

A REVIEW OF THE APPLICATION OF THE GC-MS ANALYTICAL TECHNIQUE IN DETERMINING TRACE POLYAROMATIC HYDROCARBONS (PAHS) IN DIFFERENT FIRE RESIDUE SAMPLES.

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Abstract

The study reviewed three cases which involve the analyses of different fire residues. The first case involved the burning of cloths that were contaminated with three home based inflammable compounds such as the nail polish remover solution, camphor body lotion and liquid perfume. The second case experimented using the cigarette smoke residues that were generated under the ISO smoking conditions. Lastly, the case involved fire residues collected from an unused burned farming land.

Polycyclic aromatic hydrocarbons (PAHs) are a group of toxic organic compounds that may be used as forensic source identifiers for contamination in arson cases and are released through incomplete combustion occurring under low oxygen conditions. Solid-phase extraction (SPE) and liquid-liquid extraction (LLE) methods were used to isolate and purify the analytes by removing possible interfering substances from the sample matrices and to dilute and/or pre-concentrate the trace analytes for the purpose of improving their detectability during analysis by the gas chromatographic - mass spectrometry (GC-MS).

Acenaphthylene and Fluorene hydrocarbon species were detected in all three residue samples of nail polish remover, camphor and perfume. Benzo(k)fluoranthene was present in the nail polish remover and camphor residue samples; benzo(b)fluoranthene was found in camphor residue samples; while dibenzo(a,h)anthracene and benzo(g,h,i)perylene species were detected only in the perfume residue samples.

Benzo(b)fluoranthene and benzo(a)pyrene were detected in burned farmland residue samples while the reconstruction of the scene helped to identify the plastic component of the tool used in setting fire. The limit of quantification (LOQ) of 16 PAHs species ranged from non-detectable levels for the dibenzo(a,h)pyrene and other three compounds to 108.17 ng/CPD² for naphthalene per 20 cigarettes per CFP standard smoked under ISO smoking regimes.

The study found the laboratory analytical results reported to be admissible in court within the South African jurisdiction.

Keywords: PAHs; Trace organic analytes; Fire residues; LLE; SPE; GC-MS; Admissibility of laboratory results.

1. Introduction

There is no doubt that scientific evidence is admissible in law if it is well authenticated. In the article there is no concerted attempt that will be made to enumerate the various types of scientific evidence or to describe their current reception in courts. A number of excellent recent articles have already done so.^{1,2}

The present paper, by contrast, is an excellent attempt to look at scientific evidence as a generic type of evidence, at the decisional process which leads to its admission or exclusion. No claim of novelty is made for much that is included. Nevertheless, at least two considerations suggest that a broad treatment of the questions affecting the admissibility of the scientific evidence is in order.

Trace evidence includes very small material, which may not be visible to the naked eye and require specialized scientific techniques to identify, isolate and collect for assessments purpose. Trace material

¹Ahrens, *Scientific Evidence and the Law: Verification of Verbal Testimony and Psychological Proof*, 13N.Y.L. F, 612 (1967).

²Boyce, *Judicial Recognition of Scientific Evidence in Criminal Cases*, 8 Zitah L. rev 313 (1963)

left or transferred and found around crime scenes may include chemicals, biological fluids and stains, hair and fibres, paint chips, soil, broken glass fragments, as well as residues of gunshots, prints, drugs and fires debris.

Laboratory analytical processed involves isolating elements of interest from sample matrices so that they may be identified and measured against standards of similar elements. However, this step is often challenging and requires robust approaches that would not be affected by the variations of different parameters and conditions.³

The aim of the study is two-fold, first is to assess the conditions that would make laboratory test results of trace evidence admissible in law. Secondly, is to assess the selectivity and specificity of the GC-MS analytic technique in determining PAHs in different fire residues.

