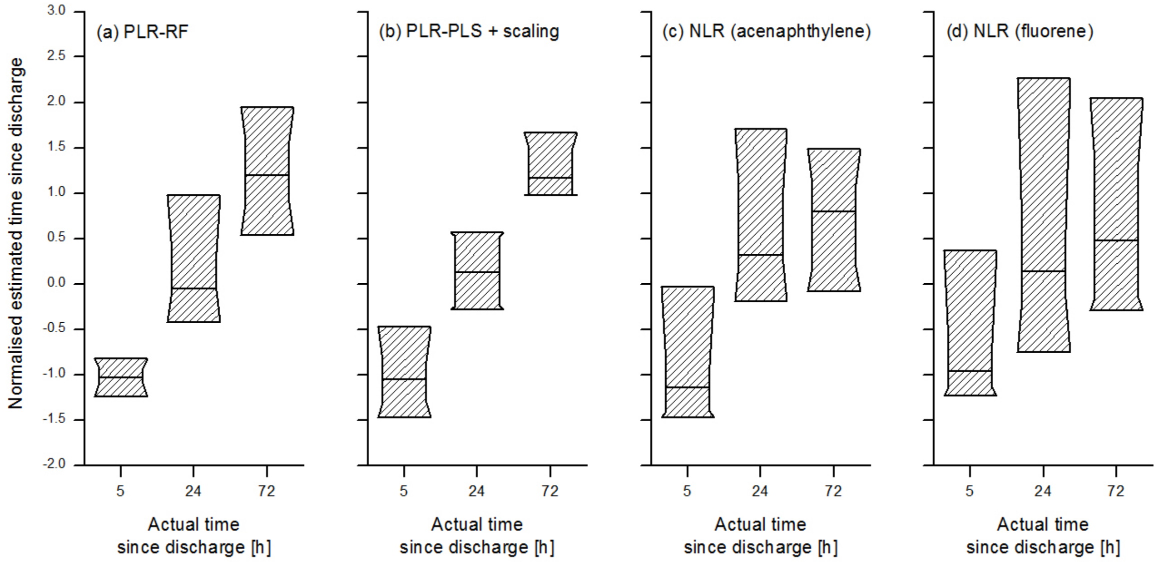


Figure E.5 *– Notched boxplots (10/25/75/90 percentiles) of the distribution of times since discharge estimated by the best multivariate and univariate regression models as a function of three actual cartridges ages. Estimates were centred and normalized to their standard deviation to improve visualisation.*

E.3.4. Differentiation of cartridges at different ageing stages

Even if time-since-discharge estimation attempted on cartridges older than 48 h were found to be significantly inaccurate, it is noteworthy that analyses performed at distinct ageing stages seemed to present differentiable distributions of estimates and thus also the potential to be mutually discriminated. For the sake of illustration, Figures E.5a-b show the distributions of the time-since-discharge estimates obtained through the RF and PLS models as a function of three actual cartridge ages (i.e., 5 h, 24 h and 72 h). As it can be noted, groups were generally separated. Particularly, 5h-old cartridges could easily be differentiated from 24h- and 72h-old ones, independently from the multivariate model considered. A slight overlap between results obtained on cartridges aged for 24 h and 72 h was observed for the RF model, while they were clearly differentiable for the PLS model.

Analysis of raw PLR-normalised data by principal components analysis (PCA), which is as unsupervised modelling method, gave similar findings (Fig. E.6). In fact, good separation between ages was achieved on PC2, after which further separation within age classes was achieved by additionally exploiting PC1. Thus, the results supported the hypothesis that analyses carried out on cartridges at different ageing stages actually convey sufficiently divergent chemical information to be discriminable from each other. More generally, they point toward the possibility of, at least, identifying recently fired from older cartridges even if an accurate estimation of the time since discharge is not more attainable. In this regard, it is interesting to note again the complementarity of the two tested chemometric techniques. Indeed, separation between cartridges at different ageing stages was slightly better with PLS than RF.

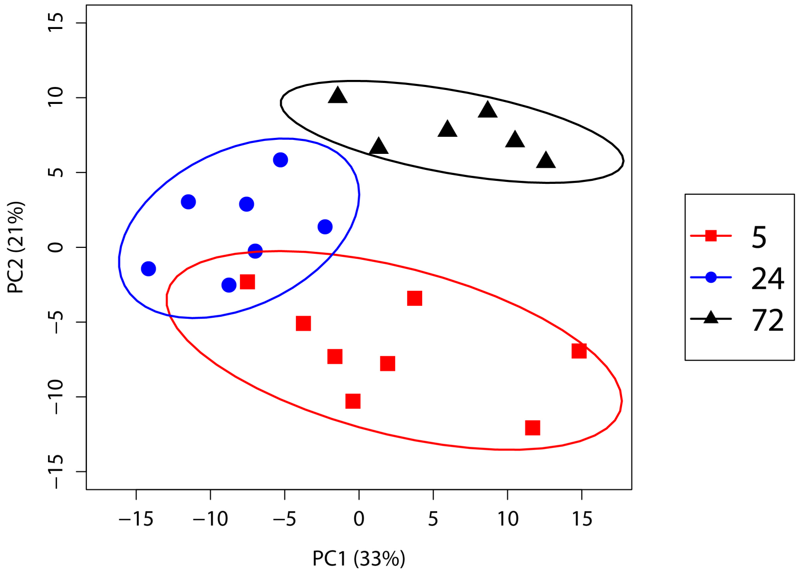


Figure E.6 *– Principal component analysis (PCA) of the two STD datasets after PLR normalization (all 210 ratios included). Explained variance of each principal component (PC) is reported in brackets.*

E.3.5. Comparison to univariate regression models

For the purpose of comparison, estimation accuracy obtained by the selected multivariate models was assessed against that achievable with traditional univariate regression approaches. In this regard, NLR was used as reference univariate method. Thus, between-runs RMSEs for all univariate NLR models ranged from 20.3 to 26.2 h (corresponding to pyrene and indene, respectively), while coefficients of determination (R^2) never surpassed 0.588 (the highest being acenaphthylene; see ESM for detailed performance characteristics). These metrics were considerably worse than those obtained from most multivariate models, consequently also denoting worse global regression performance.

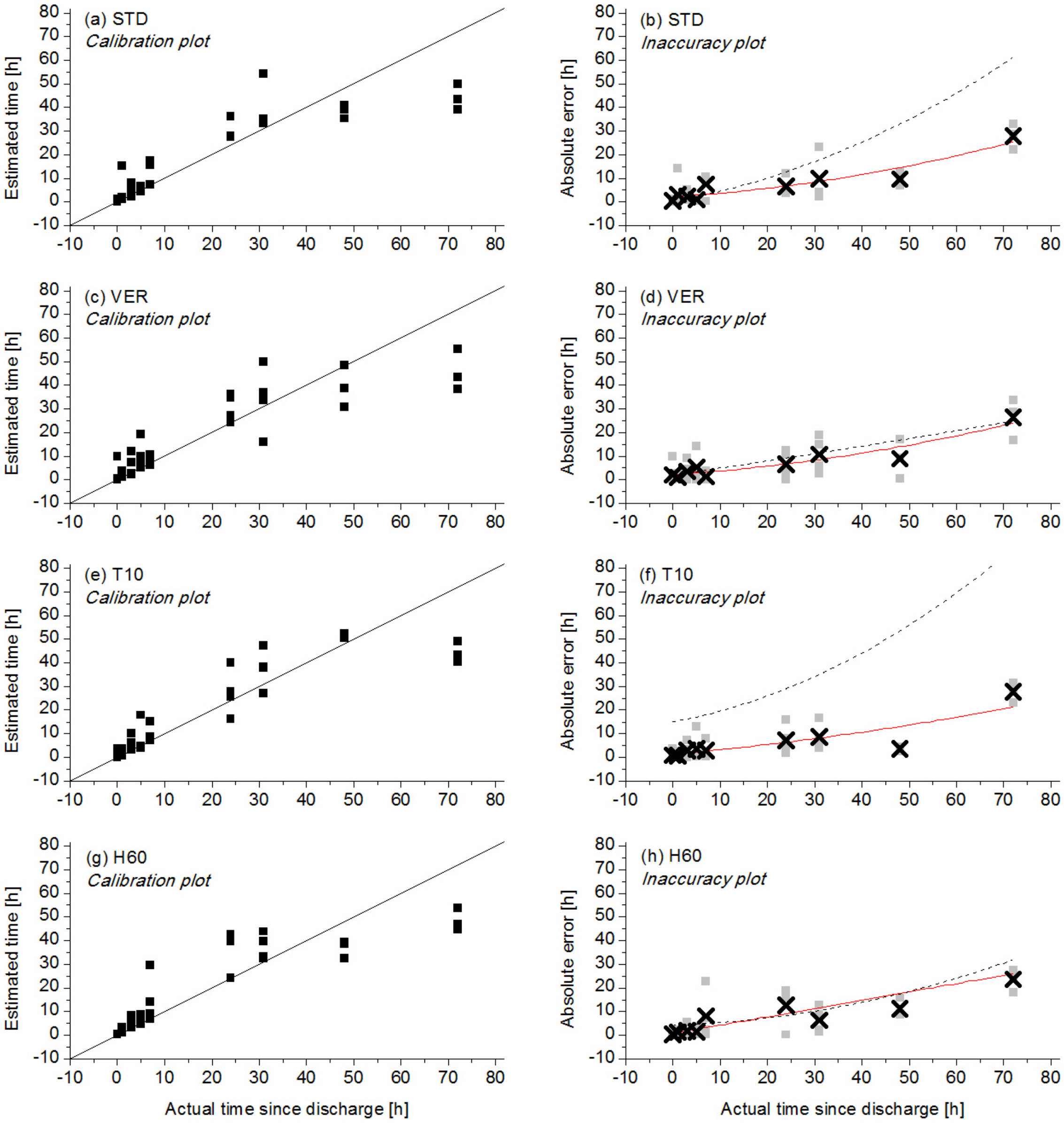


***Figure E.7** – Calibration and inaccuracy plots obtained with PLR-RF at different storage conditions in the case where the model was trained on reference cartridges aged at the same conditions as the questioned ones (refer to ESM for complete data). For inaccuracy plots, cross-points represent local means, while overall mean tendencies (approximated by quadratic regression) are represented by solid lines. Dashed lines represent overall mean inaccuracy for univariate NLR models solely trained on the acenaphthylene data.*

Inaccuracy profiles were investigated. Values for < 10h-old cartridges attained with the two univariate models considered the best in terms of overall fitting efficiency (i.e., acenaphthylene and fluorene) were in the same order of magnitude of those of the RF model. Despite that, average inaccuracy obtained on cartridges older than 10 h was significantly higher than those of both the PLS and RF models (Table E.3 and Fig. E.4e-h). Furthermore, the distribution of time-since-discharge estimates obtained on 5h-, 24h- and 72h-old cartridges showed stronger overlapping (Fig. E.5c-d). Therefore, results support the hypothesis that univariate approaches are less appropriate for gaining helpful evidence on the ageing state of spent cases in comparison to multivariate ones.

