Chemical Profiling of Ballistic Materials

Analysis of organic gunshot residue

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A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree in Doctor of Philosophy.

January 2018

i. Declaration of originality

I, Ellen Goudsmits, certify that I have written this thesis. The original work is my own, except as specified in the acknowledgement and in the references. All the information sources and literature used are indicated in this thesis. I further certify that neither this thesis nor the original work contained herein has been previously submitted for a degree or for part of the requirements for a degree.

Ellen Goudsmits

31-01-2018

ii. Acknowledgement

iii. Abstract

Gunshot residue (GSR) is a complex chemical mixture that is created during the discharge of a firearm. Its detection and interpretation play a crucial role in the investigation of firearm incidents. Current GSR analysis is limited to inorganic GSR (IGSR), however, the evidential value could be strengthened by inclusion of organic GSR (OGSR). The present study aims to address this potential by proposing a categorisation system for relevant OGSR compounds and developing a methodology for the collection, extraction and analysis of both organic and inorganic GSR from a single sample.

The organic composition of more than 50 propellant powders has been determined and compared against more than 200 propellant compositions reported in the literature. This work has resulted in a three-tier categorisation system for OGSR compounds, which together with the current IGSR classification will provide unequivocal identification of GSR materials with the possibility of discriminating between GSR from different ammunition types.

Evaluation of MonoTrap extraction showed that this is an effective pre-concentration technique for the characterisation of propellants. Solid-phase microextraction (SPME), however, was the superior method for the extraction of OGSR compounds from various sampling media, including swabs and stubs. The optimised methodology involves GSR collection using carbon adhesive stubs followed by SPME gas chromatography – mass spectrometry (GC-MS) analysis of OGSR and subsequent scanning electron microscopy – energy-dispersive X-ray spectrometry (SEM-EDX) analysis for IGSR. This protocol has resulted in the detection of both characteristic IGSR and categorised organic compounds, demonstrating the ability to obtain a full chemical profile from a single sample. Detection of both first and second tier organic compounds provides complementary compositional information that could be used to discriminate between samples. Furthermore, this methodology requires no changes to the current sampling and IGSR analysis protocols and addresses the limited storage time of OGSR. Since GC-MS instruments are readily available in most analytical laboratories, implementation of the proposed protocol is feasible.

iv. Abbreviation list

2-NDPA	2-Nitrodiphenylamine; IUPAC: 2-nitro-N-phenylaniline
2-NsDPA	2-nitroso-diphenylamine; IUPAC: 2-nitroso-N-phenylaniline
2-NT	2-Nitrotoluene; IUPAC: 1-methyl-2-nitrobenzene
2,2'-DNDPA	2,2'-Dinitrodiphenylamine; IUPAC: 2-Nitro-N-(2-nitrophenyl)aniline
2,3-DNT	2,3-Dinitrotoluene; IUPAC: 1-methyl-2,3-dinitrobenzene
2,4-DNDPA	2,4-Dinitrodiphenylamine; IUPAC: 2,4-dinitro-N-phenylaniline
2,4'-DNDPA	2,4'-Dinitrodiphenylamine; IUPAC 2-Nitro-N-(4-nitrophenyl)aniline
2,4-DNT	2,4-Dinitrotoluene; IUPAC: 1-methyl-2,4-dinitrobenzene
2,5-DNT	2,5-Dinitrotoluene; IUPAC: 1-methyl-2,5-dinitrobenzene
2,6-DNT	2,6-Dinitrotoluene; IUPAC: 1-methyl-2,6-dinitrobenzene
3-NT	3-Nitrotoluene; IUPAC: 1-methyl-3-nitrobenzene
3,4-DNT	3,4-Dinitrotoluene; IUPAC: 1-methyl-3,4-dinitrobenzene
4-NDPA	2,4-Dinitrodiphenylamine; IUPAC: 2-nitro-N-phenylaniline
4-NsDPA	4-nitroso-diphenylamine; IUPAC: 4-nitroso-N-phenylaniline
4-NT	4-Nitrotoluene; IUPAC: 1-methyl-4-nitrobenzene
°C	Degree(s) Celsius
µg	Microgram(s)
µl	Microlitre(s)
µm	Micrometre(s)
ACN	Acetonitrile; IUPAC:
AKII	Akardite II; IUPAC: 1-methyl-3,3-diphenylurea
APCI	Atmospheric pressure chemical ionisation
ASTM	American Society for Testing and Materials
ATR	Attenuated total reflectance
CAR	Carboxen
CDR	Cartridge discharge residue
CE	Capillary electrophoresis
cm	Centimetre(s)
CW	Carbowax
DBP	Dibutylphthalate; IUPAC: dibutyl benzene-1,2-dicarboxylate
DCM	Dichloromethane
DEP	Diethylphthalate; IUPAC: diethyl benzene-1,2-dicarboxylate
DESI	Desorption electrospray ionisation

DIBP	Diisobutyl phthalate; IUPAC: bis(2-methylpropyl) benzene-1,2-dicarboxylate
DMA	Differential mobility analysis
DMP	Dimethyl phthalate; IUPAC: dimethyl benzene-1,2-dicarboxylate
DNT	Dinitrotoluene
DPA	Diphenylamine; IUPAC: N-phenylaniline
DPP	Diphenyl phthalate; IUPAC: diphenyl benzene-1,2-dicarboxylate
DVB	Divinylbenzene
EC	(compound) Ethyl centralite; IUPAC: 1,3-diethyl-1,3-diphenylurea
EC	(technique) Electrochemical detection
ECD	Electron capture detection
EDX	Energy dispersive X-ray spectrometry
e.g.	Exempli gratia; for example
EI	Electron ionisation
EPA	Ethylphenylamine; IUPAC: N-ethylaniline
ESI	Electrospray ionisation
FBI	Federal Bureau of Investigation
FDR	Firearm discharge residue
FID	Flame ionisation detection
FTIR	Fourier transform infrared
Ga	Gauge
GC	Gas chromatography
gr	Grain(s)
GSR	Gunshot residue
h	Hour(s)
HMX	Octogen; IUPAC: 1,3,5,7-tetranitro-1,3,5,7-tetrazocane
HPLC	High performance liquid chromatography
HSSE	headspace sorptive extraction
I.D.	Internal diameter
i.e.	Id est; specifically
IED	Improvised explosive device
IGSR	Inorganic gunshot residue
IMS	Ion mobility spectrometry
kV	Kilovolt

LC	Liquid chromatography
m	Metre(s)
MC	Methyl centralite; IUPAC: 1,3-dimethyl-1,3-diphenylurea
MECE	Micellar electrokinetic capillary electrophoresis
MEKC	Micellar electrokinetic capillary chromatography
MeOH	Methanol
mg	Milligram(s)
min	Minute(s)
mL	Millilitre(s)
mm	Millimetre(s)
MMSE	Monolithic material sorption extraction
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MW	Molecular weight
m/z	Mass to charge ratio
n	Non-negative integer(s); number(s)
NC	Nitrocellulose; IUPAC: [(2S,3R,4S,5R,6R)-2-[(2R,3R,4S,5R,6S)-4,5-dinitrooxy-2-(nitrooxymethyl)-6-[(2R,3R,4S,5R,6S)-4,5,6-trinitrooxy-2-(nitrooxymethyl)oxan-3-yl]oxyoxan-3-yl]oxy-3,5-dinitrooxy-6-(nitrooxymethyl)oxan-4-yl] nitrate
NDPA	Nitrodiphenylamine
nESI	Nano electrospray ionisation
ng	Nanogram(s)
NG	Nitroglycerin; IUPAC: 1,3-dinitrooxypropan-2-yl nitrate
NIST	National Institute of Standards and Technology
nm	Nanometre(s)
N,N-DPF	N,N-diphenylformamide
N-Ns-2-NDPA	N-nitroso-2-nitrodiphenylamine; IUPAC: N-nitroso-2-phenylaniline
N-NsDPA	N-nitrosodiphylamine; IUPAC: N-nitroso-N-phenylaniline
NO	Nitrogen oxide
NO_2	Nitrogen dioxide
NPD	Nitrogen phosphorus detector
NQ	Nitroguanidine; IUPAC: 2-nitroguanidine
OGSR	Organic gunshot residue

PA	Peak area
PA	(fibre type) Poly acrylate
PAH	Polycyclic aromatic hydrocarbon
PDMS	Polydimethylsiloxane
PETN	Pentaerythritol tetranitrate; IUPAC: [3-nitrooxy-2,2-bis(nitrooxymethyl)propyl] nitrate
PMDE	Pendant mercury drop electrode
ppm	Parts per million
PTFE	Polytetrafluoroethylene
Q	Quadrupole mass analyser/spectrometer
RDX	Cyclonite; 1,3,5-trinitro-1,3,5-triazinane
rpm	Rounds per minute
RT	Retention time
s	Second(s)
SbBaPb	Particle containing antimony, barium and lead
SBSE	Stir bar sorptive extraction
SCF	Super critical fluid
SEM	Scanning electron microscope
SIM	Selected ion monitoring
SIMS	Secondary ion mass spectrometry
s/n	Signal to noise ratio
SPE	Solid-phase extraction
SPME	Solid-phase microextraction
SRSE	Stir rod sorptive extraction
Std dev	Standard deviation
SWGGSR	Scientific working group gunshot residue
T	Temperature
TD	Thermal desorption
TEA	Thermal energy analysis
TLC	Thin layer chromatography
TNT	2,4,6-Trinitrotoluene; IUPAC 1-methyl-2,4,6-trinitrobenzene
TOF	Time of flight
UPLC	Ultra-high performance chromatography
UV	Ultraviolet

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1   Introduction

Between April 1st 2016 and March 31st 2017, 6,375 (non-air) firearm offences were recorded in England and Wales. The majority of these involved violence against a person (37%) and robbery (29%). The number of offences showed a 23% increase compared to the previous year, when 1,250 firearm incidents resulted in injury. [1] During the majority of these offences firearms were discharged [2]. In these cases, gunshot residue (GSR) can be collected and used as forensic evidence.

To date, GSR analysis focusses exclusively on inorganic components. Analysis of organic compounds, however, could provide valuable complementary information that could strengthen the value of GSR evidence [3-8], and potentially provide additional means to discriminate between GSR materials and environmental residues [6, 7]. The aim of the presented study is to develop a method for the analysis of both organic and inorganic compounds from a single GSR sample.

An overview of the current state of organic GSR (OGSR) analysis and the challenges associated with it is provided in chapter 2 [9]. Chapter 3 focusses on the optimisation of a GC-MS method for the analysis of OGSR compounds, which is employed to the characterisation of a wide range of unburnt propellant (chapter 4). A critical evaluation of these results in combination with relevant literature has led to the selection of target compounds for OGSR analysis, which formed the basis of further method optimisation. Several extraction methods (MonoTrap, chapter 5 and SPME, chapter 6) have been evaluated. This included the evaluation of sample collection media and an evaluation of the potential change in organic composition from unburnt propellant to GSR (chapter 7). Finally, a protocol for the analysis of both organic and inorganic compounds from a single GSR sample is developed (chapter 8) and employed to samples generated with three different firearm-ammunition combinations in order to obtain a total chemical profile.

2   Literature review

Organic gunshot residue has the potential to significantly increase the probative value of GSR evidence. The ability to include the organic information may inspire the evolution of current GSR analysis, which is solely based on inorganic compounds. The challenge is to find the optimal combination of sample collection, extraction and analysis techniques for the treatment of a given GSR sample.

Currently, more than 140 compounds associated with OGSR have been identified in the literature. There are, however, no generally accepted guidelines for selecting target analytes, which will inform sampling and analysis protocols. This review provides a critical analysis of organic compounds that may be associated with gunshot residue, in order to elucidate aspects that are worth considering when selecting or considering target analytes.

A comprehensive review of the literature concerning all aspects of sampling and analytical techniques used for the determination of these OGSR compounds is presented. Despite this area gaining increasing attention and recognition in recent years, there is still an absence of a set combination of sample collection, extraction and analysis methods that is universally optimal for the treatment of any given OGSR sample. Recent developments in both extraction and analytical methodologies employed for OGSR detection are highlighted. The main advantages and disadvantages of the sampling and analysis methods are critically discussed.

Parts of this literature review have been published [9].

2.1   Introduction

Gunshot residue (GSR) is the collective name of the complex mixture of organic and inorganic particles [10] originating from the firearm, the firearm ammunition, and from the combustion products thereof, which are produced during the discharge of a firearm [3]. GSR consists of unburnt and partially burnt particles, which can arise from ammunition primer, propellant powder, and metals from the projectile (firearm ammunition); grease, lubricants, and metals from the gun barrel (firearm); and combustion products, including smoke [3, 10, 11]. Organic compounds mainly originate from propellant powders and firearm lubricants, some products of their transformation and hydrocarbons. Inorganic compounds, such as nitrates, nitrites, and metallic particles, originate from the primer and propellant, as well as the cartridge case, projectile jacket and its core, and from the gun barrel [3, 10].

Gunshot residue, which is also known as cartridge discharge residue (CDR) or firearm discharge residue (FDR) [12], escapes through weapon openings [3] and may subsequently deposit on surfaces in the near vicinity of the fired weapon [13]. Thus, GSR could become (trace) evidence consequent to the criminal use of firearms. Its potential, however, to establish a link between the shooter, the firearm, the victim, and/or the crime scene requires careful interpretation of the evidential value of such gunshot residue. [14] It is important to realise that due to the complexity of the firing process, and the large number of parameters involved in the creation of gunshot residue, the amount and composition of GSR vary. Further diversity is promoted by the wide range of firearms and ammunition available. [13]

Present analysis methods of GSR in forensic investigations mainly focus on inorganic GSR (IGSR) analysis using Scanning Electron Microscopy (SEM) methodologies [10, 12]. Combining this information with organic GSR (OGSR) information, however, would significantly increase the probative value of GSR evidence [15], because it enables a more accurate interpretation of obtained analytical results. [3] This review discusses organic compounds that could be associated with smokeless propellant powders and gunshot residue. Recent developments in both extraction and analytical methods employed in their detection are highlighted and the main advantages and disadvantages are critically discussed.

2.2 Organic GSR compounds

Organic GSR (OGSR) compounds predominantly originate from the propellant powder [11]. Modern, smokeless powders are based around nitrocellulose (NC) as an explosive (single base powders); a combination of NC and nitroglycerin (NG) (double base powders), which increases the energy potential of the powder; or a combination of NC, NG and nitroguanidine (NQ) (triple base powders). In addition to these explosive compounds, all smokeless powders contain a number of additives, including stabilisers, plasticisers, flash suppressors, coolants, moderants, surface lubricants, and anti-wear additives. [11]

The earliest detection of OGSR materials relied on nitrate and nitrite compounds [11]. In the 1960s, nitroglycerin, as well as diphenylamine and its nitric derivatives (stabilisers) were the main compounds investigated with respect to OGSR analysis [16-20]. From the 1970s onwards, smokeless propellant powders were being analysed more frequently, expanding the range of OGSR compounds that were investigated. These compounds include nitrocellulose, additional stabilisers (centralites and resorcinol), plasticisers (phthalates and triacetin) and flash

suppressors (dinitrotoluenes, in particular 2,4-dinitrotoluene) [11, 16, 18, 21-24]. Due to work being done on the analysis of explosive residues, several explosive compounds are amongst the earliest OGSR related compounds investigated. These explosive compounds include nitroglycerin, TNT, RDX, PETN, HMX, tetryl, and (di)ethylene glycol dinitrate [25-27]. In 1982, the FBI laboratory compiled a list of 23 organic compounds that may be present in smokeless propellant powders [11], which included all of these compounds with the exception of the latter four explosives. To date, the majority of these compounds are still being considered with respect to OGSR analysis.

In a 2010 review by Dalby *et al.* [3], a more comprehensive list of 48 organic compounds that may contribute to gunshot residue (including all compounds listed by the FBI laboratory) and their sources is given. This list is repeated in a more recent (2013) review by O'Mahony and Wang [28], without the addition of any 'new' organic compounds. This seemingly indicates a general consensus on possible organic compounds associated with smokeless powders and gunshot residue to date. A list of compounds provided by Taudte *et al.* [29] in 2014, concerning the organic compounds commonly used in the manufacturing of propellant powders and primers, contains approximately 60% of the compounds listed by Dalby *et al.* [3]. The most noteworthy absent compound on the list is nitrocellulose. The new compounds predominately are expansions of the 2010 list, including additional phthalates, nitrobenzenes, and nitrates.

The compounds listed in the mentioned reviews by Dalby *et al.* [3] and Taudte *et al.* [29], are compared against several experimental studies on organic GSR compounds in table 2.1. Only studies that looked at a minimum of 10 organic compounds have been included. The majority of these studies have been reported since 2010 [3, 6, 7, 29-32]. A few studies, including one review [11], prior to 2010 have been included for the purpose of comparison [33-35]. This has resulted in a list containing close to 140 organic compounds that are associated with smokeless powders and gunshot residue. It must be noted that some of the targeted compounds have not been identified in the experimental studies (indicated in grey).

Table 2.1: Organic compounds which may contribute to gunshot residue

Compound	Experiment	Review	Compound	Experiment	Review
1,2,3-Trimethylbenzene	[27]		Carbazole	[22, 23, 27, 30]	[2, 3]
1,2,4-Trimethylbenzene	[27]		Charcoal		[2]
1,3,5-Trimethylbenzene	[27]		Chrysene	[23]	
1,3,5-Trinitrobenzene	[30]	[21]	m-Cresol	[22]	[2, 3, 21]
1,3-Dinitrobenzene	[30]	[21]	o-Cresol	[22]	[2, 3, 21]
1,2-Dicyanobenzene	[23, 27]		p-Cresol	[22]	[2, 3, 21]
1,3-Dicyanobenzene	[23, 27]		Cyclonite (RDX)	[22, 26, 30]	[2, 3, 21]
1,4-Dicyanobenzene	[23, 27]		Dextrin		[2]
1,2-Dinitroglycerin		[21]	Diamylphthalate		[21]
1,3-Dinitroglycerin		[21]	Diazodinitrophenol		[2]
1,4-Dimethylnaphthalene	[23]		Diazonitrophenol		[2, 21]
2,6-Dimethylnaphthalene	[23]		Dibutyl phthalate (DBP)	[3, 22-25, 27-30]	[2, 21]
1-Methylnaphthalene	[23, 27]		Diethylene glycol dinitrate		[21]
2-Methylnaphthalene	[23, 27]		Diethyl phthalate (DEP)	[22, 24-26, 29, 30]	[2, 3, 21]
1-Naphthalenecarbonitrile	[23]		Dimethyl phthalate (DMP)	[22, 24-26, 29, 30]	[2, 3, 21]
2-Naphthalenecarbonitrile	[23]		Dimethylsebacate	[22]	[2, 3]
2,2'-Dinitrodiphenylamine	[30]		Dinitrocresol		[2]
2,4'-Dinitrodiphenylamine	[22, 23, 25, 26, 30]	[2, 3, 21]	Dinitro-ortho-cresol		[3, 21]
4,4'-Dinitrodiphenylamine	[25, 30]	[21]	Diphenylamine (DPA)	[3, 22-30]	[2, 21]
2,3-Dimetyl-2,3-dinitrobutane	[22]		Ethyl centralite (EC)	[3, 22-30]	[2, 21]
2,3-Dinitrotoluene (2,3-DNT)	[22, 25, 28-30]	[2, 3, 21]	Ethylbenzene	[23]	
2,4-Dinitrotoluene (2,4-DNT)	[3, 22, 24-26, 28-30]	[2, 21]	Ethylene glycol dinitrate		[2, 21]
2,6-Dinitrotoluene (2,6-DNT)	[22, 24, 25, 28-30]	[3, 21]	Ethylphthalate		[2, 21]
3,4-Dinitrotoluene (3,4-DNT)	[22, 24, 25, 28]	[21]	Fluoranthene	[23]	
2,4,6-Trinitrotoluene (TNT)	[22, 26, 30]	[2, 3, 21]	Fluorene	[23, 27]	
2,4-Dinitroanisole (DNAN)	[26]		Gum Arabic		[2]
2,4-Dinitrodiphenylamine	[22, 23, 25, 26, 30]	[2, 3, 21]	Gum tragacanth		[2]
4,4-Dnitrodiphenylamine	[25]	[21]	Hexylene glycol	[27]	
2-Amine-4,6-dinitrotoluene	[26, 30]	[21]	Indene	[23, 27]	
4-Amine-2,6-dinitrotoluene	[26, 30]	[21]	Indole	[23, 27]	
2-Ethyl-1-hexanol	[23, 27]		Isoquinoline	[23]	
2-Ethylhexanal	[27]		Karaya gum		[2]
2-Ethylnaphthalene	[23]		Methyl cellulose		[2]
2-Furaldehyde	[27]		Methyl centralite (MC)	[24, 25, 29, 30]	[2, 3, 21]
2-Naphthol	[25]		Monomethyl-phthalate	[22]	[2, 21]
2-Nitrobenzene	[30]		Naphthalene	[23, 27, 30]	
3-Nitrobenzene	[30]		N,N-diphenylformamide	[30]	
4-Nitrobenzene	[30]		Nitrobenzene	[30]	[21]
2-Nitrophenylamine (2-NDPA)	[3, 22-28, 30]	[2, 3, 21]	Nitrocellulose (NC)	[3]	[2]
4-Nitrodiphenylamine (4-NDPA)	[22-26, 30]	[2, 3, 21]	Nitroglycerin (NG)	[3, 22-25, 27-30]	[2, 21]
2-Nitrotoluene	[22, 25, 28, 30]	[2, 21]	Nitroguanidine	[22, 26, 28]	[2, 3, 21]
3-Nitrotoluene	[22, 25, 28, 30]	[2, 21]	N-nitrosodiphylamine	[3, 22-26, 28]	[2, 21]
4-Nitrotoluene	[22, 25, 28, 30]	[2, 21]	Nonanal	[27]	
3,5-Dinitroaniline	[30]		Octogen (HMX)	[26]	[21]
4-Methylbiphenyl	[23]		Pentaerythritol tetranitrate (PETN)	[26]	[2, 3, 21]
4-Nitrosodiphenylamine	11, 10	[21]			
Acenaphthene	[23, 27]		Phenanthrene	[23, 27]	
Acenaphthylene	[23, 27]		Phenol	[27]	
Acetophenone	[23]		Phytane	[27]	
Akardite I (AKI)		[21]	Picric acid	[28]	[2]
Akardite II (AKII)	[23]	[2, 21]	Pyrene	[23, 27]	
Akardite III (AKIII)		[21]	Quinoline	[23, 27]	
Aniline	[27]		Resorcinol	[22, 29]	[2, 3, 21]
Anthracene	[23, 27]		Rubber cement		[2]
Benzaldehyde	[23, 27]		Sodium Alginate		[2]
Benzene	[23]		Starch		[2]
Benzo[a]pyrene	[23]		Styrene	[23, 27]	
Benzo[b]thiophene	[23]		Tetracene		[2]
Benzonitrile	[23, 27]		Tetryl	[22, 30]	[2, 21]
Benzophenone	[23, 27]		Toluene	[23]	
Benzothiazole	[23]		m-Tolunitrile	[23, 27]	
Benzylnitrile	[23, 27]		o-Tolunitrile	[23, 27]	
Biphenyl	[23]		p-Tolunitrile	[23, 27]	
Biphenylene	[23, 27]		Triacetin	[22]	[2, 3, 21]
Butyl centralite (BC)		[2, 3, 21]	Urethane	[22]	
Butyl phthalate		[2]	m-Xylene	[23]	
Camphor	[22]	[2, 21]	o-Xylene	[23]	
Carbanilide		[2, 3]	p-Xylene	[23]	

This list is not exhaustive. Some of the compounds may now be obsolete from production, but may still be in circulation.

A recent study to characterise volatile OGSR [37] was aimed at the selection of promising target compounds for determining discharge times with respect to spent cases. The detection of 168 compounds, divided into four groups, was reported: 78 explosive products, 16 smokeless powder additives, 55 compounds that presumably came from another source, and 19 unidentified compounds. Of the latter two groups only one compound, pythane, was included in table 2.1. The group of smokeless powder additives contained four extra compounds: dioctyl phthalate, diisobutyl phthalate (DIBP), and two isomers of nitrophenol. The biggest groups, explosive products, contain 34 additional compounds, including unspecified isomers.

Polycyclic Aromatic Hydrocarbons

The table shows that approximately half of the total number of compounds that have been of interest in experimental studies, are not included in any of the mentioned reviews. The majority of these additional compounds are polycyclic aromatic hydrocarbons (PAHs), such as naphthalene-related compounds, benzo[a]pyrene, and chrysene. Recently, a large number of additional PAHs and other combustions products have been identified in GSR from spent cases [30, 33, 37]. The only representative of the group of PAHs listed in table 2.1 mentioned in the included reviews is tetracene [3], although combustion products and hydrocarbons are mentioned as a source of organic gunshot residue [3]. Despite this fact, there is limited information in the literature regarding the analysis of PAHs in GSR [30, 33, 37].

PAHs are widely spread, persistent, and ubiquitous environmental pollutants [38-40], which can exist in both vapour and particle phases in the atmosphere [38]. They are present in vehicular emissions, tobacco smoke, and industrial effluent [38-40]. PAHs are universal combustion products, and are predominately formed during the incomplete combustion of organic matter such as wood, fuel, gas and coal [39, 40].

Due to the generic nature of PAHs, one could argue that the evidential value of these compounds with respect to the analysis of organic gunshot residue is very limited. It must be noted, however, that the specific studies including these compounds [30, 33] did not aim for the identification of gunshot residue based on these compounds, nor claim that these compounds are characteristic for GSR. The purpose of both studies was to investigate the time since discharge. Gallidabino *et al.* [30] found PAHs particularly suitable for this purpose, since these substances are simultaneously produced during the discharge, and are thus subjected to the same variability-introducing factors. It was expected that as a consequence of this, the PAHs present closer mutual fluctuations, and thus could be used for the normalisation of the determined aging curve.

2.2.1 Traditional OGSR compounds for GSR identification

When disregarding the PAH compounds, as well as the compounds only reported in reviews, it becomes apparent that the group of organic GSR compounds included in studies which do not focus on time since discharge, is significantly smaller than the near 140 compounds included in table 2.1. Of these studies, Dalby and Birkett [7] included a list of 32 compounds, which are all directly related to the propellant powder and its additives, and in a lesser extent to the primer mix. A number of these compounds are included in studies by Benito *et al.* [6] (a total of 18 compounds) and Thomas *et al.* [32] (a total of 21 compounds), which both focus on characteristic organic GSR compounds. Of these 32 compounds in total, however, only about 10 to 15 compounds seem to be frequently included in other studies.

This general trend is confirmed by a comparison with both recent [5, 41-46] and older [21, 47-56] studies which included less than 10 compounds per study (table 2.2), as the compounds selected in these studies appear to fall within the range of the frequently targeted compounds depicted in table 2.1.

Table 2.2: Organic GSR compounds included in studies using < 10 organic compounds

Compound	References 2010 - current	References 2000-2010	References 1990-2000	References Prior to 1990
2,3-dinitrotoluene (2,3-DNT)			[57]	
2,4-dinitrotoluene (2,4-DNT)	[42, 44, 46]	[48]	[50, 57]	[21]
2,6-dinitrotoluene (2,6-DNT)	[42]		[57]	
2-Ethyl-1-hexanol		[48]		
2-nitrophenylamine (2-NDPA)	[46]		[57]	
3,4-dinitrotoluene (3,4-DNT)			[57]	
4-Nitrodiphenylamine (4-NDPA)	[46]			
Dibutyl phthalate (DBP)	[42]		[57]	[21]
Diethyl phthalate (DEP)			[57]	
Dimethyl phthalate (DMP)			[57]	
Diphenylamine (DPA)	[42, 43, 46]	[48, 51, 52]	[57]	[21, 49]
Ethyl centralite (EC)	[43, 46]	[48, 51, 52, 55]	[57]	[21, 49]
Methyl centralite (MC)	[43, 46]	[52, 55]	[57]	
Nitrocellulose (NC)	[46]	[47]		[49]
Nitroglycerin (NG)	[43]	[47, 51]	[50, 57]	[21, 53]
N-nitrosodiphylamine (N-NsDPA)	[46]	[51]	[57]	
Trinitrotoluene (TNT)			[57]	

No noteworthy differences have been observed between the compounds included in studies since 2010 and those included before then, as is depicted in table 2.2. This table shows that the most frequently targeted compounds have been selected in both recent studies and studies dating back to before 1990. The two 2004 studies included in table 2.1 [34, 35] correspond well with the most frequently targeted compounds in that table and fit within the range of table 2.2. Furthermore, the compounds mentioned in the review by Meng and Caddy [11] in relation to experimental studies performed up until 1997, show good correspondence as well.

It must be noted, however, that the frequent inclusion of a compound in a study of OGSR does not necessarily support its value as a characteristic compound for the identification of organic GSR. The frequently used compounds dimethyl phthalate, diethyl phthalate and dibutyl phthalate, for example, are ubiquitous to indoor air, settled dust, and food. This is due to their wide application as plasticisers in a broad array of polymeric materials, and the fact that phthalates are not chemically bonded to the materials. Consequently, they are susceptible to leaching and are, therefore, readily released into the environment [58].

Meng and Caddy [11] argue in their review that nitrocellulose has limited use as marker for organic GSR, because of its widespread use in many consumer products, including paints. Despite the possibility to differentiate between NC from smokeless powders, and NC from environmental sources using size-exclusion chromatography, there are practical issues that hamper this process. This is due to the fact that differentiation would be based on the relatively high molecular range of NC, whilst the molecular mass of NC in gunshot residue tends to be degraded, thus diminishing the distinguishable molecular mass. This decreases the evidential value of NC in case degradation has taken place. [11]

Ethyl centralite and 2,4-dinitrotoluene are highlighted as respectively the most and second most characteristic compounds of organic gunshot residue [11]. It is also argued, however, that the inclusion of additional compounds increases the evidential value, even if the additional compounds are less characteristic on their own [11]. This further complicates the selection of what characteristic compounds are and may explain the relatively wide variety of additional compounds selected in the different studies discussed in this review.

Organic primer compounds

The majority of the compounds discussed are related to the ammunition's propellant powder. In addition to these compounds, OGSR compounds may also arise from primers. More specifically, Meng and Caddy [11] referred to sensitising materials used in small-arm primers, such as tetracene, pentaerythritol tetranitrate (PETN), trinitrotoluene (TNT), and tetryl.

Additionally, the primer mix may also be a source for NC and NG [3]. All of these compounds are included in table 2.1, although it is worth mentioning that all of these compounds may also originate from propellant powders [3]. The table does contain a few compounds that have been listed as primer mix compounds only in a review by Dalby *et al.* [3]. These compounds are dextrin, diazodinitrophenol, diazonitrophenol, gum arabic, gum tragacanth, karaya gum, rubber cement, and sodium alginate. Diazodinitrophenol is commonly mentioned as a non-toxic replacement for lead compounds in primers, but was already included in patents since the early 1980s, often in conjunction with tetracene [59, 60]. There are, however, numerous patents for primers that include single or multiple other organic compounds, which are not included in the table and are not listed as propellant powder components. The patents are both recent, *i.e.* since the increase in lead free, non-toxic primers; and earlier, *i.e.* 1980s and 1990s [61]. The organic compounds listed in the primer-related patents include styphic acid, tetrazene, polynitrophenylether, polynitropolyphenylene, polyvinyl acetate, hexogene, actogene and nitropentene [62]. Furthermore, a patent published in 2013 describes a primer composition comprising of red phosphorus stabilized by an acid scavenger and a polymer, which gives rise to a whole new category of organic compounds [63].

2.2.2 Other approaches to GSR identification

In addition to the analysis and identification of organic propellant and primer compounds, there have been other approaches to the identification of organic gunshot residue.

In a study by Bendrihem *et al.* [64] it was demonstrated that a projectile can wipe gun cleaning oil from the inside of the barrel. Gun cleaning oils contain long-chain fatty acids and ethyl esters thereof, which are two components that can withstand the high temperatures inside the barrel during the discharge of a firearm. Consequently, they can be carried with the projectile and subsequently be identified in the bullet wipe at much longer distances than classic GSR compounds. The authors argue that thus gun cleaning oils may be considered as GSR.

In a study aimed at the characterisation of GSR from ammunition, Gilchrist *et al.* [8] looked at the anions using anion exchange chromatography, rather than looking at the complete organic compounds. The following six anions were included in the study: lactate, acetate, formate, benzoate, phthalate, and oxalate. The authors reported that by profiling and comparing anionic content of GSR, it was possible to distinguish between the three ammunition types tested.

Another area of approach to the identification of GSR is the development of artificial markers, which could be added to ammunition in order to create a characteristic marker (further details can be found in appendix A).

The alternative approaches to the identification of OGSR, in conjunction with the different set of compounds of interest when investigating time since discharge, and the lack of differences in compounds of interest with older studies, suggests that the question of which compounds make for good, reliable characteristics depends on both the aim of the study as well as on the intended analytical technique, rather than on new insights over time.

2.3   Sampling methods

In order to enable the analysis of OGSR compounds, an efficient collection method is required. The fact that GSR can be deposited on a wide variety of surfaces in the near vicinity of the fired weapon following discharge, increases the importance of selecting the most appropriate collection method [3].

2.3.1   Collection and extraction of OGSR samples

Surfaces that may be exposed to GSR include the scene of the incident, which may be a mobile location such as the interior and/or exterior of a vehicle. GSR may also be collected from skin, hair, or clothes of individuals, who can be either a shooter, a victim, or a bystander [3, 64-67]. Different collection methods employed for these types of surfaces have been discussed previously [3, 11, 65]. A summary of these methods is given in table 2.3.

Swabbing is reported as the most common technique employed for the collection of OGSR from hands [11, 12]. The swabbing method requires the choice of a suitable solvent for the collection of OGSR. In different studies, ethanol and isopropanol are mentioned as the best performing solvents [3, 53, 68, 69]. Organic solvents are commonly used for the collection of explosives. A drawback of the use of this type of solvent is the fact that it will dissolve many other compounds as well, causing interference issues. [3] In a study by Thompson *et al.* [70] water followed by solid phase extraction (SPE) was shown to be effective for the recovery of organic explosive residues and provided much greater selectivity in most cases. A combination of water and isopropanol in combination with SPE was employed by Lloyd and King [50] for both explosives and firearms residue. In their study, SPE was performed in the container in which the

swab was returned to the laboratory. They reported that the employed extraction procedure extracted the organic residue, whilst the inorganic residue remained on the swab. This allowed for a subsequent extraction of the inorganic residue by sonication in an organic solvent, followed by membrane filtration. Consequently, the organic and inorganic fractions could be separated prior to analysis.

Dalby *et al.* [3] reported that tape lifts are the most common procedure for the collection of inorganic residues from skin surfaces. Some research has investigated the possibility of collecting OGSR via this method, which requires solvent extraction [69, 71]. A drawback reported on the use of carbon adhesives is a significant reduction in the recoveries of OGSR compounds after solvent extraction [72]. This challenge could be addressed by a novel approach to the tape lift method, which involves covering half of the carbon adhesive with parafilm and PTFE tape. This enables the simultaneous analysis of the carbon adhesive half for IGSR by SEM, and the OGSR analysis of the uncoated, PTFE tape half of the stub. [6] Benito *et al.* [6] compared this method against swabbing by spiking the swab and stub with a standard solution that was allowed to evaporate. Both spiked media were dissolved in 1 mL of methanol and the OGSR particles were subsequently extracted using the same method. The reported results showed that the recovery of the modified stub was similar or better for the majority of the 17 compounds included in the standard mix. Lower recoveries were only obtained for dimethyl phthalate, TNT, and 2-nitrodiphenylamine.

Table 2.3: Collection techniques for deposited GSR

Technique	Medium	Surface	Advantages	Disadvantages	Ref
Tape lifting	Stub with adhesive: - Carbon coated - Double sided tape	- Skin - Hair - Fabric	- Most effective - IGSR & OGSR - Cheap - Good collection efficiency - SEM compatible - Surface sampling only	- Build-up of debris - Carbon or gold coating needed after sampling of fabric - Varying reports on suitability of hair sampling (200-300 dabs needed) - Loss of stickiness due to fibres and debris	[3] [12] [65] [68] [73] [74]
Glue Lifting	Glue lifting planchet (less sticky than tape)	Hands	- Less dabs than tape lift method - Less debris than tape lift method, - Thus faster SEM - Surface sampling only	- May be ineffective for particle lifting due to lesser tackiness than tape lifts	[3] [75]
Swabbing	Cotton swab soaked in organic or aqueous solvent	- Hands - Face	- IGSR & OGSR - Aqueous solvents best - IGSR and OGSR separately extracted	- Less effective - Organic solvents require SPE - Separate extraction requires SPE	[3] [12] [65] [68] [50]
Combing	Fine tooth comb	Hair	- Particles smaller than gaps between the comb teeth collected - Nearly intact grains collected	- Difficult with curly hair	[3] [51]
Swabbing & combing	(Fine tooth) comb with solvent swabs or a damp cloth between the teeth	Hair		- More complicated	[3] [76] [74]
Vacuum lifting	Vacuum with Teflon or fibre glass filter	Clothes	- IGSR & OGSR	- Combination with tape lifting (for OGSR) - Extraction needed - Sampling depth of fabric rather than surface only	[3] [12] [54]

2.3.2 Collection and extraction of volatile OGSR compounds

Volatile OGSR compounds can be collected from human nasal tissue [3, 12], but more often it is associated with the collection of volatile compounds from spent cartridge cases and firearm barrels. The collection of OGSR compounds from cartridge cases generally involves the collection of the case itself in an airtight container [30, 33], or seal bag [8], followed by extraction in a laboratory. The use of solid-phase microextraction (SPME) is generally the method of choice for the collection of volatile OGSR compounds from the barrel of a firearm [77-79].

Solid-phase microextraction

SPME is a solvent-free variety of SPE, and employs a fine fused silica fibre coated with a polymeric substance, the sorbent phase, to extract volatile organic compounds from their matrix. [77]. The principle of the extraction is based on the partition equilibrium of analytes between the matrix and a small amount sorbent phase [30]. This technique allows the collection of (ultra-)trace levels of analytes from liquid, gaseous and solid samples, due to the fact that the analytes are concentrated onto the fibre [7, 80]. SPME has wide applications within different analytical fields because of its simplicity, efficiency and good precision. With respect to OGSR analysis, SPME is applied to the identification of OGSR compounds from spent cartridge cases [7, 41] and smokeless (propellant) powders [7]; and to the determination of time since discharge from spent cases [30, 33], as well as gun barrels [77]. The latter application is a specific advantage of the suitability of SPME to the sampling of narrow spaces, like firearm barrels [77]. The major advantage of this technique, however, is the fact that thermal desorption of the SPME fibre enables the direct transfer of the analytes into the injector of a gas chromatograph (GC) [77], eliminating the need for additional extraction steps.

Different parameters, which may be considered when selecting the appropriate SPME method, are the fibre type, the sampling time and temperature and the desorption temperature.

There are several different types of SPME fibres, which vary in both the type and the amount of sorbent phase. Fibres may be coated with a single polymeric substance such as polydimethylsiloxane (PDMS), or polyacrylate (PA); or with a combination of polymers such as PDMS/divinylbenzene (PDMS/DVB), carboxen/PDMS (CAR/PDMS), or DVB/CAR/PDMS [7, 30, 33, 36, 41, 42, 77]. The PDMS only fibres are non-polar, the PA fibres are polar, and the combined coatings are bipolar [7]. The performance of all of these fibre types in the detection of 32 OGSR compounds has been previously evaluated [7], including various quantities of PDMS sorbent in the single coated fibres. It was reported that the PDMS/DVB was the most suitable fibre type for

the extraction of OGSR compounds across the investigated ammunition types. A comparison between different, albeit less, fibre types – not including the PDMS/DVB fibre – has also been made in a few other studies which focussed on the extraction efficiency of sequential SPME [42] and on the determination of time since discharge [30, 33]. The used fibre types are shown in table 2.4, the selected fibre types are indicated in bold type. In these studies, PA was selected as the best performing fibre type. It must be noted that these studies included relatively few traditional OGSR compounds. The sequential SPME study mentioned [42] included a limited amount of five OGSR compounds (table 2.2), which limits the value of this comparison. The two studies into the determination of time since discharge [30, 33] included a significant amount of PAHs (table 2.1). This may affect the performance and thereby the suitability of individual fibre types, as it was reported that the performance of a fibre type may differ between different propellant powders [7]. This indicates that both the type of sample and the target analytes selected are variables that need to be considered when selecting an SPME fibre.

Table 2.4: SPME parameters used in OGSR analysis

Fibre type	Extraction Time	Extraction Temperature	Desorption Temperature	Ref
65µm PDMS/DVB 7µm PDMS 30µm PDMS 100µm PDMS 85µm PA 85µm CAR/PDMS 50/30µm DVB/CAR/PDMS	35 min	40°C	250°C	[7]
85µm PA	40 min	80°C	280°C	[30]
85µm PA 100µm PDMS 75µm CAR/PDMS	40 min	Room T	280°C	[33]
100µm PDMS	5 min	Room T	250°C	[41]
85µm PA 100µm PDMS 7µm PDMS	21 min	66°C	250°C	[42]
85µm PA	30 min	Room T	170°C & 200°C	[77]
85µm PA 85µm CAR/PDMS	40 min	Room T	170°C	[79]
85µm PA 100µm PDMS 7µm PDMS	60 min	30°C	250°C	[36]

A few studies have investigated the optimal sampling/extraction time. Dalby and Birkett [7] compared extraction times between 5 min and 55 min, at 10-minute increments, using a PDMS/DVB fibre, and determined that an extraction time of 35 minutes was suitable. Weyermann *et al.* [33] found a similar extraction time of 40 min adequate when using a PA fibre to compare five extraction times between 20 min and 70 min. Joshi *et al.* [41], however, selected an extraction time of 5 min from a range of six times between 1 min and 60 min; reporting that the 100 μm PDMS fibre was able to extract sufficient amounts of various target analytes at this short extraction time. It must be noted that this study only included eight target analytes, and thus this short extraction time may be insufficient when including a greater number of analytes, as has been the case in the other two studies [7, 33].

A comparison of a series of extraction temperatures in order to determine the optimal temperature has not been found. Temperatures ranging from room temperature [33, 41, 77] to 80°C [30] have been reported, usually without reporting the basis on which the temperatures were chosen. Two exceptions are the studies by Dalby and Birkett [7] and the study by Weyermann *et al.* [13]. Dalby and Birkett selected a temperature of 40°C as this temperature is high enough to volatilise the compounds of interest in the headspace of the vial, but too low to cause thermal degradation of nitroglycerin which is known to start at temperatures above 50°C. [7]. Weyermann *et al.* compared room temperature extraction with an extraction temperature of 80°C, to study the influence of temperature. It was reported that the increased temperature caused lower concentrations of some compounds, such as benzonitrile and naphthalene; but higher concentrations of compounds such as diphenylamine, fluorathene and pyrene. They also detected several additional compounds at higher temperatures, whilst other compounds resulted in unidentified spectra. They concluded that the higher temperature was undesired for their study because they felt the higher temperatures provoked diminution of signals related to some compounds of interest, and made it impossible to perform a second analysis of the cartridges. [33] In the study by Gallidabino, however, which was also aimed at the investigation of time since discharge, 80°C was selected as the extraction temperature [30]. Both studies included a significant amount of PAHs. Despite the fact that over half of the targeted compounds were included in both studies, there was still a substantial number of selected PAHs that varied between the two (table 2.1). Moreover, despite using the same instrumental methodology apart from the extraction temperature, nitroglycerin was not identified in the study by Gallidabino *et al.* [30], yet it was detected in the study by Weyermann *et al.* [33], which confirms the thermal degradation of nitroglycerin at higher temperatures suggested by Dalby and Birkett [7]. This could indicate that the selection of the target analytes may be of primary importance when

selecting the extraction temperature. However, the fact that both decreased and increased concentrations of different compounds were reported by Weyermann *et al.* [33], may pose a challenge on the selection of the extraction temperature if quantification is the objective of the study.

A comparative study of several desorption times and temperatures has not been found either. Moreover, desorption times are rarely reported in studies. Although two studies [30, 33] reported a desorption time of 5 minutes, it must be noted that in the study by Gallidabino *et al.* [30], the method reported by Weyermann *et al.* [33] was employed. The reported desorption temperatures are 250°C [7, 36, 41, 42] and 280°C [30, 33], with the exception of a 1998 study by Andrasko *et al.* [77] who used lower temperatures of 170°C and 200°C.

Although SPME could be considered a well-established extraction method for OGSR compounds, for identification purposes and for the investigation of time since discharge, not all parameters of influence have been investigated equally thoroughly. Comparative studies of the SPME fibre types and extraction times indicate that both DVB/PDMS and PA fibres are suitable for the analysis of OGSR compounds, and that an extraction time of around 35 min - 40 min may be used in combination with either fibre type. The majority of the parameters and predominantly the fibre type and extraction specifications, however, seem to be dependent on the selected target analytes.

An alternative to SPME, a novel headspace sorptive extraction (HSSE) technique, was tested for the sampling of volatile OGSR compounds from spent cases [30, 37]. This method employs a magnetic stir bar as an extracting support, coated with PDMS. The extraction is based on the same principles as SPME, however, the stir bar is coated with a larger volume (up to 110 μL) of sorbent phase than an SPME fibre (maximum of 0.5 μL [30, 81]), making this a high capacity HSSE technique. The thicker coating also leads to an extended extraction time (72 hours) compared to SPME (less than an hour).

The stir bars could be analysed by thermal desorption-gas chromatography-mass spectrometry (TD-GC-MS). A desorption ramp in which the temperature was increased from 20°C to 250°C, taking a total time of 14.3 min, was used [30, 37]; as opposed to the isocratic desorption temperatures employed during SPME analyses reported in table 2.4.

This method was compared against SPME [30]. The authors reported an increased reproducibility and effectiveness, in addition to a greater amount of compounds that could be simultaneously analysed using this novel HSSE method. It must be noted, however, that a

significant fraction - greater than 75% - of the compounds of interest were PAHs. As only nine traditional OGSR compounds have been included in this study (table 2.1), the analysis of further OGSR compounds may be useful to establish the advantages of this method in the analysis of specific OGSR compounds.

2.3.3 Solvent extraction

Solvent extraction has been employed for the extraction of OGSR compounds from smokeless (propellant) powders [7, 31, 32] and spent cartridge cases [8], as well as OGSR compounds collected on a swab or (modified) stub [5, 6, 69, 71], and from vacuum collected samples [54]. Solvent extraction involves dissolving the sample by submerging the powder or object containing the OGSR compounds in a solvent for a period of time. Dissolving the sample may be done in an aqueous solution [8, 69, 72] or in organic solvents, such as methanol [6, 7, 34, 69], dichloromethane [31, 32, 54], acetonitrile [31], methyl ethyl ketone [46], or combinations thereof [71]. This process can be aided, and thus accelerated, by stirring or (ultra)sonication [7, 8, 34]. Stirring, however, was considered to be ineffective by Zeichner and Eldar, who reported that sonication is imperative to achieving an efficient extraction [72]. This is generally followed by centrifugation, which allows for the collection of the supernatant, and filtration [7, 8, 34, 71]. Alternatively, the sample may be concentrated prior to centrifugation by blowing it to near or complete dryness, usually under a (gentle) stream of nitrogen [6, 31, 32, 72]. In the latter case, the dry sample is reconstituted in a small amount of solvent. Filtration of the sample may not be necessary. [6, 31, 32] Solvents used for reconstitution include single organic solvents such as methanol [6], and mixtures [31, 32].

Several aqueous and organic solvents have been tested for the extraction of OGSR compounds from double-sided adhesive tapes mounted on a stub [72]. The influence of sonication on the extraction efficiency at different temperatures was also investigated: at and below room temperature for organic solvents and room temperature up to 80°C for aqueous solutions. It was found that the use of organic solvents resulted in considerable interference, brought on by adhesive components from the stub and skin components from the debris picked up during the sampling of the skin. [72] This drawback has also been observed when using a swab soaked in an organic solvent for the collection of GSR from skin [3]. The interference was observed even at very low temperatures, near the melting temperature of the solvents, and with relatively short extraction times of several minutes. This problem was not observed when using aqueous solvents for the extraction. It must be noted that sodium azide (0.1% w/v) was added to the water part of the aqueous solutions to improve the stability of nitroglycerin. The

major drawback of using water as a single solvent for the extraction is the relatively long extraction time and the low extraction efficiency. For example, an extraction method of 30 min sonication at 80°C resulted in a 10% recovery for nitroglycerin. The extraction time could be decreased, whilst improving the extraction efficiency, by using a mixture of water and 10% ethanol. Further optimisation of both parameters was observed when using a 20% ethanol in water mixture. According to this study, the best extraction method for recovery of OGSR from stubs employs a water/ethanol (80/20) mixture and sonication at 80°C for 15 min, followed by a further extraction with methylene chloride and concentration by evaporation.

Solvent extraction procedures are employed in conjunction with a wide array of analytical techniques including gas chromatography-mass spectrometry (GC-MS) [7], ultra-high performance liquid chromatography-tandem MS (UPLC-MS/MS) [32], high performance liquid chromatography – ultraviolet detection (HPLC-UV) [34], liquid chromatography-quadrupole time-of-flight (LC-QTOF) [6], ion exchange chromatography [8], Raman microscopy [46], capillary electrophoresis (CE) [31] and micellar electrokinetic capillary chromatography (MEKC) [34]. The use of organic solvents, however, is both economically and environmentally disadvantageous [7]. Another disadvantage is the potential need to concentrate the sample and/or remove interfering compounds by using a clean-up method [3, 11], such as SPE, which may lead to reduced recoveries [3].

In summary, there is a wide variety of sampling and extraction techniques available for the collection of OGSR. Which collection method is the most appropriate depends on the surface to be sampled, the target analytes selected, and on the analytical method.

2.4   Analytical techniques

Analysis of OGSR has been performed since the early 1960s [34], using a wide array of analytical techniques (table 2.5). The acronyms used correspond to the techniques listed in table 2.6. Some of these techniques have been discussed in earlier reviews. Therefore, techniques that have already been considered elsewhere will only be briefly covered here. Where appropriate, for example due to less extensive coverage elsewhere, an overview of previous studies is given and new developments of these techniques, as well as new methods, will be discussed in more detail.

Table 2.5: Overview of the history of OGSR analysis

	TLC	GC-MS	GC-TEA	GC-EC	LC-EC	LC-PMDE	LC-UV	LC-MS	TOF	DESI	IMS	Raman	FTIR	EC
1960s	[16-20]	[22]												
1970s	[24, 82]	[21]						[26]						
1980s	[83]		[25, 84]	[25, 53, 83]	[49, 85-89]	[88, 89]		[27]					[49]	
1990s	[90]	[70, 77, 91]	[56, 77, 92]		[50]	[91]	[70, 93]	[70]					[94]	[95, 96]
2000s		[97, 98]	[54, 72, 78, 79, 97]				[34]	[99-101]	[102, 103]	[52, 55, 104]	[47, 48, 51, 72, 105-107]		[108, 109]	[35, 51, 110-112]
2010s		[7, 30, 33, 36, 41]						[32, 69, 113]	[6]	[32, 43, 114]	[41, 115, 116]	[5, 45, 46, 117, 118]	[117, 119]	[15, 31, 44, 120]

This list is not exhaustive, but merely provides a selection of reported techniques.

Table 2.6: Analytical techniques for OGSR detection

Type of technique	Technique	Acronym	Ref
Colour test	Colour/spot test	-	[3, 11, 12]
	Thin layer chromatography	TLC	[11, 14, 16, 18]
Spectroscopy	Fourier transform infrared	FTIR	[11, 14, 49, 94]
	Raman	-	[5, 46, 118]
Liquid chromatography	High performance liquid chromatography	HPLC	[3, 11, 14]
detector combinations	Electron capture detection	HPLC-ECD	[3, 11]
	Pendant mercury drop electrode	HPLC-PMDE	[3, 11, 89, 91, 92]
	Electrochemical detection	HPLC-EC	[11, 14, 49, 86]
	Mass spectrometry	HPLC-MS	[3, 11, 99, 100]
	Tandem mass spectrometry	LC-MS/MS	[3, 14, 32, 101]
	Ultraviolet	HPLC-UV	[3, 11, 34]
	Fluorescence detection	-	[3, 11]
Gas chromatography	Gas chromatography	GC	[3, 11, 14]
detector combinations	Electron capture detection	GC-ECD	[11, 14, 25, 83]
	Electrochemical detection	GC-EC	[11, 53]
	Thermal energy analysis	GC-TEA	[3, 11, 77, 84]
	Flame ionisation detection	GC-FID	[11, 56, 77]
	Mass spectrometry	GC-MS	[3, 11, 14, 21, 22]
	Nitrogen phosphorus detector	GC-NPD	[14, 121]
Super critical fluid	Super critical fluid	SCF	[11]
detector combinations	Ultraviolet detection	SCF-UV	[11]
	Flame ionisation detector	SCF-FID	[11]
	Electron capture detection	SCF-ECD	[11]
Mass spectrometry	Time of flight	TOF	[3, 6, 103]
	Secondary ion mass spectrometry	SIMS	[3, 29, 102, 103]
	Ion mobility spectrometry	IMS	[3, 14, 29, 105, 115]
	Desorption electrospray ionisation	DESI	[29, 55, 104]
	Focussed ion beam	-	[3]
Electrochemical detection	Capillary electrophoresis	CE	[3, 11, 31, 35, 110]
	Micellar electrokinetic capillary electrophoresis	MECE	[3, 11, 57, 122]
	Micellar electrokinetic capillary chromatography	MEKC	[3, 34]

Colour tests may be attractive due to their inexpensive, simple and rapid nature, however, the major drawback of such methods is the fact that the results are merely indicative. Consequently, these tests are used less frequently nowadays. [3, 11]

FTIR has been used as a probe for the analysis of the distribution of OGSR in and around bullet entrance holes, and to estimate firing distances [14]. It has also been used as a confirmatory technique after HPLC-UV analysis, to enable a positive identification of nitrocellulose [11, 49].