The study reviewed three published cases which involve the analyses of different fire residues that are products of three burning conditions. The first burning involves cloth that was contaminated with three commonly found inflammable compounds such as nail polish remover solution, camphor body lotion and liquid perfume. The second case experimented on cigarette smoke residue that was generated under the ISO smoking standard conditions. Lastly, the case involves fire residues collected from an unused burned farming land.

PAHs are a group of organic compounds consisting of two or more fused aromatic rings with Naphthalene ($C_{10}H_8$) being the smallest ring structure composed of two aromatic rings that can be detected by instrumental analytical techniques. They are neutral, hydrophobic nonpolar molecules with physical and chemical characteristics, which influence their volatility and solubility.⁴

Their structural variations and partitioning in air play a role in their mobility, mutagenicity, carcinogenicity and fate in the environment.⁵ The 2 to 3 ring PAHs are known as low molecular weight PAHs (LMW PAHs) while the 4 to 6 ring PAHs are known as high molecular weight PAHs (HMW PAHs).⁶ The US EPA guidance lists 16 of these hydrocarbons as priority pollutants, see Figure 1.

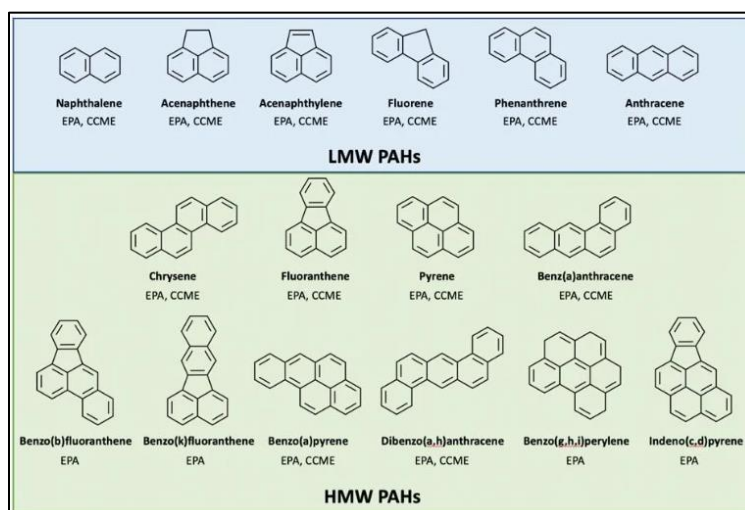


Figure 1: Structures of the 16 US-EPA Priority Pollutant.⁷

The anthropogenic sources of PAHs include incomplete burning under low oxygen conditions of organic material such as coal, wood, tobacco, wood, petroleum, refuse, tires, etc. PAHs may be used as forensic source identifiers for contamination that can occur via petroleum or anthropogenic combustion

³GC/ MDGC. What is a robust method. 11 July 2014.

<https://www.chromatographytoday.com/news/gc-mdgc/32/breaking-news/what-is-a-robust-method/30904#:~:text=One%20of%20the%20most%20widely,its%20reliability%20during%20normal%20usage>

⁴Chemistry-matters.com. *Polycyclic Aromatic Hydrocarbons (PAHs) | Chemistry Matters Chemical Expertise.*

⁵Mitra J. 2018. *Hydrocarbon Based Analysis in Arson Cases Using Inflammable Materials. Research Article Volume - 8 Issue 3 – May. DOI: 10.19080/JFSCI.2018.08.555740.*

⁶Ibid

⁷Ibid

sources. The four factors that influence the occurrence and continuation of fire are fuel, heat, oxygen and exothermic chemical reaction, and removing any of these factors will prevent, suppress or control it.⁸

2. Assessments of experimental details

The three studies experimented on three different types of fire residue matrices. The first fire residue was on clothing materials that were burnt using three accelerants that are commonly found in homes such as a nail polish remover solvent, camphor body lotion and perfume solution.⁹

A cigarette smoke residue was studied in the second case¹⁰ while the third type of fire residue was in a form of a candle which was used to initiate fire on an unused commercial land (*Sturaro et al.*, 2013). Representative samples of the three different types of fire residues were collected using commonly used scientific methods.

