

X-RAY FLUORESCENCE ANALYSIS OF GUNSHOT RESIDUE**ANÁLISIS DE RESIDUOS DE DISPARO MEDIANTE FLUORESCENCIA DE RAYOS X**Saran A.¹Gualpa J.²Much D.³Jessica Pamela Sanchez Ormachea JP.⁴Wilberger D.⁵Luciano Merini L.⁶¹ Doctora en Bioquímica, CONICET - Agencia de Investigación Científica (AIC-SR) Santa Rosa, La Pampa.² Licenciado en Biología Molecular, Agencia de Investigación Científica (AIC-SR), Santa Rosa, La Pampa.³ Licenciado en Química, CONICET - Agencia de Investigación Científica (AIC-SR) Santa Rosa, La Pampa.⁴ Licenciada en Criminalística, Agencia de Investigación Científica (AIC-SR), Santa Rosa, La Pampa.⁵ Abogado, Agencia de Investigación Científica (AIC-GA), General Acha, La Pampa - Universidad Siglo 21, Córdoba.⁶ Profesor Doctor en Bioquímica, CONICET - Agencia de Investigación Científica (AIC-SR) Santa Rosa, La Pampa.Agencia de Investigación Científica, La Pampa
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Abstract: Several analytical techniques are available for forensic scientists to perform the reconstruction of a shooting incident. Among them, the analysis of gunshot residues typical elements, as Pb, Ba and Sb, are the most frequent. Investigation of its presence on the body from the victim or a suspect can provide crucial information regarding the incident. In this work we propose the use of X-ray fluorescence for detecting gunpowder residues on the skin. The analysis was focused in the relationship of these elements, mainly on the hands and face of a handgun shooter. An experiment was performed in the forensic laboratory shooting room, using commercial carbon stubs for sampling the hand and face skin of a shooter firing a 9 mm pistol. Results indicate a clear correlation between samples taken from both hands after firing a 9 mm pistol in relationship to Pb and Ba presence. The XRF instrument proved to be a quick and easy tool to detect inorganic components as a screening for GSR on hands.

Keywords: gunshot residue (GSR), x-ray fluorescence (XRF), forensic ballistic.

Resumen: Varias técnicas analíticas están disponibles para que los científicos forenses realicen la reconstrucción de un hecho. Entre ellas, el análisis de la presencia de elementos típicos de residuos de disparos, como Pb, Ba y Sb, es lo más frecuente. La investigación de su presencia en el cuerpo de la víctima o de un sospechoso puede proporcionar información crucial sobre el incidente. En este trabajo proponemos el uso de la fluorescencia de rayos X para la detección de residuos de disparo en piel. El análisis se centró en la detección de los elementos antes mencionados, en las manos y la cara de un tirador. Se realizó un experimento en la sala de disparo (AIC-SR), utilizando stubs de carbon comerciales para tomar muestras de las manos y el rostro de él tirador antes y después de disparar una pistola 9 mm. Los resultados indican una clara correlación entre las muestras tomadas de ambas manos después de disparar una pistola 9 mm en relación con la presencia de Pb y Ba. El instrumento XRF demostró ser una herramienta rápida y sencilla para detectar componentes inorgánicos como lo son los residuos de disparo en las manos de un tirador.

Palabras clave: residuo de disparo (GSR), fluorescencia de rayos X (XRF), balística forense.

1. INTRODUCTION

Currently, an increase in the use of 9 mm pistols report has been noticed in shooting incidents where the Governmental Scientific Research Agency in Santa Rosa, La Pampa, Argentina is summoned to board the crime scene. In these incidents, several cartridges are often fired, being the cartridges containing lead based primers the most frequently found.

When a firearm is fired, the hammer fits forward and a pin strikes the primer. This creates a spark, which ignites the gunpowder and its deflagration propels the bullet out of the gun. This process is confined within the firearm which in turn has mechanism that, according to the weapon, allow to shoot continuously [1].

