The analysis of organic and inorganic gunshot residue from a single sample

Abstract

The detection and interpretation of gunshot residue (GSR) can play an important role in the investigation of firearm related incidents. Recently, the potential of organic compounds to provide an additional means to discriminate between GSR and environmental particles, in particular in cases where lead-free ammunition is used, has been highlighted. This work describes a method for the extraction and detection of complementary organic and inorganic compounds from a single GSR sample, using a methodology that makes implementation in the current standard procedure feasible. GSR samples were collected from the shooter's hands following double and single discharges, using the traditional adhesive carbon aluminium stubs. Analysis of organic compounds was performed using solid-phase microextraction gas chromatography mass spectrometry (SPME-GC-MS), followed by analysis of the traditional inorganic particles using scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM-EDX). Detection of both categorised organic GSR compounds (*e.g.* ethyl centralite, diphenylamine and 2-nitrodiphenylamine) and characteristic inorganic GSR has been achieved. Given the fact that the detected organic GSR compounds are relevant with respect to the confirmation of GSR materials, this method has successfully demonstrated the ability to obtain a total chemical profile from a single GSR sample, which has the potential to increase the probative value of GSR evidence.

Keywords: Forensic Science; Gunshot residue; Solid-phase microextraction; Scanning electron microscopy; Ballistics

1. Introduction

Gunshot residue (GSR) consists of a complex mixture of unburnt and partially burnt particles originating from the firearm, the firearm ammunition, and from combustion products, which are produced during the discharge of a firearm. The main sources of organic GSR (OGSR) compounds are ammunition components (*e.g.* propellant powder) and combustion products [1]. There are over a hundred organic compounds with a possible association to GSR [1, 2], many of which can be found in environmental and occupational materials [1, 3]. A small selection of around twenty of these compounds have recently been highlighted as OGSR compounds that have a potential relevance to the confirmation of GSR materials [4].

When a firearm is discharged, GSR escapes through weapon openings and may subsequently deposit on surfaces in the near vicinity of the fired weapon [5, 6]. As a result, GSR could become evidence consequent to the criminal use of a firearm [7] and its detection and identification could provide valuable information in forensic investigations of the incident. Currently, the accepted forensic standard for the identification of gunshot residue is the analysis of inorganic GSR (IGSR) using scanning electron microscopy coupled with energy-dispersive X-ray spectrometry (SEM-EDX) [8, 9]. This is a non-destructive technique that provides both morphological information, as well as the elemental composition of individual particles [10, 11]. The ability, however, to extract complementary organic information could increase the probative value of GSR evidence and potentially provide an additional means to distinguish GSR from environmental residues [12], especially when lead-free ammunition is used.

Several methods for the detection of OGSR compounds from propellant powders, spent cases and actual GSR samples have been proposed (Table 1), however, a standard methodology for the collection, extraction and analysis of OGSR samples has not yet been established [1]. Limited research has been done with respect to obtaining both organic and inorganic information from a single sample. A prevalence study was performed where samples were collected from police stations and vehicles, which were then analysed using SEM-EDX and liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) respectively [13]. Another approach involved the removal of particles resembling propellant from a target cloth for Fourier Transform Infrared (FTIR) analyses, after which the cloth was stubbed to collect IGSR for SEM-EDX analysis [14].

In this work, the combination of gas chromatography coupled with mass spectrometry (GC-MS) and SEM-EDX is investigated, using solid-phase microextraction (SPME) as an extraction and pre-concentration technique for OGSR. SPME in combination with either GC-MS or ion mobility spectrometry (IMS) is commonly employed for OGSR analysis. SPME is a simple, solvent-free variety of solid phase extraction (SPE), and employs a fine fused silica fibre for the extraction of the analytes. It enables the collection of ultra-trace levels of analytes from various matrices by pre-concentrating the analytes onto the SPME fibre [12, 15]. Analysis of the entire sample is achieved by thermal desorption of the fibre directly into the IMS or GC inlet [16]. Good results have been achieved by SPME in combination with GC-MS or IMS for the analysis of OGSR compounds from samples such as propellant powders and spent cartridge cases [12, 15, 17-20]. The application of SPME to the analysis of GSR samples collected from a shooter’s hands, however, has not been reported before.

The purpose of this research was to obtain total chemical profiles of single GSR samples collected from a shooter’s hands using the traditional adhesive carbon aluminium stubs [10, 11]. The developed SPME-GC-MS method was applied to samples generated using 3 different ammunition types, discharged with a pistol and a long barrel revolver. OGSR analysis is performed first in order to minimise any potential losses of OGSR compounds due to storage [21], followed by SEM-EDX analysis of IGSR.