Consistent storage conditions

Actual time [h]	***STD***		***VER***		***T10***		***T40***		***H60***		***H90***	
	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***
1	2.3	230 %	2.3	231 %	1.6	155 %	1.5	147 %	1.0	102 %	1.6	155 %
5	2.9	58 %	2.9	58 %	2.3	46 %	2.1	42 %	2.4	49 %	3.0	61 %
24	6.8	28 %	6.7	28 %	6.4	27 %	5.8	24 %	9.1	38 %	10.2	42 %
48	14.5	30 %	14.0	29 %	13.1	27 %	12.2	25 %	17.6	37 %	19.1	40 %
72	25.4	35 %	24.0	33 %	21.3	30 %	20.5	28 %	26.1	36 %	28.1	39 %

Inconsistent storage conditions

Actual time [h]	***STD***		***VER***		***T10***		***T40***		***H60***		***H90***	
	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***	I_t ***[h]***	***%***
1	1.8	183 %	3.6	360 %	0.8	80 %	18.0	1804 %	0.5	52 %	2.5	254 %
5	2.5	50 %	4.1	83 %	3.2	63 %	18.3	366 %	1.8	36 %	3.9	78 %
24	6.5	27 %	7.8	32 %	16.5	69 %	19.7	82 %	8.4	35 %	10.3	43 %
48	13.7	28 %	15.1	31 %	38.2	80 %	21.3	44 %	18.0	37 %	18.5	38 %
72	23.2	32 %	25.4	35 %	65.3	91 %	23.0	32 %	29.0	40 %	26.6	37 %

Table E.4 *– Estimated inaccuracy (It) and relative percentage to the actual time since discharge (%) obtained with PLR-RF at different storage conditions, in the cases where the model was trained on reference cartridges aged at the same or different conditions as the questioned ones, respectively. In the case of inconsistent conditions, the reference model was trained on cartridges aged at "standard" conditions. Inaccuracy values were approximated using a quadratic model. Abbreviations for ageing conditions can be found in Table E.1.*

E.3.6. Robustness to storage conditions

As shown, decrease rates of volatile GSR compounds could be affected by different influential factors. Thus, a major concern was to determine if differences in estimation accuracy could equally be encountered between models which were trained using the same regression method but on cartridges aged at distinct storage conditions. This was tested by refitting the RF model on datasets from cartridges aged at distinct ageing circumstances, and by successively determining estimation accuracy on left-out observations.

No significant shifts in estimation accuracy were observed between all the trained models (Table E.4 and Fig. E.7). Obtained within-run RMSEs were indeed comparable in all the situations, and ranged from 8.6 to 13.4 h.

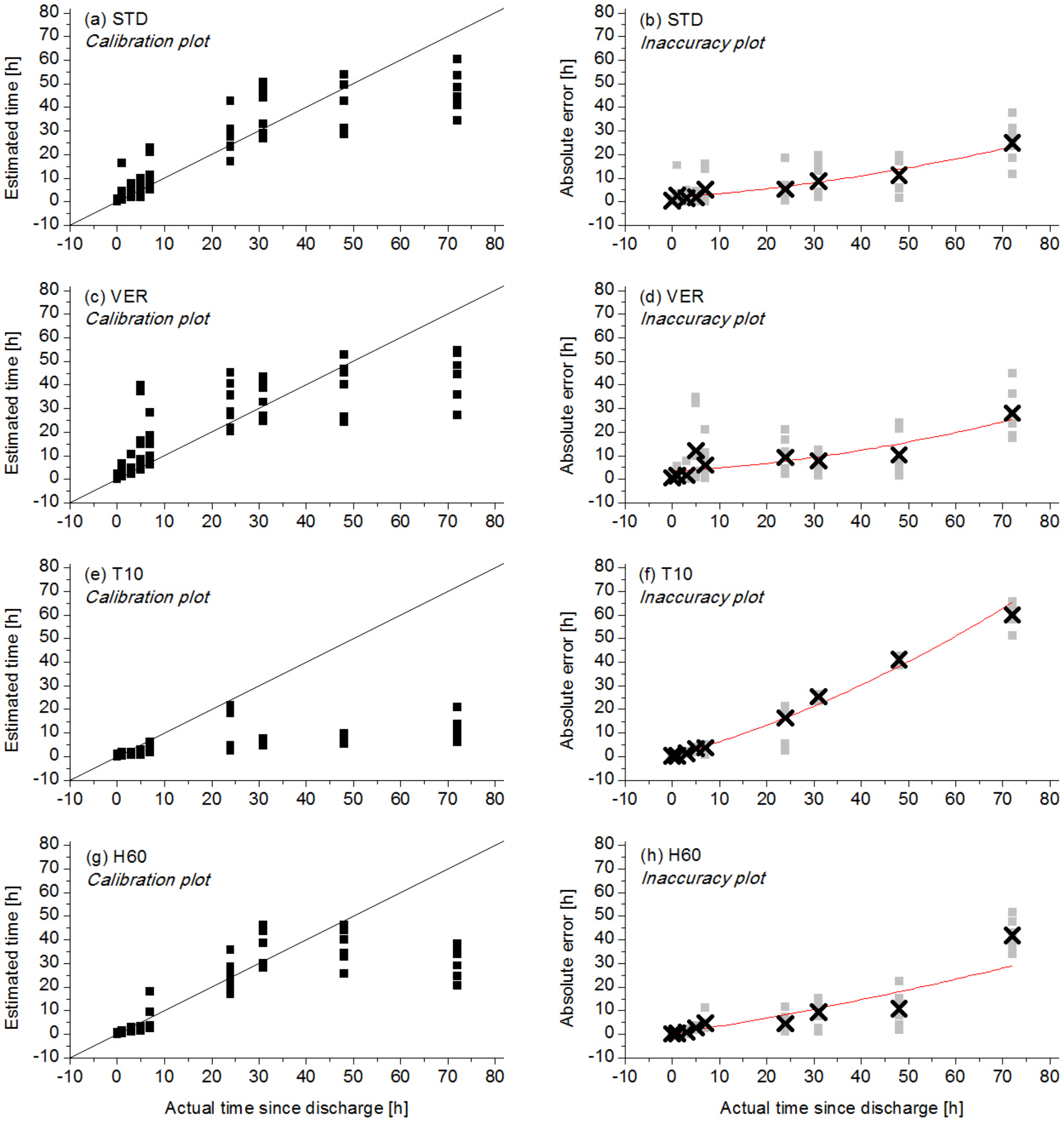


***Figure E.8** – Calibration and inaccuracy plots obtained by PLR-RF at different storage conditions in the specific case where the model was trained on reference cartridges aged at different conditions as the questioned ones (refer to ESM for complete data). Particularly, the reference model was trained on cartridges aged at "standard" conditions. For inaccuracy plots, cross-points represent local means, while overall mean tendencies (approximated by quadratic regression) are represented by solid lines.*

The inaccuracy profiles were also very similar, proving a good robustness of the selected RF approach at different storage conditions. In this regard, it should be noted that PLR normalisation could have had a significant role in preventing shifts in estimation accuracy, as the decrease rates of most ratios were found to be less affected by influential factors than their respective composing molecules (Fig. E.2e-f). The same tests were carried out on univariate NLR, but highly variable results were observed depending on the storage condition investigated (Fig. E.7). For example, using NLR trained on acenaphthylene data, RMSEs ranged from 11.8 h for cartridges stored in vertical position to 41.6 h for those stored at 10 °C.

E.3.7. Effect of differences between reference and questioned cartridges

Even if above results are definitely important for the development of a generalised interpretative approach, the main problem in real forensic cases lies in fact that the exact storage conditions encountered by a specific questioned cartridge on a crime scene are rarely known. Thus, differences between the storage conditions applied to reference materials and those actually persisting during the ageing of the questioned cartridges are very likely, making any statistical model trained on reference data not properly pertinent for time-since-discharge estimation in a particular case. The consequences of these situations were investigated by refitting the RF model on datasets from cartridges aged at distinct ageing circumstances, and by successively determining estimation accuracy on datasets from cartridges aged at different conditions.

Suboptimal accuracies were noted in many cases, especially when differences in storage temperature were introduced. Figure E.8 and Table E.4 show some examples. Thus, when time-since-discharge estimation was attempted on cartridges aged at 10 °C using a model trained with data acquired at 25 °C, outcomes were affected by strong inaccuracies which globally under-estimated the true values. In contrast, predicted times since discharge for cartridges aged at 40 °C (15 °C above the training dataset) were mostly over-estimated (mainly within the first hours of ageing). It could anyway be noted that consequences were more limited when differences in cartridge position and relative humidity were introduced.

ANOVA of the RMSEs estimated on all the validation datasets further confirmed these observations. Thus, very limited evidence to supporting the hypothesis that differences in cartridge position between the training and validation datasets are significant on the estimation accuracy of the models were provided ($p\text{-value} = 0.111$). On the contrary, results moderately supported the hypothesis that differences in the relative humidity can have an impact ($p\text{-value} = 0.021$), and strongly the hypothesis that differences in storage temperature have a significant effect ($p\text{-value} < 0.001$).

