IMPROVING DETECTION AND IDENTIFICATION METHODS FOR VOLATILE ORGANIC EXPLOSIVES

By

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Certificate of Authorship and Originality

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DEDICATION

TO MY AUNT AURELIA

COM MUITO AMOR À MINHA TIA LELÉ, A PESSOA MAIS DOCE QUE TIVE O PRAZER DE CONVIVER EM MINHA VIDA.

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List of Abbreviations and Symbols

1D	One Dimensional
2D	Two Dimensional
2-NT	Ortho-Nitrotoluene
4-NT	Para-Nitrotoluene
ANFO	Ammonium Nitrate Fuel
ANN	Artificial Neural Networks
BGE	Background Electrolyte
C^4D	Capacitively Coupled Contactless Conductivity Detection
CAR	Carbowax
CE	Capillary Electrophoresis
CI	Chemical Ionisation
CIT	Cylindrical Ion-Trap
CIT-MS	Cylindrical Ion-Trap - Mass Spectrometer
CMC	Critical Micellar Concentration
CTAB	Cetyltrimethylammonium Bromide
CW/DVB	Carbowax-Divinylbenzene
DDNP	Diazodinitrophenol
DI	Direct Immersion
DMDNB	2,3-dimethyl-2,3-dinitrobutane
DNA	Deoxyribonucleic Acid
DNT	2,4-Dinitrotoluene
DVB	Divinylbenzene
EDDs	Explosive Detection Dogs
EI	Electron Ionization
EOF	Electroosmotic Flow
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
GC-ECD	Gas Chromatography - Electron Capture Detector
GC-MS	Gas Chromatography - Mass Spectrometry
GC×GC	Two Dimensional Gas Chromatography

GC×GC-TOFMS	Two Dimensional Gas Chromatography - Time of Flight Mass Spectrometry
GSR	Gunshot Residue
His	Histidine
HMDs	Home-made Devices
HMTD	Hexamethylene Triperoxide
HMX	Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine
HPLC	High Performance Liquid Chromatography
HS	Headspace
ICAO	International Civil Aviation Organization
IEDs	Improvised Explosive Devices
IMS	Ion Mobility Spectrometry
IR	Infrared
LED-IF	Light Emitting Diode Induced Fluorescence
LIF	Laser Induced Fluorescence
LOAR-1	Lab-on-a-Robot-1
LOAR-2	Lab-on-a-Robot-2
LOAR-3	Lab-on-a-Robot-3
LOC	Lab-on-a-Chip
LOD	Limit of Detection
m/z	Mass-to-charge
MEKC	Micellar Electrokinetic Chromatography
MES	2-(N-morpholino)ethanesulfonic Acid
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NB	Nitrobenzene
NFSTC	National Forensic Science Technology Center
ng	Nanograms
NICI	Negative Ion Chemical Ionization
NMR	Nuclear Magnetic Resonance
NSWPDU	New South Wales Police Dog Unit
PA	Polyacrylate
PDMS	Polydimethylsiloxane

PDMS/DVB	Polydimethylsiloxane/divinylbenzene
PETN	Pentaerythritol Tetranitrate
PICI	Positive Ion Chemical Ionization
pg	Picogram
ppb	Parts per billion
ppt	Parts per trillion
QIT	Quadrupole Ion Trap
RDX	Cyclotrimethylenetrinitramine
RNA	Ribonucleic Acid
RSD	Relative Standard Deviation
SDS	Sodium Dodecyl Sulfate
SERS	Surface Enhanced Raman Spectroscopy
SPE	Screen-Printed Electrodes
SPME	Solid Phase Microextraction
TATP	Triacetone Triperoxide
Tetryl	2,4,6-tetranitro-N-methylaniline
TNB	1,3,5-trinitrobenzene
TNT	2,4,6-trinitrotoluene
TOF	Time of Flight
TOFMS	Time of Flight Mass Spectrometry
µg	Micrograms
UV	Ultraviolet
UV-vis	Ultraviolet – Visible Spectroscopy
VOC	Volatile Organic Compound

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ABSTRACT

Although numerous chemical detection methods have been posited and tested for portability and detection of explosives, to date no method has solved the simultaneous issue of speed, reliability, selectivity and sensitivity. In order to advance the chemical and biological detection of explosives as screening tools in search areas, it is necessary to understand the key volatile organic compounds (VOCs) produced and detected by explosives. This thesis aimed to investigate a range of chemical detection methods, including portable and benchtop, to gain a better understanding of the VOCs produced in the headspace of commonly utilised explosives. The first stage of this project focused on the investigation of a previously reported capillary electrophoresis (CE) system coupled to an oscilometric detector (C4D) but with limited success. The second stage of this project focused on the study of commercially-available techniques. A lab-on-a-chip (LOC) was repurposed and successfully used to detect explosive residues in liquid and vapour samples. The Agilent 2100™ Bioanalyzer showed recovery rates of 29, 45 and 75 % for the three nitrate explosives investigated. A transportable gas chromatography-mass spectrometry (GC-MS) system was also tested, however due to several issues presented the instrument was not able to perform headspace analysis. Instead, a benchtop GC-MS and a two dimensional gas chromatograph (GC×GC) coupled to a time of flight mass spectrometer (TOFMS) were investigated. The conventional GC-MS method proved to be inefficient for headspace profiling, whereas the GC×GC-TOFMS was successful in separating and detecting the key VOCs from explosive samples. 2,3-dimethyl-2,3-dinitrobutane (DMDNB), 2,4-dinitrotoluene (DNT), and 1,3-dinitrobenzene (DNB) were identified as the most significant VOCs and subsequently used in the final stage of this project to compare the chemical detection methods with biological detection methods. Accredited explosive detection dogs (EDDs) were exposed to varying concentrations of the three significant VOCs. The study demonstrated that the dogs increased their response over time and with exposure to the standards, demonstrating a learning curve to the target odour. This study has demonstrated comparable sensitivity between EDDs and the benchtop GC×GC-TOFMS method, however canines are still considered the most effective real-time method for screening of explosives, due to their speed and selectivity over large areas. This thesis has advanced our understanding on the VOCs that comprise

the odour profile of explosives and will assist with the future enhancement of chemical and biological detection methods.

Keywords: explosive vapours, volatile organic compounds, chemical detection methods, microchip-CE, gas chromatography, mass spectrometry, headspace analysis, explosive detection dogs.

Chapter 1: **INTRODUCTION**

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1.1 **EXPLOSIVES**

Due to recent terrorist activities and the improper use of commercial and homemade explosives, the detection of concealed explosives has become an issue of considerable global importance. While terrorism is not new to the international community, an increasing number of terrorist attacks during the last decades has generated an increased demand for rapid, sensitive, and reliable methods for detecting hidden and trace explosives [1].

Traditional chemical explosives commonly used by the military, industry and mining are typically composed of four primary elements: carbon, oxygen, nitrogen, and hydrogen [2]. Examples of these types of explosives include 2,4,6-trinitrotoluene (TNT), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), pentaerythritol tetranitrate (PETN), and nitrocellulose which are all nitrogen based organic compounds. Nitration plays a major role in the manufacturing of these explosives through a substitution or double exchange reaction in which one or more NO_2 groups of the nitrating agent replace one or more groups (usually hydrogen atoms) of the compound being nitrated [3]. These explosive compounds are considered secondary (high) explosives because they cannot be detonated readily by shock or heat as with primary explosives. Their detonation requires a shock produced by a primary explosive, such as lead azide, mercury fulminate, or tetrazene. Historically, the majority of explosive detection techniques focused on these traditional compounds since they were the explosives most commonly utilized in terrorist incidents [2].

Non-traditional chemical explosives such as improvised explosive devices (IEDs), home-made devices (HMDs), and inorganic explosives can produce similarly violent exothermic reactions but do not comprise the classic compositions of military explosives [2]. Some newly synthesized energetic materials that contain more nitrogen than carbon by weight have shown desirable explosive properties. The high nitrogen content of these novel compounds often leads to high densities, a quality which is known to liberate large amounts of energy from very small quantities of material [4-7]. Terrorists have used these compounds for their attacks due to the easy access, manufacturing procedure, and destructive power generated by these substances [2].

1.1.1 Classification

The majority of chemical explosives can be characterised into two main categories depending on their explosion velocity, namely high or low explosives [8]. High explosives comprise the majority of military, commercial and improvised explosives such as dynamite, TNT, PETN, cyclotrimethylenetrinitramine (RDX), 2,4,6-tetranitro-N-methylaniline (Tetryl), and ammonium nitrate fuel oil (ANFO) [9, 10]. The explosion propagates through a detonation reaction generating a shock wave, which travels through the material at supersonic speeds of kilometres per second [11]. In contrast, low explosives are used primarily as propellants because they tend to exert a rapid pushing effect rather than a shattering effect like high explosives. The explosion propagates through a deflagration reaction that burns through the material at subsonic speeds of centimetres per second. They are composed of a mixture of a fuel and an oxidant [11].

High explosives can be further categorised based on their sensitivity, as primary, secondary or tertiary explosives [11]. Primary explosives are extremely sensitive to stimuli such as a spark, friction or heat, and are easily detonated [12]. These types of explosives are mainly used as detonators or triggers for less sensitive explosives, such as secondary explosives, which are more stable and require a greater input of energy to initiate the reaction [13]. Examples of primary high explosives include lead azide, lead styphnate, mercury fulminate, diazodinitrophenol (DDNP), tetrazene, as well as some improvised explosives such as hexamethylene triperoxide (HMTD) and triacetone triperoxide (TATP) which have no commercial uses [9]. Secondary explosives comprise the explosives commonly used in the mining and demolition industry, such as PETN, Composition C-4, TNT and RDX [9]. Tertiary explosives are so insensitive to shock that they cannot be reliably detonated by practical quantities of primary explosive, and instead require an intermediate explosive booster of secondary explosives [12]. These are often made by low cost materials and mainly used in the mining industry, but have also been used for terrorist attacks due to the easy access to large amounts of precursors such as nitrate fertilizer [14].

Explosives can be further classified by: chemical structure, such as nitroaromatics, nitrate esters, nitramines, peroxides, acid salts and aliphatic nitrates [3]; use, such as military and commercial explosives; composition, such as plastic and cast explosives; characteristics, namely booster and main charge; and manufacturing procedures, such as industrial or

improvised [9, 11]. However different classifications and discordances often occur, especially when classifying mixtures such as plastic and cast explosives [9, 10]. A list of some common high explosives, known uses and classification can be seen in Table 1-1.

Table 1-1: Common high explosives, their acronyms and uses, classified by sensitivity and chemical structure

EXPLOSIVE	ACRONYM	SENSITIVITY CLASS	CHEMICAL CLASS	USE
2,4,6-Trinitrotoluene	TNT	Secondary	Nitroaromatic	Military and excavation
1,3,5-Trinitrobenzene	TNB	Secondary	Nitroaromatic	Military and mining
2,4,6-Trinitrophenylmethylnitramine	TETRYL	Secondary	Nitramine	Booster explosive
Cyclotrimethylenetrinitramine	RDX	Secondary	Nitramine	Military
Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine	HMX	Secondary	Nitramine	Military and oil and gas industry
Pentaerythritol tetranitrate	PETN	Secondary	Nitrate ester	Military
Triacetone triperoxide	TATP	Primary	Organic peroxide	Improvised explosive devices
Ammonium Nitrate Fuel Oil	ANFO	Tertiary	Mixture	Mining, Civil construction, Improvised explosive devices

1.2 CHEMICAL DETECTION OF EXPLOSIVES

Explosives detection is a cornerstone of both preventative and investigative examinations. The difficulties in the analysis of explosives are numerous, as they are comprised of a wide variety of chemical compounds and are inherently dangerous to study. Further confounding these efforts is the differences in pre- and post-blast examination of explosives and the range of techniques for particle analysis versus vapour analysis. There are a variety of technologies currently available and others under development that target trace detection of explosives in liquid, solid, and vapour samples. Confirmatory techniques include high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) commonly coupled to fluorescence or electrochemical detection

systems, as well as gas chromatography (GC) combined with mass spectrometry (GC-MS), electron capture (GC-ECD) or luminescence detection [2, 15]. Currently, the most widely deployed explosives screening technique is ion mobility spectrometry (IMS), which relies primarily on detection of particles present on clothes, luggage or other personal items [16].

This study was only interested in the chemical and biological detection of volatile organic compounds (VOCs) in the headspace of pre-blast explosives. Headspace detection of pre-blast explosives is hampered by their low vapour pressures and as a result, the key VOCs present in the headspace are still not well understood [17]. Nitramines commonly have the lowest volatility amongst chemical explosives, for example Tetryl has a vapour pressure of 7.41×10^{-12} atm at room temperature (25 °C), while nitroaromatics such as TNT and TNB have slightly higher vapour pressures of 9.15×10^{-9} and 2.00×10^{-8} atm, respectively [18]. The diffusion of vapours from explosives is dependent on the temperature and airflow around the object; as the temperature increases, the vapour pressure also increases. Hence, some explosives can yield a vapour pressure four times higher at 100 °C than they can at 25 °C [17].