2. Materials and Methods

2.1 GSR sample collection

GSR samples from 9 mm American Eagle and 9 mm Federal Premium were generated on the Merseyside Police range using a Glock 17 self-loading pistol. GSR samples from Alliant Unique were generated using self-loaded .38 rounds of ammunition, which were discharged using an Alfa long barrel .38/.357 revolver at the Grange Pistol and Rifle club range, Liverpool, UK.

All samples were collected at *t = 0* using 12 mm carbon tabs mounted on aluminium stubs (Agar Scientific, Essex, UK). The palm and back of the hand were sampled with at least 50 dabs, paying special attention to the webbing and upper surfaces of the thumb and index finger. Separate stubs were used to sample the right and left hand. Samples were collected following two discharges (n = 3) for each ammunition type, whereby the shooter was standing upright holding the firearm with both hands. For the American Eagle ammunition samples were collected following single discharges (n = 6) in a standing position. Before each test firing, the shooter’s hands were cleaned thoroughly using isopropanol wipes. Blank samples were taken both from the shooter’s hands and the sampler’s hands. All samples were kept on ice until return to the laboratory where the samples were stored in a freezer at -18°C.

Unburnt propellant samples were also collected, by pulling the bullets using a kinetic hammer.

2.2 SPME extractions

A 65 µm polydimethylsiloxane/divinylbenzene (PDMS/DVB) fibre (Sigma Aldrich, Bellefonte, PA, USA) was used for SPME extractions. The sample, a stub or a single grain of unburnt propellant, was transferred to a glass 10 mL headspace vial with a PTFE/silicone septum cap (Sigma Aldrich, Bellefonte, PA, USA) and pre-conditioned in an oven (Nabertherm) for 30 minutes at 80°C to allow the volatiles to enter the headspace. The extraction was performed in the oven at 80°C for 35 minutes [12, 17]. Thermal desorption of the analytes was accomplished in the injection liner at 250°C [12]. Blank stubs were also analysed to ensure no OGSR compounds were present.

Before every extraction the fibre was conditioned for 20 minutes in the GC inlet at 250°C [12], whilst the oven temperature was ramped to 150°C, and a blank run was carried out to ensure no carry-over or contamination occurred.

2.3 GC-MS analysis of OGSR

Chromatographic analysis, optimised from the method reported by Dalby & Birkett [12], was performed on an Agilent 6890N Network GC system, equipped with a J&W scientific HP5-MS UI (30 m x 0.25 mm x 0.25 µm) column. A 0.75 mm I.D. SPME injection sleeve (Supelco, Bellafonte, PA, USA) was used to prevent band broadening. Sample introduction was performed in splitless mode without a solvent delay. The initial oven temperature was 50°C, which was increased in four temperature ramps: 10°C/min to 100°C, 5°C/min to 180°C held for 2.50 min, and 30°C/min to subsequently 200°C held for 2.50 min and 300°C held for 2 min. A flowrate of 1.2mL/min was maintained.

The GC was coupled to an Agilent 5975B Inert MSD system using electron ionisation (EI). In full scan mode, masses were scanned from m/z 40 to 500. Parameters of the developed selected ion monitoring (SIM) are provided in Table 2. Mass spectra for recorded peaks were further evaluated using the NIST database (NIST Mass Spectral Search Programme Version 2.0). Limits of detection determined for the OGSR compounds detected in the samples were in the range 1-50 ng (Table 2).

2.4 SEM-EDX analysis of IGSR

Following OGSR analysis, samples were coated with a conductive layer of carbon using a Quorum Technologies Q150T ES Rotary pumped carbon coater. Analysis was performed with an SEM-EDX FEI Quanta 200 (Table 3). Oxford INCA GSR analysis software was used for the automatic identification of GSR materials, which were confirmed using manual acquisition and verification of characteristic particles, as defined by the ASTM and SWGGSR guidelines [10, 11].