E.3.8. Relative importance of ratios on time-since-discharge estimation

The RF algorithm involves an internal scoring method which automatically rank predictors as a function of their relative impact on the fitting procedure. Results of this method has thus been used also to assess the importance of each ratio obtained through PLR normalisation for characterizing the ageing of spent cartridges. Of the 210 ratios obtained from all the possible pairwise combinations between retained analytes, a total of 8 ratios generated from different combinations of 8 target compounds were found to be particularly significant in every trained RF model. These were (in order of decreasing importance):

(1) acenaphtylene / phenanthrene,
(2) acenaphtylene / anthracene,
(3) acenaphtylene / acenaphtene,
(4) fluorene / anthracene,
(5) acenaphtylene / 1-naphthalenecarbonitrile,
(6) indole / fluoranthene,
(7) fluorene / phenanthrene,
(8) acenaphtylene / fluoranthene.

At a closer inspection of their ageing profiles, it could be remarked that all these ratios decreased over time similarly to the signals of the single composing molecules (see examples in Figure E.3d-f). Nonetheless, as already noted in a previous work [4], their decrease rates were often slower and yielded more linear ageing curves. This could partially explain the better regression performances averagely obtained after PLR normalisation in comparison to other pre-treatment approaches.

Concerning the single compounds, acenaphthylene appeared in 5 of 8 most influent ratios. Indole, phenanthrene, anthracene and fluoranthene were each involved in 2 ratios, while acenaphthene, 1-napthalenecarbonitrile and fluorene were each in only 1 ratio. It is interesting that all 8 substances are characterised by boiling points higher than 254 °C (the lowest being indole). Moreover, re-fitting the PLR-RF model using these compounds exclusively, yielded a mean between-run RMSE of 10.3 h, which was not significantly different from that obtained using the full-variables set (RMSE = 10.1 h). Therefore, even if different compounds provided complementary evidences on time since discharge, most of the pertinent information to estimate the time since discharge seemed to be gathered by the least volatile molecules. This may be explained by their slower evaporation and diffusion, allowing for a more comprehensive characterization of cartridge ageing. Even if strongly exploited in the literature, naphthalene was not highlighted as particularly significant for characterizing the ageing of the chosen 9 mm cartridges, probably because of its too rapid disappear in this kind of specimens which also makes it little informative for dating purposes.

E.4. Discussion of the results in a forensic perspective

The purpose of this work was to assess the potential of providing helpful evidence about the time since discharge of handgun cartridges through the analysis of their headspace. Findings showed that, on tested ammunition, inference of the time since discharge is actually feasible with sufficient accuracy up to 48 h of ageing, if storage conditions are known. While this might be considered a modest achievement, it should be noted that most firearm-related incidents are discovered and/or reported in the first hours after their commission, and the possibility of estimating the time since discharge up to this threshold might already be considered a valuable evidence for investigative purposes. Indeed, such information might help in reconstructing the course of events through situating a shooting in time or to allow identifying those specimens which are most likely to have been fired during the alleged offence (in order, for example, to recognise imported evidences and/or to focus only on pertinent specimens for further examination). In addition to this, results on tested ammunition were also promising on the possibility of discriminating dating stages in order to, at least, identifying cartridges which were not recently fired (i.e., in the last 48 h). This might equally be helpful in a legal perspective, as it would keep the possibility of differentiating between relevant and irrelevant cartridges at a crime scene and/or discriminating alternative hypotheses brought forth by the prosecution and the defence. For example, analysis of cartridges might help in discriminating the following hypotheses [16]:

- H_p: the questioned cartridge was shot during the commission of the crime, ca. 5 h before sampling.
- H_d: the cartridge was fired prior to the commission of the crime for a legitimate reason, more than 72h before sampling.

Nonetheless, accuracy in estimation was found to be dependent on the quality of the reference material, and especially on its degree of consistency in rapport to the questioned cartridges which should be dated. In this regard, using the same type of cartridges and applying the same storage conditions as those experienced by the questioned cartridges seems to be required at this stage of research. In fact, results showed that ageing profiles are significantly influenced by the storage temperature and, even if to a less extent, to the cartridge position and relative humidity. Additionally, composition/calibre of the cartridge might also provide significative influences, as previous researches showed a dependence between the GSR composition and type of ammunition

[15]. Air flows and light might also influence the results. These observations lead toward two important conclusions. Firstly, that a case-by-case approach should be adopted in interpreting data. Using general reference materials, storage conditions and/or interpretative models does not seem to be a reliable strategy. Secondly, that acquiring knowledge about the environmental conditions occurring at the crime scene before the questioned cartridge(s) is collected is mandatory. In this regard, it is acknowledged that a main source of problems in application to real cases lies in the fact that storage conditions experienced by questioned cartridges during their ageing are not always immediately inferable during crime scene investigation. Nonetheless, strategies can be applied in order to collect such information. Meteorological data and/or post-crime measurements could be helpful to the purpose. The experimental approach systematically used in forensic entomology could be considered and, eventually, adapted for fitting with the particular requirements of the forensic investigation of firearm-related crimes [24]. A climatic chamber can then be used to simulate specific storage conditions on reference material (e.g., temperature, relative humidity, airflow, light, position).

Concerning more generally the approach, it was noted that enhanced results could be obtained by simultaneously assessing all the available chemical information in a unique multivariate model instead of interpreting single compounds at time. This implies the use of a multi-residue analytical method. Being a relatively exhaustive extraction technique, HSSE was found very helpful in implementing this. However, it is acknowledged that it is invasive and, thus, a few practical technique for caseworks. For example, it does not allow analysing an enclosed specimen without re-opening the collection vial or implementing more complex capping solutions. In this regard, SPME would be more suitable thanks to its needle-based design. Further research should thus be carried out in order to evaluate the possibility of adapting a similar multi-residue approach to SPME, or of developing novel less invasive techniques.

E.5. Conclusions

In this paper, the potential of headspace analysis for estimating the time since discharge of spent 9 mm cartridges was investigated using chemometrics. Ageing kinetics of 29 GSR compounds was firstly studied. Most of them demonstrated a significant decrease over time, with generally rapid decrease in the first hours since discharge. Despite this, the actual decrease rates were dependent on the compounds respective boiling points, indicating that different compounds could provide complementary evidence about the time since discharge.

In an attempt to implement all these sources of information into a single time-since-discharge estimation model, 6 multivariate regression techniques were evaluated (i.e., PLS, MARS, NN, RF, KNN and SVM) and combined with different pre-treatment approaches (a total of 384 models were tested). Pairwise log-ratios normalisation (PLR) in combination to RF and PLS presented particularly good performance in comparison to other fitted models and univariate approaches. Particularly, the accuracy of the present outcomes demonstrated enhanced potential for estimating the time since discharge up to 48 h of ageing and/or to differentiate recently fired from older cartridges (e.g., less than 5 h compared to more than 1 – 2 days), under controlled storage conditions. The least volatile molecules, such as acenaphtylene, seemed to play an important role in the estimation performance of multivariate models. Effects of varying storage conditions between the training and validation datasets were also tested. While changes in cartridge position and relative humidity were found to be marginally significant, differences in temperature were prone to introduce more significant errors on the time-since-discharge estimates.

Consequently, obtained results showed that useful information for dating purposes could actually be extracted from the analysis of the volatile fraction of GSR in spent cartridges prior to forensic mark examination. In this regard, a high-capacity, multi-residue method, such as that proposed in this work, combined with appropriate chemometric tools provides more accurate results than any previous methodologies. However, some limitations must be taken into account. Particularly, a case-by-case approach should be applied for collecting and interpreting the data, and strategies to minimize differences in ageing conditions should be developed in actual caseworks. Further research should now focus on the implementation of the proposed approach in practice. This might require adapting a less invasive headspace extraction (applicable on-site by untrained personal). Other type of cartridges should also be investigated to evaluate the influence of ammunition on the results.

Acknowledgements

This work has been generously supported by the Swiss National Foundation (Grant no. PP00P1_123358). The authors would like to thank Prof. Pierre Esseiva and Emanuele Sironi for their valuable advice (University of Lausanne, CH), as well as Natalia Pawlowska (University of Lausanne, CH), Laurence Hamdan and Thomas Miller (King's College London, UK) for proofreading early drafts of the article.

Bibliography

1. Heard, B.J., *Handbook of firearms and ballistics*. 2nd ed. 2008, Hoboken, NJ: John Wiley & Sons.
2. Andersson, C. and J. Andrasko, *A novel application of time since the latest discharge of a shotgun in a suspect murder.* Journal of forensic sciences, 1999. **44**(1): p. 211-213.
3. Bridgemon, R.R., *Cartridge case aging.* AFTE Journal, 1986. **18**(1): p. 49-52.
4. Gallidabino, M., et al., *Development of a novel headspace sorptive extraction method to study the aging of volatile compounds in spent handgun cartridges.* Analytical Chemistry, 2014. **86**: p. 4471-4478.
5. Andrasko, J., T. Norberg, and S. Stahling, *Time since discharge of shotguns.* Journal of Forensic Sciences, 1998. **43**(5): p. 1005-1015.
6. Andrasko, J. and S. Stahling, *Time since discharge of spent cartridges.* Journal of Forensic Sciences, 1999. **44**(3): p. 487-495.
7. Andrasko, J. and S. Stahling, *Time since discharge of rifles.* Journal of Forensic Sciences, 2000. **45**(6): p. 1250-1255.
8. Andrasko, J. and S. Stahling, *Time since discharge of pistols and revolvers.* Journal of Forensic Sciences, 2003. **48**(2): p. 307-311.
9. Wilson, J.D., J.D. Tebow, and K.W. Moline, *Time since discharge of shotgun shells.* Journal of Forensic Sciences, 2003. **48**(6): p. 1298-1301.
10. Weyermann, C., et al., *Analysis of organic volatile residues in 9mm spent cartridges.* Forensic Science International, 2009. **186**: p. 29-35.
11. Chang, K.H., et al., *Gunshot residue analysis and its evidential values: a review.* Australian Journal of Forensic Sciences, 2013. **45**(1): p. 3-23.
12. Dalby, O., D. Butler, and J.W. Birkett, *Analysis of gunshot residue and associated materials - a review.* Journal of Forensic Sciences, 2010. **55**(4): p. 924-943.
13. Meng, H.-H. and B. Caddy, *Gunshot residue analysis - a review.* Journal of Forensic Sciences, 1997. **42**(4): p. 553-570.
14. Romolo, F.S. and P. Margot, *Identification of gunshot residue: a critical review.* Forensic Science International, 2001. **119**: p. 195-211.
15. Gallidabino, M., F.S. Romolo, and C. Weyermann, *Characterization of volatile organic gunshot residues in fired handgun cartridges by headspace sorptive extraction.* Analytical and Bioanalytical Chemistry, 2015. **407**(23): p. 7123-7134.

