

LC-MS method development and comparison of sampling materials for the analysis of organic gunshot residues

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Abstract

This study aimed at developing a LC-MS method to compare the efficiency of various sampling materials for the collection and subsequent analysis of organic gunshot residues (OGSR). Seven sampling materials, namely two “swab”-type and five “stub”-type collection materials, were tested. The evaluation of sampling materials was systematically carried out by first analysing blank extracts of the materials to check for potential interferences and determining matrix effects. Based on these results, the best four materials, namely cotton buds, polyester swabs, a tape from 3M and PTFE were compared in terms of collection efficiency during shooting experiments using a set of 9 mm Luger ammunition. It was found that the tape was capable of recovering the highest amounts of OGSR. As tape-lifting is the technique currently used in routine for inorganic GSR, OGSR analysis might be implemented without modifying IGSR sampling and analysis procedure.

Keywords

Firearm discharge residue; sample collection; swab; stub

29 1. Introduction

30 Criminal investigations involving the discharge of a firearm often necessitate the detection of gunshot
31 residues (GSR) to link an individual to an incident. While GSR have also been used to estimate distance
32 of firing or identify bullet holes, providing evidence of this link remains a major goal in this field of
33 forensic science [1]. Gunshot residues are formed during the discharge of a firearm and can be
34 categorized as inorganic (IGSR) or organic GSR (OGSR) [2]. During the discharge, GSR not only
35 spread in the direction of the bullet, but also backwards leading to deposition of particles on the face,
36 hands and clothing of the shooter and to some extent on by-standers [3]. In practice, the analysis of
37 IGSR using Scanning Electron Microscopy Energy-dispersive X-ray spectroscopy (SEM-EDX) is
38 currently the method of choice in most forensic laboratories. However, the introduction of heavy metal-
39 free or “non-toxic” ammunition on the market can potentially lead to false negatives emphasizing the
40 need for the characterization of OGSR to potentially reinforce the evidential value of GSR [4]. OGSR
41 mainly originate from propellant and are composed of unburnt and partially burnt gunpowder particles.
42 Depending on their explosive content, gunpowders are classified as single base containing only
43 nitrocellulose (NC), double base containing NC together with nitroglycerine (NG) or triple base
44 containing NC, NG and nitroguanidine [1]. In addition to explosives, all smokeless powders also contain
45 a number of additives, such as stabilizers, plasticizers or flash inhibitors that endow the powder with
46 specific properties. Some of these additives might have alternative sources, such as phthalates that are
47 found in plastic products, in building materials or even in cosmetics [5]. Diphenylamine (DPA), a
48 common stabilizer in explosives and gunpowders, is also used in the perfumery, as an antioxidant in the
49 rubber and elastomer industry, or to prevent scald of apple and pear crops [6]. However, the reaction of
50 DPA with nitric degradation products from NC- and NG-containing explosives produces nitrated DPA
51 derivatives specific to OGSR [7]. Consequently, the presence of a single analyte, *e.g.* DPA, recovered
52 from a sample collected on a suspect has very low relevance, as a number of alternative sources are
53 possible. Nonetheless, the detection of several organic compounds combined with a positive IGSR
54 analysis may yield a significant evidential value.

55

56 Although many analytical methods were proposed for the quantitation of organic components in
57 gunpowders, few studies considered specifically the detection of organic GSR. Spectroscopic techniques
58 such as Raman spectroscopy [8-10] or Fourier transformed infrared spectroscopy [11] have been used,
59 but only qualitative results could be obtained and no identification of the various OGSR compounds was
60 possible. Ion mobility spectrometry (IMS) [12, 13] has the advantage of producing results in a matter of
61 seconds and enables on-site analysis, but it is a screening method and further confirmatory analysis is
62 required. Mass spectrometry (MS) [14-16] provides identification together with the advantage of very
63 fast results, however, as no previous separation is performed, matrix effects are a considerable issue
64 impacting the sensitivity of the technique. A way to lessen matrix effects is to couple an electrophoretic

65 or chromatographic separation step to mass spectrometry detection. Capillary electrophoresis [17-21] in
66 micellar electrokinetic chromatography mode can separate neutral compounds and demonstrated an
67 interesting potential, however with some detection limit issues due to the small capillary diameter and
68 injection volumes. Gas chromatography has been applied to OGSR analysis using various detectors,
69 such as thermal energy analysis (TEA) [22, 23], nitrogen-phosphorus detector (NPD) [24] or mass
70 spectrometry [25]. Nevertheless, thermolabile compounds such as nitroglycerine and
71 nitrosodiphenylamines are degraded by the high temperatures required by GC experimental conditions.
72 Finally, the most promising approach seems to be liquid chromatography (LC) coupled to MS. In 2007,
73 Laza *et al.* proposed a protocol targeting diphenylamine and derivatives as well as centralites using
74 swabbing and solid phase extraction preconcentration [26]. A few years later, Thomas *et al.* presented
75 a method for quantitation of organic compounds in gunpowders using LC-MS/MS, but the method was
76 not tested on OGSR analysis [27]. Recently, Benito *et al.* published a procedure able to quantify OGSR
77 with an original collection stub able to sample both inorganic and organic GSR using sample
78 preconcentration by evaporation under N₂ [28]. And Taudte *et al.* used artificial neural networks to
79 develop a UHPLC method for detection of 32 analytes and applied it to OGSR using UV detection [29].