3. Results and discussion

3.1 Effect of stub heating

Given the fact that the acquiring a full chemical profile of organic and inorganic GSR requires the stubs to be heated prior to SEM analysis, the influence of stub heating on IGSR detection must be considered. For this purpose GSR samples were collected from the shooter's hands following the discharge of a single round (standing position, firearm held in both hands), and cut in half for analysis. Guidelines by the ASTM and SWGGSR state that it is sufficient to analyse a portion of the stub's surface, given the fact that GSR is collected randomly across the surface of the stub and does not tend to cluster [10, 11, 22]. Analysis of two unheated stub halves of one sample (1L) confirmed this; the difference between the two stub halves was 3 particles or 4%. For all other stubs, one half of each sample was subjected to heating as per the SPME procedure before IGSR analysis was carried out. The number of characteristic particles detected on each half is shown in Table 4.The percentage indicates the relative value of characteristic particles detected on the stub half compared to the total on the whole stub.

Similar particle depositions were detected on most stubs (1L, 2L and 2R), of which sample 2L had a greater number of characteristic particles on the half that was subjected to heating. These results suggest that heating the stub does not have a significant adverse effect on the subsequent IGSR analysis. Therefore, SPME-GC-MS analysis followed by SEM-EDX detection appears to be a viable option for obtaining a total chemical profile of GSR samples.

3.2 Analysis of unburnt propellant

The organic composition of the test-fired ammunition has been determined by extracting single grains of unburnt propellant (Figure 1). The organic compositions across the different ammunition types are similar, however, AKII was only detected in Federal Premium propellant. In two of the three Alliant Unique grains 2,4-DNDPA was detected.

3.3 OGSR analysis

The results of the organic profile obtained from GSR samples collected after two discharges (n = 3) for three firearm-ammunition combinations is shown in Figure 2. The peak area of DPA obtained from the American Eagle ammunition is shown on the primary axis (left-hand side), the peak areas of the other compounds are shown on the secondary axis. Figure 2 shows that all stabilisers that were detected in a single grain of 9 mm American Eagle and Alliant Unique propellant (Figure 1) were also detected in their respective gunshot residues. Only 2,4-DNDPA, which was detected in two of the three single grains of Alliant Unique, was not detected. DPA was the only stabiliser detected in the 9 mm Federal Premium propellant. It is hypothesised that this may be caused by a more complete combustion process. Experiments involving the analysis of burnt propellant rather than GSR showed that burnt 9 mm Federal Premium propellant resulted in the lowest recovery for all stabilisers. The grains of this propellant were very small compared to the other propellants, which generally indicates that the propellant burns faster [23].

In addition to the compounds shown in Figure 2, phthalates (DBP and DIBP) were detected, but due to their generic nature, they were not considered further. Variation seen in a number of OGSR components is mostly likely due to the complexity of the firing process, which leads to the creation of varying amounts and compositions of gunshot residue [5].

The results of the organic profile obtained from GSR samples collected after single discharges (n = 6) of American Eagle ammunition is shown in Figure 3. The peak area of DPA is shown on the primary axis (left-hand side), the peak areas of the other compounds are shown on the secondary axis.

Figure 3Figure 3 shows similar peak areas for DPA and 2-NDPA on both of the shooter’s hands, however, 2-NDPA was only detected in half of the samples. In total, a greater amount of EC was detected on the left hand, but EC was not detected in samples 2 and 3 for the left and right hand respectively. In only two samples collected from the left hand was 4-NDPA detected, leading to the low peak area for this compound overall on the left hand.

The peak areas of DPA, EC, and 2-NDPA obtained from the shooter’s right hand are comparable for both double and single discharges. The peak areas of 4-NDPA were greater following a single discharge. The lack of increase in GSR when doubling the number of discharges has been reported in literature before, and has been attributed to the inherent heterogeneous nature of the deposition rather than the result of the sampling and extraction protocol [24, 25].

3.4 Total chemical profile

The results of the OGSR analysis have been compared against a recently proposed categorisation system for OGSR compounds [4]. The results of both double and single discharges are combined with results obtained from IGSR analysis in Table 5. Only characteristic inorganic particles (containing lead, antimony, and barium (PbSbBa)) are taken into account.

These results show that more than 100 characteristic inorganic particles were detected in all samples. For two propellants, additional categorised OGSR compounds were detected. EC is a first category compound, owing to its very strong association with GSR and very restricted applications unrelated to GSR. DPA and its nitro-derivatives are second category compounds, due to their strong association with GSR in conjunction with less restricted applications unrelated to GSR [4]. With the detection of both categorised OGSR compounds and characteristic IGSR, the acquisition of a complementary organic and inorganic GSR profile has been accomplished. The detection of first and second category OGSR compounds demonstrates the ability of OGSR to strengthen the value of GSR evidence. The absence of categorised OGSR compounds in Federal Premium GSR samples suggest that the OGSR composition in GSR may be more variable than the IGSR composition.

