Extraction Efficiency of the Sequential Solid Phase Microextraction of Gunshot Residues from Spent Cartridges

Kah Haw Chang [a], Ahmad Fahmi Lim Abdullah [a*]

[a] *Forensic Science programme, School of Health Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan*

ABSTRACT: Gunshot residues, produced after shooting activity, have acquired their importance in analysis due to the frequent happening of firearms involving crimes. These residues are commonly detected from spent cartridges and the potential of this kind of evidence to be analysed repeatedly and non-destructively was investigated in this work. Solid phase microextraction was performed to extract the headspace composition of spent cartridges using 85 µm polyacrylate fiber at 66 °C for 21 min. Organic compounds, *i.e.* naphthalene, 2,6-dinitrotoluene, 2,4-dinitrotoluene, diphenylamine and dibutyl phthalate were detected from the spent cartridges by gas chromatography-flame ionization detection technique. Our results of sequential SPME procedure showed that no change in the resulting GC profiles, indicating that the protocol did not deplete the compounds to a significant extent. It was suggested that the protocol is non destructive and allows for multiple sampling. Our findings did not allow the development of the time since discharge profile from the spent cartridges involving the heating of sample due to the potential loss of organic compounds to the environment. To conclude, the spent cartridge sample can still be analysed repeatedly and non-destructively, giving that the cap of the vials was not removed.

Keywords: forensic science, gunshot residue, solid phase microextraction, sequential extraction, spent cartridge

Introduction

After a shooting activity, discrete and characteristic burnt, partially burnt and unburnt combustion products with a combination of constituents from primers, bullet, cartridge case and/or firearm itself are produced, collectively known as gunshot residue (GSR) [1-3]. Nowadays, Firearm related crimes are among the major concerns to public safety, and the availability of firearms has contributed to cases of armed violence and homicides in some regions of the world [4-6]. GSR has acquired important role and its analysis has have resulted in revealing vital trace evidence establishing criminal activities involving the use of firearms [3]. In most firearm related cases, spent cartridges are frequently found at the crime scenes. Therefore, searching for the spent cartridge could be worthwhile for investigation which proved the occurrence of a firing activity.

During a forensic investigation, it would be preferable if a sample can be analysed repeatedly and non-destructively as may be warranted when the sample has to be analysed again using some other techniques. In this study, we performed the analysis of GSR from spent cartridges subsequent to firing activity through the application of headspace SPME and chromatographic technique [7]. The extraction efficiency of the sequential SPME of GSR of these spent cartridges was investigated, if multiple samples can be taken from the same cartridges especially during forensic investigation. All experiments in this study were carried out in the University Sains Malaysia (USM) Forensic Science Laboratory.

Materials and Method

Shooting and sampling

SME 9 mm ammunitions (Batch SME 5-02) were shot using a semi-automatic pistol P-99 Walther® (Serial number RMP 61077) at Royal Malaysia Police Shooting Range in Gunong, Kelantan state, Malaysia. Sampling was performed at Royal Malaysia Police Shooting Range in Gunong, Kelantan state, Malaysia. Individual spent cartridges were sampled immediately upon shooting and kept in 10 mL headspace vials with screw caps (Supelco, Bellefonte, PA).

Standards and solvent

Diphenylamine (DPA) and naphthalene were sourced from Sigma-Aldrich (St. Louis, MO). 2,4-dinitrotoluene (2,4-DNT) and dibutyl phthalate (DBP) standards were purchased from Merck (Whitehouse Station, NJ). 2,6-dinitrotoluene (2,6-DNT) was purchased from Cerilliant (Round Rock, TX). The standard solutions were prepared in analytical grade dichloromethane (DCM) purchased from Merck (Whitehouse Station, NJ). Stock standards for above compounds were prepared at 1.0 mg/mL and the working standards were diluted to 200 µg/mL.

