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A Comparative Study of SEM-EDX and ICP-MS Detection Based on Gunshot Residue Originated from AK-47 and M16 Rifles

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Abstract: This is a comparative study on Gunshot Residues (GSR) originated from AK-47 and M16 rifles by SEM-EDX and ICP-MS. The GSR samples were obtained from multiple body parts of the shooter (hand, cloth and helmet) with elapsed times, as well as from multiple shooting occasions. The SEM-EDX data established the different shapes and dimensions of the GSR samples and the presence of "unique particles". These particles had different elemental combinations with elements such as Pb, Ba and Sb. An automated search system was used for counting the number of 3-elements (Pb, Ba and Sb) called "characteristic particle". The samples collected from M16 showed higher number of characteristic particles than that of the AK-47. The existence of characteristic particles ranged from zero to big numbers and were different for different collecting positions. Right-lower arm gave the highest number of particles. The increase in number of shots led to an increase in single-element particles such as Pb and Sb. Number of particles was independent of the time between firearm shooting and sample collection. ICP-MS analysis showed that the heavy metal concentration increased with the number of shootings for the same source. Sb, Ba and Pb concentrations varied with the various parts of sources. The concentrations of GSR particles decreased when the elapsed time increased. These results concluded that SEM-EDX can establish the unique particles and their elemental composition and that the ICP-MS can quantify the precise concentration of heavy metals in the GSRs.

Keywords: Gunshot Residues, Long Rifle, AK-47, M16, SEM-EDX, Unique Particle, ICP-MS

Introduction

Due to the ever-spreading problem of firearms in an act of violence as well as the increasing acts of terrorism today, control over firearm is an important need of society. The gun problem in Thailand requires serious attention for controlling the use of firearms. Since 2004, violence by firearms has been majorly reported from 3 provinces in the deep south of Thailand. In addition, Thailand has the highest rate of gun-related deaths in Asia (other 73 provinces are safe and no violence) and twice as many gun deaths as in the United States (U.S.) (Wotton, 2016). Various firearms have been used in incidents reported widely across Thailand. Table 1 summarizes the number of cases from 2014 to 2018, collected from the Central Information Technology Center, Royal Thai Police (CITC, 2019). For the years 2014-2015, only the total number of revolver cases were reported. From 2016 onwards, registered revolver and non-registered revolver cases (illegal firearms) were reported separately (CITC, 2019).

Table 1: Government statistical data obtained for firearms in Thailand (CITC, 2019)

Year	Revolver	Rifle	Total	
2014	33708	689	34397	
2015	29949	139	30088	
Year	Registered	Out of register	Rifle	Total
2016	6978	19957	783	27718
2017	3532	18262	664	22458
2018	6517	26099	732	33348

Table 1 data also includes the rifle cases. In Thailand, an AK-47 and M16 rifles can be used legally by government officers and are forbidden for civilian use. From Table 1, it is evident that the significant use of illegal firearms is a national menace, which is embarrassing as well as a challenge to the forensic police. Forensic analysis of gunshot cases involves comprehensive chemical analysis of Gunshot Residues (GSR). The identification and documentation of GSR will be helpful in establishing whether the gunshot was fired from guns. It will also help in establishing the bullet entry hole and the firing distance. However, there is not much literature on GSR from rifles in Thailand or worldwide. Hence, for a better understanding of the composition of GSR from AK-47 and M16 long rifles, we report here the results of GSR samples collected from Thai forensic police and related officers.

Theory of GSR

GSRs or cartridge Discharge Residues (CDR) are particulates expelled from a firearm when the bullets are discharged (Michael and Lucien, 2011). Combustion product residues include unburned and burned components of powder or primer, cartridge case surface, bullet and firearm lubricants (Taudte *et al*., 2016). This residue has metals such as Ba, Pb and Sb, i.e., the major components of the primer mixture of a bullet cartridge. The residue discharges from an available opening of the gun from any available opening in the form of a vaporous plume which then solidifies into fine particles and settles on surrounding surfaces to form the GSRs (Michael and Lucien, 2011).

Based on their nature, GSRs can be classified as organic or inorganic. Organic GSRs (hereafter called OGSRs) come from the propellant powder which involves explosives and additives. Explosives includes nitroglycerine, while additives comprise stabilizers, coolants, plasticizers, flash inhibitors, deterrents and other elements used for improving the powder performance. Stabilizers includes Ethyl Centralite (EC), Diphenylamine (DPA) and/or Methyl Centralite (MC). OGSRs can be identified on the hands with elaspsed post discharge times, even after the loss from evaporation and skin permeation. Inorganic GSRs (IGSRs) consist of elements such as Sb, Ba and Pb (ASTM, E1588-10e1, 2010).

The important job of forensic examination is to identify and classify the GSRs. It should be determined whether GSRs can be observed in a sample procured from the hands of the suspect or clothes obtained from the crime scene. Another job of forensic examination is to compare the traces obtained from the crime scene with the micro-fiber traces obtained from a suspect's dress and determine their source of origin (ASTM, E1588-10e1, 2010).

History of GSR Analyzation

The study on GSRs was started in 1993 by Teorodo Gonzalez, Mexico Police City Laboratory (Meng and Caddy, 1997; Romolo and Margot, 2001). The dermal nitrate or paraffin test, also known as color and spot testing was the first method to be reported to determine entrance wounds or bullet holes or test for GSRpresence (Tugcu *et al*., 2006). Although the main issue of using this method is its presumptive nature, it is still employed in a few countries for establishing the presence of GSRs (Martiny *et al*., 2008).

The major analytical methods for detecting primer residues include Atomic Absorption Spectrophotometry (AAS), Neutron Activation Analysis (NAA) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS). As a bulk analysis method, the NAA method has been used to determine various elements such as Ba, Sb (major), Cu and Au (minor) found in IGSR (Capannesi and Sedda, 1992). Capannesi and Sedda also used the NAA method to detect the 13 trace elements present in jacketed bullet and lead core. NAA has also been used to determine firing distances (Krishnan, 1974a) as well as GSRs on the shooter's hands (Pillay *et al*., 1974; Krishnan, 1974b; Rudzitis and Wahlgren, 1975; Krishnan, 1967; Kilty, 1975). However, NAA had several drawbacks. This method is expensive as well as time-consuming (Schowoeble and Exline, 2000) and cannot be used to analyze Pb. In addition, this methodology requires trained personnel for performing the analysis, a nuclear reactor to serve as a neutron source and irradiated samples (Romolo and Margot, 2001).

Conventional AAS was used to detect Pb in GSRs samples; however, it was inadequate for analyzing Sb and Ba (Krishnan, 1974a). This method was modified by adding electro thermal atomizers such as graphite tube furnace, tantalum and carbon rod to enhance the analysis efficiency of Sb and Ba in the samples of GSR (Romolo and Margot, 2001). The samples were collected using a swab technique by dripping 5% nitric acid onto the cotton tip (Cooper *et al*., 1994; Koons *et al*., 1987). Ravreby (1982) used flameless and flame AAS to analyze GSRs collected from bullet holes. The results established that Sr, Zn, Cu, Sb, Pb, K, Ni, Ba and Fe [obtained from the paint on the bullet tips of tracer rounds] and Sn originated from the bullet, case, primer and firearms. These results helped in the identification of the type of

bullet used. The main drawback of using AAS methods described by Koons and his co-workers was the incomplete extraction of Sb. Though Pb and Ba showed complete extraction from collection swabs, control experiments showed only 60-70% extraction (Koons *et al*., 1987). This can be because of enhanced absorbance of Ba by different matrix constituents and the variable absorbance-time profiles for Sb (Koons *et al*., 1988). The effectiveness of AAS in GSR analysis was also discussed by Aleksandar (2003).

Koons *et al*. (1988) used ICP with Atomic Emission Spectroscopy (AES) to detect the presence of Ba in swabs. For the element Ba, ICP/AES showed more sensitivity than AAS and can be ascribed to reduce interference from the constituents of common swab, good accuracy and precision and wide linear dynamic range. Koons also reported successful application of ICP-MS to analyze GSRs originating from primers (Koons, 1998). This method had greater detection limits when compared with Graphite Furnace-AAS (GFAAS) and ICP-AES and exhibited faster analysis than GFAAS. MS used in this method facilitated the detection of different isotopes of Sb, BA and Pb.