4. Conclusion

In this work the generation of a total chemical profile from a single GSR sample has been accomplished using a combination of SPME-GC-MS and SEM-EDX. The successful application of the optimised SPME-GC-MS method was demonstrated with respect to the detection of OGSR compounds relevant to the confirmation of GSR materials. In all samples characteristic IGSR was detected, in combination with known propellant stabilisers. For two firearm-ammunition combinations first category OGSR compounds, *i.e.* EC, were identified. The results have shown no adverse effect of the employed method on the subsequent detection and confirmation of IGSR particles.

The strength of the proposed method is the practical applicability for real casework. The fact that the sample collection and IGSR analysis were performed according to the current standard method, enables the implementation of the proposed method without alterations except for cool storage of the sample. Furthermore, this method accounts for the limited storage time associated with OGSR compounds, and enables carbon coating the sample where necessary. It is recognised that due to the small sample size further work is still required before this method could be used as a robust and reliable technique to strengthen the value of GSR evidence. Further work by the authors will focus on applying the method to a greater range of ammunition types.

5. References

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Tables and Figures

Table 1: Methods employed for the sampling, extraction and analysis of OGSR compounds

Sample	**Sampling method**	**Extraction**	**Analytical technique**	**Ref**
Propellant powder	-	Solvent extraction	GC-MS, UPLC-MS/MS, CE, MEKC, Raman	[12, 26-30]
Propellant powder	-	SPME	GC-MS, IMS	[12, 15, 18]
Propellant powder	-	MMSE	GC-MS	
Propellant transfer	Wipe	-	IMS	[31]
GSR; gun barrel	-	SPME	GC-TEA, GC-FID	[32-34]
GSR; spent cartridges	-	SPME	GC-MS, IMS, GC-FID, GC-TEA	[12, 15, 19, 20]
GSR; spent cartridges	-	Stir bar	GC-MS	[2, 17]
GSR; spent cartridges	-	Solvent extraction	Anion exchange chromatography	[35]
GSR; target cloth	Tape lift method	-	ATR-FTIR, Raman	[36, 37]
GSR; target cloth	-	-	Raman	[29]
GSR; objects	Stub	Solvent extraction	LC-MS/MS	[13]
GSR; skin	Swab	-	IMS	[38, 39]

GSR; skin	Swab	Solvent extraction	LC-QTOF, UHPLC-MS, LC-MS/MS	[24, 40, 41]
GSR; skin	Stub	-	DESI-MS	[42]
GSR; skin	Stub	Solvent extraction	LC-QTOF, UHPLC-MS, LC-MS/MS	[24, 40, 41]

Table 2: SIM method parameters[1]

Group	**Compounds**	**Acronym**	**Parent ions (m/z)**	**Product ions (m/z)**	**SIM detection start time (min)**	**LOD for compounds detected (ng)**
1	Ethylphenylamine	EPA	121.10	106.1, 77.1	6.50	
2	Camphor		152.1	108.1, 95.1, 81.1	7.23	
3	2-Nitrotoluene	2-NT	137.1	120.1, 91.1, 65.1	7.56	
	3-Nitrotoluene	3-NT	137.1	91.1, 65.1		
	4-Nitrotoluene	4-NT	137.1	91.1, 65.1		
4	Triacetin		-	145.1, 103.0, 43.1	11.00	
	Nitroglycerin	NG	-	151.0, 76.0, 46.1		5
5	Dimethyl phthalate	DMP	194.0	163.1, 77.1	14.00	
	2,6-Dinitrotoluene	2,6-DNT	182.0	165.0, 89,1, 77.1,		
	2,5- Dinitrotoluene	2,5-DNT	182.0	63.1,		
	2,3- Dinitrotoluene	2,3-DNT	182.0	165.0, 89.1, 63.1		
	2,4- Dinitrotoluene	2,4-DNT	182.0	165.0, 135.1		
	3,4-Dinitrotoluene	3,4-DNT	182.0	165.0, 89.1, 63.1		
				63.1, 89.1		
6	Diethyl phthalate	DEP	222.10	177.1, 149.0	17.70	
7	Diphenylamine	DPA	169.1	168.1, 84.0, 51.1	18.00	1
8	Methyl centralite	MC	240.1	134.1, 106.1, 77.1	21.00	
	Carbazole		167.1	166.1, 139.1,		
9	Diisobutyl phthalate	DIBP	-	223.1, 149.0, 57.1	23.70	1

