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The potential of using laser ablation inductively coupled plasma time of flight mass spectrometry (LA-ICP-TOF-MS) in the forensic analysis of micro debris

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Abstract

The majority of crimes result in the generation of some form of physical evidence, which is available for collection by crime scene investigators or police. However, this debris is often limited in amount as modern criminals become more aware of its potential value to forensic scientists. The requirement to obtain robust evidence from increasingly smaller sized samples has required refinement and modification of old analytical techniques and the development of new ones. This paper describes a new method for the analysis of oxy-acetylene debris, left behind at a crime scene, and the establishment of its co-provenance with single particles of equivalent debris found on the clothing of persons of interest (POI).

The ability to rapidly determine and match the elemental distribution patterns of debris collected from crime scenes to those recovered from persons of interest is essential in ensuring successful prosecution. Traditionally, relatively large amounts of sample (up to several milligrams) have been required to obtain a reliable elemental fingerprint of this type of material [R.J. Walting , B.F. Lynch, D. Herring, J. Anal. At. Spectrom. 12 (1997) 195]. However, this quantity of material is unlikely to be recovered from a POI. This paper describes the development and application of laser ablation inductively coupled plasma time of flight mass spectrometry (LA-ICP-TOF-MS), as an analytical protocol, which can be applied more appropriately to the analysis of micro-debris than conventional quadrupole based mass spectrometry. The resulting data, for debris as small as 70 μm in diameter, was unambiguously matched between a single spherule recovered from a POI and a spherule recovered from the scene of crime, in an analytical procedure taking less than 5 min.

Keywords: Crime scene evidence; Micro evidence; Laser Ablation; Time of flight ICP-MS

1. Introduction

Modern forensic investigation often involves the analysis of, and interpretation of data from, microscopic samples of physical evidence. This is due to the growing forensic knowledge and awareness of criminals, resulting in them being extremely careful not to leave evidence behind at the scene of crime [2].

Research described in this paper focuses on the development of a robust analytical protocol for the chemical analysis of micro evidence recovered from specific scenes of crime where an oxy-acetylene gas cutter had been used. The use of oxy-acetylene to cut steel results in the generation of spherical air borne debris (spherules) which is available both at the scene of crime and often, because of its potentially small size, also recoverable from the clothing of perpetrators, making association of the two possible. The spherical particles are formed when the melted steel condenses from fume generated at the heating site. The spherules generated vary significantly in size with the larger particles being found at the scene and smaller to microscopic particles, which may become air borne on the persons of interest (POI). The relationship between the size of the debris and its chemical analysis forms part of this

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investigation, which focuses on debris less than 500 μm in size as anything larger is less likely to be recovered from a POI.

There are a variety of traditional methodologies available to establish the elemental signature of this debris, energy dispersive X-ray spectrometry (XRS) [3], X-ray fluorescence (XRF) [4], X-ray diffraction (XRD) [5], flame atomic absorption spectrometry (FAAS) [6] and inductively coupled plasma atomic emission spectrometry (ICP-AES) [4,5] being the most common. Poolman and Pistorius [5] illustrated the potential of using ICP-AES to discriminate between different batches of the same steel type and between oxy-acetylene generated debris from different sources. However, these techniques lack the sensitivity required to discriminate between concentrations of elements present at sub-ppb levels in the original material. In general, these techniques are only capable of being used to discriminate between relatively widely differing types and batchers of steel and have limited use when discrimination is required between the elementally similar batches of steel used for a specific application such as safes.

The refinement of industrial manufacturing processes and stringent quality control requirements predicated by more defined end user specifications, has resulted in the gross elemental variations of the source steels being minimal and, therefore, requiring even more sensitive methods to potentially find differences using ultra-trace levels of elemental constituents to achieve differentiation. The only technique capable of providing enough sensitivity for this level of discrimination is solution based ICP-MS [7,8]. The investigation carried out by Poolman and Pistorius [5] indicated that the minimal sample mass required to obtain robust results was 90 mg. In order to obtain this mass from both a crime scene and a person of interest a large number of spherules would be required, leading to the possibility of cross contamination and homogenization problems of different steel sources in the same analytical sample. Consequently, if solution ICP-MS is used it is difficult to eliminate the possibility that the debris recovered from a person of interest is not co-provenanced with the debris from the crime scene.

In order to overcome problems associated with mixed provenance samples, a laser ablation system can theoretically be coupled to an ICP-MS and used to determine the elemental composition of a single spherule. In addition, laser ablation facilitates the direct analysis of samples in their solid form eliminating the necessity for dissolution. Significantly less sample is also required for analysis in comparison to the solution based methods. Watling et al. [1] successfully used the LA-ICP-MS technique to obtain accurate and robust data for elemental distribution in debris generated from cutting steel. The results were used successfully to assign a provenance to individual samples, making it possible to link persons of interest to specific crime scenes. However, the equipment used in this investigation required that reasonably sized pieces of debris (>1000 μm diameter) were essential if provenance was to be established from a single piece. Pieces of this size are noticeable on a person of interest and as such would almost certainly be removed by perpetrators soon after the crime has been committed.

It has been established in the literature that analyzing precious metals such as gold to obtain elemental profiles which can be used to assign provenance and link persons of interest to stolen gold or artifacts is valid in prosecution [9,10]. Consequently, if this type of analytical protocol is to be taken into a realistic crime scene investigation situation, it is necessary to develop a robust analytical procedure for significantly smaller pieces of debris. The use of laser ablation inductively coupled plasma time of flight mass spectrometry (LA-ICP-TOF-MS) was investigated as a potentially valuable technique to obtain reproducible results from such small samples. Balcerzak [11] provides a detailed review of TOFMS with specific reference to the two different types of ICP-TOF-MS systems, the LECO Renaissance[1] and the instrument used for this research the GBC Optimass 8000.[2] The Balcerzak review also details analytical applications of the TOF-MS systems ranging from the determination of lead isotope ratios in wine and the analysis of lubricating oils to coupling a gas chromatograph (GC) to the system enabling the determination of organolead species below 2 pg mL^{-1} in rain water.

The primary difference between the widely used quadrupole based ICP-MS and the ICP-TOF-MS system is the method by which the isotopes of the elements are separated and their concentration determined. The quadrupole system uses a series of voltages applied to four rods to sequentially separate the ions relative to their mass to charge ratio. The time of flight mass spectrometer separates and counts ions using the principle that that the lighter ions will travel faster than larger ions under the same applied kinetic energy. This difference between the two instruments results in the TOF-MS analyzer being capable of scanning the entire Periodic Table at theoretical rates of up to 30,000 Hz (practically nearer 20,000 Hz). In comparison, the quadrupole system is able to determine a single element in a given period, with maximum statistically useable scan rates of approximately 10 Hz. The overall spectral acquisition speed of the TOF-MS analyzer exceeds that of the quadrupole ICP-MS system by at least two orders of magnitude [11].