Zeichner *et al*. (2006) studied Pb isotope ratios in IGSR by using ICP-MS. Levels of Pb isotope were potentially useful in crime scenes such as a shootout situation where several types of firearms and bullets were discharged. Via isotope distribution, ICP-MS could also determine the source of primer. This report also presented the possible link of bullet hole to the firearm from which the bullet was discharged. They also reported the problem of "Pb memory," i.e., the presence of Pb from previous firings. This Pb was also detected in residues of subsequent discharges even after the firearm was cleaned thoroughly. This "Pb memory" reduced the association level between the residues collected from ammunitions that were fired (bullet and case) and residues obtained from the barrel of the firearm.

The above-mentioned analytical methods NAA, AAS and ICP-MS involve bulk analysis and do not have the specificity necessary for detecting GSR in the field of forensic science. The findings showed only the presence of Pb, Ba and Sb, which could also be originated from the environment. For example, Pb and Ba were found in the emissions from combustion and paint, respectively (Wolten *et al*., 1979a).

Qualitative methods used to identify GSRs include Scanning Electron Microscopy with Energy Dispersive analysis by an X-ray detector (SEM-EDX). The microscopic technique of SEMemploysan electron beam to visualize the object. The SEM technique has a magnification in excess of 100,000 times and high resolution. The EDX system is used to analyze the elements of GSR particles. Detailson SEM-EDX principles and its application in IGSR analysis are provided elsewhere and, therefore, have not been included in the present study (Romolo and Margot, 2001; Martiny *et al*., 2008; Wolten *et al*., 1979a). For the last 4 decades, the SEM-EDX method is being used in forensic science laboratories globally (Elad *et al*., 2013). The principle and techniques of SEM-EDX for GSRs have been developed since 1968. The words "characteristic," "consistent with GSR" and "unique" were coined by Wolten *et al*. (1979b) and reported the characterization of GSR from several handgun cartridge samples. GSR particles were classified by its elemental composition, morphology and size. They established that four compositions, i.e., Ba-Ca-Si with traces of S, Pb-Sb-Ba, Ba-Ca-Si with trace of Pb if Cu and Zn are absent and Sb-Ba, observed in only GSRs were considered "characteristic". The "consistent with GSR" but not unique included the following composition: Pb-Ba, Pb-Sb, Pb, Ba if S is absent or present only as a trace and Sb (rare). The "unique" particle was considered if the particles in a GSR sample were found to be spheroidal. Wolten *et al*. (1979a) reported that the particulate matter collected from different commercial and industrial workers had no resemblance with GSR and "characteristic" particles were not detected. These results were expanded and corroborated by Garofano *et al*. (1999), who confirmed the uniqueness of Sb, Ba and Pb particles for IGSRs.

The advantage of SEM-EDX detection is its ability to morphologically and chemically analyze individual particles of IGSR. Aleksandar (2003) commented that SEM-EDX helps in confirming whether the particles originated from firearm discharge. However, SEM-EDX alone cannot confirm whether a particular person discharged a weapon.

The disadvantages of SEM-EDX in the detection of IGSRs were summarized by Wallace and Keeley (1979). The major disadvantage of using SEM-EDX was the longer detection time for locating the particle due to large sampling area. This problem can be eliminated by enhancing the concentration of the sample and sampling efficiency. In this regard, White and Owens (1987) replaced the SEM manual search with automated search systems for primer discharge residue particles. The introduced automated systems were helpful in locating the particles by analyzing their elemental composition. The performance of GSR automated systems was validated by standard test samples. The standard sample, artificial GSR-like samples were used to achieve GSR Proficiency Test (PT) and as quality assurance measures in forensic science laboratories (Niewoehner *et al*., 2003). At present, most forensic laboratories use SEM-EDX to analyze IGSRs derived from the primer (ASTM, E1588-10e1, 2010; Elad *et al*., 2013; Wallace and Keeley, 1979; White and Owens, 1987; Wolten *et al*., 1977; Costa *et al*., 2006).

Another aspect of GSR analysis include modern firearm and nontoxic bullet. The lead-free bullet types do not have Pb compounds present in primers. These primer types have been developed to avoid Pb poisoning. The findings of some studies on GSRs produced by lead-free bullets are summarized below. In 1980, Dynamit Nobel AG (founded by Alfred Nobel, Swedish chemist, in the year of 1865) developed a new bullet to minimize airborne lead level called Sintox (Hagel and Redecker, 1986). Recently, new bullet from Geco Sinoxid™, Geco Sintox™ and Hirtenberger Lead Free™ were analyzed, compared and reported by Niewoehner and Wenz (1999). The major elemental components of the lead-based bullet were Ba, Sb and Pb in Geco Sinoxid™, whereas the major elemental components of lead-free bullet were Sb and Ba in Geco Sintox™ and Sr in Hirtenberger Lead Free™. These three types of bullets produced particles with unique morphologies. There are limited studies that have described particles similar to those produced when lead-free bullet is fired from different sources (Abrego *et al*., 2014). Although studies on the types of bullets and different firearms (piston or revolver) are well established, only a few studies are known with long rifles.

In general, the success of GSR analysis and interpretation of a crime scene depended on factors such as methods of collecting samples, collecting position on the shooter, elapsed time after shooting, etc. Several techniques can be used for collecting GSR samples; thus, it is essential to select the most appropriate technique for ensuring maximum sampling efficiency. Inorganic residues are collected from skin surfaces using the tape lift procedure (Romolo and Margot, 2001). This procedure can also be used for collecting samples from hands, hair (Zeichner and Levin, 1993) and vehicles (doors, seat backs and seats, windows and helmet) (Shaffer and Yi, 1999). Zeichner and Eldar (2004) extracted OGSR from tape stubs by analyzing the samples using SEM-EDX. Tape lifting is advantageous when the particles settle on the surface of a material. However, this technique cannot be used for collecting GSRs from clothing due to restricted sampling area, loss of tape stickiness and presence of cloth fibers and other unwanted particles. The presence of unwanted particulates makes SEM analysis more difficult (Andrasko and Pettersson, 1991).

Water, acetone, water⁄Solid Phase Extraction (SPE), 2-butanol: Methanol (1:3) and 5% of HNO_3 were used as solvents to extract the OGSR from the shooter. Twibell *et al*. (1982) studied 8 different aqueous-based solvents to extract Nitroglycerine (NG) from a person's hand handling explosives and compared their efficiency. NG stability in the solvent used and the quantity of interfering materials obtained from the hands using cotton swabs were also examined. In this study, the partial purification of extracts using thin-layer chromatography before analyzing the samples caused the aqueous solvents to yield the best recoveries. In the absence of purification, the microorganisms that grew in the solutions rapidly degraded OGSRs. Ethanol provided the most stable, consistent and complete recovery.

GSR deposits on a person and in a crime-scene are continuously lost in normal activities. The time period within which GSR should be retained is difficult to generalize (Meng and Caddy, 1997). Therefore, it is essential to understand the effect on elapsed time of GSR particles on clothing, helmet, hands and other materials of sample collection. This can help to determine whether a sampling is necessary, particularly in those cases where the suspect has discharged a firearm at a particular period before apprehension) (Dalby *et al*., 2010). GSR may be destroyed or diminished if the sample collection of residues or movement detection is delayed or the body has been washed prior to autopsy (Molina *et al*., 2007). At every 1 to 3 h post discharge from a firearm, the numbers of GSR particles undergo rapid loss (Dalby *et al*., 2010). However, studies have also reported the presence of GSR particles 5 days after discharge from a firearm (Blakey *et al*., 2018).

Increasing the sensitivity and selectivity of both OGSR and IGSR has been a promising research for gaining information about any given sample. Analysis of trace elements or compounds using a combination of the above-said techniques with macroscopic or microscopic analysis of particle or grain morphologies would be an ideal approach for sample analysis.

SEM/EDS or ICP-MS

Only few studies on comparative study of gunshot residues by using SEM/EDS and ICP-MS were reported in the literature. Steffen *et al*. (2007) presented the collected data of gunshot residue samples. By using the SEM/EDX technique, they classified the samples into three groups using the analysis-software: 'Consistent with gunshot residue', gunshot residue characteristic' and environmental particles. It is difficult to differentiate between GSRs and similar environmental particles. Isotope ratio measurements based on ICP-MS detection were used as a supplementary feature to SEM/EDX technique, to lower the risk of misclassification.