80

81 Some research groups tried to implement a procedure able to characterize both inorganic and organic
82 GSR collected on the hands of a shooter. Three main approaches were proposed:

- 83 • The first one was to simultaneously analyse IGSR and OGSR with the same technique, as was
84 presented by Morales *et al.* using capillary electrophoresis [21]. They targeted 11 organic and
85 10 inorganic GSR compounds and were able to detect residues collected with a cotton swab.
86 However, sensitivity remained a limitation.
- 87 • The second possibility was to analyse sequentially IGSR and OGSR from the same sampling
88 material. An early study was conducted with examination of primer residues by SEM/EDX
89 followed by the analysis of propellant residues (NG and 2,4-dinitrotoluene) on a double-side
90 adhesive coated stub using GC-TEA and IMS [23]. This was further developed for samples
91 collected with a standard carbon stub using DESI-MS for OGSR and SEM-EDX analysis of
92 IGSR afterwards, but the limits of detection were too low for real samples [16]. Recently, a
93 sequence using GC-MS for OGSR followed by laser induced breakdown spectroscopy for IGSR
94 was proposed for samples collected using cotton swabs [25].
- 95 • The last approach, introduced by the group of Barrio, proposed to divide a traditional collection
96 stub in two with one half covered by carbon tape for IGSR and the other half covered by PTFE
97 for OGSR collection [28, 30]. This methodology enables the analysis of both halves of the stub
98 in parallel. In their first publication using this concept [30], the analytical techniques were
99 scanning laser ablation and inductively coupled plasma-mass spectrometry for IGSR and Raman
100 spectroscopy for OGSR. However, it seems probable that the routine method in place for the

101 analysis of IGSR will be difficult to modify. Indeed, the sampling method proves to be very
102 practical and SEM-EDX is well implemented in most forensic laboratories around the world.
103 Consequently, a good OGSR sampling method should be able to collect both types of residues
104 simultaneously with the same device and be compatible with SEM-EDX analysis. In this way,
105 the concept proposed in their second article [28] using the modified stub for parallel analysis of
106 OGSR and IGSR using LC-MS/MS and SEM-EDX, respectively, may be more promising for
107 practical implementation.

108
109 With regard to IGSR collection, tape lifts, vacuum lifts and swabbing are the most popular techniques
110 [2]. In the field of explosives where swabbing is commonly used for sample collection, sampling
111 materials were extensively studied. Four swabbing materials were compared for recovery of organic and
112 inorganic residues and cotton balls proved to be the most effective [31]. Another study concluded that
113 Teflon and Nomex[®] materials were the most promising, even if tape-lifting was also investigated [32].
114 However, in the field of OGSR, except for Zeichner *et al.* [23] who compared different tapes and Benito
115 *et al.* [28] who compared their designed stub with a cotton swab, a systematic study is still lacking.
116 Consequently, the present work aimed at comparing the efficiency of various sampling materials for the
117 analysis of OGSR. To the best of our knowledge, it is the first time that sampling devices are investigated
118 in detail for further quantitation of OGSR by LC-MS. Seven sampling materials, namely two “swab”-
119 type and five “stub”-type collection materials, were tested in this work. The investigation started with
120 the development of a simple and robust LC-MS method able to separate and quantify molecules typically
121 found in gunpowders, such as diphenylamine or ethylcentralite. The evaluation of sampling materials
122 was then systematically carried out by first analysing blank extracts of the materials to check for
123 potential interferences with the target analytes. Next, matrix effects were also determined for each
124 material. Based on these results, the best materials were finally compared in terms of collection
125 efficiency during shooting experiments using a set of 9 mm Luger ammunition. Composition of OGSR
126 was also compared to gunpowder from the same batch to evaluate which compounds are more likely to
127 be recovered from the hands of a shooter after discharge.

128

129 **2. Material and Methods**

130 *2.1. Chemicals*

131 Water containing 0.1 % formic acid, methanol, formic acid, and acetonitrile were of LC–MS grade and
132 were purchased from Sigma-Aldrich (Buchs, Switzerland). Ten OGSR compounds were targeted in this
133 study (Table 1). Diphenylamine was from Fluka (Buchs, Switzerland). Ethylcentralite, N-
134 nitrosodiphenylamine, 4-nitrodiphenylamine, akardite II, 1,3-diphenylurea, N’N-diphenylformamide
135 and dibutyl phthalate were obtained from Sigma–Aldrich (Buchs, Switzerland). 2-nitrodiphenylamine

136 was from Alfa Aesar (Karlsruhe, Germany). Methylcentralite was purchased from MP Biomedicals
 137 (Illkirch, France).
 138

139 **Table 1:** Compounds of interest and MS/MS parameters for QTrap instrument

Compound	Parent ion (m/z)	Product ion	Declustering potential [V]	Collision energy [V]
Akardite II (AK II)	227.1	170.1	120	27
		91.9		36
1,3-diphenylurea (1,3-DPU)	213	94	100	25
		77		48
Methylcentralite (MC)	241.2	134.1	125	24
		105.9		36
N’N-diphenylformamide (N’N-DPF)	198.1	92	130	30
		65		54
Ethylcentralite (EC)	269.2	147.9	120	20
		120		33
2-nitrodiphenylamine (2-nDPA)	215.1	197	80	14
		180.1		23
4-nitrodiphenylamine (4-nDPA)	215.1	197.8	60	18
		167.1		47
Diphenylamine (DPA)	170.1	93	200	32
		66		58
N-nitrosodiphenylamine (N-nitrosoDPA)	199.1	169	60	15
		66		30
Dibutyl phthalate (DBP)	279.2	205	90	11
		149		19

140

141 2.2. UHPLC-MS

142 The experiments were carried out using two different LC-MS systems. Both used an Agilent Infinity
 143 1290 ultra-high performance liquid chromatography (UHPLC) from Agilent Technologies. Both
 144 instruments were equipped with a binary pump with a maximum delivery flow rate of 5 mL/min, an
 145 autosampler, and a column compartment thermostated at 40°C. Separation was performed with Kinetex
 146 core-shell columns from Phenomenex (2.6 µm, 2.1 mm × 100 mm), using C18 and biphenyl
 147 selectivities. SecurityGuard ULTRA cartridges with the adequate selectivity were used as pre-columns.

148 The first UHPLC system was coupled with an Agilent 6530 Quadrupole Time-of-Flight mass
 149 spectrometer (Q-TOF/MS) equipped with an Agilent Jet Stream (AJS) ESI source from Agilent
 150 Technologies. Electrospray ionization was operated in positive mode. The [M+H]⁺ of the target
 151 compounds were defined as the ions of interest. The following source parameters were used: the drying
 152 gas temperature was set at 300°C and 8 L/min. The nebulizer gas was set at 35 psi, and the sheath gas

153 was set at 11 L/min and 350°C. The capillary and nozzle voltages were adjusted to 3500 V and 1000 V,
154 respectively. The fragmentor was set at 100 V. Data were collected from 100 to 400 m/z at a scan rate
155 of 4 spectra/sec. Data acquisition, treatment and instrument control were monitored using Mass Hunter.