An effective sample preparation technique was developed for the analysis of hydrocarbons from the three fire debris samples of nail polish remover solvent, camphor body lotion and liquid perfume, with fire accelerants that are commonly found in homes.¹¹ Weighed fire debris samples of the three inflammable materials were collected, instantly sealed in airtight glass bottles which had been pre-washed with n-hexane and then transported to the laboratory for analysis. After extensive sample preparations in the laboratory, the ash sample was loaded into quartz crucible pre-washed with n-hexane and covered with aluminium foil until further processing and analysis. Standards containing 16 PAHs species were prepared in ethylene chloride.

Jeffrey et al. (2018) conditioned the 3R4F and the CFP reference cigarettes to prepare PAH calibration solutions, a mixture of native and deuterium (D)-labelled PAHs, internal standards and base-modified silica cartridges for sample preparation, and glass fibre filter pads. The stock standard solutions were in toluene/isooctane solvent mixtures.

Samples were collected a few hours after the fire had quenched following the physical evidence method of locating the point of origin of the fire and physical indicators left by fire on the stones, soil, logs and vegetation.¹² A green coloured plastic bottle which could have been an incendiary bomb or tool was found about 29 m outside the burned area.

2.1 Sample extractions, clean-up and analysis

On arrival in the laboratory, samples were prepared for the purpose of isolating, extracting and quantifying the analyte compounds from the different sample matrices. Common sample extraction methods for each matrix are listed in the Table 1.

Extraction methods involves the treatment of samples to free trace levels analyte from matrices. Organic solvents of different polarities were used to isolate, purify or clean-up to remove possible interfering substances and dilute or pre-concentrate the trace analytes to improve their detectability before analysis.

Table 1: Sample extraction methods for different sample matrices.

Sample Matrices	Extraction Methods
Air	- Separatory funnel uses liquid-liquid extraction (LLE) - Solid-phase extraction (SPE) - Soxhlet uses liquid-liquid extraction (LLE)
Aqueous	- LLE

⁸*ibid*

⁹*Mitra J.* 2018. *Hydrocarbon Based Analysis in Arson Cases Using Inflammable Materials*. Research Article Volume - 8 Issue 3 – May. DOI: 10.19080/JFSCI.2018.08.555740.

¹⁰*Jeffery J, Songin MCK, Pettit M, Pettit K and Wright C.* Optimized method for determination of 16 FDA polycyclic aromatic hydrocarbons (PAHs) in mainstream cigarette smoke by gas chromatography–mass spectrometry. *Chemistry Central Journal* (2018) 12:27 <https://doi.org/10.1186/s13065-018-0397-2>

¹¹*Mitra* (2018).

¹²*Sturaro A, Vianello A, Denti P and Rella R.* 2013. *Fire debris analysis and scene reconstruction*. *Science and Justice* 53. 201-205. ScienceDirect.

www.elsevier.com/locate/scijus

	- SPE
Solids	- SPE - Ultrasonic - Rotary Evaporator (LLE)

Once samples collected from scenes where the fire allegedly started reached the laboratories, standard scientific processes were used to prepare them for analyses.¹³ *Jeffery et al (2018)* used a two-phase TELOS solid phase extraction (SPE) method with a column conditioned with cyclohexane, while *Sturaro et al., 2013* performed sample extractions by a solid phase micro-extraction (SPME) technique which allow the introduction of the fibre into the headspace vial that contain sample aliquots and it will be ready for analysis. *Matrid (2018)* used liquid-liquid solutions in extraction glassware. SPE is known to be efficient in extracting and isolating PAHs analytes from difficult sample matrices which cannot be quantified directly due to their ultra-low levels.^{14,15}