HPLC-UV can be used as a fast screening technique [11]. LC-MS and LC-MS/MS are useful tools for both the identification and quantification of OGSR compounds. Limits of detection of the latter technique have been reported in the low nanomol per litre range for diphenylamine and related compounds, which corresponds to microgram levels. Sample concentration and purification may be necessary, which can be achieved with SPE. [3]

Gas chromatography has been combined with several different detectors (table 2.6) for OGSR analysis. The main advantage of GC analysis is the possibility for thermal desorption. In combination with SPME, the direct transfer of the pre-concentrated compounds from the fibre into the GC inlet eliminates the need for additional extraction steps. [77] It should be noted that thermal desorption-GC is only applicable to thermally stable volatiles and semi volatiles [3]. For example, nitrocellulose, the main component of modern smokeless powders, is incompatible with GC analysis due to the insufficient volatility of the compound [3, 11]. It may accelerate column deterioration, if injected as a major component [3]. Thermal instability of compounds, such as nitrate esters, poses analytical challenges. Nitrate esters are frequently encountered in GSR, but their thermal instability and tendency to decompose on improperly prepared columns hampers GC analysis of these compounds. This is particularly true for PETN. In addition, GC has been reported to be unsuitable for the analysis of stabilisers such as n-nitrosodiphenylamine, because denitrosation to diphenylamine may occur under the high temperatures involved. [3, 11] For thermally stable (semi)volatile compounds, however, GC is sensitive, highly selective, rapid, and enables qualitative and quantitative analysis [11]. GC in combination with TEA is reportedly most commonly employed for OGSR analysis [3]. GC-TEA increases the high sensitivity and selectivity of gas chromatography. Moreover, it has been reported not to require purification of vacuumed samples for the analysis of trace amounts of OGSR. [11] Detection limits in the low nanogram range have been achieved for dinitrotoluene-compounds. Detection limits of several nanograms per compound have been reported for GC-MS, which is believed to be less sensitive than TEA. [3] These limits, however, are one order of magnitude lower than the detection limit mentioned for LC-MS [3] and comparable to SPME-IMS [105]. Hence GC-MS has

frequently been used in recent OGSR analyses, and the majority of the detected organic compounds associated with OGSR (table 2.1) has been detected from propellant powder and spent cases using this technique [7, 30, 33]. GC can also be coupled to IMS to enable the separation of complex mixtures [115].

A major advantage of TOF-SIMS is the ability to analyse both inorganic and organic compounds [3, 103]. It has been reported, however, to be unsuitable for more volatile compounds such as nitroglycerin, due to the high-vacuum conditions inside the instrument [3]. Different ionisation techniques for MS detection and their relation to OGSR analysis have been discussed by Taudte *et al.* [29].

CE can provide rapid, high-resolution separation of complex mixtures. Although electrically neutral compounds, such as those found in OGSR, cannot be separated by conventional CE [11], it has been used for the analysis of both inorganic and organic GSR with limited success. Pre-concentration enabled the detection of OGSR, however, it was concluded that separate runs for the inorganic and organic components may be a better option. As an alternative option, GC analysis of the OGSR compounds was suggested. [3] Alternatively, MECE allows the separation of electrically neutral compounds [11]. Limits of detection achieved by MECE for dinitrotoluenes and nitrodiphenylamines are in the low picogram range for standard solutions [3]. MEKC in combination with UV detection is reported to be an interesting screening technique, due to the fact that it has a broader range of detected analytes, better suitability for diode array detection, and lower operation costs than HPLC-UV. [3]

2.4.1 Further development of analytical techniques

Several of the techniques highlighted in table 2.6 have been further developed since the publications of the mentioned reviews [3, 11, 14, 29], and other techniques have since then expanded into OGSR analysis. The developments are not limited to a specific type of technique; significant progress has been made with methods including IMS, HPLC-MS, DESI and Raman. This indicates that a generic analytical approach for the analysis of OGSR has not yet been established.

Ion mobility spectrometry

Ion mobility spectrometry (IMS) is recognised as one of the most sensitive and robust techniques for explosive detection [47], and has been reported as a good complementary technique to GC-TEA [3]. IMS has great advantages including enhanced sensitivity and selectivity, a very fast response time, low detection limits, and field employability [47]. Despite this, relatively little investigation into the applicability of this method to the analysis of GSR has been undertaken.

This may explain the limited extent to which IMS is considered in the discussed reviews. Some studies in the early 2000s, however, have reported the use of IMS for the detection of OGSR [47, 54, 72, 123].

Previously, Colón *et al.* [47] used IMS for the detection of three different smokeless powders, on several different surfaces, using a collection filter in combination with thermal desorption for the sample collection and introduction. The detection was based on the presence of nitroglycerin, but it was unknown whether the IMS system could differentiate between explosive NG, and NG used for medical purposes. Nitrocellulose and nitrate were also detected. Neves *et al.* [123] used ion trap mobility spectrometry for the detection of smokeless powders based on ethyl centralite. The performance of IMS for the detection of GSR compounds, collected after firing tests, was studied by Zeichner *et al.*, who demonstrated the feasibility of OGSR analysis with IMS from vacuum collected samples [54], and from double-side adhesive-coated stubs [72].

A significant improvement of the applicability of IMS to the analysis of volatile OGSR, was the development of an interface enabling the combination of IMS with SPME, which was reported in 2005 [105]. This method employs thermal desorption of the SPME fibre, and thus makes optimal use of the advantages of SPME. The detection limits for several compounds achieved by the SPME-IMS system were compared to standard IMS using a filter for sample introduction. The detection limits for the standard IMS system were around 20 ng for most compounds, however, for 2,4-dinitrotoluene the detection limit was above 100 ng and for 2,6-dinitrotoluene even above 600 ng. The detection limits reported for SPME-IMS were below 1 ng for all tested compounds. Only tetryl was detected by standard IMS, but not by SPME-IMS. [105] A drawback of this method is the fact that the high desorption temperature and the length of time required to heat the interface, cause substantial power consumption. Consequently, despite the portability of the analytical instrumentation, the method is inconvenient for field analysis, as field-employed equipment is typically battery-operated. [106] This led to the development of a new, energy-conserving interface, which increased the feasibility of field analysis with SPME-IMS [106]. Another attempt to increase the suitability of SPME-IMS for field analysis, involved a different approach to selecting target analytes. The previously mentioned studies [105, 106] focused on the analysis of the parent molecules of explosives. A problem encountered in these studies was the inability to collect some compounds from the headspace using SPME, due to a lack in volatility of the compounds. In a study by Lai *et al.* [107] the target analytes selected were so called odour signature compounds; volatile odour chemicals associated with the explosive. Limits of detection are reported in the low nanogram range.

Although this may enable SPME-IMS to compete with canines when it comes to rapidly searching large areas for concealed explosives, in this scenario SPME-IMS is used as a screening tool. [107] Further development of this approach by Joshi *et al.* [48] involved the analysis of odour signature compounds that are characteristic for smokeless powders. This gives rise to the potential of a simultaneous screening and confirmatory technique. It must be noted, however, that only four target analytes have been included in this study, and the results are thus still only presumptive identifications of OGSR [48].

The first study using SPME-IMS for the detection of volatile and semi volatile additives of smokeless powders, for the purpose of identifying target compounds for vapour phase detection of smokeless powders, has been performed in 2011 by Joshi *et al.* [41]. It must be noted that prior to IMS analysis, volatile OGSR compounds from smokeless powders were analysed and identified using GC-MS. Although composition profiles obtained with both methods are provided, a clear comparison or performance discussion is not provided as the aim was to provide the list of compounds. The advantage of IMS over GC-MS mentioned, is the decreased desorption and analysis time. [41] With respect to IMS, it is reported that all peaks are sufficiently resolved, however, it must be noted that only eight target analytes have been detected using IMS. Separation of compounds may be an issue when a greater number of target analytes is present. A possible solution for this issue could be the use of a differential mobility analysis (DMA), which is a specific IMS configuration, although it was not tested specifically for OGSR compounds. The difference between both methods lies in the separation in time of drift (IMS), versus separation in space (DMA), which facilitates the improvement in resolving powder and sensitivity. [124] Implications for the field-portability of this adaptation are not considered. Alternatively, IMS has been combined with GC (GC-IMS) to provide the separation of complex mixtures [115]. Although this inhibits field analysis, a reduction in false positive occurrences was demonstrated in a study by Cook *et al.* [115]. In this study, 100 samples containing a combination of explosives and interferents were analysed with both IMS and GC-MS. The amount of false positives was reduced from 21 when using IMS, to one when using GC-IMS.

These studies seem to confirm the potential for the use of IMS in the analysis of OGSR. The advantages specific to IMS, especially the field-portability and near instantaneous analysis speeds, make IMS particularly suitable as a rapid, on site screening technique. Two anticipated difficulties of this technique are the potentially insufficient separation power for complex mixtures, or when a greater amount of target analytes is to be included in the analysis; and the lesser suitability as a confirmatory technique, as suggested by the study by Cook. With respect to the first issue, however, a recent study by Arndt *et al.* [125] employed IMS for the analysis of

gunshot residue collected from the shooter's hands using a swab. The identification of GSR was based predominantly on the presence of DPA, which was absent in approximately 100 blank samples. [125] Bell and Seitzinger [116] employed IMS to the differentiation of shooters from non-shooters, by targeting OGSR compounds in swabs collected from the hands of 73 individuals. The use of IMS in this study was presumptive, rather than conclusive. [116] This further confirms the strength of IMS as a rapid and viable screening tool.

Ultra-high performance liquid chromatography tandem mass spectrometry

A further development of LC-MS is the use of ultra-high performance liquid chromatography (UPLC). In a study by Thomas *et al.* [32] UPLC was employed for the separation of 21 OGSR compounds, providing faster separation and increased resolution. Moreover, an optimised tandem MS method enabled the detection of both positive and negative ions, allowing the analysis of all target analytes in a single run. This was achieved by employing two ionisation sources: electrospray ionisation (ESI) in both positive and negative mode, and atmospheric pressure chemical ionisation (APCI) in negative mode, and switching between them at high speeds. This resulted in the detection of 18 of the target analytes in a total run time under 8 minutes. [32]

Desorption electrospray ionisation

The major advantages associated with desorption electrospray ionisation (DESI) are its capability of direct analysis of solid surfaces without the need for sample preparation [29, 55], and the compatibility with portable mass spectrometers [55]. These advantages, in conjunction with the real time analysis capability of DESI-MS, its simplicity, and the high throughput, give rise to a potential screening application of this technique. Furthermore, the potential of DESI-MS to supply structural information in real time [55] could enable the combined function as a screening and confirmatory technique, possibly even in a single run.

Zhao *et al.* [55] used DESI-MS/MS successfully for the detection of subnanogram levels of OGSR compounds, based on the presence of methyl centralite (MC) and ethyl centralite (EC), from several solid surfaces including a human hand. They reported no interference from the tested surfaces and were able to detect OGSR for up to 12 hours, and hands could be washed at least six times. The only disadvantage mentioned is the fact that the DESI source contains a high voltage component, which is potentially harmful to the analyst. Appropriate shields and interlocks were required to prevent accidental contact, which may make this piece of equipment more suitable for use in contained environments. It should be noted that the detection in this

study is based on merely two OGSR components. The evidential value of this technique would significantly increase with the inclusion of several additional OGSR compounds. The authors stated, however, that the capability for this is present, based on previous studies that used DESI-MS for the detection of for example diphenylamine (DPA) and its nitration products from propellant powders [55, 104].

This potential is somewhat confirmed by the detection of MC, EC and DPA from smokeless powder by nanoESI-MS/MS (nESI-MS/MS) in a later study [52]. These compounds were also detected in OGSR from cotton cloths, however, interference which was most likely due to the presence of detergent was observed in the analysis of machine-washed and dried cloths.

Morelato *et al.* [43] reported the detection of MC, EC, and DPA by DESI-MS on adhesive stubs typically used for the analysis of IGSR compounds by scanning electron microscopy-energy dispersive X-ray (SEM-EDX) detection. They found that the DESI-MS analysis did not significantly interfere with the SEM-EDX detection, enabling the analysis of OGSR and IGSR from a single sample, albeit by different techniques. As a disadvantage, they reported the relatively high detection limits, which are due to the characteristics of the stub.

A possible solution to this problem is suggested in a later publication by Morelato *et al.* [114] and involved a collection and preconcentration step developed by Venter *et al.* [126]. This surface sampling technique decouples desorption from analysis, to enable the collection of the spray onto a suitable secondary surface. Subsequent analysis can be performed by direct ambient ionisation mass spectrometry (as is the case with standard DESI-MS), or by other techniques, such as GC-MS and UV spectroscopy.

Raman spectroscopy

The application of Raman spectroscopy to the analysis of OGSR compounds was first reported in 2012 [46, 118]. It was successfully used for the detection of MC, EC, dinitrotoluene, DPA, and its nitration products [46, 118]. The authors reported that the GSR spectrum showed high similarity with the spectrum of the unfired ammunition, which enabled the GSR to be traced back to the ammunition used. Other substances, which might be confused with GSR, such as sand, dried blood, or black ballpoint ink, were easily distinguishable from GSR, confirming the screening capability [46].

In another study, Raman spectroscopy was used in conjunction with a statistical analysis, which demonstrated that the obtained spectra could provide highly accurate identifications of ammunition calibre-firearm pairs when subjected to the statistical classification analysis. This

study was performed from the point of view that the specific firearm parameters are responsible for the combustion process and that the chemical composition of specific ammunition is dependent upon the calibre, and that as such this calibre-firearm pair would determine the subsequent GSR product. The authors reported the potential for a rapid, portable, solventless and selective alternative for GSR identification, while providing a statistical and chemical link between the suspect and the crime scene. [118] In order to further improve the statistical discrimination of GSR, complementary spectroscopic date from Raman and FTIR spectroscopy were combined into a single dataset, in a later study by Bueno and Lednev [117].

Abrego *et al.* [5] reported a micro-Raman spectroscopy method for the analysis of OGSR. The total analysis time, including the parallel analysis of IGSR with another technique, was 2 hours due to the fact that the observation of the GSR particles via optical microscopy for the subsequent analysis by Raman spectroscopy was performed manually. A decrease in the analysis time is expected if this step can be automated using image recognition software.

2.4.2 Full chemical profiling

The ability to combine organic and inorganic GSR information would significantly increase the probative value of GSR evidence [7, 15, 29]. Consequently, several attempts to realise this have been undertaken. The analytical instrumentation generally used for the analysis of either OGSR or IGSR, however, presents two major challenges. Firstly, there is the inability of techniques to analyse both organic and inorganic compounds, which gives rise to the need for the analysis of a single sample by multiple techniques. Secondly, there is the destructive nature of most analytical techniques, which hampers sequential analysis of the same sample.

One proposed solution is the use of modified stubs in which half of the stub's surface is used for the analysis of IGSR and the other half for OGSR analysis. This approach was used by Abrego *et al.* to analyse both halves simultaneously with Raman microscopy (OGSR) and scanning laser ablation-inductively coupled-mass spectrometry (IGSR) [5], and by Benito *et al.* for the simultaneous analysis with LC-QTOF (OGSR) and SEM-EDX (IGSR) [6].

Another approach is the analysis of OGSR with a non-destructive technique, which allows subsequent IGSR analysis. OGSR analysis with DESI-MS followed by SEM-EDX for IGSR analysis, as suggested by Morelato *et al.* [43], is an example of this.

The final option is the development of an analytical technique that can analyse both organic and inorganic compounds. So far, three analytical techniques have been described for this purpose: electrochemical detection [15], and Raman spectroscopy [118] and FTIR spectroscopy [119].

The electrochemical detection of IGSR and OGSR proposed by Vuki *et al.* [15] includes four metals (IGSR) and three OGSR compounds. Their method employs electrochemical devices that are described as sensitive, compact, low power, and easy to use, and thus particularly attractive for field analysis. The results were rapidly generated in a single scan for both organic and inorganic compounds, which was reported to be an information-rich, inorganic/organic electrochemical fingerprint. [15] It should be noted, however, that due to the absence of an evidence-based consensus on what characteristic OGSR compounds are, the inclusion of merely three OGSR compounds is likely to be insufficient to provide an accurate, reliable GSR fingerprint. Consequently, the combination of merely NG and DPA, with heavy metal constituents (lead, antimony, and zinc), which is reported as particularly specific for GSR identification [15], is questionable. The authors reported to aim for the inclusion of a few more compounds, but potential coelution is expected to pose challenges [15]. This suggests that this method may not be suitable when more compounds are included to decrease the chances of false positives and strengthen the reliability of the results. Moreover, it is important to note that this method has only been used for the detection of standard mixtures, rather than for actual gunshot residue. Consequently, the complexity of a real GSR mixture may already pose significant challenges for this method. Despite the fact that the method is described as an on-the-spot field identification of individuals firing a weapon [15], the highlighted issues indicate that this method may be have more potential as an initial screening technique, in which case analysis with a confirmatory technique may be necessary.

Simultaneous detection of IGSR and OGSR using spectroscopic techniques has also been reported [118, 119], however, a limited amount of GSR compounds have been included in these studies. Raman spectroscopy was used for the analysis of an unknown amount of OGSR compounds, predominantly nitrate esters and nitrotoluenes. Although the reported results were promising as they enabled differentiation between GSR from two ammunition-firearm combinations, it was unknown which specific characteristics resulted in the differentiation. [118] FTIR spectroscopy [119] was also based on a small, undefined number of compounds. Possibly only one OGSR compound, 2,4-dinitrotoluene, may have been included. Consequently, further development of these spectroscopic methods is required, before they can contribute to GSR analysis with any substantial degree of reliability. This further development should be focused on the inclusion of a wider array of (O)GSR compounds, and identification of the compounds on which differentiation between samples can be evaluated.

2.4.3 Overview of developments

The many different types of techniques which are still investigated with respect to applicability to OGSR analysis demonstrates - as previously mentioned - that no generic analytical approach to the analysis of OGSR has been established to date. Table 2.7 contains a brief overview of the advantages and disadvantages of the recent analytical developments discussed. A greater amount of progress has been made with the MS based techniques included in this table, whilst applications of EC, Raman spectroscopy, and FTIR spectroscopy still require development before they can compete successfully with the other methods.

Table 2.7: Advantages and disadvantages of recent analytical developments in OGSR analysis

Technique	Advantages	Disadvantages	Ref
SPME-GC-MS	Simultaneous extraction and pre-concentration Simple method No solvents required Applicable to solid, liquid and gaseous samples Over 70 OGSR compounds already detected Confirmatory technique	Laboratory based technique Relatively slow (around 30 min) Unsuitable for non-volatiles	[7, 30, 33, 41, 42]
UPLC-MS/MS	Relatively fast (8 min) Better resolution than HPLC-MS Positive and negative ions in single run Around 20 compounds already detected Confirmatory technique	Laboratory based technique Not applicable to airborne samples Laborious sample preparation Solvents needed	[32]
IMS	Rapid (seconds) Real time analysis Portable/field deployable Structural information Compatible with SPME & swipe method Low detection limits Simple method	May be unsuitable for complex mixtures More false positives than GC-MS	[41, 47, 48, 105-107, 115, 123, 124]
DESI-MS	Rapid (seconds) Real time analysis Portable/field deployable Structural information No separate sample prep or collection method required Subsequent SEM-EDX on same sample possible Simple method	May be unsuitable for complex mixtures Only four OGSR compounds tested Not applicable to airborne samples	[43, 52, 55, 114, 126]
Raman/FTIR	Non-destructive OGSR and IGSR	Laboratory based technique Further development needed	[5, 46, 119]
EC	OGSR and IGSR in a single run Potentially field deployable Rapid Sensitive Simple method	Not yet tested on GSR Only four OGSR compounds included Potential peak overlap when adding compounds	[15]

2.4.4 Analytical methodologies for OGSR analysis

A wide array of collection, sampling and extraction methods has been considered, as well as an equally wide range of analytical techniques. A universally optimal method, however, is not available.

The analysis method as a whole should be selected based on case-by-case requirements. These criteria include but are not limited to: the surface to be sampled, the target analytes selected, the purpose of the analysis, and the compatibility amongst all aspects of the method, *i.e.* collection method, sampling/extraction method, and analytical technique. The possibilities and limitations of each available method for every step should be taken into account when selecting the method. An overview of complete methods used in the literature discussed in this review, based on the sample type, is given in table 2.8.

Table 2.8: Utilised methods for the sampling, extraction and analysis of OGSR compounds

Sample	Sampling method	Extraction	Analytical technique	Ref
Propellant powder	-	Solvent extraction	GC-MS, UPLC-MS/MS, CE, MEKC, Raman	[7, 31, 32, 34, 46]
Propellant powder	-	SPME	GC-MS, IMS	[7, 33, 41]
Propellant transfer	Wipe	-	IMS	[47]
GSR; gun barrel	-	SPME	GC-TEA, GC-FID	[77-79]
GSR; spent cartridges	-	SPME	GC-MS, IMS, GC-FID, GC-TEA	[7, 33, 42, 56]
GSR; spent cartridges	-	Stir bar	GC-MS	[30, 37]
GSR; spent cartridges	-	Solvent extraction	Anion exchange chromatography	[8]
GSR; target cloth	Tape lift Method	-	ATR-FTIR, Raman	[118, 119]
GSR; target cloth	-	-	Raman	[46]
GSR; objects	Stub	Solvent extraction	LC-MS/MS	[71]
GSR; skin	Swab	-	IMS	[125, 127]
GSR; skin	Swab	Solvent extraction	LC-QTOF, UPLC-MS, LC-MS/MS	[6, 69, 113]
GSR; skin	Stub	-	DESI-MS	[43]
GSR; skin	Stub	Solvent extraction	LC-QTOF, UPLC-MS, LC-MS/MS	[6, 69, 113]

Another area of interest is that of on-site screening techniques (further information can be found in appendix A).

2.5   Summary

This review has highlighted several aspects with respect to OGSR compounds and the analytical techniques and methodologies used in their detection, worthy of consideration.

The first aspect that should be considered is which OGSR compounds will be the target analytes. As illustrated by the generated list of nearly 140 compounds associated with OGSR, making a selection of compounds that are to be included in the study is inevitable. It appears, however, that there is no general consensus on what characteristic OGSR is. Which compounds make for good, reliable characteristics depends on the aim of the study, as well as on the analytical technique intended for their detection. Despite the fact that the selection of target analytes directly influences the evidential value of the investigation and results, there are no generally accepted guidelines for the selection of target analytes. Extracting as much information as possible, however, will increase the probability of accurate interpretation and increases the evidential value of the analysis.

Consequently, the inclusion of both organic and inorganic GSR is favourable, which poses two main challenges on the analytical technique used for the detection: the inability of techniques to analyse both organic and inorganic compounds, which gives rise to the need of the analysis of a single sample by multiple techniques; and the destructive nature of most analytical techniques, which hampers sequential analysis of the same sample. Possible solutions may be provided by sampling/extraction techniques that enable the separate, yet simultaneous analysis of the OGSR and IGSR of half of the sample, such as a modified tape lift method. OGSR analysis can be performed using laboratory based techniques such as GC-MS and UPLC-MS/MS, which are capable of separating complex mixtures; or field deployable techniques such as IMS and DESI-MS, which enable rapid, on-site analysis. Another possibility is the use of a non-destructive technique for the analysis of the organic compounds, such as DESI, to allow for subsequent IGSR analysis of the same sample. Improvements in detection of OGSR and IGSR compounds with a single analysis have also been made, utilising electrochemical detection, Raman spectroscopy and FTIR spectroscopy. Further development, however, and inclusion of a more substantial number of (O)GSR compounds is required to gain any meaningful evidential value. The same has to be said about the recently developed on-site screening techniques. The use of more specific techniques, with stronger confirmatory properties, such as IMS or DESI-MS, may offer a better alternative for screening purposes.

This review has highlighted that there is a wide array of analytical techniques available, together with corresponding sample collection and extraction techniques, which are suited for the sampling of different types of (O)GSR samples. The difficulty in selecting an appropriate

analysis method lies in the many variables that affect the performance of each technique. A selection of target analytes needs to be made first and foremost, but just as important is the compatibility of the selected method as a whole. Consequently, the choice for an optimal method in a given situation calls for a case-by-case approach, in which the purpose of the investigation should be the predominant factor.

3 OGSR analysis using gas chromatography – mass spectrometry

The aim of the work discussed in this chapter is to optimise a GC-MS method for the analysis of selected OGSR compounds. This chapter provides an introduction to the working principle of GC-MS. Details of the analytical instrumentation and the optimised method are provided.

3.1 Gas chromatography – mass spectrometry

Gas chromatography – mass spectrometry (GC-MS) is currently one of the major techniques used in OGSR analysis [7, 30, 33, 36, 41]. GC accomplishes the separation of chemical components in complex mixtures, which are subsequently ionised to enable MS detection and identification.

3.1.1 Gas chromatography

Chromatography is an analytical technique that makes use of a mobile phase and a stationary phase for the separation of (complex) mixtures. Compound separation is achieved through differences in migration rates amongst the sample components as they travel through the system. Gas chromatography is a type of column chromatography, in which a gaseous mobile phase transports the sample through a column containing the stationary phase. [128, 129]

A schematic representation of a GC system is shown in figure 3.1. A liquid or gaseous sample is typically introduced via a micro-syringe, after which the sample is quickly evaporated by the high inlet temperatures. A constant flow of chemically inert carrier gas transports the sample through the fused silica capillary column. The column, coated on the inside with the stationary phase and on the outside with polyimide, is formed as a coil in order to fit inside the oven. [80, 129, 130]
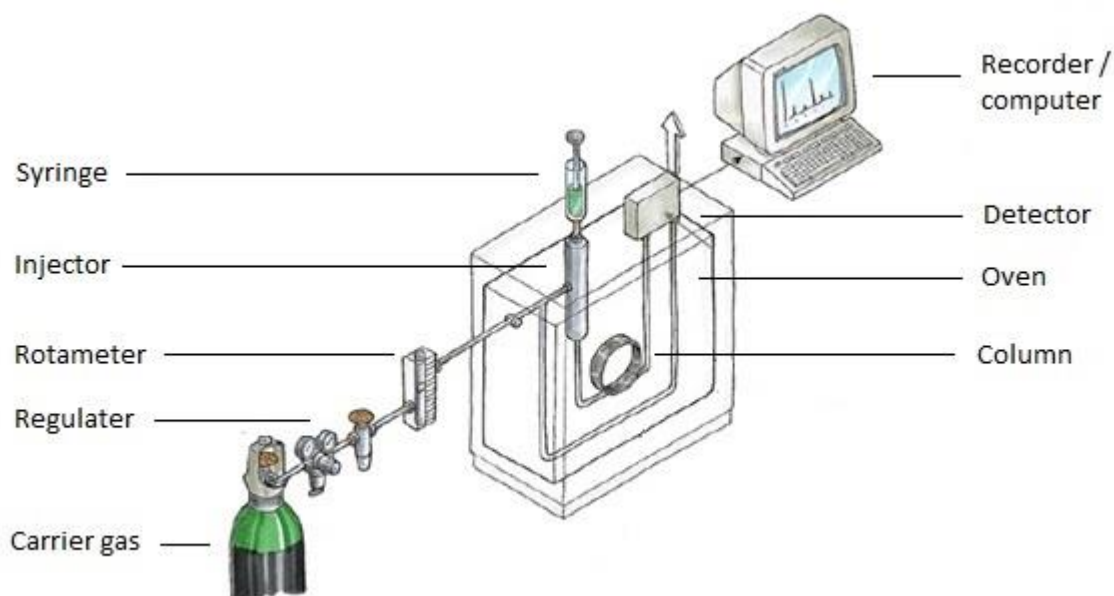
Figure 3.1: Schematic GC system

Separation principle

The separation of compounds is based on differences in boiling point, solubility, or sorption. Sorption is the process whereby a solute transfers from the mobile phase into the stationary phase. [80] This requires a degree of compatibility between the solutes and the stationary phase, *i.e.* solubility. The extent of the solubility is determined by the relative polarities of the column's stationary phase and the compounds. For polarity the 'like dissolves like' principle applies, *i.e.* polar compounds have a higher affinity for one another and a lesser affinity with non-polar compounds, and vice versa. Increased affinity increases the interaction the compounds have with the stationary phase, leading to increased retention times; compounds with little affinity for the stationary phase elute from the column first (figure 3.2). [129, 130] The oven temperature profile enables the separation of compounds with similar polarities based on differences in boiling point, and increasing the temperature reduces the retention times [80, 129, 130]. Retention times can also be reduced by increasing the flow rate of the mobile phase [129].
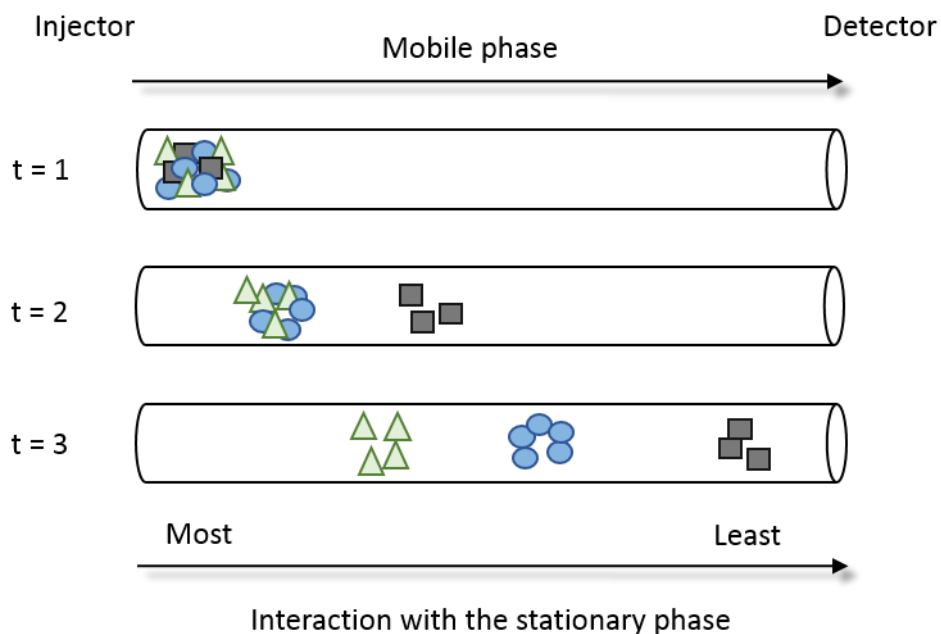
Figure 3.2: Schematic representation of the separation principle inside the column

The detector responses are recorded in a graph, the chromatogram, as a function of the elution time [131]. Figure 3.3 shows a schematic representation of the chromatogram for the separation of the three compounds shown in figure 3.2. The time from the compound's injection onto the column until it reaches the detector, is referred to as the retention time (RT) [131]. The retention time of a compound can aid in the identification of a compound, the areas under the peaks provide a quantitative measure of the amount of each compound [128].
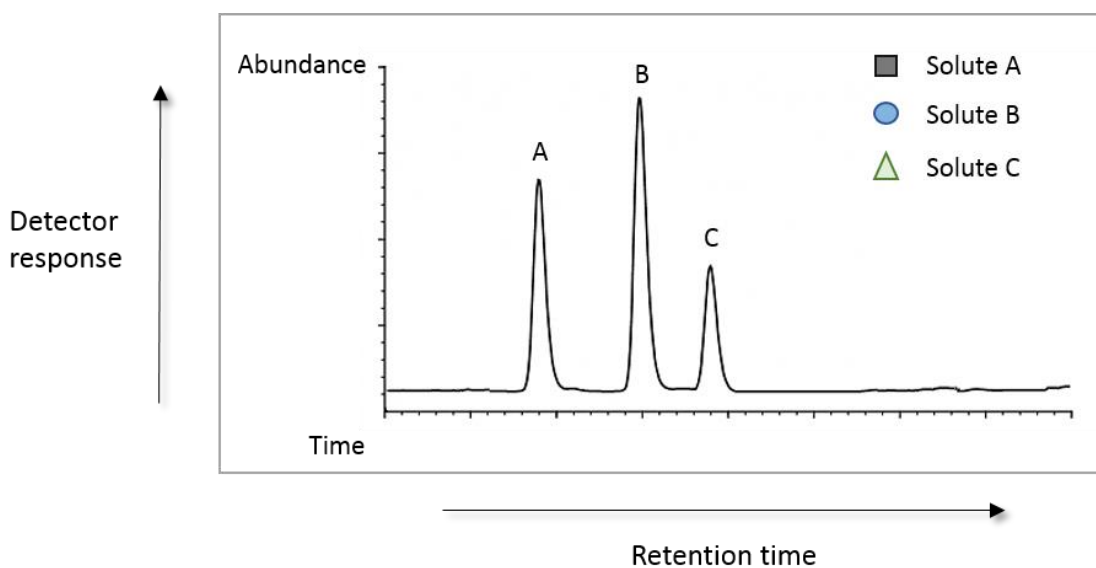
Figure 3.3: Schematic of gas chromatogram

Relationship between retention time and migration rate

During chromatographic separation, analytes are continually moving back and forth between the stationary phase and the mobile phase. The analytes are carried forward when they reside in the mobile phase, but remain virtually stationary during the time spent in the stationary phase. Consequently, the rate of migration of each analyte is determined by the proportion of time it spends in the mobile phase, or in other words, by its distribution ratio D (equation 3.1). [80]

Equation 3.1: $$D = \frac{C \text{ stationary phase}}{C \text{ mobile phase}}$$

where D is the distribution ratio of a given analyte and C the concentration of that analyte in either the stationary or mobile phase. [80, 131]

From this equation can be derived that the greater the distribution ratio of a given analyte is, the slower the progress of the analyte through the column and the longer its retention time [80].

In column chromatography, an analyte can be characterised by the partition ratio (or partition coefficient K – equation 3.2) [128].

Equation 3.2: $$K = \frac{C_S}{C_M}$$

where K is the partition coefficient of a given analyte, and C_S and C_M the (molar) concentration of that analyte in the stationary phase and mobile phase respectively. [128, 131]

Ideally, K is constant over a wide range of analyte concentrations, so that C_S is in direct proportion to C_M [128]. A value for K of zero suggests that the analyte is eluted from the column without being retained (*i.e.* an unretained species). A high value for K suggests a large retention volume is needed, resulting in a long retention time. [80]

In order to relate an analyte's migration rate to its partition coefficient, it is expressed as a fraction of the velocity of the mobile phase (equation 3.3):

Equation 3.3: $$\bar{v} = \bar{\mu} \times \text{fraction of time analyte spends in mobile phase}$$

where $\bar{v}$ is the average linear analyte migration rate and $\bar{\mu}$ the average linear velocity of the mobile phase. [128, 131]

The values of $\bar{v}$ and $\bar{\mu}$ can be calculated by dividing the column length by respectively the retention time of the analyte and the retention time of an unretained species. The latter is equal to the time it takes the mobile phase to travel through the column and reach the detector. [128]

The fraction of analyte in the mobile phase equals the average number of moles of this analyte in the mobile phase at any instant, divided by the total number of moles of analyte in the column (equation 3.4). [128]

Equation 3.4:
$$\bar{v} = \bar{\mu} \times \frac{\text{moles of analyte in mobile phase}}{\text{total moles of analyte}}$$

The total number of moles of analyte in the mobile phase is equal to C_M multiplied by V_M, the volume of mobile phase in the column. Similarly, the total number of moles of analyte in the stationary phase is equal to C_S times the volume of the stationary phase V_s. Using this information, equation 3.4 can be rewritten as shown in equation 3.5. [128]

Equation 3.5:
a) $\bar{v} = \bar{\mu} \times \dfrac{C_M V_M}{C_M V_M + C_S V_S}$
b) $\bar{v} = \bar{\mu} \times \dfrac{1}{1 + C_S V_S \div C_M V_M}$

When combining the latter equation with equation 3.2, an equation can be derived that expresses the rate of analyte migration as a function of its partition coefficient and as a function of the volumes of the stationary and mobile phases (equation 3.6). The volumes of mobile phase and stationary phase can be estimated from the method by which the column is prepared. [128]

Equation 3.6:
$$\bar{v} = \bar{\mu} \times \frac{1}{1 + K V_s \div V_M}$$

In summary, the above equations have shown how the partition coefficient of a given analyte in combination with experimental parameters (*i.e.* the column and mobile phase flow rate) determine the retention time of that analyte. The shape of the analyte peak in the resulting chromatogram is the result of diffusion taking place as the analyte travels through the column. The processes and effect of diffusion will be discussed next.

Relationship between diffusion and band broadening

The transfer of analytes through the column can be visualised as bands that move down the column. Diffusion is the process whereby analytes migrate from that band with a high concentration, to a part of the column with a lower concentration. The rate of this migration is proportional to the concentration differences between these areas, as well as the diffusion coefficient (D_M) of the analyte. [128] The diffusion coefficient is a measure for the mobility of an analyte in a given medium. It is a constant, which is equal to the velocity of migration under a unit concentration gradient. [128, 131]

Several types of diffusion may take place inside a chromatographic column [128]. Longitudinal diffusion relates to the diffusion of analytes within the mobile phase, caused by the local concentration gradients [80, 128]. It results in the migration of the analyte from the concentrated band to areas with a lower concentration on either side of the band, causing the Gaussian (or normal error curve) peak shape in the chromatogram [128]. The width of the peak is in part determined by the total amount of diffusion occurring during migration through the column [80]. The magnitude of the longitudinal diffusion is largely determined by and directly proportional to the diffusion coefficient of the analyte in the mobile phase. This means that diffusion increases for analytes with a greater diffusion coefficient. It is inversely proportional to the linear velocity of the mobile phase, meaning that its effect increases as the flow rate decreases. [128] This is due to the fact that more time spent in the column allows for more time for the diffusion to occur [128, 131]. Consequently, the width of a peak is expected to increase for analytes with a long retention time [128]. At low flow rates, diffusion into the stationary phase also contributes significantly to this effect, which increases with column length [80].

The other factor determining the peak width is the rate of mass transfer between the stationary phase and the mobile phase. This factor relates to the finite time taken for analytes to move between the two phases. [80] It consists of the mass transfer coefficient C for the stationary phase (C_S) and the mobile phase (C_M). When the stationary phase is a liquid film immobilised on a solid support, such as the column used in this work, C_S is directly proportional to the square of the thickness of the film. This is due to the fact that, on average, in a thicker film analytes have to travel farther to the surface of the stationary phase, where transfer to the mobile phase can occur. C_S is inversely proportional to the diffusion coefficient of the analyte residing in the film (D_S), given the fact that a lower D_S equals a slower rate of mass transfer. Therefore, both D_S and C_S reduce the average frequency at which analytes reach the interface where transfer to the mobile phase can occur, resulting in wider peaks. This effect is known as band broadening or peak broadening. [128]

C_M is also inversely proportional to the diffusion coefficient of the analyte. It is directly proportional to the mobile phase velocity. [128] This suggests that an increase in either C_M or the mobile phase velocity reduces peak broadening. Peak broadening in the mobile phase may still occur, particularly in packed columns and is referred to as Eddy diffusion. This does not play a significant role in open tubular columns such as used in this work. In these columns, however, a similar effect arises from static volumes of mobile phase that occur in the pores of the porous solid support that are not completely filled by the stationary phase film. The analytes have to diffuse through these static volumes, before transfer can occur between the moving mobile phase and the stationary phase. This slows down the exchange process and thereby results in peak broadening. [128]

Peak broadening also increases with increasing temperature. This is due to the fact that a low temperature reduces the distribution coefficient D_M, which in turn reduces the longitudinal diffusion. [128]

Relationship between diffusion and column efficiency.

The influence of these factors (diffusion and mass transfer rate) on the chromatographic separation, or on a chromatographic column, is referred to as column efficiency [128]. Two related terms that are widely used as quantitative measures of the efficiency are the number of theoretical plates (N) and the plate height (H). The origin of these two terms can be found in fractional distillation, where a separation column consists of several interconnected bubble-cap plates at each of which slightly different vapour-liquid equilibria are established. This facilitates the separation of different fractions containing closely related hydrocarbons at each plate. This plate model can account for both the Gaussian shape of chromatographic peaks and the factors influencing difference in analyte migration rates. A crucial difference, however, is that given the dynamic nature of chromatographic separation insufficient time is allowed for an equilibrium to be established. Despite the theoretical nature of plates and thus plate heights in chromatography, these terms are still used in relation to column efficiency. Essentially, the number of theoretical plates is proportional to the column length, and column efficiency increases as the number of plates increases and the plate height decreases. [128, 129]

Efficiency N is related to the analyte retention time and its peak width measured in terms of standard deviation assuming an ideally Gaussian-shaped peak [80] (equation 3.7).

Equation 3.7: $$N = 16\left(\frac{T_R}{W}\right)^2$$

where N is the number of theoretical plates, T_R the retention time of the analyte peak, and W the peak width at the base of the peak. The values for both T_R and W can be obtained from a chromatogram. High efficiency columns will have values for N of many (hundred)thousands. [80, 128, 131]

The plate height (H) can be thought of as the length of column that contains the fraction of the analytes between L (a unit of column length in cm) and L − σ (the standard deviation). Since for a Gaussian curve 68% of the area under the curve lies between L ± σ, H would contain 34% of the analyte. The value for H can be calculating according to equation 3.8. [128]

Equation 3.8:
$$H = \frac{\sigma^2}{L}$$

where L is the length of column (stationary phase) in cm and σ^2 the square standard deviation (or variance) in cm^2. Consequently, the unit of H is cm. [128, 131]

H can also be obtained by dividing the column length (L) by N. High efficiency columns will have values for H of less than 1 mm. [80, 128, 131]

An attempted to define column efficiency in terms of diffusion and mass transfer effects is known as the van Deemter equation, and uses H in its expression (equation 3.9).

Equation 3.9:
$$\text{a)} \quad H = A + \frac{B}{\bar{\mu}} + C\bar{\mu} \qquad\qquad \text{b)} \quad H = \frac{B}{\bar{\mu}} + C\bar{\mu}$$

where A represents Eddie diffusion (which is not relevant to the column used in this work and thus may be omitted), B represents longitudinal diffusion, C represents mass transfer rate, and $\bar{\mu}$ represents the average linear velocity of the mobile phase. [80, 128]

This equation shows that longitudinal diffusion decreases with increased flow rates, whilst the total effect of the mass transfer rates of the stationary phase and mobile phase combined increases. Experimental values of H can be plotted against the flow rate of the mobile phase for a given analyte and a set of experimental conditions, to produce a hyperbolic curve showing an optimum flow rate for maximum efficiency (figure 3.4). The position of this optimum varies per analyte, consequently, the most efficient flow rate for a particular sample is a matter of compromise. [80]
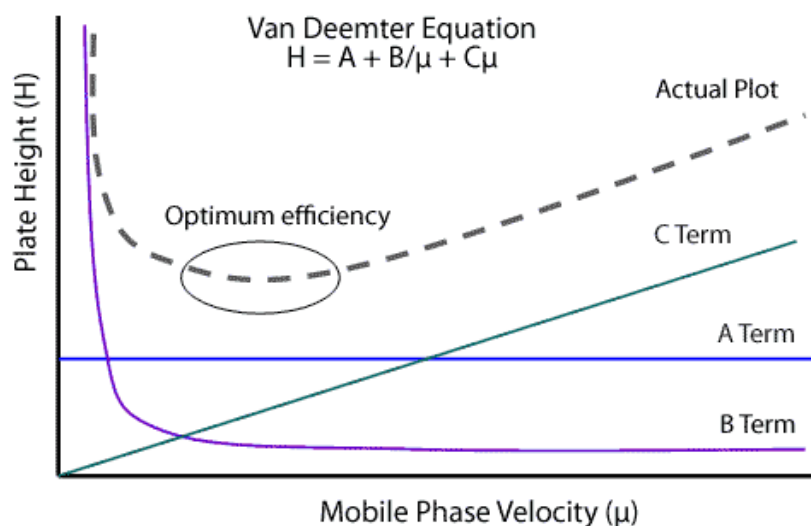
Figure 3.4: Van Deemter curve (dashed line) [132]

3.1.2 Mass spectrometry

Mass spectrometry is the most powerful detection method for chromatography, providing both qualitative and quantitative information and high sensitivity [133]. A mass spectrometer consists of three parts: 1) an ionisation source, which ionises and fragments the molecules in the sample; 2) a mass analyser, which selects the ions for detection based on their mass to charge (m/z) ratios; 3) the detector, which detects and registers the ions after amplifying their signal to a detectable current that is then recorded in a mass spectrum [134-136]. The mass spectrum provides structural information, which can be used for the identification of the detected compounds [133, 137, 138].

Ionisation source

Electron ionisation (EI) is the classic approach to ionisation in organic mass spectrometry [135, 139], and ideal for interfacing with GC due to its requirement for a gas phase sample [140].

In the ionisation source, gas phase sample components are ionised by bombarding energetic electrons onto the neutral molecules, which causes the removal of electrons from these molecules and thus the formation of ions (figure 3.5).

The electrons that are bombarded onto the sample are emitted by a heated filament [133, 139, 140] located just outside the ion source [134, 140]. This active filament is heated by the adjustable AC emission current it carries. It also has an adjustable DC bias voltage, which determines the energy of the electrons (usually – 70 eV) [134]. The emitted electrons are directed into and across the ionisation chamber by a magnetic field. These high-energy electrons interact with the sample molecules, ionising and fragmenting them. [134, 139, 140]
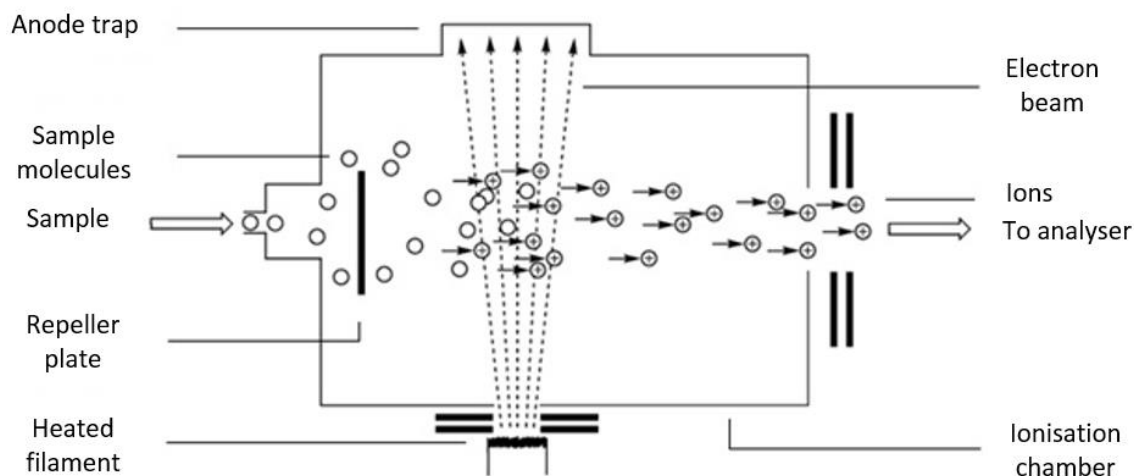
Figure 3.5: Electron ionisation [140]

Each emitted electron is associated to a wave, whose wavelength λ is given by equation 3.10:

Equation 3.10:
$$\lambda = \frac{h}{mv}$$

where *m* is its mass, *v* its velocity, and *h* Planck's constant (a physical constant of quantum mechanics expressing the ratio of the energy of one quantum of radiation to the frequency of the radiation, and approximately equal to 6.626176 x 10^{-34} J·s). This wavelength is 1.4 Å for a kinetic energy of 70 eV. The electrons are directed across the path of the gaseous sample entering the ionisation chamber, and will interact with a molecule if their wavelength is close to the length of the bonds within the molecule. This will cause the wavelength to be disturbed and become complex. If one of the wave's frequencies has an energy corresponding to a transition in the molecule, an energy transfer can occur. [139] This could lead to various (vibrational and rotational) electronic excitations within the molecule [136, 139]. If the energy transfer exceeds the ionisation energy of that molecule an electron can be expelled from that neutral, creating a positively charged ion. [135, 139] This is represented by equation 3.11:

Equation 3.11:
$$M + e^- = M^{+\bullet} + 2e^-$$

where M is the neutral molecule, e⁻ the emitted electron, M⁺• the positively charged molecular ion with a radical, and 2 e⁻ the emitted electron and the electron expelled from the neutral molecule. [133, 135, 136, 138] Depending on the molecule and the energy of the primary electron, double and triple charged ions may be formed. These, however, are generally low in

abundance in EI. [135] After ionisation the molecular ion ($M^{+\bullet}$) typically has enough residual energy to break into fragments [133].

This ionisation process typically leads to the formation of positive ions. Electron attachment, resulting in negatively charged ions, is possible, however, these ions are relatively unstable and fragment easily. [136]

The formed ions are then pushed out of the ionisation chamber by the repeller, the charge of which directs the ions out of the source through a series of lenses [133, 134]. These lenses concentrate the ions into a tight beam that travels through a hole in the draw out plate in the wall opposite the GC-MS interface, and into the draw out cylinder. Both the draw out cylinder and the body of the ion source housing the ionisation chamber are slotted to enable the vacuum to remove carrier gas and un-ionised sample molecules and fragments. [134] The ion beam then continues to the mass analyser, which distinguishes the ions based on their mass-to-charge (m/z) ratio. [134, 140]

Mass analyser

The ionised sample enters the mass analyser, a single quadrupole, through the entrance lens. The quadrupole separates ions according to their m/z ratio, by only allowing ions of a selected m/z ratio to pass through and reach the detector. The mass analyser thus acts effectively as a mass filter. [133, 134, 141]

The quadrupole is a fused silica (quartz) tube [133, 134, 141], coated with a thin layer of gold [134]. It has four long slots across the length of the tube, creating four segments in opposing pairs (figure 3.6) [134]. A complex electrical field, necessary for the mass selection, in created between the segments [134, 141]. Opposing segments are connected to one another, forming a pair, whilst adjacent segments are electrically isolated [134]. A combined direct current (DC) and radio frequency (RF) signal is applied to the two pairs of segments. [134, 138] A positive voltage is applied to one pair, and a negative voltage to the other pair [134, 141]. A positive ion entering the quadrupole will be attracted to the negative segment. If the potential of the segment changes sign before the ion hits it, the ion will change direction. Thus a stable trajectory is created, which leads the ions through the quadrupole towards the detector. [141]

At a particular RF voltage, only ions with a specific m/z ratio will have a stable trajectory and pass through the quadrupole. The other (non-resonant) ions collide with the segments and are lost before they reach the detector [133, 138, 141]. Therefore, the magnitude of the RF voltage determines the m/z ratio of the ions that pass through the quadrupole. Rapidly varying

the RF voltage will select ions with different m/z ratios to reach the detector. [134, 138, 141] The ratio of the DC to RF voltage determines the resolution, *i.e.* widths of the mass peaks. [134]
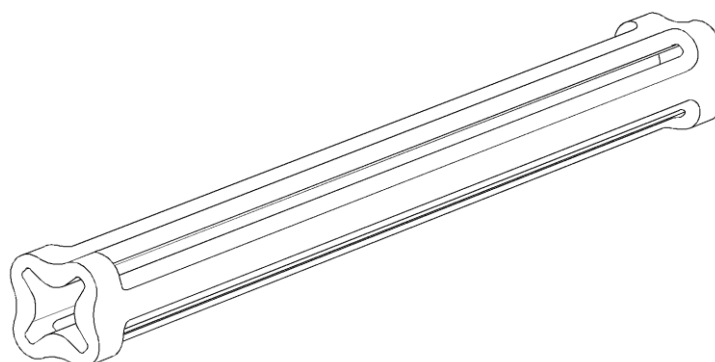
Figure 3.6: Quadrupole mass analyser [134]

Detector

The detector, a high-energy conversion dynode coupled to an electron multiplier, is located at the exit of the quadrupole. The detector ion focus directs the beam of ions exiting the mass analyser into the conversion dynode. It is positioned off axis to the centre of the quadrupole to minimise signals due to for example, electrons coming from the ion source. [134] The conversion dynode operates at − 10,000 V [134] and an ion striking the conversion dynode causes the emission of several secondary particles, which can be positive (for negative ions striking the dynode at a positive voltage), negative (for positive ions striking the dynode at a negative voltage), electrons and neutrals [142]. These secondary particles are then converted to electrons at the first dynode [142], and the electrons are accelerated by a voltage gradient into the electron multiplier [134, 142], which is the actual detector [133].

The continuous dynode electron multiplier has the shape of a horn, which carries a voltage of up to − 3000 V at its opening, attracting the electrons emitted by the conversion dynode, and 0 V at the other end, accelerating the electrons towards the end of the horn [134, 142]. The inner surface consists of a semiconducting metallic oxide. The secondary particles/electrons emitted by the conversion dynode strike the inner walls of the electron multiplier horn, which multiplies their signal through a process called secondary electron emission. When a charged particle (ion or electron) strikes the inner surface of the horn, it causes secondary electrons to be released from atoms in the surface layer. The number of electrons ejected depends on the type of incident of the primary particle, its energy and the characteristics of the incident surface. The released electrons accelerate further into the electron multiplier due to the increasingly positive potential gradient. Due to its shape, the

electrons do not travel far before they strike the inner surface of the horn again, thereby causing the emission of more electrons. Thus, a cascade of electrons is created, which multiplies on each interaction with the wall and finally results in a measurable current at the end of the horn where the electrons are collected by the anode (figure 3.7). [134, 142, 143] The number of electrons reaching the anode is multiplied by ~ 10^4 - 10^6 [133, 141]. The current collected by the anode is proportional to the number of secondary particles striking the cathode.