Deposition of both, inorganic and organic traces, occurs on proximate surfaces surrounding the firearm discharge event and is commonly referred as Gun Shoot Residues (GSR). These surfaces include the hands, chest, shoulders and face of the shooter, however, many variables affect the amount and pattern of deposited residues. Most of

the inorganic constituents present in GSR and used as tracers come from the primer mixture, which main components are lead styphnate, used as initiator, barium nitrate, used as oxidant, and antimony sulfide, used as fuel [2].

Gunshot residues experts use methods involving visual inspection of the garment and skin, as well as chemographic tests to visualize traces around possible bullet entry holes. In the forensic ballistic lab like, combined Scanning Electron Microscopy/Energy-Dispersive X-ray spectroscopy (SEM/EDX) is the gold standard method, although other supporting techniques as Fourier transform infrared spectroscopy (FTIR) are also frequently used. Combination of these methods provides valuable information on the elemental composition and distribution of gunshot residue [3]. Nevertheless, due to the cost and trained operator requirements, SEM/EDX technology is not always available.

On spite of its potential to provide multi elemental profile, the use of X-ray fluorescence (XRF) in the GSR analysis has not being widely employed yet. Due to its broad range of detectable elements and simplicity on quantification, this technique is a powerful analytical multi elemental tool. The total reflection of primary X-ray photons on the quartz carrier, containing a thin film of the sample, results in higher excitation efficiency, thereby resulting in lower detection limits [4].

The aim of the Governmental Scientific Research Agency forensic lab is to explore the chances in which XRF can be used to generate useful information to investigate a shooting incident. In this paper, the possibilities of using the XRF M4-Tornado as a GSR screening tool and collecting data for a database either for following criminal trends or for reverse lookup functions are explored. The influence of environmental and occupational sources of cross contamination was also assessed.

2. MATERIALS AND METHODS

2.1 Materials and sampling procedure

Adhesive carbon stubs (REVELER, COASIN) were used to collect samples. Elemental baseline of all the stubs used in the experiment was measured with the M4-Tornado XRF before being used. The sampling method involved tapping the adhesive carbon stub over the area of interest, until the tackiness has almost gone (ca. 20 taps).

To avoid contamination, one hour before the shoots the shooter was instructed to carefully wash her hands. Then, samples obtained from the hands and face of the expert shooter were defined as “blank” samples (Blank stubs 1 to 3). At the moment of firing, the shooter was asked to grip the weapon with both hands. Also, an environmental baseline sample was collected by exposing the stubs to shooting room ambient 1 hour before the shoots (Blank stub 4).

In order to reproduce the characteristics of a real case, one shooting round was performed with a Bersa Thunder 9 semiautomatic pistol, using a 9 mm Luger (124 gr., Magtech/CBC) ammunition available at the Ballistic Section Laboratory (Governmental Scientific Research Agency, La Pampa). GSR samples were collected from both hands and face (cheekbone) of the shooter (Sample stubs 5 to 7).

Also, a stub was left 30 min inside the shooting room during the procedure, at a distance of 1 m from the shooter, in order to assess GSR deposition (Sample stub 8). Sample identification and time of sampling is presented on Supplemental Table 1.

2.2 XRF analysis

Once the stubs were collected, the X-ray fluorescence measurements were performed on a XRF spectrometer M4-Tornado Bruker. This system is equipped with a MCB 50-0.7G Rh X-ray tube with a 0.2 mm Be-window. The tube, unless mentioned otherwise, was operated at 30 kV voltage, with a current of 550 μ A. A collimator equipped with a series of interchangeable filters (none, 12.5 μ m Al, 100 μ m Al, 630 μ m Al, 100 μ m Al + 25 μ m Ti and 100 μ m Al + 50 μ m Ti + 25 μ m Cu) results in a spotsize of approximately 2.5 mm. During the point measurements, no filters were

used and data was collected for 30 live seconds. The 30 mm² Bruker Nano GmbH Xflash 430 – PA detector with a take-off angle set at 51 degrees is capable of maximum countrates of over 300.000 cps combined with an energy resolution < 145 eV for Mn-K α . During the loading of samples, these were focused with the aid of an optical system made up of two color cameras operating at 10x (14.7 mm x 11.0 mm) and 100x (1.47 mm x 1.1 mm) magnification. Measurements were performed under low vacuum (20 mbar) conditions. The hypermap imported and processed was performed by means of the M4-Tornado software. Depending on the sample size pixel resolution, have been 25 μ m and 100 μ m respectively.