For further sample preparation, an intensive extraction process using an ethanol-toluene solvent mixture was applied, and the results were purified through the SPE process, which efficiently removed the PAHs analytes from the matrix artefacts for the purpose of making quantification of individual species possible. A PAHs in 3R4F ISO MCS standard reference material was used.¹⁶

The role of chromatographic techniques is to separate organic components of mixtures, identify them by comparing the retention times (t_R) of analyte against that of the standard constituent and quantify them by measuring the peak area of separated species. Separation happens when the sample mixture is transported by the mobile phase through the column and interact with the solid stationary phase that is fixed in the column. The analyte or sample constituents that favours the stationary phase will spend more time in the column, while the constituents that prefer the mobile phase are eluted first.

In all these cases, GC-MS techniques were adopted as an analytical instruments of choice. This separation method use a gas mobile phase and a solid stationary phase that is packed in a column that depends on the diameter, length, size of packing material, polarity and purpose. Most stationary phases for the GC are selective and specific when they separate PAHs species in sample mixtures. Also, a thorough, carefully prepared and purified sample solution is required to improve the detectability of the PAHs in a low-resolution mass spectrophotometer.¹⁷ The main components of a GC-MS techniques are shown in Figure 2.

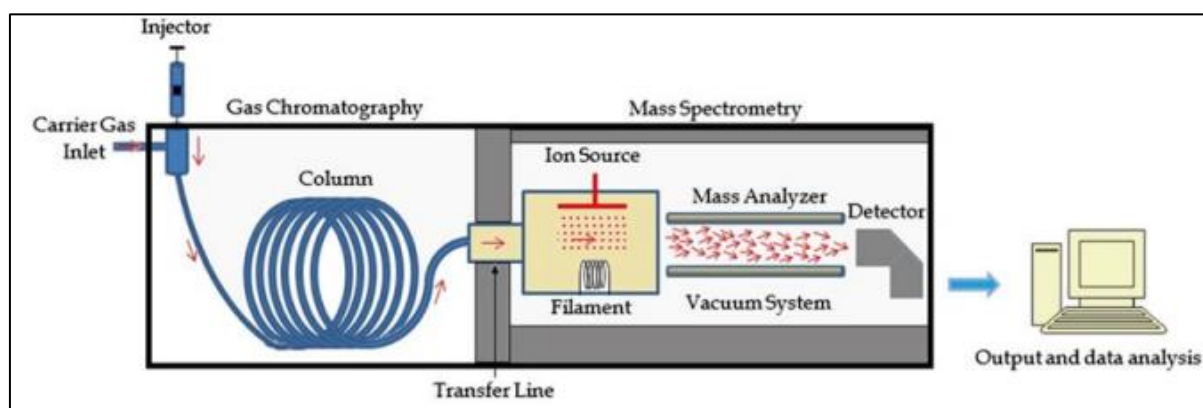


Figure 2: Schematic diagram of the main components of GC-MS instruments.¹⁸

¹³*Ibid*

¹⁴*Jeffrey et al (2018)*.

¹⁵*Moja SJ and Mtunzi F. 2013. Application of solid phase extraction (SPE) method in determining polycyclic aromatic hydrocarbons (PAHs) in river water samples. J. Environ. Chem. Ecotoxicol. Vol. 5(11), pp. 278-283, December. DOI: 10.5897/JECE2013.0294. ISSN 2141-226X ©2013 Academic Journals <http://www.academicjournals.org/JECE>. 278 – 283.*

¹⁶*Jeffrey et al (2018)*.