Rayana A. Costa and her co-worker studied the gunshot residue originated from a clean range ammunition of a 0.38 caliber revolver and a 0.40 caliber pistol by using colorimetric test, SEM/EDX and ICP-MS as a function of the number of shots. They reported that the SEM showed "unique particles" for the clean range ammunition GSR, in contrast to the literature. Elemental compositions of the "unique particles" were reported by EDX. The results primarily identified Al, C, Cl, Cr, Cu, O, Fe, K, Mo, S, Si, Sr, Ti and Zn. They suggested that the main elements detected were Cu and Zn that were obtained from Cu\\Zn alloy used

in the cartridge. ICP-MS was found to be sensitive, rapid, efficient and selective technique that can be used to determine the amount of isotopes of Ba^{138}, Sb^{121} and Pb^{208}. They also reported that ICP-MS provided positive results for Ba, Pb and Sb, with maximum concentrations of 10.9 $\mu g \cdot L^{-1}$, 4.20 $\mu g \cdot L^{-1}$ and 0.119 $\mu g \cdot L^{-1}$, respectively, as well as for Zn, Ti, Sr, Mo, Cr, Cu and Al (Costa *et al*., 2016)

GSRs Analyzation: Situation in Thailand and the Objectives of This Research

The GSRs used in this research were taken from long rifles (AK-47 and M16). In Thailand, cases involving long rifles are less frequent and considered as a case of violence. Until now, Thailand's forensic officers have investigated GSRs by using the AAS and ICP-MS technique. The SEM-EDX technique is an alternative method for the examination of GSRs. SEM-EDX technique is widely used in global forensic laboratories and the artificial GSR-like samples for proficiency test and quality assurance control are easily available. The objective of this research was to compare the analysis between quantitative ICP-MS technique and qualitative SEM-EDX technique for investigating the GSR samples collected from AK-47 and M16 rifles.

Materials and Methods

The GSRs examined in this study were obtained from shooting by volunteers at the office of police forensic science, center 2 (Chonburi, Thailand). AK-47 (model: Modified Kalashnikov Automatic rifle Fig. 1a) and M16 (Fig. 1b) long rifles were fired and the corresponding GSR samples were collected. Bullets corresponding to the M16 long rifle and AK-47 were 5.56×45 mm NATO (Fig. 1c) and 7.62×39 mm Russian (Fig. 1d), respectively. For a comparative propose, GSR samples were collected at different conditions and from different parts of volunteers (right hand, left hand, cloth and helmet), different number of shoots (1, 3, 6 and 9 rounds) and at different elapsed times (1, 3, 6 and 9 h after 3-round shooting). The equipments used in this research are represented in Fig. 1.

According to the literature (Brozek-Mucha *et al*., 2003), GSR samples collected from the hands of those people who had no contact with firearms (police officers working in the building containing a shooting gallery) were negative. Thus, for this study, data were collected from volunteers who ordinarily had no contact with firearms and used both hands to hold their guns when firing either one, two or three rounds of test shots. Before the experiments, the volunteers had no or minimal contact with the surroundings of the shooting station. Sample collection was done from volunteer's hands, cloths and helmet.

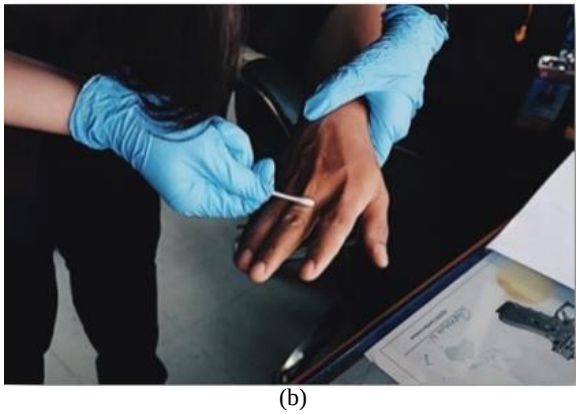
Sample Collections

Two GSR collection techniques were used. (i) Tape lifting technique by a GSR kit, was used for SEM-EDX analysis. Details of this technique are described elsewhere (Zeichner and Levin, 1993; Molina *et al*., 2007; Zeichner and Levin 1997). The GSR kit was a double-side adhesive carbon tab attached on one side to aluminum stubs. The GSR kit was pressed onto the skin many times covering the whole sample area. When holding a rifle, the following parts of the body were exposed to airborne particles: Outside surfaces of fingers, areas around the crook of the thumb and two arms and hands. The particulate materials were also detected in the folds of the skin. This process is shown in Fig. 2a.

Swabbing technique was used for ICP analysis (Cooper *et al*., 1994; Koons *et al*., 1987). A plastic shaft cotton bud was used to collect the GSRs from shooting volunteer. First, cotton bud was dipped in 5% of HNO_3 and then rolled over on the volunteer's hand. This process was repeated over the area of interest (hands, cloths and helmet) until the solvent (5% of HNO_3) was nearly evaporated. A blank sample was prepared by dipping the bud in plain 5% of HNO_3. All cotton buds were cut and stored in a refrigerator at 4°C. This process is shown in Fig. 2b.

Fig. 1: Instruments used in the experiment (a) AK-47, (b) M16, (c) bullets and (d) set of GSR kit

(a)



(b)

Fig. 2: Sample collection techniques: (a) GSR kit, (b) swabbing techniques

Table 2: Operational conditions and parameters of ICP-MS

Nebulizer spray chamber	Cyclonic glass with shield
Nebulizer	Mein hard concentric. Type C
Radio frequency power	1550 W
Auxiliary gas flow rate	1.20 L·min^{-1}
Plasma gas flow rate	16.00 L·min^{-1}
Nebulizer gas flow rate	1.04 L·min^{-1}
Replicates	3
Isotopes	Pb^{208}, Ba^{138}, Sb^{121}, Sb^{123}, Al^{27}, Ti^{47}, Ti49, Mo^{95}, Mo^{96}, Mo^{98}, Cr^{52}, Zn^{66}, Cu^{63}, Cu^{65} and Sr^{88}

Samples collected from the back of the hand were mainly around the thumb, forefinger and the webbed area in between. Samples collected from the palm of the hand were mainly from the large portion of the palm first, followed by the back of hand including fingers. All the samples collected were stored in rigid containers individually to avoid accidental contamination from the exposed surfaces.

Qualitative Analysis via SEM-EDX

SEM-EDX is commonly used to analyze IGSRs (Wolten *et al*., 1977). GSR samples on the stubs were directly moved to the SEM chamber by using forceps. No other processing of sample was done. An automated GSR analysis was performed at the Central Institute of Forensic Science (CIFS, Thailand) using a TESCAN MIRA3 XMH SEM. This SEM contained super atmospheric thin window Oxford instruments Si (Li) EDX detector and an Oxford instruments INCA 350 EDX system. The INCA GSR package was used to perform GSR automated search. This analysis was based on a four-stage process. First, the samples were moved to the SEM instrument with a predetermined field and the program automatically searched for particles of defined properties. Subsequently, the whole stub area was divided into rectangular frames and were analyzed. The size and number of the frames depended on the applied scanning resolution and magnification. Next, a Backscattered Electron (BSE) image of that field was acquired. The program's initial setting defined the stub positions and the standards of cobalt and rhodium for establishing the BSE signal range. This was followed by the performing an EDX spectrum of BSE image of bright particles present in the field. The chemical classes of particles were defined based on the list of contributing elements, mainly Pb, Ba and Sb and their composition range (between 0% and 100%). Finally, the particles were sorted into compositional groups. The fraction of GSR particles was ~1 mm in size.

Quantitative Analysis via ICP-MS

Before the ICP-MS analysis, the samples collected using the swabbing technique were stored in 15 mL-sized polypropylene tubes. The LSX-213 Octopole Reaction System ICP-MS was used to investigate the existence of organic GSRs. Table 2 shows the operating conditions of the equipment. Initially, 2 mL of nitric acid (HNO_3) was added in all tubes at 10% (v/v), followed by dilution using 18.2 MΩ.cm ultrapure water to obtain 10 mL volume. The samples were then placed in an ultrasonic bath at 25 kHz for 20 min and then heated in water bath at 100°C for 1 h.