16. Gallidabino, M., et al., *Estimating the time since discharge of spent cartridges: a logical approach for interpreting the evidence.* Science & Justice, 2013. **53**(1): p. 41-48.
17. Miller, J.N. and J.C. Miller, *Statistics and chemometrics for analytical chemistry*. 5th ed. 2005, Harlow, UK: Pearson Education Limited.
18. Filzmoser, P. and B. Walczak, *What can go wrong at the data normalization step for identification of biomarkers?* Journal of Chromatography A, 2014. **1362**: p. 194-205.
19. Dieterle, F., et al., *Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures: application in ^{1}H NMR metabonomics.* Analytical Chemistry, 2006. **78**: p. 4281-4290.
20. Andersson, K., et al., *Development of a harmonised method for the profiling of amphetamine VI: evaluation of methods for the comparison of amphetamine.* Forensic Science International, 2007. **169**: p. 86-99.
21. Lociciro, S., et al., *Cocaine profiling for strategic intelligence, a cross-border project between France and Switzerland, part II: validaiton of the statistical methodology for the profiling of cocaine.* Forensic Science International, 2008. **177**: p. 199-206.
22. Gallidabino, M., F.S. Romolo, and C. Weyermann, *Time since discharge of 9 mm cartridges using headspace sorptive extraction (HSSE), part 1: comprehensive optimisation and method validation.* Submitted to Forensic Science International, 2016.
23. Tangmose, S., et al., *Age estimation in the living: transition analysis on developing third molars.* Forensic Science International, 2015. **257**: p. 512.e1-512.e7.
24. Wyss, C. and D. Cherix, *Traité d'entomologie forensique*. 2006, Lausanne, CH: PPUR.

APPENDIX F

Estimating the time since discharge of spent cartridges: a logical approach for interpreting the evidence

Published in:

Science & Justice, **2013**, 53(1), 41-48.

Authors: Gallidabino, M., Weyermann, C., Romolo, F. S., & Taroni, F.

F. Estimating the time since discharge of spent cartridges: a logical approach for interpreting the evidence

Abstract

Estimating the time since discharge of a spent cartridge or a firearm can be useful in criminal situations involving firearms. The analysis of volatile gunshot residue remaining after shooting using solid-phase microextraction (SPME) followed by gas chromatography (GC) was proposed to meet this objective. However, current interpretative models suffer from several conceptual drawbacks which render them inadequate to assess the evidential value of a given measurement. This paper aims to fill this gap by proposing a logical approach based on the assessment of likelihood ratios. A probabilistic model was thus developed and applied to a hypothetical scenario where alternative hypotheses about the discharge time of a spent cartridge found on a crime scene were forwarded. In order to estimate the parameters required to implement this solution, a non-linear regression model was proposed and applied to real published data. The proposed approach proved to be a valuable method for interpreting aging-related data.

F.1. Introduction

Determining the time since discharge of firearms or spent cartridges would be very useful in the forensic investigation of firearm-related cases [1, 2]. For this purpose, several methods were previously proposed in the literature. Simpler approaches focused on the evaluation of physical characteristics like the thickness of the rust or dust layer on the inner surface of firearm barrels [3-5]. Modern techniques, on the other hand, are based on the chemical analysis of the gaseous and volatile compounds composing the organic gunshot residue (GSR) [3-13].

The GSR is the residue formed during the discharge of a firearm. It is a complex and heterogeneous mixture composed of a variety of chemical species, the majority of which are gaseous and volatile products generated by the cartridge explosion [14, 15]. After the shot, these products stay mainly in the inner atmosphere of barrels and cartridges, and they quantitatively decrease over time due to physicochemical processes, such as diffusion through air and adsorption on metallic surfaces. Knowing that the residual quantity could be very informative for dating purposes, recent developments proposed to sample organic GSR compounds by solid phase microextraction (SPME) and analyze them using gas chromatography (GC) [10-13]. These methods showed promising results to follow compound diminution in a wide range of firearms and spent cartridges [1, 2, 16-19] and was even applied in casework [1, 2].

Although several works reported the analysis of organic GSR compounds for dating purposes, the issue of age inference from the obtained analytical results was only superficially addressed. In simple terms, SPME/GC analyses of barrels and cartridges provide qualitative and semi-quantitative data (in the form of chromatograms) about the compounds remaining in their inner atmosphere at the moment of extraction. Information about the time that has elapsed since discharge can be evaluated from some selected aging indicators such as the presence and/or the residual quantity of specific compounds (e.g.: naphthalene). In literature, the present trend is to incorporate these indicators in investigative frameworks and then infer temporal propositions about the discharge time. However, this typical approach suffers from several statistical and conceptual drawbacks.

The main objective of this paper is therefore to develop an innovative and reliable framework for assessing the evidential value of organic GSR analyses in discriminating between temporal propositions regarding the discharge. To reach this objective, a logical approach based on the assessment of likelihood ratios (LRs) was proposed as recently suggested by different authors [20-23], and its use to discriminate between competitive hypotheses on discharge time will be shown. The paper is organized as follows. Sections 2 and 3 will introduce the hypothetical scenario and the analytical background which will be the core of the subsequent discussion. In Section 4, current interpretative approaches will be discussed in more detail. Section 5 will present the proposed evaluative method based on the LR approach. The application of this model will be shown in Section 6. Empirical problems concerning the estimation of some relevant parameters needed for implementation will also be presented. Section 7 will develop statistical solutions to overcome these difficulties. Discussion and conclusion will be presented in Sections 8 and 9, respectively.

F.2. Hypothetical scenario

One evening in the woods, the body of a young man was found in a pool of blood with a gunshot-compatible wound. A spent cartridge (caliber 9mm Parabellum) was discovered close to the dead body. The autopsy estimated the time of death at about 8 hours before the discovery. The cause of death was a heavy hemorrhage due to the gunshot wound, and a bullet was extracted from the thorax. After some time, a suspect was arrested. A 9mm Parabellum pistol and some cartridges were seized at the suspect's apartment. According to the examination of the firearm experts, the observations carried out between the questioned and the comparison cartridge cases strongly support the hypothesis that the questioned cartridge was fired with the suspect's weapon rather than with another, unknown pistol; however, the bullet was too damaged, and no useful comparison could be undertaken. With regard to the results, the suspect did not deny that he fired the questioned cartridge; he claimed however that it was already at the scene due to a shooting game the morning before the discovery of the corpse (i.e., about 32 hours earlier).

F.3. Organic GSR analysis of the spent cartridge

In the situation presented, the main issue for the court is to determine if the cartridge discharge was or was not simultaneous with the commission of the crime so as to support or reject its relevance and, indirectly, the culpability of the suspect. In order to carry out useful analyses, we assume that, before sending the questioned cartridge to firearm experts, it was immediately sealed in a hermetic vial preventing gas escape [18, 19]. A single analysis was immediately performed in the laboratory using SPME/GC [11, 17]. The chromatogram of the extracted analytes yielded the quantitative data on several organic GSR compounds including naphthalene, a polycyclic aromatic hydrocarbon which is often produced by the incomplete combustion of gunpowder [2, 24-26] and previously proposed for dating purposes [2, 10, 11, 19]. The peak area of naphthalene was therefore selected as a suitable aging indicator, and a specific value (say q = 28.00 a.u.[1]) was observed. The question that should be asked now is: how can we use this result to help the court make a decision?

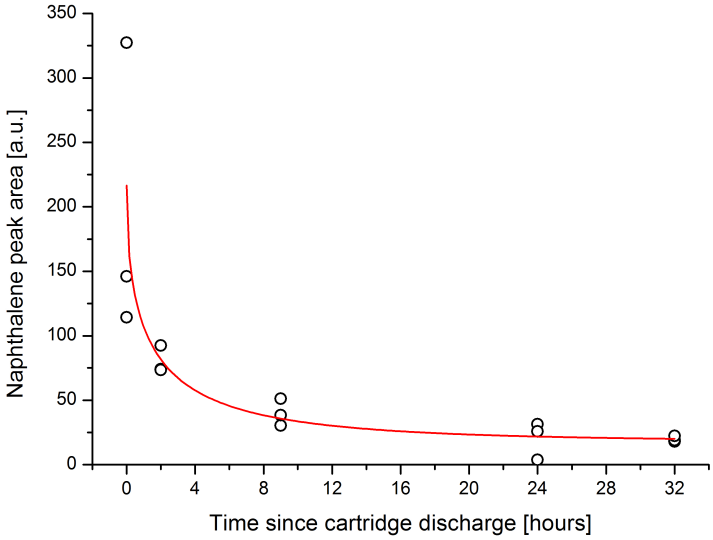


Figure F.1 *– Decrease of the naphthalene peak area measured through the SPME/GC analysis of some reference cartridges (data from Table F.1). The scale of the vertical axis is the chromatographic peak area divided by 1000. The central line represents the mean tendency curve.*

F.4. Current methodologies for the interpretation

In the literature on discharge dating, the interpretation of organic GSR analyses has always been treated as a comparative process in which the measurements on the questioned cartridge are

---

[1] In this work, peak areas are expressed as the absolute ion count divided by 1000. Units are thus arbitrary.

weighed against a reference calibration curve [2, 10, 16]. In this way, a particular observation can be correlated to a discharge time. The use of arbitrary pre-established thresholds was previously proposed in order to define intervals in which the real discharge time is most likely to have occurred [2, 10]. This solution was given mainly for dealing with the variability due to factors influencing the aging kinetics (i.e., the temperature). Considering the previous scenario (q = 28.00) and the hypothetical aging profile reported in Figure F.1, the calibration method would lead to the inference that "the cartridge discharge dates back to 13.7 hours before the discovery of the body", while the threshold method allows to conclude that "the discharge time is older than 8 hours".