In order to ensure the quality of the results, spectrum energy calibration was performed before each analysis batch, by using copper (Cu) and zirconium (Zr) metal (SN0178, Bruker® calibration standard). A steel standard (SN0163, Bruker® calibration standard) containing Fe, Cr, Ni, Mo, Mn, Cu and V was used to check the precision and accuracy of the method.

2.3 Statistical Analysis

Principal component analysis (PCA) was performed to reduce the original data space dimensionality and facilitate perception of the relevant information by visual inspection. In this way, XRFs raw data was imported into Matlab environment [5]. Autoscaling pre-processing and PCA model were performed by using PCA Toolbox [6], from Milano Chemometrics and QSAR research Group. The number of principal components used in the PCA model was based on eigenvalue versus number of PC plots. Additionally, the analysis of the loading values was used to find the most relevant characteristic for the grouping of samples, according to their similarities. The final model was created using the three first principal components (PC) that explain more than the 91% of the accumulated variance.

3. RESULTS AND DISCUSSION

All commercial stubs (1 to 8) were examined by XRF before used revealing the presence of traces of Mn, Fe, Ni, Ga and Cr and V in their composition. In figure 1, the spectrums of all commercial stubs used for the experiment are showed. Janssen et al. [3] reported that Al, Cu and trace amounts of Pb and Bi were frequently present as components of the stubs used at The Netherlands Forensic Institute. In that sense, stub composition depends largely on the manufacturer. Furthermore, LabTech S.R.L. reported that impurities as B, Mg, Al, Si, Ca or Fe can be found in the composition of their EM-Tec high purity carbon.

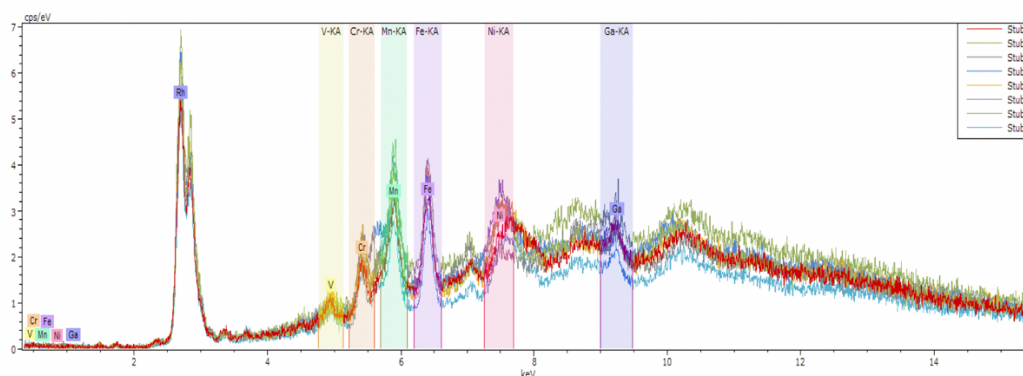


Figure 1. M4-Tornado XRF spectrum of commercial carbon stubs used at the AIC.

Figure 2 shows the comparison between the spectra of blank stubs (samples taken before the shoots) and sample stubs (samples taken after the shoots). Carbon commercial stubs background (Figure 1) were subtracted from blank and sample stubs spectra. Some elements like Ca, S, Si, Zn, Fe and Cu were found on the samples taken from both hands and cheekbone before shoot (Figure 2 A, B and C). These elements have been already described as components of the skin tissue by Inoue et al. [7]. While, high signals of Al, Si, K and Ti were found only in cheekbone samples, taken before and after the shoot (Figure 2 C). These elements can be associated to the shooter make up composition.

Melquiades et al. [8] reported the presence of these elements in different cosmetics branches. Shooting room environmental baseline samples, taken before shoot, did not present any difference with clean stubs (Figure 1), indicating the absence of trace elements in the atmosphere of the shooting room. While, only light elements (Al, Si and S) were deposited on the stub after shoot (Figure 2.D).

