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

Anne-Laure Gassner and Céline Weyermann

Institut de Police Scientifique, University of Lausanne, Batochime, 1015 Lausanne, Switzerland

Corresponding author: Anne-Laure.GassnerPerruche@unil.ch

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

1. Introduction

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

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

or chromatographic separation step to mass spectrometry detection. Capillary electrophoresis [17-21] in micellar electrokinetic chromatography mode can separate neutral compounds and demonstrated an interesting potential, however with some detection limit issues due to the small capillary diameter and injection volumes. Gas chromatography has been applied to OGSR analysis using various detectors, such as thermal energy analysis (TEA) [22, 23], nitrogen-phosphorus detector (NPD) [24] or mass spectrometry [25]. Nevertheless, thermolabile compounds such as nitroglycerine and nitrosodiphenylamines are degraded by the high temperatures required by GC experimental conditions. Finally, the most promising approach seems to be liquid chromatography (LC) coupled to MS. In 2007, Laza *et al*. proposed a protocol targeting diphenylamine and derivatives as well as centralites using swabbing and solid phase extraction preconcentration [26]. A few years later, Thomas *et al*. presented a method for quantitation of organic compounds in gunpowders using LC-MS/MS, but the method was not tested on OGSR analysis [27]. Recently, Benito *et al*. published a procedure able to quantify OGSR with an original collection stub able to sample both inorganic and organic GSR using sample preconcentration by evaporation under N_2 [28]. And Taudte *et al*. used artificial neural networks to develop a UHPLC method for detection of 32 analytes and applied it to OGSR using UV detection [29].

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

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

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

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

2. Material and Methods

2.1. Chemicals

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

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

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

2.2. UHPLC-MS

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

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

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

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

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

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

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

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

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

2.3. Sampling

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

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

2.4. Shooting sessions

Shooting sessions were carried out in an indoor shooting range in a specific building sector, apart from the laboratory. The same pistol was used for all experiments, a semi-automatic 9 mm Parabellum Sig Sauer P226. The cartridges were 9 mm Luger from Geco and Sellier&Bellot. The shooter was asked to wash his hands before coming inside the shooting range and was not allowed to touch any surface except for the firearm at the time of firing. Another person was in charge of loading the gun. Then, the shooter was asked to fire one time and was sampled outside the shooting range by a person waiting also outside. After sampling, he was asked to wash carefully his hands again before starting the procedure once more. The firearm was not cleaned between shots. For hand sampling by swabbing, the swabs were moistened with ethanol and the hand surface was scrubbed repeatedly. With the stubs, 50 dabbings were applied to the hand following recommendations from Zeichner *et al*. [33].
For gunpowder analysis, cartridges from the same batch as those discharged were dismounted. 10 mg of powder was weighed, extracted in MeOH following the protocol above, diluted and analysed by LC-MS, showing the potential discrimination between the powders and indicating the compounds expected in residues.