However, these are considered inadequate from both a statistical and a conceptual point of view. Firstly, no work considered the measurement errors in their interpretative models. This is particularly problematic because the discharge time estimation is an inductive inferential process which is naturally uncertain [27, 28]. Secondly, some ambiguities still exist about the collection of the reference data. Although almost all the authors agreed that they should be acquired from case-related material (i.e., the same firearm/ammunition system stored in the same conditions as the one used for perpetuating the investigated crime), few solutions were proposed in case the relevant comparison material and/or sufficient circumstantial information about storage conditions are not available. In these situations the use of a "standard" set of data (i.e., a set of analyses performed on arbitrarily predetermined cartridges at laboratory conditions) is generally proposed with a "prudent interpretation" [2]. Finally, it should be noted that the proposed interpretative methodologies are actually investigative frameworks whose implicit purpose is to infer the best explanation from the observations on the questioned cartridge [29, 30]. However, information on the lapse of time since discharge are rarely used for investigative purposes: contextualizing the discharge on a time scale usually becomes an issue when the relevance of the evidence is contested by the suspect during his defense [20]. At this trial stage, different scenarios explaining the facts have already been formulated by the parties, and it would be of the greatest interest to test them rather than advance new propositions. An impartial approach may therefore be preferred [31].

F.5. A logical approach for interpreting analytical results

The LR-based logical approach has gained considerable importance in the interpretation of forensic data [32-34], and applications in firearm-related [35-42] as well as dating-related domains [20-23] have been reported. Under an LR-based interpretative framework, the role of the scientist is to assess the probability of a given evidential element under different alternative hypotheses: the ratio between these probabilities is known as the LR. From a conceptual point of view, this approach is thus a balanced, robust and transparent method for the assessment of the evidential value [31]. The LR is also a useful metric because it gives information about which hypothesis is supported by the observations on the questioned material as well as the force of this inference [32, 33].

The formulation of the hypotheses depends on the circumstances of the case. In this paper, we focus on the case where a suspect admits to having fired the questioned cartridge but he contests the proposed discharge time. In such a situation, two hypotheses about the course of the events (one from the prosecutor and one from the defense, named respectively T_p and T_d) can therefore be suggested as follows:

- T_p: the questioned cartridge was fired at the same time as the commission of the crime with the suspect's firearm and ammunitions.
- T_d: the questioned cartridge was fired prior to the commission of the crime with the suspect's firearm and ammunitions.

The expert's role is therefore to assess the probability of observing q (i.e., the naphthalene peak area observed on the questioned cartridge) given respectively the prosecutor and the defense hypotheses; the LR (defined with the letter V) is given by the ratio of these two likelihoods:

$$V = \frac{p(q|T_p)}{p(q|T_d)} \qquad \text{Eq. F.1}$$

If V is greater than 1, it can be said that the value q (based on analytical results) supports the prosecutor's hypothesis T_p. If V is smaller than 1, the evidence supports the defense proposition T_d. It should be noted that it is not necessary that one of the advanced propositions perfectly explains the measurement q: each probability composing the LR can assume values smaller than 1. In order to quantify V, the determination of the relative magnitude between numerator and denominator is thus sufficient [37].

Consider now that q is a particular observation of Q: the unknown quantity of naphthalene. This variable is continuous because q can assume any value between the limits delimited by the definition of the aging parameter. For the sake of simplicity, it is assumed that Q is normally distributed, so $Q \sim N(\mu;\sigma^2)$. Therefore, if the values of the distribution parameters (the mean μ and the variance σ^2) are known, the density for a given $Q = q$ is provided by the following density function:

$$f_Q(q|\mu,\sigma^2) = \frac{1}{\sqrt{2\pi\sigma^2}} \exp\left[-\frac{(q-\mu)^2}{2\sigma^2} \right] \qquad \text{Eq. F.2}$$

From a practical point of view, it should be noted that the more recent is the discharge time, the greater is the amount of organic GSR compounds remaining in the spent cartridge (and vice-versa for longer intervals). Consequently, the distribution parameters for Q depends on the hypothesis that has been put forth, and the formula in Equation F.1 can be substituted by the following definition [37, 43]:

$$V = \frac{f_Q(q|\mu_p,\sigma_p^2)}{f_Q(q|\mu_d,\sigma_d^2)} \qquad \text{Eq. F.3}$$

where μ_p, μ_d, σ_p^2 and σ_d^2 are the parameters characterizing the distribution of the chosen aging parameter under each of the two given propositions.

F.6. Estimation of the distribution parameters in an ideal situation

F.6.1. Parameter estimation

From a general point of view, the exact values of the different parameters which are needed to implement a specific probabilistic model are unknown, and their determination is the main practical problem. The easiest way is to estimate them from a set of reference data is through conventional frequentist methods [37, 40, 44, 45]. In this case, attention must be given to the fact that, by using point estimates of the true parameters, the obtained value for V shall also be treated as a point estimate of the likelihood ratio (hereafter, $\hat{V}$) [45, 46]. Considering the Equation F.3, two sets of estimates are needed in order to calculate $\hat{V}$, ($\hat{\mu}_p$ and $\hat{\sigma}_p^2$; $\hat{\mu}_d$ and $\hat{\sigma}_d^2$), both defining the distribution of Q under a given hypothesis. Two series of experiments can thus be planned with the reference firearm and ammunition[2]: the spent cartridges belonging to the two groups are then analyzed after the intervals defined by the propositions T_p and T_d, respectively. The estimates $\hat{\mu}_p$, $\hat{\mu}_d$, $\hat{\sigma}_p^2$ and $\hat{\sigma}_d^2$ are provided by determining the sample means and variances of the two groups of measurements.

F.6.2. Case scenario example

In the previous scenario, the victim's death occurred about 8 hours before the discovery of the body: consequently, assuming that the cartridge was sampled one hour after the discovery, the prosecutor's proposition would be that the suspect's firearm and ammunition were used to shoot the spent cartridge 9 hours before its seizure on the crime scene ($T_{p=9h}$). However, the suspect pretended that cartridge

---

[2] If hypotheses are formulated as above, it is very important to use the correct comparison material given that the cartridge batch and the employed firearm are supposed to be known. Anyway, it is acknowledged that it is not always so in real casework. Evaluation of the evidence when firearm and ammunition are treated as stochastic variables is not treated in this paper, but it is possible and will be discussed in future works.

discharge occurred 32 hours before the discovery of the corpse in a situation uncorrelated with the crime ($T_{d=32h}$). For the present discussion, we neglected the effect of the environmental conditions at the crime scene, as well as the uncertainty about the suspect testimony and the medico-legal conclusions[3]. Estimates for the distribution of Q under each hypothesis can thus be inferred from two series of shots (analyzed 9 hours and 32 hours, respectively, after the discharges of the cartridges) with the firearm and ammunition seized from the suspect's apartment. Considering that naphthalene peak area was selected as a reliable aging indicator, shooting experiences for the considered scenario are summarized in Table F.1 (data are provided by the work of Weyermann *et al.* on 9mm Parabellum ammunition [17]).

Time after discharge [h]	*Observed naphthalene peak area [a.u.]*			***Estimates [a.u.]***	
	Cart. #1	*Cart. #2*	*Cart. #3*	$\hat{\mu}$	$\hat{\sigma}^2$
0	327.26	114.23	146.10	**195.86**	**13202.42**
2	92.36	73.97	73.35	**79.89**	**116.68**
9	30.30	51.26	38.43	**40.00**	**111.70**
24	31.33	25.83	3.65	**20.27**	**214.66**
32	18.01	19.05	22.23	**19.76**	**4.85**

Table F.1 *– Naphthalene peak areas measured through the SPME/GC analysis of some reference cartridges at different times after discharge. These data are drawn from the work of Weyermann et al. [17]. Values represent integrated peak areas of the corresponding chromatographic peaks divided by 1000.*

Remember that the analysis of the questioned cartridge cases produced a naphthalene peak area of q = 28.00 a.u. It is possible to calculate the LR associated with this observation by using the function F.2 in Equation F.3 and the estimates calculated in Table F.1:

$$\hat{V} = \frac{f_Q\left(q = 28.00 | \hat{\mu}_{p=9h} = 40.00, \hat{\sigma}_{p=9h}^{2} = 111.70\right)}{f_Q\left(q = 28.00 | \hat{\mu}_{d=32h} = 19.76, \hat{\sigma}_{d=32h}^{2} = 4.85\right)} = \frac{1.98 \cdot 10^{-2}}{1.65 \cdot 10^{-4}} \approx 120 \qquad \text{Eq. F.4}$$

Given the measurements on the reference material, this result means that the naphthalene peak area q observed on the SPME/GC chromatogram of the questioned cartridge is estimated to be about 120 times more likely if the discharge occurred 9 hours before its sampling on the crime scene rather than if it occurred 32 hours before, thus supporting the prosecutor's hypothesis that the discharge of

---

[3] Clearly, these variables will represent additional sources of uncertainty which should be considered in the evaluation of the measurements in real cases. However, more research is actually needed in order to model their effects.

the questioned cartridge is approximately at the same time as the commission of the crime. It is important to note that a lower q value would have supported the defense proposition. For example, with q = 17.00 a.u., a $\hat{V}$ of about 0.043 is obtained, which indicates that the observation is approximately 23 times (=1/$\hat{V}$) more likely under $T_{d=32h}$ than under $T_{p=9h}$. Figure F.2 provides a graphical representation of the two estimated density distributions exploited in the assessment of the present scenario. It should be noted that, from a geometrical point of view, the $\hat{V}$ associated to a particular observation q actually corresponds to the relative height of the two curves at this value. In this case, it is moreover evident that, for q values greater than about 25.00 a.u., the height of the distribution of Q given $T_{p=9h}$ is always greater than its height given $T_{d=32h}$: thus, the prosecution's proposition is always supported with regard to the defense's alternative for $q > 25.00$ a.u.