The detector responses are shown in a mass spectrum as peaks at a certain m/z value, where the current is converted to abundance on the y-axis [135, 138]. The highest peak in the mass spectrum thus represents the most abundant ion detected, and is referred to as the base peak. Mass spectra are typically normalised, meaning that the height of the base peak is set at 100% abundance and the heights of the other peaks are scaled accordingly [133, 135, 137, 138]. The mass spectrum provides structural information, which can be used for the identification of the detected ions [133, 137, 138].
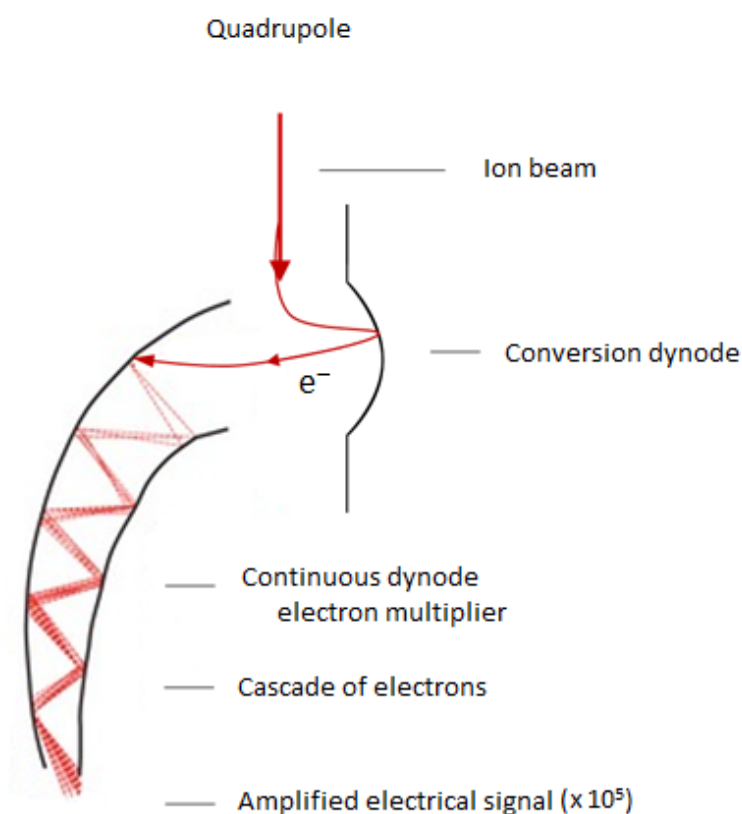
Figure 3.7: Continuous dynode electron multiplier (adapted from [144])

Fragmentation and the mass spectrum

The ionisation process resulted in the formation of $M^{+\bullet}$, positively charged molecular ions with a radical (equation 3.13). After ionisation, this molecular ion typically has enough residual energy to break into fragments [133], which may further breakdown themselves. Fragmentation leads to the ejection of a neutral particle (N) and the formation of a fragment ion ($A^{+\bullet}$ or A^+) [133, 135, 137, 140]. The ejected neutral particle may be a radical ($N^{\bullet}$) or a neutral molecule (N) (equation 3.12 and equation 3.13 respectively). [137, 140]

Equation 3.12:
$$M^{+\bullet} \rightarrow A^+ + N^{\bullet}$$

Equation 3.13:
$$M^{+\bullet} \rightarrow A^{+\bullet} + N$$

Further decomposition may occur if the fragment ion has sufficient excess of internal energy, and leads to the formation of new fragments (B^+, C^+, etc.) (figure 3.8). This process continues until there is insufficient excess of internal energy in any one ion for further reaction, or until the end of the short ionisation window (~ 10^4 s [145]) has been reached and the formed ions are detected. [137] In addition, new fragments may also be formed by undergoing re-arrangement [140].
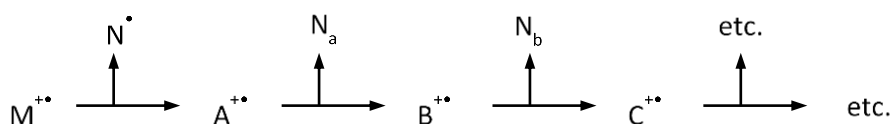
Figure 3.8: Fragmentation pathway [137]

Such a series of decompositions, which can be elucidated from a mass spectrum, is referred to as a fragmentation pathway. A molecular ion, or any of its fragments, may decompose by more than one pathway. The various fragmentation pathways together form a fragmentation pattern that is characteristic for that (molecular) ion. [137]

During any one time interval, molecular ions with various excesses of internal energy are produced by the ionisation process. This initial range in energies combined with the short time interval between ionisation and detection causes the presences of ions ($M^{+\bullet}$, A^+, B^+, etc.) in an amount determined by their individual rates of formation and decomposition, and by the internal energy imparted to M. Their relative amount is measured, and displayed in a mass spectrum as abundance. [137] Mass spectra are typically normalised, meaning that the height

of the base peak is set at 100% abundance and the heights of the other peaks are scaled accordingly [133, 135, 137, 138]. The largest m/z value often belongs the molecular ion, the intact ionised molecule [146]. The mass spectrum of methyl centralite is shown in figure 3.9 as an example, with the fragmentation pathway resulting in the peaks with the highest abundance highlighted.
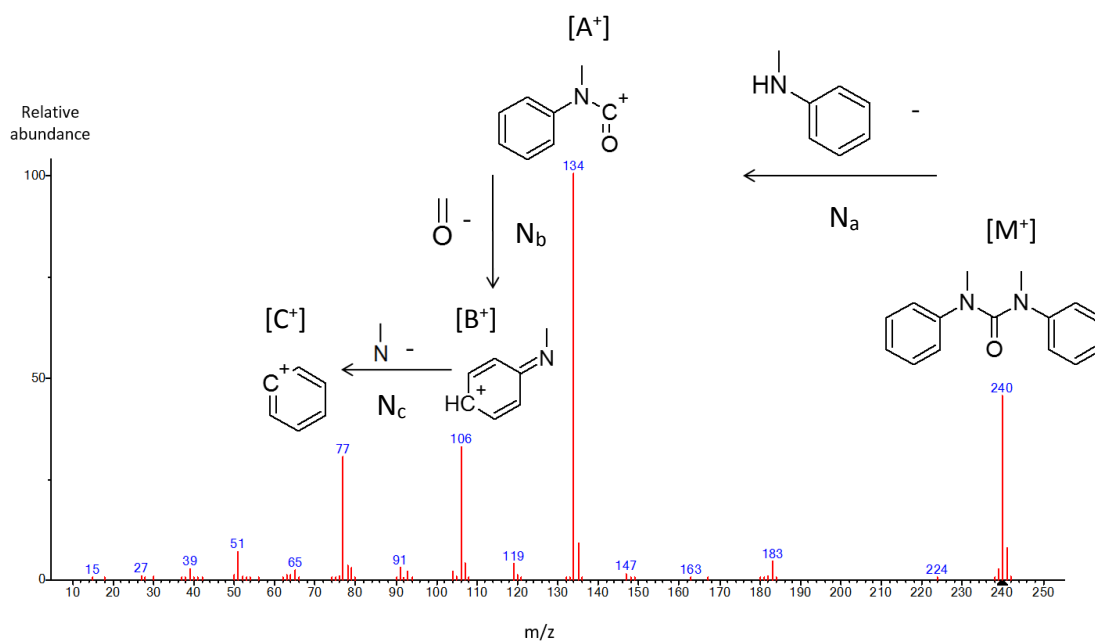
Figure 3.9: Mass spectrum of methyl centralite

Due to the extensive fragmentation that occurs, EI is often referred to as a hard ionisation technique. The fact that the fragmentation pattern of a molecule provides structural information of that molecule, makes EI a very popular and widely used technique. [136]

3.1.3 Selection of GC parameters

Several parameters need to be considered when setting up a GC method, including the column to be used, the carrier gas, the carrier gas flow rate, the split mode, the inlet temperature and the oven temperature profile. Table 3.1 shows the GC parameters chosen for OGSR analysis reported in the literature.

Table 3.1: GC parameters for OGSR analysis using SPME

	[7]	[30]	[33]	[41]
Inlet T	250°C	280°C	280°C	280°C
Split mode	Splitless	Splitless	Splitless	Split (5:1)
Carrier gas	Helium	Helium	Helium	Not mentioned
Column flow	1 mL/min	1.2 mL/min	1 ml/min	2 mL/min
Column	HP-5MS	HP-5MS	DB-1MS	DB-5MS
Length	30 m	30 m	30 m	50 m
I.D.	0.25 mm	0.25 mm	0.25 mm	0.25 mm
Thickness	0.25 μm	0.25 μm	0.30 μm	1 μm
Oven T				
Initial	50°C	40°C for 2 min	50°C for 1min	40°C for 1 min
Ramp 1	6°C/min to 200°C for 2 min	10°C/min to 100°C	8°C/min to 280°C for 5 min	15°C /min to 200°C for 1min
Ramp 2	20°C/min to 300°C	5°C/min to 280°C for 10 min		15°C/min to 240°C for 6.50 min
Ramp 3				25°C/min to 270°C
Ramp 4				5°C/min to 280°C for 4 min

All methods used a non-polar column. The stationary phase of the HP-5MS column is composed of (5%-phenyl)-methylpolysiloxane; DB-5MS of 5% phenyl arylene polymer, which is equivalent to the HP5-MS phase; and DB-1MS consists of 100% dimethylpolysiloxane, which is the least polar of these columns. [147] All columns had an internal diameter (I.D.) of 0.25 mm, which provides high column efficiency with a good capacity. Narrower columns have a low capacity [80, 129, 147, 148], whereas columns with a greater internal diameter are suitable for larger samples than required here (> 2 μL). Short columns (*i.e.* 10 m - 15 m) are used in combination with internal diameters smaller than 0.25 mm and are suitable for samples with few analytes. [147, 148] Long columns (*i.e.* 50 m - 60 m) are expensive and cause long analysis times, and are thus generally only used when sufficient compound separation cannot be obtained using shorter columns. Consequently, a column length of 25 m – 30 m is generally used [129, 147]. The standard film thickness used for such a column is between 0.18 μm and 0.25 μm. [147] A film thickness of 1 μm is typically used for columns with larger internal diameters (*i.e.* 0.45 mm –

0.53 mm) [80, 147]. The commercially available columns that fall within these parameters (non-polar, 25 m – 30 m column length) all have an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The DB-1MS and DB-5MS column are available in 25 m and in 30 m, the HP-5MS column is only available in 30 m. [147]

Since all methods are used in combination with SPME, the inlet temperatures are determined by the fibre type selected.

Three of the methods use splitless injection, the other method uses split mode with a low split ratio. In splitless mode, the compounds spend more time in the inlet liner, and thus need to be refocused on the column to prevent band broadening. This can be achieved by using an initial column temperature that is around 10°C lower than the solvent boiling point. In this study, the OGSR standards are made up in methanol, which has a boiling point of 64.7°C. A starting temperature of 40°C to 50°C would therefore be a good initial oven temperature. The oven temperature profile, along with the column flow, determine the compound separation and thus need to be optimised for the OGSR compounds of interest.

Method [30] is an optimisation of method [33]. Based on this information a HP-5MS column (dimensions 30 m x 0.25 mm x 0.25 µm) is selected. Helium is used as the carrier gas, and splitless mode is adopted. In order to keep the method compatible with SPME extractions, the inlet temperature is chosen based on the selected fibre (chapter 6). The oven temperature profile and flow rate will be optimised from both method [7] and method [30], as the same column was used in these studies.

3.2   Methodology

The analytical methodology discussed involves the optimisation of the temperature profiles for the separation of OGSR compounds based on standard mixtures. Retention times of known OGSR compounds are provided.

3.2.1   Solvents and standards

Akardite II (AKII), camphor, carbazole, dipenylamine (DPA), 4-nitrodiphenylamine (4-NDPA), 2,4-dinitrodiphenylamine (2,4-DNDPA), ethylphenylamine (EPA), ethyl centralite (EC), dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), diisobutyl phthalate (DIBP), nitroglycerin (NG) in acetonitrile (ACN) (1 mg/mL), 2-nitrotoluene (2-NT), 3-nitrotoluene (3-NT), 4-nitrotoluene (4-NT), 2,3-dinitrotoluene (2,3-DNT), 2,4-dinitrotolune (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT), 3,4-dinitrotoluene (3,4-DNT), and triacetin were purchased from

Sigma Aldrich (Bellefonte, PA, USA). Ethyl centralite (EC) in ACN (500 µg/mL) (only used for OGSR standard 1), 2-nitrodiphenylamine (2-NDPA), and 2,5-dinitrotoluene (2,5-DNT) were obtained from LGC Standards (Middlesex, UK). Methyl centralite (MC) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). A PAH calibration mix including 10 µg/mL of acenapthene, acenaphthylene, antracene, fluorathene, fluorene, naphthalene, and pyrene in ACN was purchased from Sigma Aldrich (Bellefonte, PA, USA). The molecular structures of these compounds can be found in appendix B.

Stock solutions and working standards were prepared in analytical grade methanol purchased from Sigma Aldrich, (St Louis, MO, USA).

OGSR standard 1 contained 20 OGSR compounds in various concentrations in order to achieve similar detector responses. This was necessary to enable the accurate evaluation of the separation of the compounds, and thereby the effective optimisation of the temperature profile. The required concentrations per compound were determined experimentally and were as follows:

- o 25 µg/mL of camphor, 3-NT, EPA, DPA, DMP, DEP, DBP, DIBP, and MC;
- o 37.5 µg/mL of carbazole, 2,4-DNT and 3,4-DNT;
- o 50 µg/mL of 2-NDPA, 4-NDPA, and 2,4-NDPA;
- o 62.5 µg/mL of triacetin, 2,3-DNT, and 2,6-DNT;
- o 100 µg/mL of EC;
- o 250 µg/mL of NG.

OGSR standard 2 included 23 OGSR compounds at a concentration of 25 µg/mL. In addition to the compounds listed for standard 1, it included 2-NT, 4-NT, and 2,5-DNT. Solid EC was used in this standard.

AKII was obtained later and a single standard of 25 µg/mL in methanol was prepared.

3.2.2   GC-MS instrumentation and conditions

Chromatographic analysis was performed with an Agilent Technologies 6890N network GC system, equipped with an Agilent 7683 Series autosampler. An Agilent J&W Scientific HP5-MS UI (30 m x 0.25 mm x 0.25 µm) column was used. Helium was used as the carrier gas, the injection volume was 1 µL, and a solvent delay of 1.80 minutes was used. Injections were performed in splitless mode, using a purge flow 40 mL/min at 0.75 min, unless stated otherwise.

An inlet temperature of 250°C was maintained for all experiments. The optimisation of the oven temperature profile is discussed in section 3.3.1.

The GC was coupled with an Agilent 5975B Inert MSD system using electron impact (EI) ionisation. The transfer line between the column and the MS was kept at 280°C. Masses were scanned from m/z 40 to 500. Mass spectra for recorded peaks were further evaluated using the NIST database (NIST Mass Spectral Search Program Version 2.0).

3.2.3   Optimisation of the temperature profile

Two chromatographic methods reported in the literature for the analysis of OGSR compounds were compared [7, 30]. The details of these methods can be found in table 3.1 ([1] and [2] will be referred to as method A and method B respectively).

An aliquot of standard 1 was first ran using these two methods: both resulted in the complete or partial co-elution of the same compounds: 2,3-DNT and 2,4-DNT; 3,4-DNT and DEP; carbazole and MC; and DBP and 2-NDPA respectively. The optimisation of the temperature profile thus focussed on these compounds.

Improvement of the compound separation was attempted by adjusting hold periods and temperature ramps. Hold periods were extended or introduced, and the temperature at which they started were varied to determine the optimal temperature for the separation of the target compounds. After each modification, the standard mixture was rerun. The results were evaluated primarily on the visual assessment of the chromatograms. If improved separation of the target compounds was observed, the other compounds was assessed to ensure their separation had not been compromised. Finally, attempts were made to reduce the run time, predominately by removing or reducing hold periods. Full details of the temperature profiles assessed can be found in appendix C.1.

3.2.4   Retention time determination

Two chromatographic methods (differing in oven temperature profile) have been used for further work. The retention times of relevant OGSR compounds using these two methods have been determined using standard 2. This standard was run with each method in triplicate to determine the intravariation. The intervariation was determined by running the standard on three different days (day 1, day 2 and day 14). Relative standard deviations (std. dev.) have been calculated for both methods, and were then converted to percentages of the mean values. The mean percentage was also calculated to enable a quick comparison.

A standard mix containing several PAHs at a concentration is 25 µg/mL was also analysed to determine whether their retention times would interfere with the detection of known OGSR compounds.

3.3 Results and discussion

The optimisation of the temperature profile based on two reported chromatographic methods is discussed and the corresponding retention times are provided.

3.3.1 Oven temperature optimisation

Two chromatographic methods reported in the literature were compared. Method A [7] uses a flow rate of 1 mL/min and has a total run time of 32.00 min, method B [30] employs a flow rate of 1.2 mL/min and has a total run time of 54.00 min, despite the consistently reported 46 min [30, 33]. Figure 3.10 shows the chromatograms of standard 1 obtained with these methods. The poorest resolution is observed for 2,3-DNT and 2,4-DNT, 3,4-DNT and DEP, and carbazole and MC for both methods, which both employ a continuous temperature ramp across these compounds (6°C/min for method A and 5°C/min for method B). Figure 3.11 shows the mid-section of these chromatograms, including all the highlighted compounds, in more detail.
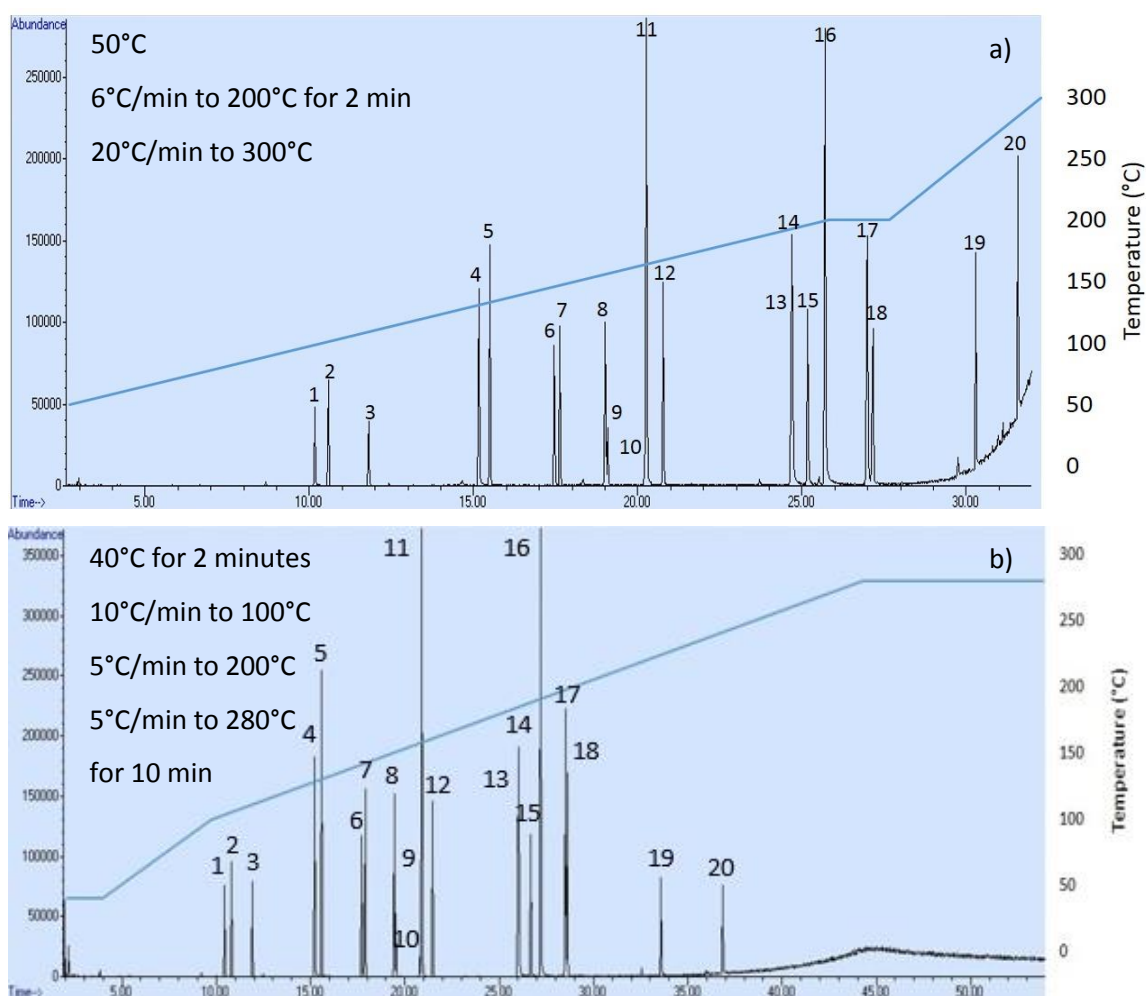
Figure 3.10: Chromatogram of a) method A, and b) method B: 1. EPA, 2. Camphor, 3. 3-NT, 4. Triacetin, 5. NG, 6. DMP, 7. 2,6-DNT, 8. 2,3-DNT, 9. 2,4-DNT, 10. 3,4-DNT, 11. DEP, 12. DPA, 13. Carbazole, 14. MC, 15. DIBP, 16. EC, 17. DBP, 18. 2-NDPA, 19. 4-NDPA, 20. 2,4-DNDPA

Figure 3.10 shows that both methods have a good compatibility with the compounds present in OGSR standard 1. For method A this was expected due to the fact that standard 1 contained 16 of the compounds that this method was original employed to [7]. Method B was reported in combination with a standard that contained over 50 compounds and predominantly PAHs. Only eight of the compounds present in standard 1 were present in the reported standard [30]. In practice, method B has only two ramps due to the fact that the latter two ramps are the equivalent of one ramp of 5°C/min from 100°C to 280°C, as illustrated in figure 3.10. Changing the third ramp to 10°C/min results in a total run time of 46 minutes, which corresponds with the run time reported in the literature [30, 33]. This change takes effect shortly after 2-NDPA has eluted from the column. Consequently, this change has no impact on the separation of the first 18 compounds, including the section that is targeted for optimisation.
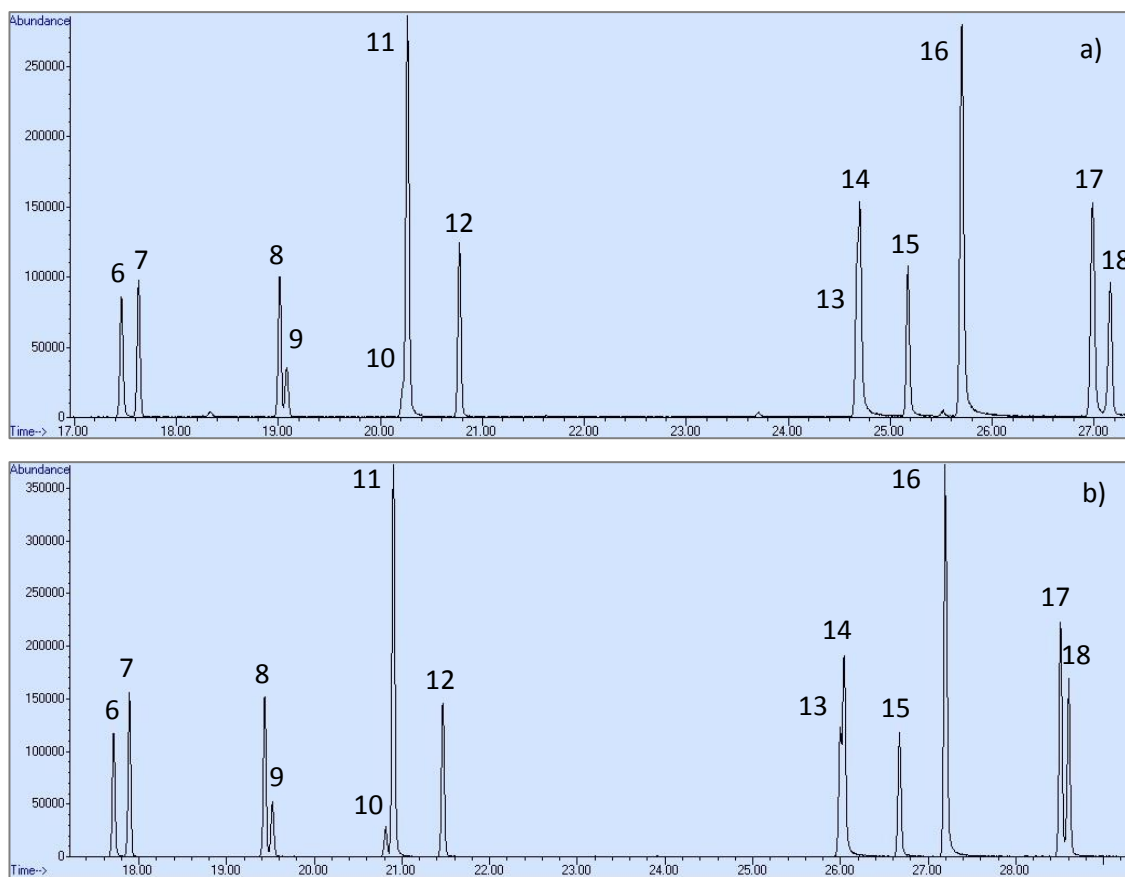
Figure 3.11: Mid-section chromatogram of a) method A and b) method B

Figure 3.11 shows that for method A peaks 10 (3,4-DNT) and 11 (DEP), and peaks 13 (carbazole) and 14 (MC) co-elute. Peaks 8 (2,3-DNT) and 9 (2,4-DNT) partially co-elute. Method B performed slightly better, as some separation of the peak tops is observed for both peaks 10 and 11, and peaks 13 and 14. Peaks 17 (DBP) and 18 (2-NDPA), however, partially co-elute using this method. Optimisation was predominantly aimed at improving the separation between carbazole and MC. For this purpose ramps were modified and holds were added.

The final optimisation of both methods is shown in figure 3.12, method A is shown both with a flow rate of 1 ml/min (a) and an increased flow rate of 1.2 ml/min (b), method B was employed with its original flow rate of 1.2 ml/min (c).

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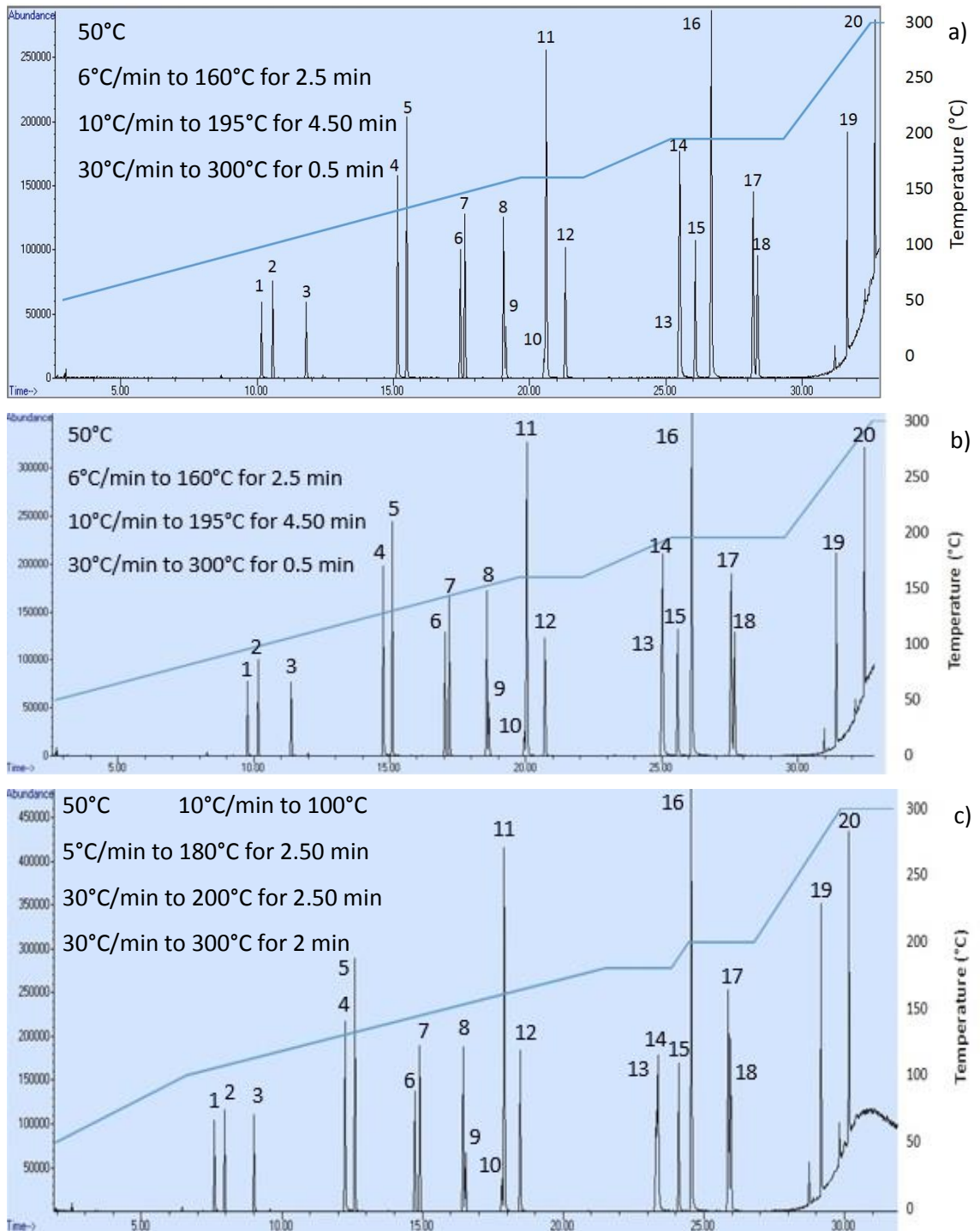
Figure 3.12: Chromatogram of optimisation of a) method A flow rate 1 ml/min; b) method A flow rate 1.2 ml/ min, and c) method B flow rate 1.2 ml/min: 1. EPA, 2. Camphor, 3. 3-NT, 4. Triacetin, 5. NG, 6. DMP, 7. 2,6-DNT, 8. 2,3-DNT, 9. 2,4-DNT, 10. 3,4-DNT, 11. DEP, 12. DPA, 13. Carbazole, 14. MC, 15. DIBP, 16. EC, 17. DBP, 18. 2-NDPA, 19. 4-NDPA, 20. 2,4-DNDPA

The run time of all three methods is similar, however, 2,4-DNDPA eluted relatively close to the end of the run time for method A. To prevent loss of this compounds if a slight shift in retention time would occur, a slightly extended final hold would be recommended.

No real differences in the separation of the DNTs was achieved. A closer comparison of the effect that changing the flow rate of method A had on the separation of 3,4-DNT and DEP, and carbazole and MC is shown in figure 3.13.
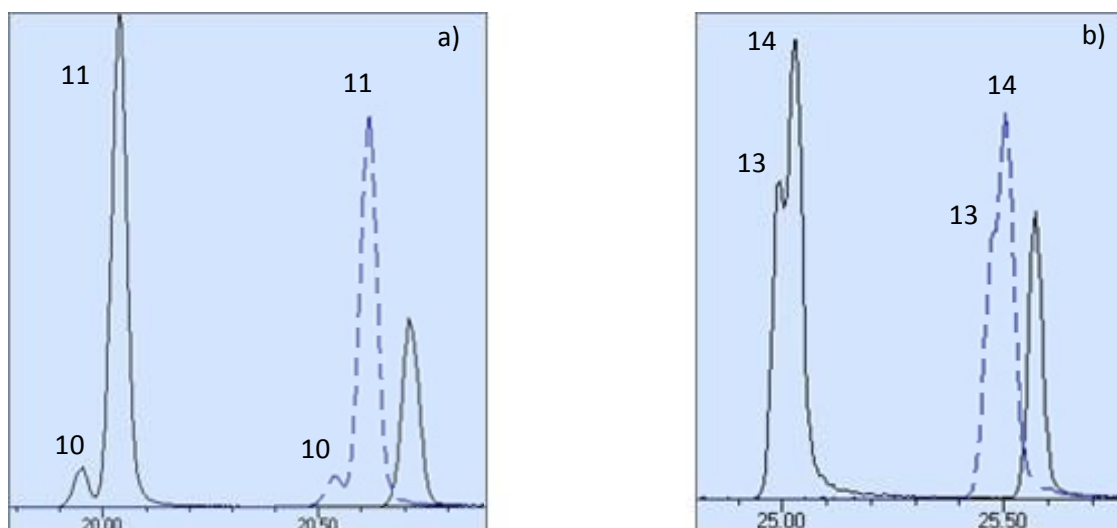
Figure 3.13: Comparison of the separation of a) 3,4-DNT (10) & DEP (11), and b) carbazole (13) & MC (14), Continuous line: flow rate is 1.2 mL/min, dashed line: flow rate is 1 mL/min

A slightly better separation was observed for both pairs of co-eluting compounds using the increased flow rate (continuous line). The first two compounds, 3,4-DNT (10) and DEP (11), resulted in two separate peak shapes. Some separation of the tops of the peaks of carbazole (13) and MC (14) was observed, however, these compounds still co-elute.

A comparison of the separation achieved for these co-eluting compounds with the optimised method A using a flow rate of 1.2 ml/min, original method B, and the optimisation of method B is shown in more detail in figure 3.14 (3,4-DNT and DEP) and figure 3.15 (carbazole and MC). The peaks shown in these figures have been obtained by overlaying the chromatograms of all three methods in a single chromatogram, the full chromatogram can be found in appendix C.2.
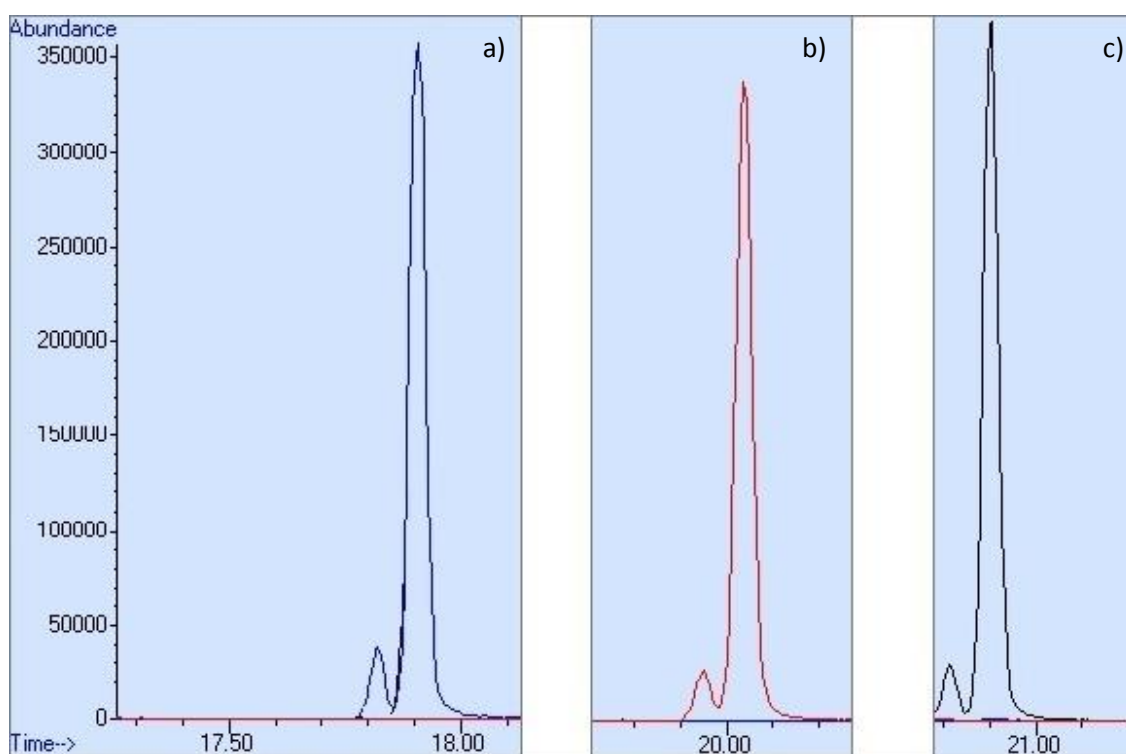
Figure 3.14: Comparison of 3,4-DNT and DEP separation with a) method B optimised, b) method B original, and c) method A optimised (flow rate: 1.2 mL/min)

Figure 3.14 shows that the poorest separation has been achieved with method B (b), and the best separation with the optimisation of this method (a). The differences, however, are relatively small. Consequently, the method choice will be predominantly determined based on the separation achieved between carbazole and MC.
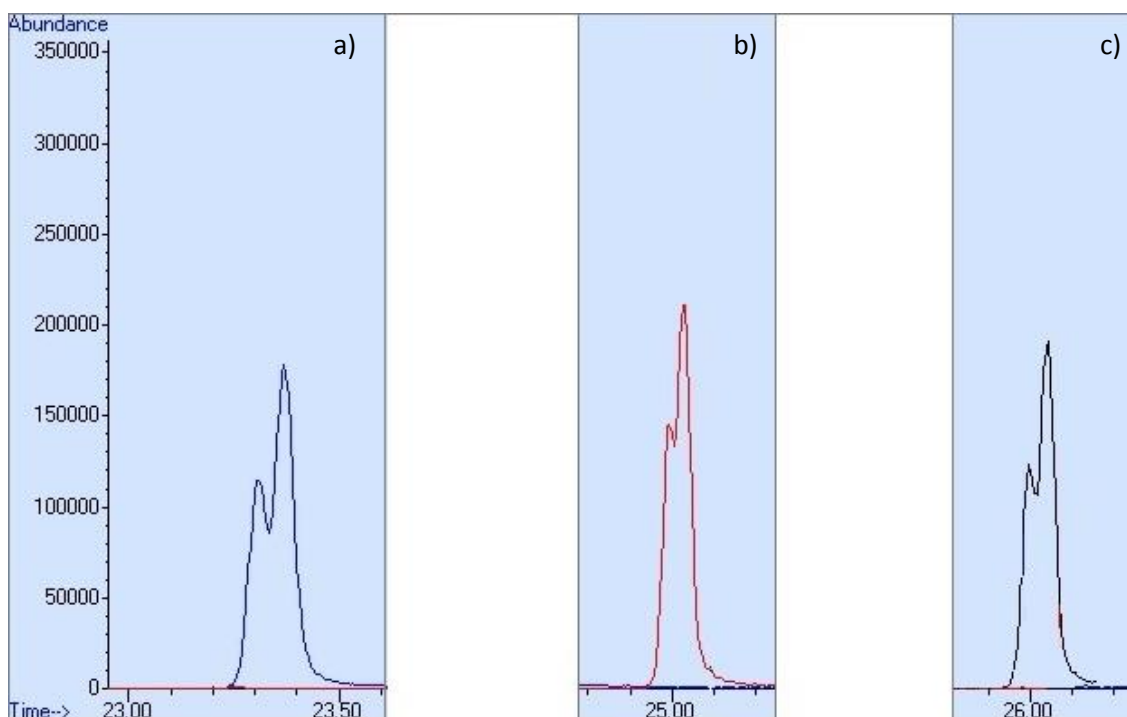
Figure 3.15: Comparison carbazole and MC separation with a) the optimisation of method B, b) the original method B, and c) the optimisation of method A (flow rate: 1.2 mL/min)

In accordance with the above results, method B (b) has resulted in the poorest separation between carbazole and MC. Although these peaks still co-elute, a significantly clearer distinction can be made between the two peaks using the optimisation of method B (a), which enables a clear determination of the retention time of both compounds. Given the fact that quantification was not an objective, the partial co-elution of these compounds was acceptable. Consequently, this optimisation of method B has been selected as the preferred chromatographic method.

3.3.2 Retention time determination

Due to the nature of the work two chromatographic methods were used; *i.e.* method A and the optimisation of method B, which will be referred to as method C henceforth. The retention times of relevant OGSR compounds using these two methods have been determined by running standard 2 in triplicate with each method over a period of 14 days. The obtained retention times and their relative standard deviations (intervariation) are shown in table 3.2.

Table 3.2: Retention times OGSR compounds - Method A & C

	Compound		RT method A (min)	Std dev A (%)	RT method C (min)	Std dev C (%)
1	Ethylphenylamine	EPA	10.158	0.01	7.604	0.01
2	Camphor	-	10.564	0.02	7.962	0.03
3	2-Nitrotoluene	2-NT	10.943	0.01	8.266	0.01
4	3-Nitrotoluene	3-NT	11.790	0.01	9.005	0.02
5	4-Nitrotoluene	4-NT	12.183	0.02	9.360	0.03
6	Triacetin	-	15.144	0.01	12.226	0.03
7	Nitroglycerin	NG	15.480	0.00	12.581	0.03
8	Dimethyl phthalate	DMP	17.422	0.00	14.703	0.01
9	2,6-Dinitrotoluene	2,6-DNT	17.611	0.00	14.886	0.01
10	2,5-Dinitrotoluene	2,5-DNT	18.384	0.02	15.746	0.02
11	2,3-Dinitrotoluene	2,3-DNT	18.993	0.00	16.424	0.01
12	2,4-Dinitrotoluene	2,4-DNT	19.066	0.02	16.513	0.03
13	3,4-Dinitrotoluene	3,4-DNT	20.198	0.01	17.800	0.02
14	Diethyl phthalate	DEP	20.240	0.00	17.880	0.01
15	Diphenylamine	DPA	20.753	0.01	18.444	0.01
16	Carbazole	-	24.651	0.03	23.271	0.08
17	Methylcentralite	MC	24.678	0.04	23.321	0.00
18	Diisobutyl phthalate	DIBP	25.152	0.01	24.076	0.01
19	Ethylcentralite	EC	25.673	0.01	24.526	0.01
20	Dibutyl phthalate	DBP	26.954	0.01	25.843	0.01
21	2-Nitrodiphenylamine	2-NDPA	27.125	0.00	25.929	0.01
22	4-Nitrodiphenylamine	4-NDPA	30.296	0.03	29.175	0.03
23	2,4-Dinitrodiphenylamine	2,4-DNDPA	31.582	0.01	30.178	0.01

For method A the mean standard deviation was 0.01% for both the intra- and the intervariation, for method C this was 0.01% and 0.02% respectively.

AKII and a calibration mix containing several PAHs were obtained later, and thus their retention times have only been determined for method C (table 3.3). The PAHs were selected based on literature highlighting compounds that could potentially be useful for time since discharge analysis [30, 33]. None of these retention times overlapped with the retention times of known OGSR compounds. The standard deviations fell within the same range as the above compounds, with a mean deviation of 0.01%.

Table 3.3: Retention times AKII and PAHs - Method C

	Compound		RT method C (min)	Std dev C (%)
24	Akardite II	AKII	26.978	0.00
25	Naphthalene	-	8.573	0.02
26	Acenaphthylene	-	14.370	0.01
27	Acenapthene	-	15.172	0.01
28	Fluorene	-	17.370	0.03
29	Phenanthrene		21.600	0.01
30	Antracene	-	21.822	0.03
31	Fluorathene	-	27.409	0.01
32	Pyrene	-	28.026	0.00

It should be noted that the retention times are based on the average retention times recorded after the method development. The retention times changed throughout the course of this project due to degradation of the stationary phase, trimming of the column ends during routine maintenance and troubleshooting, and replacement of the column. To account for the resulting shifts, a standard was analysed after any maintenance was carried out on the instrument and the retention times were recorded.

Trimming the column ends is not expected to have a significant effect on the separation, due to the fact that the resolution is a function of the square root of the column length. Therefore, removing 1 m of the column (30 m length) will only reduce the resolution by 1.7%; typically, only 10 cm is removed from the column during routine maintenance. [129, 148]

3.4 Conclusion and further work

The optimised GC-MS method (method C) offered some improvement in compound separation without extending the run time. There was no overlap in retention time with some PAHs reportedly associated with OGSR. The method employed an initial oven temperature of 50°C, which was increased in four ramps: 10°C/min to 100°C, 5°C/min to 180°C held for 2.50 min, 30°C/min to 200°C held for 2.50 min, and 30°C/min to 300°C for 2 min. The flow rate was 1.2mL/min.

Due to the fact that good separation was achieved for a wide range of OGSR compounds, including nitrotoluenes, diphenylamines and centralites, this method is expected to be applicable to a wide range of ammunition types. It may, therefore, also be useful for the analysis of explosive materials, in particular improvised explosive devices (IEDs) [149, 150].

Further work will focus on techniques for extraction of OGSR compounds from standards and unburnt propellant, including propellant characterisation.

4 Characterisation of unburnt propellant powder

The aim of this chapter is to characterise the organic composition of a range of propellant powders from different calibres and manufacturers, and to evaluate these compounds in terms of their importance with respect to aiding GSR confirmation. An overview of the components of a round of handgun ammunition is given, and a list of the propellants obtained during this study is provided.

4.1 Ammunition components

Propellant powder is the main source of OGSR compounds [11], and originates from the firearm ammunition. This work is limited to ammunition for small arms – firearms that can be carried by an individual – and includes both handguns (*e.g.* pistols and revolvers) and shoulder guns (*e.g.* rifles) [151]. The rounds of ammunition used for these types of firearms generally consist of the following components (figure 4.1):

- Primer mix in a primer cup, which acts as the fuse;

- Propellant powder, the fuel;

- Cartridge case, which holds all ammunition components;

- Projectile, also referred to as the bullet.
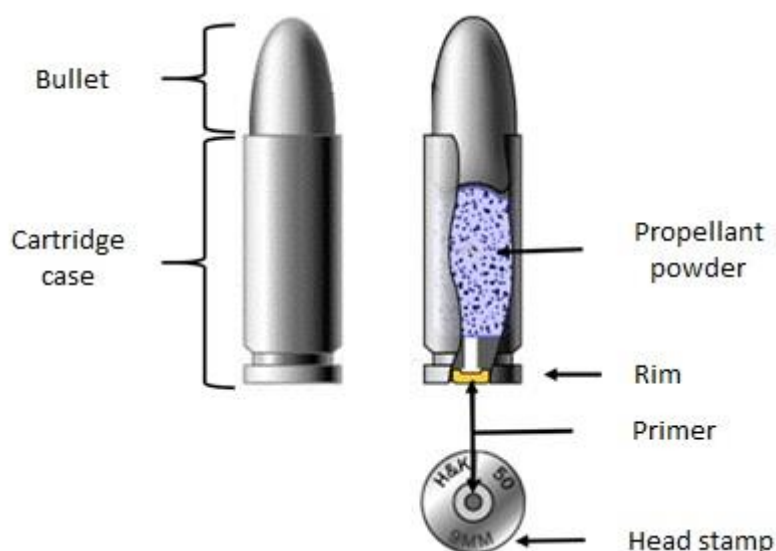
Figure 4.1: Round of handgun ammunition (adapted from [152])

The primer mix is an easily ignitable explosive, which ignites when struck by the firing pin. Centre fire rounds have the primer cup in the middle (figure 4.1), whereas rim fire rounds have the primer in the rim of the case. The primer mix in turn ignites the propellant powder, which is an energetic explosive. [10, 11, 153-155] As it burns, it creates expanding gasses that are contained inside the cartridge case. This enables a pressure build up, and thus provides the kinetic energy necessary to propel the bullet out of the case and out of the barrel of the gun. [10, 11, 153, 155, 156]

The calibre of a firearm is based on the barrel diameter and gives a rough indication of the bullet diameter (the bullet calibre), as the bullet is usually slightly bigger than the barrel diameter. The calibre is generally given in inches or millimetres. Some common calibres of handgun and rifle ammunition are provided in table 4.1. The calibre of a shotgun is generally expressed in bore or gauge (ga). A further explanation of handgun and shotgun calibres is provided in appendix D). More information on different bullets and cartridge features, as well as marks that may be left behind by the firearm can be found in the literature [151, 157, 158].

Table 4.1: Common handgun and rifle calibres

Calibre (mm)	Calibre (inch)	Handguns	Rifles
5.56 mm	.22	.22	5.56 mm or .223
7.62 mm	.308	.308	7.62 mm or .308
7.65 mm	.38	.38	-
9 mm	.38 spl*, .357	9 mm, .357, or .38 spl*	-
10.9 mm	.44	.44	-
11.43 mm	.45	.45	-

* spl stands for special. A .38 special may also be referred to as a .357

The main focus of this research is handgun ammunition, which includes propellant used for self-loading ammunition. Handguns have remained the most commonly used non-air firearms types in England and Wales since 2008/09, accounting for 42% of non-air firearm offences in 2016/17. Although shotguns account for only 10% of the firearm offences, they are fired more frequently during the commission of the offence. [1]. Therefore, some shotgun ammunition has been collected as well. Only a few types of rifle ammunition have been included, given the fact that these account for around 1% of the offence in 2016/17 [1].

4.1.1   Propellant powder

In order to produce a great volume of gas and thus generate the pressure necessary to expel the bullet from the case, the propellant powder needs to burn quickly [10, 11, 156, 158]. The burn rate is influenced by the shape and size of the powder grains, and to a lesser extent by chemical treatment/surface coating [156, 158]. Some examples of unfired propellant particle shapes are shown in figure 4.2 [156]. Figure 4.3 shows macroscopic images of some of the propellant powders used in this study.

Figure 4.2: Examples of unfired propellant particle shapes [156]:

1. Disc flake, 2. Perforated disc flake, 3. Irregular flake, 4. Quadrilateral flake, 5. Grain, 6. Ball, 7. Stick, 8. Single perforated stick, 9. Flattened ball.
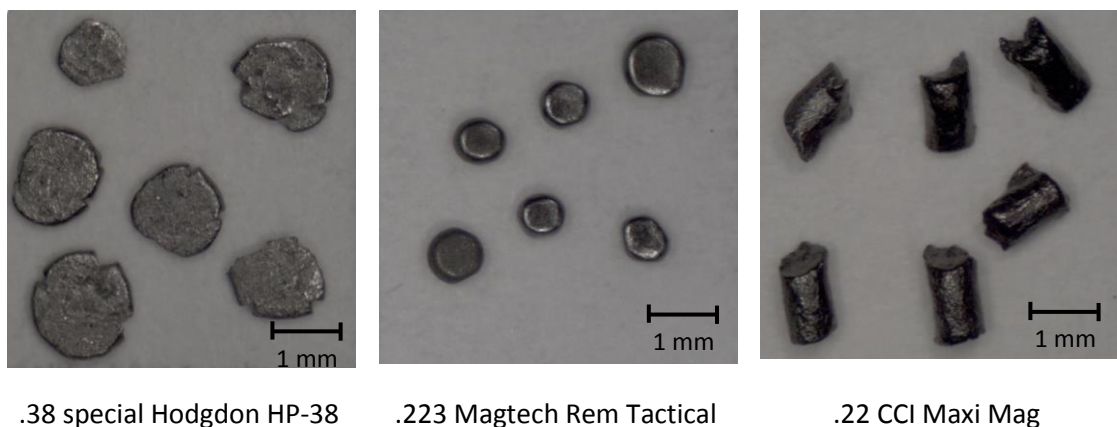
.38 special Hodgdon HP-38   .223 Magtech Rem Tactical   .22 CCI Maxi Mag

Figure 4.3: Macroscopic images of some of the propellant powders used in this study

In general, smaller grains burn faster as more surface area is exposed [156]. Propellant powders for pistol and shotgun ammunition tend to be in the form of thin discs flakes or quadrilateral (square) flakes (figure 4.3, Hodgdon propellant), which offer a large surface area for burning to

take place. They maintain the same exposed surface area until they are completely consumed. This makes for fast burning propellant powder, suitable for firearms with short barrels. Most propellant powders for rifles, which typically have longer barrels, take the form of near-spherical grains (balls) or small solid cylinders (sticks) (figure 4.3, .223 Magtech and .22 CCI propellant respectively). These shapes expose reducing surfaces areas as their diameter decreases during the burning process, resulting in a slower burning powder. [158]

Under ideal circumstances all the propellant powder would be consumed by the burning process and be converted into gasses [11, 156], creating the maximum kinetic energy. In reality, however, GSR also contains unburnt and partially burnt particles [10, 11, 156]. Therefore, the characterisation of unburnt propellant could potentially contribute to the evaluation of OGSR materials.

The accurate analysis of the additive in smokeless powders is often an integral part of the investigations of improvised explosive devices (IEDs) and the evaluation of OGSR [149]. Propellant powders consist of one or more explosive compounds (*e.g.* NC, NG), and a number of additives to improve the properties of the powder. [11, 154, 159] A list of some propellant additives and their function is provided in table 4.2 (molecular structures can be found in appendix B); these are amongst the compounds of interest this research will focus on.

Table 4.2: Propellant powder additives and their function [11]

Type of additive	Function	Compounds used
Stabilisers	Increasing chemical stability by reacting with the explosive's decomposition products	Diphenylamines [150] + its nitro-derivatives Centralites (EC, MC) [150] Akardite (AKII)
Plasticisers	Provide strengthened flexibility to the grains	Phthalates (DBP [150], DEP [154], etc.) Triacetin Nitrotoluenes [159]
Flash suppressors	Produce nitrogen gas to dilute muzzle gasses	Dinitrotoluenes (mainly 2,4-DNT) [150] Nitroguanidine
Burn rate modifiers	Slowing down burn rate of NC	Dinitrotoluenes [154, 160, 161]

4.2 Methodology

The characterisation of unburnt propellant powder was accomplished using methanol extraction

4.2.1 Acquisition of propellant powder

A list of propellant powder obtained during this project is provided in table 4.3 – table 4.6. Most of the propellant was provided in the form of rounds of ammunition. The bullets were pulled using a kinetic hammer and the propellant powder was decanted in plastic seal bags. Propellant from rim fire ammunition was collected by pulling the bullet with a pair of pliers. Shotgun shells were cut open to obtain the propellant. At recreational shooting clubs self-loaded centre fire ammunition is used; such propellant powder was provided in a plastic vial or seal bag.