156 The second UHPLC system was hyphenated to a triple quadrupole mass spectrometer (5500 QTrap)
157 from ABSciex. Electrospray ionization was operated in positive mode. The [M+H]⁺ of the target
158 compounds were defined as the precursor ions, and quantification was obtained from the SRM
159 measurements. MS/MS parameters are given in Table 1. The following source parameters were used:
160 the desolvation temperature was set at 500°C, the nebulizer gas at 60 psig, the turbo gas at 50 psig, the
161 curtain gas at 25 psig. The IonSpray voltage was adjusted to 5500 V. Data acquisition, treatment and
162 instrument control were monitored using Analyst software.

163 Two different MS instruments were chosen due to their complementary features. Indeed, a QTOF can
164 be used in scan mode to detect all components in a defined mass range and has a great potential to
165 identify unknown compounds and evaluate the presence and magnitude of co-eluting interferences. A
166 QTrap, used as a triple quadrupole instrument, is limited to the transitions defined in the method, thus
167 to known compounds. However, its sensitivity is normally better than that of a QTOF.

168 The organic mobile phases were independently prepared by adding 0.1% formic acid to acetonitrile and
169 methanol respectively. Water with 0.1% formic acid was used as aqueous phase. Screening methods
170 were first used to test the 2 (columns) x 2 (organic mobile phase) conditions. Standard gradient methods
171 were used at this stage to evaluate analyte separation: at a flow rate of 0.4 mL/min, gradient started at
172 35% ACN and 50% MeOH. The initial mobile phase composition was kept constant for 1 min and then
173 increased constantly up to 100% organic mobile phase at 7 min.

174 Methods were then optimized and the final methods were as follows. With the C18 column and
175 acetonitrile mobile phase, gradient elution followed the method: 35% B (from 0 to 0.5 min), 35–80% B
176 (in 5.5 min), and 80-100% B (in 1 min). The injection volume was 5 µL and the mobile phase flow rate
177 was set at 0.25 mL/min. With the biphenyl column and methanol mobile phase, the final method was
178 the following: 55% B (from 0 to 0.5 min), 55–80% B (in 5.5 min), 80-100% B (in 0.5 min). The injection
179 volume was 5 µL and the mobile phase flow rate was set at 0.4 mL/min.

180 Semi-quantitative determination of sample concentration was performed using the QTrap instrument
181 and the C18 column. Calibration standards from 0.1 to 20 ng/mL (8 levels, n = 2), except for 1,3-DPU
182 for which the concentration range was from 0.02 to 4 ng/mL, were injected in the system to draw a test
183 calibration curve and estimate the concentrations of the samples collected from the hand. In the case of
184 DPA, only samples from 1 ng/mL up to 20 ng/mL were considered, as its limit of detection was higher

185 than for the other target analytes. Solvent blanks were also injected to check for potential
186 contaminations.

187 2.3. Sampling

188 Various sampling materials were investigated, namely swabs and stubs. DNA cotton buds type 150C
189 were from Copan (Italy) and ESD polyester swabs from ITW Texwipe (Netherlands). Carbon tape
190 coated stubs were from Plano (Germany). This collection device consisted of a metal stub coated with
191 a carbon adhesive tape inserted in a plastic vial with a screwed cap. Other materials that can be coated
192 on the same metal stub were also studied. Carbon tape 12 mm in diameter was provided from Agar
193 Scientific (UK), double sided tape 665 and double sided tape for posters from 3M (USA).
194 Polytetrafluoroethylene (PTFE, 19 mm x 0.2 mm) was purchased from Bisan (Poland).

195 Blank extracts (n = 3) for each material were prepared by adding 1 mL MeOH to a vial containing the
196 sampling material. The vials were ultrasonicated during 15 minutes at ambient temperature and then
197 centrifuged. Matrix effects (n = 5) were evaluated by comparing a standard mixture spiked in MeOH
198 with the same mix spiked in the material extract prepared following the same protocol as the blank
199 extracts. The evaluation was carried out at 100 ppb with the QTOF instrument and 10 ppb with the
200 QTrap. The so-called matrix effect is the ratio of the peak area in the extract to the peak area in MeOH.

201

202 2.4. Shooting sessions

203 Shooting sessions were carried out in an indoor shooting range in a specific building sector, apart from
204 the laboratory. The same pistol was used for all experiments, a semi-automatic 9 mm Parabellum Sig
205 Sauer P226. The cartridges were 9 mm Luger from Geco and Sellier&Bellot. The shooter was asked to
206 wash his hands before coming inside the shooting range and was not allowed to touch any surface except
207 for the firearm at the time of firing. Another person was in charge of loading the gun. Then, the shooter
208 was asked to fire one time and was sampled outside the shooting range by a person waiting also outside.
209 After sampling, he was asked to wash carefully his hands again before starting the procedure once more.
210 The firearm was not cleaned between shots. For hand sampling by swabbing, the swabs were moistened
211 with ethanol and the hand surface was scrubbed repeatedly. With the stubs, 50 dabbings were applied
212 to the hand following recommendations from Zeichner *et al.* [33].