1.2.1 Portable Instruments

The need for fast detection of explosives has generated a demand for rapid, sensitive and reliable methods, which can be made rugged and portable for field analysis [19-21]. Numerous analytical methods have been tested for detection of explosives [22], chemical threats [23], and breakdown products in air, water and soil [24], but to date no method has solved the simultaneous issue of speed, reliability, selectivity and sensitivity [19, 22, 25, 26]. The development of portable instrumentation has gained increasing interest because it is independent of the laboratory infrastructure and is able to operate at the location where the sample is collected [27]. Portable instruments including detectors based on conductometry [28], potentiometry [29], Raman scattering [30], UV-vis spectroscopy [31], nuclear magnetic resonance (NMR) spectroscopy [32], terahertz spectroscopy [33], mass spectrometry (MS) [1], ion mobility spectrometry (IMS) [34] and infrared (IR) spectroscopy [35], have been recently reported.

Instruments such as the IMS and IR or Raman spectroscopy detection based platforms have been widely researched for miniaturization and portability and are currently widely used in airports, with proven parts per billion detection limits [22]. Other notable portable

techniques developed for gas and vapour sampling are colorimetric and fluorescence quenching tests, chemiluminescence sensors, and MS devices. All of these techniques have portable instrument representatives currently on the market for the detection of hazardous materials in gas and vapour samples. These instruments are marketed as having parts per trillion (ppt) detection limits, but their real limits of detection (LODs) are not well publicized. Literature reports would indicate that average LODs of 100 ppb in vapour samples are achievable, based upon well published mass limits of detection conducted in laboratory studies [2, 15]. However these studies are based on samples being extracted and analysed by swipes coupled to thermal desorption systems, which does not provide a direct comparison to non-contact vapour sample extraction (pre-concentration) and analysis.

IMS instruments are widely used in routine detection techniques due to their ability to characterise the sample both qualitatively and quantitatively, as well as the very low detection limits that are often attainable [34]. IMS characterises a sample through the mobility of ions within the gas-phase of the instrument whilst an electric field is applied. The sample vapours are ionised at atmospheric pressure before introduction into a drift tube. The drift times are related to the mass of the ions and by determining the m/z ratio, it is possible to identify components within the sample through comparison with known standards. For these reasons, investigation into enabling the miniaturisation and portability of IMS apparatus for field deployment has increased over the years [36]. However, shortening the length of the drift tube and miniaturisation of the ionisation source would likely be associated with a corresponding decline in sensitivity [37]. It is also important to note that response and detection using IMS sampling requires contact with the surface of interest (i.e. swabbing) [34], and when used for vapours and gas analysis needs more than one pre-concentration step to perform its analysis. IMS presents the same setback for real time analysis as MS techniques (discussed below) in which it requires ionization of the analytes. The analyte ionization in a complex real life scenario can be problematic due to competitive ionization pathways with interferents, which can lead to a reduction in selectivity and sensitivity.

For IR spectroscopic methods, samples are passed through an infra-red beam, allowing certain groups to absorb at specific wavelengths, thus producing a distinctive spectrum [22]. Fourier transform infrared (FTIR) spectroscopy is capable of scanning all IR frequencies simultaneously rather than individually (as with more traditional IR

spectroscopy methods) and has previously been used for the detection and identification of explosive particles [35, 38]. The explosive particles present are able to be identified using a spectral library, however previous studies have demonstrated a non-specificity for this method's library, which did not contain spectra of common explosives such as TNT and 2,4-dinitrotoluene (DNT) [38]. FTIR metal surface probes have been utilized for topographical analysis and low detection limits have been reported, ranging from 160 to 400 $ng.cm^{-2}$ for TNT, Tetryl, PETN , DNT and HMX [35]. Sensitivity and specificity are currently problematic for this method, particularly when analysing complex mixtures which produce overlapping bands in the spectra. Current work is attempting to address these challenges [22].

Raman spectroscopy measures the vibrational transitions in a sample through the collection and analysis of scattered photons once the sample has undergone laser excitation. The resulting spectra can offer a fingerprint of the item under analysis that can identify individual components of the sample. This technique has been studied for its potential as an explosives detection method due to the near instantaneous results and the possibility for samples to be analysed at a distance from the instrumentation [39, 40]. The major problem with Raman scattering for chemical analysis is that it is a weak process whereby only 0.1% or less of the photons fraction incident on a sample are Raman shifted in wavelength while the rest are elastically scattered [41]. This hinders the Raman sensitivity and the particles identification [22]. This issue was addressed with the development of surface enhanced Raman spectroscopy (SERS), which displays much higher sensitivity as it uses surface-induced resonance rather than electronic transitions intrinsic to the molecule [24]. However, the issue with particle identification of explosives still remains, affecting the reliability of this method.

Luminescence based sensors for detecting explosive compounds may be described as utilising either direct or indirect detection methods. Direct detection techniques utilise any fluorescence which the sample may emit itself or through inducement with a chemical reaction. Indirect detection involves the implication of explosives being present through their effect on a fluorescent material such as, for example, via quenching [42]. Quenching techniques have previously been considered in the literature as a subjective method that suffers from field interferents and still requires a confirmatory analysis [43]. Colorimetric tests have also been used extensively as rapid screening tests for explosives, and in 2011 were described as the most common test for post-blast analysis [8]. In general, these tests

are portable and easy to use but lack sensitivity, reliability and reproducibility when compared to other more robust methods [22]. However, they have not been extensively tested on pre-blast explosives or attempted with vapour analysis.

Electrochemical methods are used to detect compounds by measuring their potential difference [44] and have several key qualities which present portability characteristics, such as low cost, fast analysis, minimal sample manipulation, high sensitivity, easy miniaturization and the existence of commercially available battery-powered equipment [45, 46]. Electrochemical techniques were employed for the detection of electroactive explosive compounds such as TNT and other nitroaromatic compounds [47, 48]. Non electroactive explosives such as TATP and HMTD can also be detected with this technique by converting the peroxide explosives into H_2O_2 by simple UV irradiation or acid treatment [49]. However, standard electrochemical methods have also some disadvantages, which cause complications for field deployment and use of this technique [48], such as difficulties for electrodes handling and need for frequent calibration due to poor stability [50, 51]. An alternative to this method is the development of screen-printed electrodes (SPEs) [52, 53], which have been used to identify the presence nitro-explosives by a simple voltametric scan towards the negative potential [47]. However this method requires the use of electrolytes to dilute the required sample, method which hampers the field use of this technique [48].

Various forms of mass spectrometry (MS), such as single quadrupole [54, 55], ion trap [56, 57], time-of-flight (TOF) [58, 59], and tandem-based (MS/MS) [60, 61] have been used for the detection of explosive compounds. Several studies have demonstrated an array of portable MS based instruments capable of detecting explosives with a limit of detection lower than 1 ng [62-64]. MS is often cited as a major contributor to the technology that makes it possible for experts to monitor and detect explosives and explosive residues [2]. Classification of compounds through MS is performed by ionization which allows their separation based on mass-to-charge (m/z) ratios. However, the size and cost of mass spectrometers has previously presented a drawback to their use in the field. Consequently, major progress has been made in miniaturizing MS instruments [65, 66] [67]. For example, organic and inorganic explosive samples have already been detected in the picogram range using a miniaturised ion trap instrument [68]. Other research has focused on increasing the reliability of MS platforms for explosive detection [69]. Like IMS, the main setback for MS detection systems is that it requires

ionization of the analytes which can be troublesome in a real time scenario due to competitive ionization pathways with field interferents [22, 70].

Field deployable instruments must be small, cost efficient, rugged and be able to perform real time detection of hazardous materials among interfering field activities [23]. Lab-on-a-chip (LOC) devices have promising features for the development of portable instruments such as the compact size and portability, quick analysis time, low cost, small sample and reagent consumption, and the possibility to integrate with other systems [71]. These devices result from the development of a technique known as microfluidics [72], which consists of pumps, valves, flow sensors, separation capillaries, and chemical detectors integrated on a single substrate or as compacted modules [73]. LOC devices are portable and capable of performing extremely fast, cost-effective separations [74], and represent an attractive alternative for the rapid analysis of explosives [75, 76]. The integration of capillary electrophoresis (CE) into LOC devices allows for the fast analysis of chemicals with low reagent consumption [23]. This technique presents itself as a viable alternative to eliminate complex procedures as it does not require ionization of the analytes. The sensitivity of this technique still requires considerable improvements, which can be achieved by coupling with laser induced fluorescence (LIF) detectors. LIF is selective to nitro-containing analytes, improving the selectivity for target analytes over interferents. Moreover, compared to other fluorescence quenching technologies, as chemical and colour sensors LIF holds an advantage as the separation allows for another dimension of selectivity leading to a potentially enhanced sensitivity. For these reasons, LOC devices are one of the new portable technologies investigated in this study.

1.2.2 Benchtop Instruments

While portable instruments are desirable for the rapid screening of explosives in large areas such as airports and stadiums, these instruments still suffer from many drawbacks that means, in some instances, a confirmatory analysis is still required to confirm the presence and concentration of explosives in an environment. Confirmatory analyses must be carried out in accredited laboratories for quality assurance and quality control reasons. Advances in technology to improve the sensitivity, selectivity, and reliability of benchtop analytical instrumentation for the detection of explosives has increased dramatically, particularly after the World Trade Center attack in New York City on September 11, 2001. Numerous analytical methods have been tested for the detection of explosives and

their breakdown products in air, water and soil [22, 24]. Current separation techniques routinely employed by forensic scientists for the analysis of explosives are outlined in section 1.2. Of these techniques, CE and GC-MS were selected for this study as they are capable of analysing pre-blast explosives in vapour samples [76-79].

1.2.2.1 Capillary Electrophoresis (CE)

CE is an analytical technique used to separate electrically charged molecules based on their electrophoretic mobility. The separation occurs in a silica capillary tube connected between two buffer reservoirs under the influence of an electric field formed through the application of a difference of potential [80]. The capillary wall in general has a negative electric charge allowing the formation of a double layer between the wall of the capillary and the positive ions of the electrolyte. After the high voltage is applied by the electrodes (positioned at the ends of the capillary), migration of the ions and the electrolyte towards the cathode takes place. This movement of the electrolyte is called electroosmotic flow (EOF) [80]. The compounds injected into the capillary migrate differently within the electrolyte. Cations migrate towards the negative electrode (cathode), while the anions migrate towards the positive electrode (anode). Figure 1-1 demonstrates a schematic of CE operation [81]. The major advantages of CE over other detection systems are its wide selectivity range, high resolution with very low sample consumption, and the basic instrumentation needed to set up this system, which makes it adaptable to miniaturisation without losing its functionality and accuracy [23, 27, 77, 82].

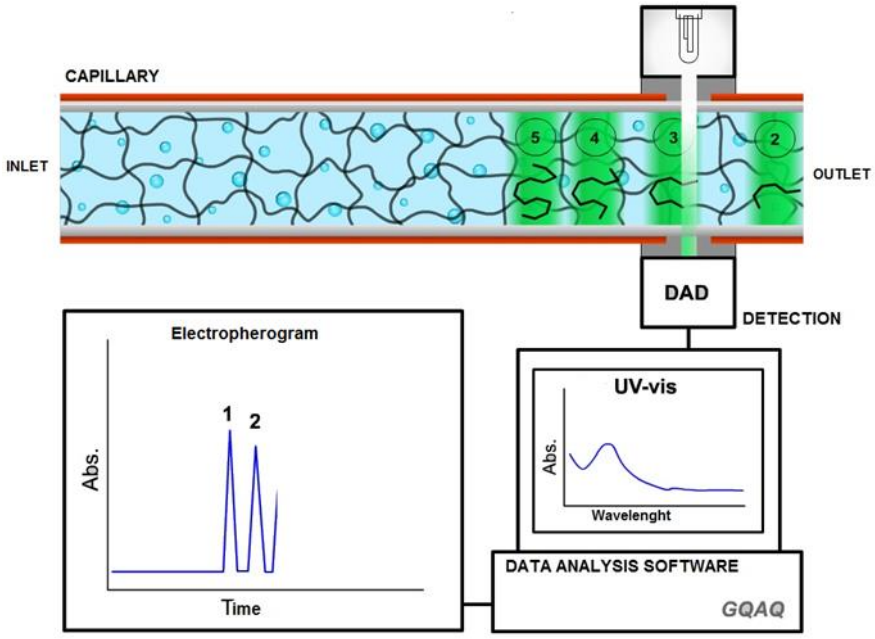

Figure 1-1: Schematic diagram of capillary electrophoresis separation and diode array detection (DAD). Figure adapted from Tavares, 1996 [81]

Neutral molecules such as explosives generally migrate with the same velocity as the electroosmotic flow and cannot be separated by conventional capillary electrophoresis as they are not electrically charged [75]. To allow the separation of non-charged molecules, surfactants are added to the background electrolyte (BGE) in a method known as micellar electrokinetic chromatography (MEKC), first described in 1984 by Nakagawa and further studied by Terabe *et al.* [83]. The method relies on the formation of ionic micelles around a non-charged analyte, which will migrate through the capillary allowing the migration time to be measured by the detector of choice [84]. Figure 1-2 demonstrates a schematic of the MEKC process. The micelles in the MEKC method can be described as a form of pseudo-stationary phase and their hydrophobic tails and hydrophilic heads enables the solvation of non-water soluble material [84].