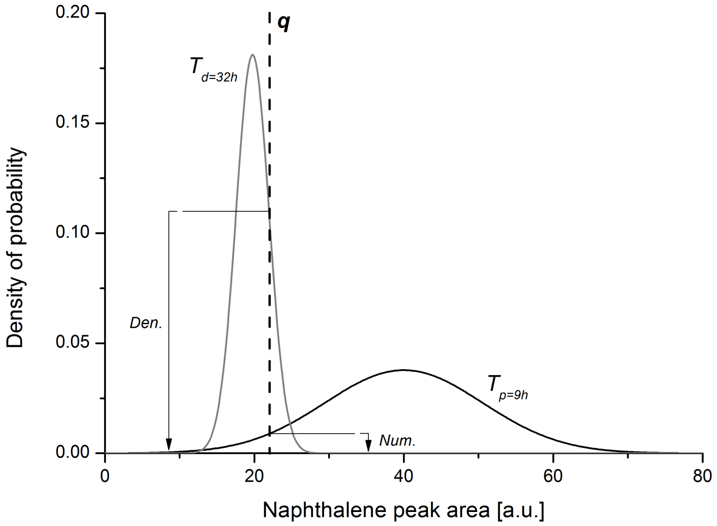


Figure F.2 *– Example of density distributions for the naphthalene peak area at different intervals after discharge. These distributions were inferred from the data summarized in Table F.1. From a geometric point of view, the estimated likelihood ratio ($\hat{V}$) associated to a particular measurement q is the ratio between the heights of the two curves at this value ($\hat{V}$ = num./den.).*

F.6.3. Practical issues

Estimating parameters from the direct analysis of comparison material at the given discharge times could be a good approach in ideal situations. However, two problems could arise in real cases. First, on the basis of some new pieces of information gathered during an intermediate investigative stage, it is possible that both parties change their explanations about the events, requiring a further evaluation of the evidence under new revised hypotheses. For instance, the validity of the lapse of time after the victim's death may be questioned, and the defense may ask to evaluate the measurements on the questioned cartridge under another prosecutor's proposition. Secondly, many environmental factors may influence the GSR aging kinetics in real cases (e.g., the temperature and the rate of air flow) and the actual state of these factors may be uncertain. Both of these issues would need further comparison shots and analyses to be conducted in order to estimate the distribution parameters for different intervals after discharge and/or different environmental conditions. However, one must also take into account the actual limitations of a real case:

- the case-related comparison material is never unlimited (i.e., comparison cartridges seized from the suspect may be insufficient to perform all the required analyses);
- the available time and money to produce expert opinion is also limited (i.e., manipulations shall be selected as a function of their relevance).

A statistical tool to manage these difficulties is therefore necessary. For this purpose, a parametric regression model is proposed in the following sections.

F.7. A regression model for the estimation of parameters

F.7.1. Parametric non-linear regression model

In general, a parametric regression model is a statistical tool which describes the relationship between two or more variables through parametric equations [47]. In its simple univariate form, the model is composed of a stochastic response variable (in this case, the selected aging parameter Q) and a non-stochastic predictor variable (the time interval, say t, after the discharge of the cartridge). Assuming a true regression relationship between these variables, it is presumed that the response Q is the sum of a systematic part (described by the mean response μ_t) and a random part (the measurement's random error ε_t on the mean response), whose magnitudes depend on t [48, 49]; formally:

$$Q = \mu_t + \varepsilon_t \qquad \text{Eq. F.5}$$

Assuming moreover that the error ε_t is normally distributed, that is $\varepsilon_t \sim N(0;\sigma_t^2)$, it is deductible that $Q \sim N(\mu_t;\sigma_t^2)$ for any time interval t after the discharge. Thus, using such a model, it is virtually possible to estimate the mean and the variance of Q at any t if the relationship between these variables is known.

The mean response μ_t and the variance of the measurement error σ_t^2 are two unspecified functions of t. Yet, the mean response μ_t can be approximated by a regression function $f(t,\theta)$ which depends on a series of regression parameters $\theta = \{\theta_1, \theta_2, \ldots, \theta_n\}$, so that $\mu_t = f(t,\theta)$. Regarding the variance of the measurement's error σ_t^2, it is generally assumed to be homogeneous throughout the considered domain of the predictor variable (a situation called homoscedasticity) [50]. Nevertheless, a preliminary observation of Table F.1 (which reports real data obtained from the SPME/GC analysis of spent cartridges) already offers contrary observations to this assumption. Thus, it is more rigorous to approximate σ_t^2 by a variance function $g(t,\theta,\tau)$ that depends on the generic parameters θ as well as on the specific regression parameters $\tau = \{\tau_1, \tau_2, \ldots, \tau_m\}$ (a situation called heteroscedasticity), so that $\sigma_t^2 = g(t,\theta,\tau)$ [50]. Regression parameters θ and τ involved in the definition of the model are still undetermined but they can be estimated on the basis of some analyses carried out on a set of comparison cartridges. The main difficulty lies in the fact that the functions $f(t,\theta)$ and $g(t,\theta,\tau)$ must be specified *a priori*.

The definition of an effective set of functions may depend on the chosen aging parameter. For the sake of demonstration, Figure F.1 shows the evolution of the naphthalene peak area as a function of time for a 9mm Parabellum ammunition. It is obvious that the relationship between Q and t is non-linear: in fact, the decrease of the peak area is particularly rapid in the first period after discharge, and then it becomes stable. On the basis of the published literature, this seems to be the case for all organic GSR compounds in a spent cartridge or firearm, regardless of the caliber [2, 10-13, 17, 19]. The disappearance of these compounds is a complex process, which involves different phenomena. The diffusion is however the largest contributor, and a reliable equation to approximate naphthalene decrease may be derived from the diffusion theory. Inspired by several works studying the diffusion of volatile molecules [23, 51-54], the following equation was used as a regression function:

$$f(t,\theta) = \theta_1 + \theta_2 \cdot e^{-\theta_3\sqrt{t}} \qquad \text{Eq. F.6}$$

where θ_1 and θ_2 are two size constants (for $t \rightarrow \infty$, θ_1 represents the minimal value of the considered aging parameter; for $t = 0$, the sum $\theta_1 + \theta_2$ represents its maximal value), and θ_3 is a characteristic curve constant which is proportional to the rate of decrease of the aging parameter. It should be noted that this function is intrinsically non-linear [47].

Furthermore, it could be observed from Figure F.1 that the measurement error fundamentally decreases over time, and this indicates the heteroscedasticity of the data. The literature reported similar trends for several organic GSR compounds in small gun cartridges [17]. For other situations however, the behaviors are unknown because of the general lack of error bars on the published aging profiles. For modeling variance inhomogeneity, a power-of-the-mean function is generally used [49, 50]:

$$\begin{aligned} g(t,\theta,\tau) &= \tau_1^2 \cdot (\mu_t)^{\tau_2} \\ &= \tau_1^2 \cdot f(t,\theta)^{\tau_2} \end{aligned} \qquad \text{Eq. F.7}$$

θ_1, θ_2, θ_3, τ_1 and τ_2 are parameters whose real values are unknown but estimable. Starting from a series of comparison shots carried out at different times after discharge (such as those represented in Figure F.1), the parameter estimation can easily be performed by the maximum likelihood method and a computerized iterative resolution algorithm [47, 49, 50].

Time after discharge [h]	*Hypothesis*	***Estimates [a.u.]***		*Densities*			
		$\hat{\mu}$	$\hat{\sigma}^2$	*q = 17.00 a.u.*	*q = 28.00 a.u.*	*q = 39.00 a.u.*	*q = 50.00 a.u.*
4	$T_{p=4h}$	**57.69**	**398.76**	2.50×10^{-3}	6.61×10^{-3}	1.29×10^{-2}	1.85×10^{-2}
9	$T_{p=9h}$	**35.73**	**150.24**	1.01×10^{-2}	2.67×10^{-2}	3.14×10^{-2}	1.65×10^{-2}
20	$T_{d=20h}$	**23.39**	**63.38**	3.63×10^{-2}	4.24×10^{-2}	7.32×10^{-3}	1.88×10^{-4}
32	$T_{d=32h}$	**20.04**	**46.30**	5.31×10^{-2}	2.96×10^{-2}	1.21×10^{-3}	3.62×10^{-6}

Table F.2 *– Interpolated estimates for the mean and variance of Q (i.e., the distribution of the naphthalene peak area) given different intervals after discharge. These estimates were obtained by applying the non-linear regression model F.8. The right side of the table shows the probability densities associated with some selected measurements q at the different intervals after discharge.*

Scenarios	***I***		***II***		***III***		***IV***	
	$T_{p=4h}$ **vs.** $T_{d=32h}$		$T_{p=9h}$ **vs.** $T_{d=32h}$		$T_{p=4h}$ **vs.** $T_{d=20h}$		$T_{p=9h}$ **vs.** $T_{d=20h}$	
Measurements	$\hat{V}$	$log_{10}\hat{V}$	$\hat{V}$	$log_{10}\hat{V}$	$\hat{V}$	$log_{10}\hat{V}$	$\hat{V}$	$log_{10}\hat{V}$
q = 17.00 a.u.	0.05	-1.33	0.19	-0.72	0.07	-1.16	0.28	-0.55
q = 28.00 a.u.	0.22	-0.65	0.90	-0.04	0.16	-0.81	0.63	-0.20
q = 39.00 a.u.	10.66	1.03	25.98	1.41	1.76	0.25	4.29	0.63
q = 50.00 a.u.	5124.79	3.71	4564.41	3.66	98.89	2.00	88.07	1.94

Table F.3 *– Estimated likelihood ratios ($\hat{V}$) and related logarithmic values associated with some selected measurements q under different scenarios. $\hat{V}$ was obtained by applying the Equation F.3 and the data in Table F.2.*

F.7.2. Case scenario example

To illustrate the utility of this statistical model, suppose that the investigators of the previous example were interested in the evaluation of the observed *q* with regard to several pairs of hypotheses. In fact, the hypotheses that the discharge occurred 4 hours and 20 hours before the discovery of the body were additionally forwarded by the parties after the presentation of new circumstantial information. Additional analyses were conducted with the available reference material at adequate times after the discharges, and the naphthalene peak areas were measured (Table F.1 and Figure F.1). The estimation of the regression parameters was performed using "R" statistical software and the above definitions. The following equations were obtained for the regression model $Q = \mu_t + \varepsilon_t$, where $\varepsilon_t \sim N(0;\sigma_t^2)$:

$$\begin{gathered} \hat{\mu}_t = 17.945 + 198.641 \cdot e^{-0.805\sqrt{t}} \\ \hat{\sigma}_t^2 = 0.321^2 \cdot \hat{\mu}_t^{2.037} \end{gathered} \qquad \text{Eq. F.8}$$

where t is expressed in hours. Although no analysis was performed at 4 hours and 20 hours after the discharge of the reference cartridges, using F.8 it is possible to interpolate distribution parameters for Q at those times. Table F.2 shows the interpolated estimates corresponding to all the hypotheses forwarded by the parties ($T_{p=4h}$, $T_{p=9h}$, $T_{d=20h}$, $T_{d=32h}$), as well as the corresponding densities for the previous measurement q = 28 a.u. carried out on the questioned cartridge. For the sake of illustration, other hypothetical measurements are also reported (q = 17.00 a.u., 39.00 a.u. and 50.00 a.u.). Table F.3 summarizes the $\hat{V}$ obtained through the analysis of different pairs of propositions (scenarios I to IV). We observe that, for a given measurement q, the magnitude of $\hat{V}$ clearly depends on the considered pair of hypotheses. Generally, greater discrimination is obtained for the propositions in scenario I ($T_{p=4h}$ vs. $T_{d=32h}$). This is normal considering that the estimated distributions for q given $T_{p=4h}$ and $T_{d=32h}$ are only slightly overlapping in comparison with other scenarios (Figure F.3) and thus less "similar". In fact, for the scenario where the distributions are the most overlapping (i.e., scenario IV, $T_{p=9h}$ vs. $T_{d=20h}$), the discrimination between hypotheses is globally weaker.