Analytical XRF signals corresponding to L line of Ba and M line of Pb were used as the best discriminating variables to identify GSR (Fig 2A and B, *1 and *2). It was not possible to measure the presence of Sb with M4-Tornado XRF because of the interference of high amounts of Ca present on the samples. The Ca $K\alpha$ line ($K\alpha_1 = 3.6923$) interferes with Sb $L\alpha$ ($L\alpha_1 = 3.6038$). We were capable of elucidate, through the spectra comparison, that Ca contributions not only comes from the skin but also from the GSR minor components. Hence, even in those surfaces where skin interference could be avoided, the Ca contribution from the GSR is enough to overlap the Sb K line, which is a limitation of the technic. Ferreira et al. [2] reported the same issues of skin interference measuring this element by Total Reflection X-Ray by Fluorescence of GSR in samples collected with swabs.

The Pb MAB line ($MAB = 2.342$) with S $K\alpha$ line ($K\alpha_1 = 2.309$) and Ba $L\alpha$ ($L\alpha = 4.465$) with Ti $K\alpha$ line ($K\alpha_1 = 4.510$) undergo similar interference, however, non-overlapping peaks were selected in order to perform quantification as is recommended by Masatsugu Ogasawara et al. [9]. Using Rh tube, it is not possible to access the Ba and Sb K lines and the Pb L lines due to the high instrument background.

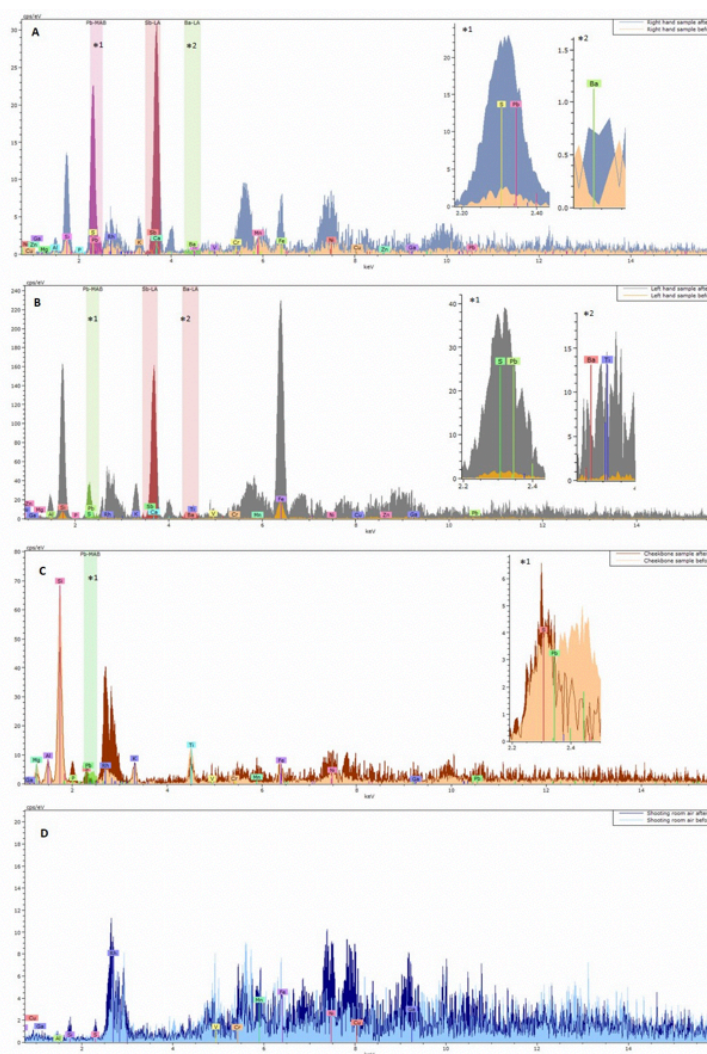


Figure 2. GSR measured on carbon stubs sample before and after firing a 9 mm pistol. A) Right hand. B) Left hand. C) Cheekbone. D) Shooting room air.