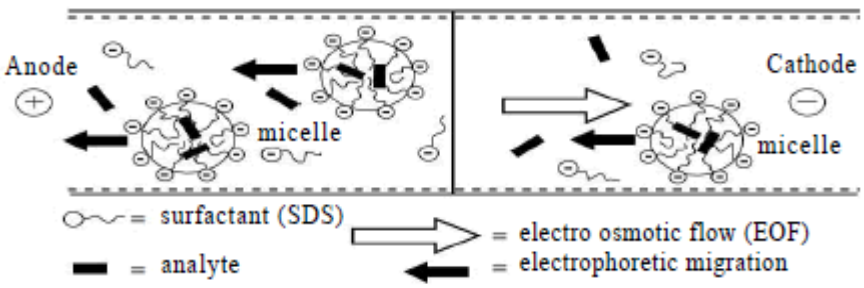

Figure 1-2: Schematic of a MEKC separation process. Figure adapted from Raja *et al.,* 2004 [85].

MEKC has been used for the detection of explosives and gunshot residues for several decades [86]. The first reported experiment using MEKC with explosives was able to separate 26 organic gunshot and explosive constituents in under 10 minutes and detect these compounds using an UV detection system [87]. A comprehensive separation of 24 nitroaromatics and nitramines was carried out in under 12 minutes through the addition of organic modifiers in the buffer, which altered the permittivity of the buffer and lowered the EOF. The detection wavelength was also optimized for the UV detector [88].

In another study, a sodium phosphate and sodium dodecyl sulfate (SDS) electrolyte enabled the separation of 14 nitrate explosives in under 11 minutes with limits of detection below 1 ppm. The detection was also optimized comparing the LOD of 4 different wavelengths [89]. A more complete study examined how artificial neural networks (ANN) can impact the separation of 12 explosive compounds by capillary electrophoresis. The selectivity of the separation was manipulated by varying the concentration of the surfactant SDS and the pH of the electrolyte, while maintaining the buffer concentration at 10 mM sodium tetraborate. The concentration of SDS and the electrolyte pH were used as input variables and the mobility of the explosives were used as output variables for the ANN. In total, eleven experiments were performed based on a factorial design to train a variety of ANN architectures. A product resolution response surface was constructed based on the predicted mobilities of the best performing ANN. The separation was further improved by changing the capillary to an extended cell detection window and reducing the diameter of the capillary, which provided a more efficient separation without compromising detection sensitivity [90]. More recently 25 organic explosives including peroxides such as TATP were separated in less than 17 minutes. This was the first time that CE was applied to the identification of improvised explosives. Limits of detection below 0.5 ppm were achieved by performing a pre-capillary complexation step, which provided the ability to simultaneously detect both organic and inorganic components of gunshot residue (GSR) and explosives when combined with the micellar phase [86].

1.2.2.2 Gas Chromatography – Mass Spectrometry

GC-MS is a fundamental technique for forensic analysis. GC separates compounds based on differences in volatility and solubility in the liquid, solid and gaseous phases [91]. After separation, the molecules are ionized as they sequentially enter the ion source. The

ionisation techniques used are electron ionisation (EI) and chemical ionisation (CI), both with the purpose of producing charged species that are later analysed according to their mass to charge ratio (m/z) [92].

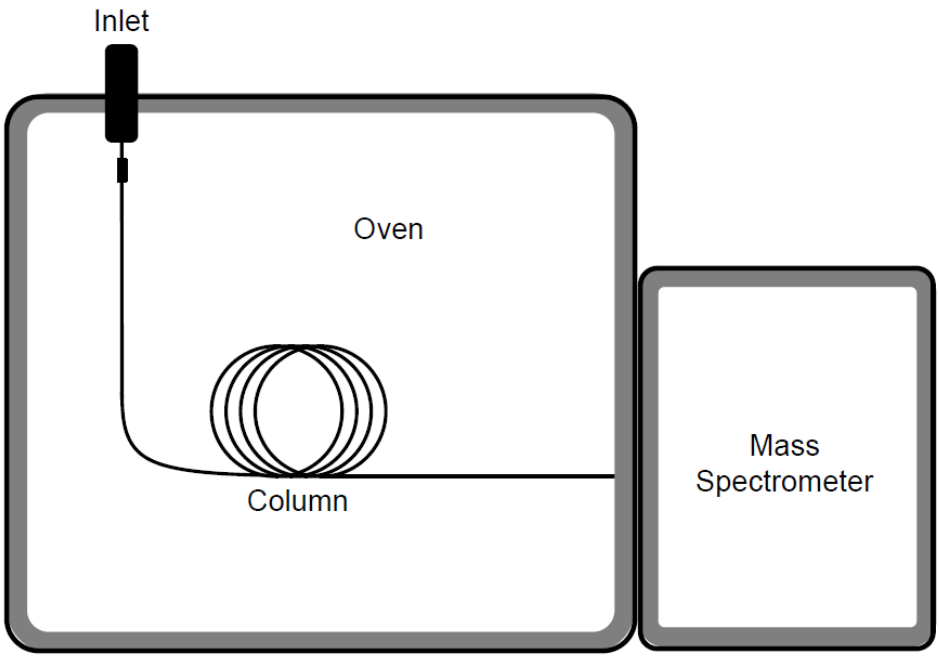

Figure 1-3: Schematic of a GC-MS design showing the inlet for sample introduction, column used for separation, and the detection system. With permission from Perrault, 2015 [93]

Electron ionisation (EI) is a widely used technique characterized by the bombardment of the target molecule with an electron, producing unstable positive ions (molecular ions), which are then fragmented into more stable ions. As a result of this energy interaction, the m/z and the abundance of characteristic fragments are obtained for the compound being analyzed. The charged fragments are transferred to the mass detector, thereby obtaining the analyte identification via a unique fingerprint of ion fragments [94]. Chemical ionisation (CI) is typically used to improve the yield of the pseudo molecular ion or increase sensitivity, especially with halogenated compounds. The technique uses a charged reactive gas to transfer the charge to the compound. These charged species are more stable than the ions formed in EI [95].

The correct ionization mode can enhance selectivity and significantly lower detection limits for the analysis of explosives by GC-MS. The detection limits for the analysis of liquid injections of organic explosives and related compounds by gas chromatography-mass spectroscopy utilizing EI, negative ion chemical ionization (NICI) and positive ion

chemical ionization (PICI) have been compared [96]. The detection limits determined for nitroaromatics, nitrate esters and nitramines proved to be lower using NICI than any of the other studied detection methods, with the exception of RDX. The lowest detection limit for RDX was achieved with the PICI ionization [96].

GC-MS has been successfully applied for forensic analysis since its development [97]. Besides MS, various kinds of detectors may be coupled to a GC for the analysis of explosive materials [25, 98-100]. Explosives from different classes have been detected in mixtures with limits of detection below the picogram level with the use of benchtop instruments coupled to TEA and ECD detectors [101-103]. A method for the vapour analysis of nitroaromatic explosives by GC-MS was also described with detection limits in the picogram levels [104]. This method made use of solid phase microextraction (SPME) for the headspace sampling and high volumes of samples were injected into the instrument to achieve these values. SPME is considered the optimal method for sampling volatiles in explosives when coupled with GC-MS [79, 105-109]. Notably, GC-MS also has the advantage of being adaptable to a field-portable method and has been recently investigated for its applicability to explosives, fire and counterfeit drug investigations. For this reason, a transportable GC-MS was also investigated as part of this study [110].

1.2.2.2.1 Solid Phase Micro-Extraction

Solid-phase micro extraction (SPME) involves the use of a retractable fused silica fibre which is coated with an adsorbent or absorbent film made of a polymer, which is the active site for the extraction process. Various polymer film phases are available such as polydimethylsiloxane (PDMS), divinylbenzene (DVB), polyacrylate (PA) or carbowax (CAR), which differ from each other by their polarities and film thicknesses. The SPME needle functions like a syringe and is placed inside a special holder. The SPME holder contains a plunger that when pushed will expose the fibre. The extraction occurs by direct immersion (DI) into an aqueous solution or by exposing the fibre into the headspace (HS) above a solid sample. The fibre is exposed for a sufficient time until equilibration between the phases is achieved [111]. In this process, the organic analytes migrate to the adsorbent/absorbent surface by affinity. Once equilibration is achieved, the fibre is retracted into the SPME holder, allowing for safe transport [112]. The SPME holder is introduced into the injection port of the GC where the fibre is once again exposed, allowing the volatile compounds to be thermally desorbed from the fibre under the flow

of the mobile phase gas [113]. Figure 1-4 outlines a headspace extraction using the SPME needle and holder.

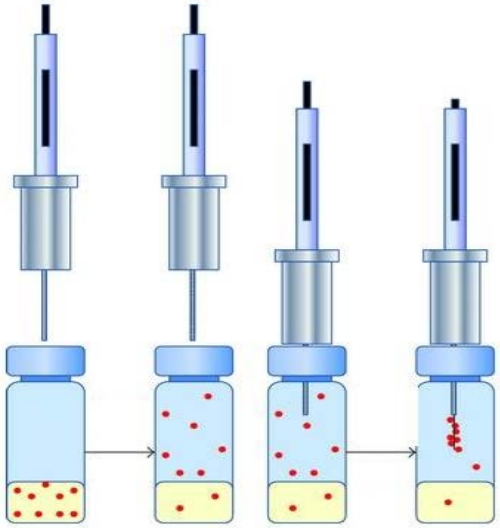

Figure 1-4: SPME extraction using the headspace mode. Figure adapted from Indelicato *et al.,* 2014 [114].

Due to its speed, ability to be automated, potential to avoid interferents, proven limits of detection when compared to other extraction techniques, and multiple sampling capability, SPME has been widely used for the collection of VOCs [105]. When optimising SPME conditions several factors must be considered, such as mode of extraction, fibre type, extraction time, extraction temperature, and desorption temperature.

The determination of the mode of extraction is dictated by which types of molecules are to be detected. Where the target analytes are volatile the optimal mode is headspace (HS) extraction, while direct immersion (DI) extraction is best suited for less-volatile, hydrophobic compounds [79]. Explosives have been previously extracted via DI-SPME [105], although several studies have successfully achieved explosive vapour extraction via HS-SPME [107, 115-118]. HS-SPME has the advantage of identifying trace volatile components in samples without the need for long extraction times, dynamic flow or heating the samples, all of which can change the ratios of components in the odour signature [106].

Choosing the appropriate polymer type to be used for the analysis of explosives is important. The selectivity of the fibre coating means that while PDMS/DVB allows for

good recovery overall when analysing multiple explosive compounds, polar compounds such as the nitroaromatic DNT are best extracted with a polar fibre (e.g. CW/DVB) [105]. The fibre that may be most suitable in laboratory studies may require longer desorption and clean-up times, while the fibre better suited to field experimentation can have rapid sampling and desorption times. When comparing the range of fibres available, several studies have found PDMS to be the optimal fibre for field conditions, while CW/DVB was preferred for laboratory experimentation [106, 119]. It is clear that different compounds require different fibre types and, in some cases, multiple fibres need to be used for optimal extraction of the VOCs.

The optimal extraction time will be dependent on the fibre type, target compound, extraction temperature and analyte chemistry. As extraction time increases, the heavier molecules displace the lighter molecules, and thus a compromise on the extraction time must be made [115].

The extraction and desorption temperature are additional parameters that should be considered for optimal use of SPME. The extraction temperature is the temperature at which the SPME fibre is exposed to the sample, while the desorption temperature is the temperature the GC inlet is set to in order to thermally desorb all analytes from the SPME fibre and into the GC column. While the vapour pressure, and thus volatility, of the explosive compound increases at a higher temperature, the adsorption onto the fibre coating may not increase at higher temperatures [111]. In order to achieve narrow chromatographic peaks, rapid desorption needs to occur, hence a higher temperature is desired [105]. However, it has to be noted that explosive compounds are inherently unstable and degrade easily at elevated temperatures, for example Tetryl a common nitramine rapidly degrades to picric acid at temperatures above 90 °C [120].

1.2.2.3 GC×GC-TOFMS

Two dimensional gas chromatography (GC×GC) is a more advanced GC instrument which has been applied in many fields such as food and flavour [121], environmental studies [122], metabolomics [123], petroleum products [124], decomposition odour profiling [125, 126] and forensic analysis [127, 128]. Analysis of several compounds of forensic interestwere described using comprehensive two-dimensional gas chromatography GC×GC coupled to different detectors [129]. Moreover the GC×GC was coupled to a time of flight – mass spectrometer (TOFMS) for the analysis of explosives [118]. Comprehensive two-dimensional gas chromatography methods are usually employed to provide a significant increase in the chromatographic separation resolution, allowing for the analysis of complex matrices that contain hundreds or thousands of analytes [130]. The GC×GC capabilities are of particular interest for the analysis of explosives [129]. The main components of the explosives compounds are not thermally stable and have considerably low vapour pressures, presenting major challenges for obtaining an accurate representation of the volatile signatures [18]. Moreover, explosives samples present a complex mixture of VOCs that are present in the headspace as well as in the surrounding environment, making it difficult to completely separate and profile the analytes of interest by conventional methods [78].