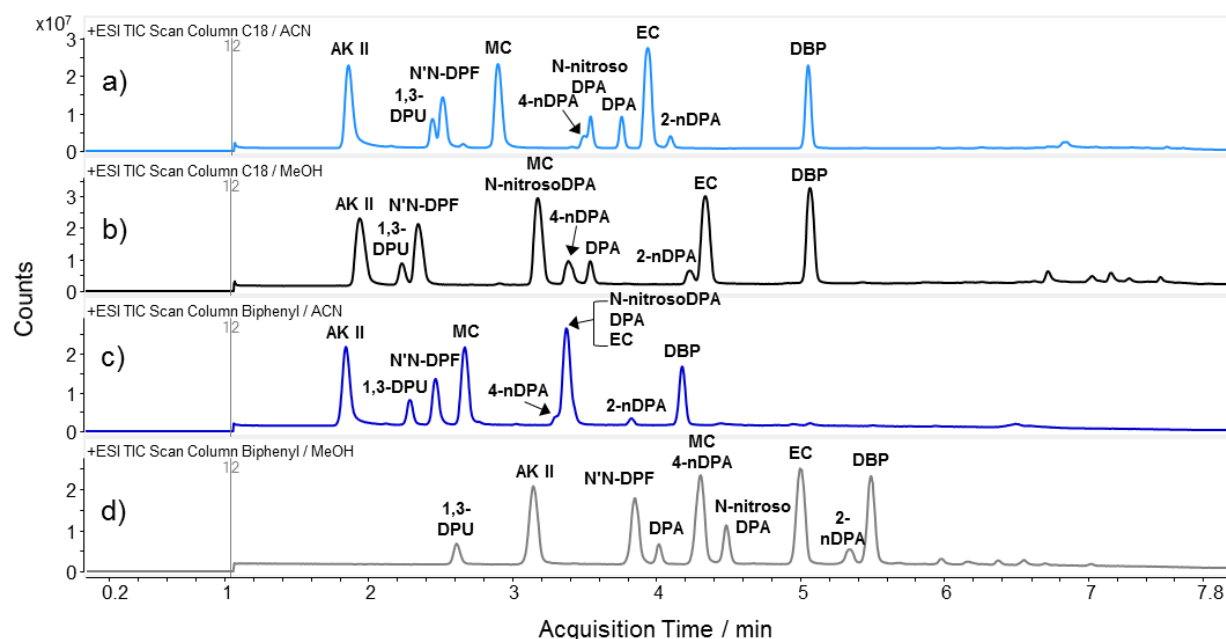
213 For gunpowder analysis, cartridges from the same batch as those discharged were dismantled. 10 mg
214 of powder was weighed, extracted in MeOH following the protocol above, diluted and analysed by LC-
215 MS, showing the potential discrimination between the powders and indicating the compounds expected
216 in residues.

217

218 3. Results and Discussion

219 3.1. Method development

220 Two column selectivities and two organic mobile phases were investigated for separation of the analytes
221 of interest, producing a set of four conditions to be tested on the QTOF instrument. C18 and biphenyl
222 stationary phases were selected since OGSR molecules are both lipophilic and aromatic. To the best of
223 our knowledge, it is the first time that a biphenyl column is used for OGSR analysis. Acetonitrile (ACN)
224 and methanol (MeOH) containing 0.1% formic acid were selected as organic components of the mobile
225 phase, whereas water with 0.1% formic acid was used as aqueous phase. ACN and MeOH were selected
226 because they are commonly used in LC-MS and have relatively low toxicity. Formic acid was added to
227 both aqueous and organic solutions to promote ionization and to keep a constant proportion of acid along
228 the chromatographic run. Consequently, the composition of the mobile phase is very simple and robust
229 as pH does not have to be adjusted. Standard gradient methods were used at this stage to rapidly evaluate
230 analyte separation. In three conditions out of four, most of the molecules could be separated by
231 chromatography (Figure 1).



232
233 **Figure 1.** Screening of the conditions for separation of 10 standards using the QTOF on a) C18 column with ACN mobile
234 phase, b) C18 column with MeOH mobile phase, c) biphenyl column with ACN mobile phase and d) biphenyl column with
235 MeOH mobile phase. Flow rate was 0.4 mL/min and gradient was from 35% for ACN and 50% for MeOH up to 100%.

236
237 When no baseline resolution was obtained between two molecules, they could nevertheless be
238 distinguished by mass spectrometry. Selectivity was thus considered sufficient with both mobile phases
239 using the C18 column and with MeOH using the biphenyl column. In the case of the combination
240 “biphenyl column-ACN”, 4-nDPA, DPA, EC and N-nitrosoDPA could not be resolved

241 chromatographically. This can be explained by the fact that π - π interactions are inhibited by acetonitrile
 242 [34]. Despite co-elution, these molecules were separated in MS. However, considering the low number
 243 of molecules to separate, co-elution of four molecules seemed unacceptable. Finally, one method was
 244 further optimized for each column, the first using the C18 column with ACN and the second using the
 245 biphenyl column with MeOH as described in the Material and Methods section. Flow rate and gradient
 246 were modified to improve resolution, retention time distribution and solvent consumption. For the C18
 247 column, ACN was chosen over MeOH as no co-elution of compounds happened. It is interesting to note
 248 that the order of elution varied with the column and solvent. It seemed thus beneficial to carry out the
 249 whole interference study using two column selectivities since interferences might also be affected by
 250 experimental conditions.

251 These two methods were then applied to the determination of limits of detection (LOD) with the two
 252 LC-MS systems. These were obtained by using decreasing concentrations of a standard mixture of the
 253 analytes of interest. The LOD was defined here as the concentration equivalent to a signal-to-noise ratio
 254 of three. As expected, the QTrap instrument was between 2 and 100 times more sensitive than the QTOF
 255 mass spectrometer depending on the analyte (Table 2). Indeed, triple quadrupole-type instruments are
 256 renowned for improved sensitivity in trace analysis compared to QTOF, which are more adapted to
 257 screening and identification of unknown compounds. DPA and its degradation products had slightly
 258 higher LOD than the other compounds especially with the QTOF. No significant difference was
 259 observed between columns with the QTRAP, but it seemed that limits of detection were slightly better
 260 using an ACN-based mobile phase than a MeOH-based for the QTOF. The instruments showed excellent
 261 sensitivities and allowed detection of low pg amounts of OGSR for the QTOF and even sub-pg amounts
 262 for the QTrap.