Table 4.3: Obtained police ammunition

Supplier	No.	Calibre	Ammunition type
Nottinghamshire	1.	9 mm	Magtech, 9 mm Luger, 124 grain (gr) (training)
Police	2.	.308	Winchester, .308 Federal Tactical rifle, 165 gr
	3.	.223	Magtech, .223 Rem Tactical, 63 gr
Merseyside	4.	9 mm	Federal Premium, 9 mm Federal Tactical rifle, centre fire, 124 gr (operational)
Police			
	5.	9 mm	American Eagle, 9 mm, centre fire, 115 gr (training)
	6.	.223	Federal Premium .223 Federal Tactical rifle, centre fire, 65 gr (operational)
	7.	.223	Federal Premium .223 Federal Tactical rifle, centre fire, 55 gr (training)

Table 4.4: Obtained handgun and rifle ammunition

Supplier	No.	Calibre	Ammunition type
Merseyside	8.	.45	PMC, .45 Auto, centre fire, 230 gr
Police	9.	.44	Samsom, .44 REM, centre fire, 180 gr
Seized	10.	.38	CBC Wadcutter, .38 special, centre fire
ammunition	11.	.38	Norma, .38 special, centre fire, 158 gr
	12.	.38	Silvalube, .38 special, centre fire
	13.	.38	Sinoxid Dynamit Nobel Wadcutter, .38 special
	14.	.38	Winchester, .38 special, centre fire
	15.	.38	Winchester, +P .38 special, centre fire, 125 gr
	16.	.38	Winchester, Western .38 special, centre fire, 158 gr
	17.	.357	CBC, .357 Magnum, centre fire, 158 gr
	18.	.357	PMC, .357, centre fire, 158 gr
	19.	9 mm	CBC, 9 mm luger, centre fire
	20.	9 mm	CCI, 9 mm luger, centre fire
	21.	9 mm	Howitzer, 9 mm luger, centre fire
	22.	9 mm	PMP, 9 mm Parabellum, centre fire, 115 gr
	23.	9 mm	Sellier & Bellot, 9 mm luger
	24.	9 mm	Winchester Western, 9 mm luger, centre fire
	25.	.22	Magtech, .22 rimfire, longrifle, 40 gr
	26.	.22	CCI Maxi Mag, .22 Magnum rimfire
Phoenix Shooters	27.	.22	Remmington, .22 rimfire
Associations,	28.	.223	Barnaul, .223, centre fire, 55 gr
Deeside	29.	.303	HXP, .303, centre fire
	30.	.308	Nato Meng 290002 7.62mm, centre fire
	31.	.357	Sellier & Bellot, .357, centre fire
Altcar Rifle club,	32.	.308	Bisley Target L42A3, 7.62mm, centre fire
Merseyside			
Wellington Rifle	33.	.22*	Long rifle Eley, sub sonic, rim fire, 40 gr
and Pistol Club,			
Skipton			
Grange Pistol and	34.	.38	PPU, SWC .38 special, centre fire, 158 gr
Rifle Club, West			
Kirkby			

Table 4.5: Obtained propellant for self-loading ammunition

Supplier	No.	Ammunition type
Grange Pistol and Rifle Club,	35.	Alliant, Unique
West Kirkby	36.	Alliant, Unique (former Hercules)
	37.	Alliant, Bullseye
	38.	Hodgdon, Universal
	39.	Hodgdon, Titewad
	40.	Ramshot, True Blue
	41.	Vihtavuori, N320
Wellington Rifle and Pistol Club, Skipton	42.	Hodgdon, HP-38
Altcar Rifle club, Merseyside	43.	Vihtavuori N140

Table 4.6: Obtained shotgun shells

Supplier	No.	Calibre	Ammunition type
Merseyside Police	44.	12 ga	Baikal 12 ga 70 mm, 6 shot
seized shotgun shells	45.	12 ga	RSW/GECO Rottweil 12 ga, 6.5 shot
	46.	20 ga	Winchester 20 ga 75 mm, 4 shot
Phoenix Shooters Association	47.	12 ga	Eley Alphamax, lead shot
Deeside	48.	12 ga	Eley Grand Prix, lead shot
	49.	12 ga	Eley Grand Prix, Bismuth alloy shot
	50.	12 ga	Express Hevishot, steel shot
	51.	12 ga	GMS Impact, steel shot
	52.	12 ga	Gyttorp HP Steel, steel shot

4.2.2 Solvents and standards

Akardite II (AKII) and N,N-diphenylformamide (N,N-DPF) were purchased from Sigma Aldrich (Bellefonte, PA, USA). Individual standards of 25 µg/mL were prepared in analytical grade methanol and analysed using direct injection to confirm their retention times and mass spectra.

4.2.3 Methanol extractions

Methanol extractions were carried out in 14 mL glass vials (Samco), by adding 2 mL of methanol to 100 mg of propellant powder. Samples were then placed in an ultrasonic bath for one hour. None of the powders dissolved completely, most of them remained on the bottom of the vial as white to colourless pellets. Some propellant, mainly from shotgun ammunition and including all

quadrilateral flakes, formed a thick gelatinous layer on the bottom of the vial with the methanol layer on top. The supernatant was transferred to Eppendorf cups and centrifuged for 15 minutes using a bench-top mini centrifuge. The supernatant was then filtered using 0.45 µm PTFE filters and transferred to GC vials for analysis [7]. Samples were analysed in triplicate and blank runs were performed in between all samples to ensure no carry-over occurred.

4.2.4   GC-MS method

The details of the used GC-MS instrumentation and conditions are provided in section 3.2.2, a summary of the method is provided in table 4.7.

Table 4.7: Summary of GC-MS method

Oven profile (C)	Ramp (°c/min)	Hold Temperature (°C)	Hold time (min)
50°C	10	100	0
	5	180	2.50
	30	200	2.50
	30	300	2.00
Injection mode	Splitless		
Solvent delay	1.80 min	**Scan mode**	Full scan
Flow rate	1.2 mL/min	**Scan range**	m/z 40 - 500

4.3   Results and discussion

Characterisation of all propellant powders is discussed, in particular the two propellants selected for further work: Hodgdon HP-38 and Magtech .223 Rem Tactical.

4.3.1   Characterisation

A total of 52 propellant powders, from 31 different brands (*e.g.* Magtech, Hodgdon etc.), were characterised. Self-loading propellant, various calibres of handgun and rifle ammunition, including police ammunition and target practice ammunition (wadcutter), as well as shotgun ammunition were included. Most of the ammunition was American, but some propellant from different countries was analysed too, including UK, Scandinavian, Serbian, Russian, Korean, Israeli, Brazilian and Belgium ammunition.

In 51 out of the 52 propellant powders known OGSR compounds were detected. Only hydrocarbons could be detected in Eley Grand Prix shotgun ammunition with lead shot. A possible explanation could be that this ammunition was very old. The owner indicated that the manufacturing of this ammunition had been discontinued as it had been replaced by Eley Alphamax, and it was suspected that this round might be too old to be discharged. Given the fact that it only concerned one propellant powder of which the manufacturing has been discontinued, further investigation of its composition was not undertaken.

Seven further OGSR compounds were detected in the propellants. Akardite II (AKII) and N,N-diphenylformamide (N,N-DPF) were detected in respectively nine and 22 propellants across all ammunition types. Therefore, standards of both compounds were obtained to confirm the identification of these compounds. Vihtavuori N320 contained 2,2'-DNDPA and 2,4'-DNDPA. Oxamide and 2,4,6-trinitrotoluene (TNT) were both present in .223 Barnaul propellant. An additional phthalate, diphenyl phthalate (DPP), was detected in .38 Norma, .38 Winchester and 9 mm CBC propellant. Gyttorp HP Steel contained two further phthalates as major compounds, but these could not be identified without reference standards. Mass spectrometry data suggested these compounds were diisopentyl phthalate and dipentyl phthalate.

AKII is a stabiliser that may be used as an additive in propellant powder and is thus also associated with OGSR materials [3, 4, 9, 11, 29]. N,N-DPF has only been mentioned in conjunction with OGSR in the literature on a few occasions [36, 113], but its function in propellant powder was not reported. Consequently, this compound cannot be considered to be an important OGSR compound at this time. The DNDPAs are both derivatives of DPA, which are likely formed during NC decomposition. TNT is a known explosive compound used in propellant powder as a sensitiser [3, 29]. Oxamide is reportedly a stabiliser for nitrocellulose preparations [162, 163] and a burn rate modifier used in nitrocellulose-based propellant, such as solid rocket propellants [162, 164, 165]. It acts as a coolant or burn rate suppressant due to the fact that its thermal decomposition is an endothermic process [165]. The molecular structures of the additional compounds can be found in appendix B.

The characterisation of the 51 propellant powders is shown in figure 4.4. Propellants from the same brand (*e.g.* Magtech) are indicated with the same letter, and where relevant, the calibre of the ammunition is included. Given the fact that the two unidentified phthalates were major compounds, they are included as "phthalates" along with minor compound DEP, which was not present in the Gyttorp HP Steel propellant. DNTs contain 2,3-DNT, 2,5-DNT and 3,4-DNT, which were all minor compounds. The DNDPAs are not included as their peak areas are too small to be represented in the figure.
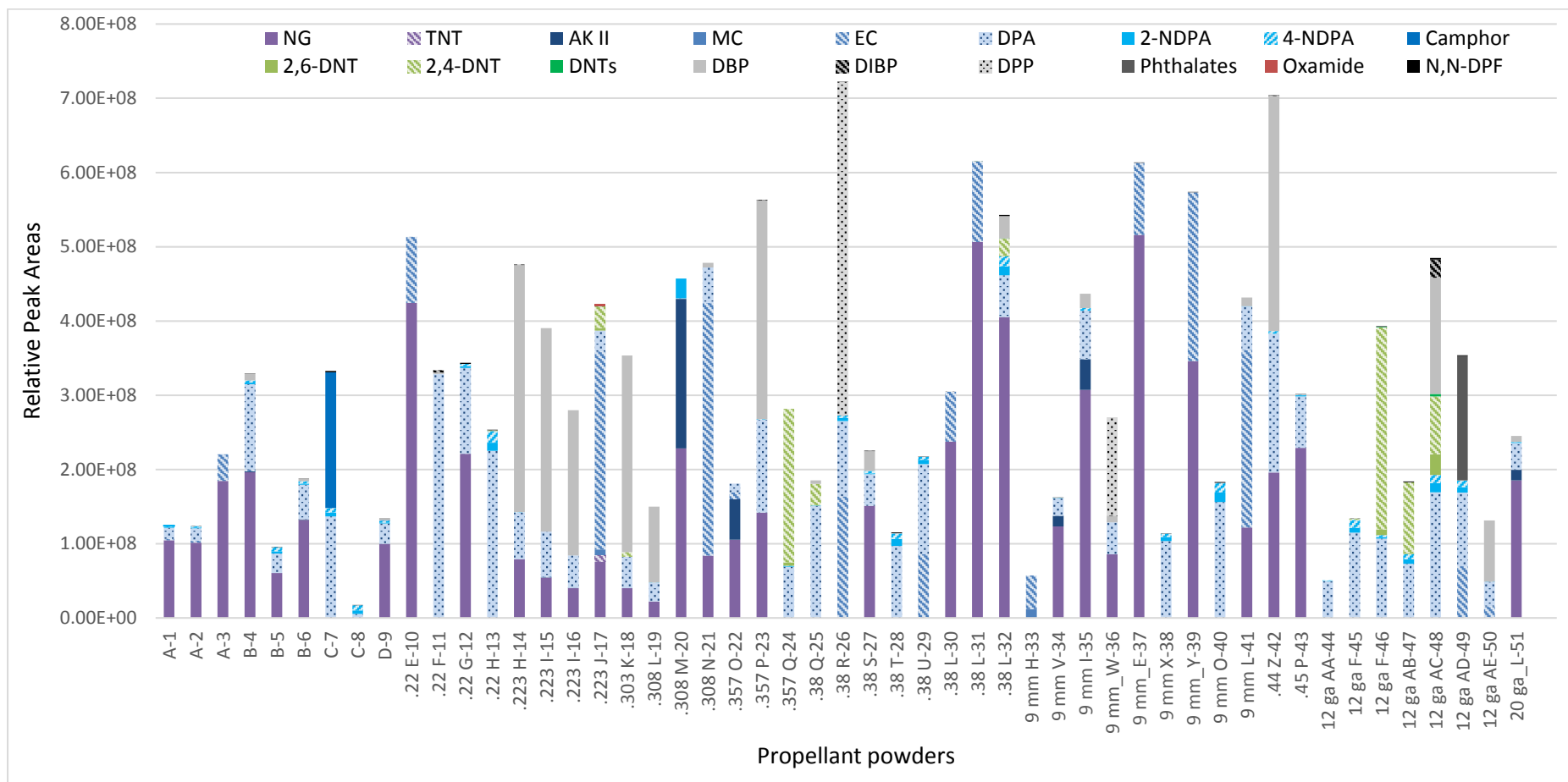
Figure 4.4: Characterisation of propellant powders using methanol extraction

The characterisation of the various propellants was analysed in order to evaluate potential patterns in ammunition/propellant brands or within calibres. This focussed on three key groups of OGSR compounds: presence of NG, the combination of stabilisers present, and the presence of DNTs.

NG was not detected in 18 out of the 51 propellants, suggesting these are single base powders (only containing NC). This included seven of the eight shotgun propellants. TNT was only detected in one of the propellants, in combination with NG.

A breakdown of the various combinations of stabilisers is shown in figure 4.5. It clearly shows that the most frequently detected combination of stabilisers is that of DPA, 2-NDPA and 4-NDPA. This combination was predominantly detected in single base propellants (13 of the 18), and in only three propellants that contained NG. The most common combination of stabilisers in the analysed handgun ammunition also included EC. In both of these stabiliser combinations, one propellant powder was found that also included camphor. Figure 4.5 further shows a variety of other combinations; only some smaller trends were observed. AKII was detected in 9 propellants, but only once without the presence of EC. MC was only detected in combination with EC, and NDPAs were only present in combination with DPA. None of these trends was limited to a certain brand or calibre.

■	EC
▨	DPA
▨	DPA & EC
▨	DPA, EC & AKII
■	DPA, 2-NDPA, 4-NDPA
▨	EC, DPA, 2-NDPA, 4-NDPA
■	AKII, EC, DPA, 2-NDPA, 4-NDPA
▨	DPA, NDPA
■	AKII/EC, DPA, NDPA
◕	MC, EC, (DPA)
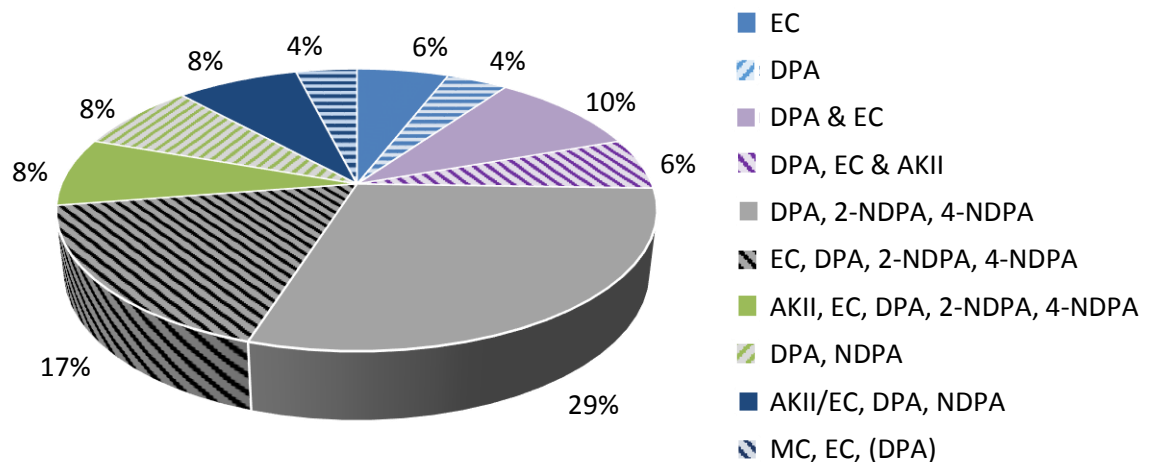
Figure 4.5: Breakdown of the combination of stabilisers present across of propellants

DNTs were found in various propellants, but not in any of the 9 mm calibres or in self-loading propellants. Approximately half of the shotgun ammunition contained DNTs and each resulted in the greatest peak area for 2,4-DNT, which was the only DNT to be detected by itself. In three of these, 2,6-DNT was also present. In seven of the handgun ammunitions DNTs were also found, across several calibres, both with and without NG present, and with different combinations of

stabilisers. The minor DNTs (2,3-DNT, 2,5-DNT and 3,4-DNT) were only present in combination with 2,4-DNT and 2,6-DNT.

A summary of the general patterns observed per propellant type is given in table 4.8. These trends are limited to the presence of NG and the stabilisers, of which MC and AKII are relatively rare. Some similar compositions have been identified both within calibre/brand types as across brands.

Table 4.8: Summary of general trends of OGSR compounds across different propellant types

Propellant type	Frequently present	Rarely present	Similar compositions
Self-loading	DPA (9/9)	AKII (2/9)	2/3 Alliant & Ramshot
	EC (6/9)	MC (0/9)	2/3 Hodgdon
Shotgun	DPA & NDPAs (8/8)	AKII (1/8)	2/2 Eley, Express Hevishot
		MC (0/8)	& GMS Impact
		EC (3/8)	
9 mm	NG (6/9)	AKII (2/9)	Howitzer and Sellier &
	EC (6/9)	MC (1/9)	Bellot
	DPA (8/9)		
.38	NG (6/8)	AKII (0/9)	
	EC (5/8)	MC (0/9)	
	DPA (7/8)		

Within the self-loading propellant powder, only Vihtavuori could be distinguished from the others based on the absence of NG (figure 4.6). The Alliant propellants were relatively similar to one another and to Ramshot propellant. Apart from the absence of NDPAs in one of the Alliant powders, it also had different ratios of EC and DPA. A greater difference was observed within the Hodgdon propellants, with only two of them containing AKII and EC.
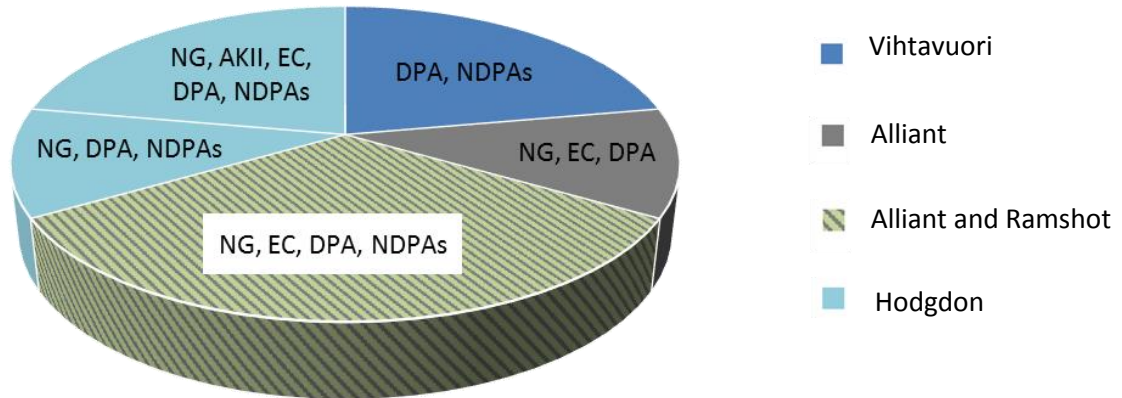
Figure 4.6: Evaluation of composition of self-loading propellant

Four of the shotgun propellants were relatively similar in terms of the OGSR compounds present. Two of these were Eley, the other two were different brands. All shotgun propellants contained DPA and NDPAs.

The majority of the 9 mm propellants contained NG, and all stabilisers except for camphor and oxamide were detected in at least one sample. Four propellants contained phthalates. Howitzer and Sellier & Bellot, both single base propellants, contained the same combination of OGSR compounds.

Similar to the 9 mm, the majority of .38 propellants were single base. Five propellants contained EC and seven contained DPA, five of which were a combination of DPA, 2-NDPA and 4-NDPA. Three of the propellants contained DNTs: CBC, Winchester +P and Winchester Western. The third Winchester propellant did not contained DNTs.

Comparison within brand types showed that the three .38 Winchesters all contained different ratios of DNTs, different combinations of stabilisers and only one contained a phthalate.

Of the three Magtech propellants of different calibres (.22, .223 and 9 mm) only one contained NG and phthalates, two contained EC, two contained DPA, and one contained DNTs.

In summary, some trends and patterns were recognised across the different calibres, such as the absence of DNTs in 9 mm ammunition and the absence of NG in the majority of the shotgun ammunition. In general, it appears to be more likely that single base propellants have DPA, 2-NDPA and 4-NDPA as stabilisers, whilst NG containing propellants tend to also contain EC. Despite this fact, these results clearly demonstrate that it is difficult to determine the calibre or brand of the propellant solely by the combination of OGSR compounds that are present. A database of characterised propellants could potential be useful to create indicative shortlists of similar propellants. Although some propellants cannot be distinguished from one another due

to lack of specificity in the OGSR composition, the absence of major trends across the propellants suggests that linking of samples may be possible for some propellants.

4.3.2   Hodgdon HP-38 and Magtech .223

Two propellants were selected for further work: Hodgdon HP-38 propellant and Magtech .223 Rem Tactical. This choice was made based on the availability (of sufficient quantities) of the propellant powder and the presence of at least three different OGSR compounds per powder.

Figure 4.7 and figure 4.8 show the chromatogram of the methanol extractions of the Hodgdon HP-38 and Magtech .223 propellant respectively. The chromatogram of Hodgdon HP-38 shows all known OGSR compounds (not including DPF), whilst only the three major compounds are visible in the chromatogram of the Magtech propellant; the abundance of the minor compounds was significantly lower. The complete list of compounds detected in both propellants and their average peak areas (displayed in standard form for ease of comparison) and standard deviations are shown in table 4.9.
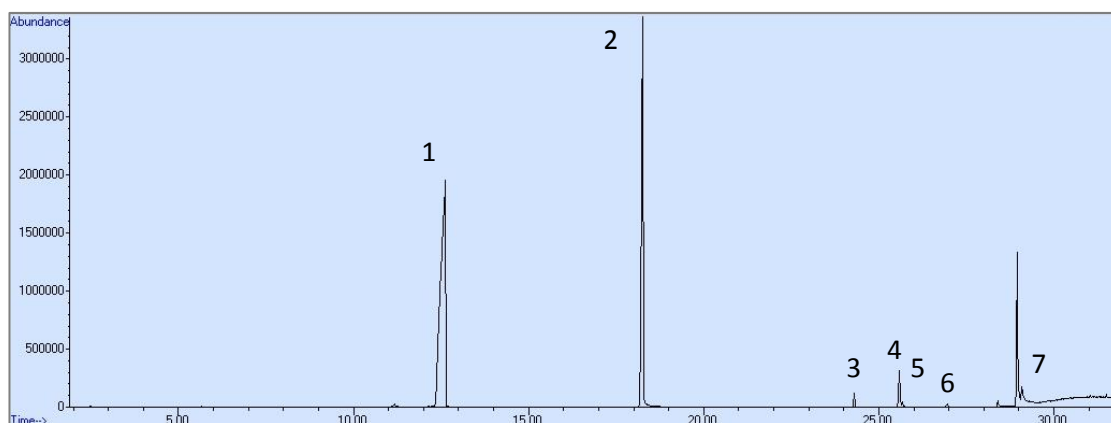
Figure 4.7: Hodgdon HP-38 propellant: 1. NG, 2. DPA, 3. EC, 4. DBP, 5. 2-NDPA, 6. AKII, 7. 4-NDPA
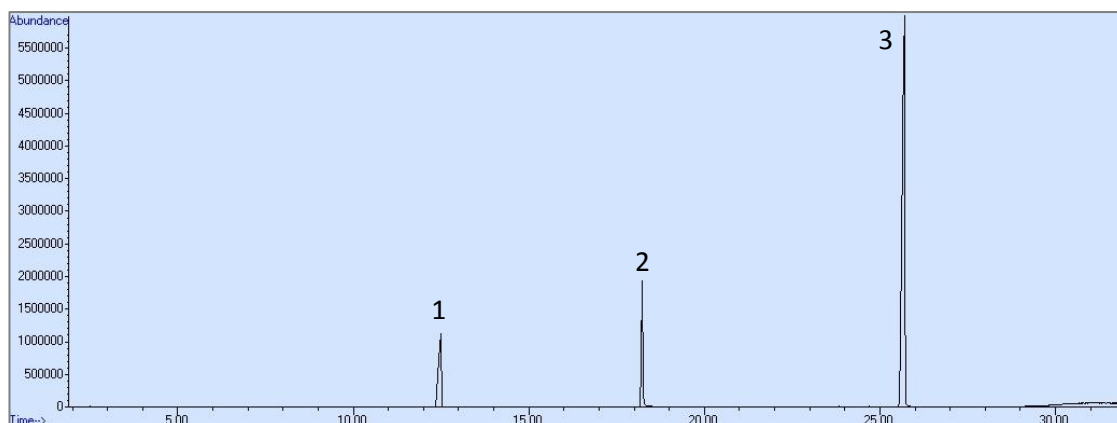
Figure 4.8: .223 Magtech propellant: 1. NG, 2. DPA, 3. DBP

Table 4.9: Mean peak areas (PAs) and standard deviations of Hodgdon HP-38 and .223 Magtech

Compounds	Hodgdon HP-38		Magtech .223	
	Mean PA (n =3)	Std. dev (%)	Mean PA (n =3)	Std. dev (%)
NG	1.96E+08	4.46	5.99E+07	34.61
DPA	1.15E+08	3.27	5.40E+07	26.49
DIBP	-	-	3.02E+05	35.99
EC	3.01E+06	5.63	1.53E+05	34.73
DBP	9.06E+06	4.11	2.83E+08	24.34
2-NDPA	1.95E+06	26.56	-	-
AKII	8.52E+05	8.46	-	-
4-NDPA	3.16E+06	6.35	4.74E+05	13.14
N,N-DPF	1.14E+05	31.58	4.03E+04	42.00

Both propellant powders have a different composition; a different combination of OGSR compounds has been detected, with varying relative abundances between the compounds. Good qualitative repeatability was obtained for each propellant, and a good chromatographic separation was observed for all detected compounds. Furthermore, low standard deviations were obtained for most compounds in Hodgdon HP-38 propellant samples. The yields of the .223 Magtech propellant were lower, and resulted in relatively high standard deviations. This is possibly due to the fact that the .223 Magtech propellant appears to have a coating, which might hamper the release of OGSR compounds [7].

The identification of all compounds was confirmed using standard solutions. These two propellants will be used for the evaluation of further extraction techniques, in which the discussion of N,N-DPF will be limited given the fact that it is not a known OGSR compound.

4.4 GSR as forensic evidence

There appears to have been a resurgence of interest in the analysis and detection of OGSR materials in recent years. Aspects focussed on include characterisation of organic compounds in (smokeless) propellant powders [6, 32, 37, 41], time since discharge estimation [30], persistence of OGSR on skin [125, 166], shooter/non-shooter differentiation [116], and potential contribution to the confirmation of GSR materials [4, 127]. Research focussed on different compounds depending on the research aim; most of the characterisation work looked at additives in unburnt propellant powder [6, 37], whilst time since discharge estimations tend to focus on combustion products [30, 33]. There are currently more than a 100 organic compounds with a possible association with OGSR [9, 37] (table 2.1). Many of these compounds, however, can be found in environmental and occupational materials [3, 9], thus raising the question of their detection as being useful and relevant in regards to the interpretation of forensic evidence. This is particularly true with respect to the confirmation of GSR materials.

Currently, for the detection and confirmation of GSR materials in forensic casework scanning electron microscopy (SEM) with energy dispersive X-ray spectrometry (EDX, also referred to as EDS) detection is employed [3, 12-14]. This is a non-destructive technique that provides both morphological information and the metallic composition of individual particles [167, 168]. The method is well established, and guidelines by the ASTM [167] and forensic science working groups (*e.g.* SWGGSR [168]) provide protocols for sample treatment, analysis, and the interpretation of the results.

The confirmation of GSR materials via its inorganic constituents, however, suffers from the introduction of 'lead-free' or 'non-toxic' ammunition developed for health and environmental reasons [5, 6]. These types of primers complicate the unambiguous confirmation of GSR materials [5-8], which is currently based on these metallic compounds [167, 168]. This calls for an approach based on other compounds than the traditional inorganic residues, to further strengthen the evidential value of GSR evidence. A potential alternative is the determination of OGSR compounds [5-8], which could provide valuable, complementary information and potentially provide additional means to discriminate between GSR materials

and environmental residues [6, 7]. Information equivalent to the classification of IGSR compounds, however, is currently more ambiguous for OGSR.

In this work, the compounds included in the standards have been carefully selected to cover a wide range of compounds (centralites, diphenylamines, nitrotoluenes, phthalates, etc.) and inclusion of relevant compounds is ensured by the analysis of propellant powders. But there appears to be no general consensus on which organic compounds have a forensic relevance to the confirmation of GSR materials.

4.4.1 'Characteristic OGSR'

The confirmation of GSR materials based on inorganic compounds currently relies on particle analysis, *i.e.* the evaluation of constituent elements within a particle [167, 168]. OGSR analysis is generally performed using so called bulk sample methods [167] (*e.g.* chromatography [6, 7, 32, 37]). Consequently, detected compounds could potentially originate from different, unrelated sources, which stresses the importance of a careful selection and evaluation of characteristic compounds.

Due to this generic use of bulk sample methods, and to a lesser extend the variability in GSR compositions, it becomes evident that for the confirmation of GSR materials via its organic constituents a combination of compounds should be detected. Consequently, 'characteristic OGSR' should be defined as:

a combination of organic compounds associated with gunshot residue, which are not generally found in the (occupational) environment.

Similar to the current ASTM guidelines [167], the detection of what is defined as characteristic OGSR would not imply the guilt of a suspect by default, but merely the presence of OGSR materials. The (weight of the) evidence always needs to be evaluated in the context of the case.

In order to determine which OGSR compounds have the potential to contribute to the confirmation of GSR materials it is imperative to define transparent selection criteria.

Selection criteria OGSR compounds

The majority of OGSR compounds originate from the ammunition components (*e.g.* propellant powder and primer mix), or are (combustion) products created during the discharge of a firearm

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[9]. Due to the complexity of the firing process the composition of GSR may vary [13], and as a result the compounds created during the discharge of a firearm are not necessarily reproducible. Furthermore, many of these compounds, such as naphthalene and other polycyclic aromatic hydrocarbons (PAHs), although present in OGSR materials, are also universal combustion products [38-40]. These compounds thus have a limited forensic relevance with respect to the confirmation of GSR materials.

The forensic relevance of individual compounds, however, is imperative due to the loss of correlation between compounds, resulting from the bulk sample analysis rather than particle analysis. In order to re-establish a correlation between the detected compounds and GSR materials, it is of primary importance that the compounds considered have a known origin related to ammunition or GSR, and that only identified compounds will be considered. The forensic relevance of compounds further depends on the strength of the association with GSR materials (*e.g.* are the compounds frequently detected in ammunition components, or only sporadically?), and the significance of the detection of the compounds (*e.g.* do the compounds have a limited or widespread occupational and/or environmental prevalence?).

In summary, the criteria that need to be considered in the selection of suitable compounds that could potentially provide complementary evidence with respect to the confirmation of OGSR materials are:

1. Compounds should have a known origin (*e.g.* ammunition components);
2. Compounds should have a strong association with the ammunition components;
3. Compounds should have a limited occupational and environmental prevalence.

4.4.2 Criterion 1: known OGSR origin

Many of the compounds that are created during the discharge of a firearm are universal combustion products [38-40], and as such not unambiguously linked to GSR. Consequently, these compounds do not satisfy the criteria of a known OGSR origin and will not be considered with respect to the confirmation of GSR materials in this study.

The detection of OGSR compounds originating from ammunition components, such as propellant powder, however, can provide consistent and repeatable results. Furthermore, characterisation of a large number of propellant powders has been reported in the literature [169], providing a compound selection that could be representative for a wide range of ammunition. Such data for organic compounds in primers is currently more ambiguous. Due to firearms legislation, collecting unburnt primer alongside unburnt propellant powder could not

be achieved. Therefore, the selection of compounds will predominantly focus on compounds originating from the propellant powder in order to meet the criteria of a known origin.

4.4.3   Criterion 2: Strong association

A 136 organic compounds are currently associated with OGSR [9], many of which are linked to ammunition components [3]. In order to investigate which OGSR compounds could potentially provide complementary evidence with respect to the confirmation of GSR materials the organic compositions of over 200 propellant powders reported in the literature have been evaluated. Table 4.10 gives a summary of OGSR compounds detected and the frequency with which they were detected in the various studies. This has resulted in a short list of 20 compounds that abide by the first two selection criteria, and therefore may be promising compounds for OGSR classification.

Table 4.10: Characterisation of propellant powders and spent cases (adapted from [4])

Reference	[33]	Unpublished data[1]	[7]	[32]		[41]	[37]	[5]	[21]	[93]	[169]	[57]
Type of Sample	Spent cases		Propellant powder									
Number of Samples	n=2	n=2	n=6	n=4	n=13	n=65	n=9	n=5	n=33	n=2	n=106	n=38
2,4-Nitrodiphenylamine								2				
2,4-Dinitrotoluene				3		~ 28		1	13	2	22	15**
2,6-Dinitrotolulene										1	6	
2-Nitrodiphenylamine	2		5		10	~ 33	2	1		2	38	
4-Nitrodiphenylamine	2		5	2	9	~ 24		2		2	38	
Dibutylphthalate	2		2	2	4		5		12		35	10
Diethylphthalate												
Diphenylamine	2	1	5	3	12	62	8	5	27	1	71	32
Ethyl centralite	2	2	5	2	10	~ 31	5	4*	11	1	54	8
Ethylphenylamine		1				1						
Methyl cellulose								1				
Methyl centralite		1	1			~ 5		4*			2	5
Nitroglycerin	2		4	3	10		8		27	1	89	22
N-Nitrosodiphenylamine					9			2		1	75	
Akardite II			2					2				
Triacetin		1										
Carbazole	1		1									
3-Nitrotoluene						2						
4,4-Dinitrodiphenylamine	2											
2,4,6-Trinitrotoluene								1		1		

~ Approximate numbers were interpolated from a diagram, exact numbers were not included in the paper.

* Method used could not distinguish between EC and MC, hence it is unknown which centralite is present.

**Dinitrotoluene isomers (2,3-DNT, 2,4-DNT, 2,6-DNT, 3,4-DNT) were grouped together.

[1] Goudsmits, E.; Sharples, G.P.; Birkett, J.W. (2015), unpublished experimental data.

4.4.4 Criterion 3: Limited prevalence

Evaluating the potential of compounds to provide complementary evidence with respect to the confirmation of the presence of OGSR materials, requires accurate information on their occurrence in the daily and occupational environment [99].

Centralites rarely exist in the normal environment [55] and their use is reportedly restricted to ammunition [6]. Ethyl centralite (1,3-diethyl-1,3-diphenylurea), as well as akardite II (1-methyl-3,3-diphenylurea), is an additive in (double-base) propellant powders for rockets [11, 99], but no other data on the occurrence in the daily environment was found [99].

Nitroglycerin and nitrocellulose are both used in pharmaceutical preparations [55, 99]. Nitrocellulose also occurs in lacquers, varnishes and celluloid films [55, 99] and in printing [99].

The only other application of 2,4-dinitrotoluene found is the presence in several azo dyes [170].

Diphenylamine is predominantly used as a stabiliser in NC containing explosives and propellants [170]. It is the most commonly present organic compound in GSR samples [171] and in propellant powders as shown in table 4.10, often as one of the most abundant peaks [125]. It must be noted, however, that DPA is a compound from the third European Union list of priority pollutants [170] and has wide applications. It is used in rubber products, the food industry, dyes, explosives, plastics, pharmaceuticals, the agricultural sector, perfumery, elastomer industry and in photography chemicals [55, 99, 170]. DPA is found in soil and groundwater, and it occurs naturally in onions, leaves of black and green tea, further plants and the peel of citrus fruits [170]. Despite this, reports on non-GSR-related contamination are inconsistent; contamination has been observed [11], but no mention of false positives due to DPA contamination has been made in several studies [99, 125, 172]. It is known that DPA reacts with nitric and nitrous acids that result from the degradation of NG and NC, transforming DPA into its mono-, di-, and tri-nitrated-derivatives [6, 150] (figure 5.19). These derivatives have been reported to be characteristic to smokeless powders [6]. Consequently, despite the presence of DPA on its own not being significant due to its wide applications, relevance may be attributed to its presence in conjunction with its nitrated-derivatives [11, 99].

2-Nitrodiphenylamine and 4-nitrodiphenylamine are added to smokeless powders as stabilisers as well [41]. Other applications of 2-NDPA include its use in several azo dyes and in US Navy fuel for torpedoes and other weapon systems [170]. 4-NDPA may also be a compound in azo dyes, and it is an intermediate for the production of antioxidant additives for rubber products [170].

Of the phthalates particularly dibutyl phthalate is frequently associated with OGSR materials. Phthalates are, however, ubiquitous to indoor air, settled dust and food. This is due to their wide application as plasticisers in a broad array of polymeric materials and the fact that phthalates are not chemically bonded to the materials. Consequently, they are susceptible to leaching and are, therefore, readily released into the environment [58].

Apart from being universal combustion products, PAHs are in general persistent and ubiquitous environmental pollutants [38-40].

Due to the wide prevalence of phthalates and PAHs, these compounds are unsuitable for use in the confirmation of GSR materials [173]. They may be used, however, to differentiate between different propellant powders or GSR samples, or for time since discharge studies [30, 33].

Population studies

Data on the occurrence of OGSR compounds in the environment is incomplete without a thorough population study, in which data is obtained on the actual prevalence of these compounds in the environment. Some population studies with respect to the prevalence of organic explosive compounds have been performed [174-176]. A few of these compounds are also relevant to GSR materials, namely NG, trinitrotoluene (TNT) and (di)nitrotoluenes including 2,4-DNT.

Samples in these population studies were taken from locations such as airports, vehicles, and government and public buildings. None of these compounds were found in 333 samples collected throughout the United States [174], or in 255 samples taken in and around London [175]. Of the 493 samples taken from Manchester, Birmingham, Glasgow and Cardiff, only 2 were positive for nanogram levels of NG (Glasgow taxi floor and a wardrobe in a hotel in Cardiff), and only 1 sample was positive for nanogram levels of 2,4-DNT (the back of an X-ray machine in the search area at Glasgow Airport) [176]. From 255 samples collected from police vehicles and police custody suits in and around London only 15 samples were positive for nanogram levels of NG [175].

With respect to OGSR compounds, a study has been performed sampling the hands of 100 individuals from the general population. OGSR compounds studied included NG, DNT's including 2,4-DNT, DPA and some of its nitrated derivatives, centralites and phthalates. Despite detection limits in the picogram range no OGSR compounds were detected [177]. Population studies focusing on OGSR compounds from public areas, such as reported above, are currently not evident in the literature.

4.4.5 Compound selection

The analysis of the data has highlighted that many organic compounds currently associated with GSR have limited forensic relevance with respect to the confirmation of GSR materials. This hampers the effective use of OGSR as complementary evidence to IGSR information.

A first step towards the effective inclusion of OGSR compounds to the confirmation of GSR materials is made in the form of a proposed categorisation system (table 4.11), which organises the compounds with the most forensic relevance into three different categories based on the discussed criteria.

Category 1 contains the compounds with highest forensic relevance, *i.e.* these compounds have a very strong association with OGSR and their detection is significant due to the very restricted applications that are unrelated to OGSR.

Category 2 contains compounds that are strongly associated with OGSR, based on analysis of over 200 propellant powders (table 4.10). The usage of these compounds, however, is less restricted and thus more applications unrelated to OGSR may exist. This reduces the significance of their detection due to their (potential) occupational and environmental prevalence.

Category 3 contains compounds to which the lesser restriction of usages, and thus a reduced significance of detection may also apply. In addition, although these compounds are associated with ammunition components (see table 4.10), they are detected less often and thus have a reduced association. Further OGSR compounds may be added to the third category if deemed necessary and if they meet the set criteria.

Table 4.11: Categorisation system for OGSR compounds [4]

Category	Description	Compounds	Function
1	Compounds that are very strongly associated with GSRs with very restricted applications unrelated to GSR	Ethyl centralite	Stabiliser
		Methyl centralite	Stabiliser
		Nitroglycerin	Explosive
		Nitroguanidine	Explosive
2	Compounds that are strongly associated with GSRs, but which have less restricted applications unrelated to GSR	Akardite II	Stabiliser
		2,4-Dinitrotoluene	Flash suppressor
		2-Nitrodiphenylamine	Stabiliser
		4-Nitrodiphenylamine	Stabiliser
		Diphenylamine + nitrated-derivatives	Stabiliser
3	Compounds that are associated with GSR, but which are detected less frequently and have less restricted applications unrelated to GSR	Nitrocellulose	Explosive
		Other nitrotoluenes (2-NT, 3-NT, 4-NT, 2,3-DNT, 2,5-DNT, 2,6-DNT, 3,4-DNT,	Plasticisers / Flash suppressors & modifiers
		TNT)	Sensitiser
		Other diphenylamine derivatives (EPA and 2,4-DNDPA, N-NDPA etc.)	Stabilisers
		Triacetin	Plasticiser

This system contains a few exceptions based on table 4.10, due to the fact that the overall perceived forensic relevance is the leading factor for the categorisation, and not any one criterion by itself.

Despite being absent in table 4.10 due to the lack of detection in published work, NC and NQ are included in the system, due to the fact that they are base compounds of propellant powder. NC is present in single, double and triple base powders. This high association cancels out the low experimental association based on able 4.10. The lesser restrictions of applications that are not related to OGSR warrants the inclusion of NC in category 3. NQ is only present in triple base powders, but it is included in Category 1 due to its very limited (reported) applications

unrelated to GSR. The latter is the same reason for including akardite II to Category 2, despite its low experimental association.

Dibutyl phthalate has a relatively high association to OGSR materials, however, due to the generic use of bulk sample analysis for its detection, its wide-spread prevalence excludes it from the proposed categorisation system. Similarly, DPA is only included in conjunction with its nitrated-derivatives due to its relatively high occupational/environmental prevalence.

With continual changes being made to ammunition compositions, such a categorisation system will need to be kept under constant review to add or remove compounds based on analysis and manufacturer information. Furthermore, improvements in OGSR detection and/or population studies on the occurrence of OGSR compounds in public areas or the environment may further inform this system.

It should be noted that the aim of this categorisation system is to highlight OGSR compounds with forensic relevance with respect to the confirmation of GSR materials, and to potentially provide a backbone for a classification system including organic gunshot residue. Consequently, in the current forensic setting, this system may be used to complement inorganic GSR information; it is not suggested as a replacement of the existing standard.

Data from further population studies focussing on the OGSR compounds highlighted in this paragraph, could subsequently be used to optimise the proposed selection of OGSR compounds.

4.5   Conclusion and further work

The results have demonstrated that methanol extraction of relatively large quantities of propellant (*i.e.* 100 mg) is a suitable technique for the characterisation of the organic composition, including both major and minor compounds. Several further OGSR compounds have been detected, of which AKII, oxamide, and TNT are the most notable.

Although the characterisation of unburnt propellant has shown some general trends in OGSR composition, it clearly demonstrated that it is difficult to determine the calibre or brand of a propellant sample solely by the combination of OGSR compounds that are present. The absence of clear patterns does leave the potential open for linking specific samples to one another, which might provide supporting information in some cases.

In combination with the organic compositions reported in literature, this data has informed a categorisation system for OGSR compounds with respect to the confirmation of GSR

materials. The proposed three-tier system enables the classification of GSR materials based on OGSR compounds. This, together with the current classification system based on IGSR materials [167, 168] could provide unequivocal identification of GSR materials with the possibility of discriminating between GSR from different ammunition types.

Compositional analysis of smaller propellant samples will require the use of extraction/pre-concentration techniques. In the following chapters, two extraction techniques will be evaluated, initially using standards to eliminate sample variation. Subsequently, two propellant powders containing a range of OGSR compounds will be extracted in order to determine the most promising method for OGSR analysis of actual GSR samples, which may contain unburnt and partially burnt propellant [3, 11].

5 Application of MonoTrap to the extraction of OGSR compounds

The aim of this chapter is to investigate whether MonoTraps can be used for the extraction of OGSR compounds. A description of the four MonoTrap adsorptives evaluated is given, and the extraction procedures for both headspace and on-solvent extraction of standards and unburnt propellant are discussed.

5.1 MonoTrap

MonoTrap is a very hydrophobic adsorptive made of monolithic material [178], and as such is an example of a monolithic material sorption extraction (MMSE) technique [179]. Monolithic material consists of rigid macroporous polymers prepared by bulk polymerisation in a closed mould [179]. The material is created by polymerisation of a monomer mixture with a porogenic solvent, forming a highly porous polymer bed [180]. Inorganic monolithic material, which is used for MonoTrap, mainly consists of porous high-purity silica [178, 179]. Monolithic material is widely used as a stationary phase in liquid chromatography [180-182]. Other applications include its use as a column for anion-exchange chromatography [183], in tube SPE [181], in needle extraction [182, 184] and stir bar/stir rod sorptive extraction (SBSE/SRSE) [180, 185, 186]. MonoTraps are employed for a wide range of biological applications, including the extraction of flavour and aroma compounds [187], characterisation of odorants [188], and determination of plant hormones [189]. It has wide uses in separation science and in (bio) catalysis [181], and is used for sample preparation in drug and pharmaceutical analysis [190].

MonoTrap has an increased surface area (150 m^2/gram or more), which is provided by the network of interconnected pores in the silica frame [178, 180, 191] (figure 5.1). Moreover, due to the network of pores, with sizes in the low micrometer range, this material possesses very good permeability. Consequently, high analyte migration rates are achieved. [178, 180, 191] In the case of SRSE, this results in significantly faster extraction times for stir bars coated with monolithic materials (up to a few hours [180, 185]) than for stir bars coated with a thick layer of another material, such as PDMS (up to 72 hours [30]). Consequently, MonoTrap combines the increased capacity due to the thick layer achieved by a stir bar, with high analyte migration rates due to the network of pores. It is suitable for non-polar to polar compounds [191], and it requires only 200 µL of solvent to perform the extraction, which is both environmentally and economically advantageous [7].

Through pore

Mesopores dotted on the structure
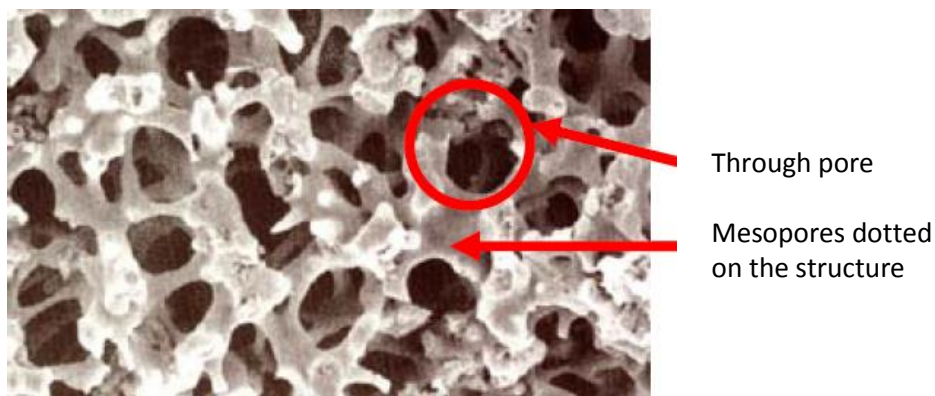
Figure 5.1: Enlarged view of a section of Monotrap [178]

MonoTrap is available in a silica type, and in a type in which the silica frame contains activated carbon that acts as an adsorptive medium. This type of MonoTrap has octadecyl-groups conjugated to its frame [178]. Both the silica and the carbon type are available in disc and rod configuration (figure 5.2). In this chapter, the performance of MonoTrap for the extraction of OGSR compounds is assessed. A comparison of MonoTrap against SPME is provided in chapter 6.

Figure 5.2: MonoTrap silica disc, silica rod, carbon rod and carbon disc

5.2 Methodology

All four MonoTraps can be used for on-solvent and headspace extraction. Both procedures involve extraction of OGSR compounds from the sample and recovery of these compounds from the Monotraps (desorption) in separate steps. On-solvent extraction required dilution of the methanol solutions with ultra-pure water to ensure the required buoyancy of the MonoTraps.

5.2.1 Solvents, standards and propellants

The standards used are listed in section 3.2.1, all standards were made up in methanol.

The stock solution of OGSR standard 1, containing 18 OGSR compounds (concentrations ranging from 25-62.5 µg/mL) was diluted with ultra-pure water (1:4). The resulting solution (standard 1a) contained:

- o 5 µg/mL of camphor, 3-NT, EPA, DPA, DMP, DEP, DBP, DIBP and MC;
- o 7.5 µg/mL of carbazole, 2,4-DNT, and 3,4-DNT;
- o 10 µg/mL of 2-NDPA, 4-NDPA, and 2,4-DNDPA;
- o 12.5 µg/mL of triacetin, 2,3-DNT and 2,6-DNT.

OGSR standard 2 contained 25 µg/mL of the above compounds in addition to 2-NT, 4-NT, 2,5-DNT, EC and NG. This solution was diluted with ultra-pure water, resulting in a final concentration of 5 µg/mL for all compounds in a matrix of methanol and ultra-pure water (1:4).

Desorption of the MonoTrap adsorptives was performed with analytical grade methanol (Sigma Aldrich, St Louis, MO, USA), acetone, dichloromethane, ethyl acetate or hexane (Fisher Scientific, Loughborough, UK).

Propellant extractions were performed on Hodgdon HP-38, .223 Magtech, and .357 PMC ammunition, details are provided in section 4.2.1.

5.2.2 MonoTraps

MonoTrap adsorptives – silica disc, silica rod, carbon disc, and carbon rod – were purchased from GL Sciences Inc. (Tokyo, Japan). Procedures for headspace extraction, on-solvent extraction and post-extraction desorption of the MonoTraps are provided. Background levels of the MonoTrap adsorptives were obtained by extracting a mixture of methanol and ultra-pure water. Due to the fact that MonoTraps are reported to be single use adsorptives, these blanks served as procedural blanks; sample extractions had to be performed with new, unused adsorptives.

5.2.3 Headspace extraction

Headspace extractions of standards (1 mL) were carried out by suspending the MonoTrap in the vial's headspace using the provided holder (figure 5.3). The holder was pierced through the prepped septum in the vial cap provided. The MonoTrap was suspended about 1 cm below the septum. Headspace extractions of unburnt propellant powder (100 mg and 10 mg) were performed by placing the MonoTrap directly on top of the propellant in a closed 14 mL glass vial (Samco), ensuring that half of the adsorptive was in contact with the powder. The vial was then

placed in an oven (Nabertherm) at 80°C for 3 hours as per the manufacturer's recommendations [192]. Optimisation of the headspace extraction procedure (10 mg of propellant) was performed using a Raven incubator (LTE Science, UK) to enable overnight extraction.
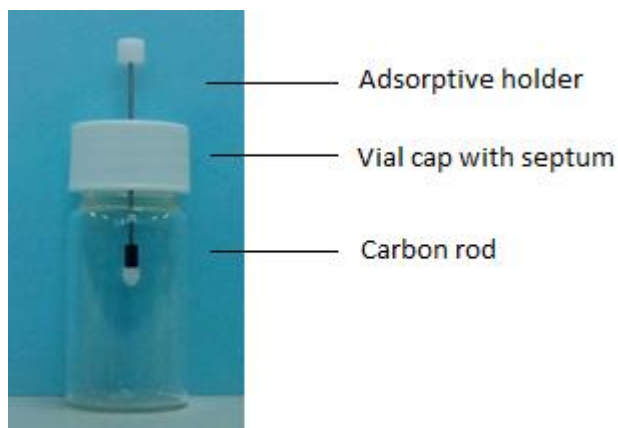
Figure 5.3: Set up for headspace extraction with carbon rod

5.2.4   On-solvent extraction

On-solvent extraction was carried out in 14 mL glass vials (Samco) to which the sample (1 mL standard or the propellant extract) and small flea (5 mm x 2 mm Teflon Spinbar® magnetic stirring flea, Sigma Aldrich, St Louis, MO, USA) were added. Propellant extracts were obtained by sonicating 100 mg propellant powder in 2 mL of methanol for 1 hour. Ultra-pure water was added to obtain a 50:50 mixture. The MonoTrap was then carefully lowered onto the solvent, and care was taken to keep its top-facing surface dry to ensure its buoyancy. The vial was placed on a stirrer/hotplate (Stuart, heat-stir US 152) at 60°C for 30 minutes, during which the sample was agitated by stirring it at approximately 500 rpm as per the manufacturer's recommendations [178].