For selecting isotopes Al^{27}, Ba^{138}, Cr^{52}, Cu^{63}, Cu^{65}, Mo^{95}, Mo^{96}, Mo^{98}, Pb^{208}, Sb^{121}, Sb^{123}, Sr^{88}, Ti^{47}, Ti^{49} and Zn^{66}, the standard conditions mentioned in the ICP-MS software were used. The analytical curve was created using a set of five points of the following concentrations: 10; 20; 40; 80; and 100 µg·L^{-1}. This was followed by the determinations of correlation coefficient (R^2), Limits Of Detection (LOD) and Quantification (LOQ) and recovery (%R). LOD and LOQ were determined based on the International Union of Pure and Applied Chemistry using Equations (1) and (2):

$$LOD = 3 \times (s / a) \quad (1)$$

$$LOQ = 10 \times (s / a) \quad (2)$$

where, s is the standard deviation of data and a is the slope of a graph (McNaught and Wilkinson, 1997).

Results and Discussion

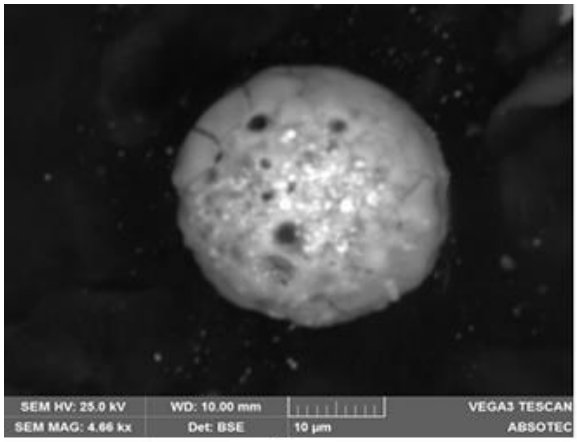
Qualitative Analysis via SEM-EDX

Figure 3 shows the SEM-EDX image of a typical "unique particle" obtained from the first-round of the AK-47 (Fig. 3a) and the M16 (Fig. 3b) rifles and the images were similar to ones reported by Brozek-Mucha (2007). Typically, the GSR samples possess regular or distorted spherical-like shape. Occasionally, objects similar to sponge fragments or spherical shell fragments and spheres aggregates of various sizes were also observed. The particle shown in Fig. 3a was ~20 µm in diameter, while the one in Fig. 3b was ~60 µm. The range of dimension of GSR particles (1-100 µm) distinguished GSR from the environmental solid particles (>100 µm) (Brozek-Mucha, 2007).

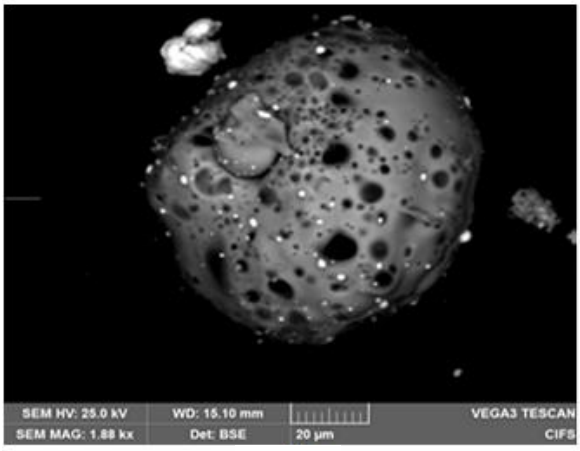
The GSR particles detected in the collected samples were classified based on their elemental content (Meng and Caddy, 1997). Figure 4 shows the EDX spectrum of the unique particle. This spectrum clearly showed the characteristic elements Sb, Ba and Pb. The presence of unique particle with characteristic elements indicated that this sample was gathered from the shooter's hands. Figure 4 shows the overlapping signals of calcium with antimony as well as titanium with barium.

The EDX spectrum (Fig. 4) shows the elements found in GSR particles collected from first-round of AK-47. They were C, Ca, O, Pb, Cl, Sb, As, Ba and Fe (Fig. 4). Al, Cu, K and Si originated from the primer mixture. N, C and O corresponded to the nitrocellulose. Presence of Al, Ni, Cu and Zn can be attributed to the bullet, cartridge case, metal jacket and bullet coating as they were usually made of brass (70% Cu: 30% Zn) (Zadora and Brozek-Mucha, 2003). Low intensity signals of Ca, Cl, Cr, S and Ti elements were also detected.

GSR particles were examined by the GSR automated search program. Figures 5 and 6 show the existence of characteristic particle collected from 1, 3, 6 and 9-round of the AK-47 and M16, respectively.



(a)



(b)

Fig. 3: SEM images of the typical "unique particle": (a) AK-47 and (b) M16

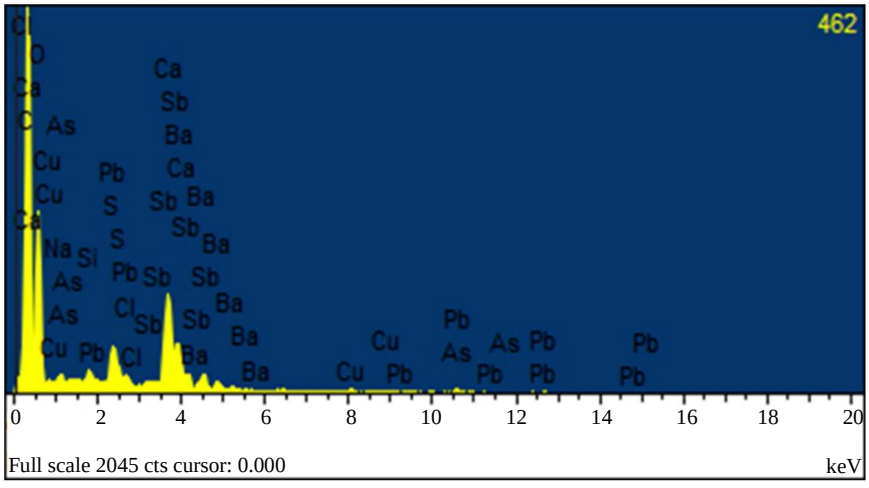


Fig. 4: The EDX spectrum of elemental compositions from GSRs

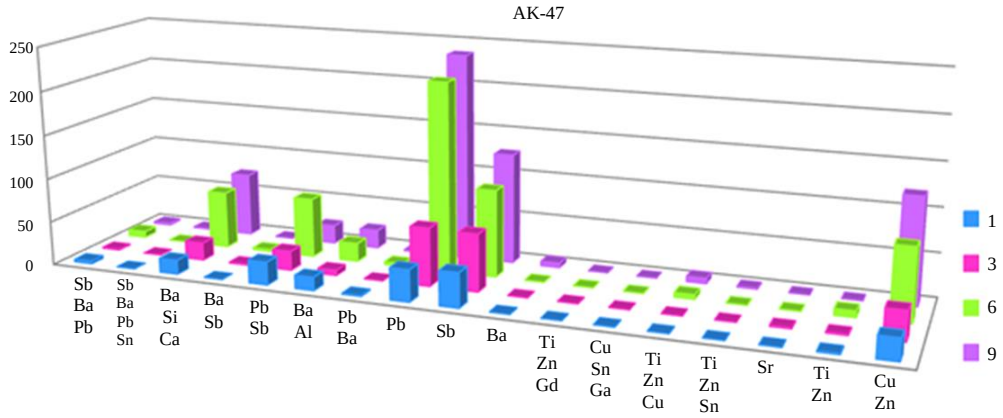


Fig. 5: Numbers of particles as a function of number of shots collected from the AK-47