263

264 **Table 2:** Limits of detection determined with two instruments and two columns. BP: biphenyl. Values are given in ppb

	Agilent QTOF 6530		ABSciex 5500	
	C18 column	BP column	C18 column	BP column
1,3-DPU	0.1	1	0.004	0.004
AK II	0.1	0.5	0.01	0.01
N'N-DPF	0.1	0.5	0.02	0.02
DPA	1	2	0.5	1
4-nDPA	1	2	0.02	0.02
N-nitrosoDPA	2	5	0.02	0.5
EC	0.1	0.5	0.01	0.01
2-nDPA	2	5	0.02	0.02
MC	0.1	1	0.01	0.05

265

266

267 3.2. Sampling materials and matrix effects

268 Different types of materials for sampling of a shooter's hand were studied and the interferences inherent
269 in their own composition were evaluated. Seven materials classified as swab- or stub-type were selected
270 according to what was proposed in the literature (Table 3).

271

272 **Table 3:** Sampling materials investigated in the study

Sampling materials	Type
Cotton bud	Swab
Polyester swab	Swab
Carbon tab	Stub
Carbon tape	Stub
3M tape	Stub
3M poster tape	Stub
PTFE	Stub

273

274 Stubs would be more interesting for practical purposes as they provide the possibility of collecting both
275 IGSR and OGSR simultaneously, even if swabs have the advantage of collecting less skin debris and
276 producing less interferences than tapes during solvent extraction. First, blanks of the intact materials
277 were extracted in MeOH and analyzed to determine the potential presence of target analytes or
278 interferences in the extract. As the sensitivity of the QTRAP was better than the QTOF, this evaluation
279 was mainly carried out with this instrument and only rapidly checked with the QTOF. For most of the
280 materials, all blank samples were considered as “clean” since the target molecules were absent from the
281 sampling devices and no interference was discovered at expected retention times and masses. However,
282 DBP was found in all extracts, as well as in blank solvent samples. The presence of DBP in blanks might
283 stem from the plastic of pipette tips or tubes from the LC-MS system. This type of contamination is
284 quite common and potential sources are actually difficult to avoid. Consequently, DBP was removed
285 from the set of target molecules, as its ubiquity makes it difficult to quantify accurately. Results showed
286 that the DNA cotton buds and the PTFE film presented no interferences at all. With polyester swabs,
287 only a minor peak just before the retention time of DPA was observed using the C18 column, but it was
288 sufficiently resolved so as not to hinder the detection of DPA. With both 3M tapes, the results were
289 satisfactory, as only a small peak of 1,3-DPU was detected. This molecule is not of prime interest in the
290 detection of OGSR, so it could simply be removed from the set of molecules if necessary. Carbon tapes,
291 traditionally used for IGSR sampling, turned out to be less good than other tested materials. Carbon tabs
292 showed the presence of a strong peak of EC in all the blanks extracts analyzed with both columns.
293 Contamination problems were suspected, so experiments were repeated to confirm the results. However,
294 even with carbon tabs from another lot, the peak of EC was still present, whereas no EC was present in
295 solvent blanks. Due to the intensity of the peak, the molecule was probably inserted during the carbon

296 tape fabrication and was not due to contaminations from our lab. The other carbon tape from Agar
297 Scientific also showed a lot of unrepeatable interferences and contaminations. Due to the highly variable
298 interference results, it was concluded that such tape can be very easily contaminated in the lab and was
299 thus discarded from our sampling assortment.

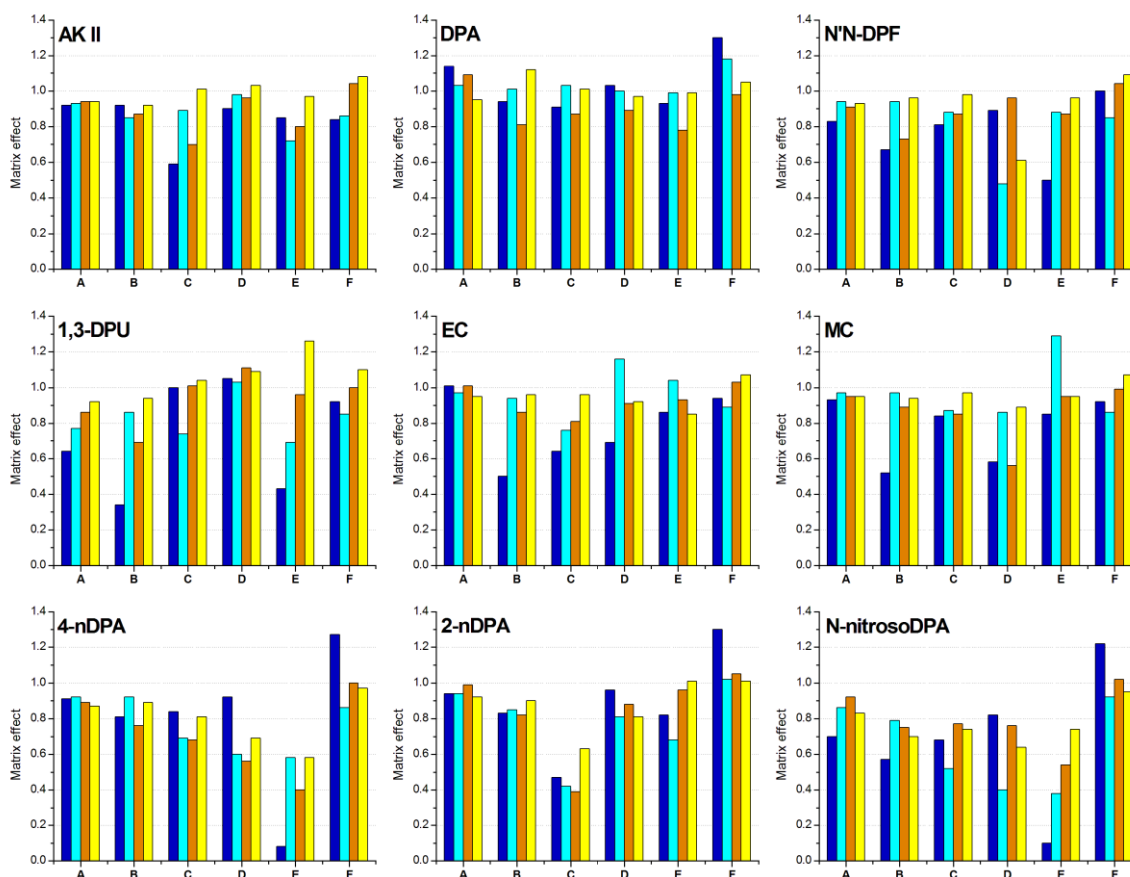
300 The next step was to determine the matrix effects produced by the sampling materials. Indeed, as their
301 composition is relatively complex and the concentrations involved are quite high relative to OGSR, the
302 molecules originating from the sampling material could hinder detection by competing with the analytes
303 for ionization, the so-called matrix effect. To measure the effect of the matrix, the peak areas of the
304 target analytes spiked into matrix extracts were compared to peak areas of standard solutions as
305 commonly performed in bioanalysis.