¹⁷*Ibid*

¹⁸*Emwas AHM, Al-Talla ZA, Yang Y, and Kharbatia NM. Gas Chromatography–Mass Spectrometry of Biofluids and Extracts Article in Methods in molecular biology (Clifton, N.J.) · February 2015 DOI: 10.1007/978-1-4939-2377-9_8*

2.2 GC-MS and analytical conditions

Sturaro (2013) performed all the analyses using a GC-MS system composed of an HP 6890 GC coupled with an HP 5973N quadrupole mass analyzer. An HP 5MS capillary column containing stationary phase and a helium gas mobile phase were used to separate sample constituents. Temperature programming was used with temperatures 45 °C for 3 mins, 10 °C for a min and 250 °C for 15 mins. The injector temperature was set at 250 °C and the injects were performed under split less conditions for under a minute. The MS was used in SCAN acquisition mode with mass ranging from 50 to 300 Da and a scanning rate of 5.56 scan/s to detect the ions produced by the electron impact ionization at 70 eV. The temperature of the ion source was set at 230 °C.

The vials containing sample aliquots were heated to volatilize species of interest, which were then released into the headspace and adsorbed on to the PDMS fibre for analyses by desorption process while being carried out of the GC component after separation and to be quantified by the MS.¹⁹ Sample residues presumed to contain traces of wax, were treated with a procedure proven to identify chemical trace components of candles and the extracted liquid phase was analysed to identify components of candle wax such as palmitic, stearic acid methyl ester and saturated hydrocarbons by GC-MS technique.²⁰

Mitra (2018) used an Agilent 6890N GC equipped with an Agilent 7683B Injector, a 30mm x 0.32mm Hewlett-Packard-5 (HP-5MS) capillary column, coated with 5% phenyl - methyl siloxane (film thickness 0.25 micrometre) and an Agilent 5975 Mass Selective Detector (MSD) was used to separate and quantify the PAH compounds.

The samples were injected in the split less mode at an injection temperature of 230 °C, the transfer line temperature was 300 °C under vacuum. The interface temperature was 230 °C. The column temperature was initially held at 70 °C for 1 minute, then heated to 150 °C at 8 °C per min, then heated to 250 °C at 5 °C per min and to 300 °C at 7 °C per min and held for 5 mins. The temperature of the detector was set for 230 °C while the helium mobile gas was used at a constant flow rate of 1 mL/min.

Jeffrey (2018) used a single quadrupole GC-MS Agilent technique for the analysis of MCS of 3R4F and CFP reference cigarettes generated under ISO 3402 intense smoking regimes, see Table 2.

Table 2: GC conditions used for analysis of PAHs in mainstream smoke.

GC separation conditions	
<ul style="list-style-type: none"> • Injection • Injection volume • Carrier gas • Column • Oven temperature programme 	<ul style="list-style-type: none"> • Multimode (PTV) injection, splitless mode • 2 µl • Helium; 1 ml/min (50 min), then 2 ml/min (until the end of the analytical run) • Agilent DB-EUPAH (60 m × 250 mm × 0.25 µm) • 50 °C (0.8 min), ramp 45 °C/min to 200 °C, ramp 2.5 °C/min to 225 °C, ramp 5 °C/min to 266 °C, ramp 14 °C/min to 300 °C, ramp 10 °C/min up to 320 °C (48 min). Total run time 74.762 min

2.3 Comparison of the results and discussions

All the results were based on extracts that were divided into three aliquots to ensure consistency of the sample and to minimise possible variations during analyses by GC-MS. The samples were found to contain different amounts of acenaphthylene and fluorene poly aromatic species, but with the same retention time and different abundance of quantification / confirmation ions.²¹ It was recommended that these inflammable materials that are fire accelerants and are commonly found in homes should be stored in cool areas away from possible fire ignitors as they emit harmful and inhalable hydrocarbon pollutants.

¹⁹*Sturaro et al., (2013).*

²⁰*Ibid*

²¹*Mitra (2018).*

The data demonstrate that the optimised sample preparation strategy for GC-MS analysis was a robust analytical approach that allow for the complete determination of measurable PAHs species from cigarette smoke residues with significant levels of total particulate matter. Table 3 shows the main attributes of the reference cigarette residues used in the study.²²

Table 3: The main attributes of the 3R4F Kentucky reference cigarette.