The advantage of the very rapid data acquisition time across the entire Periodic Table is the ability to more accurately determine the composition of fast transient signals resulting in improved elemental determination for extremely small samples. This advantage was further emphasized in research carried out by Leach and Heiftje [12] who developed a method for the rapid identification of alloy samples using single shot LA-ICP-TOF-MS. These authors were able to use this technique to accurately determine relative concentrations of 15 elements, present in concentrations above 0.1% by mass, to successfully classify 33 alloy samples.

[1] Leco Corporation, 3000 Lakeview Avenue, St. Joseph, MI 49085-2396, USA.

[2] GBC, Monterey Road, Dandenong, Vic., Australia

The research detailed in the current publication, builds on work undertaken by Watling et al. [1] and Leach and Heiftje [12]. There has been no attempt to quantify results and the interpretational protocol is based on the comparison of elemental distribution patterns. The paper details the development of an analytical protocol, based on the use of LA-ICP-TOF-MS, for the analysis of micro spherules of steel debris generated during the oxy-acetylene cutting of safes. The method enables a comparison of elemental data for single spherules, recovered from clothing of a POI with equivalent data for debris recovered from a crime scene, to facilitate establishment of potential co-provenance. Analytical precision and the robustness of interpretational protocols are also discussed.

2. Experimental

This investigation details the analysis of samples, which, potentially, would take the form of physical evidence from a crime scene. Consequently, all accepted crime scene procedures and collection protocols were followed closely in sample collection and analysis. The experimental method involved sample generation, using an oxy-acetylene gas cutter, sample collection, sample preparation and separation, sample classification using optical and scanning electron microscopy and sample analysis using LA-ICP-TOF-MS.

Pieces of the doors of three steel safes, obtained from a local scrap metal dealer, were used for this experiment. Attempts to obtain safe material from specific suppliers were unsuccessful as the manufacturers wished to retain propriety information of steel composition. Additionally, the purchase of individual safes for experimental procedures was not economically viable, especially as it is envisaged that significantly more safes will be used in future experiments. The three different safe doors obtained simply provided different source material to enable the modeling of a crime scene scenario, where a safe had been cut using oxy-acetylene, and where spherules would be expected to be produced. These safes are referred to as A–C. Each safe was cut open using an oxy-acetylene gas cutter and samples collected from the resulting crime scene. In addition, the oxy-acetylene torch operator was fitted with a clean shirt, for each safe cutting procedure, which was collected after each respective cutting. All samples, which were taken were imaged using both optical and scanning electron microscopy.

A representative sample of the material recovered from each of the three crime scenes (A–C) was taken, and sieved through silk screens and divided into three size fractions, >63 < 150 μm, >150 < 250 μm, and >250 < 500 μm. A representative portion of each of these size fractions was prepared for LA-ICP-TOF-MS by mounting the material on Perspex discs using cyanoacrylate glue (superglue).

The shirts worn by the operator (POI) were sampled, in the conventional manner, using a sticky Perspex disc. These samples were then analyzed using the LA-ICP-TOF-MS system, using two different analytical protocols based on the analysis of either ten spherules or a single spherule. The 10 spherule ablation protocol involved the ablation of 10 spherules per analysis over an acquisition time of 400 s while the single spherule method involved the ablation of a single spherule with an acquisition time of 40 s. In both protocols, significant care was taken to ensure minimal ablation of the Perspex disc beneath the sample.

A general set of LA-ICP-TOF-MS parameters used during this investigation are detailed in Table 1. It should be noted that these are not the fixed parameters utilized for this form of analysis. The LA-ICP-TOF-MS system is optimized daily by tuning the instrument while ablating a NIST 610 glass standard.

3. Instrumentation

GBC Optimass 8000 Inductively Coupled Plasma Time of Flight Mass Spectrometer was used throughout the analytical procedures.

Table 1
General set of parameters for the LA-ICP-TOF-MS system

VG UV microprobe laser		ICP		TOF-MS	
Laser power (mJ)	1.5 per shot	Skimmer (V)	−1000	*X* position (mm)	9.9
Pulse rate (Hz)	10	Extraction (V)	−1100	*Y* position (mm)	0.2
Spot size (μm)	50	Z_1 (V)	−700	*Z* position (mm)	−0.1
Ar flow (L/min)	1	*Y* mean (V)	−100	Nebulizer flow (L/min)	1.030
		Y deflection (V)	−2	Plasma flow (L/min)	10.000
		Z lens mean (V)	0	Auxillary flow (L/min)	1.000
		Lens body (V)	−180	Power (W)	1000
		Fill (V)	−40		
		Fill bias (V)	0.00		
		Fill grid (V)	−32.0		
		Pushout plate (V)	545		
		Pushout grid (V)	−395		
		Blanker (V)	200		
		Reflectron (V)	764		
		Multiplier gain (V)	3300		

UV Microprobe: Frequency Quadrupled 266 Nd:YAG UV Laser;
Phillips XL 30 Scanning Electron Microscope.

4. Results and discussion

4.1. Physical characteristics and morphology of oxy-acetylene cutting debris

An investigation into the morphology of the generated debris from the oxy-acetylene gas cutting of steel has confirmed the observations made by Poolman and Pistorius [5] who describe its structure as spherical (Fig. 1).

4.2. Chemical analysis of oxy-acetylene cutting debris using LA-ICP-TOF-MS

4.2.1. 10 Spherule LA-ICP-TOF-MS

While this protocol may be considered to suffer from the same problems (mixing of sample sources) as previous methods, it is nonetheless valid if multiple sources of the debris can be excluded. Furthermore it is necessary to undertake this work to establish if there are differences in elemental signatures resulting from the use of multiple samples and if the statistical match from single sample work is reduced. The reproducibility of the LA-ICP-TOF-MS technique was investigated by analyzing debris created from the same source. The effects of debris size were also investigated during this experiment. That is, debris generated from Safe B from the size fractions >63 < 150 μm, >150 < 250 μm, and >250 < 500 μm was analyzed and elemental distribution data plotted (Fig. 2).

The results illustrated in Fig. 2 indicate that while some elements, often present at extremely low concentrations, do show minor variations, in general there is exceptional comparison of replicate analyses proving the method to be reproducible. In addition, there is a significant variation in the elemental profiles of the three safes, facilitating an unambiguous identification of each by analysis of the debris produced. In order to illustrate that the size fraction has little effect on the determined elemental distribution patterns, data for all the different size fractions were compared (Fig. 3).