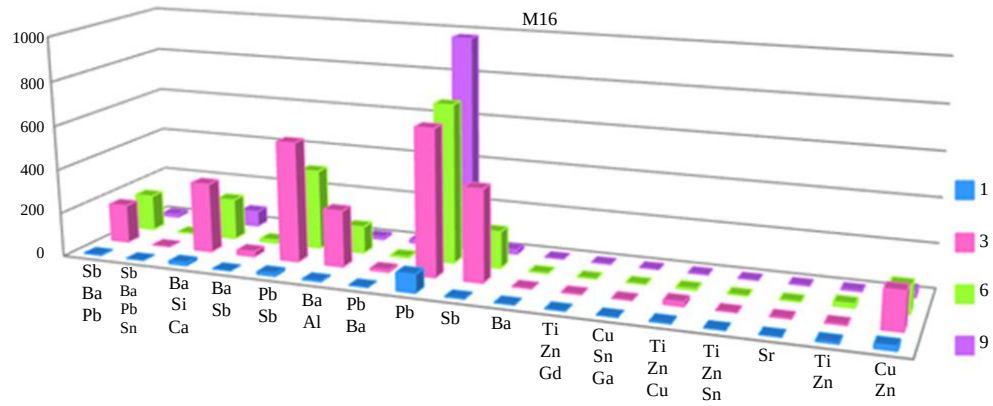


Fig. 6: Numbers of particles as a function of number of shots collected from the M16

Samples collected from the M16 showed higher number of "characteristic particle" than that of the AK-47. Further, the increase in number of shots led to an increase in single-element particles such as Pb and Sb. In Fig. 5, one- and two- component particles called "indicative" were also noticed. Indicative particles were also a characteristic of GSR. Particles containing only one component such as Sr, Pb, Sb, Ba and Zn can be assigned to the projectile, case, its jacket and the barrel. However, these can also be found in subjects that used daily and, thus, cannot be used as an evidence of firearm shooting (Zadora and Brozek-Mucha, 2003).

A 3-round shot was used to study the GSR particles as a function of elapsed times. Figure 7 and 8 show the number of element particles as a function of the elapsed time of the samples collected from the AK-47 and the M16, respectively. The number of particles varied from zero to large numbers and did not depend on the time between the time of shooting and the sample collection time. This can be attributed to the fact that after the volunteer used the firearm (shoot the rifles), they lived a normal life. Therefore, the IGSRs may have lost from the volunteer's activities such as washing, doing work, etc., (Meng and Caddy, 1997). IGSR retention on the shooter's hands varied significantly from normal activities. The maximum recovery times for particles collected from shooter's hands ranged from 1 to 48 h (Kilty, 1975).

The numbers of "characteristic particle" as a function of collecting position of the shooters were detailed in this section. Figures 9 and 10 show the plot of numbers of particle varied from zero to large numbers (greater than 100) versus the collecting positions of the shooter. Right-lower arm had higher number of particles than the left-upper arm. Particles were also observed on the helmet. These results were not unexpected. During the discharge of a rifle, the shooter wears a helmet and uses both of his arms to hold the rifle. Difference in the number of particles were observed between AK-47 and M16. M16 gave a small number of particles than that of AK-47. Both rifle's discharge modes were different and were dependent on the CDR mechanism, geometry of the rifle and a bullet's primer.

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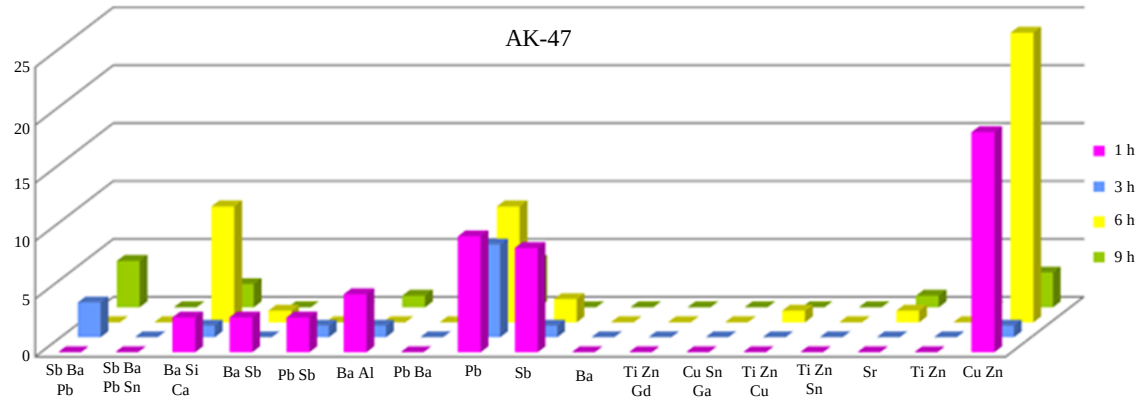

Fig. 7: Numbers of particles as a function of elapsed time collected from AK-47

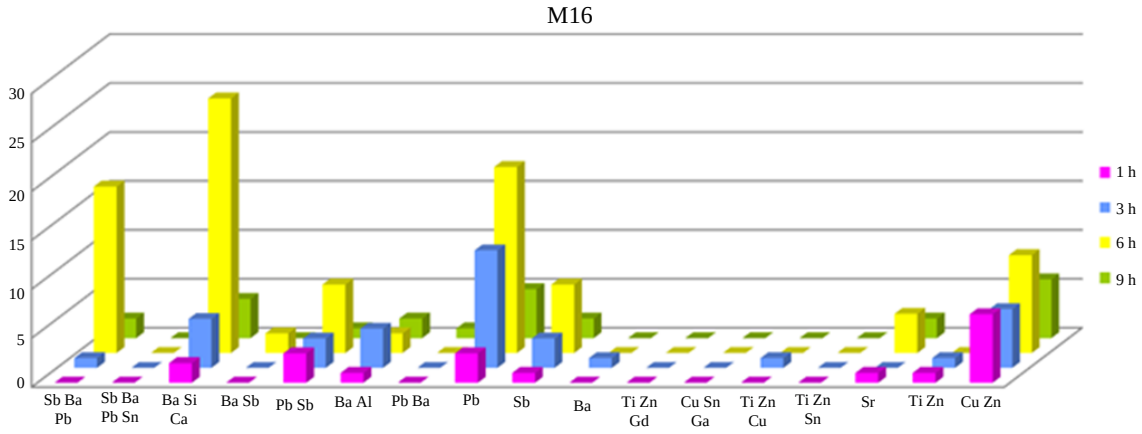

Fig. 8: Numbers of particles as a function of elapsed time collected from M16

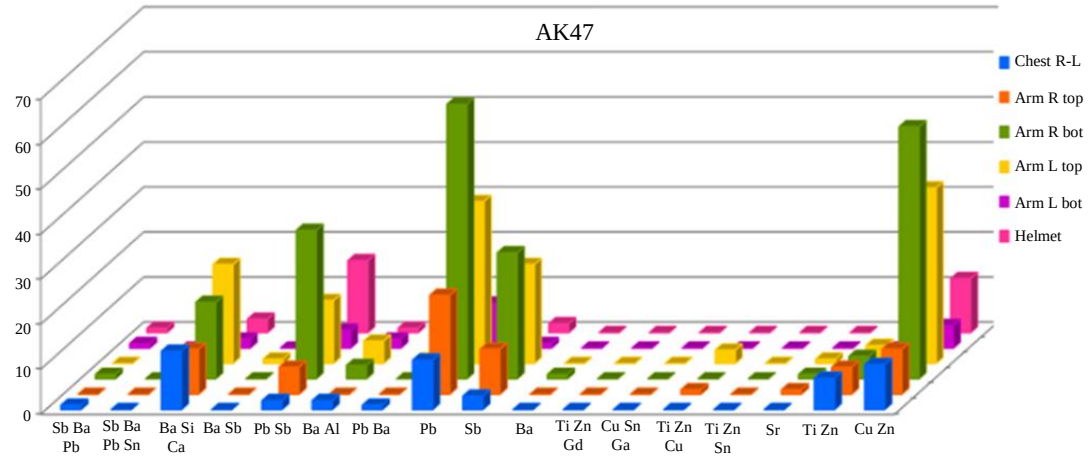

Fig. 9: Numbers of particles as a function of collecting position collected from AK-47. (Chest R-L = collected from shooter's cloth, Arm R top = collected from shooter's upper-right arm, Arm R bot = collected from shooter's lower-right arm, Arm L top = collected from shooter's upper-left arm, Arm L bot = collected from shooter's lower-left arm)