When compared to other techniques, GC×GC methods have a much higher separation resolution which is an advantage for the headspace analysis of explosives [118]. For example, the most commonly used explosive system, the IMS [131] cannot provide a comprehensive VOC profile from one sample as it targets the active agents only, which limits the ability to exploit the entire volatile signature of the analysed sample [117]. Conventional (1D) GC-MS methods are able to provide a non-targeted approach that can generate a global characterization of the detectable volatile signature [78], but lack the resolution power to separate and identify all chemical species, especially where co-elution of compounds occurs [132]. GC×GC-TOFMS is able to provide a comprehensive, non-target analysis of the VOCs present in explosive vapours due to its superior peak capacity (i.e. the number of peaks that will fit in the space of a chromatogram) resulting from the introduction of a second column in series with a different stationary phase, enabling two distinct mechanisms of separation (Figure 1-5). The first and second dimension columns are joined by a modulator which allows eluent from the first column to be focused onto the second column in short pulses, thus sharpening the peaks by reducing their width (e.g.

peaks are typically 100 msec in width) and increasing their peak heights. Narrower peaks are readily detected by the fast scanning detectors commonly employed with multidimensional GC such as the TOFMS. When compared to 1D GC-MS, the GC×GC-TOFMS system not only provides increased peak capacity but enhanced resolution, sensitivity, selectivity, improved characterisation of dynamic range, and a range of software features that enable an abundance of possibilities for data analysis [132]. For this reason, it was compared to 1D GC-MS in this study for the analysis of explosive VOCs. Figure 1-5 demonstrates a schematic of the GC×GC instrument design.

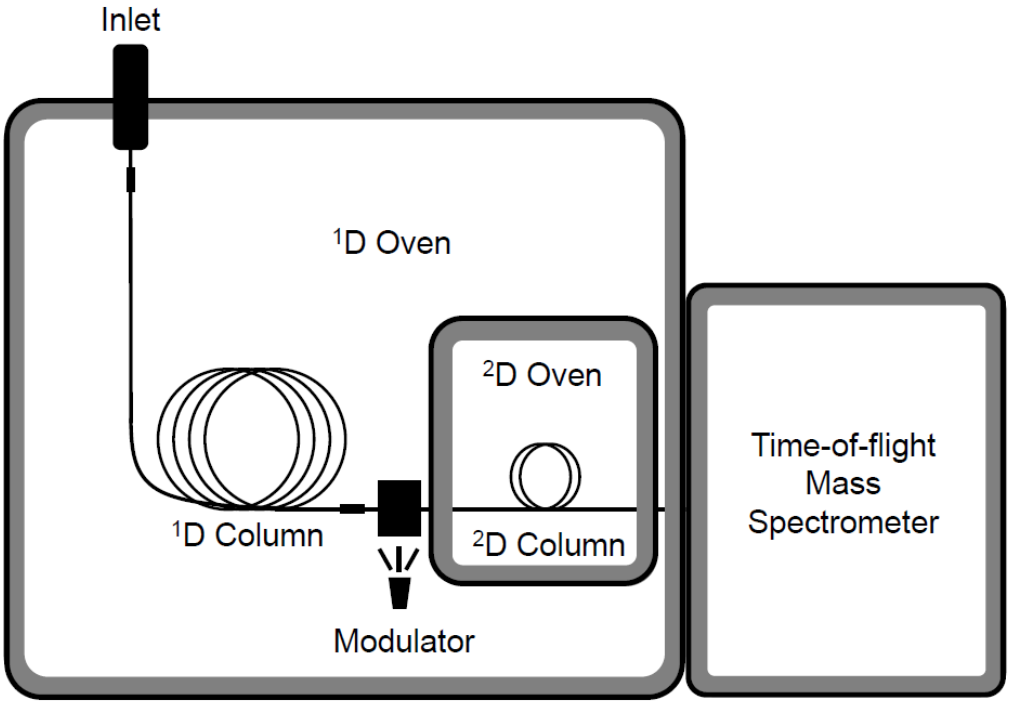

Figure 1-5: Schematic of a GC×GC design showing first and second dimension columns in series, with the modulator located between the two columns. Figure adapted with permission from Perrault, 2015 [93].

1.3 BIOLOGICAL DETECTION OF EXPLOSIVES

Various biological detectors for explosives have been studied over the years with *Canis familiaris*, better known as the domestic dog, the most widely deployed detector to date. In addition to canines, other animals and plant species have been proposed as alternative methods of biological explosive detectors, however these alternatives have not been validated and still present several limitations.

A research project in Tanzania presently trains African giant pouched rats to detect the explosive components of land mines. Reports indicate that rats may be capable of detecting comparably low levels of explosive VOCs as dogs with advantages including their small size and low cost, but with more challenging training and retrieval aspects [133, 134]. Truffle-detecting pigs were also considered for land mine detection, however it was discovered that their weight was sufficient to trigger the devices [1]. Bees have also been studied as explosives biological detectors. It has been demonstrated that bees are capable of detecting explosive odours at concentrations below those of most instruments and comparable to dogs [135]. The bees can be traced to the source or used to survey areas by examining chemical residues brought back to the hive. Their advantages are that they can be trained quickly and will not detonate the mines. However, their limitations are that they do not fly at night, in heavy rain, or in temperatures below 9 °C [1].

1.3.1.1 Explosive Detection Dogs (EDDs)

Perhaps the most well-known and widely employed biological screening tool for the detection of volatile explosives is the domestic dog, *Canis lupus var. familiaris*. Scent-detection dogs play a central and critical role in many law enforcement agencies [136]. They are used in investigations involving the detection and tracking of numerous VOC's of forensic interest. For example, detection canines have been used for detecting illicit drugs [137, 138], land mines [139, 140], guns, ignitable liquid residues [141-143], explosives [107], clandestine burials [144], and controlled goods such as illegally imported food, currency and wildlife products [145]. Dogs have also been used for tracking purposes, locating offenders, searching for missing persons [106] and disaster victims, as well as locating human remains and tracking blood [146, 147]. They are an excellent screening tool for explosive detection, and a complementary detection method for confirmatory laboratory methods [17]. Canines can be used to rapidly screen people, luggage and cargo [148], but still require the aid of confirmatory methods to identify the explosives detected. However, using dogs to screen dangerous areas such as clandestine laboratories and demilitarized areas with potential undetonated explosives poses both a chemical and biological hazard to the animal and the handler.

Detection dogs undergo extensive training to detect target odours with a high degree of specificity. Due to the variety of materials used to construct explosive devices, explosive

detection dogs (EDDs) need to be trained on a range of representative odours. This has initiated numerous studies into the effectiveness of different training methods and the effectiveness of various training odours [107]. Training EDDs typically involves presenting them with the target odours of interest to condition them to recognise the odour signature and provide a positive alert. During training, target odours are placed among a range of distractor odours, which are odours that the canine should not alert to but which are likely to be encountered in their working environment [139]. The effectiveness of EDDs is dependent on many factors, including level and type of training, the animals own temperament and physiology, environmental conditions and the ability of the handler [149-151].

EDDs provide several advantages over chemical detection methods, including their agility [30], their ability to rapidly and thoroughly search large areas [150, 152], their olfactory sensitivity that allow them to detect and discriminate between target and non-target substances even at low concentrations [142, 153], and their scent-to-source capabilities that allow them to pinpoint areas of highest concentration [151, 153, 154]. Due to these advantages, the deployment of EDDs is unlikely to decrease in favour of field portable instrumentation in the near future. However, a study of the literature also reveals several limitations for EDD deployment. For example, successful detections are highly variable from canine to canine [149, 155], and depend significantly on their training [154], hormonal and behavioural changes, possible infections, illness [151, 154], and environmental conditions [150, 156]. Additionally, EDDs are expensive to train [151, 152], and require long breaks when deployed [147]. They can also suffer from olfactory fatigue, which is the loss of sensitivity and selectivity due to repeated and prolonged exposure to the same odour [149, 156].

While the deployment of scent-detection canines is widely accepted in the forensic and law enforcement communities, questions regarding their accuracy, reliability, and validity have been raised and are regularly debated [151, 157, 158]. Research regarding the accuracy of individual detection canines in a range of detection scenarios can illustrate considerable variability, with some authors reporting successful detections ranging between 40 % and 100 % [142, 149, 150, 155]. In general, canines are reported to perform with high accuracy, given the wide range of scenarios presented [107, 149, 150, 159, 160]. The published accuracy of EDDs is between 87.8 % and 93.8 % for searches in an uncontrolled outdoor space [160]. When assessing the canines' performance, both the true

positive rate and the false positive rate need to be taken into account, as a dog may have a high true positive rate and miss very few targets, but also have a high false positive rate thus making the accuracy reported misleading [159]. Studies have shown that approximately 10% of alerts made by deployed canines were unnoticed, or not acted upon by the handler [149, 150]. However, it needs to be noted that in these studies, it is the researcher that reports, and thus interprets, these missed alerts where the canine displayed behaviour not typical of a positive alert [150]. Experienced handlers are required to recognise a proper canine response, however there is no reported method of assessing what constitutes an uncertain or potential response as it may differ from the dog posture to slight changes in behaviour [159].

Due to the low volatility of explosives, in 1996 the US Congress passed the Anti-Terrorism Bill that requires the addition of detection taggants to plastic explosive compounds [161]. A detection taggant is a solid or liquid vapour emitting substance added as a marker to an explosive material to facilitate discovery by EDDs before detonation [117]. The International Civil Aviation Organization (ICAO) has designated the detection taggant compounds as para-nitrotoluene (2-NT), ortho-nitrotoluene (4-NT), 2,3-dimethyl-2,3-dinitrobutane (DMDNB), and ethylene glycol dinitro (EGDN) [162]. These compounds were selected because they are not commonly found in nature, do not hinder the explosive properties of the tagged explosive, do not present a significant environmental hazard, and continue to odourise at a steady rate for 5 to 10 years [163].

The use of canines as a method of detection of explosives is well established worldwide [1]. The lack of data regarding optimal training protocols hampers the reliability of canine detection, leading to successful challenges in court regarding the admissibility of evidence obtained with the assistance of canines [164]. Challenges facing the field of canine detection include the limited ability to evaluate canine performance with standardized methods and calibration standards. Unlike instrumental methods, it is difficult to determine detection levels and perform a calibration of the canines' ability to locate scientifically valid quality control checks. When assessing the detection limits of EDDs the detection threshold is defined as the lowest concentration of target odour where at least 50% of the dogs in the study produce an alert [136]. The literature claims that EDDs are able to detect parts-per-trillion (ppt), but there are numerous differences between published reports [164]. Variations in the ways in which the tests were

conducted, different training practices and dog breeds, as well as possible contamination issues may be the causes for these differences [159].

The detection threshold for nitroglycerin, a known volatile explosive has been reported in the range of 10 ppb, while the threshold for 2,3-dimethyl-2,3-dinitrobutane (DMDNB), a common taggant of plastic explosives, has been reported at 500 ppt [106]. In a study designed to determine a canine's response to landmines, the dogs were able to detect 1 ppb of DNT in the headspace [165]. Overall the literature shows that canines are very sensitive to odours but the limits of detection will differ between different compounds and between individual animals [159]. Notably, canines preferentially employ olfaction over vision when detecting a target, even when in full light. The presence or absence of light does not appear to influence their detection ability [160].

Currently, EDDs still represent the fastest, most versatile, reliable, real-time explosive detection device available. Chemical detection methods, while they continue to improve, generally suffer from a lack of efficient sampling systems, selectivity problems in the presence of interferents, and in some cases limited mobility [25]. However, relying on dogs for real time detection of explosives in risk areas such as clandestine laboratories and demilitarized areas can be dangerous as they cannot identify the target compound and there can be a significant threat to both the dog and handler's life when accessing these areas. For this reason, it is necessary to continually develop and improve both chemical and biological detection methods for pre-blast explosives in air.

1.4 PROJECT AIMS AND JUSTIFICATION

One of the greatest challenges in explosives detection, is the discovery and identification of trace explosive VOCs in air, particularly in large areas such as airports and stadiums. Due to the low volatility of most explosives, the key VOCs available for detection are still not well understood. This lack of knowledge has hampered the ability to develop new methods or enhance current methods for the chemical or biological detection of pre-blast explosives, particularly in terms of screening capability.

This thesis aimed to investigate a range of chemical detection methods, both portable and benchtop, that have the potential for identifying a comprehensive VOC profile of commonly utilised explosives. It did not intend to focus on those methods already being used commercially (e.g. IMS) as to date, these methods have not been useful for

characterising the VOC profile of explosives since they focus on targeted analysis of specific compounds. Since biological detection (i.e. dogs) is currently the optimal method for rapid detection of explosive VOCs, this thesis initially focused on portable chemical detection techniques that may be able to demonstrate similar capabilities.

Early studies in this thesis focused on CE with two distinct detection devices, namely UV and capacitively coupled contactless conductivity detection (C^4D), as they had the potential to be miniaturised and incorporated into a remote device in the future. Results of these studies led to the investigation of a microchip-CE-UV instrument and subsequent comparison with a transportable GC-MS which is a more commonly utilised method for VOC analysis. Due to the current limitations that were identified for these range of portable instruments, later studies in this thesis focussed on benchtop instruments in order to satisfactorily characterise the VOC profiles of a range of commonly utilised explosives. These methods involved GC-MS and GC×GC-TOFMS in an attempt to provide enhanced capabilities to comprehensively characterise the VOC profile.

Finally, it was of interest to compare the enhanced chemical detection methods with the more commonly utilised biological detection method i.e. explosive-detection dogs (EDDs). This was achieved by identifying the key VOCs present in the headspace of explosives and testing the most abundant compounds with specially trained EDDs to compare the sensitivity and selectivity of the chemical analysis (i.e. analytical instrumentation) with the biological analysis (i.e. EDDs). Ultimately, this thesis aimed to advance the current knowledge base of explosive VOCs to enhance the development of future techniques for the rapid detection and identification of pre-blast explosives in forensic investigations.