The results illustrated in Figs. 2 and 3 are results obtained from debris generated from Safe B. The results for Safes A and C are similarly reproducible and confirm the observation that the size of the debris has little to no effect on the elemental distribution patterns.

Having established the reproducibility of the method it is fundamentally important that differentiation between debris generated from differing safes or steels is possible for the technique to have potential in criminal investigations. Data confirming the ability to differentiate between the debris generated from the three different safes, using the 10 spherule LA-ICP-TOF-MS protocol, are illustrated in Fig. 4.

The ability to assign and discriminate provenance of the different sources has been illustrated using a ternary diagram (Fig. 4c) and a discriminant plot from a principal component analysis (PCA) (Fig. 4b).

The ternary plot represents the direct comparison of relationship between the three components in a system. The positioning of a data point anywhere within the ternary plot indicates its relative percentile inter-association of the three components defined in the diagram. It is also possible, as indicated by the figure adjacent to the analyte in the diagram, to increase or decrease the value of a component by a specific amount. The ability to increase or decrease the influence of the different components makes it possible to include and compare analytes within the ternary system, which vary significantly in abundance [13]. This is the case illustrated in Fig. 4c where vanadium is in significantly more abundant than dysprosium and gadolinium.

The PCA has been carried out on the entire dataset using a correlations matrix approach within the Microsoft Excel-Stat package. The correlation PCA method, mean centres and root mean sum of squares (RMSS) scales the data for each element. The resulting scatter plot of the first two principal component scores (discriminant plot) is illustrated in Fig. 4b.

The results illustrated in Fig. 4 indicate clearly the potential of the method to be used to discriminate between debris of differing provenance and to co-associate debris from the same source. The grouping illustrated within the discriminant plot and ternary diagram indicates good reproducibility for the 10 spherule LA-ICP-TOF-MS technique making it possible to co-provenance equivalent debris together with good separation from unrelated material thus making unambiguous discrimination possible.

Despite the success of this method the effectiveness of this specific protocol is dependant on there being 10 spherules

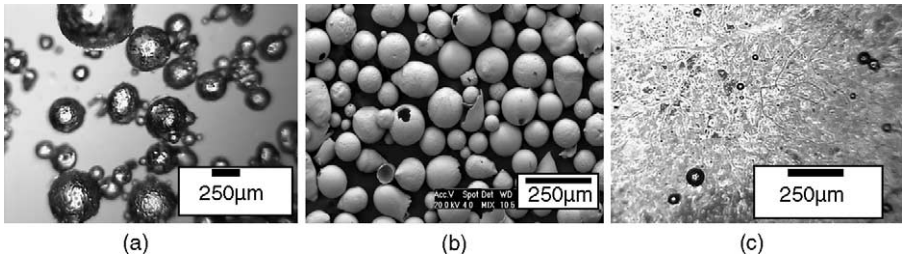
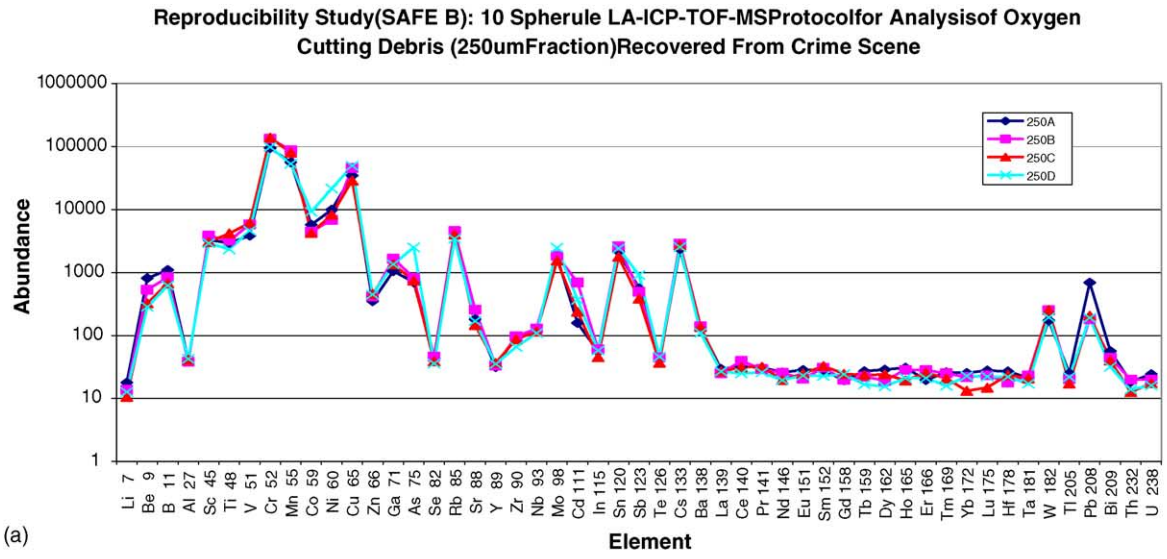
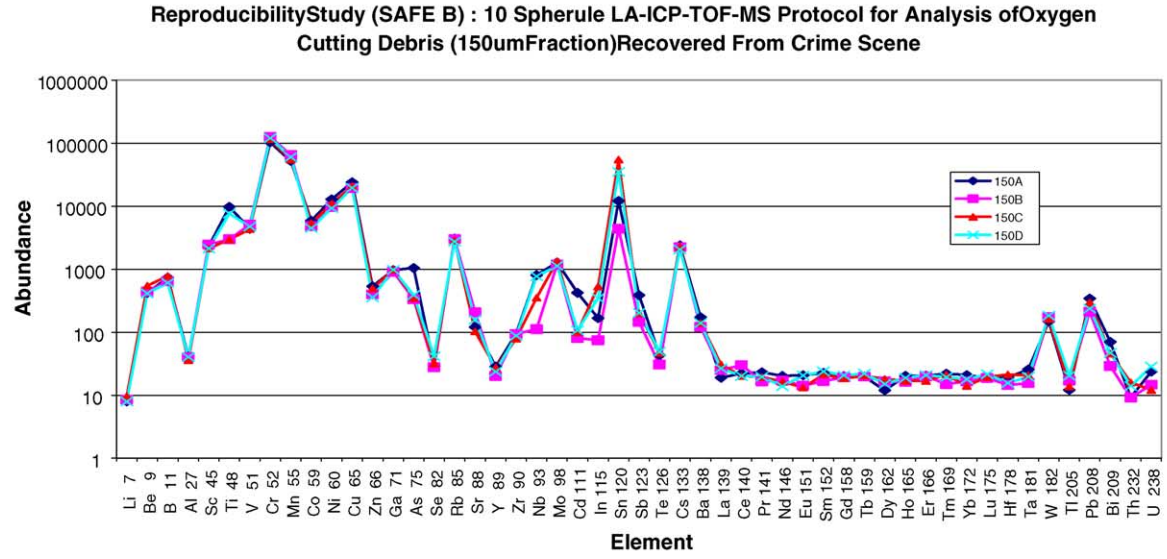
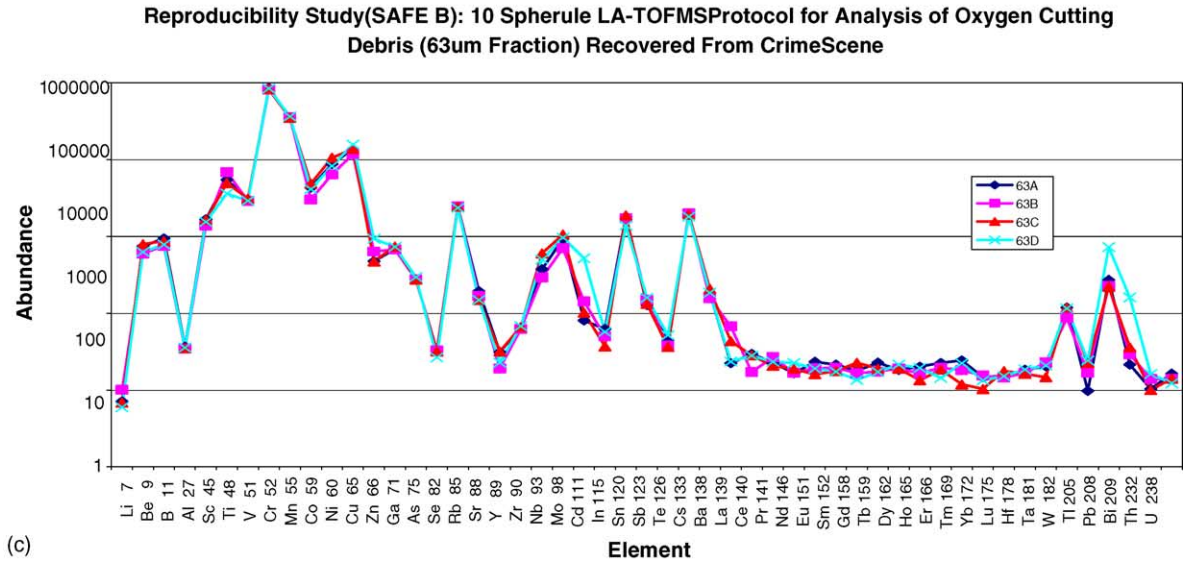