5.2.5   MonoTrap desorption

After completion of the extraction procedure, the MonoTrap was removed from the vial and carefully dried. Analyte desorption was accomplished using 200 μL of solvent, initially methanol. Rods were desorbed in a GC vial with a fixed 300 μL insert (Chromacol ltd., Herts, UK), discs were desorbed in the provided MonoTrap extraction cups. In both cases the MonoTrap was submerged in the solvent, which could penetrate the absorptive. The extraction cup was lowered onto a vial filled with ultra-pure water and then capped (figure 5.4), the capped GC vial was placed in a beaker filled with ultra-pure water. The vials were placed in an ultrasonic bath

for five minutes at ambient temperature. Finally, the cap and the absorbent were removed and as much of the solvent as possible was transferred to a GC vial (Chromacol ltd., Herts, UK) containing a 150 µL glass insert with polymeric feet (Agilent Technologies, Santa Clara, CA, USA).
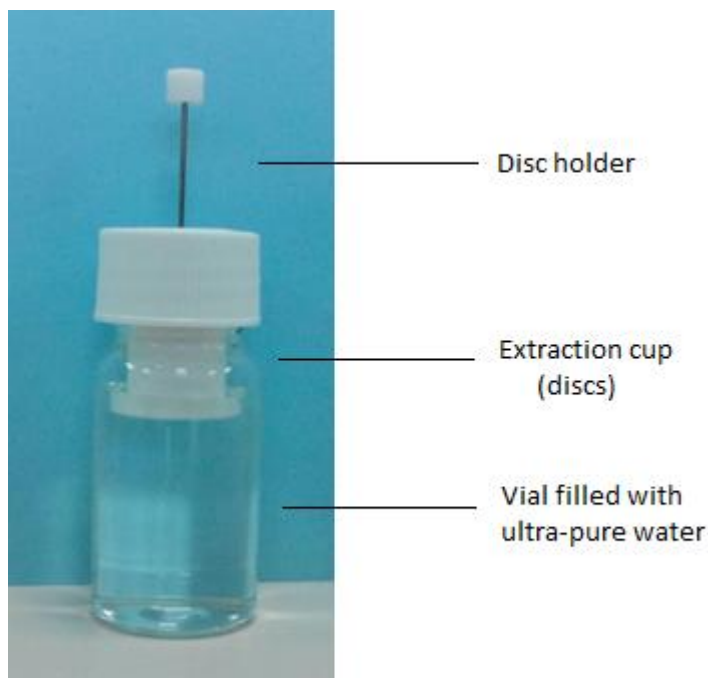
Figure 5.4: Set up for disc extraction

5.2.6   GC-MS instrumentation and conditions

The details of the GC-MS instrumentation are provided in section 3.2.2. Optimisation of the MonoTrap headspace extraction was performed using an Agilent 7890A GC system coupled with an Agilent 5977A mass spectrometer. Samples were introduced using an autosampler (Agilent 7683B) via splitless mode with a solvent delay of 3 min. Mass spectra were evaluated using the NIST database (NIST Mass Spectra Search Program version 2.2).

Both chromatographic method A and C were used during this work (table 5.1), table 5.2 in the next section details which method was used per section. Methanol blanks were run between all samples.

Table 5.1: Summary of GC-MS method

Oven profile (A)	Ramp (°c/min)	Hold Temperature (°C)	Hold time (min)
50°C	6	200	2.00
	20	300	0
Flow rate (A)	1 mL/min		
Oven profile (C)	**Ramp (°c/min)**	**Hold Temperature (°C)**	**Hold time (min)**
50°C	10	100	0
	5	180	2.50
	30	200	2.50
	30	300	2.00
Flow rate (C)	1.2 mL/min		
Injection mode	Splitless	**Scan mode**	Full scan
		Scan range	m/z 40 - 500

5.2.7 Optimisation parameters

Investigation of the limiting factors of on-solvent extraction of standard 2 was performed using the silica disc. An extended extraction time of 1 h and desorption using dichloromethane were compared against the initial extraction parameters (30 min extraction and methanol desorption).

In order to investigate the extent of desorption achieved by dichloromethane, sequential desorption of the MonoTraps was performed on extracts of unburnt propellant. The dichloromethane and methanol extracts were analysed separately.

Optimisation of headspace extraction was performed on 10 mg propellant using the best performing MonoTrap: the carbon disc. All extractions were carried out at 80°C, and unless stated otherwise an extraction time of 3 hours and a desorption procedure involving methanol and 5 min sonication were performed. The extraction parameter investigated was the extraction time, ranging from 3 to 18 hours. The main focus of the optimisation, however, was the desorption procedure. Five different solvents were tested for the desorption of the MonoTraps: methanol, dichloromethane, acetone, ethyl acetate and hexane. Combined solvent systems were also investigated, using ratio mixtures of 25:75, 50:50, and 75:25 of both methanol and dichloromethane, as well as acetone and dichloromethane. The optimal sonication time was determined over a range of 5 to 60 min using the best performing solvent system (25:75 acetone and dichloromethane). The desorption volume was kept at 200 µL for all experiments. The final optimised method was used for the extraction of a single grain of two different propellants: 9 mm federal premium and .357 PMC.

Semi-quantitative comparison of the results was based on the recorded peak areas. Mean peak areas and standard deviations were calculated in order to gain a preliminary indication of the repeatability achieved with each MonoTrap. The results were plotted in graphs with the standard deviations represented by error bars.

A summary of the samples and MonoTraps used for each stage is given in table 5.2, with reference to the sections in which the results are discussed.

Table 5.2: Summary of samples and MonoTraps used per section

Section	Sample	MonoTrap	Extraction method	Desorption solvent	GC method
5.3.1	Blank	All	Headspace + on-solvent	Methanol	A
5.3.2	Standard 1a	All	Headspace + on-solvent	Methanol	A
5.3.3	Standard 2	Silica disc	On-solvent	Methanol / DCM	C
5.3.4	Standard 2	All	On-solvent	DCM	C
5.3.5	Hodgdon HP-38	All	On-solvent	Methanol	A
5.3.6	Hodgdon HP-38 .223 Magtech	All	Headspace	Methanol	A & C
5.3.7	Hodgdon HP-38	Carbon disc	Headspace	Various	C

5.3 Results and discussion

Section 5.3.1 contains the results of the blank extractions of all MonoTraps using headspace and on-solvent extraction. Section 5.3.2 discusses the initial assessment of the extraction capability of the MonoTraps, which is performed on standard 1a in order to eliminate sample variation and gain insight in potential preferential extraction of certain OGSR compounds. Initial investigation of key factors influencing the final recovery is performed on the complete standard (section 5.3.3), followed by a comparison of all MonoTrap types (section 5.3.4). On-solvent and headspace extraction of unburnt propellant samples are discussed in sections 5.3.5 and 5.3.6 respectively, followed by optimisation of the procedure using best performing MonoTrap in section 5.3.7.

5.3.1 Background levels

All MonoTraps showed comparable background levels with very few peaks for both headspace and on-solvent extraction (figure 5.5 and figure 5.6 respectively).
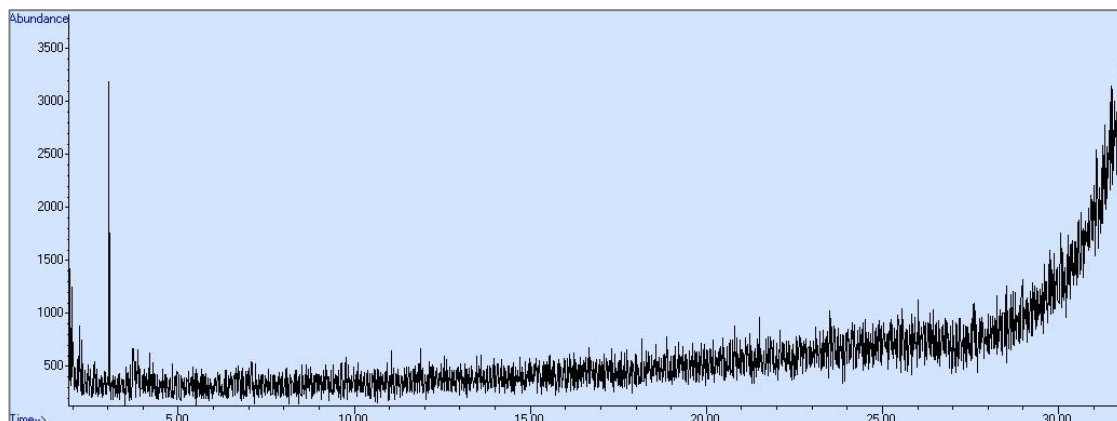
Figure 5.5: Headspace blank silica disc
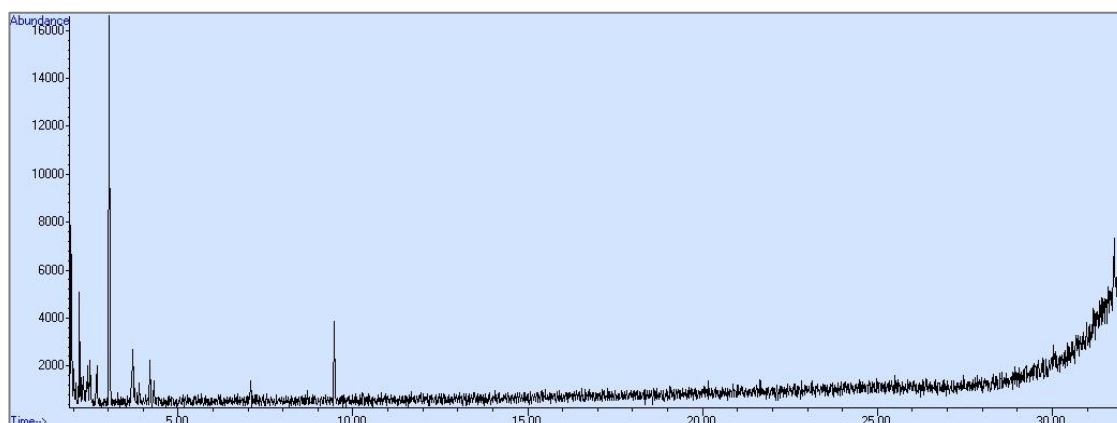
Figure 5.6: On-solvent blank silica disc

The headspace blanks (figure 5.5) showed no additional extraneous peaks; the only peak in the chromatogram is also present in system blanks, and thus not related to the extraction. This peak is therefore also present in the blanks obtained using on-solvent extraction (figure 5.6). Additionally, some small siloxane peaks can be observed in the first 10 minutes of the run time. Due to the fact that these compounds elute relatively early and in low abundance, no adverse impact is expected from the background levels.

The chromatograms of the silica discs are given as an example; similar chromatograms with no additional peaks were obtained for all four MonoTraps.

Contamination of the adsorptives during storage was prevented by keeping the MonoTraps in the original packaging in the fridge away from any propellant or GSR samples.

5.3.2   Assessment of extraction capability

The ability of the various MonoTraps to extract the selected OGSR compounds was investigated using standard 1a, which was prepared to achieve similar abundances across the compounds. Both chromatograms and peak area data were analysed to determine if preferential extraction of any compounds occurred.

Headspace extraction

Only three of the 18 compounds present in standard 1a were extracted from the headspace: camphor, 3-NT, and DPA (figure 5.7). The concentration of these compounds was 5 µg/mL – the lowest concentration in the standard mixture. The carbon rod only yielded two compounds, not including DPA.
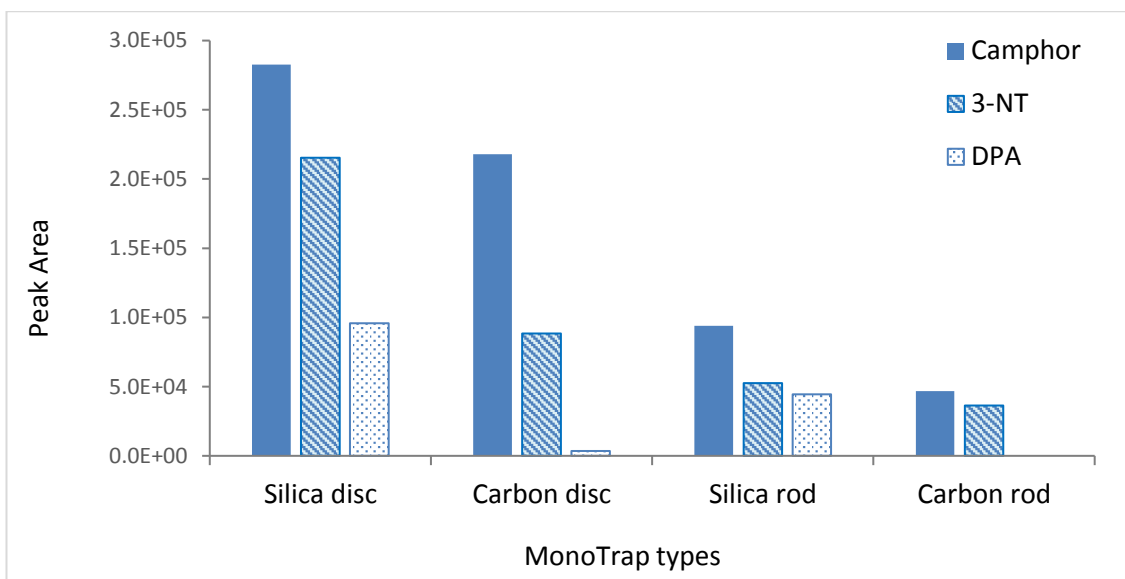
Figure 5.7: Comparison peak areas obtained with headspace extraction of standard 1a (n = 1)

On-solvent extraction

On-solvent extraction of OGSR standard 1a achieved the detection of 16 compounds. Only triacetin and 3,4-DNT were not detected. The concentration of these two compounds in the standard were 12.5 µg/mL (highest bracket) and 7.5 µg/mL (second lowest bracket) respectively. The chromatogram obtained using the silica disc is shown in figure 5.8. Similar results were achieved using the other MonoTraps.
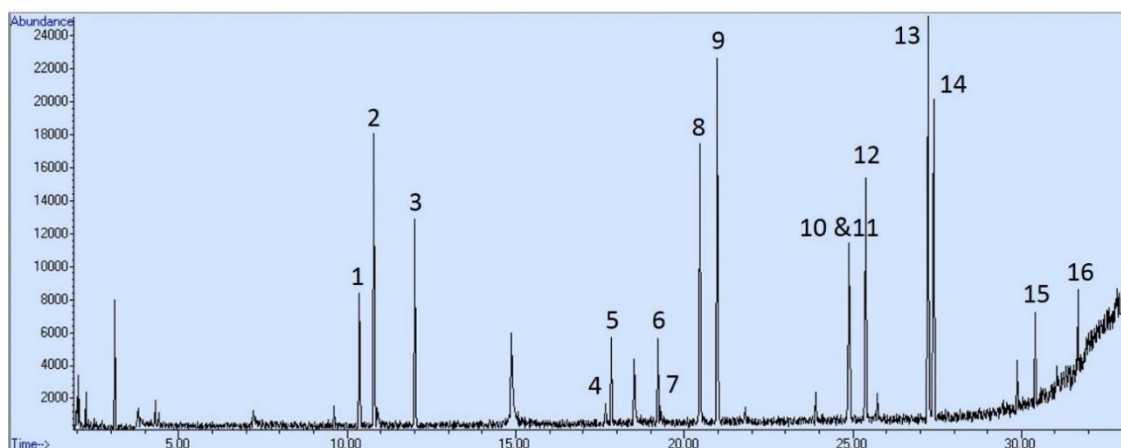
Figure 5.8: Chromatogram OGSR standard 1a - on-solvent extraction with silica disc: 1. EPA, 2. Camphor, 3. 3-NT, 4. DMP, 5. 2,6-DNT, 6. 2,3-DNT, 7. 2,4-DNT, 8. DEP, 9. DPA, 10. Carbazole, 11. MC, 12. DIBP, 13. DBP, 14. 2-NDPA, 15. 4-NDPA, 16. 2,4-NDPA.

A relatively good separation was achieved using chromatographic method A. Only the DNTs (4 and 5, and 6 and 7) are not baseline resolved, and carbazole (10) and methyl centralite (11) co-eluted. It should be noted that despite the clear presence and sharp peak shapes of the other compounds, the abundances (vertical axis) are relatively low.

The chromatogram shows that similar detector responses (*i.e.* similar abundance) were obtained for most compounds, as was expected for this standard. Smaller peaks, however, were obtained for all dinitrotoluenes (4-7) and for both 4-NDPA (15) and 2,4-DNDPA (16). This suggests that MonoTraps may be less suitable for the extraction of DNTs. Given the fact that 2-NDPA resulted in one of the highest peaks, the relatively poorer results for 4-NDPA and 2,4-DNDPA are likely to be related to the increased baseline (and noise) observed towards the end of the chromatogram as a result of the high oven temperature (300°C). This temperature was necessary to enable to elution of these compounds within a reasonable timeframe. The peak areas achieved with all MonoTraps are shown in figure 5.9.
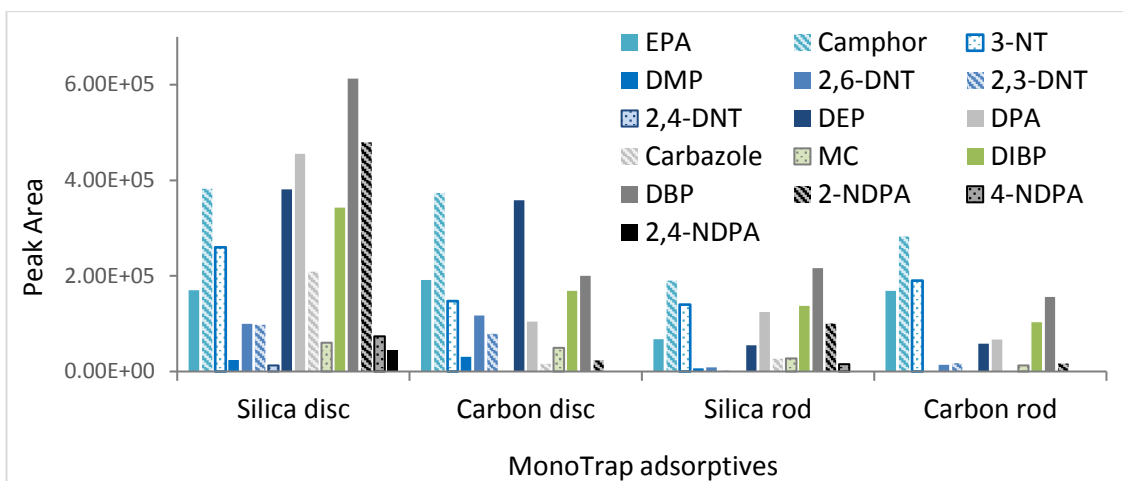
Figure 5.9: Peak areas achieved with on-solvent extraction of standard 1a

This graph clearly demonstrates that the discs outperformed the rods in terms of the overall OGSR compounds extracted from a liquid sample. This was expected based on the colour of the MonoTrap extracts after desorption; the extracts of the discs were slightly lighter yellow than the standard solution, whereas the extracts of the rods were colourless. A limited extraction capability of the rods could be due to the limited surface contact with the sample; less than a quarter of the rod was in direct contact with the liquid sample during extraction, compared to half the disc. Submerging the rod to increase the surface area in contact with the sample resulted in a very poor extraction, this was also supported by the manufacturer's information [178].

Both discs showed comparable peak areas for the first eight compounds shown in figure 5.9. A closer comparison of the peak areas per compound (appendix E) shows that the carbon disc performs slightly better for EPA and 2,6-DNT, whereas the silica disc performs better for four other compounds. This effect is more significant for the latter eight compounds shown in figure 5.9, which clearly resulted in greater peak areas for the silica disc.

Despite the fact that greater peak areas were obtained with the discs for almost every compound compared to the rods, the silica adsorptives performed better qualitatively. Both the silica disc and rod extracted more OGSR compounds (16 and 14 respectively) than the carbon adsorptives (13 and 11 respectively) (table 5.3).

Table 5.3: OGSR compounds that were not detected in MonoTrap extracts

	Triacetin	3,4-DNT	2,4-DNT	2,4-DNDPA	4-NDPA	DMP	Carbazole
Silica disc	x	x					
Silica rod	x	x	x	x			
Carbon disc	x	x	x	x	x		
Carbon rod	x	x	x	x	x	x	X

These results illustrate that the carbon rod achieved the poorest results overall. The silica disc extraction clearly resulted in the detection of the greatest number of OGSR compounds and achieved the greatest peak areas across these compounds.

The results have also shown consistently lower peak areas for DNTs, carbazole, MC, 4-NDPA and 2,4-DNDPA across all MonoTraps. This could indicate a reduced extraction capability for these compounds, or a reduced recovery from the MonoTrap adsorptives. This is investigated a little further in the next section.

5.3.3 Investigating limiting factors of on-solvent extraction

In order to investigate whether the main limiting factor was the extraction or the recovery of OGSR compounds, two different desorption solvents and an extended extraction were compared. This was done using solvent extraction of standard 2 with the silica disc, given the superior performance of this MonoTrap. Initially, an extraction time of 30 minutes was used in order to compare methanol (MeOH) and dichloromethane (DCM) for desorption of the silica disc. Subsequently, the extraction time was increased to one hour. These results are shown in figure 5.10. Of the 23 compounds present in the standard at a concentration of 5 µg/mL only nitroglycerin and triacetin have not been detected in the extracts.
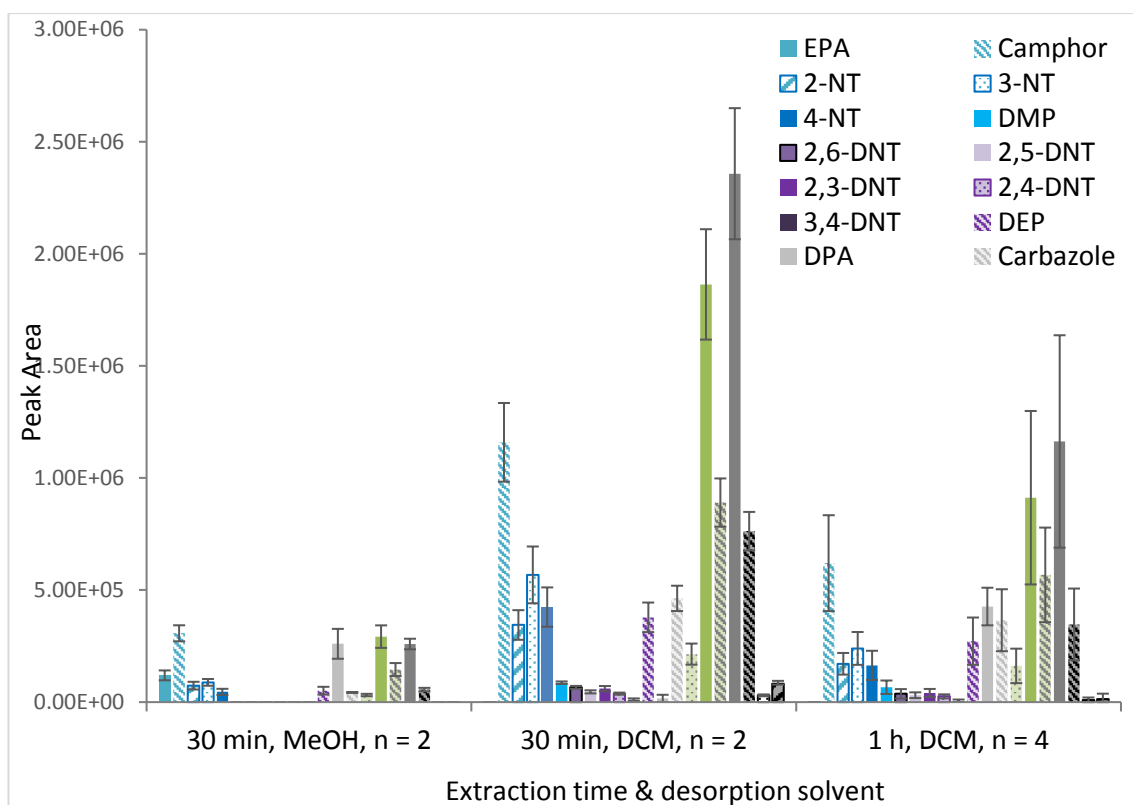
Figure 5.10: Comparison extraction parameters of on-solvent extraction (silica disc, standard 2)

This graph clearly shows that dichloromethane performs better than methanol, both qualitatively and in terms of peak areas, for almost all compounds. The results obtained with methanol are comparable to the results from the silica disc shown in figure 5.9. As expected, DNTs, 4-NDPA and 2,4-DNDPA were no longer detected due to the low concentration of these compounds in standard 2. The reduced concentration of several OGSR compounds, including DNTs, carbazole and the nitrodiphenylamines, had little effect on the detection of these compounds using dichloromethane for desorption. Methanol desorption was only better for the recovery of EPA, which was not detected in the dichloromethane extracts, and DPA, which was only present in the dichloromethane extracts as a very small peak in comparison to the methanol results. This could indicate dichloromethane is less suitable for the desorption of these compounds. It should be noted that this does not apply to the nitro-diphenylamines.

The results of the increased extraction time show a decrease in peak areas for most compounds except for DPA, for which the greatest peak areas were obtained with this method. It is hypothesised that the extended extraction time improves the extraction of DPA from the sample, and that the amount of DPA desorbed by dichloromethane increases with an increased amount of DPA present in the disc before desorption. This effect is not observed for the other diphenylamine-related compounds, including EPA.

DPA can be considered to be an important compound for this study due to its frequent association with OGSR [3, 6, 7, 11, 29-34]. The fact, however, that a 30-minute extraction time followed by desorption with dichloromethane produced better results for almost all other compounds, makes this the preferred method. These results also suggest that optimisation of desorption parameters may have a much greater effect on the recovery than the extraction parameters, and that the ideal parameters may vary across compounds. In order to investigate whether these parameters also vary between silica and carbon, or discs and rods, dichloromethane desorption is first employed to all MonoTraps.

5.3.4   Performance comparison based on standards

The performance of the different MonoTraps using 30-minute extraction and dichloromethane desorption was evaluated using on-solvent extraction of standard 2 (figure 5.11).
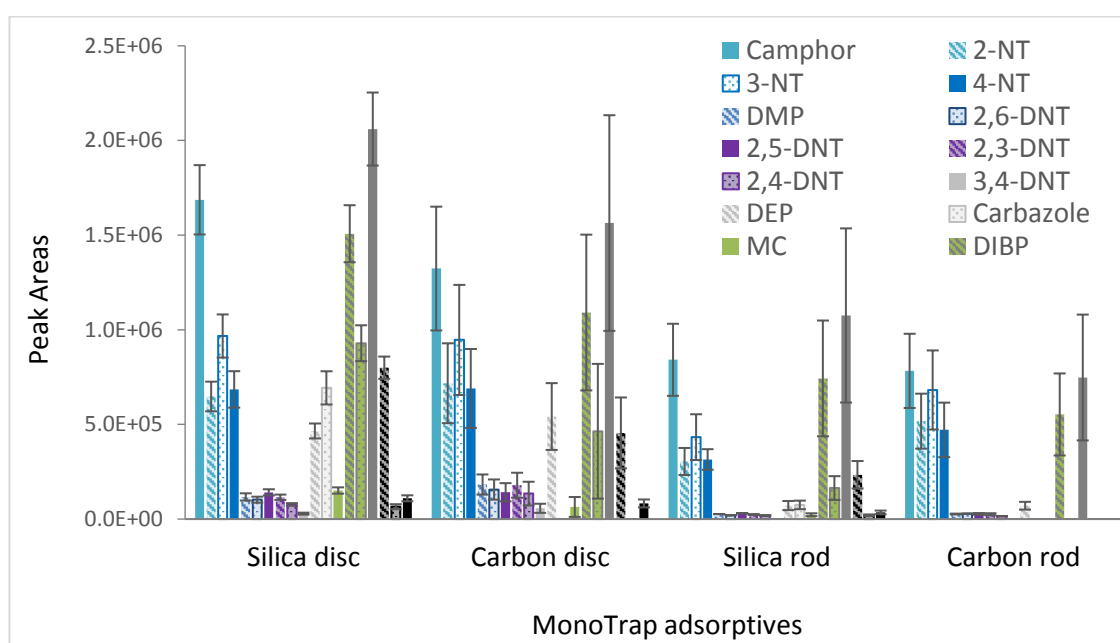
Figure 5.11: Comparison of the mean peak areas achieved for the extraction of standard 2

This standard contained five additional compounds to standard 1a: 2-NT, 4-NT, 2,5-DNT, EC and NG. NG was the only added compound that was not detected in any of the extracts. Given the fact that NG is structurally more similar to triacetin than to DPA and EPA (figure 5.12), it is suspected that dichloromethane is not the cause of this. The fact that NG was not detected in any of the extracts could be cause for concern because double base propellant powders are based around NC and NG [11], making NG a compound of significant relevance for OGSR

detection. EC, another important compound [6, 11, 21], was extracted by all MonoTrap types, except for the carbon rod. The (di)nitrotoluenes were detected in all extracts.

| NG | Triacetin | DPA | EPA |
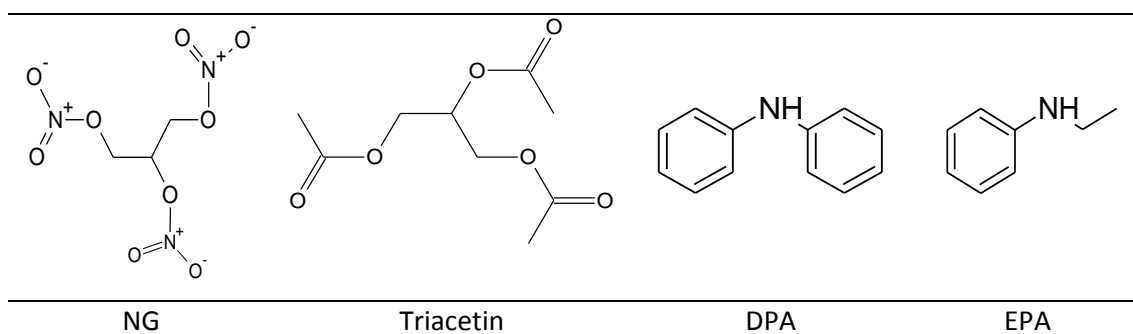
Figure 5.12: Molecular structures of NG, triacetin, DPA, and EPA

Figure 5.11 clearly shows an improvement in both the number of OGSR compounds detected and the peak areas obtained in comparison with the methanol extracts (figure 5.9). The relative performances across all MonoTraps appear to have remained the same. The carbon disc resulted in better peak areas for the DNTs, but all of these were also extracted by the silica disc. The silica disc achieved the greatest peak areas for the latter eight compounds (from MC onwards) and was able to extract the most compounds. The silica rod still extracted one compound (4-NDPA) more than the carbon disc, but the peak areas remained lower for all other compounds. The carbon rod consistently performed the poorest, both qualitatively and in terms of peak areas.

A preliminary evaluation of the repeatability of the peak areas across all compounds is shown in table 5.4. The rods have a similar repeatability, despite the fact that the carbon rod extracted four compounds less. The repeatability obtained with the carbon disc is much poorer, which appears to be largely due to the high standard deviations for both centralites. Without these two compounds, however, the mean standard deviation across all other compounds only decreased to 17%. The silica disc appears to produce the most repeatable results. The repeatability of individual compounds does not appear to be directly linked to the material (silica or carbon) or the configuration (disc or rod), as no similarities were observed between the compounds with either the highest or the lowest standard deviation. Based on these results, this method is not (yet) applicable to full quantitative analysis.

Table 5.4: Preliminary repeatability evaluation based on peak areas

	Silica disc	Carbon disc	Silica rod	Carbon rod
Mean std dev	4.68%	23.15%	10.97%	10.63%
Highest std dev	12% Carbazole	> 65% MC, EC	25% MC, 2,4-DNDPA	> 35% DIBP, DBP
Lowest std dev	< 1% 2,3-DNT, 2,4-DNT	12% camphor	< 1% camphor	1% 2,6-DNT, 4-NT

In summary, the results have shown that three MonoTrap types, not including the carbon rod, have potential as a pre-concentration technique for OGSR compounds. Based on the obtained results, it was expected that on-solvent extraction in combination with dichloromethane desorption would yield the best results. The significant increase in peak areas across most compounds for all MonoTraps, which was achieved by changing the desorption solvent, has demonstrated that the choice of desorption solvent is crucial for the recovery of OGSR compounds. It suggests that optimisation of the desorption parameters could further improve the results. Given the fact that the recovery varies per compound, a careful selection of compounds needs to be made to ensure a relevant optimisation procedure. Therefore, further optimisation will be done using propellant powder.

5.3.5 On-solvent extraction of propellant

In order to investigate whether further OGSR compounds, including EPA and DPA, could be detected, sequential desorption using dichloromethane followed by methanol was performed on propellant extracts. This method was first tested with the silica disc, the carbon disc and the silica rod; the carbon rod was excluded due to previous poor extraction performance. Despite the fact that these extractions were performed in duplicates, neither of the disc desorptions resulted in a sufficient amount of dichloromethane to be transferred to the GC vial. It is suspected that both the evaporation of the more volatile dichloromethane during sonication and the nature of the sample play a role in this. Unlike the clean liquid standards, the methanol extracts of the propellant were suspensions; the propellant powder was left in the vial during MonoTrap extraction and the agitation of the sample prevented some of the particulate matter to settle on the bottom. Some particulates adhere to the MonoTrap itself during the extraction, and could not be removed due to the fragile nature of the Monolithic material. Therefore, they caused issues in the removal of the extract post desorption. Desorption of the silica rods did yield sufficient quantities for GC analysis. This could be due to the fact that fewer and smaller particles were able to transfer onto the rods and the fact that desorption of rods is performed

in long narrow inserts. Consequently, most of the desorption solvent reached above the height of the rod, which allowed for the easy transfer of most of the solvent. Desorption of the discs, however, is performed in cups, causing the solvent to form a thin film. This makes the collection of the extract more difficult and promotes the evaporation of the solvent during sonication. This effect was also observed for the clean liquid samples; less of the dichloromethane extract was recovered and greater variability in the amount recovered was observed, compared to the methanol recoveries.

Subsequent desorption with methanol was performed in the same cup/insert, without prior cleaning or removal of the particulates. Sufficient amounts of methanol extract, however, could be recovered for analysis. These results are shown in figure 5.13, including the results of dichloromethane extract of the silica rod performed before the methanol desorption.
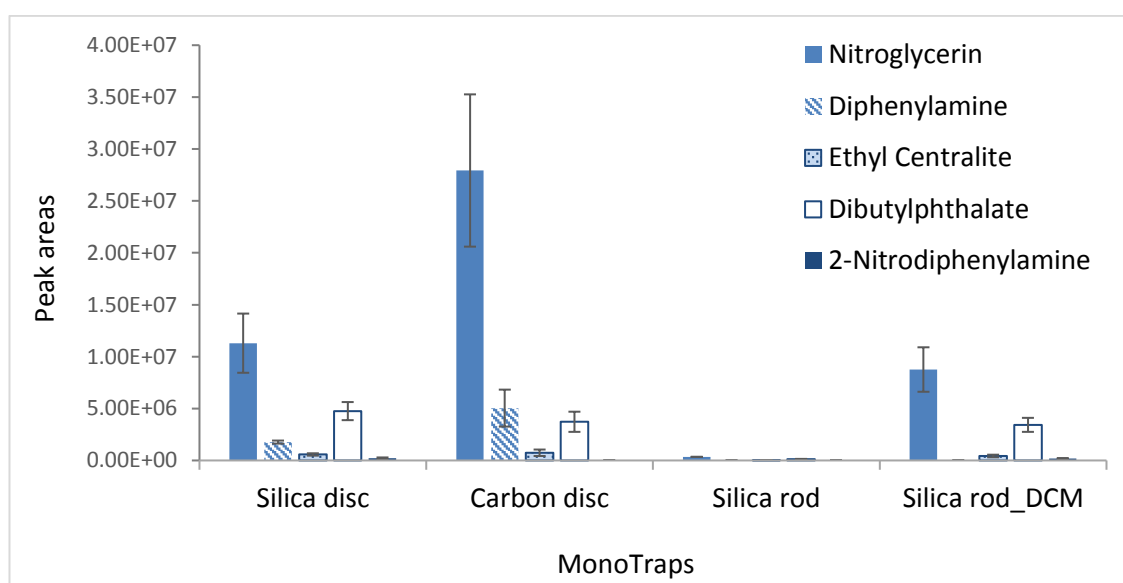
Figure 5.13: Results of on-solvent extraction of Hodgdon HP-38 propellant: methanol extracts silica disc, carbon disc, and silica rod, and dichloromethane extract of the silica rod (performed before methanol desorption) (n = 2)

The second desorption with methanol resulted in the detection of several compounds other than DPA. This suggests a poor recovery of the OGSR compounds from the MonoTraps using the manufacturer's recommendations (5 min sonication in 200 µL of solvent).

The highest peak areas for NG, DPA and EC were detected in the methanol extracts of the carbon disc, however, it was the only extract in which 2-NDPA was not detected. The silica disc methanol extracts were they only extracts in which five compounds were detected (not including AKII, 4-NDPA and N,N-DPF) and it achieved higher abundances than the silica rod. As

expected, DPA was the only compound not detected in the dichloromethane extract of the silica rod, however, it was not detected in its methanol extracts either. Given the low recovery of all compounds in this extract compared to the methanol extracts of the MonoTrap discs, it is suspected that less analytes may have been extracted with the silica rod. This is congruent with the results obtained from the extraction of standards.

It was hypothesised that the evaporation of dichloromethane and the particle interference could hamper the effective recovery of OGSR compounds by the solvent. Therefore, further testing was done in attempt to improve this method. Using the silica disc, several changes to the extraction protocol were investigated that aimed at reducing the interference of the particulate matter. These steps included varying the ratio of methanol to water in the vial, transfer of the methanol propellant extracts to clean vials before adding the MonoTrap, and simultaneous methanol and MonoTrap extraction by adding the MonoTrap to the sample prior to sonication (1h) to extract OGSR compounds from the propellant. Simultaneous methanol and MonoTrap extraction was also undertaken with the silica rod, as differences in buoyancy behaviour were observed between the two configurations. Although some methods seemed better than others, no satisfactory improvement was achieved.

Overall, these results suggest that the use of MonoTrap is less suitable for on-solvent extraction of suspensions, in particular when using very volatile solvents for desorption.

5.3.6   Headspace extraction of propellant

Based on the above results, single MonoTrap desorption with methanol was employed on the initial headspace extractions. The chromatograms of the extractions of the Hodgdon HP-38 and the .223 Magtech propellant performed with the carbon discs are shown in figure 5.14 and figure 5.15 respectively.
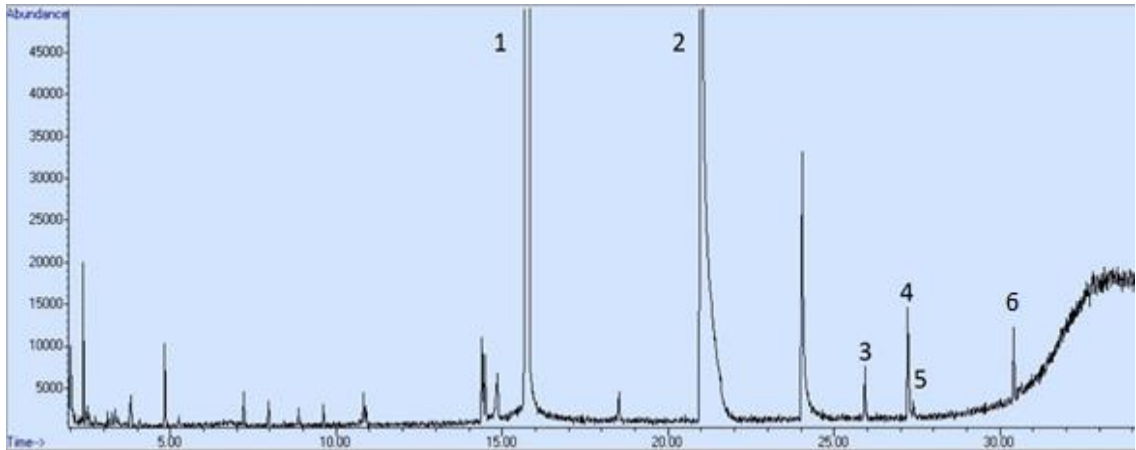
Figure 5.14: Chromatogram headspace extraction Hodgdon HP-38 propellant with carbon disc (chromatographic method A): 1. NG, 2. DPA, 3. EC, 4. DBP, 5. 2-NDPA, 6. 4-NDPA
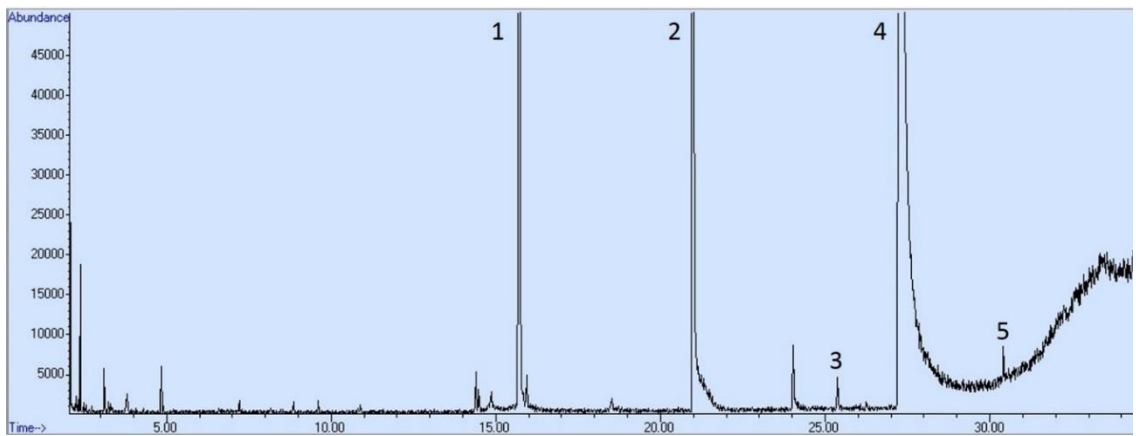
Figure 5.15: Chromatogram headspace extraction Magtech .223 Rem Tactical propellant with carbon disc (chromatographic method A): 1. NG, 2. DPA, 3. DIBP, 4. DBP, 5. 4-NDPA

The chromatograms clearly show the major compounds in both propellant powders (NG and DPA for Hodgdon HP-38, and NG, DPA and DBP for .223 Magtech), which are consistent with the major compounds detected via methanol extraction. In addition, most minor compounds were detected as well. Only AKII (Hodgdon HP-38) and DIBP (.223 Magtech) were not detected in the MonoTrap extracts.

The major compounds of both propellants were extracted with all four MonoTraps. A comparison of the peak areas obtained with all types is shown in figure 5.16 for Hodgdon HP-38 and in figure 5.17 for .223 Magtech. In both graphs, the major compounds are displayed on the primary (left-hand) axis and the minor compounds on the secondary axis.
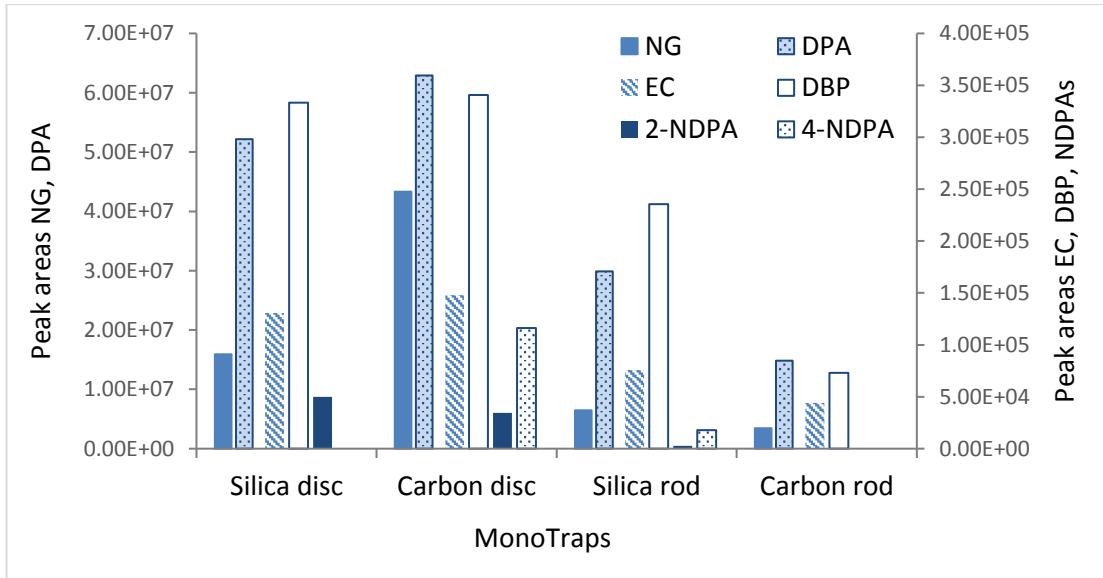
Figure 5.16: Peak areas for all MonoTraps for headspace extraction Hodgdon HP-38 (n= 1)
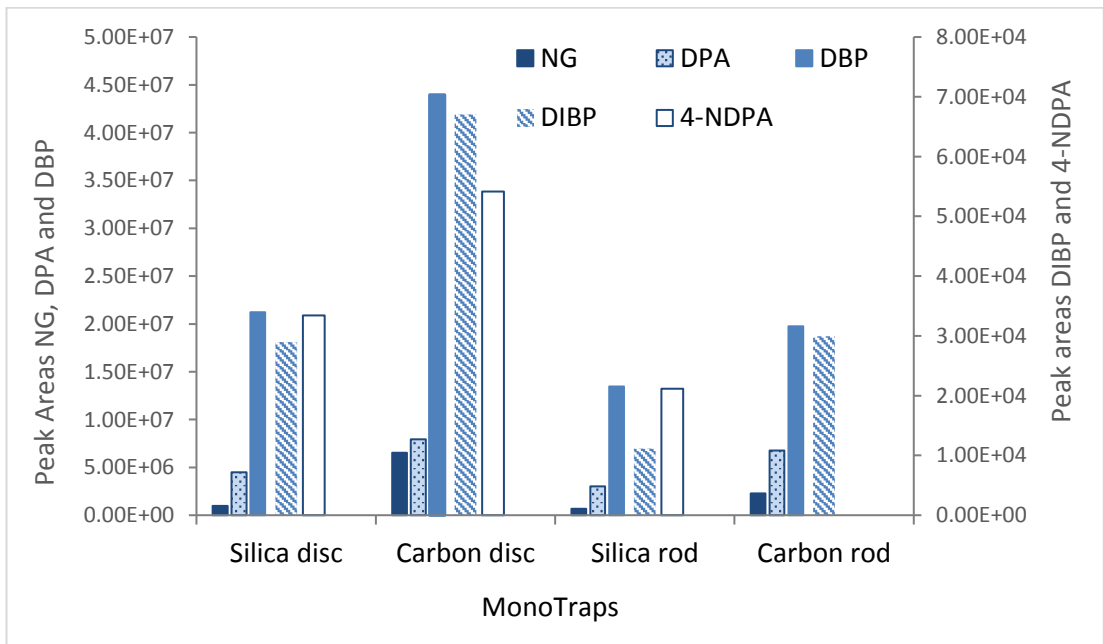
Figure 5.17: Peak areas of all MonoTraps for headspace extraction of Magtech .223 Rem Tactical (n = 1)

The headspace MonoTrap extractions produced similar results to the methanol extractions of the propellant powders, particularly for the major compounds in each powder. DPA and NG respectively were the major compounds extracted from Hodgdon HP-38 propellant. Their peak areas were much higher than those of the other four OGSR compounds present (figure 5.15), which were all extracted using the carbon disc and to a lesser degree with the silica rod. The carbon disc yielded the greatest peak areas for all compounds, except for 2-NDPA for which the

greatest peak area was detected in the silica disc extract. In contrast to the results obtained from the extraction of standard solutions, the silica disc extracted one compound, 4-NDPA, less than the carbon disc. The only OGSR compound that was not detected in the MonoTrap extracts, was AKII. Analysis of a standard including AKII did show that this compound was suitable for MonoTrap extraction. Methanol extractions yielded the lowest peak areas for AKII, which could suggest that it is present in lower quantities in the propellant.

DBP, DPA and NG respectively, were clearly present as major compounds in the .223 Magtech propellant (figure 5.17), and comparable peak areas were obtained across all types. The achieved peak areas of DIBP and 4-NDPA varied according to MonoTrap type, and 4-NDPA was not detected in the carbon rod extracts. EC was the only compound that was not detected in any of the extracts. This may be the result of the coating that the Magtech propellant grains appear to have, which could also have led to the lower overall abundances detected for the .223 Magtech propellant compared to the Hodgdon HP-38 propellant for both methanol and MonoTrap extractions.

It was noticed that the headspace extracts of all MonoTraps were considerably cleaner than those of the on-solvent extraction, which potentially allows for the use of more volatile solvents, such as dichloromethane, for desorption. Further optimisation (section 5.3.7) of the headspace MonoTrap extraction was performed using Hodgdon HP-38 propellant, since it contained more compounds of interest. It focused on the carbon disc extractions, given the fact that it achieved the greatest number of OGSR compounds and the greatest peak areas across all compounds.

5.3.7 Optimisation of MonoTrap headspace extraction

The optimisation process focused on one extraction parameter (the extraction time), and two desorption parameters (the desorption solvent and the sonication time).

Comparison extraction times

The extraction time was varied at a constant temperature of 80°C and four different times were investigated, ranging from the manufacturer's recommendations of 3 hours to an overnight run. (figure 5.18). The desorption process was kept at methanol desorption via 5 min sonication.
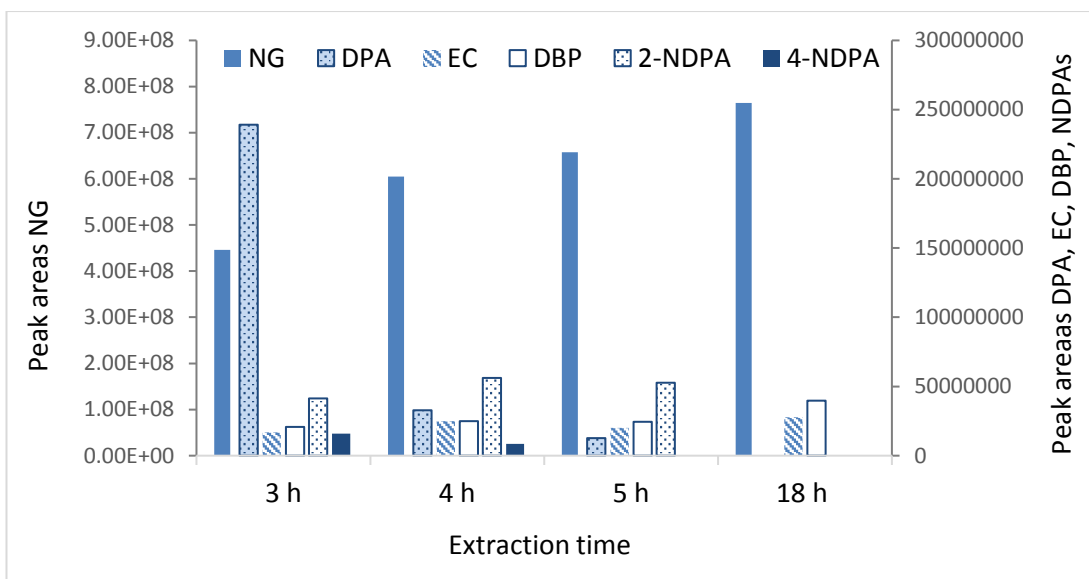
Figure 5.18: Optimisation of extraction time (n = 1)

This figure shows that the peak area of 2-NDPA initially increased, but after 18 h it was no longer detected. The peak areas of DPA and 4-NDPA steadily decreased as the extraction time was increased. This is in accordance with work done by Bohn et al. [193-195] in which the concentration of DPA and its nitro-derivatives were studied as a function of time and temperature. NC and NG are known to decompose at elevated temperatures, resulting in loss of NO and NO_2 groups. These groups accelerate further decomposition of NC and NG in an autocatalytic process. DPA functions as a stabiliser by reacting with these nitrogen oxides, thereby preventing them from reacting with NC and NG. [99, 150, 193, 194] This leads to the formation of nitro-derivatives of DPA, which act as stabilisers themselves [150, 193, 195]. The pathway for the formation of these nitro-derivatives of DPA was described by West et al. [150], which is shown in figure 5.19. The major products of this reaction are N-nitroso-diphenylamine (N-NsDPA), 2-NDPA and 4-NDPA. N-NsDPA and the other nitroso-intermediates, 4-nitroso-diphenylamine (4-NsDPA) and N-nitroso-2-nitrodiphenylamine (N-Ns-2-NDPA), are generally reported to degrade in the GC inlet, which explains why they were not detected. [150] As demonstrated by Bohn et al., the formation of DPA-derivatives accounts for the steady decrease of DPA and the initial increase of 2-NDPA. The experiments also demonstrated the subsequent decrease of the nitro-derivatives of DPA at elevated temperatures (65-90°C) over time. [193-195] It is likely that 2-NDPA and 4-NDPA were already present to some extent in the propellant prior to the extraction, given the fact that these compounds were also detected using methanol extraction, which involved no significant temperature increase.
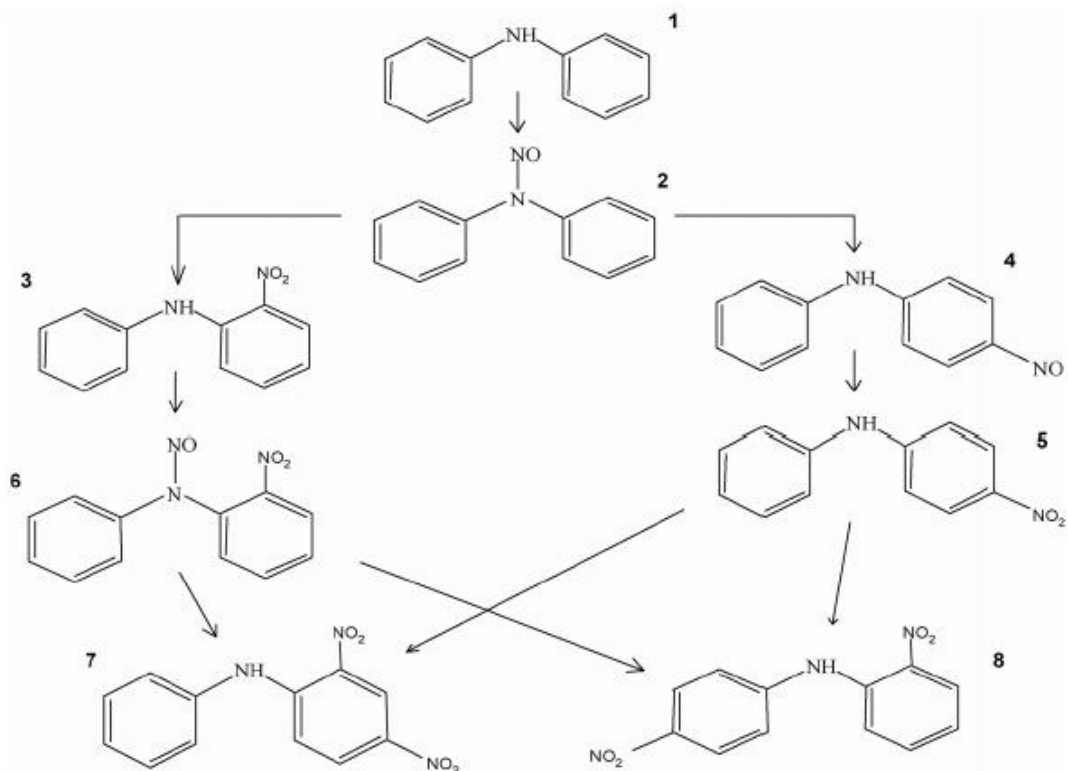
Figure 5.19: Dominating reaction routes for nitrosation of DPA in aging propellant powder: 1. DPA, 2. N-NsDPA, 3. 2-NDPA, 4. 4-NsDPA, 5. 4-NDPA, 6. N-Ns-2-NDPA, 7. 2,4-DNDPA, 8. 2,4'-DNPDA [150]

The continuous increase in NG, however, suggests that the mechanism of extraction may also play a role as opposed to further decomposition of NG. With SPME extractions, it has been observed that increasing the extraction time favours less volatile compounds and results in a reduction of more volatile compounds. It is possible that this principle also applies to the MonoTraps. Without the ability to assess the yields of NC, however, it cannot determine which of these factors is the (predominant) cause of the observed trend.