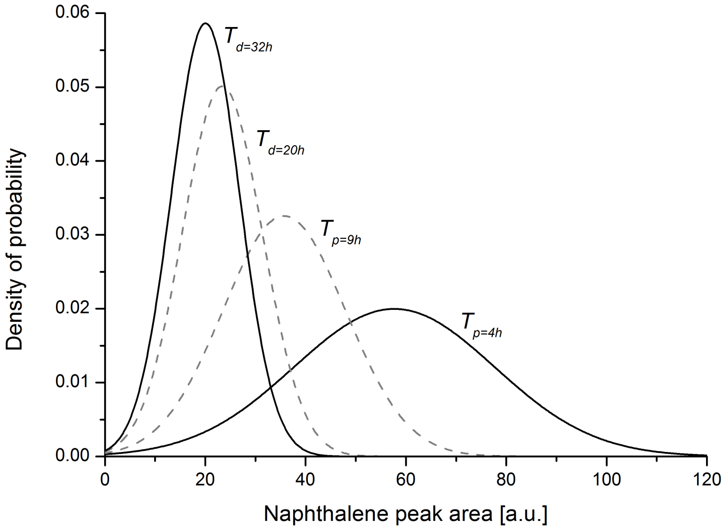


Figure F.3 *– Examples of density distributions for Q (i.e., the chromatographic peak area of naphthalene) estimated with the regression model F.8 at four intervals after discharge. It is evident that the distributions for* $T_{p=4h}$ *and* $T_{d=32h}$ *are less overlapped with respect to the distributions for* $T_{p=9h}$ *and* $T_{d=20h}$.

F.8. Discussion

The proposed approach allows the analysis of any scenario forwarded by the parties on the basis of the same set of reference data. This is possible because distribution parameters for the naphthalene peak area are estimated by the regression model. Thus, it is no longer necessary to perform specific analyses for any new hypothesis. However, it should be noted that distribution parameters at a specific time after discharge estimated with the regression model may not perfectly match the same parameters directly estimated from a group of measurements at the same time. For example, we can observe that the parameters estimated from three individual measurements carried out 9 hours after the discharge are $\hat{\mu} = 40.00$ and $\hat{\sigma}^2 = 111.70$ (Table F.1), while the same estimates calculated with the corresponding regression model are slightly different: $\hat{\mu} = 35.72$ and $\hat{\sigma}^2 = 150.49$ (Table F.2). Anyway, assuming that the regression functions are correctly specified, the latter values shall be considered more valid as a consequence of the fact that the model is estimated on the basis of a larger number of contributing measurements.

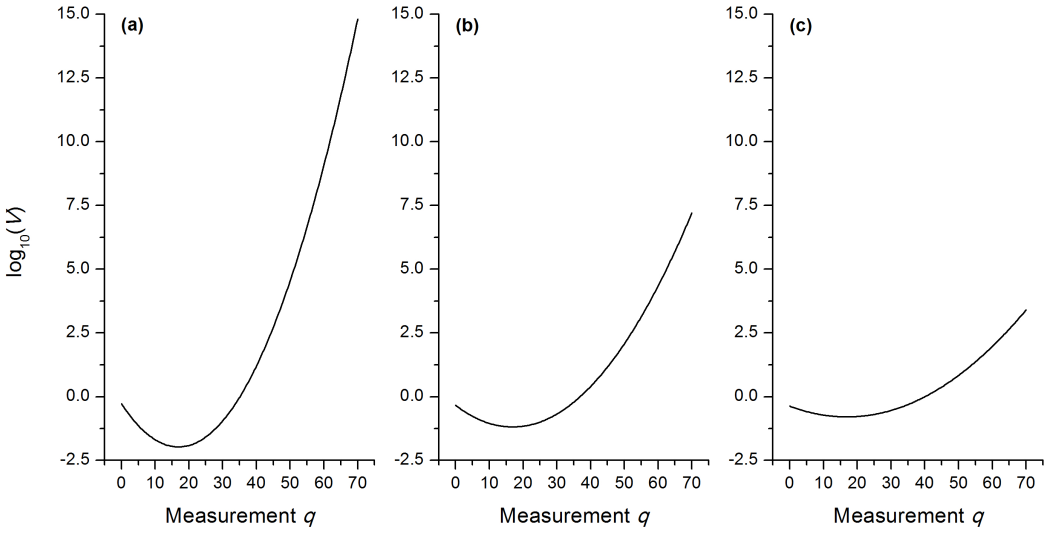


***Figure F.4** – Estimated likelihood ratio ($\hat{V}$, $T_{p=4h}$ vs. $T_{d=20h}$) as a function of different observed measurements q (in this case, the chromatographic peak area of naphthalene) assuming respectively a low (a), an average (b) and a high (c) expected variability of the observations. $\hat{V}$ is reported as its logarithmic value. These simulations were performed by modifying the estimated regression parameter τ_1^2 in the model F.8.*

By plotting $\hat{V}$ as a function of q, as in the different cases depicted in Figure F.4, other interesting observations could be drawn. Firstly, it should be noted that $\hat{V}$ always reaches a minimum value for which the evidence maximally support the defense hypothesis T_d. However, it never has a well-defined maximum, and very large values supporting the prosecutor's hypothesis T_p are theoretically possible. Secondly, the probative force associated to a large q value in favor of T_p is generally greater than the contribution of a small q value in favor of T_d. In fact, the increase of $\hat{V}$ over the neutral value of 1 ($\log\hat{V} = 0$) is more rapid than its decrease below this threshold (see also Table F.3). These observations are coherent since they reflect the intrinsic uncertainty about the weaker extracted quantities of organic GSR compounds. In fact, small q values have two reasonable explanations: a sufficiently long time has passed between discharge and analysis (the small extracted quantity is thus due to a true decrease of naphthalene) or the shot is recent but only a small quantity of compounds was produced (the small extracted quantity is due to a large deviation from the mean, which is still probable considering the large distributions of q for the shorter discharge times). On the contrary, large q values are fundamentally explained only by a short interval since discharge. This shows that an LR-based approach easily allows one to proportionally weigh all the possible explanations in the final result. Anyway, it should be pointed out that very high residual amounts of compounds are always very improbable under any hypothesis, and the probability of obtaining a large value for $\hat{V}$ is thus greatly limited.

A further investigation of the current model reveals that, for a given pair of propositions, the final magnitude of $\hat{V}$ mainly depends on the measurement q carried out on the questioned material (evidence characteristics), the expected aging profile of the selected indicator (mean tendency) and the expected variability of the observations (deviation from the mean). This last factor merits further discussion. In fact, it is demonstrable that a better discrimination between the given hypotheses is obtained for a smaller expected variability of the observations. Figure F.4 simulates an increasing variability of the observations, and the evidence value is obviously higher when the standard deviation is minimized. This factor thus has a large influence on the evaluation of the evidence. However, up to now, few works have shown error bars on the aging profiles, and even fewer performed systematic studies to establish what its real range is. This is because the variability of the observations is often assimilated to the measurement variance (i.e., the precision of the analytical method), which is merely perceived as a validation parameter unrelated to the interpretation of the evidence. In addition to the measurement variance, the variability of the GSR's initial composition also contributes to the total variability of the observations, and further studies are thus essential. Several replicas of the same reference analyses are generally needed to correctly assess the evidence in a particular case. It is also interesting to note that, while the expected variability of the observations is moderate (i.e., the analytical method is not very repeatable and/or the GSR's initial composition is highly variable), the given hypotheses could always be discriminated to a certain degree. A low variability is thus not necessarily needed to assist the court in its decision, even if they would allow maximizing the contribution of the physical evidence.

Finally, note that the considered case is a very simple scenario, merely elaborated to introduce the possibility of applying a LR-based perspective in the interpretation of dating-related data. There is no claim of generalization of the proposed model. In fact, real cases are generally more complex. Particularly, serious problems affect the evaluation of organic GSR compound analyses found in real casework, such as uncertainties about storage conditions and the circumstances surrounding the discharge, as well as the inaccessibility to relevant reference material. All of these factors actually constitute additional sources of uncertainty and were not addressed in this contribution. However, a further benefit of applying a probabilistic evaluative perspective is that all of these factors could be treated as additional stochastic variables and implemented in the model. Future works should consider this objective. Moreover, completely Bayesian inferential methodologies can be adopted instead of frequentist parametric estimation methods [46, 55] and this would be particularly useful to statistically learn parameters from previous experiments and cases [36, 56, 57]. Applications to other dating-related forensic fields should also be promising.

F.9. Conclusion

Estimating the time since the last discharge of a firearm or of a spent cartridge can be useful in specific situations. A novel, logical approach to interpret the data obtained by SPME/GC using likelihood ratios was thus proposed in this contribution. A probabilistic model was developed and applied to a hypothetical scenario where the discharge time of a questioned cartridge found on the crime scene was questioned.