Fig. 1. Optical microscopy (a) and SEM (b) images of the oxy-acetylene cutting debris recovered from the scene of crime and optical images of material recovered from shirt of person of interest (c).



(a)





(c)

Fig. 2. Reproducibility study for the 10 spherule LA-ICP-TOF-MS analysis protocol. (a–c) Illustrates the elemental distribution patterns for the >250 < 500 μm, >150 < 250 μm, and >63 < 150 μm size fractions, respectively. *Note:* These are results obtained from debris generated from Safe B.

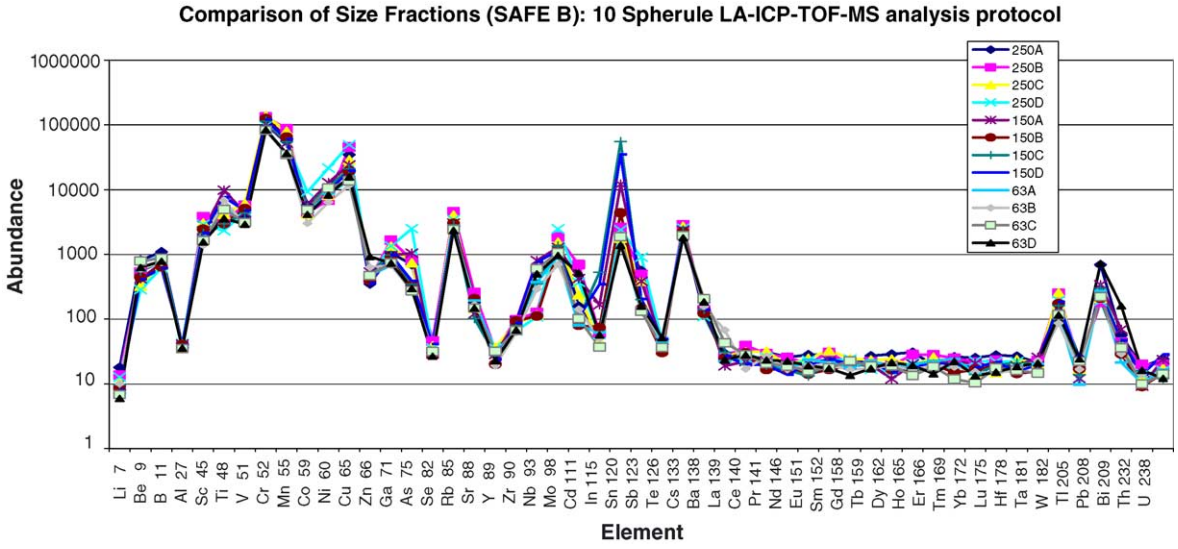


Fig. 3. Comparison of elemental distribution patterns of different size fractions of the debris generated from Safe B using the 10 spherule LA-ICP-TOF-MS analytical protocol.

