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

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Highlights

- An HSSE method for the analysis of 29 compounds in spent cartridges was optimised.
- Design of experiments was used to optimise extraction and desorption conditions.
- The final method presented notable performances and a short runtime.
- Use of deuterated standards allowed effective repeatability improvement.

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 paper aimed to more 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.

Keywords (max. 6): forensic science, firearm, gunshot residue, sorptive extraction, design of experiments, time since discharge

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 or after to the occurrence of the alleged crime [2, 3]. If such allegations are forwarded, estimating the time since discharge might 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) [5-9]. An especially heterogeneous mixture, GSR is composed of metallic micro-particles, unburnt or partially burnt smokeless powder flakes and explosion products [10-13]. 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) [14-18]. Given their significant vapour pressures, these are volatile and disappear over time after the cartridge is fired through evaporation and diffusion phenomena [15]. Application of solid phase micro-extraction (SPME) as a sampling technique to recover and analyse these explosion products was first suggested by Andrasko et al. [19] in 1999, following the encouraging results obtained on shotguns [16]. Specifically, the original protocol involved extracting the residual amounts daily from the internal atmosphere of the questioned spent cartridge until the total disappearance of any response after gas chromatographic analysis [19]. Naphthalene and an unidentified decomposition product of nitrocellulose (referred as “TEA2”) were then exploited as target analytes to make a decision about the time elapsed since discharge. However, while partial ageing curves could be obtained using this multiple-sampling procedure, the underlying premise relied on the fact that SPME did not significantly modify the cartridge’s internal atmosphere [20]. Subsequent studies proved otherwise for small calibres [17], 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 later evaluated by Weyermann et al. [17], 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. Hence, no reliable approach is currently available to deal with these kind of appraisals.

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 [16]. 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 possibility of using headspace analysis of spent handgun cartridges to provide helpful dating evidence through the use of 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 (instead of a single-analyte) 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 [21], 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 [15, 21]. Thus, 29 compounds known to be released during a cartridge discharge were selected as targets on the basis of previous studies [15, 21] 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) [22-25], 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.

2. Material and methods

2.1. Materials

Based on previous research [15, 21], 29 target compounds known to be interesting target analytes for dating were selected (Table 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. Table S1 in Electronic Supporting Material (ESM) shows the list of all these compounds, complete with manufacturer information. 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).

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.

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).

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.

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.

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.

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.

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.

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 [22]. 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 [25].

Lack-of-fit and regression significance were evaluated through Snedecor's F-test as described by Ferreira et al. [25]; curvature was tested through Student's t-test as suggested by Box et al. [22]. 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.

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 [26]. Selectivity was evaluated by injecting 24 molecules known to be commonly co-released with target compounds during the discharge of handgun cartridges [26], 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 [27]. They were defined as the lowest amounts able to generate an S/N ratio ≥ 3 after background correction [28]. 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.

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.

3. Results and discussion

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 1. These were selected in order to cover the main classes of compounds often present in volatile GSR [15, 21].

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. 1a), demonstrating an improvement by a factor of 2 (approximately) in comparison to the previous published approach [15, 21]. 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 Fig. 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 MS scan mode, in place of the previous scan-based method [15, 21]. One target ion and two qualifiers were thus selected for each analyte (Table 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.

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. 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).

Globally, peak shapes and resolution were not affected by changing the solvents, but there was significant variation in the amounts detected (signal intensity). Figure 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.

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 [29-31]. Contrariwise, the best coating volume was empirically investigated. Figure 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. 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.

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 2). After the data were 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.

For the purpose of illustration, Figures 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 [15, 21]. 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. 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.

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 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.

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 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. 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 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}.

3.5. Analytical performance characteristics

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

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 [15]. 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 [30-32]. 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 [15]. 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.

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 actually 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 1).

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 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.

3.6. Application to real samples

The optimised method was finally applied to real samples. Figure 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 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 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.

4. Conclusion

In the present contribution, 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 [15, 21]. 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 approach. Furthermore, an evaluation of the potentials and limitations of headspace analysis for estimating the time since discharge of 9 mm cartridges stored at different ageing conditions will be carried out.

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Figure 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 1.

Figure 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).

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

Figure 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.

Figure 5 – Plot of peak widths at half height ($w_{1/2}$) for select 3 target analytes as a function of cryo-focusing temperature.

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

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

Figure 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).

Analytes						S	LODs [pg]		Recoveries		RSDs (absolute peak areas)				RSDs (normalised 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*	X	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*	X	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%	21.7%	0.3%	0.2%	18.3%	28.5%

		Median					50	50	101%	82%	5.2%	5.7%	42.4%	66.8%	1.0%	2.6%	40.4%	57.7%
		Max.					500	500	118%	106%	18.0%	24.7%	69.1%	90.6%	16.0%	20.0%	66.9%	83.6%

Table 1 – Analysed target and deuterated compounds, complete with monitored ions and corresponding analytical performance characteristics with the optimised method. For target analytes, the column "deuterated standard" indicates the standard used for normalisation. "S" is the selectivity: "ok" means that no interfering compounds were identified, while the inverse applies for "X" (see the text for further details). "IP" is the intermediary precision. LODs are reported as the absolute amounts deposed in cartridges.

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 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).

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 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).

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 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.