SPME extraction from the spent cartridges

A manual SPME holder and SPME fibers coated 85 µm polyacrylate (PA) (Supelco, Bellefonte, PA) were used in the experiment. Optimised SPME protocol was as follows: incubation time, 2 min; extraction time, 21 min and sample temperature, 66 °C [7]. Spent cartridge in the vial was placed horizontally with screw cap closed tightly in an oven. The needle of the holder was then penetrated through the septum of the screw cap, exposing coated fiber to the headspace of the vial. The fiber was handled carefully to avoid contact with the spent cartridge or the wall of vial. The coated fiber was then reinserted into the needle upon extraction before gas chromatographic analysis.

Instrumental conditions

7890 A GC system equipped with FID (Agilent Technologies, Santa Clara, CA) is used. Separation of the compounds was achieved using a DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) (Agilent Technologies, Santa Clara, CA) with splitless injection technique. Purified nitrogen gas (99.9% purity) was used as the carrier gas at a constant flow rate of 0.5 mL/min. The injection port was set at 250 °C. Oven temperature programme consisted of an initial temperature of 125 °C and was increased to reach the final temperature (260 °C) at a rate of 4 °C/min. Detector temperature was set at 300 °C. Hydrogen flow, air flow and the makeup flow were set at 30, 300 and 15 mL/min, respectively. Chemstation software (Rev. B.04.02) was used for GC automation and data analysis. The retention times of reference standards were determined and compared to those appeared in the chromatogram during GSR analysis.

Sequential extraction of GSR from spent cartridges

Sequential extraction was carried out on the same spent cartridge in the same vial for a total of seven consecutive extractions. After the sampling of the spent cartridges into separate headspace vials, they were closed tightly with the screw caps. In this experiment, a total of five spent cartridges were used. The same spent cartridge was repeatedly extracted by SPME fibre to determine if there was any loss of selected volatile compounds due to multiple SPME extractions. These analyses were performed continuously on the same day to eliminate any inter-day variations. The mean peak areas of the selected volatile compounds and changes in composition (if any) were determined.

The effect of sequential extraction of spent cartridge was also studied on different days, up to a period of seven days. A total of five spent cartridges were used on each day. The first extraction was performed on day zero, and the consecutive extractions were carried out every day until the sixth day. Throughout the experiment, the same vials with spent cartridges were used and these samples were tightly capped and kept in an air-conditioned room (~20 °C) after each extraction. Multiple SPME extractions were performed, involving the piercing of SPME needle through the septum of these sampled vials without removing the screw caps. The experiment was also aimed to investigate whether a cartridge sample can be well secured inside a vial although repeated sampling was performed. Another experiment was performed following multiple SPME extractions where the spent cartridges extracted was transferred into new vials and analysed. Upon extraction and analysis, the mean peak areas of selected volatile compounds and changes in composition (if any) were determined.

Results and Discussion

Peak identification and analysis of GSR in spent cartridges

Combustion of smokeless powders in an ammunition during firing forms volatile compounds [1] in the headspace composition of smokeless powders [2]. In our work, all the compounds investigated were also the commonly used ingredients in the manufacturing of ammunition or the production of smokeless powders [1,2,8]. Utilising the same procedure and same analytical conditions for the determination of

GSR in spent cartridges as in the standard analysis, retention times for each compounds being studied can be used as the reference for identification of volatile compounds from GSR. Table 1 illustrates the retention times and the roles of target compounds in smokeless powders as well as GSR [2,8].

Table 1: Retention times and the roles of tested compounds

Retention time (min)	Name	Role
6.724	Naphthalene	Moisture displacer
10.863	2,6-DNT	Flash suppressant
12.566	2,4-DNT	Flash suppressant, plasticizer
15.081	DPA	Stabilizer
23.046	DBP	Plasticizer

During a forensic investigation, if a sample can be repeatedly analysed without any destructive outcome, it was worthwhile. Moreover, the same sample can also be used in other techniques in testing the value of the evidence. Our experiment studied the effect of sequential extraction from the same spent cartridge kept in a vial, in order to determine if there was any loss of volatile or semi-volatile compounds from the headspace composition after the first SPME extraction, and also after repeated extractions.