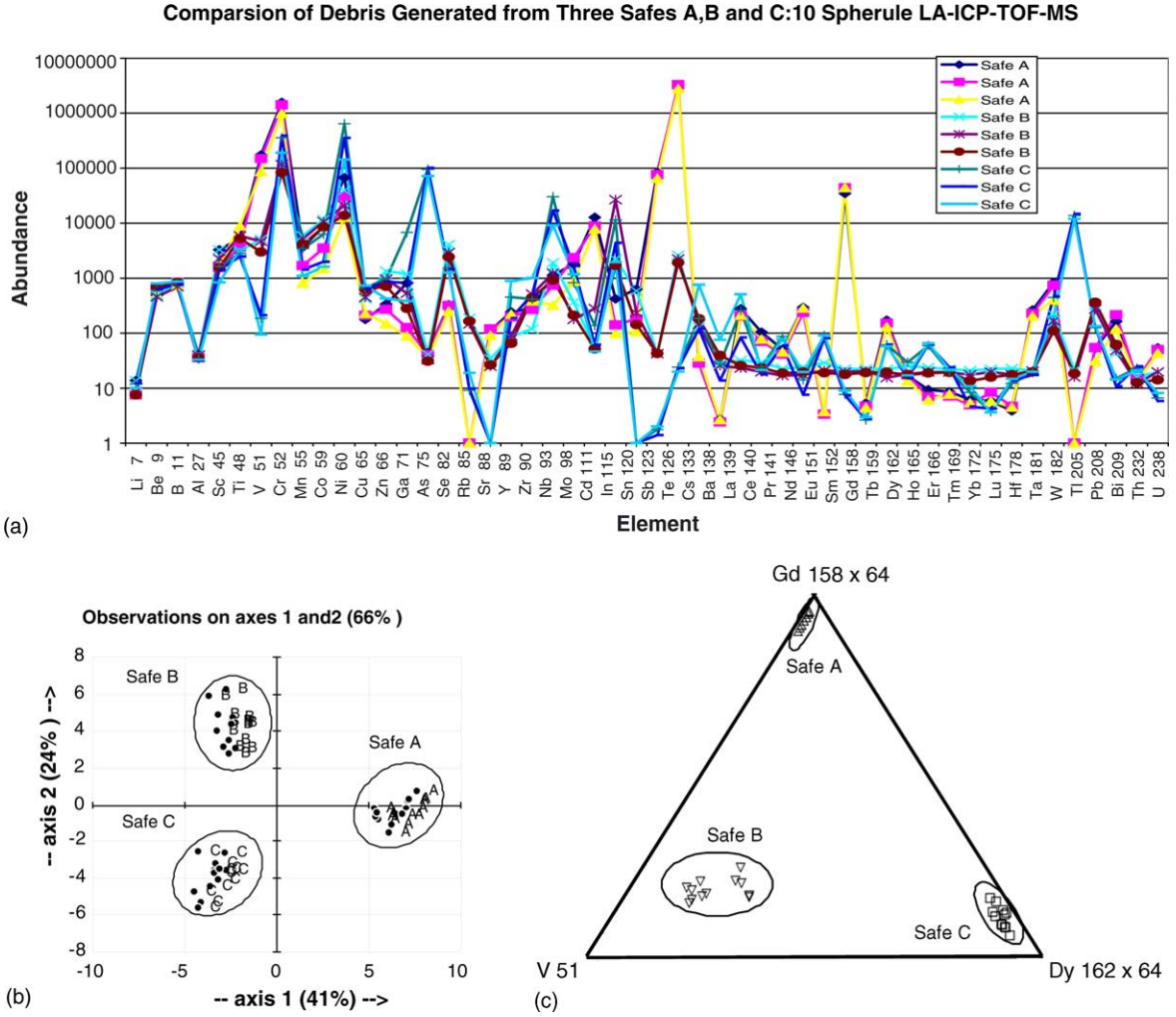
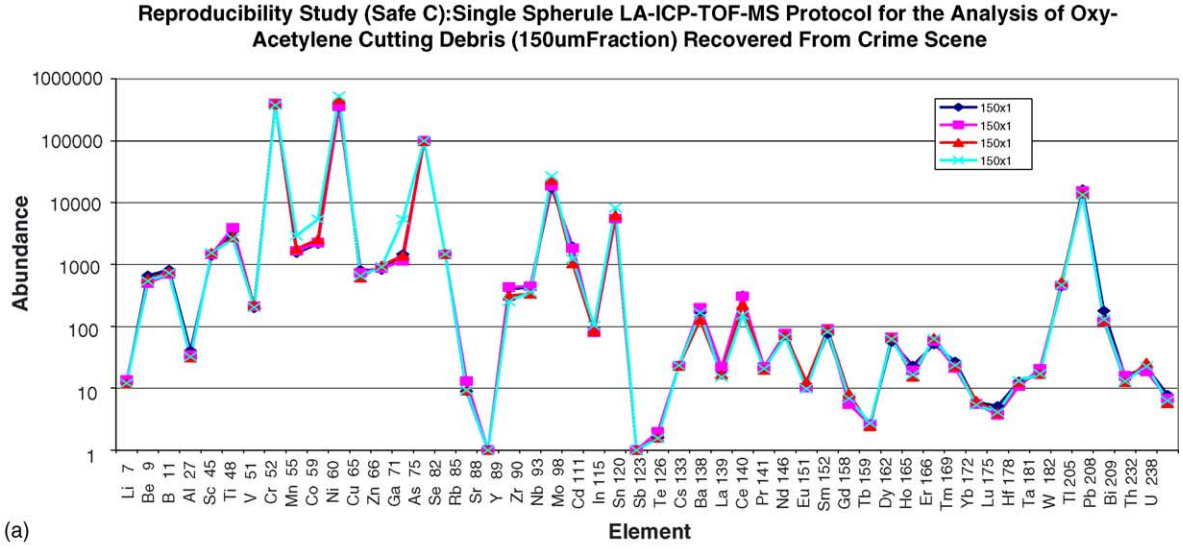
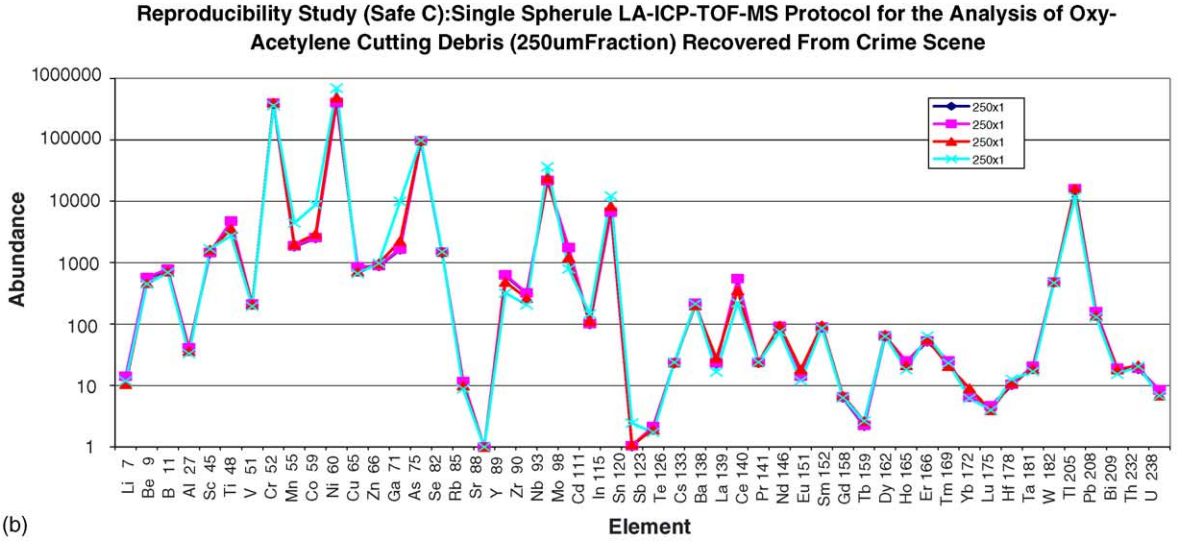