Figure 5.18 further shows a continuous but small increase in the peak area of DBP. The peak area of EC remained similar across the tested range. These results suggest that an extraction time of 4 hours may yield slightly higher peak areas for most of these compounds, however, the improvement is minimal. It could be argued that from a practitioner's point of view the shorter extraction time is superior over the small gain in peak areas, since all compounds could be clearly detected after 3 h. Moreover, creation of further NDPAs would be an undesirable side effect if linking propellants, and including relative OGSR ratios in that process, would become an objective. It demonstrates the need to employ a single analytical method to the analysis of samples that are to be compared with one another.

Due to this and the practical benefit of a shorter extraction time, an extraction time of 3 h was maintained. Given the minimal improvement, further optimisation of the extraction time was not attempted.

Optimisation desorption solvent

Previous experiments have shown that desorption with dichloromethane resulted in much greater peak areas than methanol, but was unable to desorb DPA and EPA. These compounds were desorbed by methanol, suggesting that the selection of desorption solvent is crucial and would have a significant impact on the recovery.

In the first step of the optimisation process several single solvents were tested, as well as a 50:50 mixture of methanol and dichloromethane (figure 5.20). During these experiments the standard extraction parameters (3 hour extraction at 80°C) were maintained and the desorption volume (200 µL) and sonication time (5 minutes) were kept consistent.
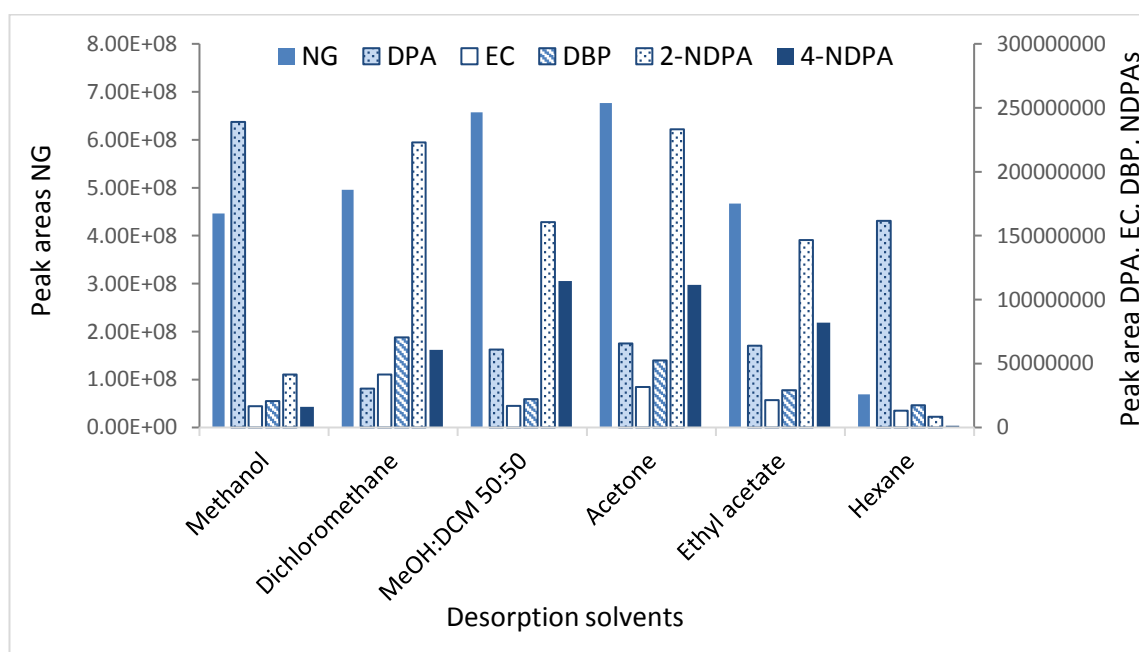
Figure 5.20: Optimisation of desorption solvent (n = 1)

Figure 5.20 clearly shows that hexane performed the poorest as desorption solvent across all compounds except for DPA. Methanol desorption resulted in the greatest peak areas for DPA, but in relatively low peak areas for EC, DBP and the NDPAs. Of the other four solvents, ethyl acetate and acetone obtained the greatest peak areas for DPA, albeit much lower than the recovery of methanol and hexane. The greatest peak areas for NG and 4-NDPA were achieved with acetone and the mixture of methanol and dichloromethane. Acetone and dichloromethane

resulted in the greatest recovery of EC and 2-NDPA. The greatest recovery of DBP was obtained with dichloromethane. This makes acetone the preferred single solvent, with dichloromethane and the mixture of methanol and dichloromethane also showing some potential.

These results confirm that the selection of desorption solvent is crucial for the recovery of OGSR compounds, and that a single solvent is unlikely to achieve the best recovery across the range of compounds. Instead, a mixture of solvents that is favourable for the recovery of different OGSR compounds is likely to be the preferred solvent system. Consequently, a further comparison of methanol and DCM ratios is performed (figure 5.21), as well as an evaluation of dichloromethane and acetone ratios (figure 5.22), as these solvent systems yielded the greatest recovery across the tested compounds.
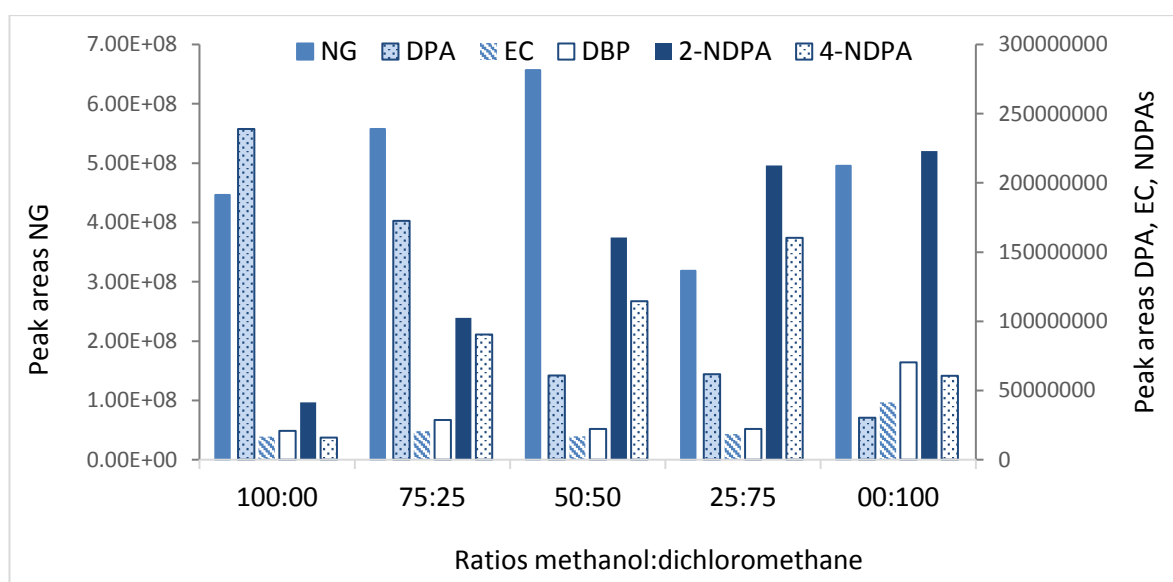
Figure 5.21: Evaluation of methanol and dichloromethane ratios (n = 1)
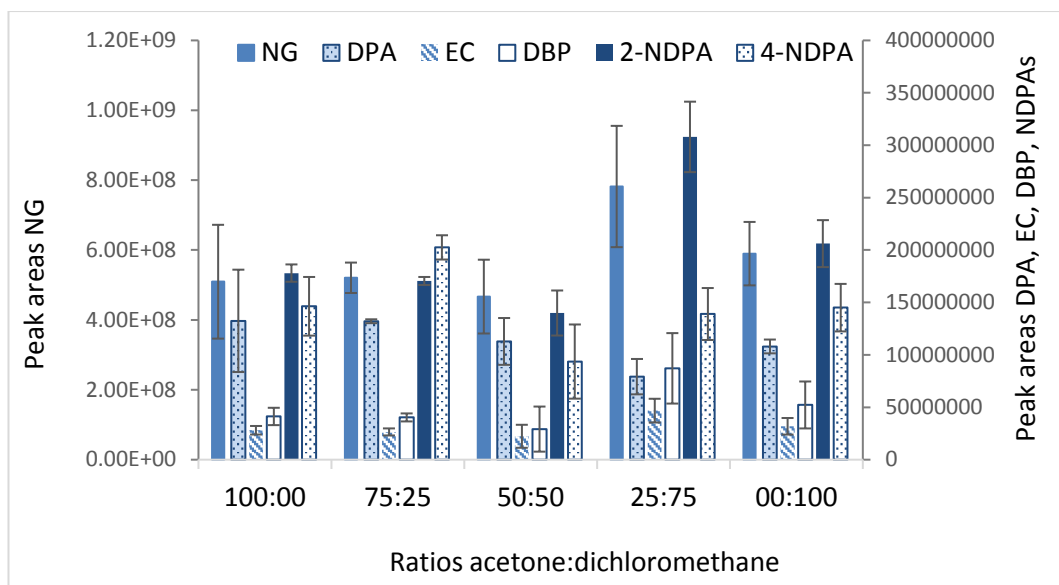
Figure 5.22: Evaluation of acetone and dichloromethane ratios (n = 3)

Figure 5.21 shows an improvement in peak areas for all ratios compared to 100% methanol, except for DPA. The greatest peak areas for NG were recovered using the 50:50 ratio. All other compounds showed an increase in peak areas as the dichloromethane part was increased. Dichloromethane as a single solvent resulted in the greatest peak areas for EC, DBP, and 2-NDPA.

The increase in peak area for most compounds as the dichloromethane part increases, was also observed for the mixture of acetone and dichloromethane. Figure 5.22 shows that a 25:75 ratio of acetone and dichloromethane achieved greater peak areas than 100% dichloromethane. It resulted in the greatest recovery of NG, EC, DBP and 2-DNPA, and in similar peak areas for 4-NDPA. Contrary to this, it resulted in the smallest peak areas for DPA, but it was still clearly detected. Consequently a desorption solvent of 25:75 acetone and dichloromethane was determined to be the best solvent system for the recovery of the range of OGSR compounds tested.

Optimisation of sonication time

The optimisation of the sonication time was evaluated for the optimised solvent system (25:75 acetone and dichloromethane) (Figure 5.23). The same extraction parameters (3 hours at 80°C) were maintained.
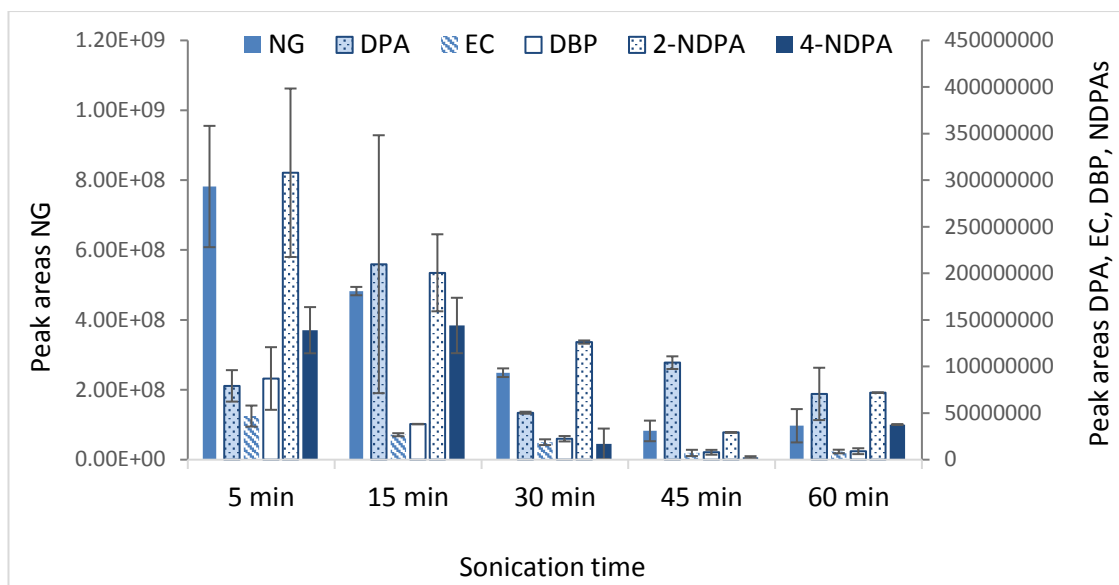
Figure 5.23: Evaluation sonication time (n = 2)

Figure 5.23 clearly shows that an extended sonication time does not improve the recovery of OGSR compounds. Except for DPA, the greatest peak areas are achieved after the recommended 5 min sonication. DPA resulted in greater peak areas after 10 min sonication, however, it was still clearly detected after only 5 min. Given the relatively high volatility of both dichloromethane and acetone, a shorter sonication time was preferred to limited solvent losses due to evaporation.

Overall, the optimisation process has shown that the ideal conditions for the extraction and desorption vary across the OGSR compounds. Therefore, the optimal method will be a compromise, and importance has to be assigned to OGSR compounds to be able to determine what the best compromise would be. Further optimisation involving more complex solvent systems or sequential desorption may eventually lead to more exhaustive recovery of the extracted OGSR compounds, however, a balance must be found between complex sample preparation and practical applicability of the method in real case work. Given the fact that significant improvement has been made with these initial steps, the optimised method will be compared against methanol extraction and SPME in section 6.3.5, before further optimisation will be considered. This optimised method employs 3 h extraction at 80°C and desorption using 200 µL of 25:75 acetone and dichloromethane and 5 min sonication.

5.4   Conclusion and further work

On-solvent extraction using the silica disc yielded the best results for the extraction of OGSR compounds from standards. For unburnt propellant, however, headspace extraction using the carbon disc achieved the best results. Optimisation of this protocol showed that the desorption solvent is the most crucial optimisation step. The highest abundances were recovered using 25:75 ratio of acetone and dichloromethane.

The optimisation of the desorption process further showed that the optimal conditions vary across the range OGSR compounds. This demonstrates the importance of selecting target compounds for analysis.

6 OGSR analysis using solid-phase microextraction

The aim of this chapter is to investigate which SPME fibre is more suitable for the extraction of OGSR compounds and to compare these results to methanol and MonoTrap extraction. The extraction principle is highlighted, and the properties of various available fibres and the rationale behind the selection of the fibre are provided.

6.1 Solid-phase microextraction

Solid-phase microextraction (SPME) was invented and developed by Pawliszyn and associates [196, 197], and has been commercially available since 1993 [198]. SPME is generally described as a solvent-free variety of SPE, and employs a fine fused silica fibre coated with a polymeric substance – the sorbent phase – to extract volatile organic compounds from their matrix [77, 81]. This description refers to the device employed in the first SPME experiment. The design of the employed SPME device is still based on the design of the first commercial device made by Supelco [198] and resembles a modified syringe [81, 199]. A schematic representation is shown in figure 6.1, which consists of two parts: the SPME holder (left) and the fibre assembly (right).
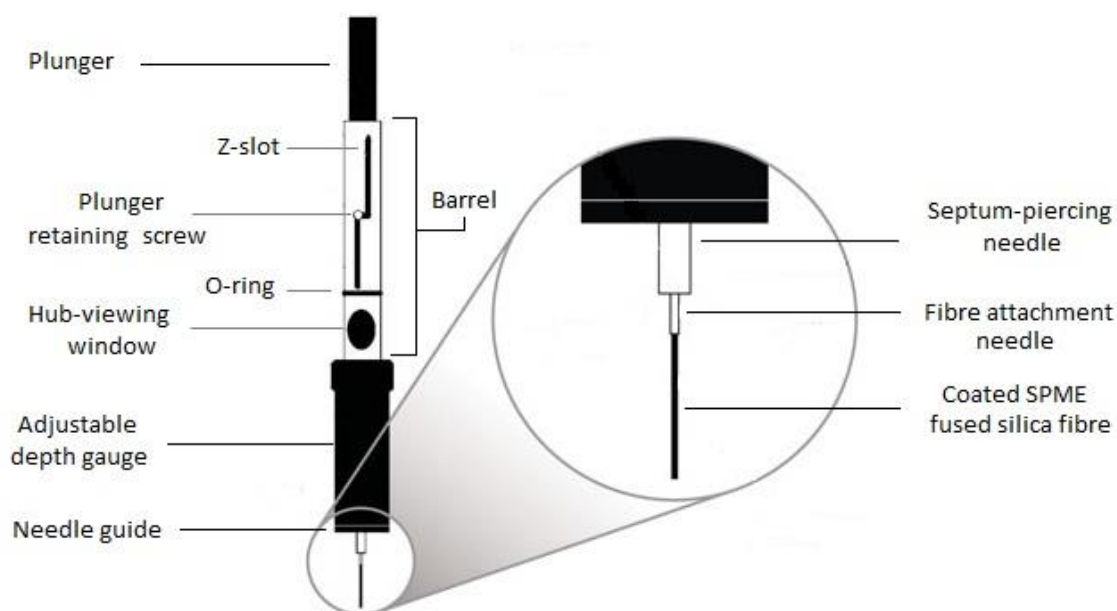
Figure 6.1: Schematic SPME device (adapted from [200])

The fibre is approximately 1 cm long and can be retracted inside the hollow septum-piercing needle, which functions as protective sheath. Mounted on the other side of the fibre configuration (not visible in figure 6.1) is a colour coded, threaded hub, which is attached to the

plunger when the SPME device is assembled. The colour of the hub indicates the type of fibre coating and can be seen through the hub-viewing window without disassembly, by lowering the adjustable depth gauge. This feeds the needle further into the depth gauge, as depicted in figure 6.1.

SPME can be used for both direct immersion (liquid and gaseous samples, figure 6.2a), and headspace sampling (solid and liquid matrices, figure 6.2b) [81, 201]. In headspace sampling – the method used during this study – analytes are extracted from the vapours derived from a sample in a partially filled, sealed container [81, 199, 202]. Consequently, in headspace sampling analytes are extracted from the headspace first, followed by indirect extraction from the sample matrix [202]. The extraction of analytes from one phase (sample or headspace) to another phase (headspace or fibre coating) is depicted in figure 6.2 using arrows.
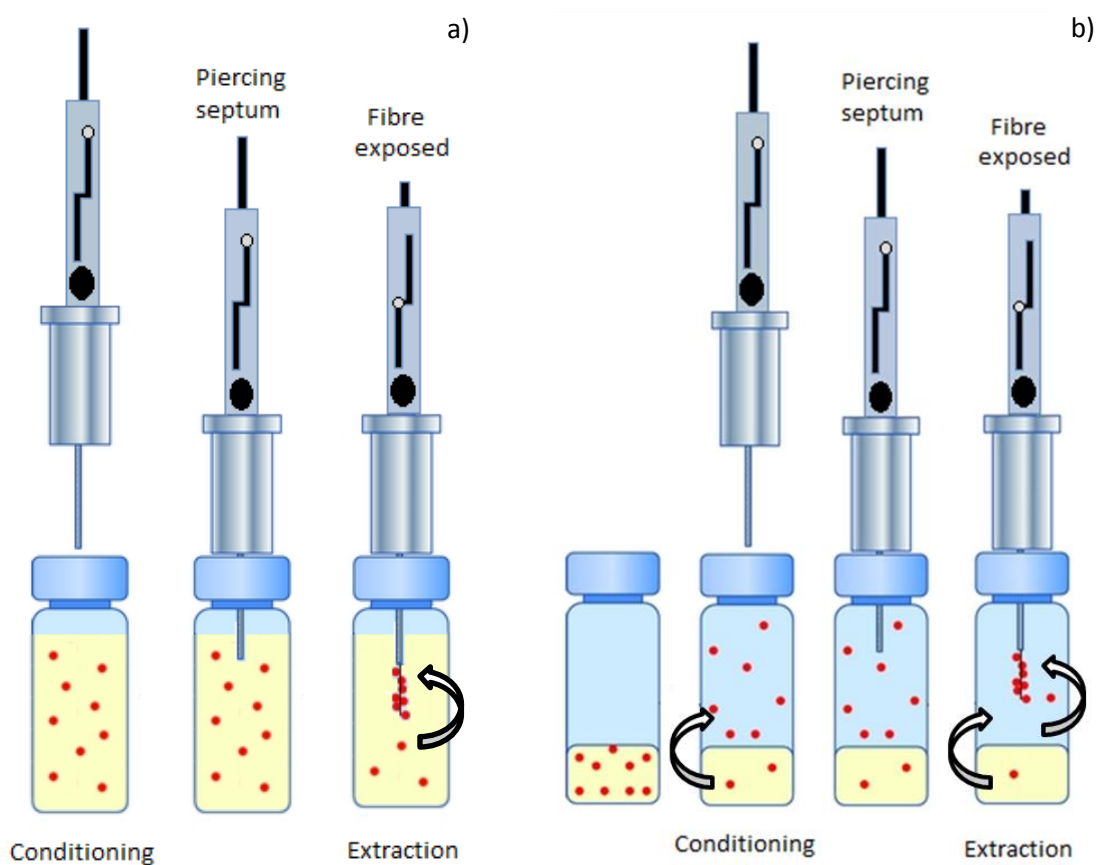
Figure 6.2: SPME extraction: a) direct immersion, b) headspace extraction (adapted from [203])

SPME allows the detection of trace levels of analytes by pre-concentrating the analytes onto the fibre and enabling subsequent analysis of all compounds collected [7, 204]. Desorption of the analytes from the fibre coating can be accomplished by desorption in a liquid or in flowing mobile phase via a modified multiport valve for liquid chromatographic techniques, or by thermal desorption for gas chromatography [81, 199].

6.1.1 Extraction principle

The principle of the extraction is based on the partition equilibrium of the analytes between the matrix and a small amount sorbent phase [202, 204]. This is particularly true for direct immersion SPME (figure 6.2a). In headspace SPME – the method used during this study – the analytes are extracted from the gas phase (headspace) equilibrated with the sample matrix. Consequently, the equilibrium constant of the analytes between the sample matrix and the fibre coating is a combination of the partition coefficients of the analytes between the sample matrix and the headspace (K_1) and between the headspace and the fibre coating (K_2) (figure 6.2b). These coefficients are dependent on the mass distribution ratio (D) of the analytes. Partition coefficients K_1 and K_2 can be derived from equation 6.1 and equation 6.2 respectively:

Equation 6.1: $$K_1 = D_1 \cdot \frac{V_1}{V_2}$$

in which D_1 is the distribution ratio of the analytes between the sample matrix and the headspace, V_1 the volume of the sample matrix (aqueous), and V_2 the volume of air/headspace.

Equation 6.2: $$K_2 = D_2 \cdot \frac{V_2}{V_3}$$

in which D_2 is the distribution ratio of the analytes between the headspace and the fibre coating, V_2 the volume of the headspace and V_3 the volume of the fibre coating. [201]

The equilibrium constant of the analytes between the sample matrix and the fibre coating (K_3) can be derived from the above equations and is given in equation 6.3:

Equation 6.3:
$$K_3 = K_1 \cdot K_2 = D_1 \cdot D_2 \cdot \frac{V_1}{V_3} = D_3 \cdot \frac{V_1}{V_3}$$

Since the equilibrium constant of an analyte depends on its mass distribution ratio, equilibrium constants will vary from compound to compound. [8] When the equilibrium is achieved, continued exposure of the fibre will not lead to an increase in analyte concentration on the fibre. Consequently, at this point the SPME extraction is typically considered to be complete. [198]

6.1.2 SPME fibres

Extracted analytes can either be absorbed or adsorbed by the fibre, depending on the nature of the coating [202]. Different SPME sorbent phases exist, which can be homogenous, pure (liquid) polymer coatings such as polydimethylsiloxane (PDMS), or porous solid particles embedded in a polymeric phase such as a PDMS/divinylbenzene (PDMS/DVB) fibre coating [205]. In the case of liquid coatings, such as PDMS, compounds are absorbed and distributed into the whole volume of the coating. Solid coatings, such as divinylbenzene (DVB) [205], adsorb compounds onto the surface of the coating, reducing the diffusion coefficient of the coating [81, 202].

The different fibre coatings available vary in polarity and may be used to extract different compounds based on the 'like dissolves like' principle. For example, PDMS coatings are non-polar and thus suitable for the extraction of non-polar analytes, whereas the polar polyacrylate (PA) coating is used for the extraction of more polar compounds. [81, 205, 206] Both PDMS and PA may be used individually as a fibre coating.

PA is a highly polar, rigid material that is solid at room temperature. Therefore migration times of the analytes in and out of the fibre coating are slower than for liquid polymeric phases, causing longer extraction times [205, 206]. PA is used for the extraction of polar semi-volatile compounds [206], especially from aqueous samples [205].

PDMS is a liquid sorbent phase, available in different thicknesses (expressed in micrometres), and the most common non-polar coating. It is used for the extraction of small volatile compounds (100 µm) and non-polar volatile and semi-volatile compounds (7 µm and 30 µm respectively) [205, 206].

Blended coatings

PDMS and carbowax (CW) can be used in coatings containing porous particles, which can be embedded in such polymeric phases [205]. Blended coatings provide the advantages from both phases. [205, 206] The use of carbowax, however, has some disadvantages, as it is soluble in

water and tends to swell. Swelling may cause the coated phase to be stripped off the fibre when it is retracted in the septum-piercing needle. It is also sensitive to oxygen at elevated temperatures, which may cause oxidation of the fibre coating and make the coating powdery and easily removable from the fibre. [205, 206]

Despite a low mechanical stability of such combined phases, coatings containing porous particles have a high selectivity [205]. The pores in the solid particles can absorb and physically retain analytes that fit into the pores. Consequently, the pore size determines which analytes are retained; as a general rule for the extraction of compounds the diameter of the pore should be twice the size of the analyte [205].

Due to the presence of pores ranging from < 2 nm to > 50 nm in size, DVB can be used for the extraction of both small and larger compounds. It has a high degree of porosity, which increases its total capacity [205]. A disadvantage of DVB is that the coating is more fragile and can be stripped off the fibre [206]. The combination of carbowax with DVB increases the molecular weight range of the analytes that can be extracted. A bipolar PDMS/DVB coating provides a slightly better retention of smaller analytes than PDMS alone. The coating has shown good selectivity for the extraction of amines and alcohols. [205]

A coating blend of carboxen with PDMS (CAR/PDMS) is similar to PDMS/DVB, but is more suitable for the extraction of small molecules [206] due to the fact that its pores go completely through the particles. This facilitates a more rapid desorption of small analytes. Whereas PDMS/DVB is ideal for trapping C_6 to C_{15} analytes, CAR/PDMS is ideal for trapping C_2 to C_{12} analytes. Larger molecules are strongly retained on the particle's surface and are difficult to desorb. [205] A blend of CAR, PDMS and DVB is particularly suitable for trace analysis [7].

An overview of some commercially available fibre coatings is given in table 6.1 [7, 205-207]. The influence of the coating thickness and stability are discussed below.

Table 6.1: Summary of SPME fibre types

Coating	Coating stability	Max T	Polarity	Target compounds
PDMS 100 μm	Non bonded	280°C	Non polar	Volatiles (MW 60-275)
PDMS 30 μm	Non bonded	280°C	Non polar	Non polar volatiles (MW 80-500)
PDMS 7 μm	Bonded	340°C	Non polar	Non polar high MW (MW 125-600)
PA 85 μm	Cross-linked	320°C	Polar	Polar semi volatiles (MW 80-300)
CW/DVB 65 μm	Cross-linked	260°C	Polar	Polar volatiles (MW 50-300)
CAR/PDMS 85 μm	Highly cross-linked	320°C	Bipolar	Gases, low MW (MW 30-225)
PDMS/DVB 65 μm	Highly cross-linked	270°C	Bipolar	Volatiles, amines, nitro-aromatics (MW 50-300)
DVB/CAR/PDMS 50/30 μm	Highly cross-linked	270°C	Bipolar	Trace analysis (MW 40-275)

Coating thickness

The thickness of the polymeric coatings ranges between 5 μm and 100 μm [199]. Thicker fibres – which have a larger coating volume – can extract and retain more analytes and consequently yield a broader linear range [81, 206]. The volume of the fibre coating can be calculated by multiplying the area of the coated surface by the thickness of the fibre, as shown in equation 6.4:

Equation 6.4: $$V = (diameter\ fibre \cdot \pi \cdot length\ fibre) \cdot thickness\ fibre$$

in which V is the volume of the fibre coating, usually expressed in mm^3 or mL [204].

Thicker fibres are advantageous for the extraction of volatile compounds with low molecular weights [205, 206], as they enable the adequate retention of these compounds, which is a problem for thin (e.g. 7 μm) coatings [206]. Large molecules, however, have slower migration rates into and out of the fibre coating than small analytes, and thus require longer extraction times when using thick (e.g. 100 μm) fibres [81, 206]. Large analytes migrate more readily into thinner coatings [206], leading to an increased extraction efficiency with decreasing film thickness [205]. Since large analytes can be retained in thinner coatings if the analytes have an

affinity for the fibre, a fibre thickness in between 7 μm and 100 μm makes the best compromise between the retention of small volatiles and the extraction of larger analytes. [206]

Bonding

The thickness of the coating affects the ability of the coating to crosslink and form bonds with the fibre support, which determines its stability [206]. The fibre support consists of fused silica of the same type of chemically inert material as used to manufacture capillary GC columns, and is very stable even at high temperatures [207]. There are three classifications used to describe the fibre bonding: non-bonded, cross-linked and bonded. Only the latter contains bonds between the coating and the fused silica support [206].

Non-bonded coatings are stabilised, but do not contain any crosslinking agents. Consequently, these coatings have less thermal stability [205] and are not solvent resistant.

Partially cross-linked fibre coatings contain cross-linking agents, such as vinyl groups, which form crosslinks between the polymer chains of the coating. This makes the coating more (thermally) stable and more solvent resistant than non-bonded coatings. [81, 205, 206]

Bonded fibre coatings are the most stable due to the fact that the cross-linking agents form crosslinks within the coating, as well as crosslinks to the fused silica support [205, 206]. Because of increasing difficulty in forming bonds within thicker coatings, bonded phases must be thin (*i.e.* 7 μm PDMS). [206]

6.1.3 Fibre selection

The efficiency of the analyte extraction and subsequent desorption from the fibre depends on several physical characteristics, which need to be considered when selecting the fibre [205, 206]:
- o Molecular weight and size of the analytes;
- o Boiling point and vapour pressure;
- o Polarity of analytes;
- o Functional groups of the analytes and the fibre;
- o Concentration range.

These characteristics need to be matched to the properties of the fibre coating, such as film thickness, polarity and porosity [206].

A fibre thickness between 7 μm and 100 μm makes for the best compromise between the retention of small volatiles and the extraction of larger analytes. A bipolar phase enables the extraction of both polar and non-polar compounds. To ensure a good capacity a liquid polymer

phase or solid particles with a high porosity would be suitable. Additionally, a cross-linked or bonded sorbent phase increases the thermal stability and solvent resistance of the fibre, which provides practical advantages.

Making a further selection based on the physical characteristics of the compounds of interest is challenged by the fact that in the study described in this work the extraction and subsequent analysis of as many OGSR compounds as possible is a key element. This requires an extraction technique capable of extracting trace amounts of both small and larger analytes of varying polarities. Consequently, a bipolar coating such as PDMS/DVB, CAR/PDMS or DVB/CAR/PDMS may offer the best solution. CAR/PDMS and DVB/CAR/PDMS are better suited for the extraction and desorption of small analytes. PDMS/DVB is also capable of extracting larger compounds efficiently. Furthermore, this coating is particularly suitable for the extraction of amines and nitro-aromatic compounds, which concerns several known OGSR compounds. Based on this theory, PDMS/DVB may be a suitable fibre coating.

The performance of several fibre types – including the three bipolar coatings, PA and PDMS coatings – in the detection of 32 OGSR compounds has been previously evaluated [7]. It was reported that PDMS/DVB was the most suitable fibre type for the extraction of the OGSR compounds across the selected ammunition types. This confirms the PDMS/DVB fibre as a suitable fibre for the purpose of this study.   The PA fibre, however, appears to be the most frequently used fibre for OGSR extraction in the literature [30, 33, 36, 42, 77, 208]. Therefore, both fibres were tested.

6.2   Methodology

Two fibres, a 65 µm PDMS/DVB fibre and an 85 µm PA fibre (Sigma Aldrich, Bellafonte, PA, USA), were compared against each other with respect to the extraction of OGSR compounds from standard solutions and unburnt propellants.

6.2.1   Solvents and standards

OGSR standard 3, including 24 OGSR compounds, was prepared in methanol. It contained 25 µg/mL of AKII, camphor, carbazole, DBP, DEP, DIBP, DMP, DPA, EPA 2-NDPA, 4-NDPA, 2,4-DNDPA, EC, MC, NG, 2-NT, 3-NT, 4-NT, 2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, 3,4-DNT, and triacetin. This was further diluted in methanol to acquire a concentration of 10 µg/mL.

6.2.2 Acquisition of propellant powders

Hodgdon HP-38 propellant for self-loading ammunition was obtained from the Wellington Rifle and Pistol club (Skipton, UK). Nottinghamshire police provided .223 Magtech ammunition, of which the bullets were pulled using a kinetic hammer and the propellant powder decanted in plastic seal bags.

6.2.3 SPME-GC-MS method

The details of the used GC-MS instrumentation and conditions are provided in section 3.2.2. The SPME fibres were pre-conditioned inside the GC inlet at a temperature of 250°C for 20 min [7]. This was done in split mode (99:1) whilst the oven temperature was ramped to 150°C to help prevent column contamination. The sample was pre-conditioned by placing the vial inside an oven (Nabertherm) at 80°C for 30 min, in order to allow the compounds of interest to enter the headspace. The extraction was performed in the same oven at 80°C for 35 minutes [7, 30]. The analytes were desorbed inside the GC inlet at a constant temperature of 250°C for 5 minutes [30] in splitless mode, before the GC run was started. A summary of the GC-MS conditions is provided in table 6.2. A 0.75 mm internal diameter SPME injection sleeve (Supelco, Bellafonte, PA, USA) was used for all SPME samples to prevent band broadening. Before the extraction of every sample, the fibre was reconditioned in the GC inlet and a blank fibre run was performed to ensure no carry-over of compounds of interest occurred.

Table 6.2: Summary of GC-MS method

Oven profile (C)	Ramp (°c/min)	Hold Temperature (°C)	Hold time (min)
50°C	10	100	0
	5	180	2.50
	30	200	2.50
	30	300	2.00
Injection mode	Splitless		
Inlet temperature	250°C	**Scan mode**	Full scan
Flow rate	1.2 mL/min	**Scan range**	m/z 40 - 500

6.2.4   Headspace extraction

Initial evaluation of the performance of the SPME fibres was done by depositing a 10 μL aliquot of standard 3 on the bottom of a 10 mL glass headspace vial with a PTFE/silicone septum (Supelco, Bellafonte, PA, USA). Repeat extractions were performed on three different days (day 1, 2, and 5) in order to assess the repeatability.

Further comparison of the two fibres was based on the extraction of 50 mg samples of unburnt propellant, using the same vials and extraction method.

6.3   Results and discussion

The results of SPME extractions of the standards will be provided first, followed by the results of propellant extractions. At the end of each section, these results will be compared against the previously discussed extraction methods.

6.3.1   Evaluation of retention time repeatability

Initial evaluation of the performance of the SPME fibres was done using standards in order to eliminate the effect of sample variation.

The chromatograms of the extraction of OGSR standard 3 (concentration 10 μg/mL) using the PDMS/DVB fibre and the PA fibre are shown in figure 6.3 and figure 6.4 respectively.
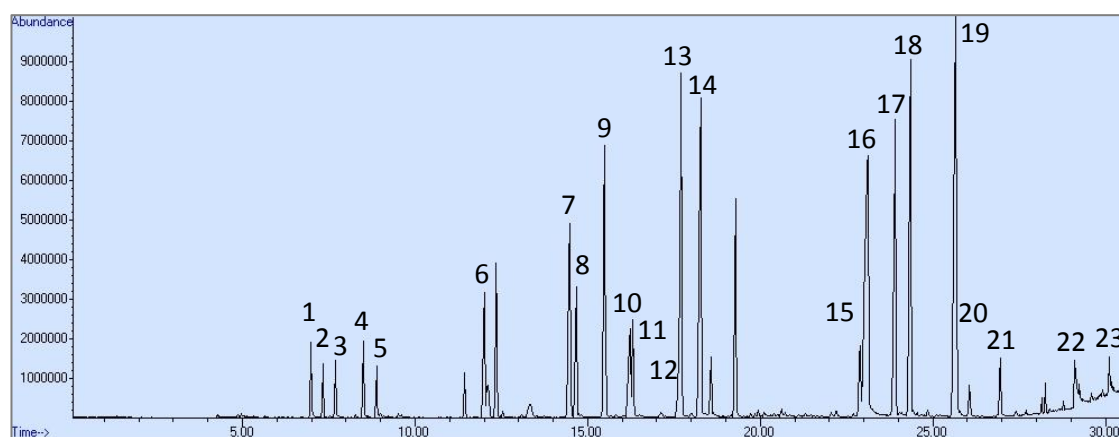
Figure 6.3: Chromatogram OGSR standard 3 extracted with a PDMS/DVB fibre: 1. EPA, 2. Camphor, 3. 2-NT, 4. 3-NT, 5. 4-NT, 6. Triacetin, 7. DMP, 8. 2,6-DNT, 9. 2,5-DNT, 10. 2,3-DNT, 11. 2,4-DNT, 12. 3,4-DNT, 13. DEP, 14. DPA, 15. MC, 16. Carbazole, 17. DIBP, 18. EC, 19. DBP, 20. 2-NDPA, 21. AKII, 22. 4-NDPA, 23. 2,4-DNDPA.
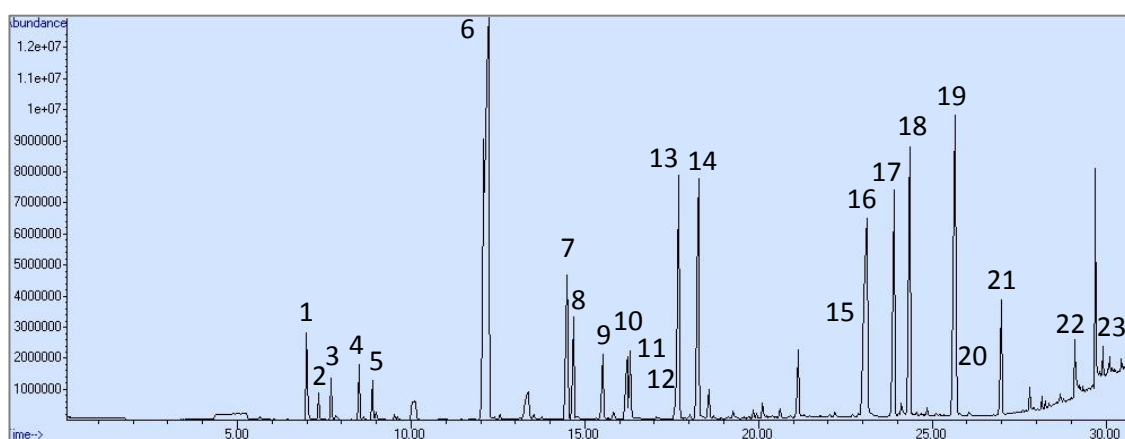
Figure 6.4: Chromatogram OGSR standard 3 extracted with a PA fibre: 1. EPA, 2. Camphor, 3. 2-NT, 4. 3-NT, 5. 4-NT, 6. 2,4-diisocyanato-1-methyl-benzene 7. DMP, 8. 2,6-DNT, 9. 2,5-DNT, 10. 2,3-DNT, 11. 2,4-DNT, 12. 3,4-DNT, 13. DEP, 14. DPA, 15. MC, 16. Carbazole, 17. DIBP, 18. EC, 19. DBP, 20. 2-NDPA, 21. AKII, 22. 4-NDPA, 23. 2,4-DNDPA.

Of the 24 compounds, only NG was not detected with either fibre. All other OGSR compounds were clearly detected following extraction with the PDMS/DVB fibre. The detection of triacetin using the PA fibre was hampered by an interfering peak that also occurred in the fibre blanks. Consequently, no accurate retention time or peak area could be determined.

The retention times and standard deviations are shown in table 6.3.

Table 6.3: Retention times OGSR compounds standard 3 using SPME (n = 3)

	Compound		PDMS/DVB RT (min)	Std dev (%)	PA RT (min)	Std dev (%)
1	Ethylphenylamine	EPA	6.989	0.04	6.998	0.03
2	Camphor	-	7.330	0.03	7.337	0.02
3	2-Nitrotoluene	2-NT	7.697	0.03	7.698	0.03
4	3-Nitrotoluene	3-NT	8.507	0.03	8.507	0.02
5	4-Nitrotoluene	4-NT	8.886	0.04	8.887	0.02
6	Triacetin	-	12.007	0.04	-	-
7	Nitroglycerin	NG	-	-	-	-
8	Dimethyl phthalate	DMP	14.492	0.05	14.498	0.06
9	2,6-Dinitrotoluene	2,6-DNT	14.679	0.04	14.683	0.03
10	2,5-Dinitrotoluene	2,5-DNT	15.526	0.06	15.524	0.02
11	2,3-Dinitrotoluene	2,3-DNT	16.233	0.05	16.226	0.02
12	2,4-Dinitrotoluene	2,4-DNT	16.319	0.05	16.314	0.01
13	3,4-Dinitrotoluene	3,4-DNT	17.617	0.00	17.617	0.00
14	Diethyl phthalate	DEP	17.717	0.05	17.718	0.02
15	Diphenylamine	DPA	18.277	0.04	18.268	0.02
16	Carbazole	-	23.046	0.08	23.070	0.04
17	Methylcentralite	MC	23.115	0.05	23.126	0.03
18	Diisobutyl phthalate	DIBP	23.916	0.04	23.915	0.01
19	Ethylcentralite	EC	24.357	0.02	24.356	0.02
20	Dibutyl phthalate	DBP	25.660	0.03	25.666	0.01
21	2-Nitrodiphenylamine	2-NDPA	25.682	0.02	25.688	0.01
22	Akardite II	AKII	26.962	0.03	26.996	0.02
23	4-Nitrodiphenylamine	4-NDPA	29.142	0.12	29.112	0.03
24	2,4-Dinitrodiphenylamine	2,4-DNDPA	30.117	0.04	30.107	0.01

The mean standard deviation of the retention times obtained with the PDMS/DVB fibre across the five days is 0.04%, for the PA fibre this is 0.02%. For both fibres the lowest standard deviation achieved is 0% for 3,4-DNT. The highest standard deviation obtained with the PDMS/DVB fibre is 0.12% for 4-NDPA, which could be due to an interfering siloxane peak. The next highest standard deviation is 0.08% for carbazole. The highest standard deviation obtained with the PA fibre is 0.6% for DMP.

Similar to the retention times obtained for direct injection (section 3.3.2) these retention times are subject to slight changes due to periodic column and inlet maintenance throughout the course of this project and were re-evaluated by analysing the OGSR standard following any maintenance on the instrument.

6.3.2   Evaluation of peak area repeatability

The peak areas achieved with both fibres following extraction of standard 3 (10 µg/mL) are shown in figure 6.5, the standard deviations are shown as error bars.
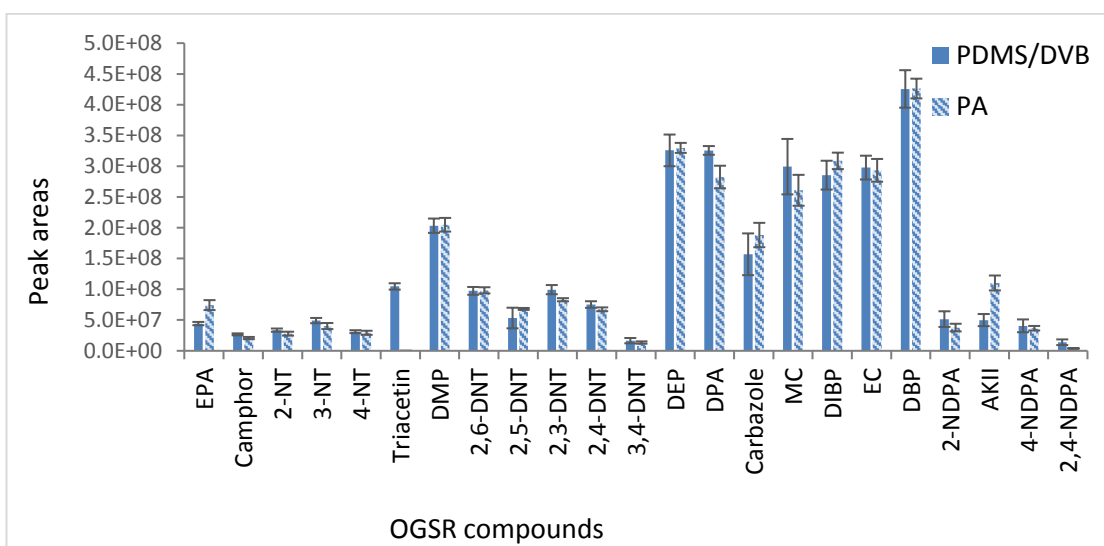
Figure 6.5: Comparison of peak areas obtained from standard 3 (10 µg/mL, n = 3)

Figure 6.5 demonstrates a good repeatability of both fibres; the average standard deviations for the PDMS/DVB and PA fibre were 12.9% and 8.5% respectively. The relative abundances for all compounds are similar between the two fibres, except for triacetin, of which no accurate peak area could be determined for the PA fibre due to an interfering peak.

It was noticed that the repeatability of the PA fibre rapidly decreased after repeated extractions. Mean standard deviations of 30% with a highest value of 70% could occur after as few as five to ten extractions. This is believed to be the result of fibre degradation, which could be observed by the black discolouration of the fibre. According to the manufacturer's information, PA fibre degradation can occur under the presence of oxygen at high temperatures, such as in the GC inlet. Frequent air and water checks were carried out in order to minimise this effect, however, a deviation of only a few percent from the optimum would already accelerate the degradation process. No adverse effect was observed on the PDMS/DVB fibres, which were used for up to 30 extractions. Consequently, the PDMS/DVB fibre is considered to be the more reliable and robust fibre.

6.3.3   Comparison with direct injection and MonoTrap extractions

The results obtained with the SPME extractions have been compared against MonoTrap extractions and direct injections of the standard. All retention times obtained with SPME were slightly shorter than the retention times obtained after liquid injections, varying from 0.7 s faster for the earliest eluting compounds to 0.07 s faster for the last compounds. Most notably is that

although MC and carbazole co-elute, MC elutes first following SPME desorption and elutes second after liquid injection. A possible explanation could be that an SPME run is not started until 5 minutes after the desorption of the compounds is started, which could provide a positive bias towards the slightly more volatile MC.

The performance of the SPME and MonoTrap methods in terms of the peak areas achieved are compared against direct injections of the standard in table 6.4. The peak area values are indicated in standard form for ease of comparison.

Table 6.4: Comparison of extraction capabilities of various methods (n = 3)

	Concentration standard	Volume	µg in sample	Mean peak area	Maximum peak area
Direct injection	25 µg/mL	1 µl*	0.025	3.10E+06	7.37E+07
MonoTrap: silica disc	5 µg/mL	1 mL	5	4.70E+05	2.36E+06
SPME: PDMS/DVB fibre	10 µg/mL	10 µL	0.10	3.11E+07	4.99E+08
SPME: PA fibre	10 µg/mL	10 µL	0.10	3.01+07	4.26E+08

* Volume for direct injection

The best performing MonoTrap for the extraction of OGSR compounds from standards was the silica disc. This extraction method, however, has resulted in the lowest overall yields and the lowest maximum peak area, despite a greater amount (in µg) present in the MonoTrap extracted samples.

Both SPME fibres achieved peak areas of one order of magnitude greater than direct injection, which is more than the difference in the total amount (per analyte) present in the SPME vial before extraction, compared to the direct injection volume. A pre-concentration step is required to detect all compounds via direct injection in standards of a lower concentration, with NG and nitro-DPAs being the first compounds to become undetectable. This clearly demonstrates that SPME is the superior method for the analysis of OGSR compounds.

6.3.4 Comparison of SPME fibre using propellant samples

Figure 6.6 shows the results of the SPME extractions (PDMS/DVB and PA fibre) of 50 mg of Hodgdon HP-38 propellant. The results of the extractions of .223 Magtech propellant are shown in figure 6.7. For both propellants, the major compounds are shown on the primary (left-hand) axis.
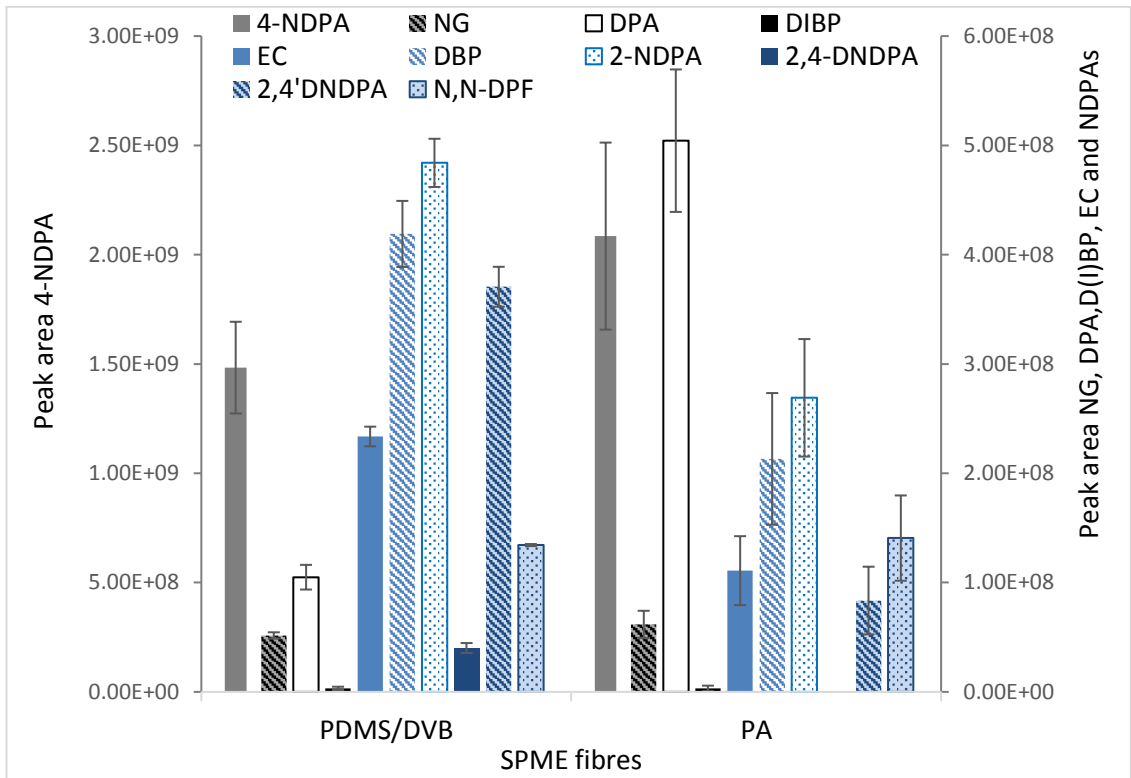
Figure 6.6: Peak areas SPME extractions of Hodgdon HP-38 propellant (n = 3)
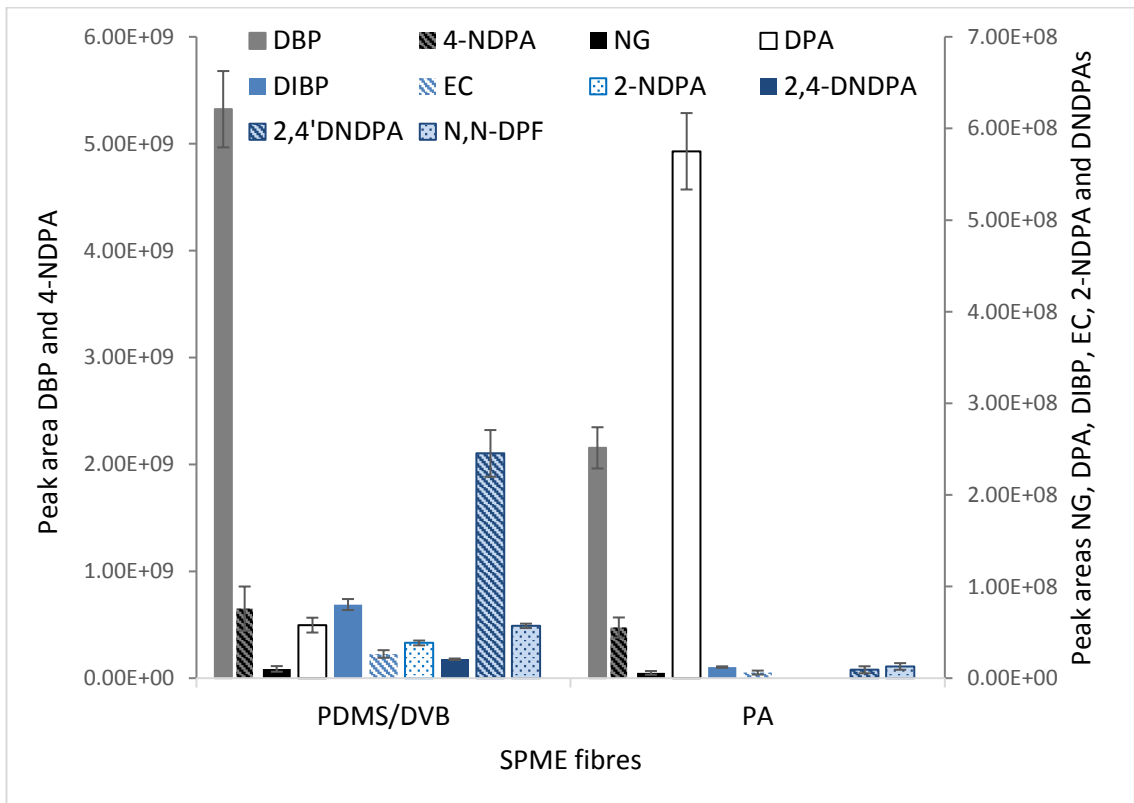
Figure 6.7: Peak areas SPME extractions of .223 Magtech propellant (n = 3)

The performance of the fibre types varied between the two propellants. For Hodgdon HP-38, the PA fibre extracted greater peak areas for 4-NDPA (major compound), NG, DPA and N,N-DPF. The PDMS/DVB fibre performed better for EC, DBP, 2,4'-DNDPA, 2-NDPA and 2,4-DNDPA, the latter two of which were not detected following PA extraction. Similar to the MonoTraps, AKII was not detected in any of the Hodgdon HP-38 extractions. DIBP, however, was extracted in this propellant, contrary to the MonoTrap and methanol results. For .223 Magtech, the PDMS/DVB fibre extracted greater peak areas for all compounds, except for DPA. The difference in DPA peak areas was one order of magnitude. N,N-DPF was extracted with both fibres from each propellant, in accordance with the methanol extractions.