Mapping comparison between both hands is presented in Figure 3. Due to the X ray beam area and the presence of background elements from skin, it was not possible to visualize an individual GSR particle. However, it was possible to observe the Pb and Ba distribution pattern. Can be observed in Fig. 3 that the distribution of Pb and Ba do not overlap completely. Brožek Mucha et al. [10] explain in their work that as the X-ray beam is penetrating the stub much deeper than the electron beam, the differences in distribution of these elements indicate the heterogeneity of the body of the particle, not only on its surface.

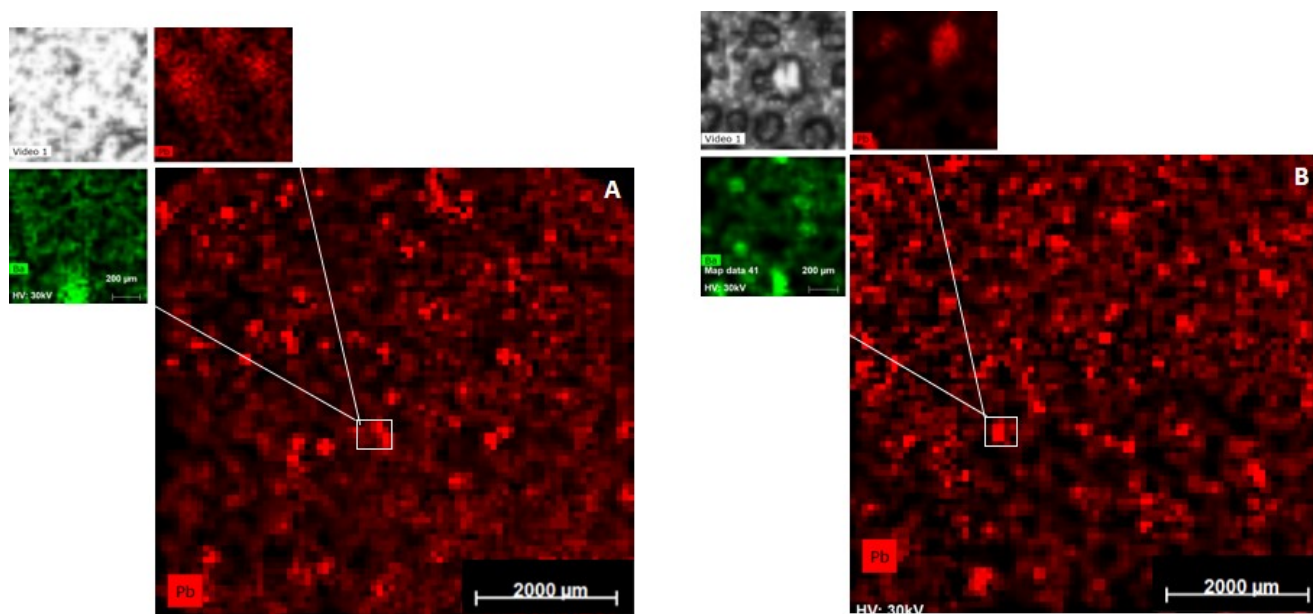


Figure 3. Mosaic image of M4-Tornado XRF maps showing the distribution of Pb and Ba in A) Right hand and B) Left hand samples taken after firing 9mm gun.

Through mathematical algorithms, the XRF software converts the raw data, reported as intensity counts per second (cps; the area under the curve) versus energy (keV), into norm concentration % of the elements on the sample (Table 1). The results show that sample stub 5, corresponding to right hand after shoot, contains the highest amount of Pb by norm. wt. % (2.18%), followed by sample stub 6 (left hand after shoot) with 1.22%. However, higher amounts of Ba by norm. wt. % were found on left hand than on right hand. On cheekbone sample (stub 7), it was only possible to quantify Pb, in lower amounts than the found on hands samples. Also, other characteristic elements from GSR as Si, Ca, and S were found on hands samples, even in higher norm. wt. % than Pb and Ba.