3. Results and Discussion

3.1. Method development

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

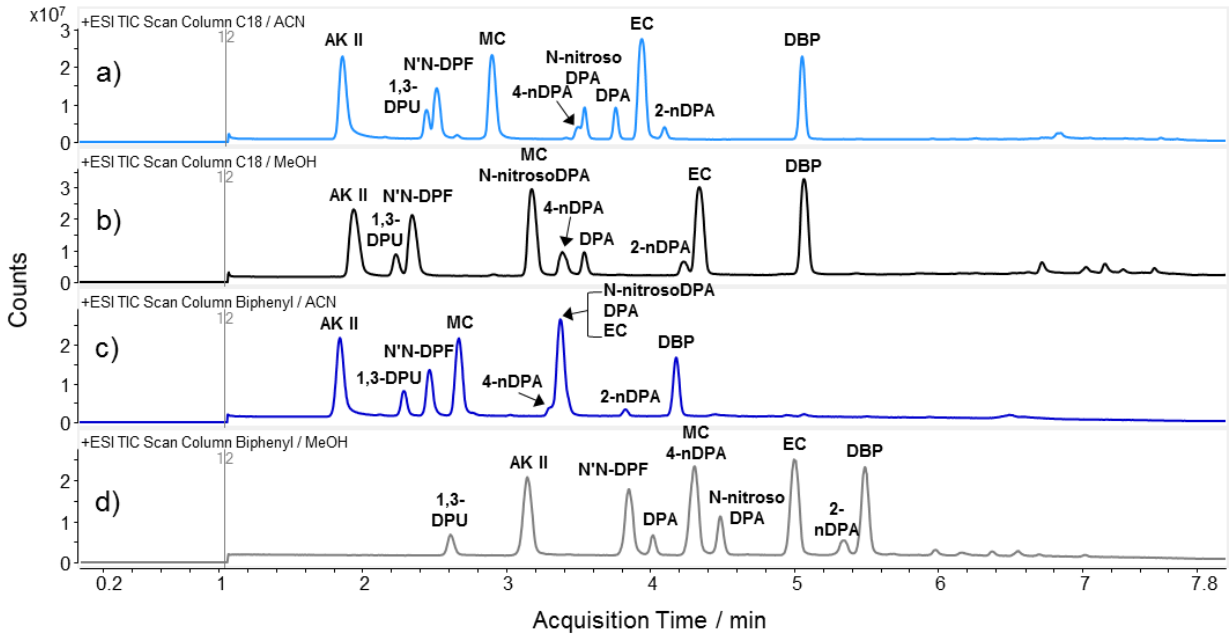


Figure 1. Screening of the conditions for separation of 10 standards using the QTOF on a) C18 column with ACN mobile phase, b) C18 column with MeOH mobile phase, c) biphenyl column with ACN mobile phase and d) biphenyl column with MeOH mobile phase. Flow rate was 0.4 mL/min and gradient was from 35%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