10	Ethyl centralite	EC	268.1	120.1, 148.1, 77.1	24.20	2.5
11	Dibutyl phthalate 2-Nitrodiphenylamine	DBP 2-NDPA	- 214.1	149.0 180.1,167.1, 169.1, 139.1	25.10	10
12	Akardite II	AKII	226.1	169.1, 168.1, 77.1	26.20	3
13	4-Nitrodiphenylamine 2,4-Dinitrodiphenylamine	4-NDPA 2,4-DNDPA	214.1 259.1	184.1, 168.1, 167.1 168.1, 167.1, 139.1	28.60	50

[1] Ions indicated in grey are repeated ions within a group.

Table 3: IGSR analysis parameters.

Parameter	Value
Accelerating voltage	25 kV
Working distance	10 mm
Magnification	x 250
Minimum size of the particle	1 µm

Table 4: IGSR analysis of heated and non-heated samples collected after single discharges

Sample no.	Sample location	Subjected to SPME	Characteristic particles	
			No. per half stub	% of whole stub
1L-a	Left hand shooter	No	40	52
1L-b	Left hand shooter	No	37	48
1R-a	Right hand shooter	Yes	99	38
1R-b	Right hand shooter	No	164	62
2L-a	Left hand shooter	Yes	244	53
2L-b	Left hand shooter	No	217	47
2R-a	Right hand shooter	Yes	162	42
2R-b	Right hand shooter	No	226	58

Table 5: Chemical profile GSR collected from the shooter's hands

Ammunition	OGSR category 1	OGSR category 2	Not classified	IGSR

American Eagle 1 discharge	EC	DPA + 2-NDPA and 4-NDPA	PbSbBa > 100 particles
American Eagle 2 discharges	EC	DPA + 2-NDPA and 4-NDPA	PbSbBa > 100 particles
Federal Premium 2 discharges	-	DPA	PbSbBa > 100 particles
Alliant Unique 2 discharges	EC	DPA + 2-NDPA and 4-NDPA	PbSbBa > 100 particles

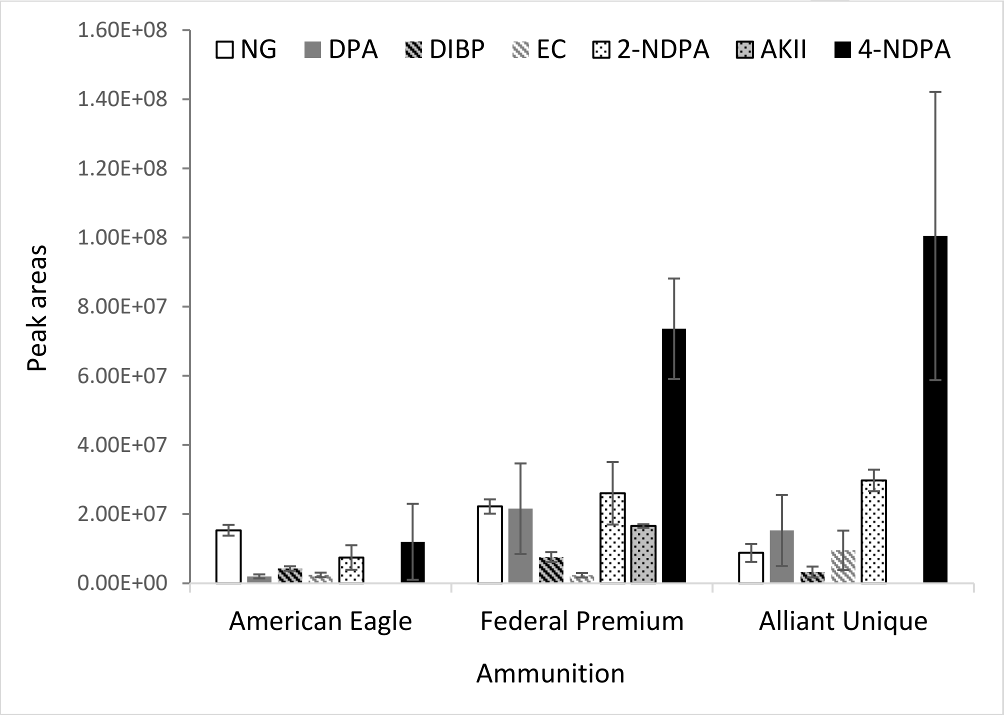

Figure 1: OGSR characterisation of single grains of propellant (n = 3)