Table 1. Semi quantitative determination of elements (norm. wt. %) carried out by M4-Tornado XRF on commercial carbon stubs (Stubs 1-8), Blank stubs (Blank stub 1-4) defined as samples obtained before shooting and Sample stubs (Sample stub 5-8) defined as samples obtained after shooting a 9 mm gun.

Samples	Mg	Al	Si	P	S	K	Ca	Ti	V	Cr	Mn	Fe	Ni	Cu	Zn	Ga	Ba	Pb
Stub 1	0	0	0	0	0	0	0	0	4,15±	9,31±	23,88	22,75	21,45	0	0	18,45	0	0
Stub 2	0	0	0	0	0	0	0	0	2,11	2,11	±2,11	±2,11	±2,11	0	0	20,61	0	0
Stub 3	0	0	0	0	0	0	0	0	2,11	±2,11	±2,11	±2,11	±2,11	0	0	12,40	0	0
Stub 4	0	0	0	0	0	0	0	0	4,44±	9,66±	24,36	24,77	24,37	0	0	12,40	0	0
Stub 5	0	0	0	0	0	0	0	0	2,11	2,11	±2,11	±2,11	±2,11	0	0	16,78	0	0
Stub 6	0	0	0	0	0	0	0	0	4,72±	9,12±	26,03	22,87	20,48	0	0	22,11	0	0
Stub 7	0	0	0	0	0	0	0	0	2,11	2,11	±2,11	±2,11	±2,11	0	0	23,24	0	0
Stub 8	0	0	0	0	0	0	0	0	2,11	2,11	±2,11	±2,11	2,11	0	0	14,38	0	0
Blank stub 1	6,13±	7,91±	16,53	1,08±	6,28±	0,04±	3,42±1	0,61±	2,55±	4,72±	13,13	9,92±	4,65±	8,30±	15,02±	7,12±	0	0
Blank stub 2	4,51±	7,70±	33,23	0,58±	7,44±	3,42±	6,80±2	0,45±	1,76±	4,09±	10,84	10,62	4,81±	0	0,08±0	5,45±	0	0
Blank stub 3	2,95	1,87	±7,80	0,11	3,22	2,19	,36	0,06	0,73	3,02	±8,65	±7,36	5,96		,14	7,46	0	0
Blank stub 4	18,19	13,31	58,06	1,96±	1,50±	3,39±	0,38±0	2,31±	0,17±	0,43±	0,86±	1,29±	0,26±	0	0,24±0	0,18±	0	0
Sample stub 5	2,85±	13,96	30,84	0,32±	8,76±	2,32±	13,21±	2,40±	0,25±	2,61±	5,71±	7,46±	1,77±	0,94±	1,42±0	2,02±	0,80±	2,18±
Sample stub 6	1,65	±4,46	±5,67	0,22	6,32	0,90	4,30	2,99	0,23	0,57	1,21	2,19	0,46	0,32	,43	0,68	1,77	0,79
Sample stub 7	3,31±	11,79	39,71	0	5,29±	3,65±	14,76±	0	0,46±	1,63±	4,29±	8,76±	1,14±	0,25±	1,08±0	1,38±	1,28±	1,22±
Sample stub 8	2,09	±1,73	±3,28	1,76	1,33	10,75			1,18	0,73	1,92	3,92	0,53	0,18	,35	0,71	0,44	0,60
Sample stub 7	17,30	12,84	51,60	1,35±	1,20±	3,62±	0,62±0	1,43±	0,10±	1,29±	2,76±	2,61±	0,77±	0	0,67±0	0,76±	0	0,74±
Sample stub 8	±2,28	±3,44	±4,94	2,03	0,74	3,67	,24	0,49	0,10	0,71	1,64	0,99	0,52		,34	0,50		0,63
Sample stub 8	0	8,45±	4,02±	0	0	0	0,77±0	0,55±	3,58±	9,34±	21,59	21,01	19,47	0	0	12,93	0	0
		2,17	2,69				,44	0,11	0,58	0,35	±2,14	±2,48	±3,91					

Values are mean ± S.E. (n=5). Stub blank 1 and Stub sample 5=Right hand; Stub blank 2 and Stub sample 6= Left hand; Stub blank 3 and Stub sample 7= Cheekbone; Stub blank 4 and Stub sample 8= Shooting room air. Limits of detection (LD) expressed in norm. wt. %. Fe 5.42, Cr 2.00, Ni 2.49, Mo 0.94, Mn 0.40, Cu 0.27, V 0.40. Values below the limit of detection were filled with zero.