Chapter 2: ANALYSIS OF EXPLOSIVES USING CE-UV AND CE-C^{4}D

Chapter 2: ANALYSIS OF EXPLOSIVES USING CE-UV AND CE-C^{4}D

2.1 INTRODUCTION

This chapter details the initial stages of the development of a remotely controlled platform hypothesised to be able to detect and identify explosives in air samples. This platform was chosen after the observation of a similar instrument that was used to analyse a series of volatile organic acids [23], which were used as analogues for chemical warfare agents. This platform could remotely access, detect and send the data to a distant control unit. This study aimed to prove the capability of a micellar electrokinetic chromatography (MEKC) method using a CE system coupled to a capacitively coupled contactless conductivity detection (C^{4}D) system to separate and detect explosives standards in a solution, with the additional intent of investigating the use of this system for detection of explosives in vapour samples.

2.1.1 Capacitively Coupled Contactless Conductivity Detection (C^{4}D)

The C^{4}D system in the axial electrode configuration was introduced in 1998 as a quantification method for capillary electrophoresis [166, 167]. It allows the detection of small inorganic ions as well as organic and biochemical species. The C^{4}D is a ruggedized and miniaturised instrument (Figure 2-1) which does not require contact with the sample, and is thus contactless [23] providing several advantages over other commonly used detectors for CE. The C^{4}D can be placed on the outside of the capillary in any region and does not require that the capillary have a detection window [168], preserving the capillary and facilitating field deployment [23]. Although organic ions can be readily quantified by conductivity measurement, the sensitivity tends to be somewhat reduced compared to inorganic ions as the charge-to-size ratios of organic ions are usually lower [169]. Nevertheless, contactless conductivity detection in CE has gained significant importance and has been widely applied in the analysis of organic ions and other species [168].

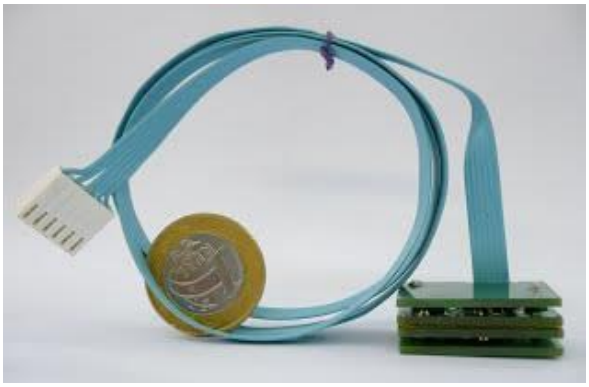

Figure 2-1: Size comparison of a C^{4}D system and a Brazilian Real coin. Figure adapted from da Silva *et al.,* 1998 [167].

C^{4}D has been extensively studied for the analysis of ions and cations [168] and is an excellent tool for the analysis of post blast explosives. It is also an ideal tool for detecting improvised explosives consisting of nitrate and per chlorate salts (ammonium, potassium or sodium) [75] since these are small ions with high mobility. Its ability to detect other pre-blast explosives has not yet been investigated and formed the focus of this chapter. A miniaturized analytical system for separating and detecting inorganic explosive residues, based on the coupling of a microchip electrophoresis with a contactless conductivity detector was described by Wang *et al.* [170, 171]. The low electroosmotic flow (EOF) achieved facilitated the rapid switching between analyses of cations and anions using the same microchannel and run buffer, resulting in the separation of seven explosive related cations and anions (ammonium, methyl ammonium, potassium, sodium, perchlorate, chlorate, nitrate) under one minute. The separation of improvised explosive-related cations was further optimised with movable C^{4}D [172, 173]. In order to detect improvised (ionic) explosives simultaneously with the TNT related compounds, the C^{4}D system was combined with an amperometric detection system in one microchip device [72]. The C^{4}D detection system presents great advantages for the development and implementation of a portable technique, however, further investigation is required to prove the efficacy of this system with nitro-based explosives and pre-blast analysis.

Prior to the evaluation of the use of the CE-C^{4}D system, a well described MEKC method for the analysis of explosives standards using CE-UV [86, 90] was optimised using a benchtop instrument. The results achieved with these experiments were the starting point for the design of a novel method for the detection of nitro-based explosives using the CE-C^{4}D system.

2.1.2 Lab-On-A-Robot

In 2008, Berg and collaborators [174] described the first integrated system capable of performing remote analysis of air samples using microchip-CE. The system called lab-on-a-robot-1 (LOAR-1) had all the necessary items for remote analysis, including collecting gas samples, performing injection, separation and detection, and sending the data to a distant control unit. However, several electrical connections were exposed, the chip was loosely mounted on top of the platform, the graphical user interface was complex, and the autonomy was severely limited by the size of the batteries included. Based on this prototype, Costa *et al.*, 2012 developed two new versions, branded as LOAR-2 and LOAR-3 following the original instrument created in 2008 [23].

The last and more advanced prototype, LOAR-3, was a fully remote controlled electrical system equipped with a window for sampling and a microchip-CE separation system capacitively coupled to a contactless conductivity detector, also called an oscilometric detector [23]. The integrated remote activated platform was able to detect organic acids in air samples, demonstrating the capacity of the system to perform the first remotely controlled analysis of organic acids using CE-C^4D.

The following experiments formed the basis for establishing a portable device for detection of explosives vapours by first optimising a MEKC method using liquid standards and testing it with a CE-C^4D detection system with the long-term intent of developing a portable instrument similar to LOAR-3 if successful.

2.2 EXPERIMENTAL

2.2.1 Instruments

The experiments were conducted using an Agilent CE G1600AX Capillary Electrophoresis System (Agilent Technologies, Germany) and analysed using an Agilent Chemstation Software. The analyses were carried out at a temperature of 25°C with an applied voltage varying between +20 kV, +25 kV, and +30 kV. Fused-silica capillaries with internal diameters of 50.0 and 75.0 μm ID (Polymicro Technologies) of varying lengths were employed for the experiments. Sample introduction was by hydrodynamic pressure injection varying between 20 and 50 mbar for times ranging from 5 to 15 s. An

UV diode array detector measured the absorbance at wavelengths of 195 nm, 200 nm, 215 nm, 254 nm, and 300 nm.

The C^4D detector was brought to Australia from the University of Sao Paulo, Brazil by Dr. Lucas Blanes and tested in several studies prior to this experiment. The system comes with its own data analysis software, which was used for all the data collection and analysis process. The detector was installed in the same 50 μm ID capillary used for the fast analysis and charged from a wall power plug (220 V). As there was no need to turn off the UV detector, it was possible to watch and compare the experiments with two different detectors at the same time. The UV detector demonstrated that the system was functional and that the separation of the analytes was occurring. The detector presented a clear signal and baseline.

2.2.2 Reagents

Explosive standards were purchased from AccuStandard (New Haven, CT, USA) at a certified concentration of 1000 μg/mL in acetonitrile, methanol or acetonitrile/methanol as available, and diluted in buffer according to the experimental requirements. The full list of tested explosives with its abbreviation and chemical classification is shown in Table 2-1. Analytical grade acetonitrile and methanol were purchased from ChemSupplies Pty Ltd (Gillman, SA, Australia). Sodium dodecyl sulphate (SDS) was purchased from Sigma–Aldrich (St. Louis, MO, USA) and sodium tetraborate was purchased from Honeywell Fluka (Switzerland). Other chemical reagents used, such as sodium phosphate, β-cyclodextrins, MES, histidine, and acetate were acquired from ChemSupplies Pty Ltd (Gillman, SA, Australia). The internal standard 2-naphthol (99.0% certified purity) was obtained from Dr Ehrenstorfer (Augsburg, Bavaria, Germany). Ultrapure grade water (18.2 MU cm^{-1}) was obtained from a Sartorius 611 water purification system.

Stock solutions of 100 mM SDS and 100 mM sodium tetraborate were prepared with Milli-Q water, and were diluted to the appropriate concentration desired in the experimental buffer. Each buffer was degassed and filtered using a 0.22 μm nylon filter, supplied by Rowe Scientific (Minto, NSW, Australia).

The effective mobility for each of the explosives was calculated by establishing the migration time of each analyte and the migration time of the electroosmotic flow (EOF).

The peak apex determined the analyte migration times. As previously reported in the literature, the start of the EOF peak was used as the migration time for the electroosmotic flow [90].

Table 2-1: Abbreviation and classification of selected explosives analysed using CE-UV

Explosive	Abbreviation	Chemical Classification
2-Nitrotoluene	2-NT	Nitroaromatic
3-Nitrotoluene	3-NT	Nitroaromatic
4-Nitrotoluene	4-NT	Nitroaromatic
2,3-Dinitrotoluene	2,3-DNT	Nitroaromatic
2,4-Dinitrotoluene	2,4-DNT	Nitroaromatic
2,6-Dinitrotoluene	2,6-DNT	Nitroaromatic
3,4-Dinitrotoluene	3,4-DNT	Nitroaromatic
2,4,6-Trinitrotoluene	TNT	Nitroaromatic
2-Amino-4,6-Dinitrotoluene	2-A-4,6-DNT	Nitroaromatic
4-Amino-2,6-Dinitrotoluene	4-A-2,4-DNT	Nitroaromatic
Nitrobenzene	NB	Nitroaromatic
1,3-Dinitrobenzene	DNB	Nitroaromatic
1,3,5-Trinitrobenzene	TNB	Nitroaromatic
Octohydro-1,3,5,7- tetranitro-1,3,5,7- tetrazocine	HMX	Nitramine
Hexahydro-1,3,5-trinitro-1,3,5- triazine	RDX	Nitramine
2,4,6,N-Tetranitro-N-methylaniline	Tetryl	Nitramine
Pentaerythyritol Tetranitrate	PETN	Nitrate Ester
Triacetone Triperoxide	TATP	Peroxide

Linearity, reproducibility and limits of detection (LOD) were measured throughout the course of the experiments. The standards were injected in a range of concentrations from 2 to 40 ppm in triplicates on three different days. The LODs were calculated as 3.3σ/slope of the calibration curve where σ = error in the slope, while the limits of quantification (LOQ) were based on 3.3×LOD [175].

2.3 RESULTS AND DISCUSSION

2.3.1 CE-UV

After reviewing the literature, a background electrolyte (BGE) consisting of a sodium tetraborate buffer and SDS as the micellar phase was chosen to conduct the experiments [86, 88, 90, 176]. This BGE was chosen due to its successful prior applications and ease of preparation, the pH value of the solution (9.2) and the fact that it does not require any

further adjustments [86, 88, 90]. Initial analyses were performed with 18 standards from which target analytes were chosen according to their availability, importance and elution pattern. The concentrations of both sodium tetraborate and SDS were varied to achieve optimal separation of all 18 explosive standards separately. The optimised BGE conditions and separation method was applied to achieve the detection of a mixture of 13 explosive standards in a single base line, as can be seen in Figure 2-2. The effect of the temperature on the separation was also investigated. Prior studies suggest that the critical micellar concentration (cmc) is temperature-dependent [176-178], hence selectivity and resolution can be affected by subtle temperature changes. Most MEKC studies reviewed used 25 °C as the optimum temperature and no studies have exceeded 30 °C [86, 88, 90, 176]. This is an important factor to consider as it means that any portable instrument for implementation of these methods needs to be able to regulate the temperature.

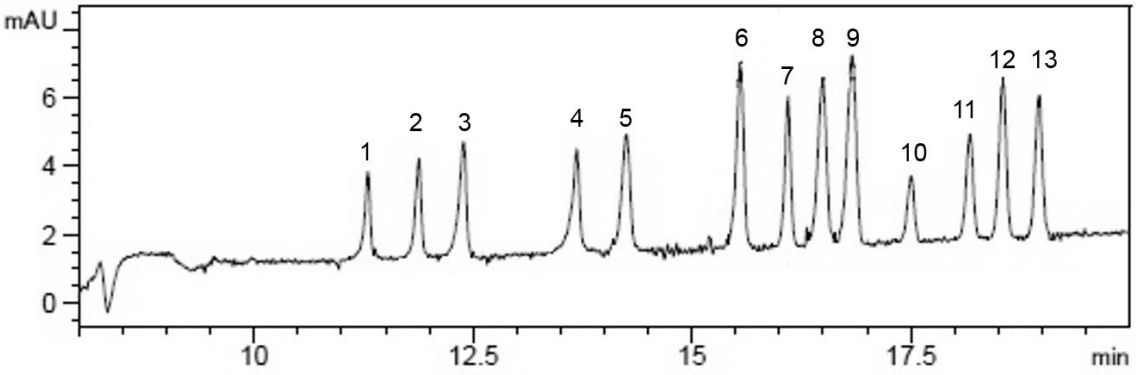

Figure 2-2: Electropherogram of the separation of 13 organic explosives at 10 ppm using the optimised method for CE separation and UV detection. 75 µm ID fused-silica capillary, length 78.5 cm, 72 cm to detector. BGE composed of 25 mM sodium tetraborate, 75 mM sodium dodecyl sulfate, pH 9.2. Sample injection at 50 mbar for 12 s. Run conducted with the + 25 kV voltage applied at 25 ºC. The wavelength shown is 200 nm.