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

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

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

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

3.2. Sampling materials and matrix effects

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

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

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

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

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

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

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

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

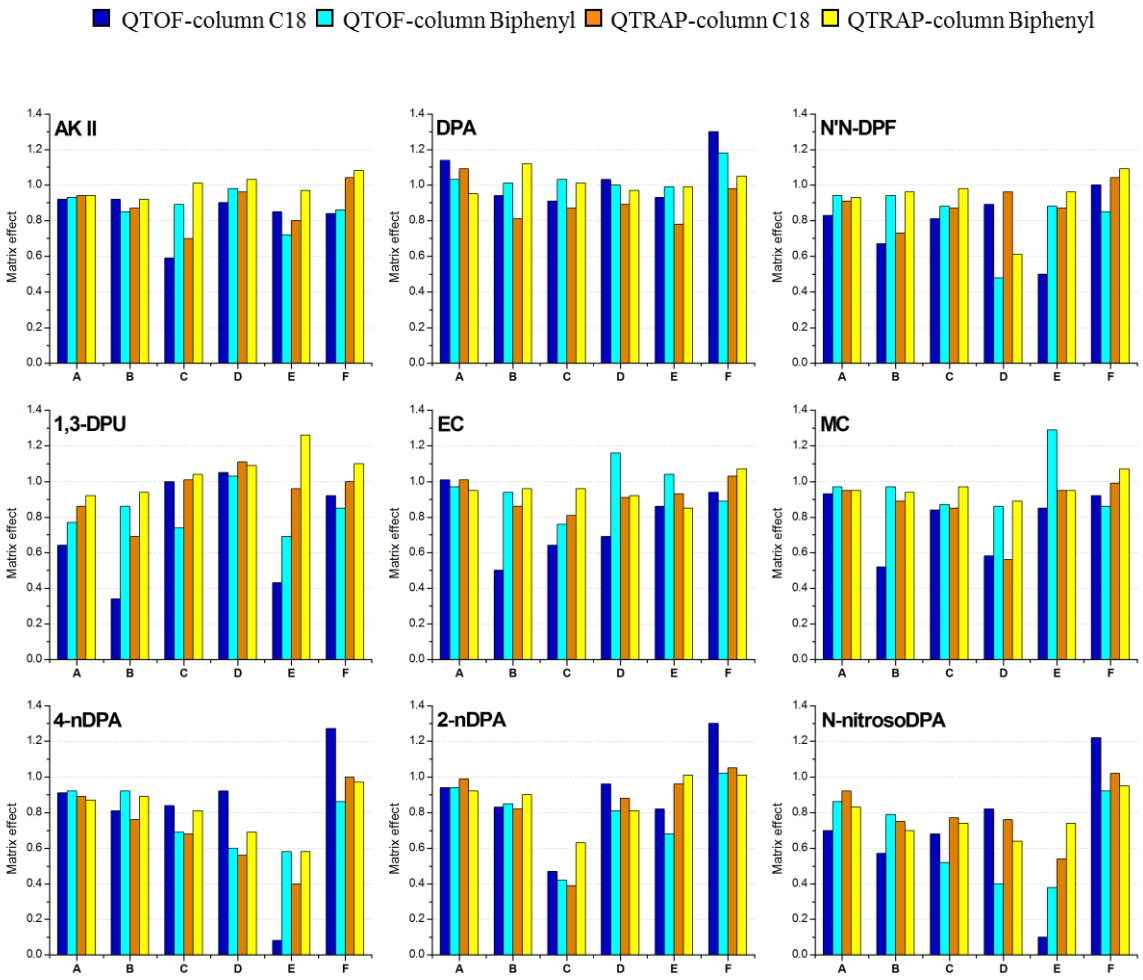


Figure 2. Matrix effects (n = 5) estimated with the QTOF and the QTrap using C18 and biphenyl columns. The matrix effect value is the ratio of the peak area of a molecule in the sampling media extract to the peak area in a standard solvent. The letters on the horizontal axis are: A = cotton buds, B =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