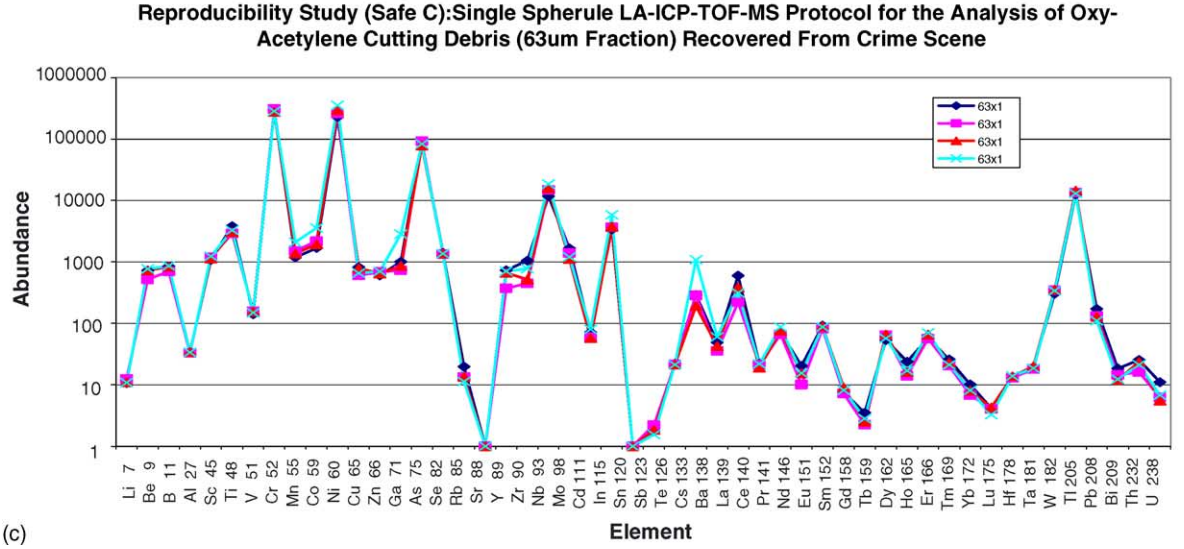
Fig. 4. Discrimination between oxy-acetylene debris generated from three different safes. (a) Illustrates the elemental distribution pattern, (b) the discriminant plot from a principal component analysis, and (c) a ternary discrimination diagram.



(a)



(b)



(c)

Fig. 5. Reproducibility study for the single spherule LA-ICP-TOF-MS analysis protocol. (a–c) Illustrates the elemental distribution patterns for the >63 < 150 μm, >150 < 250 μm, and >250 < 500 μm size fractions, respectively. *Note:* These are results obtained from debris generated from Safe C.

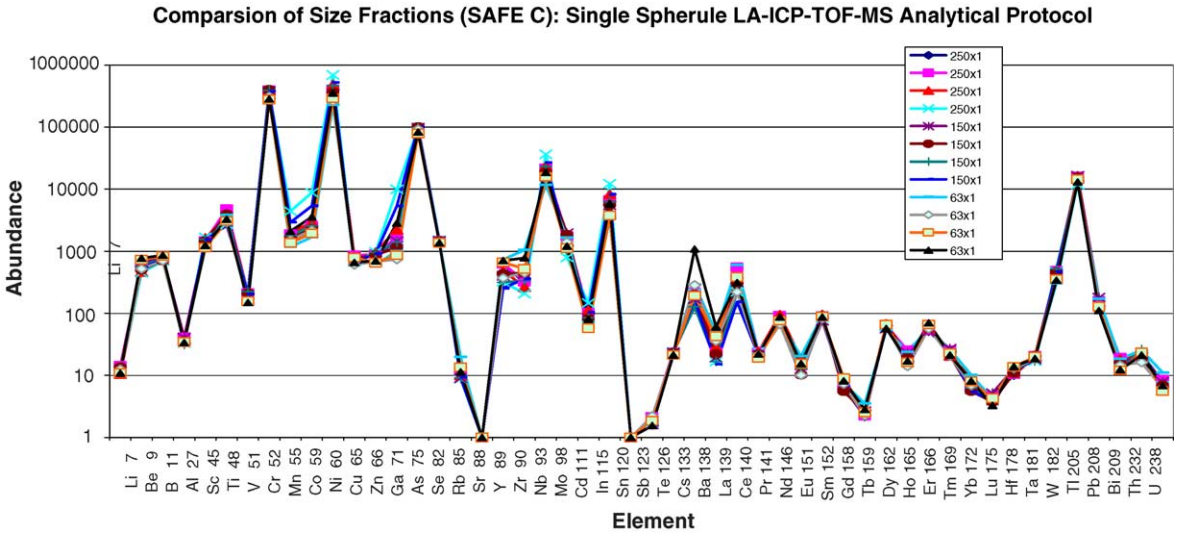


Fig. 6. Comparison of elemental distribution patterns of different size fractions of the debris generated from Safe C using the single spherule LA-ICP-TOF-MS analytical protocol.