The PDMS/DVB fibre consistently resulted in lower standard deviations of the peak areas, however, due to expected heterogeneity of the sample this cannot be attributed to the fibre performance with certainty. The PDMS/DVB fibre did perform significantly better in the extraction of the .223 Magtech propellant, extracting more OGSR compounds and greater peak areas than the PA fibre. Based on these results, and the fibre-degradation issues of the PA fibre (discussed in section 6.3.2), the PDMS/DVB fibre is considered to be the most suitable fibre type for further work.

6.3.5   Comparison extraction methods for propellant

The final comparison of the extraction methods is performed on propellant extracts given the fact that these provide a more realistic surrogate to OGSR samples, which may contain unburnt and partially burnt propellant particles. The comparison is based on extractions of Hodgdon HP-38 propellant (n = 3) using the best of the extraction methods: methanol extraction, for MonoTrap the carbon disc optimised method and for SPME the PDMS/DVB fibre. The data has been normalised in relation to sample size (figure 6.8).
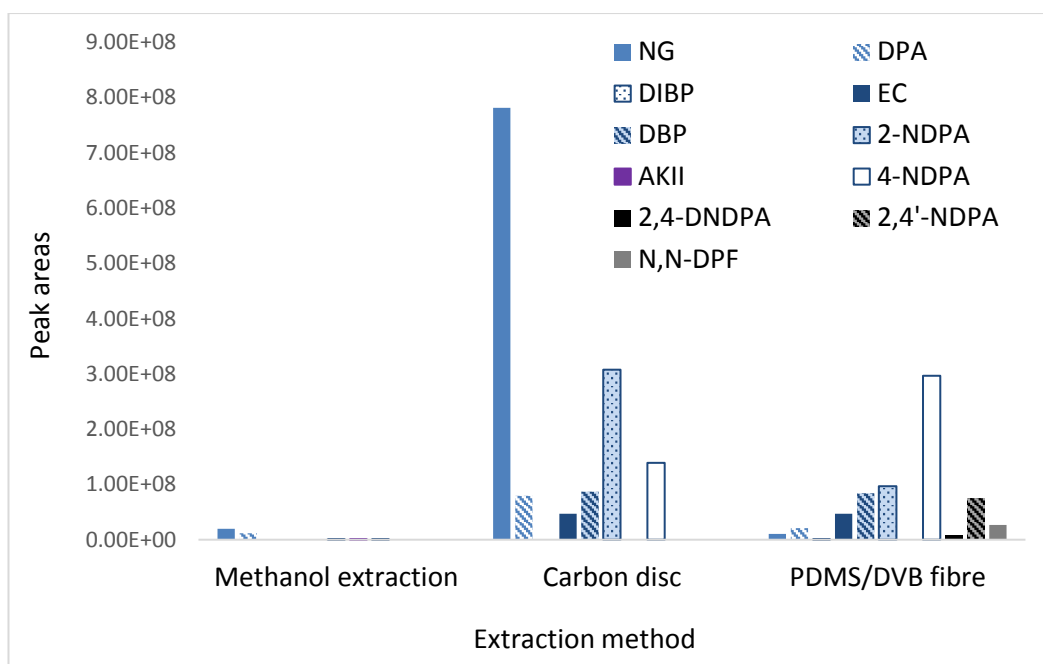
Figure 6.8: Comparison of methanol extraction, MonoTrap disc and PDMS/DVB fibre (n = 3)

As expected these results clearly show that methanol extraction without a pre-concentration step obtained the lowest peak areas for all compounds. It was, however, the only method with which AKII was detected. The optimised MonoTrap method using the carbon disc resulted in the greatest peak areas for NG, DPA, and 2-NDPA. Similar peak areas were obtained for EC and DBP using the carbon disc and the PDMS/DVB fibre. This fibre achieved the greatest peak areas for 4-NDPA and N,N-DPF, and it was the only method with which DIBP, 2,4-DNPA and 2,4'-DNDPA were detected.

The SPME extractions thus resulted in the detection of more OGSR compounds than methanol and MonoTrap extractions for both propellants. 2,4-DNDPA and 2,4'-DNDPA were detected in both propellants, and 2-NDPA was now also detected in the .223 Magtech propellant. Given the fact that these nitro-derivatives of DPA are formed as a result of NC decomposition, it is possible that their levels have increased due to the exposure to 80°C during extraction. West et al. mentioned 2-NDPA and 4-NDPA as major products of this reaction and suggested that further reaction with nitrogen oxides results in formation of 2,4-DNDPA and 2,4'-DNDPA [150]. Both of these are only reported as degradation products of DPA and NC reactions and not as additives of propellant powders [6, 36, 150, 209]. If these compounds were the result of the extraction temperature, they would be expected to be present in the MonoTrap extracts as well given the prolonged exposure to the same temperature. This, however, was not the case, which suggests that the detection of the additional compounds may be due to the superior extraction capability of SPME over MonoTrap, rather than the result of degradation during extraction. This

is supported by the fact that the majority of the NDPAs were only extracted with the PDMS/DVB fibre, which is particularly suitable for the extraction of amines and nitro-aromatic compounds.

Apart from the additionally detected NDPAs, both SPME fibres extracted a different combination of major compounds from each propellant. Methanol and MonoTrap extractions revealed that the major compounds of Hodgdon HP-38 and .223 Magtech are DPA and NG, and DBP, DPA, and NG respectively. The SPME results indicated respectively 4-NDPA, and 4-NDPA and DBP as the major compounds of these propellant types. This indicates that the extraction method has a significant influence on the ratios of the extracted compounds and this should be taken into consideration when comparing samples.

Overall, the results suggest that when sufficient quantities of propellant powder are available, methanol extraction would be the best method for characterisation. It was the only method with which AKII was detected, and the formation of nitro-derivatives of DPA, and loss of DPA itself in the process, is limited.

If smaller quantities are available for characterisation, the optimised MonoTrap method is the better pre-concentration step, given the peak areas it has achieved across all OGSR compounds. Problems with on-solvent extraction, however, indicate that it may be difficult to adapt this method to actual GSR samples, which is not expected to be a problem for the SPME method. Therefore, the performance of both extraction media were tested by analysing single grains of various propellants. This was done to mimic the low concentration of OGSR compounds expected to be present in real OGSR samples (up to 178 ng following 1-3 discharges [166]) as opposed to 10 mg of unburnt propellant. Grains from two different propellants were extracted with both methods. MonoTrap extraction using the optimised method resulted in the detection of NG and DPA from the 9 mm Federal Premium propellant. No compounds could be detected in the extract of the .357 PMC propellant. SPME extraction, however, resulted in the detection of seven OGSR compounds including NG and DPA from the 9 mm Federal Premium propellant. Four compounds were detected following the SPME extraction of .357 PMC. These results, which will be further discussed in section 7.3.3, clearly demonstrate that SPME with the PDMS/DVB fibre is more suitable for the trace analysis required for the detection of OGSR compounds from GSR samples. Furthermore, the straightforward implementation of this method to GSR samples makes this the extraction technique of choice for further work.

6.4 Conclusion and further work

SPME is the preferred method for the extraction of OGSR compounds from standards, with the PDMS/DVB fibre being the most robust and reliable fibre type. It should be noted, however, that consistent detection of NG was not achieved in any of the standard extracts (MonoTrap or SPME) whilst it was consistently detected via direct injection of the standard and in all propellant extracts.

The results have demonstrated that characterisation of propellant and analysis of trace levels of OGSR compounds are two different arenas. For larger quantities of propellant (*e.g.* 100 mg), methanol extraction is the preferred choice due to its ability to extract AKII and limited formation of (D)NDPAs. MonoTrap is suitable for characterisation of smaller quantities (*e.g.* 10 mg), given the fact that it accurately reflects the major compounds in propellants and produces good peak areas.

The results have clearly shown that to enable a proper comparison, the same extraction technique should be employed to all samples. Methanol extraction and the optimised MonoTrap method were found to be unsuitable for extracting OGSR compounds from small quantities of propellant (*e.g.* single grains). SPME using the PDMS/DVB fibre, however, was able to extract several OGSR compounds from single grains. This in combination with the fact that the SPME method can be applied to the OGSR samples collected with various media, such as swabs or stubs, without altering the method makes this the most suitable method for further work. Moreover, SPME can be applied to spent cases and gun barrels [33, 56, 77-79], giving it the greatest potential for linking propellant compositions to OGSR samples from for example a firearm, victim, or suspect.

A requirement for such linking is that the differences in compositions survive the firing process. Therefore, the next chapter will focus on a preliminary evaluation of the change in organic composition from unburnt propellant to GSR. To enable this, a sample collection method for OGSR from a shooter's hands is optimised.

7   Optimisation of sampling and detection of OGSR materials

The aim of this chapter is to optimise a method for collection and detection of OGSR samples, and to investigate potential change in OGSR composition from unburnt propellant to GSR.

7.1   Analysis of GSR materials

GSR samples are typically collected using carbon adhesive tabs mounted on stubs. These may be carbon coated to prevent charging of the sample, and achieve an effective SEM analysis [167, 168]. Definitive information on the classification of inorganic particles for SEM-EDX analysis is described in guidelines by ASTM [167] and SWGGSR [168]. The classifications indicate whether particles are deemed as being characteristic or indicative of GSR. Particles characteristic of GSR are defined as particles that are most likely associated with the discharge of firearm, and thus have a composition that is rarely found in particles of any other source. [167, 168] Indicative particles may be associated with the discharge of a firearm, but could also originate from other, unrelated sources [167]. These particles are referred to as being 'consistent with GSR' by SWGGSR [168]. The classification also takes into account contamination from environmental sources (*e.g.* lead particles), which may not be relevant to the confirmation of GSR materials [168].

Which combination of elements constitutes characteristic GSR depends on the type of primer (table 7.1). The most common primers for handgun ammunition are based on the *sinoxid* formulation, containing lead sthypnate, antimony sulphide and barium nitrate.

Table 7.1: Classification of IGSR compounds relevant for confirmation of GSR materials [167, 168]

Type of primer	Characteristic particle compositions	Indicative particle compositions
Sinoxid	- Lead, barium and antimony	- Barium, calcium and silicon (sulphur) - Antimony and barium (zinc, sulphur) - Lead and antimony - Barium and aluminium - Lead and barium
Antimony-free	- Lead, barium, calcium, silicon and tin	-
Lead-free	-	- Barium, calcium, silicon (sulphur) - Antimony and barium (zinc, sulphur) - Barium and aluminium
Non-toxic	- Gadolinium, titanium and zinc - Gallium, copper and zinc	- Titanium and zinc - Strontium

In contrast to IGSR analysis, there appears to be no set combination of sample collection, extraction and detection methods for OGSR analysis [9]. Moreover, the detection of complementary organic and inorganic GSR compounds from a single sample is particularly challenging. It requires the optimisation of a method that is not only capable of OGSR detection, but that is also compatible with the current method of IGSR analysis [167, 168] to increase the practical applicability of the method to actual casework.

Recently, several attempts have been made to develop a sample and extraction methodology for GSR, which enables the analysis of both inorganic and organic compounds from a single sample (table 7.2). These methods employed [69, 71], or intended to employ [113], SEM-EDX for IGSR analysis, followed by OGSR extraction. The focus of this chapter is the OGSR analysis, the combination with IGSR analysis is discussed in chapter 8.

Table 7.2: OGSR analysis of combined methods

Sampling	OGSR (solvent) extraction	OGSR analysis	Ref
Stubs	5.5 mL acetone	UPLC-MS	[69]
Wipes	5 mL methyl tert-butyl ether		
	5 min sonication, blown to dryness, reconstituted		
	in 200 μL solvent		
Stubs	Solvent extraction: 2 mL methanol (40%) acetone	LC-MS	[71]
	(40%) and acetonitrile (20%), 25 min sonication		
Stubs / swabs	Solvent extraction: 1 mL methanol, 15 min	LC-MS	[113]
	sonication		

In this work both swabs and stubs are evaluated for the collection of OGSR compounds, followed by SPME-GC-MS analysis. In the case of swabs, swabbing solvents may be used to improve OGSR collection. Table 7.3 provides an overview of swabbing solvents used for the collection of OGSR compounds from hands.

Table 7.3: Swabbing solvent used for OGSR collection from hands

	Solvent	Ref
Dry swab	-	[174]
Single solvents	Methanol	[6]
	Ethanol	[113, 127, 175]
	Isopropanol	[116]
	Acetone	[125]
Solvent mixtures	Ethanol 50%, water 50%	[176]
	Isopropanol 70%, water 30%	[69]

Regardless of the sample collection device used, some background levels are expected to be present. Potential interferences form the sampling device and in particular the sample matrix (*e.g.* the hands of a shooter) could complicate the analysis.

The analysis of OGSR compounds is further complicated by the low concentration of OGSR compounds expected to be present. The total amount of OGSR deposited on the hands of a shooter following the discharge of a firearm (1-3 rounds) is suggested to be approximately 10 ng [6, 69] up to 90-178 ng [166] (average of 4 OGSR compounds detected per sample). In order to improve the sensitivity of the OGSR compound detection and address the issue of potentially high background levels, a selected ion monitoring (SIM) method could be implemented.

7.2 Methodology

The optimisation of the collection, extraction and analysis methodologies for OGSR compounds has been performed using both standards and GSR samples. Three different sample collection media were tested, including stubs and two types of swabs. An SIM method was developed and compared against the full scan mode.

7.2.1 Solvents and standards

OGSR standard 4, including 22 OGSR compounds, was prepared in analytical grade methanol (Sigma Aldrich, St Louise, MO, USA). It contained 25 μg/mL of AKII, camphor, carbazole, DIBP, DMP, DPA, EPA 2-NDPA, 4-NDPA, 2,4-DNDPA, EC, MC, 2-NT, 3-NT, 4-NT, 2,3-DNT, 2,4-DNT, 2,5-DNT, 2,6-DNT, 3,4-DNT.

7.2.2 Acquisition of propellant powders

Alliant Unique propellant powder for self-loading ammunition was obtained from the Grange Pistol and Rifle club. Merseyside Police provided propellant from 9 mm American Eagle, 9 mm Federal Premium, .357 PMC and .357 CBC ammunition, which was obtained by pulling the bullets using a kinetic hammer and decanting the unburnt propellant.

7.2.3 Acquisition of GSR samples

GSR samples were obtained using two different firearm-ammunition combinations. GSR samples from 9 mm American Eagle ammunition were generated on the Merseyside Police range using a Glock 17 self-loading pistol. GSR samples from Alliant Unique propellant were generated by discharging self-loaded .38 rounds using an Alfa long barrel .38/.357 revolver at the Grange Pistol and Rifle club. The choice for a long barrel firearm was made in order to comply with the UK firearms laws (Firearms (Amendment) Act 1997 (chapter 5), Extension to section 5 (Part I, chapter 27) of the Firearms Act 1968: Weapons subject to general prohibition).

GSR samples were collected from the shooter's hands at time *t = 0 h* after discharge of two rounds of ammunition. Sample collection was performed using medical cotton swabs with wooden shafts, (Deltalabs, Barcelona, Spain) or polyester swabs with plastic shafts (Tx761 X100 Alpha, Fisher Scientific, Loughborough, UK), moistened with approximately 1 mL of methanol and ultra-pure water (50:50). After sampling, the heads of the swabs (± 2 cm) were removed and placed in an 1.5 mL Eppendorf extraction cup. Carbon adhesive tabs (12 mm) mounted on

aluminium stubs (Agar Scientific, Essex, UK) were also used for sample collection. Spent cases were collected in plastic seal bags. All samples were kept on ice until return to the laboratory where the samples were stored in a freezer at -18°C.

7.2.4 Qualitative comparison of OGSR compounds from various samples

In order to investigate whether it is possible to link GSR to its unburnt propellant using OGSR compounds, SPME extractions were performed on 10 mg and a single grain of unburnt propellant, 10 mg of burnt propellant, OGSR from spent cases and OGSR collected from the shooter's hands.

Burnt propellant samples were obtained by placing 10 mg of propellant powder in a line in a porcelain well-plate, and lighting it with a gas lighter using an electric ignition. The residue was then scraped off the well-plate using a clean spatula and transferred to a glass 10 mL headspace vial for SPME extraction.

7.2.5 SPME-GC-MS method

All samples were transferred to glass 10 mL headspace vials for SPME extraction. The details of the used GC-MS instrumentation and conditions are provided in section 3.2.2, the SPME GC-MS method is described in section 6.2.3. In summary, the PDMS/DVB fibre and sample were pre-conditioned at 250°C for 20 min and 80°C for 30 minutes respectively, followed by 35 min extraction at 80°C. A summary of the GC-MS conditions is provided in table 4.7. Details of the employed SIM method are provided in table 7.5, retention times were adjusted following maintenance using standard solutions.

Table 7.4: Summary of GC-MS method

Oven profile (C)	Ramp (°c/min)	Hold Temperature (°C)	Hold time (min)
50°C	10	100	0
	5	180	2.50
	30	200	2.50
	30	300	2.00
Injection mode	Splitless		
Inlet temperature	250°C	**Scan mode**	Full scan
Flow rate	1.2 mL/min	**Scan range**	m/z 40 - 500

Table 7.5: Details SIM method; ions indicated in blue are already included in the group

Group	Compounds	Parent ions (m/z)	Product ions (m/z)	Start time (min)
1	EPA	121.10	106.1, 77.1	6.50
2	Camphor	152.1	108.1, 95.1, 81.1	7.00
3	2-NT	137.1	120.1, 91.1, 65.1	7.40
	3-NT	137.1	91.1, 65.1	
	4-NT	137.1	91.1, 65.1	
4	Triacetin		145.1, 103.0, 43.1	10.60
	NG		151.0, 76.0, 46.1	
5	DMP	194.0	163.1	14.00
	2,6-DNT	182.0	165.0, 89,1, 77.1	
	2,5-DNT	182.0	165.0, 89.1	
	2,3-DNT	182.0	165.0, 135.1	
	2,4-DNT	182.0	165.0, 89.1, 63.1	
	3,4-DNT	182.0	63.1, 89.1	
6	DPA	169.1	168.1, 84.0, 51.1	17.80
7	MC	240.1	134.1, 106.1	21.00
	Carbazole	167.1	166.1, 139.1,	
8	DIBP		223.1, 149.0, 57.1	23.40
9	EC	268.1	120.1, 148.1	23.90
10	2-NDPA	214.1	180.1, 167.1	25.00
11	AKII	226.1	169.1, 168.1	26.20
12	4-NDPA	214.1	184.1, 167.1	28.60
13	2,2'-DNDPA	259.1	196.1, 167.1,	29.60
	2,4-DNDPA	259.1	167.1, 139.1	
	2,4'-DNDPA	259.1	260.1, 167.1	

7.3 Results and discussion

The optimisation of the SIM method was performed on both standards and GSR samples; collection media were compared using GSR samples only.

7.3.1 Single ion monitoring

Phthalates that co-eluted with known OGSR compounds (DEP and DBP) were not included in the SIM method, as their detection is not is significant due to their widespread prevalence [4].

Full scan and SIM resulted in overall peak areas in the same order of magnitude and comparable average standard deviations (6.50% for full scan and 5.17% for SIM) for the standards. SIM enabled the determination of the peak areas of NG, MC and 2-NDPA, which was hampered in full scan mode by co-eluting compounds. Moreover, 2-NDPA was often not detectable in GSR samples using full scan mode, but was successfully identified using the SIM method.

For the analysis of the OGSR samples, simultaneous SIM and full scan mode was employed to enable the detection of further compounds in the full scan chromatogram, such as DBP, which was a major compound in many propellants.

7.3.2 Comparison of collection media

No known OGSR compounds were detected in the extracts of the blank cotton swabs. Phthalates were detected in the blank extracts of the polyester swab (DIBP and DBP), the carbon adhesive stub (DIBP) and in the cotton swab left overnight in the Eppendorf cup (DIBP and DBP). This confirms that caution should be taken in interpreting the presence of phthalates in a sample [4].

Several swabbing solvents were investigated, including ethanol, acetone and isopropanol, however, methanol was the only solvent that was not extracted by the PDMS/DVB fibre. To prevent fibre saturation by the solvent, a mixture with methanol (methanol and ultra-pure water (50:50)) was selected as the swabbing solvent. This combination enables the collection of both organic and inorganic residues [176]. Additionally, the methanol water mixture is more skin-friendly and remains moist for longer, which enables a more thorough sampling of the entire surface.

The ability of both swabs to collect IGSR was compared based on single discharges of 9 mm American Eagle ammunition with a Glock 17 pistol. The shooter's hands were first sampled using a moistened swab and subsequently with a stub, which was analysed to determine the number of characteristic IGSR particles left behind after swabbing. Both the left and the right hand were sampled (n = 1) with each swab (table 7.6)

Table 7.6: IGSR left after swabbing

Swab type	Sample surface (n = 1)	No. characteristic particles
Cotton	Right hand shooter	87
	Left hand shooter	70
Polyester	Right hand shooter	32
	Left hand shooter	9

In comparison to stub sampling only, which yielded an average of almost 300 particles per hand for single discharges with this firearm-ammunition combination, both swabs appear to be capable of collecting IGSR. Table 7.6 indicates that more characteristic particles were left behind by the cotton swab than the plastic swab. It was hypothesised that this could be due to the application the two swabs were designed for; the cotton swab was designed for DNA sampling and the polyester swab for the capture of dust and small particles in a clean room, making the material and/or fibre structure of the polyester swab potentially more suitable for GSR collection [113]. Moreover, initial tests of the recovery of OGSR from spiked swabs indicated a greater recovery of OGSR from the polyester swabs. It is suspected that the loose, double layer of polyester enables the release of OGSR compounds more easily that the tight, compact cotton swab.

The first comparison of the three collection media was performed by collecting GSR samples from the shooter's right hand following discharged of 9 mm Federal Premium ammunition using a Glock 17 pistol. Samples were collected following two sequential discharges in an attempt to reduce sample variability. The results of the OGSR analysis per collection medium (n = 3) are shown in figure 7.1.
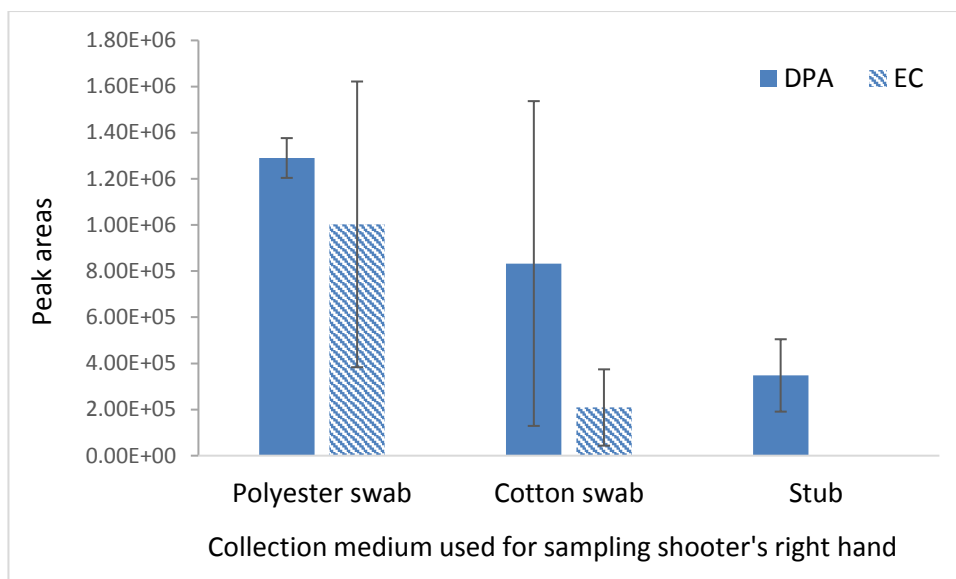
Figure 7.1: Comparison peak areas OGSR collected after discharge of two 9 mm rounds (n= 3)

The polyester swab yielded the best results due to the fact that EC was consistently detected in its extracts. EC was only detected to satisfactory levels (3 times s/n ratio) in two of the samples collected with the cotton swab. This could be due to variability in the amount and/or composition of the GSR created during the discharge [13]. EC was not detected in any of the stub extracts. Although three repeats provide only limited information in such a complex sample, it was suspected that the absence of EC could be due to interfering peaks with m/z 77, which was included in the initial SIM method as a peak of EC. This peak is likely caused by a benzene ring, a structure that is common in generic combustion products. Therefore, m/z 77 was removed from the SIM method to enable a clearer detection of EC.

In addition to these OGSR compounds, DIBP and DBP were detected in the full scan chromatogram of every sample, including the samples collected using a stub that did not have DBP in its blanks. This correlates with the presence of DBP as a major compound in the 9 mm Federal Premium propellant (unburnt).

The optimised SIM method was then applied to GSR samples generated by two discharges of an Alfa long barrel revolver with .38 special rounds that were self-loaded with Alliant Unique propellant. The results of the OGSR analysis per collection media (n = 3) is shown in (figure 7.2).
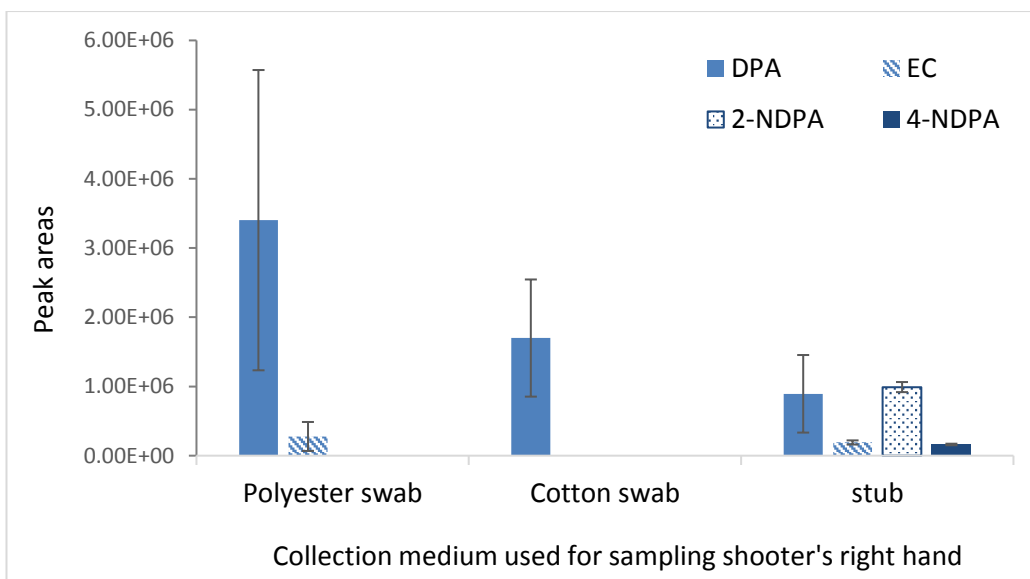
Figure 7.2: Comparison peak areas OGSR collected after discharge of two .38 spl rounds (n= 3)

Figure 7.2 shows that the polyester swab yielded the greatest peak areas for DPA and EC, similar to the 9 mm discharges. EC was not detected in two out of the three samples collected with the cotton swab, suggesting that this swab is less suitable for the collection of this compound. The greatest number of OGSR compounds were detected when using the stub for GSR collection. The additionally detected nitro-derivatives of DPA strengthen the value of the DPA detection with respect to the confirmation of GSR materials [4]. In contrast to the swab extracts, DBP was not detected in the stub extracts, which corresponds to the absence of DBP in the unburnt Alliant Unique propellant. Consequently, only sample collection using stubs can be used to determine whether DBP is a component of the propellant power.

Given the expected variability in the amount and composition of GSR due to the complexity of the firing process and the large number of parameters involved in the creation of gunshot residue [13], the semi-quantitative differences are not of a sufficient order of magnitude to contribute to differentiating between the potential of each collection device. Therefore, the detection of the greatest number of OGSR compounds makes the stub the preferred collection medium.

7.3.3 Qualitative comparison of OGSR compounds from various samples

In order to investigate whether differences in OGSR compounds observed in unburnt samples can survive the firing process, unburnt and burnt propellant samples were compared. All samples were extracted using SPME; characterisation of the propellant was done on 10 mg samples. This sample was used as a reference sample for the comparison of the OGSR

composition against both single unburnt grains and 10 mg burnt propellant. The results of two .357 propellant samples (n = 3) are shown in figure 7.3 and figure 7.4.
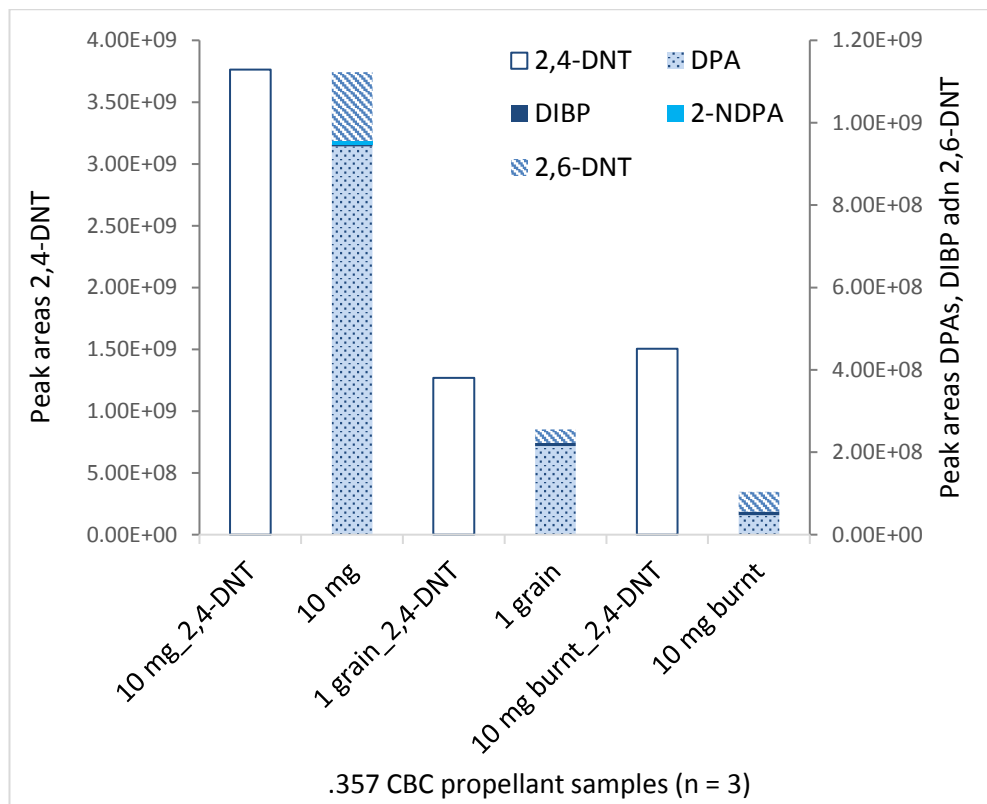
Figure 7.3: Comparison unburnt and burnt .357 CBC propellant samples

The relative levels of 2,4-DNT in all three samples of .357 CBC propellant were significantly higher than the peak areas of the other OGSR compounds present and are shown on the primary axis. The 10 mg unburnt sample only included one more OGSR compound, namely 2-NDPA. Both the single grain and the 10 mg unburnt sample showed the other four compounds and in similar relative abundances to the reference sample: 2,4-DNT, DPA, 2,6-DNT and DIBP respectively.
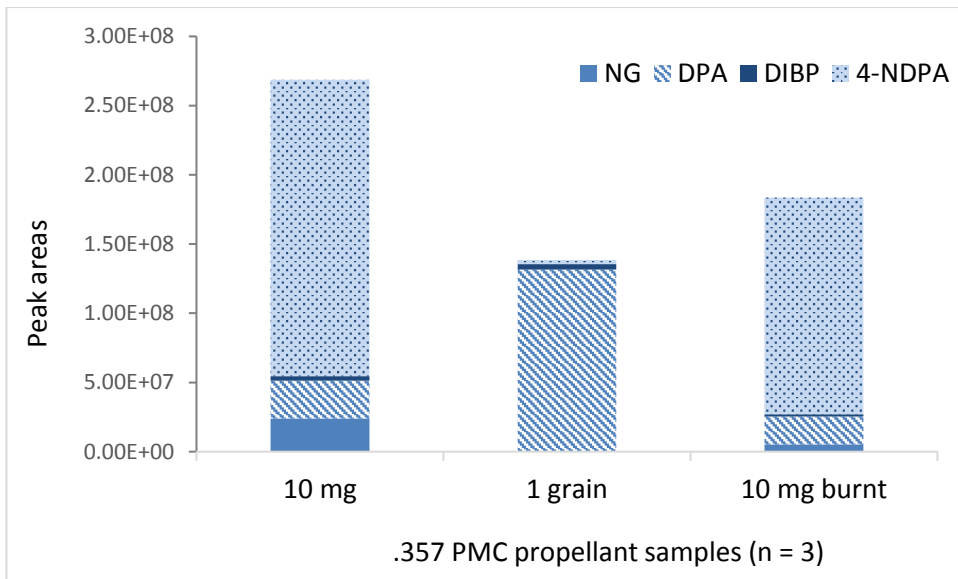
Figure 7.4: Comparison unburnt and burnt .357 PMC propellant samples

The unburnt and burnt 10 mg samples of the .357 PMC propellant showed the presence of the same four OGSR compounds in similar relative abundance. The single grain samples, however, did not show the presence of NG and showed DPA as the major compound of the propellant. Given the fact that OGSR samples may contain both unburnt and partially burnt particles, this result could complicate the comparison of OGSR with unburnt propellant.

In order to investigate this, OGSR samples collected from the shooter's hand and from spent cases are included in the analysis of two further propellant samples (figure 7.5 and figure 7.6).
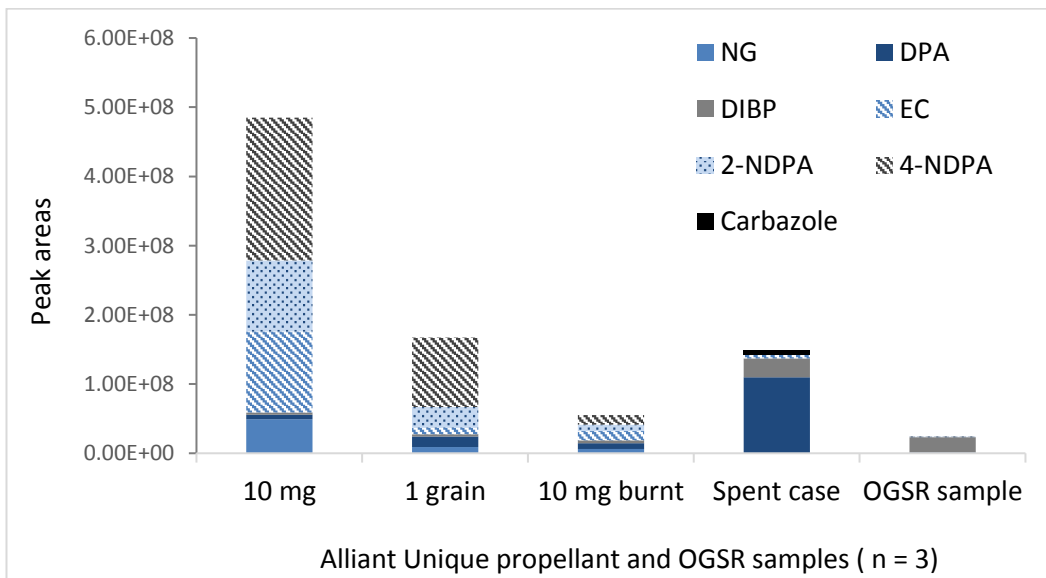
Figure 7.5: Comparison Alliant Unique propellant and OGSR samples

Figure 7.5 shows a similar trend in composition of the propellant samples, including the relative abundance of the present compounds. This composition is similar to the OGSR collected from the shooter's hands, which includes DPA, 2-NDPA, 4-NDPA, EC and DIBP, but not NG. The peak areas, however, are too small to be clearly shown in the graph and have different relative abundance. The spent case also resulted in different relative abundances, with the majority being contributed by DPA. Despite the fact that the spent case resulted in a greater combined peak area for all compounds, only three of the OGSR compounds (DPA, EC and DIBP) were detected in this sample. This was the only sample in which carbazole was detected.
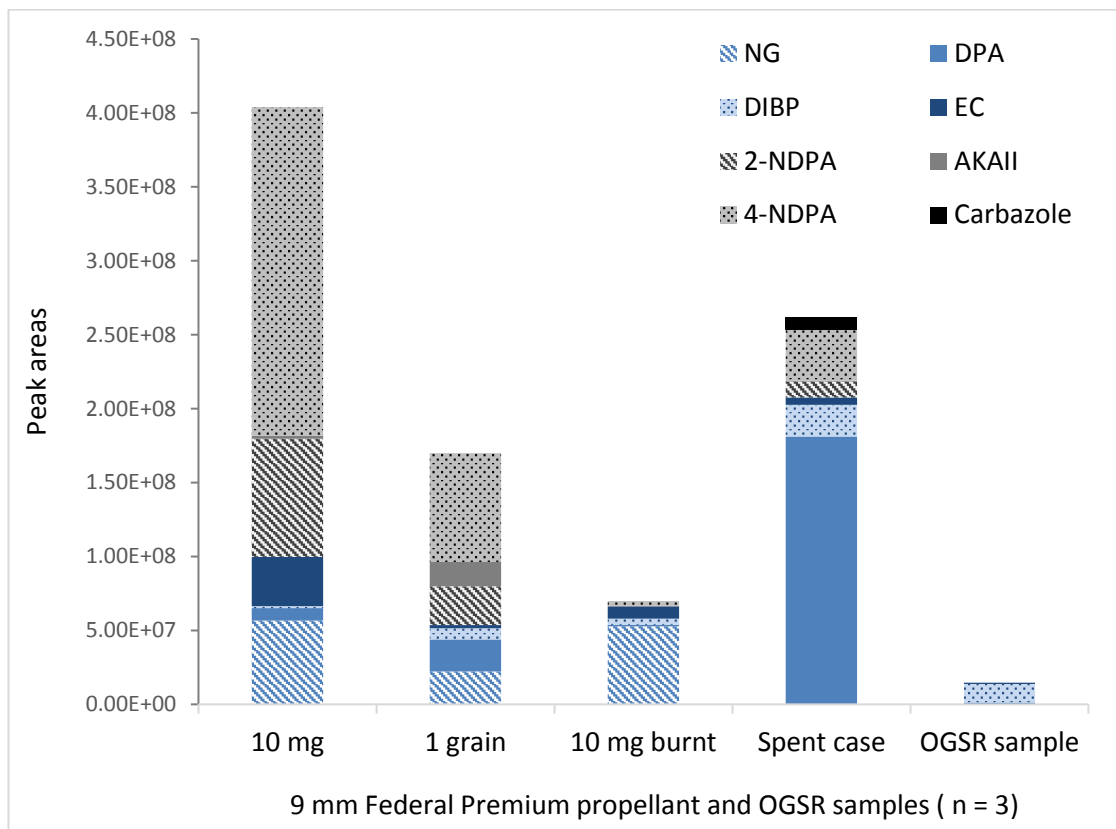
Figure 7.6: Comparison samples 9 mm Federal Premium propellant and OGSR samples

The majority of the OGSR compounds were detected in all samples, except for the OGSR samples collected from the shooter's hands. 2-NDPA and NG were not detected in the 10 mg burnt sample and the spent case respectively. Only three OGSR compounds were detected in the OGSR samples collected from the shooter's hands: DPA, EC and DIBP. Both the spent case and the OGSR sample show different relative abundances from the unburnt propellant samples, suggesting that linking of OGSR samples to unburnt propellant based on relative abundances is not possible.

When comparing the results of the each propellant to one another, only differentiation of .357 CBC burnt propellant was possible based on the presence of DNTs. Without a GSR sample, however, it is unconfirmed if these compounds can still be detected in the GSR. These results suggest that comparison of GSR samples with unburnt propellant may be better approached as an indicative method to exclude unburnt propellant based on the presence of compounds in the GSR samples.

The results consistently showed the highest abundance for DPA in the spent cases, which was present in both SIM and full scan mode in the same order of magnitude. Previous research has also shown DPA to be a major compound in spent cases [33].

Despite the GSR samples consistently having the lowest combined peak areas, some minor compounds (e.*g.* EC) were still detectable. The detection of EC and DPAs in combination with NDPAs suggests that this method can contribute to the evidential value of GSR evidence.

7.4 Conclusion and further work

These results have shown that OGSR detection using SPME-GC-MS in SIM mode can be achieved following sample collection with various collection media. Although the highest abundances were achieved with the polyester swab, the stub resulted in the detection of the greatest number of OGSR compounds. Employing a simultaneous SIM/scan mode furthermore enabled the detection of DBP using this sampling medium, as it was the only medium of the three investigated that did not have DBP in its blanks.

The composition of unburnt propellant appeared similar to burnt samples of the same propellant. Although relative abundances were similar across some samples, variations in other samples showed this to be an unreliable source for differentiation of samples. As expected GSR samples yielded the smallest peak areas overall, however, OGSR compounds were still detectable. These include EC, DPA and NDPAs. This showed some potential for the exclusion of ammunition types based on the presence of compounds in the OGSR samples, however, further investigation of the prevalence of such compounds should be carried out before this could be used with any certainty. The detection of EC and DPA with NDPAs does suggest that this method could be used to provide complementary evidence with respect to the conformation of GSR materials. This would require the developed SPME-GC-MS method to be compatible with SEM analysis for IGSR, which will be investigated in the next chapter.

8   Analysis of full chemical profile of ballistic materials

The aim of this chapter is to develop a sampling, extraction and detection protocol that enables the detection of both OGSR and IGSR from a single stub. Such a protocol looks to provide a full chemical profile of ballistic materials.

8.1   Combined analysis of OGSR and IGSR

To date, three papers have been published with respect to the combined analysis of OGSR and IGSR. Two of these papers [69, 71] focussed on the analysis of a single sample; one of these was a study of background levels of GSR in a police station [71]. The third paper involved collection of unburnt propellant particles from the target cloth, followed by IGSR collection using a stub [210]. In all papers, IGSR analysis was performed using SEM-EDX, which is currently the gold standard in GSR analysis [29, 211]. OGSR compounds were extracted from the same sample by solvent extraction of the stub following the SEM procedure [69, 71].

In contrast to this, a study of the stability of OGSR compounds on stubs showed that the highest degree of degradation occurred in the first four days of storage [212]. This suggests that OGSR analysis should be performed without delay, and that in case of sequential OGSR and IGSR analysis, OGSR analysis should be performed first to reduce storage time [212].

Recently, it has been proposed that OGSR may deposit in two different physical states: vapour and particle deposition [213]. Although data supporting this hypothesis is still missing, this would suggest that a part (the vapour deposition) of the OGSR compounds collected may be lost during SEM analysis due to the vacuum. A comparison of the stub sampling protocol with and without SEM analysis could provide some insight in the potential losses of OGSR during SEM analysis, however, this has not been investigated to date. Furthermore, the papers do not mention carbon coating the sample prior to IGSR analysis, which could hamper the subsequent extraction of OGSR compounds.

Both these problems could be avoided by extracting the OGSR compounds first. Additionally, this would address the issue of the limited storage time associated with OGSR compounds [212, 213]. To minimise the impact on the current standard method for IGSR analysis, thus increasing the practical applicability of this method to real casework, solvent extractions should be avoided.

The protocol suggested in this chapter involves sample collection using the carbon adhesive stubs, followed by OGSR analysis via SPME-GC-MS and subsequent analysis of IGSR

using the current standard SEM-EDX method [167, 168]. Before this complete protocol is evaluated, the effect of the SPME procedure on the IGSR analysis is investigated.

8.2   Methodology

SPME-GC-MS analysis of OGSR compounds collected with a stub was combined with IGSR analysis using SEM-EDX. IGSR analysis was performed by a colleague, Lauren Blakey, as part of collaborative work. Only the main parameters of this methodology are provided in this body of work.

8.2.1   Acquisition of GSR samples

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

All samples were collected at $t = 0$ using 12 mm carbon tabs mounted on aluminium stubs (Agar Scientific, Essex, UK). Samples were collected following two discharges (n = 3) for each ammunition type, whereby the shooter was standing upright holding the firearm with both hands. For the American Eagle ammunition samples were collected following singe discharges (n = 6) from both a standing and a prone position, whereby the shooter held the firearm with both hands. Before each test firing, the shooter's hands were cleaned thoroughly using isopropanol wipes. Blank samples were taken both from the shooter's hands and the sampler's hands. All samples were kept on ice until return to the laboratory where the samples were stored in a freezer at -18°C.

8.2.2   OGSR analysis

All stubs were transferred to glass 10 mL headspace vials for SPME extraction. The details of the used GC-MS instrumentation and conditions are provided in section 3.2.2, the SPME GC-MS method is described in section 6.2.3. In summary, the PDMS/DVB fibre and sample were pre-conditioned at 250°C for 20 min and 80°C for 30 minutes respectively, followed by 35 min extraction at 80°C. A summary of the GC-MS conditions is provided in table 8.1.

Table 8.1: Summary of GC-MS method

Oven profile (C)	Ramp (°c/min)	Hold Temperature (°C)	Hold time (min)
50°C	10	100	0
	5	180	2.50
	30	200	2.50
	30	300	2.00
Injection mode	Splitless		
Inlet temperature	250°C	**Scan mode**	Full scan
Flow rate	1.2 mL/min	**Scan range**	m/z 40 - 500

8.2.3 IGSR analysis

In order to investigate whether the SPME extraction at elevated temperatures affected the SEM-EDX analysis of IGSR, carbon tabs used for the collection of GSR following a single discharge (n = 2) were cut in half and mounted on two different stubs. Both halves of the first sample (left hand) underwent SEM-EDX analysis without prior heating to confirm that IGSR was spread more or less uniformly across the carbon tab. Of the other three pairs, the half labelled 'a' was first subjected to SPME extraction; the half labelled 'b' was analysed directly by SEM-EDX (table 8.2). The number of characteristic particles (consisting of lead, barium and antimony) on each stub were compared to evaluate whether IGSR analysis was affected by the heating of the stub.

Table 8.2: Samples used for investigation of effect of SPME procedure on IGSR

Sample no.	Sample location	Subjected to SPME procedure
1L-a	Left hand shooter	No
1L-b	Left hand shooter	No
1R-a	Right hand shooter	Yes
1R-b	Right hand shooter	No
2L-a	Left hand shooter	Yes
2L-b	Left hand shooter	No
2R-a	Right hand shooter	Yes
2R-b	Right hand shooter	No

IGSR analysis for the purpose of obtaining a chemical profile was performed on intact stubs following the SPME procedure for OGSR detection.

Carbon coating of the samples and IGSR analysis were performed according to the SWGGSR guidelines [168]. Instrument details and general parameters are given below.

In order to avoid charging of organic debris such as hair, fragments of epithelium, or particles of incompletely burnt propellant powder, the IGSR samples were coated with a conductive layer of carbon prior to SEM analysis. This was done using a Quorum Technologies Q150T ES Rotary pumped carbon coater.

IGSR analysis was performed using an SEM-EDX FEI Quanta 200. The process time and voltage were kept at 4 and 25 kV respectively. The spot size ($\sim$6), contrast ($\sim$34), and brightness ($\sim$90) were optimised to keep the input rate ($\sim$21) as close to 20 as possible, the acquisition rate ($\sim$14) to 15 and the deadline ($\sim$33%) below 35%. These values changed slightly between analyses, but no major divergence was observed during this work. Automatic identification of GSR materials was performed using INCA GSR analysis software, all characteristic particles contained lead, barium and antimony (table 7.1).

8.3 Results and discussion

In the proposed combined method, OGSR analysis using SPME is performed first. Consequently, the stub with carbon tab and IGSR compounds will be exposed to heat prior to SEM-EDX analysis.

8.3.1 Effect of stub heating

GSR samples collected from the shooter's hands following the discharge of a single round (standing position, firearm held in both hands) were cut in half and analysed separately to investigate the effect of stub heating on IGSR detection. To confirm similar amounts of IGSR were collected across the stub's surface, both halves of the first sample (1L) were analysed without prior heating. Of each of the three other samples one half was subjected to heating conform the SPME procedure before IGSR analysis was carried out. The number of particles detected on each half are shown in table 8.3, as well as the number of particles as a percentage of the whole stub.

Table 8.3: IGSR analysis of heated and non-heated samples

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

Guidelines by the ASTM and SWGGSR state that it is sufficient to analysis of a portion of the stub's surface, given the fact that GSR is collected randomly across the surface of the stub and does not tend to cluster [167, 168, 214]. Analysis of the two unheated stub halves of one sample (1L) confirmed this; the difference between the two stub halves was three particles or 4%.

Similar particle depositions were detected on most stubs (1L, 2L and 2R), of which sample 2L had a greater number of characteristic particles on the half that was subjected to heating. Only sample 1R showed a greater variation in the number of characteristic particles detected, which is believed to be caused by cutting the stub in unequal halves.

Overall, these results suggest that heating the stub does not have a significant adverse effect on the subsequent IGSR analysis. Therefore, SPME-GC-MS analysis followed by SEM-EDX detection appears to be a viable option for obtaining a chemical profile of GSR samples.

8.3.2   OGSR analysis

The results of the organic profile obtained from GSR samples collected after two discharges (n = 3) for three firearm-ammunition combinations is shown in figure 8.1. The peak area of DPA obtained from the American Eagle ammunition is shown on the primary axis (left-hand side), the peak areas of the other compounds are shown on the secondary axis.

Figure 8.1: OGSR collected from the shooter's right hand after two discharges

Figure 8.1 shows that all stabilisers that were previously detected in a single grain of 9 mm American Eagle and Alliant Unique propellant (chapter 7) were also detected in their respective gunshot residues. Only 2,4-DNDPA, which was detected in two of the three single grains of Alliant Unique, was not detected. DPA was the only stabiliser detected in the 9 mm Federal Premium propellant. It is hypothesised that this may be caused by a more complete combustion process. The burn tests of the three propellants showed that burnt 9 mm Federal Premium propellant resulted in the lowest recovery for all stabilisers. The grains of this propellant were also amongst the smallest grains encountered during this project, which generally indicates that the propellant burns faster [156].

In addition to the compounds shown in figure 8.1, phthalates (DBP and DIBP) were detected, however, due to their generic nature they were not further considered.

Some of the error bars showed an increase in standard deviation between the repeats. This is mostly likely due to the complexity of the firing process leading to the creation of varying amounts and compositions of gunshot residue [13].

The results of the organic profile obtained from GSR samples collected after single discharges (n = 6) of American Eagle ammunition is shown in figure 8.2. The peak area of DPA is shown on the primary axis (left-hand side), the peak areas of the other compounds are shown on the secondary axis.