The optimum BGE conditions were 25 mM sodium tetraborate, 75 mM SDS, and a pH of 9.2. The capillary temperature was held at 25 °C. As expected, the 50 µm inner diameter capillary showed better resolution under the same conditions than the 75 µm ID capillary, corroborating the literature findings [86, 90]. An initial method was optimized for a 78.5 cm total length capillary (72 cm to the detector). The samples were injected at 50 mbar of pressure for 12 seconds, and a voltage of + 25 kV was applied.

As the final goal of this project was to develop a field deployable platform, faster methods were trialled maintaining the aforementioned BGE conditions. Using a capillary with effective length of 56.0 cm (total length of 62.5 cm) the injection procedure was optimised

for hydrodynamic pressure at 30 mbar for 5 seconds and a separation voltage of + 30 kV. The faster method was able to successfully separate 11 explosive standards in a single run plus the addition of an internal standard for area normalization, as can be seen in Figure 2-3.

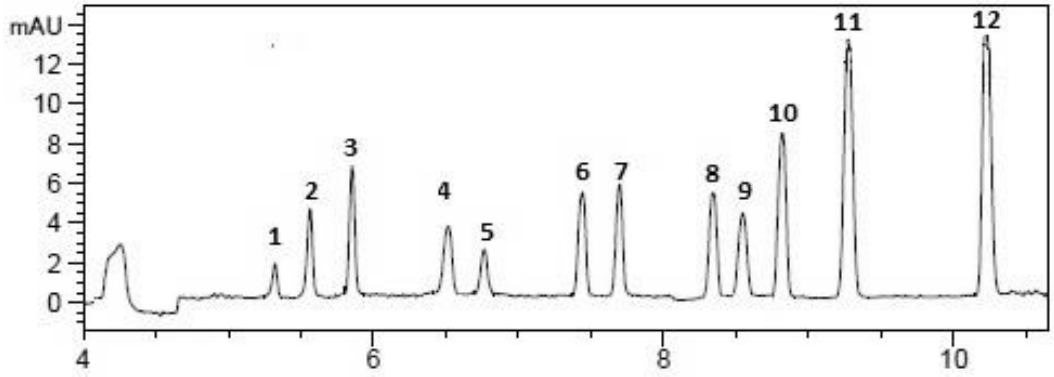

Figure 2-3: Electropherogram of the separation of 11 organic explosives and the internal standard at 20 ppm in BGE, using the optimised method for CE separation and UV detection. . 75 µm ID fused-silica capillary, length 62.5 cm, 56 cm to detector. BGE composed of 25 mM sodium tetraborate, 75 mM sodium dodecyl sulfate, pH 9.2. Sample injection at 30 mbar for 5 s. Run conducted with the + 30 kV voltage applied at 25 ºC. The wavelength shown is 200 nm. Analytes: 1) HMX; 2) RDX; 3) TNB; 4) TNT; 5) PETN; 6) TETRYL; 7) 2,4-DNT, 8) 2,6-DNT, 9) 2-NT; 10) 3-NT; 11) 3,4-DNT; 12) 2-Naphtol.

The linear range for each of these explosives was calculated over the range of 2 to 40 ppm based on normalized peak area, which is the total peak area divided by the peak area of the internal standard. Triplicate measurements of six points were used to construct the calibration curves, which showed high linearity with R^2 values between 0.97 and 0.99. When compared, the values of the normalized peak height and peak area showed considerable difference, with peak area giving a better correlation response than peak height. Therefore, only peak areas were used for quantitation. The figures of merit can be found in Table 2-2.

Table 2-2: Figures of merit for the analytes separated and detected using the MEKC for CE separation and UV detection

STANDARD	Average Retention Time (min)	R^2	LOD (ng/µL)	Minimum detectable Mass (pg)
HMX	5.47	0.97	1.35±0.7	2.02
RDX	5.51	0.98	0.74±0.5	1.11
TNB	5.87	0.99	0.69±0.4	1.03
TNT	6.49	0.98	0.74±0.5	1.11
PETN	6.79	0.98	0.74±0.9	1.11
TETRYL	7.35	0.97	1.40±0.7	2.10
3,4-DNT	7.74	0.99	0.68±0.4	1.02
2,4-DNT	8.34	0.99	0.69±0.4	1.03
2,6-DNT	8.52	0.98	0.72±0.5	1.08
2-NT	8.93	0.98	0.71±0.6	1.06
3-NT	9.25	0.98	0.70±0.6	1.05

The detection limits ranged from 0.7 to 1.5 ng/µL for all of the explosives. To calculate the minimum detectable masses, it was first necessary to calculate the volume of liquid injected, using the Poiseuille equation, which estimates the flow of liquid through a cylinder [179].

$$V = (\Delta P d^4 \pi t)/(128 \eta L)$$

ΔP is the pressure drop down the length of the cylinder (Pascals), d is the inside diameter (m) of the cylinder, t is the time the pressure is applied (s), η is the fluid viscosity (Pascal-seconds), and L is the total length of the cylinder (m). According to this equation the calculated injection volume was of the order of 1.5 nL. By multiplying the LOD for the amount injected it was possible to determine that the approximated minimum detectable masses are between 1.02 and 2.10 pg. The limits of detection and quantification, as well as the minimum detectable masses were comparable to literature findings [86, 90] demonstrating the successful reproduction of the methods developed. Intra and interday variations analysis resulted in relative standard deviation (RSD) values between 0.2 and 4 %, and 0.6 and 7 %, respectively.

2.3.2 CE-C^{4}D

Initially, mixtures of different explosives up to 100 ng/µL, with different retention times, were analysed using the optimized method for CE-UV, which consisted of a BGE composed of 25 mM sodium tetraborate and 75 mM sodium dodecyl sulfate (pH 9.2). Analyses were carried out in approximately 11 minutes on a 75 µm ID fused-silica

capillary with total length of 62.5 cm. The applied voltage was + 30 kV voltage, and the run was conducted at 25 ºC. The samples were injected at 30 mbar for 5 s. The results achieved during the CE separation were analysed in real time using the Agilent Chemsation for the UV detection and the C^{4}D system software installed on the same computer for the C^{4}D detection results. While the UV detected the standard peaks, the same was not observed using the C^{4}D detection. Hence, optimisation of the C^{4}D method was attempted.

The first optimized method for CE-UV analysis, which allowed a larger volume of sample to be injected in the column, with a slower separation process and therefore a longer acquisition time was also trialled. The method consisted of a BGE composed of 25 mM sodium tetraborate, 75 mM sodium dodecyl sulfate (pH 9.2). Analysis was carried out in the same width capillary (75 µm) but with a longer total length of 78.5 cm. Sample injection was performed at 50 mbar for 12 s, allowing for more sample to be present in the capillary for the analysis. The slowest method allowed for a better separation but the peaks were broader. The comparison between the electropherograms achieved with the UV detector and the C^{4}D showed the same results as the previous method. While the UV system was able to detect the explosive standards being separated by the CE system, the C^{4}D did not detect these standards.

A further literature review demonstrated that the pH value and composition of the BGE solutions were of considerable importance when testing C^{4}D detector, as it varies greatly with the source of the electrolytes being analysed [180]. Hence, subsequent trials focused on different BGE solutions, with pH values that could better suit the oscilometric detector. It is important to note that when surfactants are used in the preparation of the background electrolytes in capillary electrophoresis it affects both the electroosmotic flow (EOF) and the separation process. By adding other additives the effects may vary greatly, and the resulting situation may be very complex leading to the complete impediment of the separation process [181].

2.3.2.1 Phosphate Buffer

In CE separation analysis the addition of a buffer yields significant changes in the solution. The buffer prevents the pH of the BGE from changing during the run due to the electric field created [182]. In order to separate explosives, a surfactant must be added to generate the micelles that will allow the separation of neutral compounds [88]. With the

discovery of the effects of the pH on the C^4D performance, the first trial focused on lowering the pH. By keeping the same surfactant (SDS) and changing the BGE buffer from sodium tetraborate to sodium phosphate, it was possible to decrease the pH value from 9.2 to 8.1 This process has been cited in previous studies [176, 183-185]. SDS is a well-researched surfactant, and for that reason was maintained [86, 88, 90, 186].

The analysis method consisted of a BGE composed of different concentrations of sodium phosphate and concentrations varying from 50 to 80 mM SDS. The pH was confirmed to be 8.1 with the use of a Mettler Toledo© SevenCompact pH meter™. Analyses were carried out in approximately 11 minutes on a 75 µm ID fused-silica capillary with a total length of 62.5 cm. The applied voltage was + 30 kV voltage, and the analysis was conducted at 25 ºC. The samples were injected at 30 mbar for 5 s. Unfortunately, detection of the explosive standards by C^4D detection was still not achieved by changing the buffer in the BGE solution and hence, the experimental design was revisited.

2.3.2.2 Acetic acid

The use of acids in the BGE is a common practice to change the pH values of these solutions for CE analysis [182]. In order to achieve a successful separation with MEKC, the BGE pH should not be lower than 7 [86]. In 2007, Jensen *et al.* [184] made use of acetic acid to change the pH value of a BGE containing 10 mM of phosphate buffer to 7.0. Following this experiment, a new method was trialled in this study by adding acetic acid to the previous buffer until a pH value of 7 was reached. The analysis method consisted of a BGE composed of 10 mM sodium phosphate, acetic acid and 50 mM SDS. The pH was confirmed to be 7.0 with the use of a Mettler Toledo© SevenCompact pH meter™. Analyses were carried out in approximately 11 minutes on a 75 µm ID fused-silica capillary with a total length of 62.5 cm. The applied voltage was + 30 kV voltage, and the analysis was conducted at 25 ºC. The samples were injected at 30 mbar for 5 s. However, the additional change in pH was not effective and the explosive standards could not be detected by the CE-C^4D system using this method.

2.3.2.3 Flow Reversal

Positively charged polymers are commonly used to reverse the electroosmotic flow during CE analysis and are used for the detection of low conductivity analytes [169, 187-189]. Cetyltrimethylammonium bromide (CTAB) is a model example which has

elucidated the multiple effect of surfactants in an integrated way [190]. CTAB has been shown to work well with acidic solutions [182] and has been used successfully with the CE-C^{4}D system in an experiment to determine the critical micellar concentration of non-UV absorbing charged surfactants [184]. Even at concentration levels lower than 10^{-4} M, CTAB strongly reduces the cathodic EOF in bare fused-silica capillaries and converts it into anodic EOF. The magnitude and polarity of the EOF depends not only on the concentration of CTAB but also on the composition of BGEs used. The correct amount of CTAB to be employed is of utmost importance as it only enables MEKC methods when used at levels above its CMC [181].

During this trial, CTAB was used in the BGE to internally coat the capillary. The BGE was composed of 0.1 mM CTAB, 10 mM sodium phosphate, acetic acid and 50 mM SDS. The pH was confirmed to be 7.0 with the use of a Mettler Toledo© SevenCompact pH meter™. Analyses were carried out in approximately 11 minutes on a 75 µm ID fused-silica capillary with a total length of 62.5 cm. The applied voltage was -25 kV, and the analysis was conducted at 25 ºC. The samples were injected at 30 mbar for 5 s. However, the inversion of the EOF did not produce the desired effect, as no explosive standard peaks were detected using the CE-C^{4}D system.

2.3.2.4 Cyclodextrins

Cyclodextrins may serve in the place of SDS micelles as pseudostationary ligands for the resolution of cyclic nitramines [191]. Charged cyclodextrins are routinely used for the resolution of enantiomeric pharmaceuticals [192] and have been applied for the CE-based analysis of aromatic explosives [193] and nitramines [194]. A study by Luong and Guo in 1998 [193] used cyclodextrin, SDS and a borate buffer for the separation of nitroaromatic compounds by MEKC. Several subsequent studies used cyclodextrin to aid in the separation of explosive related compounds [87, 194].

During this trial, concentrations of β-cyclodextrins varying from 10 to 25 mM were added to a 25 mM borate and 55 mM SDS BGE. Analyses were carried out in approximately 11 minutes on a 75 µm ID fused-silica capillary with a total length of 62.5 cm. The applied voltage was +25 kV, and the analysis was conducted at 25 ºC. The samples were injected at 30 mbar for 5 s. The separation of the nitroaromatic compounds could not be observed using the referenced method and CE-C^{4}D system.

2.3.2.5 Other tests

According to literature reports, 2-(N-morpholino)ethanesulfonic acid (MES) and histidine (His) is another commonly used BGE solution for CE separations [168, 169]. MES and histidine have been used as a BGE since 1960, are regarded as a good buffer for presenting midrange pKa, maximum water solubility and minimum solubility in all other solvents, having minimal salt effects, minimal change in pKa with temperature, being chemically and enzymatically stable, and reasonably easily synthesized [195].

During this trial the standards were diluted in 55 mM SDS solutions, instead of the running BGE. The BGE was mainly composed of MES/His varying from 60 to 75 mM, to which different compounds were added individually per trial. MES/His was mixed to 25 mM borate and afterwards to 10 mM sodium phosphate, to which were added 0.1 mM CTAB or 25 mM β-cyclodextrins. These four BGEs were trialled separately but all the analysis were carried out the same way, in approximately 11 minutes in a 75 µm ID fused-silica capillary with a total length of 62.5 cm. The applied voltage was +25 kV (- 25 kV when CTAB was used) at 25 ºC. The samples were injected at 30 mbar for 5 s. The four solutions presented the same output with regards to the separation and detection of the nitroaromatic compounds, which could not be detected using CE-C^4D.