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

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

3.3. Gunpowder analysis and OGSR collection efficiency

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

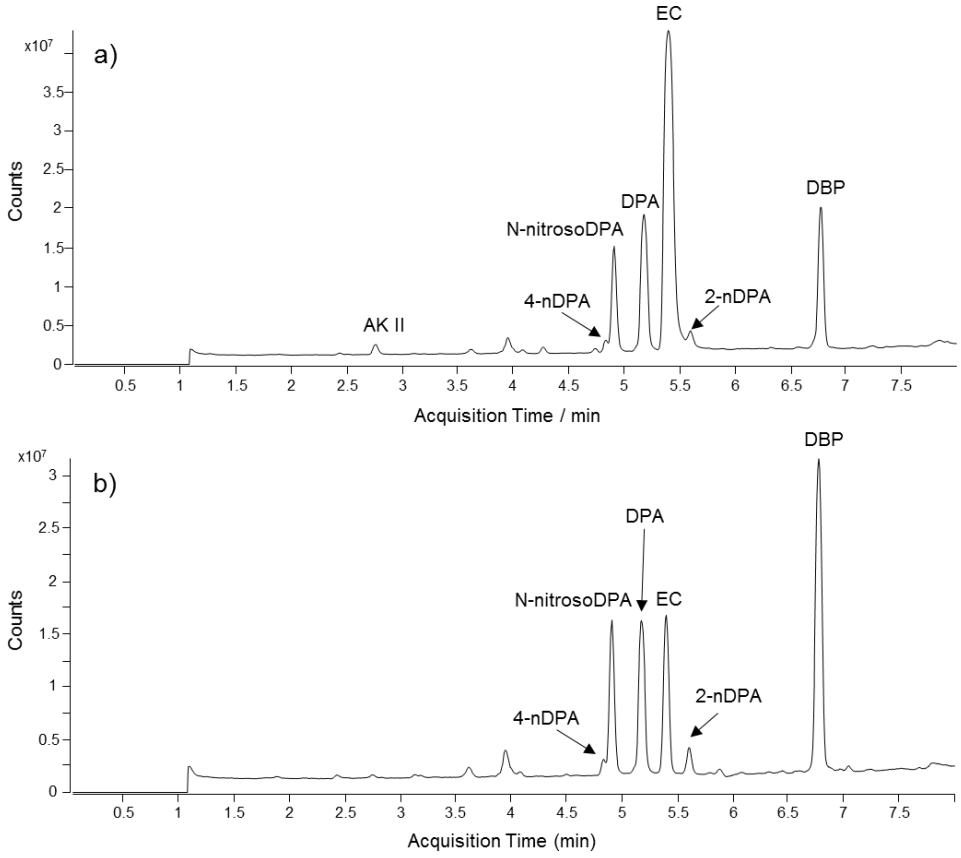


Figure 3. Gunpowder analysis: TIC data showing the main components detected by the QTOF instrument using the C18 column. a) Geco gunpowder (2 mg/mL) b) S&B gunpowder (2 mg/mL). Data were acquired between m/z 100 and 400 in TOF mode (no fragmentation).

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

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

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

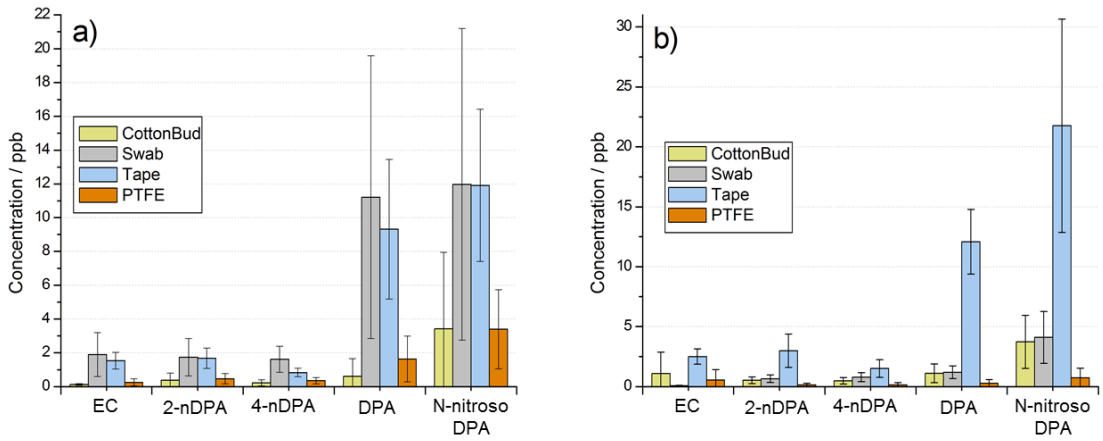
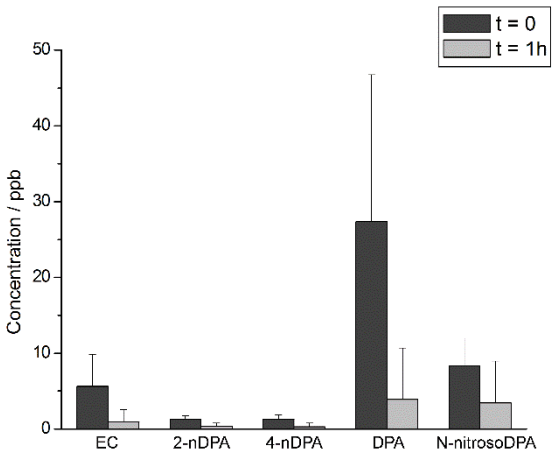


Figure 4. Comparison of the collection efficiency of the sampling materials. (n = 3). Data were acquired using the QTrap instrument and a C18 column. Ammunition: a) 9 mm Luger from Geco, b) 9 mm Luger S&B

From the results in Figure 4a, it is clear that the polyester swab and the tape have collected more residues than the cotton bud and the PTFE film. However, in Figure 4b, the tape performed far better than the other three sampling materials. Two parameters changed between the two sessions: the gunpowder and the person in charge of sampling. If comparing the materials by sampling type (swab or stub), the difference between cotton buds and polyester swabs in Fig 4a could be due to the weaving of the fibres, to the material itself and consequently to the application it was designed for. The cotton buds were planned to be used for DNA sampling and the polyester swabs for capturing dust in a clean room. Consequently, the weaving of the polyester swab is probably more adapted to OGSR collection. The 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

4. Conclusions

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

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

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