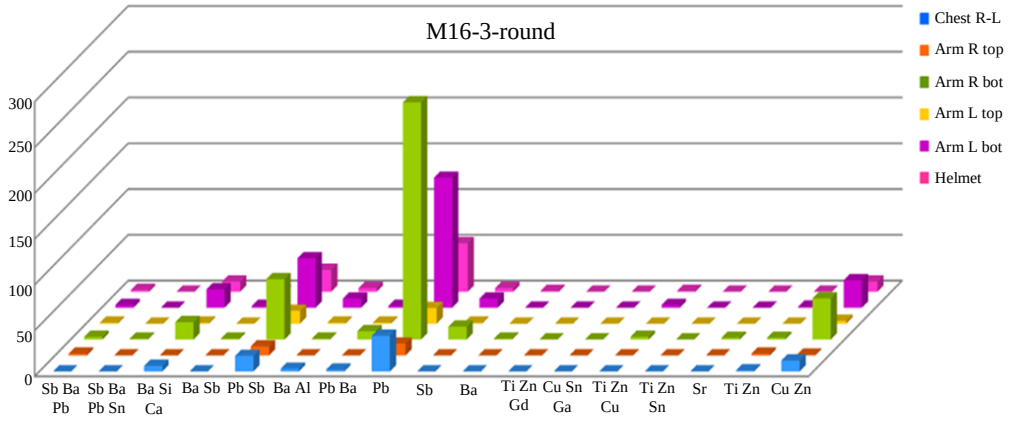

Fig. 10: Numbers of particles as dependent of collecting position collected from M16

Table 3: Figures of merit obtained from ICP-MS analysis for the quantification of Pb^{208}, Ba^{138}, Sb^{121}

Isotope	LOD (µg• L^{-1})	LOQ (µg• L^{-1})	Recovery (%)	R^2
Pb^{208}	8.72	24.65	82-101	0.9990
Ba^{138}	0.53	1.76	95-105	0.9997
Sb^{121}	1.95	6.67	82-118	0.9983

GSRs collected from volunteer's hands, immediately after shooting had the maximum concentration. Subsequently, when the sampling was done after more elapsed times, the concentration of GSRs was higher in hair, helmet and clothes than in hands.

Quantitative Analysis via ICP-MS

SEM-EDX analysis targeted organic GSRS having Pb^{208} Ba^{138} and Sb^{121}. The plot of elemental concentration (Pb, Ba and Sb) versus their signal intensities (not shown here) provided the LOD, LOQ, %Recovery and R^2. Table 3 shows the values of LOD, LOQ, %Recovery and R^2 determined by the ICP-MS analysis. It is well known that the Sb^{121} isotope has better sensitivity and accuracy than Sb^{123}.

The semi-quantitative analysis was reported in part per billion (ppb) units. The expanded uncertainties of these concentrations were calculated using a coverage factor of two (k = 2) approximating to a 95% level of confidence. Figures 11 and 12 show Sb, Ba and Pb concentrations collected from both the left and right hands as a function of difference in number of shooting for the AK-47 rifle and M16 rifle, respectively. The plots showed that right hand had higher OGSRs concentrations than that of the left hand. This can be reasoned to the position of hands when the shooter holds a rifle. Pb, Ba and Sb concentrations linearly increased with the increase in the number of shooting. In addition, concentrations of Pb were approximately ten and five times of that of Sb and Ba, respectively. Higher concentration of metals observed on the right hand than the left hand was consistent with the SEM-EDX data. In addition, these metals increased systematically as a function of the number of shots.

Concentration of Sb, Ba and Pb collected from AK-47 and M16 rifles linearly decreased with the increase in elapsed time (Fig. 13 and 14). This can be attributed to the loss of GSRs from the volunteer's activities. The decrease in Pb, Ba and Sb concentrations as function of the elapsed time were reported in the literatures (Meng and Caddy, 1997; Kilty, 1975). Presence of aluminum (Al^{27}), copper (Cu^{63}) and calcium (Ca^{40}) were also observed (not shown here).

Understanding the importance of elapsed time of GSR particles is paramount. When the elapsed time was two to three days, the presence of GSRs on the suspect was very small and was difficult to analyze by SEM-EDX technique. In such scenarios, ICP-MS is a good alternative method due to its ability of low detection limit level (ten to hundreds of parts per billions).

We established that the GSRs collected from the suspect were useful in forensic analysis. Previous literature showed that ICP-MS method was better than the ICP-AES technique for the analysis of residues originating from primers because of their superior detection limits and detection of several isotopes for each of the target elements (Pb, Ba, Sb), (Romolo and Margot, 2001; Costa *et al*., 2016). ICP-MS was effective in detecting elements, which may be present in specific bullets such as strontium in some nontoxic primers, cobalt in Nyclad™ bullets, or Cu, Ni, or Zn in jacketed bullets (Abrego *et al*., 2014). ICP-MS was also helpful in identifying the source of primer having Pb, by isotope distribution (Koons, 1998).

Use of the ICP-MS was more challenging than that of SEM-EDX as ICP-MS is a time consuming and destructive method, leaving the chemical solution to the environment. However, the adoption of a "case by case" approach to GSR analysis by forensic labs should be favored. The comparison of samples collected from a

victim, suspect, or crime scene to firearms, bullets, or cartridge cases has been reported in literatures and shown to be an effective approach within a "case by case" framework (Dalby *et al*., 2010; Romolo and Margot, 2001).

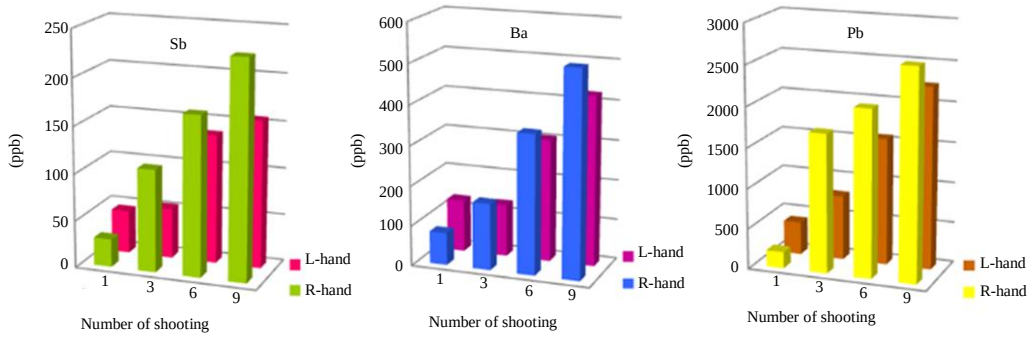

Fig. 11: Concentrations of Pb, Ba and Sb as function of number of shotsfor AK-47 (L-hand = Left hand, R-hand = Right hand)

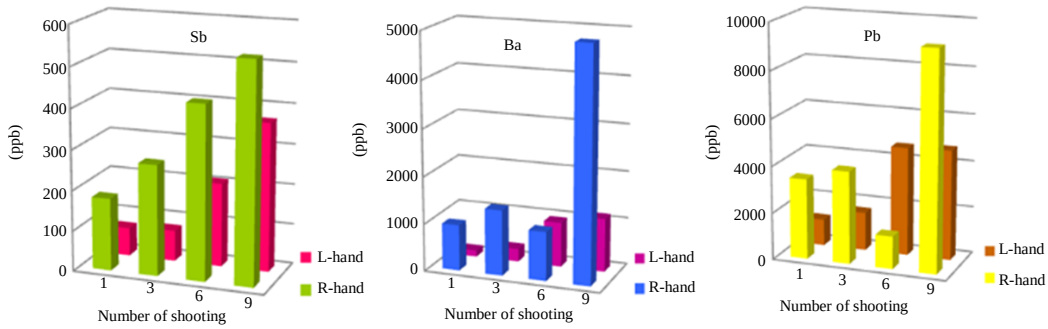

Fig. 12: Concentrations of Pb, Ba and Sb as function of number of shotsfor M16

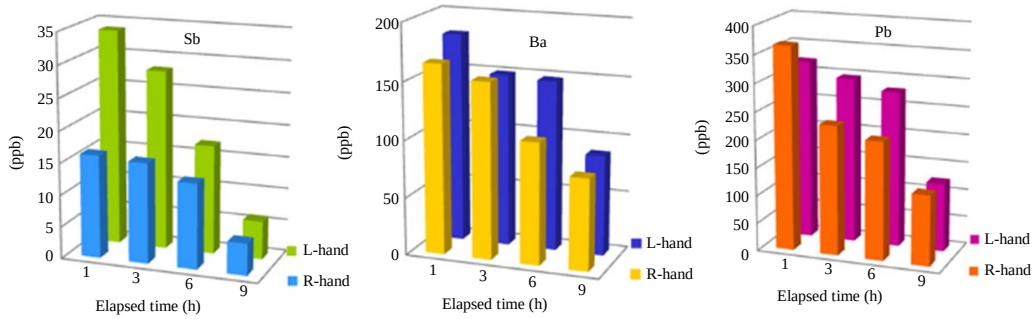

Fig. 13: Concentrations of Pb, Ba and Sb as a function ofelapsed time for the AK-47 rifle