After approximately one year of trials and based on the limited results achieved using the CE-C^4D technique, subsequent testing was abandoned to investigate alternative methods as outlined in future chapters. A summary of the rationale for testing alternative methods is outlined below.

2.4 CONCLUSIONS

A MEKC method to analyse liquid standards of explosives using a CE-UV system was reproduced as described in the literature. The reproduced method was then adapted to yield an optimised and faster method that could successfully separate and detect 11 explosives in under 11 minutes. The limits of detection, quantification, minimum detectable masses and RSDs were comparable to literature findings for liquid standards [86, 89, 90]. This shorter MEKC method with UV detection was used to investigate whether the C^4D detector is capable of detecting explosives molecules pre-blast in liquid standards, which would ultimately lead to the portable detection of nitro explosives in air samples. Unfortunately it was not possible to reproduce the same outcomes achieved with

the UV detector for the C^4D detector. The capillary and injection conditions were maintained throughout all the tests, but the BGE and running settings were changed based on literature recommendations. Each change in the optimized method aimed to successfully detect the liquid standards using the C^4D system so that subsequent trials could investigate explosive VOCs. Several replicates of each method were carried out, with different concentrations of different explosive standard mixtures, but ultimately all trials failed to detect the analytes.

As a result, it was determined that the C^4D is not capable of detecting explosive molecules in a liquid solution and would be unable to detect the same compounds in air samples. The C^4D has been shown to detect small ions, making it a promising tool for the analysis of post-blast scenarios and more studies are needed to determine if this detector could be suited for improvised explosives instead. However, based on the current results it seems unlikely that the CE-C^4D system could be used to detect explosive VOCs in pre-blast scenarios.

Since the UV detector proved to have more potential than C^4D for the detection of nitro explosives, it was decided to test a microchip-CE-UV instrument for the detection of explosive VOCs and optimally for headspace sampling as a portable detection device. It was also decided to compare this to transportable GC-MS as benchtop GC-MS is currently the preferred method for explosive VOC analysis, but portable GC-MS has had little investigation to date for explosives.

Chapter 3: THE APPLICATION OF MICROCHIP-CE AND TRANSPORTABLE GC-MS FOR THE ANALYSIS OF EXPLOSIVES

Chapter 3: THE APPLICATION OF MICROCHIP-CE AND TRANSPORTABLE GC-MS FOR THE ANALYSIS OF EXPLOSIVES

3.1 INTRODUCTION

This chapter investigates two distinct portable platforms for their capability to detect explosives in vapour samples. The instruments were chosen as they are promising, easy to use, low cost platforms and have been developed for the in-field detection of chemicals of forensic interest. The aim of this study was to test the limits of detection of these instruments in a controlled laboratory environment and determine their potential applicability for in-field detection of explosive VOCs.

3.2 THE AGILENT BIOANALYZER

Lab-On-a-Chip (LOC) devices are a result of the development of microfluidics [72], which consists of pumps, valves, flow sensors, separation capillaries, and chemical detectors integrated into compacted modules [73]. A range of different devices have been developed using this platform, ranging from single components such as flow sensors and valves for gas pressure regulation, to complex systems for chemical analyses [196]. In addition to the compact size, microfluidics have promising advantages such as portability, rapid analysis times, low cost, small sample and reagent consumption, and the possibility to integrate with other systems [71, 74]. Recently LOC devices combined to CE-UV were used for the separation and detection of nitroaromatic explosives in different samples [75, 76].

The Agilent Bioanalyzer 2100 (Bioanalyzer) (Figure 3-1) is a compact and portable commercial LOC instrument that combines CE separation and UV detection for the analysis of compounds on a microchip [197]. This instrument was developed for the analysis of DNA, RNA, proteins and fluorescent cell cytometry, but has been recently used beyond this scope for the analysis of amphetamines [197] and detection of explosives, both directly [198] and after extraction from soil samples [199]. The small size and weight, combined with the fact that the instrument does not require pumps or gas

for its operation, and the ability to perform fast chemical mixture analyses makes the Bioanalyzer a viable alternative for the screening of explosive mixtures [197, 199].

Figure 3-1: The Agilent Bioanalyzer 2100 instrument and laptop for data collection and analysis.

3.2.1 Experimental

3.2.1.1 Reagents

Nitroaromatic and peroxide explosive standards were chosen to perform the experiments. TNT, DNB, TNB, Tetryl, 3-NT, 2,4-DNT, 2-A-4,6-DNT, 4-A-2,6-DNT, and TATP were purchased from AccuStandard (New Haven, CT, USA) at a certified concentration of 1000 µg/mL in acetonitrile. Analytical grade solvents (methanol, acetonitrile, and acetone) were purchased from ChemSupplies Pty Ltd (Gillman, SA. Australia). Sodium dodecyl sulphate (SDS) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and sodium tetraborate from Honeywell Fluka (Switzerland). Ultrapure grade water (18.2 $M\Omega.cm^{-1}$) was obtained from a Sartorius 611 water purification system. DNA 1000 Dye Concentrate® (blue) was obtained from Agilent Technologies©.

3.2.1.2 Instrument

An Agilent Technologies (Santa Clara, CA, USA) Agilent Bioanalyzer 2100 was used for all the liquid and vapour experiments. The Bioanalyzer is equipped with Light Emitting Diode Induced Fluorescence (LED-IF) and Laser Induced Fluorescence

(LIF) detection systems [197], and the separations occur after an electric field is created by applying a voltage to the system. The system allows for 12 samples to be analysed in 1800 seconds. Separations were performed on the standard RNA 500 microchips®, which are manufactured in borate silica and possess 16 wells connected by microchannels with a depth of 10 µm, a width of 50 µm, and a length of 15 mm (Figure 3-2). Of the sixteen wells, twelve are used for sample analysis, while the other four are used as buffer reservoirs or waste collection. The separation channels lay between wells A4 and C4 and are filled with the background electrolyte. Wells B4 and D4 were used for waste, and all other wells were filled with samples. Each chip can be used up to three times after washing with milli-Q water and 1 M NaOH. Experiments were conducted in a controlled laboratory environment, and vapour analyses were performed in a fume hood with a controlled temperature. Data was collected and analysed using the Agilent 2100 Expert software.

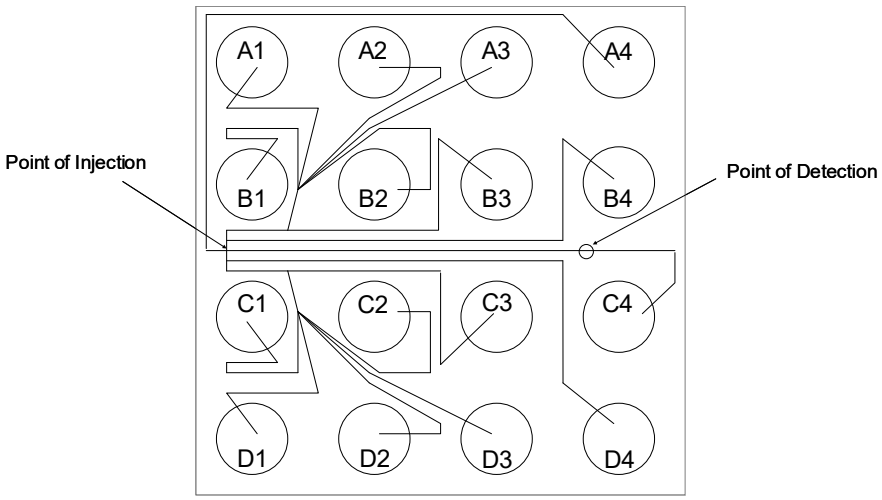

Figure 3-2: RNA 500 microchip design (actual size 17 mm^2). Figure adapted from Lloyd, 2013 [200]. The chip design and pattern belongs to Agilent technologies. All rights reserved.

3.2.1.3 Liquid Analysis

Individual stock solutions of explosive standards (1000 ng/µL) were diluted to the desired concentration in a sodium tetraborate (10mM; pH 9.2) and SDS (50 mM) buffer, and pipetted into the sample wells of the microchip. The background electrolyte used during the analysis was comprised of 10 mM sodium tetraborate (ph 9.2), 50 mM SDS, and 2% v/v of Agilent Bioanalyzer DNA 1000 dye®. The background electrolyte was sonicated for 10 minutes and filtered using a 0.25 µm syringe filter (Sartorius AG, Goettingen. Germany) prior to priming the chip. The chip was primed by pipetting 9 µl of the

background electrolyte into well C4 and applying air pressure to the well for 45 seconds with the Agilent Priming Station (Figure 3-3). For the analysis, 9 µL of single standard or standard mixture solutions diluted in buffer (10 mM sodium tetraborate and 50 mM SDS; pH 9.2) were pipetted directly into the sample well of a previously primed microchip. All other wells were filled with 9 µL of sample or buffer. After all wells were filled, the microchip was inserted into the instrument and different potentials were applied for injection and analysis. Calibration curves were constructed by pipetting 9 µl of the diluted explosive standard directly into the microchip sample wells at 1, 5, 10, 15 and 20 ng/µl. Successful detection of the liquid standards was required before attempting to analyse vapour samples as the latter represents more complex mixtures of VOCs.

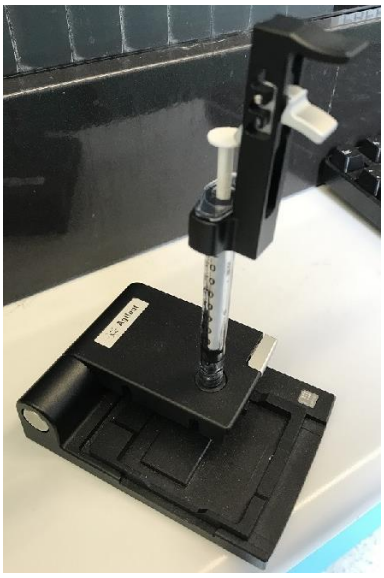

Figure 3-3: Agilent Priming Station used to apply air pressure to the wells of the microchip when priming it.

3.2.1.4 Vapour Analysis

Following the liquid analysis results (see Section 3.2.2.1), three explosive standards were chosen and diluted to the desired concentrations into organic solvents to facilitate evaporation. The solution was placed into a 2 ml glass vial. A circular 0.5 mm diameter paper chad was hole punched from a Whatman™ qualitative filter paper Grade 1 sheet and inserted in the cap of the 2 mL glass vial containing the explosive mixture. The vial was exposed to temperatures ranging from room temperature to 80 ºC for 15 minutes using a dry heat block (Figure 3-4).

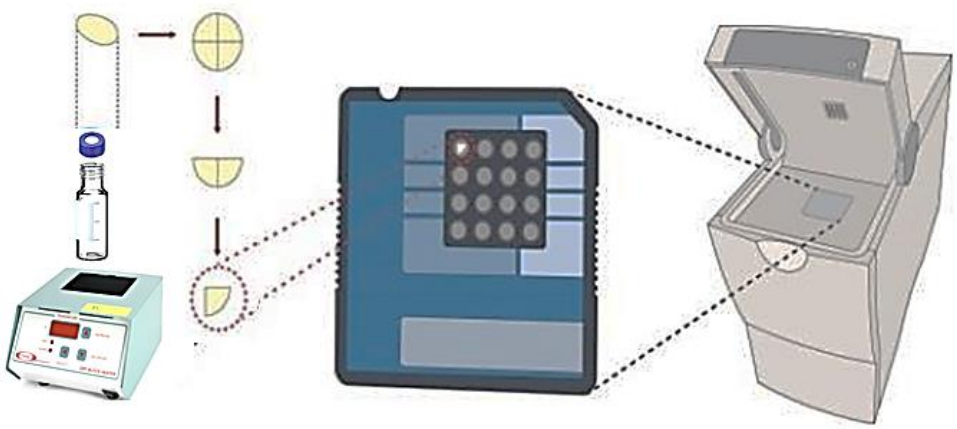

Figure 3-4: Vapour extraction and paper chad analysis method for the Bioanalyser experiments. A hole punched filter paper chad is inserted in a 2 ml vial cap. The vial, which contains explosive standards diluted in organic solvents, is subjected to heating in a dry block for 15 min. After extraction the paper chad was folded twice and inserted directly into the sample well of the microchip containing 5 µL of buffer. After insertion, 4 µL of buffer was added to the filter paper and the sample was analysed using the Agilent Bioanalyzer 2100. Figure adapted with permission from Ueland *et al.*, 2016 [199].

Methanol, acetonitrile, acetone, and water were compared as extraction solvents. Different amounts of each solvent were tested with equimolar amounts of the target explosives to determine the optimal extraction solvent, solvent volume, and temperature.

After the extraction process, the paper chad was retrieved from the vial cap, folded twice in order to fit into the sample reservoir, and placed directly into the lab on a chip injection well containing 5 µL of the background electrolyte. 4 µL of electrolyte was added to obtain a final volume of 9 µL. After all wells had been filled with 9 µL of sample or background electrolyte the microchip was inserted into the instrument for analysis.

Calibration curves were constructed using the extracted total peak area of the chromatograms. Triplicates of standard solutions at 5, 10, 20, 30 and 40 ng/µl were evaporated and analysed. The recovery rates from the paper chad were determined by dividing the total peak area at 20 ng/µl by the total area achieved during the liquid injection experiment for the same standards at the same concentration (n=4).