Parameter	Mean value (mg/cigarette)
Weight	1060
Total particulate matter (TPM)	11.0
Nicotine	0.73
Nicotine-free dry particulate matter (NFDPM)	10.27
Carbon monoxide (CO)	12.0
Puff count	9.0

For quality assurance, unfortified CFPs were extracted to provide method blank samples. For regular monitoring of analytical method performance, unsmoked/blank CFPs were fortified with native or internal standards and extracted following the SPE procedure.²³ The recoveries of native PAHs in quality control samples were calculated to obtain the percentage recovery, which were assessed for each analytical sequence to monitor the method performance. The retention times and mass/charge ratios of the PAHs species of 3 R4F ISO cigarette smoke reference are presented in Table 4.

Table 4: The PAHs constituents of the 3R4F ISO mainstream smoke reference residues.²⁴

Numbers	Ion (m/z)	PAH	GC-MS
			Retention time (min)
1	128	Naphthalene	7.8
2	136	Naphthalene-d8	7.7
3	134	13C6-Naphthalene	7.8
4	252	Benzo[a]pyrene	39.3
5	264	d12-Benzo[a]pyrene	37.1
6	256	13C4-Benzo[a]pyrene	37.3
7	278	Dibenzo[a,h]anthracene	48
8	302	Dibenzo[a,l]pyrene	63
9	302	Dibenzo[a,e]pyrene	68.8
10	302	Dibenzo[a,i]pyrene	72.6
11	302	Dibenzo[a,h]pyrene	74.9
12	314	13C12-Dibenzo[a,i]pyrene	72.6

The limit of quantification (LOQ) was calculated in ng/CFP from the analyte concentration for the MS system and the LOQ per cigarette was then estimated using the number of cigarettes smoked (Table 5). The chromatograms of the dominant species are shown in Figure 3 below. The LOQ refers to the lowest concentration of an analyte that can be determined accurately and with a high level of precision and certainty.²⁵

²²Jeffrey et al (2018).

²³Ibid

²⁴Ibid

²⁵Konieczka P, 2012. *Comprehensive sampling and sample preparation. Theory of extraction techniques. Analytical techniques for scientists. Reference work. ISBN: 987-012-381374-9. Editor in Chief. Canada. www.sciencedirect.com* .

Table 5: Comparison of LOQs for 16 PAHs achieved by GC-MS.²⁶

Number	PAHs Analytes	LOQ (ng/CPD ²)	Estimates (ng/Clg)	LOQ
1	Naphthalene	108.17	5.41	
2	Benzo[c]phenanthrene	66.80	3.34	
3	Dibenzo[a]anthracene	38.11	1.91	
4	Chrysene	49.61	2.48	
5	Cyclopenta[c,d]pyrene	60.04	3.00	
6	5-Methylchrysene	2.48	0.12	
7	Benzo[b]fluoranthene	5.08	0.25	
8	Benzo[k]fluoranthene	5.07	0.25	
9	Benzo[j]aceanthrylene	ND	ND	
10	Benzo[a]pyrene	3.03	0.15	
11	Indenol[1,2,3-c,d]pyrene	1.54	0.08	
12	Benzo[a,h]pyrene	1.48	0.07	
13	Benzo[a,l]pyrene	ND	ND	
14	Dibenzo[a,e]pyrene	0.01	0.01	
15	Dibenzo[a,l]pyrene	ND	ND	
16	Dibenzo[a,h]pyrene	ND	ND	

ND: Analyte is not detectable in the sample.

*20 Cigarettes per CFP were smoked under ISO smoking regimes.