306
$$\text{Matrix effect} = B/A \text{ (Eq. 1)}$$

307 With A the peak area obtained in standard solutions (average of 5 replicates) and B the corresponding
308 peak area for standards spiked after extraction of sampling materials (average of 5 replicates) [35]. The
309 carbon tab was also examined for matrix effects, in order to get insight into the complexity of such
310 sampling products. Matrix effects were determined with both instruments and columns, but at different
311 concentrations, namely 100 ppb with QTOF and 10 ppb with QTrap. It is expected that matrix effects
312 might be stronger at lower concentrations, but the instruments might also present different matrix effects
313 due to the different source technologies.

314 An absence of matrix effect would be characterized by a value of 1. A value superior to 1 indicates an
315 increase in analyte ionization caused by the matrix and logically a value inferior to 1 corresponds to a
316 decrease in ionization. Signal enhancement is totally acceptable when identified, so matrix effects > 1
317 do not pose a real problem. However, a decrease in sensitivity is an issue because OGSR are present in
318 traces and any reduction in sensitivity impairs chances of OGSR detection. Globally, results were
319 encouraging and mostly superior to 0.5 representing adequate sensitivity losses inferior to a factor two
320 (Figure 2). RSD for standard solutions were less than 5% and in the case of spiked samples less than
321 10%.

322



324

325 **Figure 2.** Matrix effects ($n = 5$) estimated with the QTOF and the QTrap using C18 and biphenyl columns. The matrix effect
 326 value is the ratio of the peak area of a molecule in the sampling media extract to the peak area in a standard solvent. The letters
 327 on the horizontal axis are: A = cotton buds, B = Polyester swab, C = Carbon tab, D = 3M tape, E = 3M poster tape, F = PTFE.

328

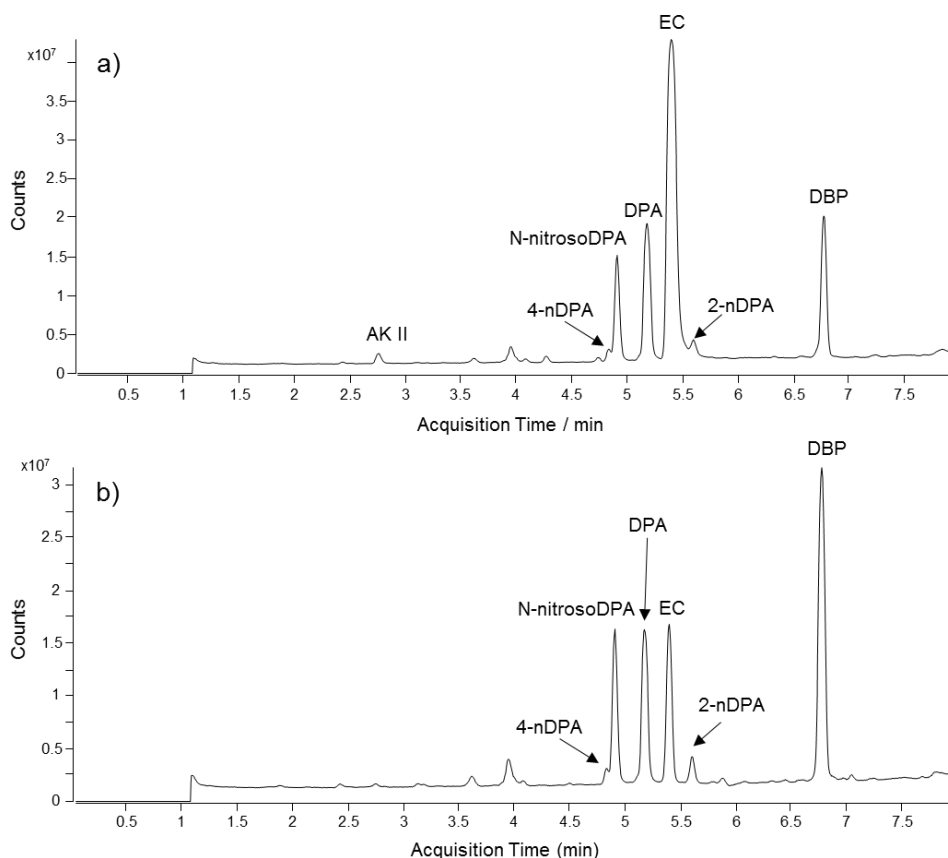
329 Some exceptions were highlighted with matrix effects leading to more than 50% loss. The 3M tape for
 330 posters (letter E in Figure 2) was considered less adapted to the analysis of OGSR than the other
 331 materials, because it induced a strong decrease in 4-nDPA and N-nitrosoDPA signals. Carbon tabs (letter
 332 C) also produced strong matrix effects for 2-nDPA. As a consequence, both 3M poster tape and carbon
 333 tabs were not investigated further. PTFE (letter F) presented the lowest matrix effects, certainly thanks
 334 to its simple composition. Cotton buds (letter A) and polyester swabs (letter B) produced values mostly
 335 over 0.8 except for 1,3-DPU and N-nitrosoDPA. Finally, 3M tape (letter D) was the best of all tapes
 336 selected in terms of matrix effects, mostly affecting the signal of MC, 4-nDPA and N-nitrosoDPA, but
 337 with values superior to 0.5. Instrument and column type can also have some influence as illustrated by
 338 the combination C18 column-QTOF that showed stronger matrix effects for 1,3-DPU, MC and EC than
 339 the 3 other combinations. In the case of tape (letter D), the signal of N'N-DPF was dependent on the
 340 column used. Thus, biphenyl column did visibly not separate a co-eluting compound that had a different

341 retention time using the C18 column. In conclusion, four of the seven candidates remained at the end of
342 this evaluation, namely DNA cotton buds, polyester swabs, 3M tape and PTFE film, and they were
343 further evaluated for their collection efficiency in shooting sessions.