In the experiment, the sequential extraction was performed consecutively on the same vial with the spent cartridge for seven times within in short interval period of time (~40 min). The results (Figure 1) show that all the volatile compounds, expected to present, were all observed in the resulting GC chromatograms.

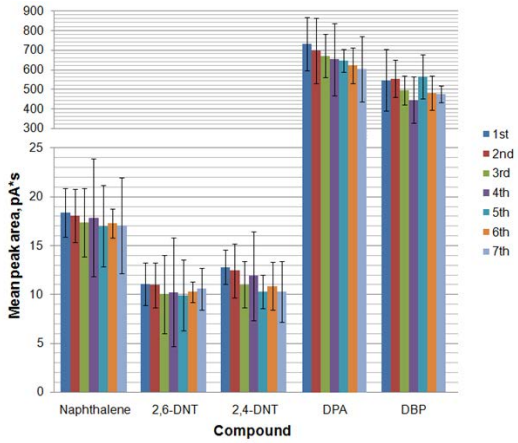

Figure 1: Sequential extraction of the headspace composition of spent cartridges on the same vial for seven times within one day

Repeated extraction with SPME technique has shown no significant decrease in the amount of selected volatile compounds compared to single extraction from spent cartridges except for the minor variations of peak areas for each selected compound throughout the seven extraction process. A downward trend in the detection of volatile compounds would be expected if depletion of compound occurred in time sequence as a result of sequential extraction. However, this was not seen in our result. It indicates that the composition of compounds in the headspace was not affected by the repeated extraction process. Therefore, the use of the same spent cartridges for replicating the analysis of the samples is possible. This finding is in contrast with that by Weyermann *et al.* (20090 [9], where the authors reported some effects of repeated sampling that contributes to decrement in the volatile compounds in the headspace composition of spent cartridge, as a result of sampling effect. In our experiments, the extracted compounds by the SPME appeared to be negligible when compared to the total amount of these compounds in the headspace, showing no significant decrement. Wilson's study supported this experimental outcome in which no significant difference was observed in the extracted amount of volatile compounds from the vials with repeated sampling and those sampled once [10].

Apart from the study of sequential extraction from the same spent cartridges on the same day, extractions on consecutive days were also studied. In this experiment, the first extraction of a spent cartridge was considered as day zero. For the next consecutive six days, the same cartridge in the same vial with sealed cap was used to perform the extraction protocol. After extraction each day, the spent cartridges in respective vials were kept at an air-conditioned room, at about 20 °C. Five different spent cartridges were used in this experiment in order to determine if there is any loss of volatile compounds into the environment when the caps of the vials were penetrated once or more than once for SPME extraction when the spent cartridges are stored for a week time. Figure 2 illustrates the sequential extraction of the headspace composition of spent cartridges on the same vial for seven consecutive days.

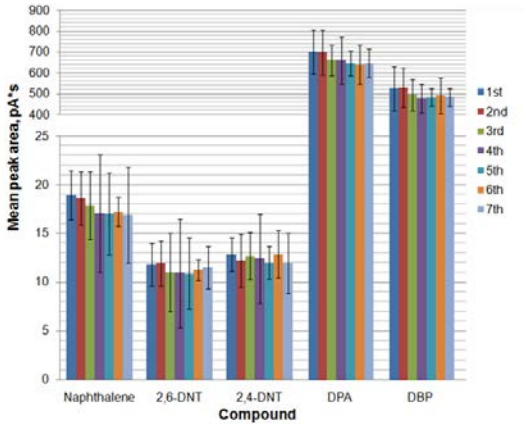

Figure 2: Sequential extraction of the headspace composition of spent cartridges on the same vial for seven consecutive days

The result (Figure 2) showed no observable decrement in the extracted amount of target volatile compounds. Throughout the extraction process over the seven days periods, the peak areas differed only by 20.0% for the compounds. Penetration of SPME needle through the PTFE septum of the cap did not contribute to the escape of these volatile compounds. The amount of the volatile compounds absorbed by the fibre of SPME appeared to be negligible compared with the total amount of headspace composition of the vial. Repeated uses of the same spent cartridges showed no effect on the results of extraction, at least for the period of one week.