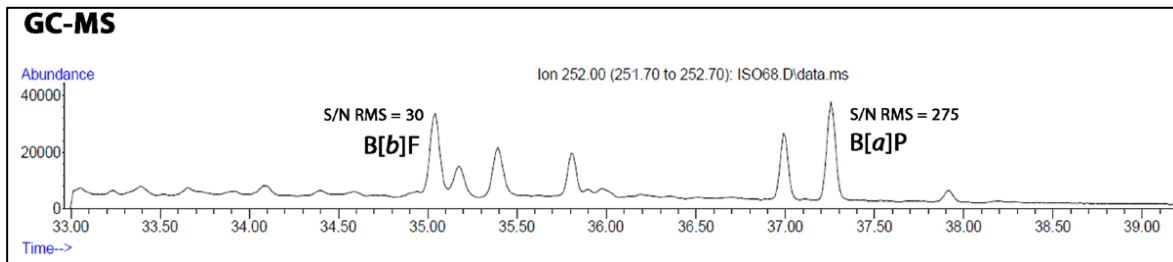


Figure 3: Benzo[b]fluoranthene and benzo[a]pyrene and their signal to noise ratios for the 3R4F ISO MCS standard.²⁷

The analytical results of the fire debris identified the component of the tool used in setting fire, Figures 4 and 5.²⁸ The GC-MS outcomes on the plastic residues clarified the arrangements of the tool components. Based on the fire scene reconstruction and the fire setting tool used, it was concluded that the arsonist intended to burn a small bush glade far from a country house and the village, where it will be near impossible not to see the fire smoke. The chosen time and the setting point are additional clues which suggest that only a small fire was required, but the area that was burned was not utilized for economic reasons.

²⁶Jeffrey et al (2018).

²⁷Ibid

²⁸Sturaro et al., (2013).

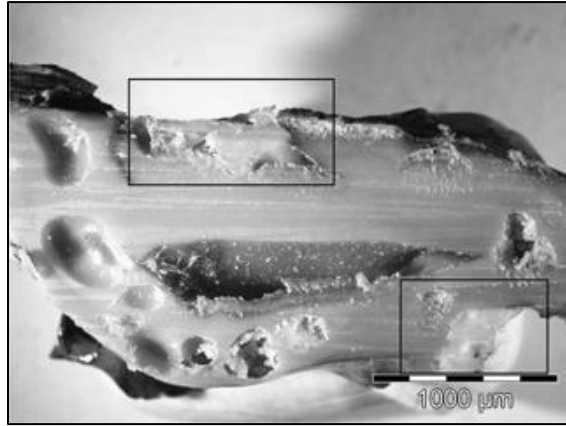


Figure 4: A microscope image of a section of plastic residue with a white material in the squares made of a flat plastic strip fused on the green surface.²⁹



Figure 5: On the left is a layout of the reconstructed incendiary tool and on the right shows the end of the laboratory effectiveness test.³⁰

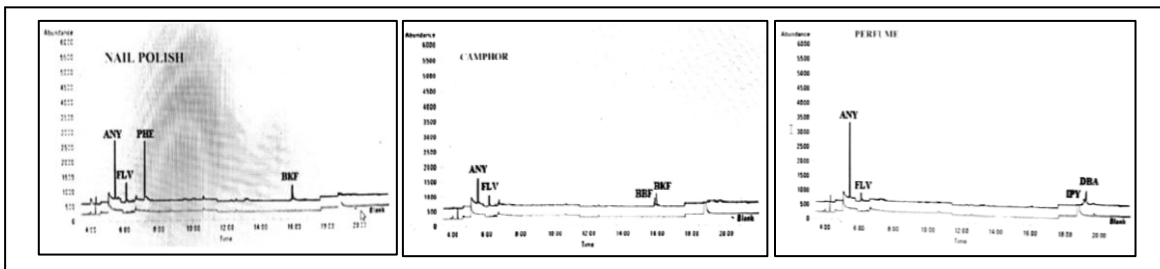


Figure 6: Chromatograms obtained from nail polish remover, camphor and perfume samples.

Figure 6 confirm the identity of the PAHs species that were present in fire residues of nail polish remover, camphor and perfume sample.³¹ However, such an assessment will be preceded by the calibration process prepared from a standard solution that contain 16 PAHs species whose retention times will be used to confirm the presence of similar species in each of the fire debris.