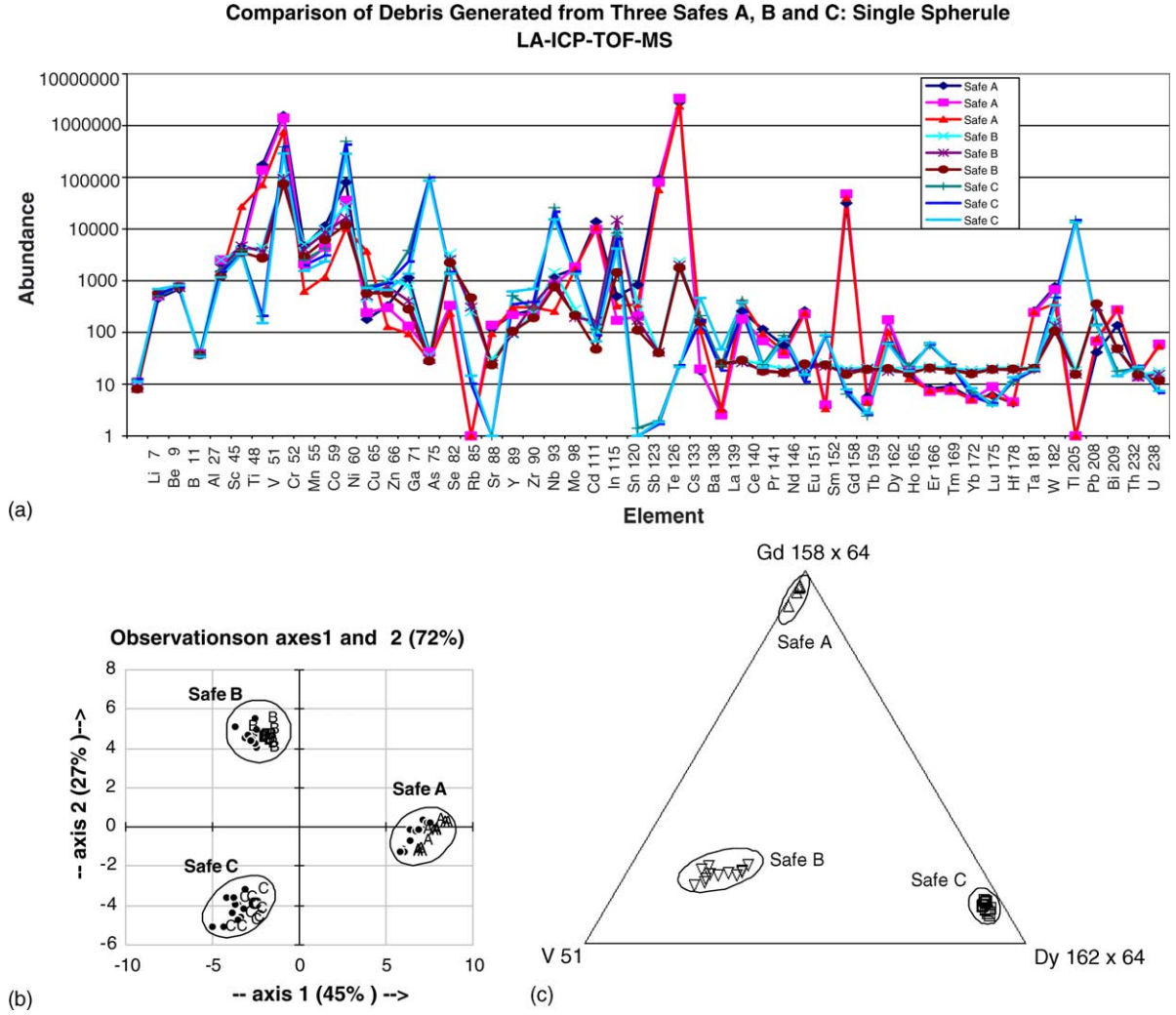


Fig. 7. Discrimination between oxy-acetylene debris generated from three different safes. (a) Illustrates the elemental distribution pattern, (b) the discriminant plot from a principle component analysis, and (c) a ternary discrimination diagram.

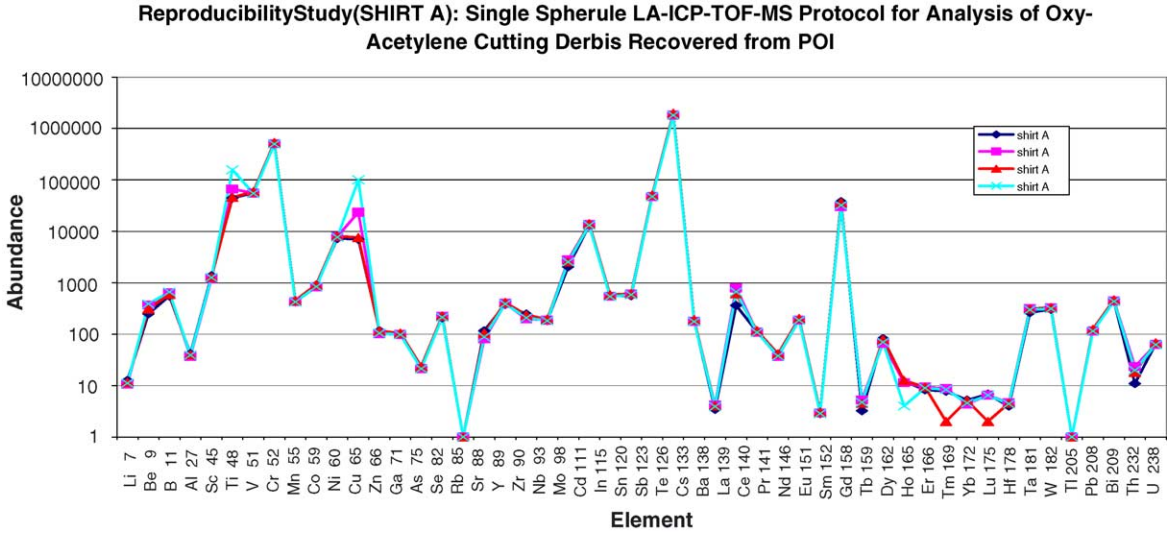


Fig. 8. Reproducibility study for single spherule LA-ICP-TOF-MS analysis of material recovered from material recovered from shirt worn while cutting Safe A.

available (more if multiple analyses are to be carried out). This leads to a possible problem when analysis is being carried out on material recovered from persons of interest. Not only is there significantly less material available but there is a possibility of contamination from spherules of a different source. Consequently the establishment of a technique based on the analysis of a single spherule is essential to avoid the potential of cross contamination and also to increase the application of the technology where recovered debris is potentially limited.

4.2.2. Single spherule LA-ICP-TOF-MS

This experiment was designed to investigate the reproducibility of the single spherule ablation method together with establishing its effectiveness for the analysis of singe debris pieces. The investigation was designed to establish if a match could effectively be made between debris recovered from a person of interest and debris collected at a crime scene. The reproducibility of the data produced from the analysis of a single spherule using LA-ICP-TOF-MS method is illustrated in Fig. 5.

In order to confirm the results obtained using the 10 spherule LA-ICP-TOF-MS protocol and establish that the size of the debris has minimal effect on the determined elemental distribution a comparison of the elemental distribution patterns for each of the size fractions is illustrated in Fig. 6.