In order to perform the PCA analysis, values below the limit of detection were filled with zero. Sample scores and variable loadings on PCs 1 and 2 are shown in Figure 4. The first four principal components explained 78.2% of the data variance. A clear correlation between samples taken from both hands after firing a 9 mm pistol is observed. The elements Ba, Ca, Pb and S had a larger contribution to group this samples. The fact that commercial stubs were close to samples taken from the shooting room air before and after the experiment, suggest that the room air is free of trace elements. In addition, even after firing a 9 mm gun, heavy elements are not deposited at a distance of 1 m from the shooter. The grouping of skin samples taken before shoots with cheekbone sample taken after shoot, allows the interpretation that the method was not sensitive enough to discriminate a one-shot GSR deposition on the face. This situation could be associated to several factors such as the firearm and ammunition used, occupational contamination of the shooter makeup and the sampling method chosen [2] (Ferreira et al., 2019).

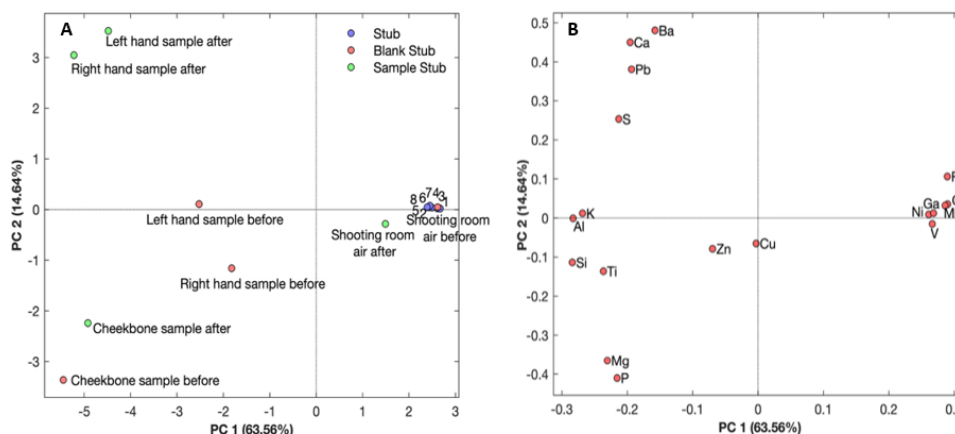


Figure 4. PC1 × PC2 biplot distribution: A) Score plot and B) Loading plot for GSR sampled with carbon stubs.

4. CONCLUSION

The XRF instrument proved to be a quick and easy tool to quantify inorganic components as a screening for GSR on hands. In this way, the use of XRF could be included as a screening and complementary method for SEM. Results not only show the elemental composition of the residues, but also the spatial distribution of Pb and Ba, which could be used in the near future for GSR patterns. In future studies the spatial distribution discriminating of ammunition and firearm will be evaluated.

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SUPPLEMENTAL FILES

Supplemental Table 1. Sample identification

Sample ID	Surface sampling	Moment of sampling
Stub 1	None	-
Stub 2	None	-
Stub 3	None	-
Stub 4	None	-
Stub 5	None	-
Stub 6	None	-
Stub 7	None	-
Stub 8	None	-
Blank stub 1	Right hand	Before shoot
Blank stub 2	Left hand	Before shoot
Blank stub 3	Cheekbone	Before shoot
Blank stub 4	Shooting room air	Before shoot
Sample stub 5	Right hand	After shoot
Sample stub 6	Left hand	After shoot
Sample stub 7	Cheekbone	After shoot
Sample stub 8	Shooting room air	After shoot