The data in Table 6 indicates the test result of cloth samples burnt with the three fire accelerants found in nail polish remover, camphor and perfume. The four different polycyclic aromatic hydrocarbons were detected in each of the samples were 0.084 mg/kg of acenaphthylene, 0.012 mg/kg of fluorene, 0.074 mg/kg phenanthrene and 0.13 mg/kg of benzo(k)fluoranthene species in the nail polish remover.

²⁹Sturaro et al., (2013).

³⁰Ibid

³¹Mitra (2018).



Table 6: Quantity detected of PAH compounds in nail polish remover sample.

Number	PAHs	Nail polish remover residue sample	Camphor residue samples	Perfume residue sample
		PAHs (mg/Kg)		
1	Naphthalene	ND	ND	ND
2	Acenaphthylene	0.084	0.036	0.11
3	Acenaphthene	ND	ND	ND
4	Fluorene	0.012	0.007	0.06
5	Phenanthrene	0.074	ND	ND
6	Anthracene	ND	ND	ND
7	Fluoranthene	ND	ND	ND
8	Pyrene	ND	ND	ND
9	Benzo (a) anthracene	ND	ND	ND
10	Chrysene	ND	ND	ND
11	Benzo (b) fluoranthene	ND	0.08	ND
12	Benzo (k) fluoranthene	0.13	0.11	ND
13	Benzo (a) pyrene	ND	ND	ND
14	Indenol (1,2,3-c,d) pyrene	ND	ND	0.017
15	Dibenzo (a,h) anthracene	ND	ND	0.047
16	Benzo (g,h,i) perylene	ND	ND	ND

Other PAHs found were 0.036 mg/kg of acenaphthylene, 0.007mg/kg of fluorene, 0.08 mg/kg benzo(b)fluoranthene and 0.11mg/kg benzo(k)fluoranthene present in samples of camphor debris, as well as the 0.11mg/kg, acenaphthylene, 0.06 mg/kg of fluorene, 0.017mg/kg of indenol(1,2,3-c,d)pyrene and 0.047mg/kg of dibenzo(a,h)anthracene detected in samples of perfume debris.

Conclusion

The GC-MS instruments were conditioned differently due to the specific circumstances created by different sample matrices in each case. The calibrated instruments were efficient in separating and quantifying the harmful polyaromatic hydrocarbons that are generally produced in arson crimes into individual species found in the three types of fire debris that were collected, treated and analysed.

Acenaphthylene and Fluorene hydrocarbon species were detected in all the three residue samples of nail polish remover, camphor and perfume. Benzo(k)fluoranthene was present in the nail polish remover and camphor residue samples; benzo(b)fluoranthene was found in camphor residue samples; while dibenzo(a,h)anthracene and benzo(g,h,i)perylene species were detected only in the perfume residue samples.

Benzo(b)fluoranthene and benzo(a)pyrene were detected in burned farmland residue samples while the reconstruction of the scene helped to identify the plastic component of the tool used in setting fire. The limit of quantification (LOQ) of 16 PAHs species ranged from non-detectable levels for the dibenzo(a,h)pyrene and other three compounds to 108.17 ng/CPD² for naphthalene per 20 cigarettes of CFP of standard smoked under ISO smoking regimes.

The analytical results were validated to confirm their accuracy, reliability and credibility based on standard methods used to collect representative samples, to preserve them, to extract analytes from the different fire residue matrices, to purify and characterize them. Since the outcomes of the three case studies were published in international scientific journals, it confirms their good standing within the scientific community. The sensitivity and selectivity of the conditioned GC-MS techniques used to separate and quantify individual PAHs from three different fire residue matrices were validated through the quality control processes, specifically reported by Jeffrey *et al* (2018).

Based on the content assessed, the study found the laboratory analytical results reported to be admissible in court within the South African jurisdiction.



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