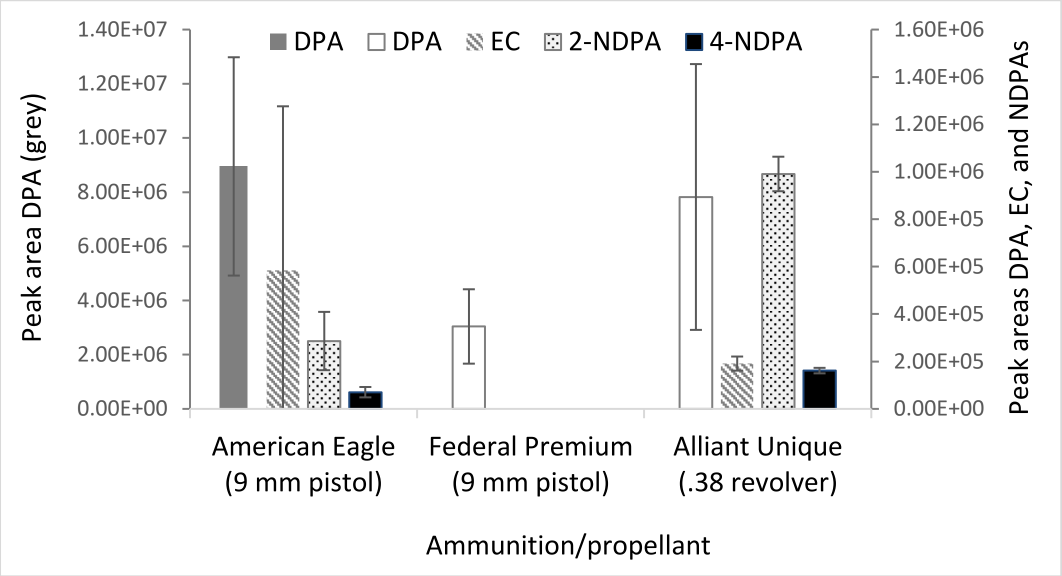

Figure 2: OGSR collected from the shooter's right hand after two discharges (n = 3)

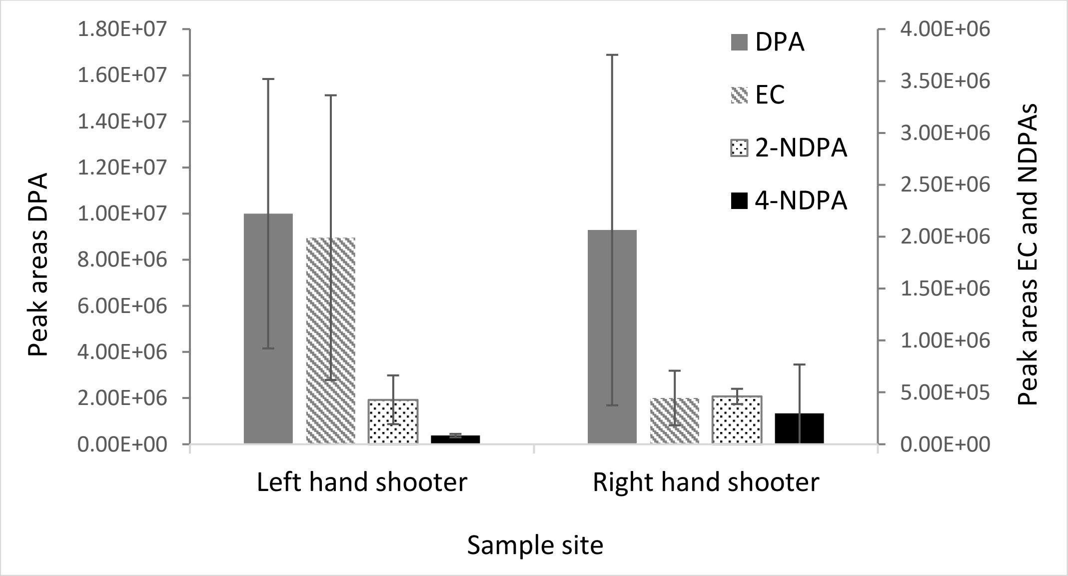

Figure 3: OGSR collected from the shooter's hands after single discharges of American Eagle ammunition using a 9 mm pistol (n = 6)