344

345 3.3. Gunpowder analysis and OGSR collection efficiency

346 Samples of unfired gunpowders, namely of Geco and Sellier&Belloc (S&B) brands, were first analysed
347 to get some insight into the compounds present and their relative amounts. The main compounds
348 detected in both gunpowders were the same, namely EC, DPA, N-nitrosoDPA, 4-nDPA, 2-nDPA and
349 DBP as shown in Figure 3. AK II, N’N-DPF and MC were also found in lower quantity in both
350 gunpowders.



351

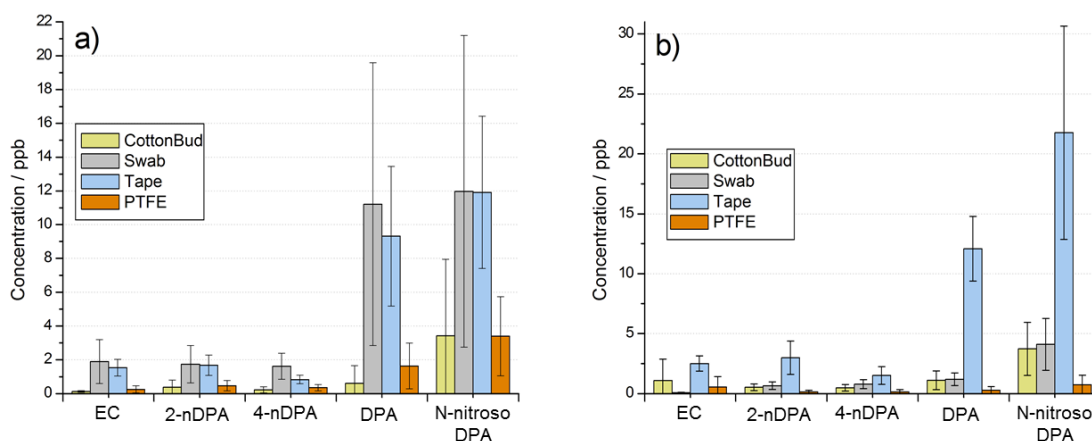
352 **Figure 3.** Gunpowder analysis: TIC data showing the main components detected by the QTOF instrument using the C18
353 column. a) Geco gunpowder (2 mg/mL) b) S&B gunpowder (2 mg/mL). Data were acquired between m/z 100 and 400 in TOF
354 mode (no fragmentation).

355

356 It is possible to determine absolute collection efficiency by spiking a surface with a known amount of
357 target molecules and then sample this surface to evaluate how much of the initial quantity can be
358 recovered. This technique is particularly useful in the evaluation of swabbing materials, as they are

359 moistened with a liquid before sampling. However, this technique is not suited to the evaluation of stubs.
360 Indeed, while it is acceptable to estimate that the liquid from the swab may act similarly with a spiked
361 sample and a real shooting sample, this approximation is not valid in the case of a stub, where no liquid
362 is used to dissolve and sample the compounds deposited on the skin surface. Consequently this step was
363 skipped to directly test the materials in shooting conditions.

364 The four selected materials were investigated during one shooting session using the same ammunition
365 batch. The shooter was sampled after one shot and three shots were performed for each material. Two
366 sessions were carried on different days to test two different ammunitions. Sampling materials were
367 compared in terms of amount of compounds that could be recovered from the hand of the shooter. Semi-
368 quantitative determination of sample concentration was performed using the QTrap instrument and the
369 C18 column because this instrument was the most sensitive. The average concentration and the standard
370 deviation of three discharges were calculated for each material and illustrated in Figure 4.



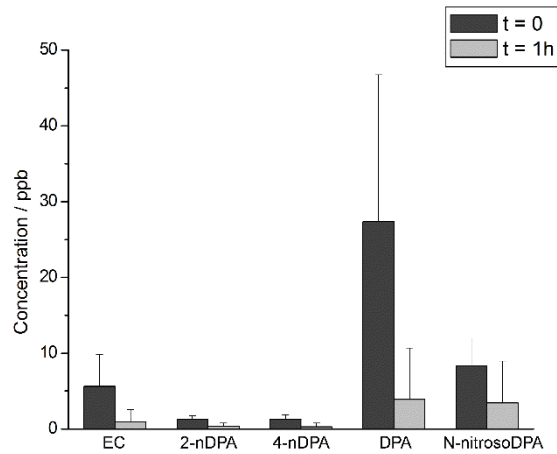
371
372 **Figure 4.** Comparison of the collection efficiency of the sampling materials. (n = 3). Data were acquired using the QTrap
373 instrument and a C18 column. Ammunition: a) 9 mm Luger from Geco, b) 9 mm Luger S&B

374
375 From the results in Figure 4a, it is clear that the polyester swab and the tape have collected more residues
376 than the cotton bud and the PTFE film. However, in Figure 4b, the tape performed far better than the
377 other three sampling materials. Two parameters changed between the two sessions: the gunpowder and
378 the person in charge of sampling. If comparing the materials by sampling type (swab or stub), the
379 difference between cotton buds and polyester swabs in Fig 4a could be due to the weaving of the fibres,
380 to the material itself and consequently to the application it was designed for. The cotton buds were
381 planned to be used for DNA sampling and the polyester swabs for capturing dust in a clean room.
382 Consequently, the weaving of the polyester swab is probably more adapted to OGSR collection. The
383 difference was not significant during the second session. Between tape and PTFE, the main difference