The parameters needed for the implementation of the model can easily be estimated from comparison shots carried out with seized reference material. A regression model was proposed for interpolating such estimates on the basis of a limited number of comparison data. This solution is adapted to the constraints of real casework (i.e., the limited availability of comparison cartridges).

The proposed approach proved to be a valuable method for interpreting aging-related data, and further developments are promising.

Acknowledgements

This work has been kindly supported by the Swiss National Foundation (Fund no. PP00P1_123358/1). The authors would also like to thank Dr. A. Biedermann and S. Gittelson from the Institut de Police Scientifique (Lausanne, Switzerland) for their precious advices and the revision of early drafts of this paper.

Bibliography

1. Andersson, C. and J. Andrasko, *A novel application of time since the latest discharge of a shotgun in a suspect murder.* Journal of Forensic Sciences, 1999. **44**(1): p. 211-213.
2. Persin, B., et al., *Évaluation de la date d'un tir.* Canadian Society of Forensic Science Journal, 2007. **40**(2): p. 65-85.
3. Meier, J., *Détermination du temps écoulé depuis le tir.* Revue Internationale de Police Criminelle, 1970. **234**: p. 22-26.
4. Sokol, J., *Die Bestimmung des Zeitpunktes, wann eine Waffe das letztemal beschossen und eine Patrone verfeuert wurde*. 1931, Bern, CH: Buchdruckerei Friedli & Co.
5. Lucas, A., *Forensic chemistry and scientific criminal investigation*. 3th ed. 1935, London: Edward Arnold & Co.
6. Price, G., *Recent advances in ballistics laboratory methods.* Journal of Forensic Science Society, 1968. **8**(2): p. 83-90.
7. Sinha, J.K., *Time of firing of shot shells.* Journal of Forensic Sciences, 1976. **21**(1): p. 171-175.
8. Voskertchian, G.P. and G.V. Pavilova, *Spectrophotometric determination of time since discharge of firearms.* AFTE Journal, 1994. **26**(3): p. 216-222.
9. Voskertchian, G.P. and G.V. Pavilova, *Spectrophotometric determination of time since discharge of firearms.* Crime Laboratory Digest, 1995. **22**(1): p. 4-10.
10. Andrasko, J., T. Norberg, and S. Stahling, *Time since discharge of shotguns.* Journal of Forensic Sciences, 1998. **43**(5): p. 1005-1015.
11. Andrasko, J. and S. Stahling, *Time since discharge of spent cartridges.* Journal of Forensic Sciences, 1999. **44**(3): p. 487-495.
12. Andrasko, J. and S. Stahling, *Time since discharge of rifles.* Journal of Forensic Sciences, 2000. **45**(6): p. 1250-1255.
13. Andrasko, J. and S. Stahling, *Time since discharge of pistols and revolvers.* Journal of Forensic Sciences, 2003. **48**(2): p. 307-311.
14. Wallace, J.S., *Chemical analysis of firearms, ammunition, and gunshot residue*. 2008, Boca Raton, FL: CRC Press.

15. Rowe, W.F., *Residues*, in *Encyclopedia of forensic sciences*, J.A. Siegel, P.J. Saukko, and G.C. Knupfer, Editors. 2000, Academic Press: San Diego, CA.
16. Rigal, O., *Analyse des résidus de tir par arme à feu en SPME GC/MS: caractérisation d'un tir*, 2007, University of Paris-Sud.
17. Weyermann, C., et al., *Analysis of organic volatile residues in 9mm spent cartridges.* Forensic Science International, 2009. **186**: p. 29-35.
18. Belaud, V., *Identification par SPME-GC-MS des résidus de tirs volatils retrouvés dans les douilles: étude du vieillissement de composés cibles*, 2008, University of Lausanne.
19. Wilson, J.D., J.D. Tebow, and K.W. Moline, *Time since discharge of shotgun shells.* Journal of Forensic Sciences, 2003. **48**(6): p. 1298-1301.
20. Margot, P., *A question of time.* Science & Justice, 2000. **40**(2): p. 64-71.
21. Weyermann, C., *Dating: document*, in *Wiley encyclopedia of forensic science*, A. Jamieson and A. Moenssens, Editors. 2009, John Wiley & Sons: Chichester, UK.
22. Weyermann, C., B. Schiffer, and P. Margot, *A logical framework to ballpoint ink dating interpretation.* Science & Justice, 2008. **48**: p. 118-125.
23. Weyermann, C., et al., *Minimum requirements for application of ink dating methods based on solvent analysis in casework.* Forensic Science International, 2011. **210**(1-3): p. 52-62.
24. Cropek, D.M., et al., *Use of pyrolysis GC/MS for predicting emission byproducts from the incineration of double-base propellant.* Environmental Science & Technology, 2002. **36**: p. 4346-4351.
25. Cropek, D.M., P.A. Kemme, and J.M. Day, *Pyrolytic decomposition studies of AA2, a double-base propellant*, 2001, US Army Corps of Engineers.
26. Cropek, D.M., P.A. Kemme, and J.M. Day, *Incineration by-products of AA2, NC fines, and NG slums*, 2001, US Army Corps of Engineers.
27. Evett, I.W., *Expert evidence and forensic misconception of the nature of exact science.* Science & Justice, 1996. **36**(2): p. 118-122.
28. Stoney, D.A., *What made us ever think we could individualize using statistics?* Journal of Forensic Science Society, 1991. **31**(2): p. 197-199.
29. Jackson, G., et al., *The nature of forensic science opinion - a possible framework to guide thinking and practice in investigations and in court proceedings.* Science & Justice, 2006. **46**(1): p. 33-44.

30. Evett, I.W., G. Jackson, and J.A. Lambert, *More on the hierarchy of propositions: exploring the distinction between explanations and propositions.* Science & Justice, 2000. **40**(1): p. 3-10.

31. Jackson, G., *The scientist and the scales of justice.* Science & Justice, 2000. **40**(2): p. 81-85.

32. Aitken, C.G.G. and F. Taroni, *Statistics and the evaluation of evidence for forensic scientists*. 2nd ed. 2004, Hoboken, NJ: John Wiley & Sons.

33. Robertson, B. and G.A. Vignaux, *Interpreting evidence: evaluating forensic science in the courtroom*. 1995, Chichester, UK: John Wiley & Sons.

34. Taroni, F., et al., *Bayesian networks and probabilistic inference in forensic science*. 2006, Hoboken, NJ: John Wiley & Sons.

35. Biedermann, A., S. Bozza, and F. Taroni, *Probabilistic evidential assessment of gunshot residue particle evidence, part 1: likelihood ratio calculation and case pre-assessment using Bayesian networks.* Forensic Science International, 2009. **191**: p. 24-35.

36. Biedermann, A., S. Bozza, and F. Taroni, *Probabilistic evidential assessment of gunshot residue particle evidence, part 2: Bayesian parameter estimation for experimental count data.* Forensic Science International, 2010. **206**(1-3): p. 103-110.

37. Biedermann, A. and F. Taroni, *A probabilistic approach to the joint evaluation of firearm evidence and gunshot residues.* Forensic Science International, 2006. **163**: p. 18-33.

38. Buckleton, J.S., et al., *An exploratory Bayesian model for firearm and tool mark interpretation.* AFTE Journal, 2005. **37**(4): p. 352-359.

39. Champod, C., et al., *Firearm and tool marks identification: the Bayesian approach.* AFTE Journal, 2003. **35**(3): p. 307-316.

40. Cardinetti, B., et al., *A proposal for statistical evaluation of the detection of gunshot residues on a suspect.* Scanning, 2006. **28**: p. 142-147.

41. Bunch, S.G., *Consecutive matching striation criteria: a general critique.* Journal of Forensic Sciences, 2000. **45**(5): p. 955-962.

42. Romolo, F.S. and P. Margot, *Identification of gunshot residue: a critical review.* Forensic Science International, 2001. **119**: p. 195-211.

43. Royall, R., *On the probability of observing misleading statistical evidence.* Journal of the American Statistical Association, 2000. **95**(451): p. 760-768.

44. Evett, I.W., *A Bayesian approach to the problem of interpreting glass evidence in forensic science casework.* Journal of Forensic Science Society, 1986. **26**: p. 3-18.

45. Nordgaard, A. and T. Höglund, *Assessment of approximate likelihood ratios from continuous distributions: a case study of digital camera identification.* Journal of Forensic Sciences, 2011. **56**(2): p. 390-402.
46. Taroni, F., et al., *Data analysis in forensic science: a Bayesian decision perspective*. 2010, Chichester, UK: John Wiley & Sons.
47. Draper, N.R. and H. Smith, *Applied regression analysis*. 3rd ed. 1998, New York: John Wiley & Sons.
48. Johnson, R.A. and D.W. Wichern, *Applied multivariate stastistical analysis*. 5th ed. 2002, Upper Saddle River, NJ: Prentice-Hall.
49. Huet, S., et al., *Statistical tools for nonlinear regression: a practical guide with S-Plus and R examples*. 2nd ed. 2004, New York: Springer.
50. Ritz, C. and J.C. Streibig, *Nonlinear regression with R*. 2008, New York: Springer.
51. Lociciro, S., et al., *Dynamic of the ageing of ballpoint pen inks, quauntification of phenoxyethanol by GC-MS.* Science & Justice, 2004. **44**(3): p. 165-171.
52. Weyermann, C., *Mass spectrometric investigation of the aging processes of ballpoint ink for the examination of questioned documents*, 2005, University of Giessen.
53. Weyermann, C., et al., *A GC/MS study of the drying of ballpoint pen ink on paper.* Forensic Science International, 2007. **168**: p. 119-127.
54. Guerrier, B., et al., *Drying kinetics of polymer films.* AlChE Journal, 1998. **44**(4): p. 791-798.
55. Gelman, A., et al., *Bayesian data analysis*. 2nd ed. 2004, Boca Raton, FL: Chapman & Hall / CRC.
56. Biedermann, A., F. Taroni, and S. Bozza, *Implementing statistical learning methods through Bayesian networks, part 1: a guide to Bayesian parameter estimation using forensic science data.* Forensic Science International, 2009. **193**: p. 63-71.
57. Biedermann, A., et al., *Implementing statistical learning methods through Bayesian networks, part 2: Bayesian evaluations for results of black toner analyses in forensic document examination.* Forensic Science International, 2010. **204**(1-3): p. 58-66.