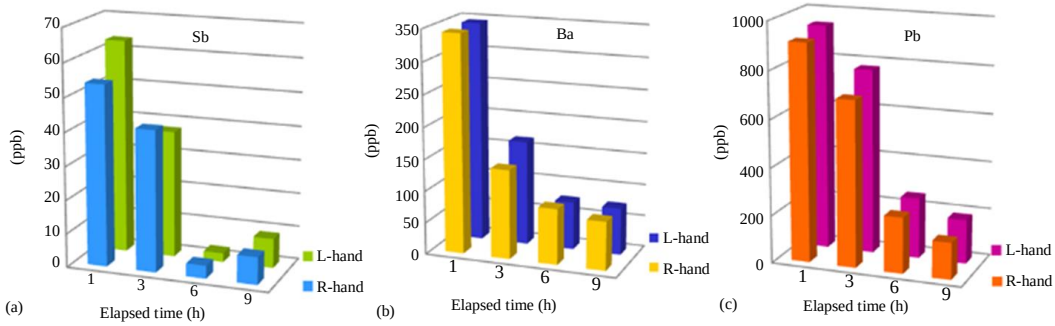

Fig. 14: Concentrations of Pb, Ba and Sb as a function of elapsed time for the M16 rifle

Conclusion

In this study, we have successfully reported the characteristic of GSRs collected from AK-47 and M16. "Unique particles" and "characteristic condition" indicated the persistence of GSRs. The different sources of collected GSR samples gave a different number of particles. Surge in the number of shooting rounds increased the GSR particles in samples. Samples collected after longer elapsed times also gave useful information. Increase in elapsed time decreased the number of GSR particles. These trends confirmed that firearm GSR interpretation principles are effective for the long rifles AK-47 and M16 used in this research.

The SEM-EDX technique was useful in investigating both "characteristic" elemental composition and identifying the various sizes and shapes of GSR samples as well as the "unique particles". Counting the unique particles and comparison with the other samples were successful by the automated system equipped with the SEM-EDX. It was confirmed that the ICP-MS technique was sensitive, rapid, efficient and selective in quantifying the isotopes ofAl^{27}, Ba^{138}, Cr^{52}, Cu^{63}, Mo^{98}, Pb^{208}, Sb^{121}, Sr^{88}, Ti^{47} and Zn^{66} in GSRs as functions of the number of shoots (n = 1, 3, 6 and 9). The presence of isotopes of Al^{27}, Zn^{66}, Cu^{63} and Sr^{88} suggested that they were the conventional markers and the most abundant species of the bullets used. Besides that, using the AK-47 long rifle, Pb in GSRs (differently to observed by qualitative SEM-EDX technique) were detected and quantified up to a maximum concentration of 2500 ppb (after 9 shots).

In this study, we concluded that SEM-EDX can identify the unique particles and ICP-MS can only measure the exact concentration of the heavy metal in the GSRs. We believe that our study may contribute to the improvement of the technology used in the GSR analysis in Thailand. In essence, SEM-EDX is a powerful tool that can be used by forensic scientists to discriminate and classify evidence material due to the determination of morphology and the elemental composition of GSRs. Moreover, chemo metric analysis methods such as ICP-MS can be used to enhance the obtained results. We believe that our findings will influence the policy planning in Thailand.

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Author's Contributions

[1]Yaowaluck Phermpornsagul: Conceptualization, methodology, writing-original draft preparation.

Suphareark Arepornrat: Validation, investigation, writing-original draft preparation.

Worasit Palkawong Na Ayuthaya: Investigation, writing-original draft preparation.

Panya Khaenamkaew: Conceptualization, validation writing-original draft preparation, writing-review and editing, supervision, project administration, funding acquisition.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Abrego, Z., N. Grijalba, N. Unceta, M. Maguregui and A. Sanchez *et al*., 2014. A novel method for the identification of inorganic and organic gunshot residue particles of lead-free ammunitions from the hands of shooters using scanning laser ablation-ICPMS and Raman micro-spectroscopy. Analyst, 23: 6232-6241. DOI: 10.1039/C4an01051e

Aleksandar, I., 2003. Is there a way to precisely identify that the suspect fired from the firearm? Forensic Sci. Int., 136: 158-159. DOI: 10.1016/S0379-0738(03)90015-6

Andrasko, J. and S. Pettersson, 1991. A simple method for collection of gunshot residues from clothing. Sci. Justice, 31: 321-330. DOI: 10.1016/S0015-7368(91)73164-2

ASTM, E1588-10e1, 2010. Standard guide for gunshot residue analysis by scanning electron microscopy/ energy dispersive X-ray spectrometry. ASTM International, West Conshohocken, PA, www.astm.org

Blakey, L.S., G.P. Sharples, K. Chana and J.W. Birkett, 2018. Fate and behaviour of gunshot residue-a review. J. Forensic Sci., 63: 9-19. DOI: 10.1111/1556-4029.13555

Brozek-Mucha, Z., 2007. Comparison of cartridge case and airborne GSR-a study of the elemental composition and morphology by means of SEM-EDX. X-Ray Spectrometry, 36: 398-407. DOI: 10.1002/XRS.990

Brozek-Mucha, Z., G. Zadora and F. Dane, 2003. A comparative study of gunshot residue originating from 9 mm Luger ammunition from various producers. Sci. Justice, 43: 229-235. DOI: 10.1016/S1355-0306(03)71782-1

Capannesi, G. and F. Sedda, 1992. Bullet identification: A case of a fatal hunting accident resolved by comparison of lead shot using instrumental neutron activation analysis. J. Forensic Sci., 37: 657-662. DOI: 10.1520/JFS13276J

CITC, 2019. Central Information Technology Center Directory Listing of files/crimes, Royal Thai Police.

Cooper, R., J.M. Guileyardo, I.C. Stone, V. Hall and L. Fletcher, 1994. Primer residue deposited by handguns. Am. J. Forensic Med. Pathol., 15: 25-327. DOI: 10.1097/00000433-199412000-00007

Costa, R.A., L.C. Motta, C.A. Destefani, R.R.T. Rodrigues and K.S.D.E. Santo *et al*., 2016. Gunshot Residues (GSR) analysis of clean range ammunition using SEM/EDX, colorimetric test and ICP-MS: A comparative approach between the analytical techniques. Microchem. J., 129: 339-347. DOI: 10.1016%2fj.Microc.2016.07.017

Dalby, O., D. Butler and J.W. Birkett, 2010. Analysis of gunshot residue and associated materials-a review. J. Forensic Sci., 55: 924-943. DOI: 10.1111/J.1556-4029.2010.01370.X

Elad, S.I., T. Tsadok and N. Levin, 2013. Optimizing FEG-SEM combined with an SDD EDX system for automated GSR analysis. X-Ray Spectrometry, 43: 29-37. DOI: 10.1002/XRS.2495

Garofano, L., M. Capra, F. Ferrari, G.P. Bizzaro and D. Di Tullio *et al*., 1999. Gunshot residue: Further studies on particles of environmental and occupational origin. Forensic Sci. Int., 103: 1-21. DOI: 10.1016/S0379-0738(99)00035-3

Hagel, R. and K. Redecker, 1986. Sintox – A new, non-toxic primer composition by Dynamit Nobel AG. Propellants Explos. Pyrotech., 11(6): 184-187. https://doi.org/10.1002/prep.19860110606

Kilty, J.W., 1975. Activity after shooting and its effects on the retention of primer residue. J. Forensic Sci., 20: 219-230. DOI: 10.1520/Jfs10268j.