384 is the stickiness of the surface significantly enhancing collection efficiency for both shooting sessions.
385 Benito *et al.* found that PTFE was superior to swabbing [28]. However, in their study PTFE was only
386 compared to cotton swabs and their results were obtained by spiking standard solutions onto the
387 sampling materials. They did not compare the sampling materials in real conditions. Our results
388 indicated that the performance of cotton buds was similar to PTFE and to some extent even better (Figure
389 4b). It is still unclear why PTFE is able to collect OGSR, as it has a practically smooth surface.
390 Electrostatic interactions might play a role in adhesion. The main benefit of PTFE over tape-lifting and
391 even swabbing is its low interference when solvent-extracting the sample. But despite the complex
392 matrix of tape and subsequent interferences, the stickiness seems to be of paramount importance.
393 Moreover, it would also be usable on hair and clothing. Besides, tape seems to be superior to swabbing
394 materials, even if the concentrations collected by polyester swabs were very close to those of tape with
395 Geco ammunition (Figure 4a). The mixed results for polyester swabs might be explained by the different
396 sampling persons, thus indicating that tape would be more practical and repeatable than swabs.
397 Furthermore, the choice between these two materials should also be based on combined sampling and
398 analysis of IGSR and OGSR, as well as practicality. For all molecules and materials, the standard
399 deviation is substantial. Two factors can explain the high variability: the intrinsic high variability
400 associated to OGSR production and deposition during discharge and the technical skill of the person in
401 charge of sampling. While the second factor can be improved by adequate training of the staff, an
402 important criteria for sampling material choice should also be the simplicity and robustness of the
403 sampling procedure.

404 Regarding the composition of OGSR in comparison to the intact gunpowders, the same compounds were
405 indeed found in both sample types. Nevertheless, in samples from the hands, only the major compounds
406 were detected. However, qualitative comparison indicated that the amount recovered of each compound
407 was not proportional. Indeed, the relative quantity of two compounds was not conserved after discharge.
408 For example, EC was the most highly concentrated compound in the Geco gunpowder, but DPA and N-
409 nitrosoDPA were recovered in higher quantities in hand samples. Similarly, EC was a major compound
410 in S&B gunpowder but was found at levels similar to 2- and 4-nDPA in OGSR. Despite the major loss
411 of EC, when comparing relative amounts of DPA and derivatives it was observed that the 2- and 4-
412 nitroDPA that were present in lower amounts than their parent molecules in gunpowders were also less
413 concentrated in the OGSR samples. In conclusion, it might be difficult to connect OGSR to their
414 respective gunpowder as the relative amounts of analytes were not preserved.

415
416 Preliminary experiments of persistence were carried out in order to show that the present method might
417 be applied to casework. The shooter was sampled three times at time $t=0$ and three times 1h after
418 shooting. The average concentration and the standard deviation of the three discharges were calculated
419 for each target compound (see Figure 5).



420

421 **Figure 5.** Comparison of the collection efficiency of tape stubs at $t = 0$ and $t = 1h$. Data were acquired using the QTrap
 422 instrument and a C18 column. Ammunition: 9 mm Luger from Geco,

423 It was still possible to detect OGSR one hour after firing a pistol. As expected, the concentrations
 424 measured after one hour were significantly lower than at $t = 0$. However, it is important to note that the
 425 five compounds of interest could always be detected. A new batch of Geco ammunition was employed
 426 in these experiments, explaining why the ratio N-nitrosoDPA/DPA collected from the hands is lower
 427 than in Figure 4a. These results indicate that preconcentration of the samples will probably be needed
 428 to improve limits of detection for sampling after longer time since discharge ($t > 1h$).

429

430 **4. Conclusions**

431

432 This study aimed at screening various LC-MS conditions to develop a robust method for the analysis of
433 OGSR and at evaluating several sampling materials for the detection of OGSR in real conditions. Two
434 instruments were employed during the study, namely a QTOF and a QTrap, to develop a method using
435 two column selectivities, C18 and biphenyl. Adequate separations were obtained with both columns and
436 LOD in the low ppb and sub- ppb range were obtained using the QTOF and QTrap, respectively. To the
437 best of our knowledge, it is the first time that a biphenyl column was employed in the field of OGSR
438 and its selectivity might be complementary to C18. Sampling devices were then investigated in detail
439 for further quantitation of OGSR by LC-MS. Seven sampling materials were evaluated: two “swab”
440 types and five “stub” types. Four materials, namely cotton buds, polyester swabs, a tape from 3M and
441 PTFE were found adequate for sampling as their composition did not interfere much with the analytes
442 of interest and matrix effects induced losses inferior to 50%. They were then compared in terms of
443 collection efficiency after shooting experiments and it was found that the tape was capable of recovering
444 the highest amounts of OGSR. Polyester swabs were too prone to the sampling procedure and varied
445 greatly from person (in charge of hand swabbing) to person. Cotton buds and PTFE, proposed in a
446 previous study, collected less OGSR.

447 Due to the high intrinsic variability associated to OGSR production and deposition during discharge, the
448 sampling procedure should also be as simple and robust as possible to avoid bias linked to sampling.
449 Furthermore, sampling material should be free of target analytes and minimize matrix effects. Regarding
450 the concentrations detected just after discharge, they were in the low ppb range and the QTrap instrument
451 was able to detect the major compounds without requiring a preconcentration step. Moreover, the
452 concentrations were largely superior to the LOD estimated for this instrument. Preliminary experiments
453 at $t = 1\text{h}$ showed lower concentrations than at $t = 0$, as expected, but detection was still possible. In
454 conclusion, with a performant QTrap-type MS instrument, OGSR can be easily detected just after
455 discharge. Further experiments must be conducted to study the transfer of OGSR and their persistence.
456 Nevertheless, this preliminary study demonstrated that with modern instrumentation and an efficient
457 sample preconcentration technique, forensic scientists might attain low pg/mL sensitivity and should be
458 able to quantitate OGSR in the few hours after discharge. Moreover, tape-lifting is the technique
459 currently used in routine, so OGSR analysis might be implemented without modifying IGSR sampling
460 and analysis procedure.

461

462

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464

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