The results illustrated in Figs. 5 and 6 have been obtained from debris generated from Safe C. The results for Safes A and B are similarly reproducible and confirm the observation that the size of the debris has little effect on the

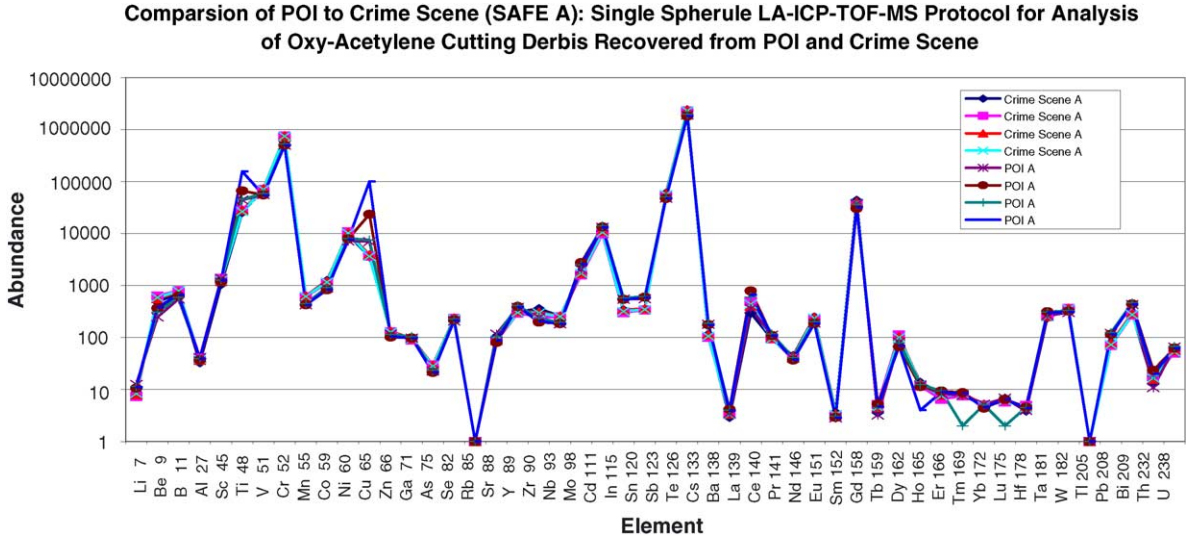


Fig. 9. Comparison of material recovered from scene of crime where Safe A is cut using an oxy-acetylene gas cutter with material recovered from shirt of a person of interest.

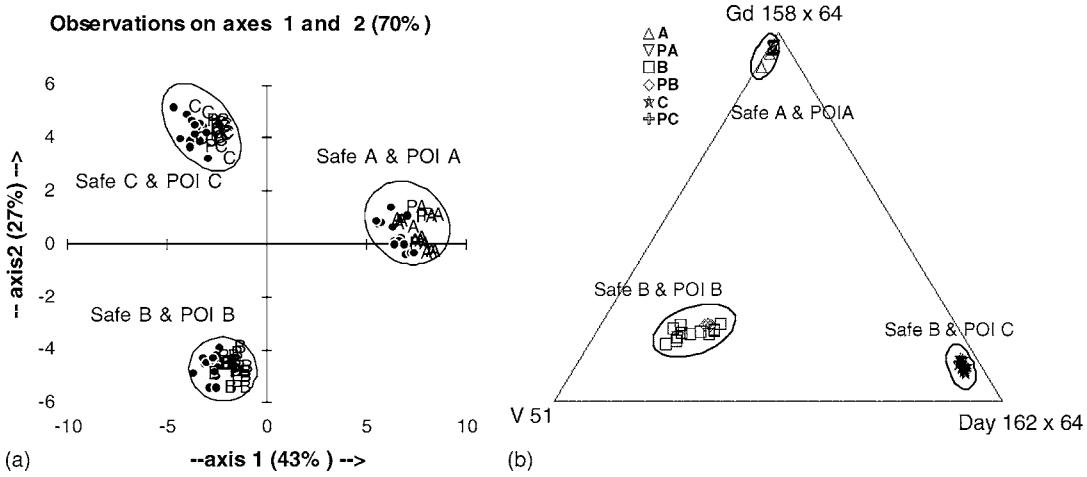


Fig. 10. Linking persons of interest to correct crime scenes using discriminant plot from PCA (a) and a ternary diagram (b).

determined elemental distribution present in this size range of debris.

Having established the reproducibility of the method it is fundamentally important that differentiation between debris generated from differing safes or steels is possible for the technique to have potential in criminal investigations. The effectiveness of this technique to provide robust data, which can be used to differentiate between the debris generated from the three safes, using the single spherule LA-ICP-TOF-MS protocol, is illustrated in Fig. 7.

The results illustrated in Fig. 7 indicate that the single spherule LA-ICP-TOF-MS analytical method can be used to obtain results equally reproducible results as those for 10 spherule alternative method. The discriminant and ternary plots (Fig. 7) illustrate the clear discrimination between the debris generated from the different safes. The close association of individual data points within individual groups indicates the reproducibility of the method.

4.2.3. Linking POI's to crime scenes

The single spherule method was utilized with success to obtain the elemental distribution pattern of the debris recovered from the shirts of the persons of interest. The ability to analyze single spherules eliminates the possibility of contamination from debris generated from a source other than that found at the scene of crime. The reproducibility of the results obtained for the analysis of debris recovered from the shirt, worn while cutting Safe A, is illustrated in Fig. 8.

The results illustrated in Fig. 8 are from debris recovered from Shirt A (corresponds to Safe A). Similar reproducible results were obtained for the material recovered from Shirts B and C. The determined elemental distribution pattern of the material recovered from the shirt of the person of interest can be compared to the material recovered from the scenes of crime (A–C). The comparison of the elemental distribution patterns of debris collected from crime scene A and recovered from the shirt of POI A is illustrated in Fig. 9, the exceptional closeness of the patterns indicating co-provenance of both sets of debris.

The ability to match persons of interest to crime scenes is further illustrated by reference to Fig. 10. The grouping evident in both the discriminant plot and ternary diagram of the crime scene (safe samples) and POI samples indicates a clear co-provenance. The presence of different groups within the diagram illustrates the ability to discriminate material of varying provenance from different crime scenes making it possible to link persons of interest with their relevant crime scene.

5. Conclusions

Using optical and scanning electron microscopy, it was confirmed that the debris generated from the oxy-acetylene cutting of steel was essentially spherical to sub-spheroidal in shape. There was notable variation in the size of the debris generated, which was expected to be reflected by variations in trace element distribution pattern. However, this supposition was incorrect with essentially identical patterns obtained for all size fractions. Reproducibility experiments for both the single and 10 spherule LA-ICP-TOF-MS analytical methods indicated a high degree of precision of the analytical data, confirming that the single spherule method was equally as effective at assigning provenance of debris as the 10 spherule method.

This research has successfully established a method for the analysis of oxy-acetylene cutting debris as little as 70 μm in size. The possibility of using the elemental distribution pattern, generated from the analysis of a single spherule, to accurately link persons of interest to crime scenes, has also been demonstrated. Consequently the previous requirement to use bulk samples, which may of necessity contain multiply sourced material, has now been overcome. Results, detailed in this paper, indicate the potential for the technique to be

effectively used in confirming or refuting co-provenance of steel micro-debris recovered from a POI and at a crime scene.

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