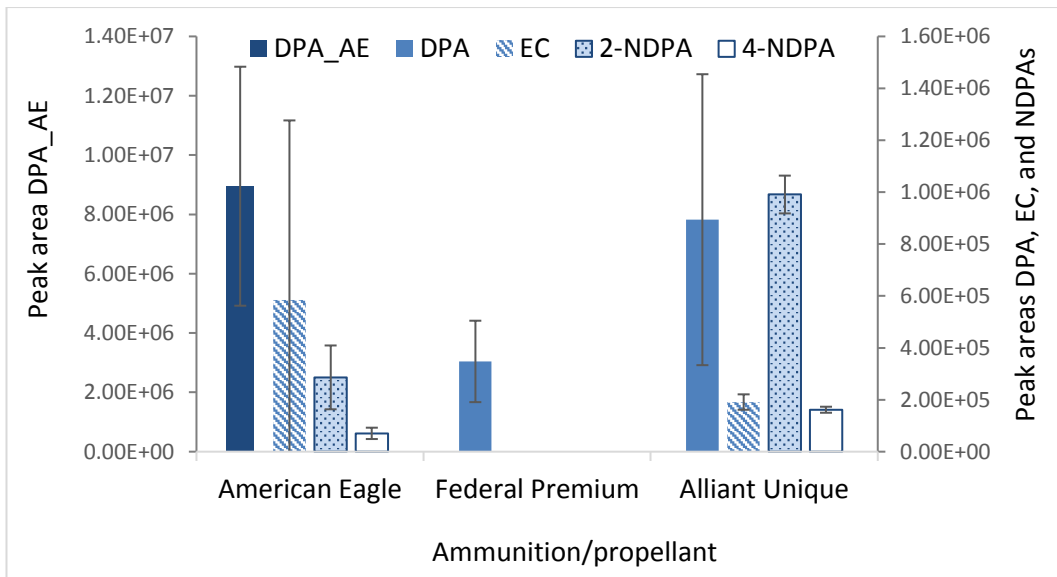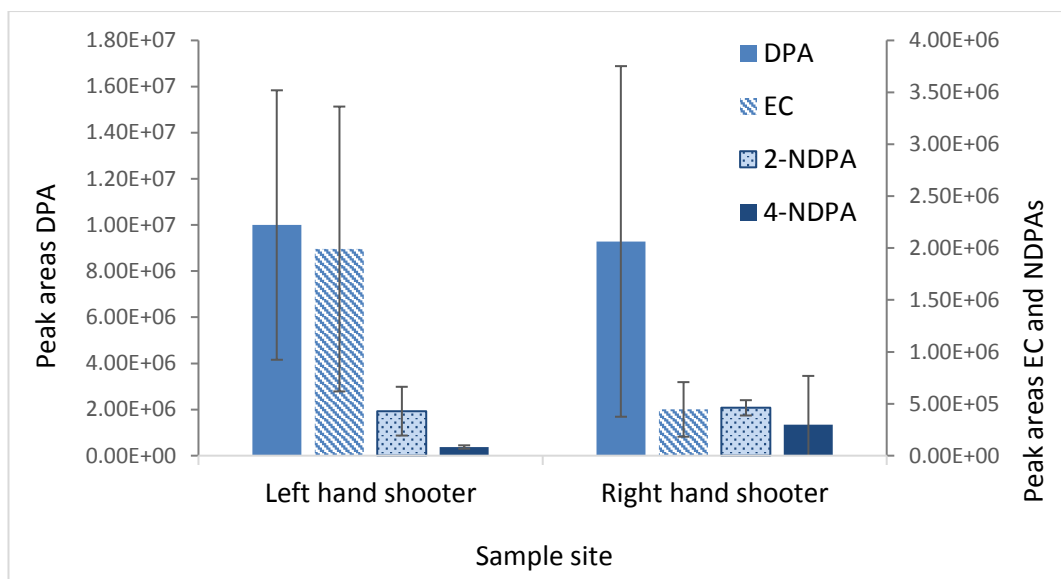Figure 8.2: OGSR collected from the shooter's hands after single discharges of American Eagle ammunition

Figure 8.2 shows similar peak areas for DPA and 2-NDPA on both of the shooter's hands, however, 2-NDPA was only detected in half of the samples. A greater amount of EC was detected on the left hand, but EC was not detected in sample 2 and 3 for the left and right hand respectively. In only two samples collected from the left hand was 4-NDPA detected, leading to the low peak area for this compound overall on the left hand.

The peak areas of DPA, EC, and 2-NDPA obtained from the shooter's right hand are comparable for both double and single discharges. The peak areas of 4-NDPA were greater following a single discharge. The lack of increase in GSR when doubling the number of discharges has been reported in literature before, and has been attributed to the inherent heterogenous nature of the deposition rather than the result of the sampling and extraction protocol [69, 166]. It is suggested that the persistence of OGSR on skin primarily depends on lipophilicity, and that the mechanisms of loss of OGSR are both evaporation and absorption by the skin [166]. The fact that samples were collected at t = 0 limited the time for these differences to exhibit. Moreover, the same two shooters performed the double and single discharges, and no pattern was observed that could be linked to either shooter. Since the discharges were performed in quick succession, it is hypothesised that the heat and pressure created during the second discharge potentially caused degradation and increased spreading of the OGSR particles that were still airborne. This could explain how similar amounts of OGSR were recovered for single and double discharges, and leaves the potential for an increase in OGSR as a greater number of rounds are discharged, or if more time is allowed between discharges.

8.3.3 Chemical profile

The results of OGSR analysis have been compared against the categorisation system proposed in table 4.11. The results of both double and single discharges are combined with results obtained from IGSR analysis in table 8.4 and table 8.5 respectively. Only characteristic inorganic particles (consisting of antimony, barium and lead) are taken into account.

Table 8.4: Chemical profile GSR of two discharges

	OGSR category 1	OGSR category 2	Not classified	IGSR
American Eagle	EC	DPA + 2-NDPA and 4-NDPA		Characteristic > 100 particles
Federal Premium	-		DPA	Characteristic > 100 particles
Alliant Unique	EC	DPA + 2-NDPA and 4-NDPA		Characteristic > 100 particles

Table 8.5: Chemical profile GSR of single discharge (American Eagle)

	OGSR category 1	OGSR category 2	IGSR
Right hand	EC	DPA + 2-NDPA and 4-NDPA	Characteristic > 100 particles
Left hand	EC	DPA + 2-NDPA and 4-NDPA	Characteristic > 100 particles

These results show that more than 100 characteristic inorganic particles were detected in all samples. For two propellants, additional categorised OGSR compounds were detected, including EC, which can strengthen the value of GSR evidence. The absence of categorised OGSR compounds in Federal Premium GSR samples suggest that the OGSR composition in GSR may be more variable than the IGSR composition. Given the relatively similar organic composition of the tested ammunition, however, it was not possible to differentiate the samples.

8.4 Conclusion and further work

The results have shown that a chemical profile of both organic and inorganic compounds can be obtained from GSR samples collected on stubs using the developed SPME-GC-MS method. The results have demonstrated that there is no adverse effect of the SPME procedure on subsequent SEM analysis. Characteristic IGSR in combination with categorised OGSR compounds were detected following one or two discharges. This demonstrates the ability to obtain a chemical profile of a single sample, and the potential of OGSR compounds to provide additional information without compromising the SEM analysis. EC and DPA in combination with both of

its nitro-derivatives were only detected in 13 out of the 51 characterised propellants in this work. This suggests that the presence of OGSR compounds provides complementary compositional information that could potentially be used to discriminate between samples. EC appears to be more suitable for the exclusion of unburnt propellant samples that lack EC, which concerns 22 out of the 51 propellants in this work. Further studies in the prevalence of OGSR in the environment should be undertaken in order to strengthen the categorisation system, which could then become a valuable tool to aid the confirmation of GSR materials by including OGSR information.

9 Conclusion and further work

This work has demonstrated that a chemical profile including both organic and inorganic information can be obtained from GSR samples using the proposed protocol. This protocol employs GSR sampling with carbon adhesive stubs, which are first analysed for OGSR using SPME-GC-MS and subsequently analysed with SEM-EDX for IGSR. Implementation of this protocol in real casework must be seen as feasible, given the fact that it requires no changes to the current sampling or IGSR analysis protocol other than storing the samples on ice once collected. Furthermore, by analysing OGSR compounds first, the limited storage time of these compounds is taken into consideration.

Detection of OGSR and IGSR compounds was achieved following discharges with three firearm-ammunition combinations. Discharges with both a 9 mm pistol and a .38 long barrel revolver resulted in the detection of several stabilisers, which are strongly associated with OGSR. Most notably are EC, of which its applications are restricted to use in ammunition, and NDPAs, which are thought to be specific to OGSR as they are formed by the reaction of DPA with the decomposition products of NC. This confirms the potential of OGSR to provide valuable complementary evidence to the traditional metallic residue. The OGSR compounds could be detected after as few as a single firearm discharge. Characteristic IGSR compounds were detected in all samples post SPME extraction, and no adverse effect of the SPME extraction on the IGSR analysis could be identified.

The achieved results are promising and show the potential for this work to be taken forward and further develop this protocol into a robust analytical methodology for the chemical profiling of ballistic materials. Some areas of further work have been identified below.

This work has focussed on a limited number of firearm-ammunition combinations, which should be expanded to ensure the applicability to a wide range of ammunition types and firearms. Including ammunition with greater differences in the organic composition of the unburnt propellant, and ammunition with lead-free primers would be of particular interest to gauge the extent of additional information OGSR can provide. This work should include a further investigation of the formation of nitro-derivatives of DPA during extraction, and possibly during delayed sampling or storage, in order to determine to what extent they could be used for some form of comparison between samples. In real cases, storage of samples prior to analysis is

inevitable, so optimal storage conditions and potential changes in compositions that may occur during storage should be evaluated.

Sampling in this work has been performed at t = 0. In order to investigate the results that can be achieved in actual casework, delayed sampling times should be investigated. Such experiments should account for normal behaviour and activity following the discharge rather than just desk related duties, and include handwashing. Furthermore, various positions of the shooter and the grip on the firearm could be investigated, and outdoor experiments should be carried out to account for the effects of wind and other weather conditions that may impact on the deposition. Further work involving sequential discharges could provide more knowledge on the accumulation, or absence thereof, of OGSR.

In addition to the shooter's hands, other areas might be suitable for OGSR collection and come less often in contact with other surfaces, limiting loss through secondary transfer. One suggestion could be sampling of the shooter's face, or potentially nasal passages. Removal of OGSR through washing also may be less likely on these surfaces. Furthermore, OGSR collection from the victim, bystanders or other surfaces at the scene of the crime could provide valuable information. For this purpose, the distance that characteristic OGSR can travel in detectable levels should be investigated.

Finally, further studies on the prevalence of OGSR compounds in the environment should be undertaken in order to build on the categorisation system. In combination with persistence studies, this could elucidate the true added value OGSR can provide to forensic investigations.

Overall, the work presented in this thesis has addressed some of the challenges associated with OGSR analysis and the desire to obtain a full chemical profile from a single GSR sample. This has led to a proposed categorisation system for OGSR compounds, which, when further developed by for example prevalence studies, may aid the move towards the inclusion of OGSR compounds in GSR analysis. A protocol for the collection, extraction and detection of both organic and inorganic compounds has been presented, which addresses several important practical implications. The protocol involves sample collection using stubs in accordance with current GSR collection protocols, followed by SPME-GC-MS analysis for OGSR compounds and subsequent IGSR analysis using SEM-EDX. This means that the OGSR analysis can be integrated with the current standard methods for IGSR collection and analysis. This in combination with the fact that GC-MS instruments are readily available in most analytical laboratories, make the actual

implementation of the OGSR analysis more likely. By analysing OGSR first, the limited storage time of OGSR compounds is taken into consideration as well.

Despite these promising results, an opportunity remains to further build on the categorisation system and to fully identify the potential of OGSR analysis for forensic casework through extensive evaluation of the persistence and prevalence of OGSR and the analysis of real-world scenarios.

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Appendices

Appendix A: Extended literature review

1. Artificial markers

Another area of approach to the identification of GSR is the development of artificial markers, which could be added to ammunition in order to create a characteristic marker for GSR. These may be luminescent markers that consist of a metallic-organic complex [1-3], the chemical composition of which is by design not commonly found in the environment or in occupational tasks [1]. Such markers could considerably simplify investigative routines by enabling the visual detection and identification of GSR at the crime scene with the aid of an ultraviolet light source [1]. Additionally, by using different tags, the markers can be used to differentiate, for example, between ammunition for civil, law enforcement, or military use [4].

A suitable marker should be thermally stable, chemically inert, have a high luminescence [2], not interfere with the ammunition's performance [5], and be of low cost [1]. Lanthanide-organic compounds meet all these criteria and are thus suitable as markers [4]. In an evaluation of the performance of these markers it was found that they remain luminescent for up to 30 months, persist on hands for about 9 hours and are only removed after 16 hand washes. It was also found that markers that were deposited on the hands post-firing could be transferred to other objects. The authors suggested that this opened new perspectives for forensic analysis by increasing the diversity of sampling surfaces. [5]

It must be noted, however, that the possibilities of further transfer from contaminated objects onto a third party – and the possible implications of this – have not been investigated, whilst the occurrence of such transfer could drastically impact on the implications of finding marker traces on an individual or object. Furthermore, the fact that these markers remain luminescent for 30 months could prove to be problematic, as it may challenge the linking of markers to specific events. Determining the time since discharge will be crucial to prove the relevance of the found marker traces to the case at hand, but this may be very difficult to establish once any amount of time of that order of magnitude has passed [6, 7]. The persistence of these markers potentially also allows the build-up of contaminated areas and objects over time, in particular if the use of markers would become common. This will cause increasing difficulties in the interpretation of such scenes and hamper the linkage of marker traces found at a scene to a specific incident, and thus potentially diminish the suitability and evidential value of these markers.

Another possible issue with the evidential value is the potential for marker compounds to be released during a discharge of unmarked ammunition following the firing of marked

ammunition with the same firearm, the so called weapon memory effect. This could also be an issue when ammunition marked with different tags is fired with the same firearm. Despite the fact that the occurrence of a weapon memory effect has been reported for both inorganic [8, 9] and organic [10] GSR compounds, investigation of these issues for the marker compounds has not been reported.

Detailed investigation of the toxicity of these metallic-organic markers is currently absent. One acute toxicity study [11] has been performed to date, for one of the markers: a europium-organic complex [3]. Toxicity after ingestion was investigated in duplicate for three rats per dose (50 mg/kg, 300 mg/kg, and 2000 mg/kg). Two of the three rats died within one hour after administration of the highest dose of 2000 mg/kg, which is the equivalent of approximately 5 mg for a 60 kg person. This would be enough marker for 160 rounds of ammunition. Despite the fact that it was found to be less toxic than inorganic compounds found in conventional ammunition, in particularly lead, acute adverse effects were observed after ingestion of the second highest dose (300 mg/kg), including disorientation, loss of motor-coordination and infrequent tremors. [11] Experiencing any of these symptoms whilst handling loaded firearms on a shooting range could have severe consequences. Compared to the highest dose, this would equate to the amount of marker used for 26 rounds of ammunition, a number that may be fired on a regular basis at a professional or recreational shooting range in a single session. Further toxicity studies will be required before the introduction of markers in ammunition can reasonably be considered safe.

Another major factor, on which the successful implementation of such markers hinges, is whether manufacturers will actually add these markers to their ammunition. Despite the fact that these markers are reported to be inexpensive, without legislation that calls for the inclusion of markers in ammunition, it is questionable whether manufacturers will add the artificial markers to their products.

2. Screening techniques

Another area of interest is that of on-site screening techniques. Growing security demands have generated the need for field-deployable, on-the-spot tools for rapid and reliable detection of gunshot residue [12, 13]. Such techniques could be employed to the rapid screening of a crowd in order to identify a subject who has loaded or discharged a firearm [12]. Two recently developed techniques are discussed.

Swipe and Scan

The so called 'Swipe and Scan'-method [12], which detects GSR based on metallic components, is a new application of a metal detection method reported in 1992 [14]. This method relies on abrasive stripping voltammetry, an electroanalytical development of voltammetry by Scholz and Lange in the early 90s.

It was based upon a preliminary mechanical transfer of trace amounts of a solid sample onto the surface of an electrode. The solid electrode consisted of a paraffin-impregnated graphite rod. Mechanical transfer of trace amounts of the sample was accomplished by gently rubbing one of the cylindrical ends across the smooth sample surface, such as a metal or crystal sheet. Once the sample was collected on the electrode, the rod was transferred to a conventional voltammetric cell for electrochemical measurement. The electrode was readily reusable after cleaning the contaminated area by rubbing it over clean abrasive paper. [14]

O'Mahony *et al.* [12] converted this method into the Swipe and Scan-method, by replacing the electrode rod with a screen-printed sensor strip. Sample collection from hands is accomplished by rubbing the sensor strip several times abrasively over the skin. Buffer solution is subsequently dropped onto the sensor strip, prior to the electrochemical measurement. The reported results demonstrated the possibility of detecting GSR on the hands of persons who fired or loaded a gun, even after hand washing. [12] No comments on the reusability of the sensor strips were made. The main limitation of this method is the fact that the detection is based on the presence of lead and copper, and to a lesser extend antimony. Therefore, it is uncertain to what extent this method can be used as an effective screening technique. Further development will be necessary to reduce the occurrence of false positives.

Forensic Finger

An improvement of the above method is the so called 'Forensic Finger', which has two major differences with the Swipe and Scan-method, making the method less cumbersome. The Forensic Finger consists of two single-use wearable fingertips (from a nitrile glove). The sensor is printed on the index finger tip, instead of on a strip, and the sample is collected by gently rubbing it across the sample surface. The tip for the thumb contains an electrochemical cell with a solid-state electrolyte, which eliminates the need for liquid handling. By bringing the two tips in contact with one another, the electrochemical cell is completed. The sample is then analysed using a field-portable electrochemical analyser. [13]

It was reported that the complete process can be carried out independently, and within four minutes. In addition to unspecified IGSR compounds, the Forensic Finger was used for the

detection of dinitrotoluenes. [13] Further investigation into the detection capability of specific inorganics and further organic compounds would be desired to improve the evidential value of such a screening.

Despite the fact that the Forensic Finger offers a few important practical improvements to on-site screening for GSR, further enhancement is still required before implementation is likely. Although no significant false positives or false negatives have been reported in either study [12, 13], the evidential value of the results is expected to benefit significantly from the inclusion of more organic compounds. In addition, an analysis time of four minutes is a great improvement from laboratory-based techniques, but is relatively slow for an on-site screening method. Moreover, with the need for two single-use finger tips per screening and an electrochemical analyser, the method still appears to be a little cumbersome and not very suitable to dynamic field analysis; the screening may take place on site, but a stationary, designated workplace may still be necessary.

Other techniques with screening potential

Due to the disadvantages highlighted in the electrochemical screening methods, the development of an on-site screening method that combines the practical requirements with an increased evidential value may lie in another type of technique. For example, further development of DESI-MS to enable the inclusion of multiple OGSR compounds could result in a more reliable, practically applicable method. Alternatively, further development of the screening capabilities of IMS could offer a better solution.

Both techniques may have the potential to become an ideal screening technique: a method that combines rapid screening with on-site confirmatory capabilities, possibly in a single run. In order to enable the confirmatory task, the number of false positives and false negatives of a DESI-MS method should be determined and reduced. This may be achieved by the inclusion of further OGSR compounds. The amount of false positives and false negatives of IMS compared to GC-IMS has already been investigated based on samples consisting of explosives mixed with interferents [15]. No difference in the amount of false negative results was observed, but IMS did have a higher amount of false positives (21 in 100, instead of 1 in 100). One could argue, however, that for the purpose of a rapid, on-site screening test the absence of false negatives is desirable over the absence of false positives as it increases the probability of including the subject, *i.e.* the shooter. It would also minimise the amount of samples that require retesting for confirmation.

IMS in combination with SPME has currently been developed as a rapid method for the screening of volatile (airborne) OGSR compounds in large spaces [16]. The generic sampling method of standard IMS, however, employs filters that are wiped across the sample surface. The reported detection limits for OGSR compounds are around 20 ng, however, for some compounds higher detection limits of above 100 ng or even above 600 ng have been reported [17]. Consequently, in order to make IMS suitable for on-site screening of deposited OGSR, development of the collection method to incorporate simultaneous pre-concentration of the sample may be desired. This would decrease the detection limits, reduce the amounts of false negatives, and possibly reduce the amount of false positives.

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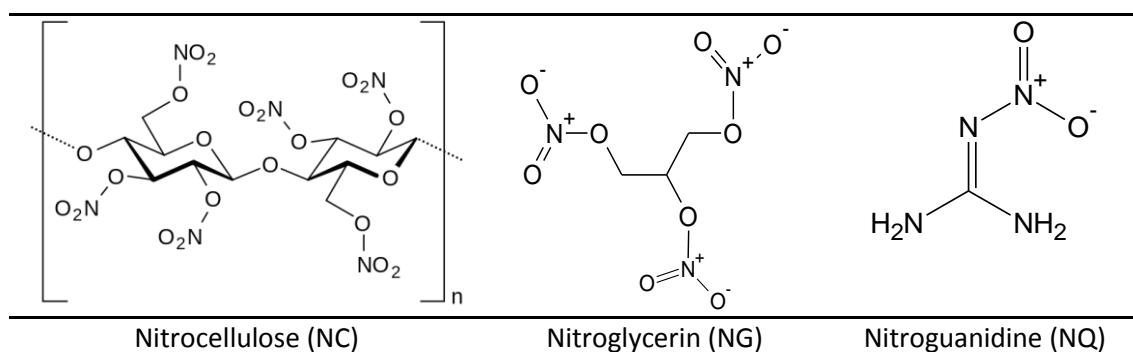# Appendix B: Molecular structures OGSR compounds

Figure B.1: Explosives / propellant base compounds
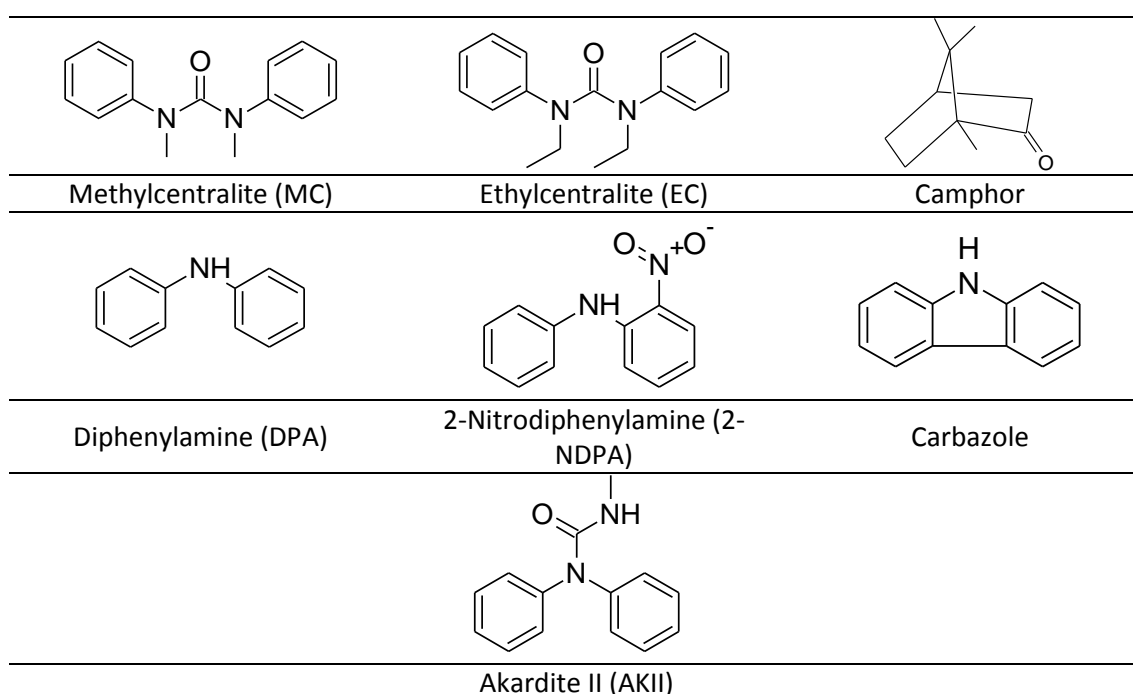
Figure B.2: Stabilisers
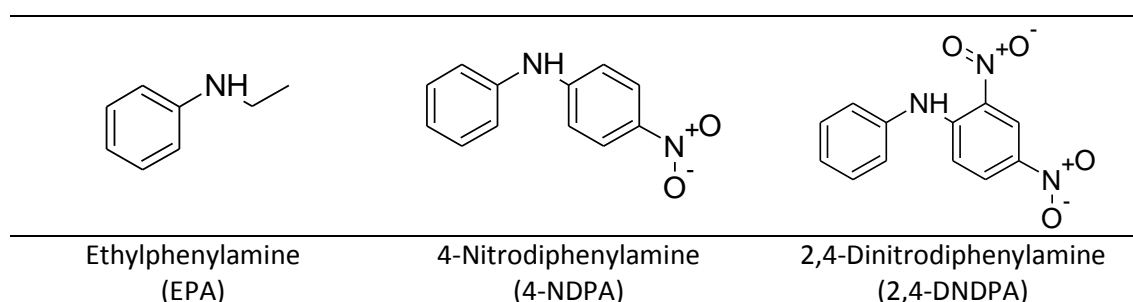
Figure B.3: Diphenylamine derivatives

| 2,4-Dinitrotoluene (2,4-DNT) | 2,3-Dinitrotoluene (2,3-DNT) | 2,5-Dinitrotoluene (2,5-DNT) | 2,6-Dinitrotoluene (2,6-DNT) | 3,4-Dinitrotoluene (3,4-DNT) |
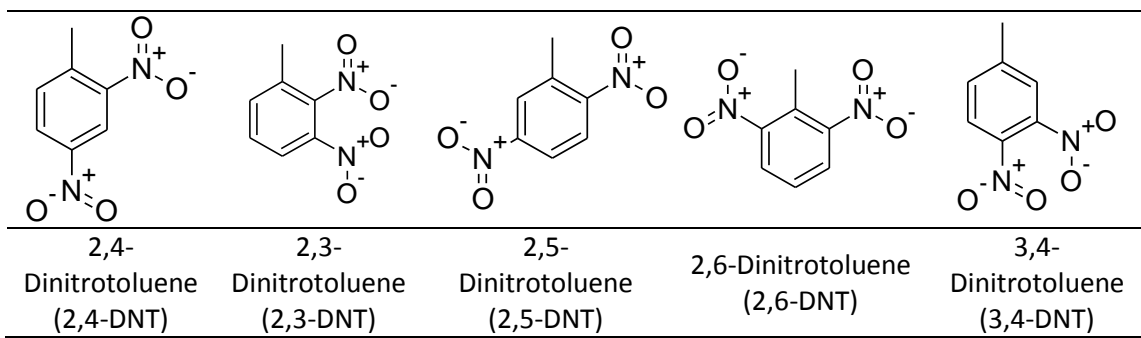
Figure B.4: Flash suppressors (mainly 2,4-DNT) and burn rate modifiers

| Triacetin | Dibutylphthalate (DBP) | Diisobutylphthalate (DIBP) |

| Dimethylphthalate (DMP) | Diethylphthalate (DEP) |

| 2-Nitrotoluene (2-NT) | 3-Nitrotoluene (3-NT) | 4-Nitrotoluene (4-NT) |

Figure B.5: Plasticisers

| Acenaphthene | Acenaphthylene | Anthracene |

| Fluoranthene | Fluorene |

| Naphthalene | Phenanthrene | Pyrene |
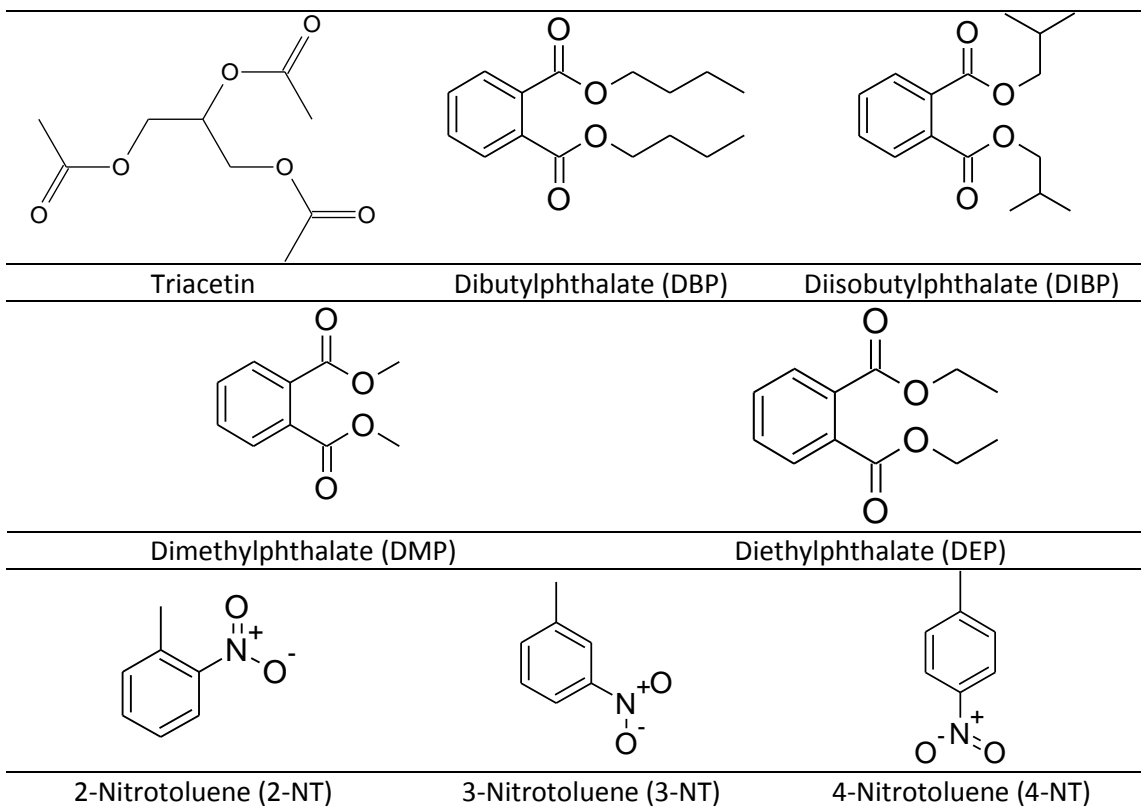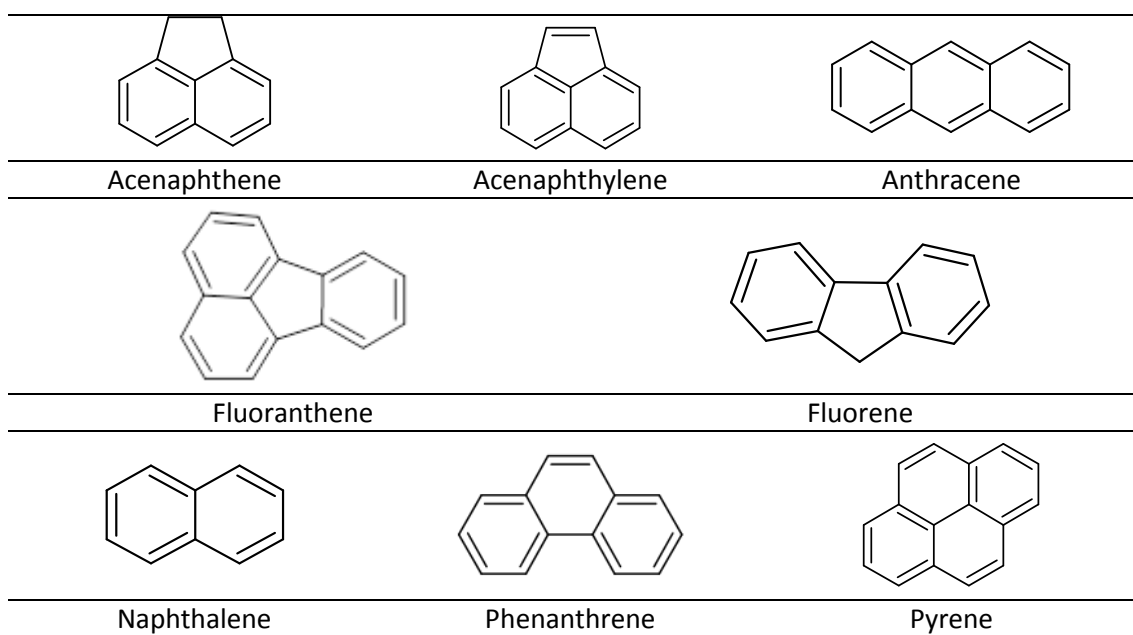
Figure B.6: Polycyclic aromatic hydrocarbons (PAHs)

1. Additional compounds

| 2,2'-dinitrodiphenylamine (2,2'-DNDPA) | 2,4'-dinitrodiphenylamine (2,4'-DNDPA) | Oxamide |
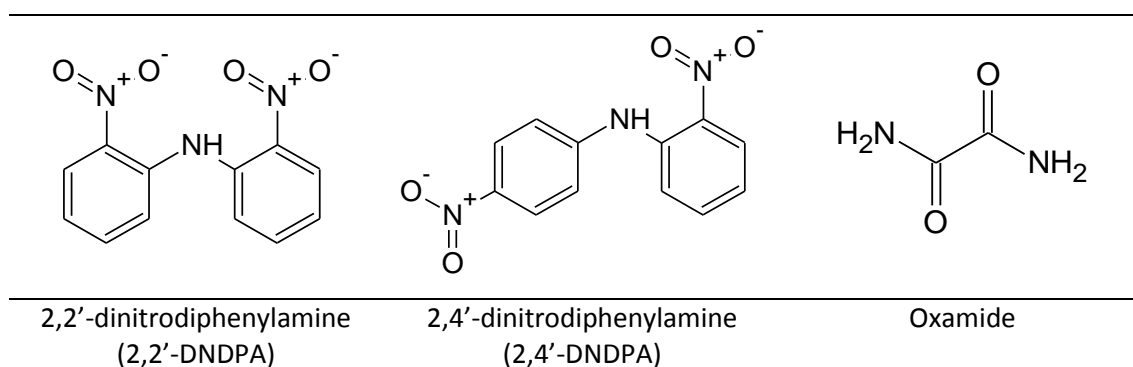
Figure B.7: Additional diphenylamine derivatives (left and centre) and stabiliser (right)

| 2,4,6-Trinitrotoluene (TNT) | Diphenylphthalate (DPP) |

Figure B.8: Additional sensitisers (left) and plasticiser (right)
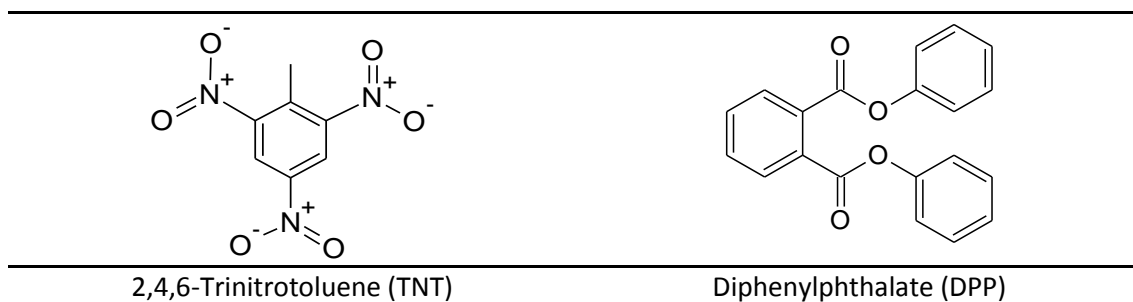
Appendix C: GC-MS optimisation

1. Details of assessed temperature profiles

Method A

Table C.1: Details of method A

Method A	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	6.00	200	2.00	27.00	Insert hold at 160°C for 2 min or at 165°C for 1.5 min (for DNT's & DEP) Next ramp at 10 C to 190 or 195C, hold for 3 min (carbazole & MC + DBP & 2-NDPA)
Ramp 2	20.00	300	0.00	32.00	Insert final hold for 1 min for 2,4-DNDPA

Table C.2: Details of method A - optimisation test 1

T1	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	6.00	160	2.00	20.83	No effect on 2,3-DNT & 2,4-DNT, some effect on 3,4-DNT & DEP but hold needs to be extended
Ramp 2	10.00	190	3.00	26.33	Carbazole is shoulder of MC peak, DBP & 2-NDPA closer together and after hold
Ramp 3	25.00	300	2.00	32.73	2,4-DNDPA at 30.9 min, so hold 1 min sufficient

Table C.3: Details of method A - optimisation test 2

T2	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	6.00	160	2.50	20.83	Separation DNTs & DEP 0.006 min and 0.110 min better, no visual improvement
Ramp 2	10.00	195	3.50	27.83	No real difference carbazole and MC, DBP & 2-NDPA slightly better
Ramp 3	25.00	300	1.00	33.03	Hold 0.50 min sufficient?

Table C.4: Details of method A - optimisation test 3

T3	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	6.00	170	1.00	21.00	2,3-DNT & 2,4-DNT out of hold, no real difference in separation
Ramp 2	10.00	200	2.00	26.00	No improvement 3,4-DNT & DEP - T2 is better - hold at 165?
Ramp 3	20.00	240	0.00	28.00	No improvement carbazole & MC
Ramp 4	30.00	300	0.50	30.50	0.50 min hold sufficient

Table C.5: Details of method A - optimisation test 4

T4	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	6.00	165	2.00	21.17	2,3-DNT in hold, 2,4-DNT out of hold; for 3,4-DNT & DEP T2 still best
Ramp 2	10.00	195	4.50	28.67	DBP & 2-NDPA ΔRT 0.164 vs 0.179 original
Ramp 3	30.00	300	0.50	32.67	

Table C.6: Details of method A - optimisation test 5

T5	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	2,3-DNT in hold, 2,4-DNT out of hold; for 3,4-DNT & DEP T2 still best
Ramp 1	6.00	160	2.50	20.83	DBP & 2-NDPA ΔRT 0.164 vs 0.179 original
Ramp 2	10.00	195	4.50	28.83	
Ramp 3	30.00	300	0.50	32.83	

Method B

Table C.7: Details of method B

Method B	°C/min	Next °C	Hold min	Run time	Remarks
Initial		40	2.00	2.00	Compared to method A:
Ramp 1	10.00	100	0.00	8.00	Better separation for DNTs & DEP +
Ramp 2	5.00	200	0.00	28.00	carbazole & MC. Not for DBP & 2-NDPA
Ramp 3	5.00	280	10.00	54.00	2,4-DNDPA at RT 36.855 min

Table C.8: Details of method B - optimisation test 1

T1	°C/min	Next °C	Hold min	Run time	Remarks
Initial		40	2.00	2.00	
Ramp 1	10.00	100	0.00	8.00	
Ramp 2	5.00	200	0.00	28.00	No advantage, optimisation from
Ramp 3	10.00	280	10.00	46.00	original

Table C.9: Details of method B - optimisation test 2

T2	°C/min	Next °C	Hold min	Run time	Remarks
Initial		40	2.00	2.00	
Ramp 1	10.00	100	0.00	8.00	
Ramp 2	5.00	180	1.00	25.00	Hold for carbazole & MC not long
Ramp 3	5.00	200	0.00	29.00	enough
Ramp 4	25.00	280	0.00	32.20	No 2,4-DNDPA detected

Table C.10: Details of method B - optimisation test 3

T3	°C/min	Next °C	Hold min	Run time	Remarks
Initial		40	2.00	2.00	
Ramp 1	10.00	100	0.00	8.00	
Ramp 2	5.00	180	3.00	27.00	Carbazole & MC better resolution (both in hold)
Ramp 3	5.00	200	0.00	31.00	DBP & 2-NDPA not fully resolved
Ramp 4	30.00	280	5.00	38.67	2,4-DNDPA at RT 34.65 min

Table C.11: Details of method B - optimisation test 4

T4	°C/min	Next °C	Hold min	Run time	Remarks
Initial		40	2.00	2.00	
Ramp 1	10.00	100	0.00	8.00	
Ramp 2	5.00	180	2.50	26.50	
Ramp 3	5.00	190	1.50	30.00	Slight improvement DBP & 2-NDPA
Ramp 4	30.00	280	3.00	36.00	2 min hold sufficient?

Table C.12: Details of method B - optimisation test 5

T5	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	To shorted time, no adverse effects observed
Ramp 1					
	10.00	100	0.00	5.00	
Ramp 2	5.00	180	2.50	23.50	
Ramp 3	10.00	190	2.50	27.00	DBP & 2-NDPA still not fully resolved
Ramp 4	30.00	280	2.00	32.00	2 min hold okay

Table C.13: Details of method B - optimisation test 6

T6	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	10.00	100	0.00	5.00	
Ramp 2	5.00	180	2.50	23.50	
Ramp 3	30.00	195	3.50	27.50	Relatively big improvement from T5,
Ramp 4	30.00	280	2.00	32.33	but not fully resolved

Table C.14: Details of method B - optimisation test 7

T7	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	10.00	100	0.00	5.00	
Ramp 2	5.00	195	4.00	28.00	195°C used for MC based on Method A-T5, but very poor resolution now
Ramp 3	30.00	300	1.50	33.00	280°C or 300°C no real difference. No 2,4-DNDPA

Table C.15: Details of method B - optimisation test 8

T8	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	
Ramp 1	10.00	100	0.00	5.00	
Ramp 2	5.00	180	5.00	26.00	Carbazole & MC ok, DBP & 2-NDPA
Ramp 3	30.00	300	0.50	31.50	poor

Table C.16: Details of method B - optimisation test 9

T9	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	T6 & T9 equally good, T9 faster (0.33 min)
Ramp 1	10.00	100	0.00	5.00	
Ramp 2	5.00	180	2.50	23.50	
Ramp 3	30.00	200	2.50	26.67	
Ramp 4	30.00	300	2.00	32.00	2,4-DNDPA at 30.165

Table C.17: Details of method B - optimisation test 10

T10	°C/min	Next °C	Hold min	Run time	Remarks
Initial		50	0.00	0.00	Based on T8:
Ramp 1	10.00	100	0.00	5.00	
Ramp 2	5.00	180	10.00	26.00	Hold extended to include DBP & 2-NDPA, but no improved resolution
Ramp 3	30.00	300	0.50	31.50	

2. Chromatograms oven profile optimisation

40°C for 2 minutes
10°C/min to 100°C
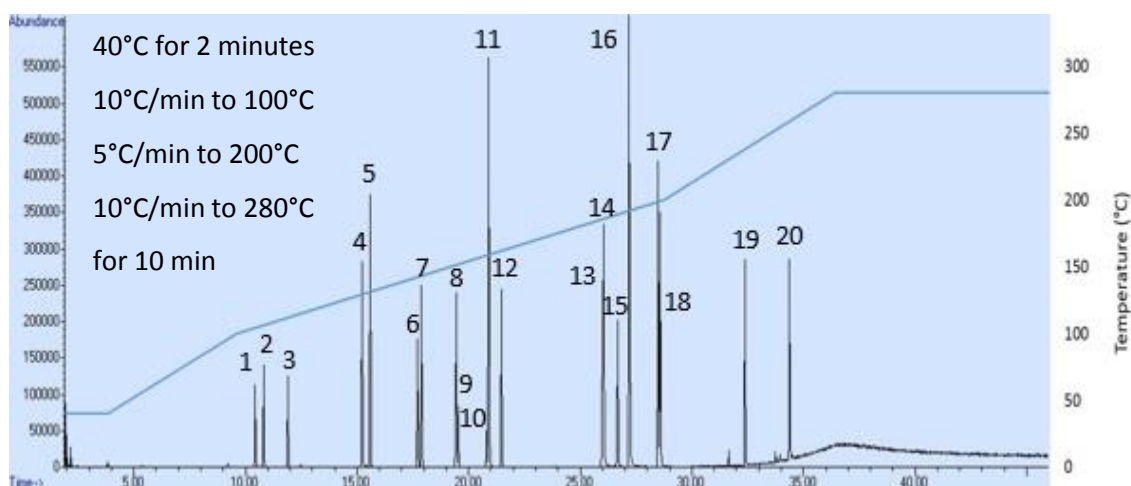5°C/min to 200°C
10°C/min to 280°C
for 10 min

Figure C.1: Chromatogram method B adjusted to 46 min run time, OGSR standard 1: 1. EPA, 2. Camphor, 3. 3-NT, 4. Triacetin, 5. NG, 6. DMP, 7. 2,6-DNT, 8. 2,3-DNT, 9. 2,4-DNT, 10. 3,4-DNT, 11. DEP, 12. DPA, 13. Carbazole, 14. MC, 15. DIBP, 16. EC, 17. DBP, 18. 2-NDPA, 19. 4-NDPA, 20. 2,4-DNDPA.
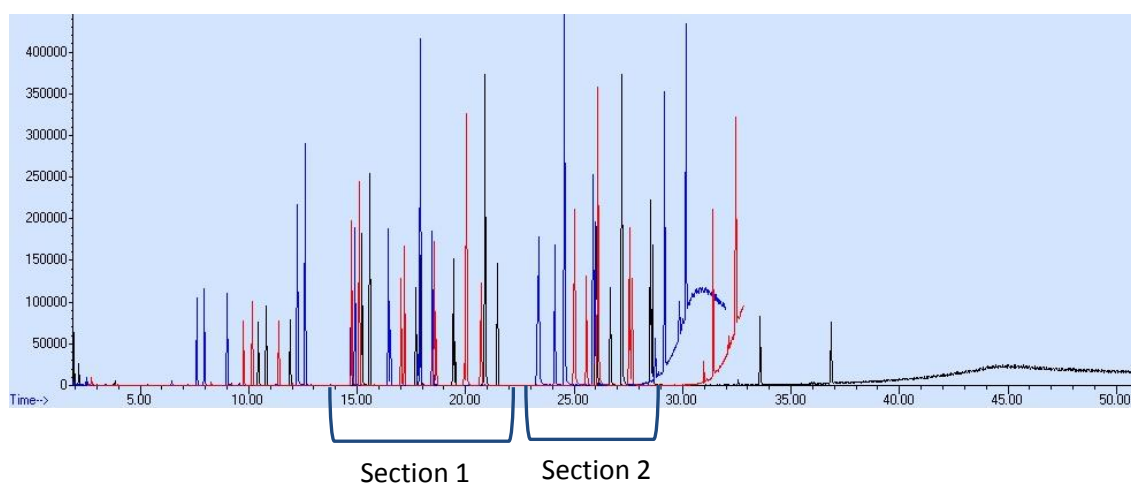

Section 1   Section 2

Figure C.2: Overlay chromatograms OGSR standard 1* - best three methods: method A – flow rate mobile phase 1.2 mL/min (red), method B (black), and method C (final optimisation method B, T9) (blue)
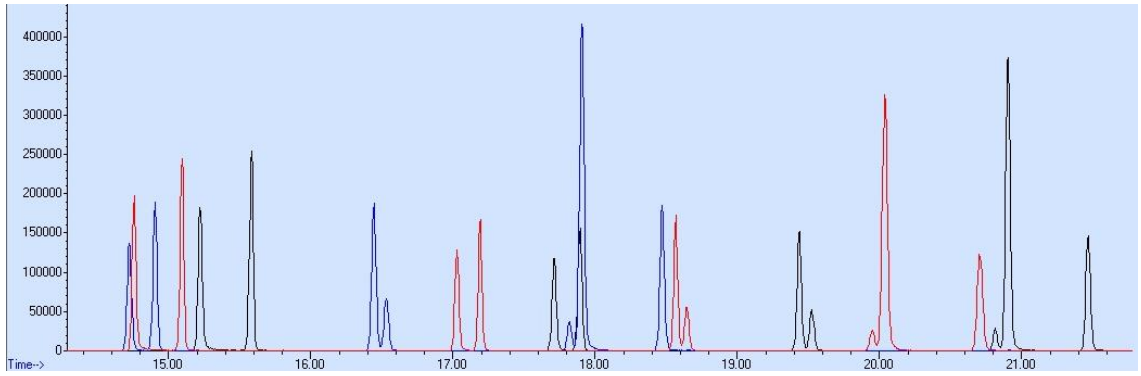
Figure C.3: Close up for figure B.6, section 1 – method A (red), method B (black), method C (blue)
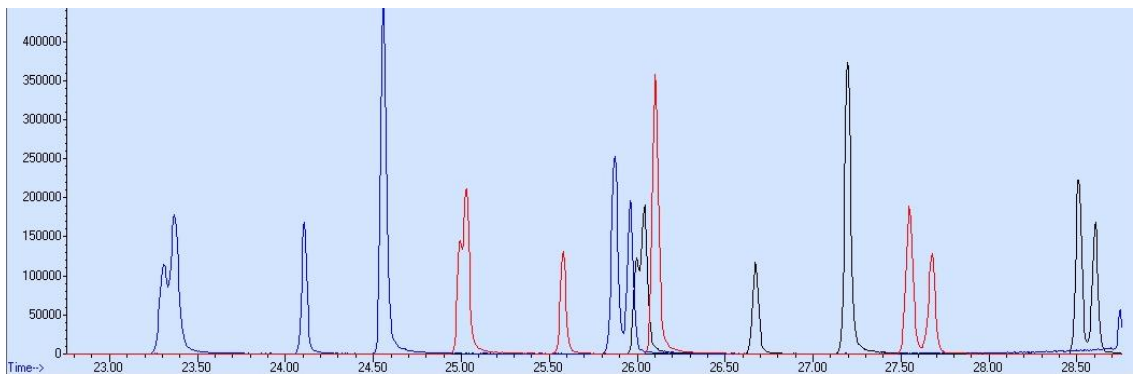

Figure C.4: Close up of figure B.6, section 2 – method A (red), method B (black), method C (blue)

Appendix D: Calibre

The inner surface of the barrel of these firearms contain a number of spiral grooves (figure D.1) known as rifling, to enable a stable flight. As the bullet travels through the barrel it is gripped by the riffling, which causes the bullet to rotate and exit the barrel in a stabilising rotating spin that prevents it from wobbling or turning over in flight. [18-20]
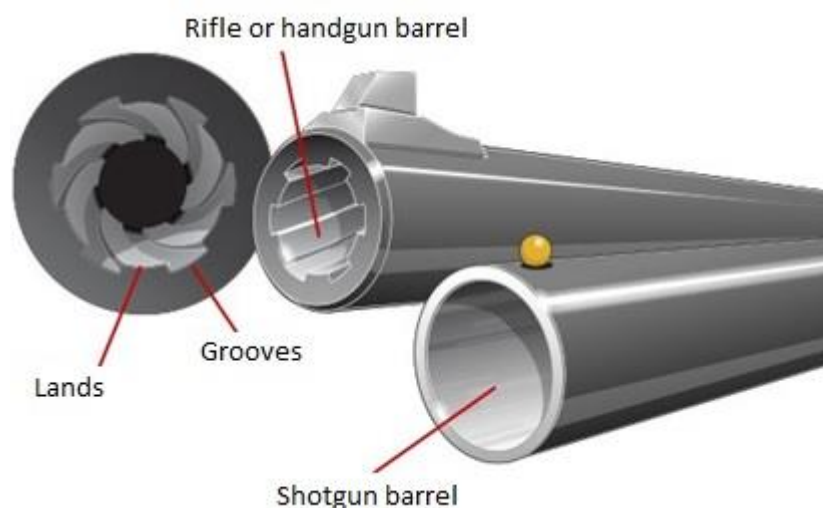

Figure D.1: Riffling of a gun barrel [21]

Most rifling has a groove depth of approximately 0.1 mm [19]. The raised areas between two grooves are called lands (figure D.1) [18-20]. The calibre of a firearm is based on the barrel diameter measured between two opposing lands. This oversimplified definition of calibre gives a rough indication of the bullet diameter (the bullet calibre), as the bullet is usually slightly bigger than the barrel diameter.

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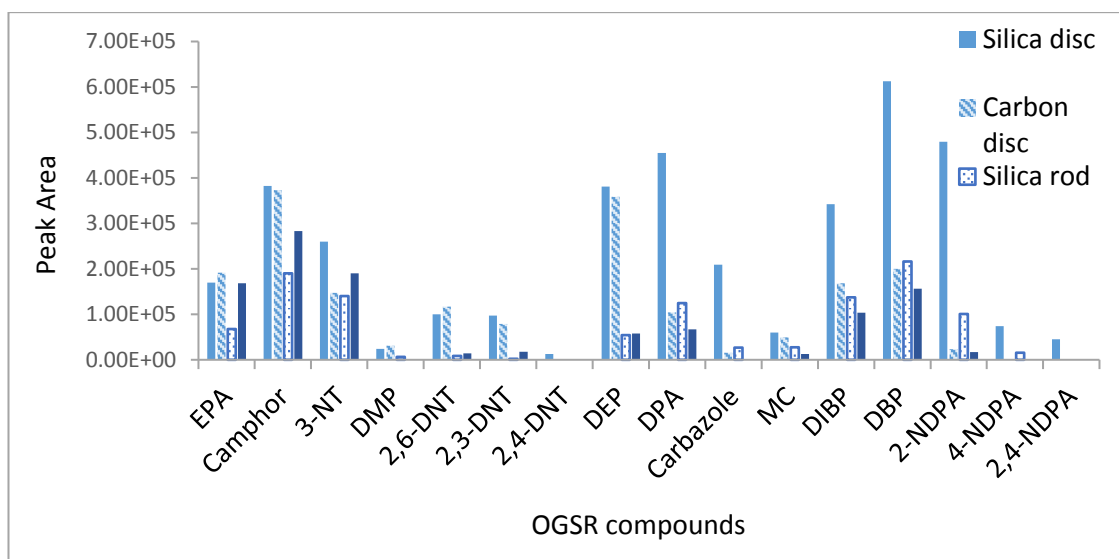
Appendix E: MonoTrap

Figure E.1: Comparison extraction efficiency MonoTrap types per compound

Appendix F: List of publications

Goudsmits, E., Blakey, L.S., Chana, K., Sharples, G.P., and Birkett, J.W., *The analysis of organic and inorganic gunshot residue from a single sample*. [In preparation, due to submit to Forensic Science International in February 2018].

Goudsmits, E., Sharples, G.P., and Birkett, J.W., *Organic compositional analysis of propellant powders using monolithic material sorption extraction (mmse) - a feasibility study.* Journal of Forensic Science and Research, 2017. **1**: p. 68-76.

Goudsmits, E., Sharples, G.P., and Birkett, J.W., *Preliminary classification of characteristic organic gunshot residue compounds.* Science and Justice, 2016. **56**(6): p. 421-425.

Goudsmits, E., Sharples, G.P., and Birkett, J.W., *Recent trends in organic gunshot residue analysis.* Trends in Analytical Chemistry, 2015. **74**: p. 46-57.