Koons, R.D., 1998. Analysis of gunshot primer residue collection swabs by inductively coupled plasma-mass spectrometry. J. Forensic Sci., 43: 748-754. DOI: 10.1520/Jfs14301j

Koons, R.D., D.G. Havekost and C.A. Peters, 1987. Analysis of gunshot primer residue collection swabs using flameless atomic-absorption spectrophotometry; a re-examination of extraction and instrument procedures. J. Forensic Sci., 32: 846-65. DOI: 10.1520/Jfs12397j

Koons, R.D., D.G. Havekost and C.A. Peters, 1988. Determination of barium in gunshot reside collection swabs using inductively coupled plasma-atomic emission-spectrometry. J. Forensic Sci., 33: 35-41. DOI: 10.1520/Jfs12434j

Krishnan, S.S., 1967. Firing distance determination by neutron activation analysis. J. Forensic Sci., 12: 471-83.

Krishnan, S.S., 1974a. Firing distance determination by atomic absorption spectrophotometry. J. Forensic Sci., 19: 351-356. DOI: 10.1520/Jfs10182j

Krishnan, S.S., 1974b. Detection of gunshot residue on the hands by neutron activation analysis and atomic absorption analysis. J. Forensic Sci., 19: 789-797. DOI: 10.1520/Jfs10469j

Martiny, A., A.P. Campos, M.S. Sader and A.L. Pinto, 2008. SEM/EDS analysis and characterization of gunshot residues from Brazilian lead-free ammunition. Forensic Sci. Int., 177: 9-17. DOI: 10.1016/J.FORSCIINT.2007.07.005

McNaught, A.D. and A. Wilkinson, 1997. Compendium of Chemical Terminology. 2nd Edn., (Blackwell Science, ISBN-10: 0865426848, pp: 450.

Meng, H.H. and B. Caddy, 1997. Gunshot residue analysis-a review. J. Forensic Sci., 42: 553-570. DOI: 10.1520/Jfs14167j

Michael, G.H. and G.H. Lucien, 2011. Shooting Incident Reconstruction. 2nd Edn., Academic Press, ISBN-10: 0123822424, pp: 440.

Molina, D.K., M. Martinez, J. Garcia and V.J. DiMaio, 2007. Gunshot residue testing in suicides: Part I: analysis by scanning electron microscopy with energy dispersive x-ray. Am. J. Forensic Med. Pathol., 28: 187-190. DOI: 10.1097/Paf.0b013e31806195e1

Niewoehner, L. and H.W. Wenz, 1999. Application of focused Ion Beam systems in gunshot residue investigation. J. Forensic Sci., 44: 105-109. DOI: 10.1520/Jfs14419j

Niewoehner, L., H.W. Wenz, J. Andrasko, R. Beijer and L. Gunaratnam, 2003. ENFSI proficiency test program on identification of GSR by SEM/EDX. J. Forensic Sci., 48: 786-793.

Pillay, K.K.S., W.A. Jester and H.A. Fox, 1974. New method for the collection and analysis of gunshot residues as forensic evidence. J. Forensic Sci., 19: 768-783. DOI: 10.1520/Jfs10467j

Ravreby, M., 1982. Analysis of long-range bullet entrance holes by atomic absorption spectrophotometry and scanning electron microscopy. J. Forensic Sci., 27: 92-112. DOI: 10.1520/Jfs11454j

Romolo, F.S. and P. Margot, 2001. Identification of gunshot residue: A critical review. Forensic Sci. Int., 119: 195-211. DOI: 10.1016/S0379-0738(00)00428-X

Rudzitis, E. and M. Wahlgren, 1975. Firearm residue detection by instrumental neutron activation analysis. J. Forensic Sci., 20: 119-124. DOI: 10.1520/Jfs10247j

Schowoeble, A.J. and D.L. Exline, 2000. Current Methods in Forensic Gunshot Residue Analysis. 1st Edn., CRC Press, ISBN-10: 1420042572, pp: 192.

Shaffer, D.K. and K. Yi, 1999. A comparison of particle transfer efficiencies of two collection methods for the identification of gunshot residue on fabric surfaces using scanning electron microscopy-energy dispersive spectrometry. Scanning Electron Microscopy, 21: 99-100.

Steffen, S., M. Otto, L. Niewoehner, M. Barth and Z. Brożek-Mucha *et al*., 2007. Chemometric classification of gunshot residues based on energy dispersive X-ray microanalysis and inductively coupled plasma analysis with mass-spectrometric detection. Spectrochimica Acta Part B: Atomic Spectroscopy, 62: 1028-1036. DOI: 10.1016/J.SAB.2007.04.005

Taudte, R.V., C. Roux, L. Blanes, M. Horder and K.P. Kirkbride *et al*., 2016. The development and comparison of collection techniques for inorganic and organic gunshot residues. Analytical Bioanalytical Chem., 12: 1-10. DOI: 10.1007/S00216-016-9357-7

Tugcu, H., C. Yorulmaz, Y. Karslioglu, H.B. Uner and S. Koc *et al*., 2006. Image analysis as an adjunct to sodium rhodizonate test in the evaluation of gunshot residues: An experimental study. Am. J. Forensic Med. Pathol., 27: 296-299. DOI: 10.1097/01.PAF.0000248739.79253.25

Twibell, J.D., J.M. Home, K.W. Smalldon, D.G. Higgs and T.S. Hayes, 1982. Assessment of solvents for the recovery of nitroglycerine from the hands using cotton swabs. J. Forensic Sci., 27: 792-800. DOI: 10.1520/Jfs12194j

Wallace, J.S. and R.H. Keeley, 1979. A method for preparing firearm residue samples for scanning electron microscopy. Scann. Electron Microscopy, 2: 179-184.

White, R. and A. Owens, 1987. Automation of gunshot residue detection and analysis by Scanning Electron Microscopy/Energy Dispersive X-Ray analysis (SEM/EDX). J. Forensic Sci., 32: 1595-1603. DOI: 10.1520/JFS11219J

Wolten, G.M., R.S. Nesbitt, A.R. Calloway and P.F. Loper, 1979a. Particle analysis for the detection of gunshot residue: II. Occupational and environmental particles. J. Forensic Sci., 24: 423-430. DOI: 10.1520/Jfs10849j

Wolten, G.M., R.S. Nesbitt, A.R. Calloway, G.L. Loper and P.F. Jones, 1979b. Particle analysis for the detection of gunshot residue, I: Scanning electron microscopy/energy dispersive X-ray characterization of hand deposits from firing. J. Forensic Sci., 24: 409-422. DOI: 10.1520/Jfs10848j

Wolten, G.M., R.S. Nesbitt, A.R. Calloway, G.L. Loper and P.F. Jones, 1977. Equipment systems improvement program-final report on particle analysis for gunshot residue detection.

Wotton, C., 2016. Thailand has a higher rate of gun-related deaths than the US. Elite+ magazine, New/Variety News.

Zadora, G. and Z. Brozek-Mucha. 2003. SEM–EDX-a useful tool for forensic examinations. Mater. Chem. Phys., 81: 345-348. DOI: 10.1016/S0254-0584(03)00018-X

Zeichner, A. and B. Eldar, 2004. A novel method for extraction and analysis of gunpowder residues on double-side adhesive coated stubs. J. Forensic Sci., 49: 1-13. DOI: 10.1520/Jfs2004022

Zeichner, A. and N. Levin, 1993. Collection efficiency of Gunshot Residue (GSR) particles from hair using double-side adhesive tape. J. Forensic Sci., 38: 571-584. DOI: 10.1520/Jfs13441j

Zeichner, A. and N. Levin, 1997. More on the uniqueness of Gunshot Residue (GSR) particles. J. Forensic Sci., 42: 1027-1028. DOI: 10.1520/Jfs14255j

Zeichner, A., S. Ehrlich, E. Shoshani and L. Halicz, 2006. Application of lead isotope analysis in shooting incident investigations. Forensic Sci. Int., 158: 52-64. DOI: 10.1016/J.FORSCIINT.2005.01.020