Our finding is in agreement with a previous research study (Wilson *et al.*, 2003) that suggested negligible effect on sequential sampling without any substantial decrement in the volatile composition in the spent cartridges. Besides, the same spent cartridge was proposed for repeated analysis at different time in order to construct a curve for estimating the time since discharge [10] but this protocol was not suitable for kinetic study in our experiment. An experiment was carried out to evaluate the possibility of compound loss if a spent cartridge was transferred to a new vial. Figure 3 illustrate the results of sequential extraction when the same spent cartridges were transferred to new vials after each SPME extraction and analysis.

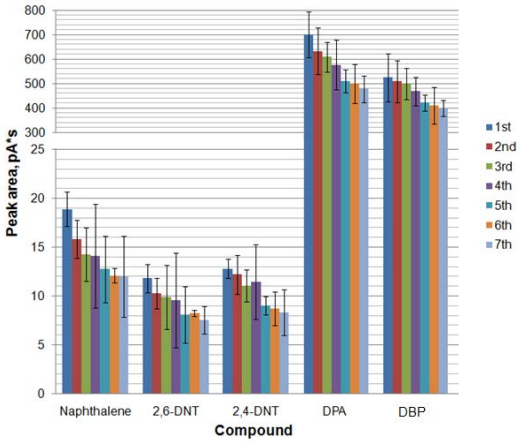

Figure 3: Sequential extraction of the headspace composition of spent cartridges with the transfer of the samples into a new vial

Contrary to the method developed by Wilson *et al.* (2003) which performed the extraction at room temperature, we introduced heat (66 °C) to the spent cartridge during the SPME protocol [10]. The optimised SPME conditions have proved effective in providing greater peak areas for the analysis of smokeless powders [7]. From Figure 3, the tested volatile compounds showed observable decreasing trend through the seven extractions. Exposure of same spent cartridge at a higher temperature to the atmosphere after each SPME extraction repeatedly could cause greater and quicker loss of volatile compounds. During kinetic study, the loss of volatile compounds might not totally be due to their kinetics, but also be affected by the extraction protocol. Therefore, our experimental outcome suggested the potential of repeated analysis of the same spent cartridge, but without the removal of the cap of a vial which would expose it to the atmosphere. Repeated sampling of the headspace composition of a spent cartridge with SPME is beneficial, especially in a forensic laboratory, as it permits multiple samples to be taken from the same spent cartridges [10]. However, our findings did not allow us to study the kinetic behaviours of volatile compounds and to develop the time since discharge profile from the spent cartridges in which these sampled cartridge cases needed to be exposed to an open air. Note that the kinetic study involved the examination of tested volatile compounds with time elapsed after shooting where the spent cartridges were left open in the headspace vials, exposing to the atmosphere.

Conclusion

The sequential sampling using SPME fibre showed that no change has occurred to the resulting GC profiles indicating that the extraction protocol did not deplete the compounds to a significant extent, suggesting that the protocol is non destructive and allows for multiple sampling. The finding did not allow the development of the time since discharge profile from the spent cartridges due to the potential loss of organic compounds to the environment. However, the spent cartridge sample can be analysed repeatedly and non-destructively, giving that the cap of the vials was not removed, and may be warranted to be analysed by other techniques such as microscopic examination and elemental analysis.

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Additional information and reprint request:
Ahmad Fahmi Lim Abdullah
Email: fahmilim@usm.my
Forensic Science Programme
School of Health Sciences
Universiti Sains Malaysia
16150 Kubang Kerian, Kelantan.