3.2.2 RESULTS

3.2.2.1 Liquid Analysis

The sample injection was performed using pinched mode, which is a two-step injection mode [201]. On the loading step, electric potentials are applied to all reservoirs confining the sample flow from the separation channel towards the waste well, avoiding diffusion and allowing the compound of lowest mobility to cross channels, while the dispensing step allows for the sample to be discharged from the sample wells to the separation channels for the analysis [202]. During the loading step, 100 V was applied to A4 and C4 sample wells as seen in Figure 3-3, to avoid sample diffusion, and a 1400 V potential difference was applied for 40 seconds for the sample dispensing. The separation was conducted using 1500 V for 100 seconds. Indirect detection was performed using LED-IF (λex = 635 nm, λdet = 680 nm).

A mixture of eight explosives was tested and all explosives were successfully separated and detected, as shown in Figure 3-5. Analyte confirmation came from spiking tests and/or previous retention time and mobility knowledge from single standard runs.

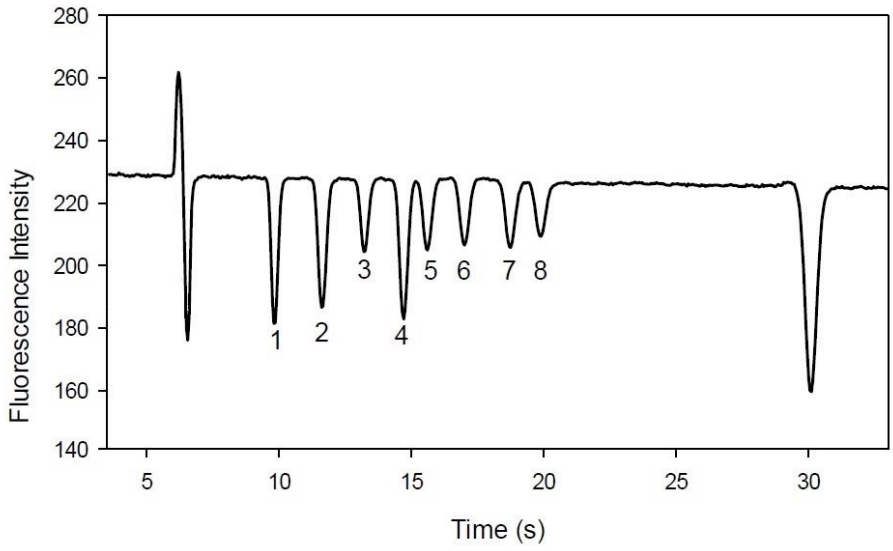

Figure 3-5: Electropherogram of the separation of 9 µl of a 20 ng/µL mixture of eight explosives diluted into buffer. The explosive mixture was pipetted directly into a sample well of the microchip. 1) TNB; 2) 1,3-DNB; 3) TNT; 4) Tetryl; 5) 2,4-DNT; 6) 3-NT; 7) 2,6-DNT, 8) 2-A-4,6-DNT. Background electrolyte (10 mM Borate: 50 mM SDS; pH 9.2), injection 1500 V for 40 s, separation 1500 V for 100 s.

Three target analytes (TNB, Tetryl and TNT) were chosen due to their retention time, sensitivity and availability, and calibration curves were constructed by pipetting 9 µL of these explosive standards directly into the microchip sample wells at 1, 5, 10, 15 and 20

ng/µL. The respective LODs were calculated as 3.3 σ/slope of the calibration curve, where σ is the slope deviation [175]. The minimum detectable masses were calculated as nine times the LOD, once 9 µL of solution was used on each sample well per trial. The minimum detectable masses found were consistent with literature findings [199], ranging from 2.32 to 3.25 ng with an average RSD of 0.55.

3.2.2.2 Vapour Analysis

Before the extraction procedures, organic solvents were added to the explosive standards in order to facilitate the evaporation. The standards were diluted in methanol, distilled water, acetone and acetonitrile separately, to investigate the best facilitator. In an optimization test, equimolar mixtures of 40 ng/µL of each explosive standard per solvent were left to evaporate for 15 minutes at room temperature and subjected to 40, 60, and 80 ºC in a dry heat block. This experiment demonstrated that for all three explosives the optimal temperature and solvent were 80 ºC and acetone, respectively. This finding contradicts other literature findings that recommend methanol as the optimal solvent for the recovery of nitroaromatic explosives [199], but is logically accepted as acetone presents the lowest evaporation point (56 ºC) when compared to methanol (64 ºC), acetonitrile (82 ºC), and water (100 ºC). This result is likely due to the higher concentration of analytes delivered per time provided by the solvent with more volatility. The higher volatility of acetone represents a higher flow of this solvent through the paper chad than the other solvents tested, facilitating the transportation of explosive residues. The use of acetone for the recovery and analysis of organic explosives post-blast is reported in one published study [203], while a review proposes the use of both methanol and acetone for explosives detection in different media [26].

Acetone was used to dilute the three target explosives (TNB, TNT and Tetryl) to the desired concentrations before the vapour extraction procedures. After the vapour extraction procedure the paper chad was folded and inserted directly into the sample well containing 9 µL of BGE, avoiding any other extraction or pre-concentration steps. Mixtures of the three target explosives were well detected and visualised using fluorescence quenching, with a clear separation of the three analytes and identification through the retention time. An electropherogram showing the comparison between the liquid and vapour analysis can be seen in Figure 3-6. Limits of detection and the minimum detectable masses for the three target explosives were calculated after construction of

calibration curves, which were built using vapour extraction from solutions at 10, 20, 30, and 40 ng/µL. The LOD was calculated as 3.3σ/slope of the calibration curve where σ = error in the slope [175]. The minimum detectable quantities were 6.03 ng, 9.99 ng, and 14.22 ng for TNB, TNT and Tetryl, respectively. Recovery rates for the target explosives were determined by comparing the peak area of the target explosives at 20 ng/µL in the liquid analysis to those achieved through the vapour analysis (n=4). The recoveries from paper chads after vapour extraction, when compared to the direct injection were 47, 75, and 29 % for TNB, TNT and Tetryl, respectively. The minimum detectable masses of the selected explosives and the recovery rates from the paper chads can be seen in Table 3-1.

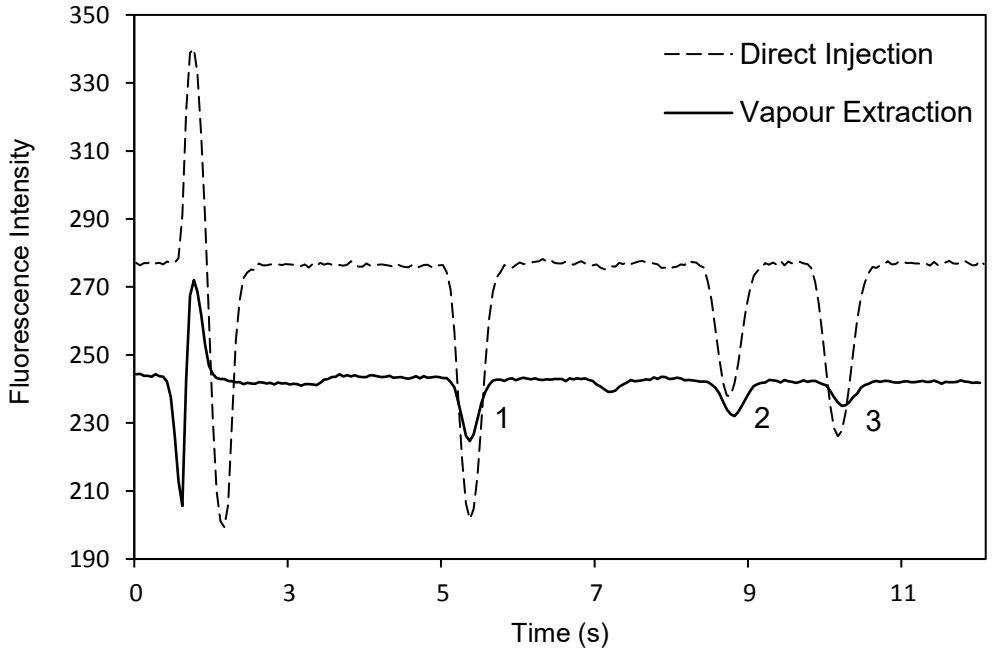

Figure 3-6: Electropherogram comparison between vapour and liquid analysis using the Bioanalyser. LOC analysis of explosive standards using direct injection of liquid standards and vapour extraction of liquid standards in filter paper. 1) TNB; 2) TNT; 3) TETRYL at 20.ng/µL. Background electrolyte (10 mM Borate: 50 mM SDS; pH 9.2), injection 1500 V for 40 s, separation 1500 V for 100 s.

The liquid and vapour analysis produced the same baseline noise, although it is more evident in the vapour analysis due to the peak size of the standards. The peak size in the vapour extraction analysis is considerably smaller compared to the liquid injection analysis. This lower yield is to be expected due to the affinity of the target molecules for the mobile phase (acetone) and stationary phase (filter paper). For vapour extraction analysis, consideration is required for sample degradation due to heating, as well as the presence of organic solvent in the filter paper, which can act as a masking agent.

Additionally, investigation of the vials post extraction showed there was a coloured residue present on the bottom indicating that it was not possible to vaporize one hundred percent of the explosive sample.

Table 3-1: Values for the average retention time and minimum detectable masses for liquid analysis and vapour extraction in the paper chad, and average recovery rates for the vapour analysis when compared to the liquid analysis (n=4)

	LIQUID ANALYSIS		VAPOUR ANALYSIS		
EXPLOSIVES	AVERAGE RETENTION TIME (S)	MINIMUM DETECTABLE MASS (NG)	AVERAGE RETENTION TIME (S)	MINIMUM DETECTABLE MASS (NG)	AVERAGE RECOVERY (%)
TNB	4.72	2.32±0.7	5.36	6.03±4.5	47.00
TNT	8.00	3.25±0.4	8.81	9.99±2.3	75.00
TETRYL	10.50	2.35±0.4	10.23	14.22±1.2	29.00

All three target explosives were successfully detected after the vapour extraction procedure, and the achieved LOD were comparable to the conventional methods used on bench CE instruments [204] proving the efficiency of microchip models. The recovery rates for the three target explosives were similar to that described in the literature using alternative methods [205], with recovery rates as high as 75 % for TNT. The lower response observed from Tetryl during the vapour extraction can be explained by several factors. The negative peaks observed in the electropherograms are due to the fluorescence quenching of the dye present on the background electrolyte [198, 206, 207], hence explosives with higher fluorescence quenching power, such as TNB and TNT may have a higher impact in these experiments than nitramines, such as Tetryl and RDX [206]. Moreover, nitramines are known to be less volatile and sensitive than the nitroaromatics [25], which significantly impacts the amount of explosive residues present in the vapour samples and explains why Tetryl was more readily detected during the liquid analysis compared to the vapour analysis.

The current method was optimized for the analysis of three target explosives, which were successfully detected, separated and identified after a fast and simple process of extraction, using low cost equipment and an innovative platform. The ability of this instrument to analyse explosive mixtures, rapidly and at relatively low costs demonstrates the potential of this method for explosive residues detection in the gas and vapour

samples. However it is important to note that given the respective LODs and considering the saturated vapour pressure for each of the three target compounds the amount of air to be sampled in a real case scenario using the current method would be extremely large. For example TNT which had a minimum detectable mass from vapour analysis calculated to 9.9 ng and a saturated vapour pressure at 25 °C of 9.15 ppb_v would need approximately 100 mL of air sampled at 100% efficiency. Considering that trace explosive vapours in the environment are generally calculated at concentrations of at least two orders of magnitude below the saturated vapour concentration, the volume needed to be sampled for the correct detection of TNT would be 10 L. Applying the same concept for the other two target analytes, a total of 4 L would need to be sampled for the correct analysis of TNB (saturated vapour pressure at 25 °C 20 ppb_v) and approximately 1400 L would have to be sampled for the correct analysis of Tetryl (saturated vapour pressure at 25 °C 0.0074 ppb_v). Those values indicate the need for a pre-concentration procedure which was not further developed in this study.

3.3 THE GRIFFIN 450^{TM} GC-MS

GC-MS detection is regarded as the gold standard for laboratory chemical identification of volatiles and semi-volatiles [208], and has been successfully used to detect explosives [22]. Over the last decade, field portable GC-MS systems have played a major role in forensic applications [209]. Field analysis requirements typically include the need for rapid assessment of the volatile organic compounds (VOCs) present, to which the GC-MS system offers the best collective analytical capability [97].

The Griffin 450^{TM} Gas Chromatograph–Mass Spectrometer (Griffin 450) (Figure 3-7), is designed to detect, identify, and confirm parts per trillion concentrations of compounds with tandem mass spectrometry (MS/MS) capability [210]. A six week study conducted by the National Forensic Science Technology Center (NFSTC) to determine whether the Griffin 450 was suitable to conduct in-field chemical analysis and identify forensically relevant compounds, such as illegal drugs, ignitable liquids, and explosives, found that it compares well with benchtop GC-MS systems [211]. For this reason, it was investigated as part of the long-term goal of identifying a portable detection and identification system for explosives VOCs.

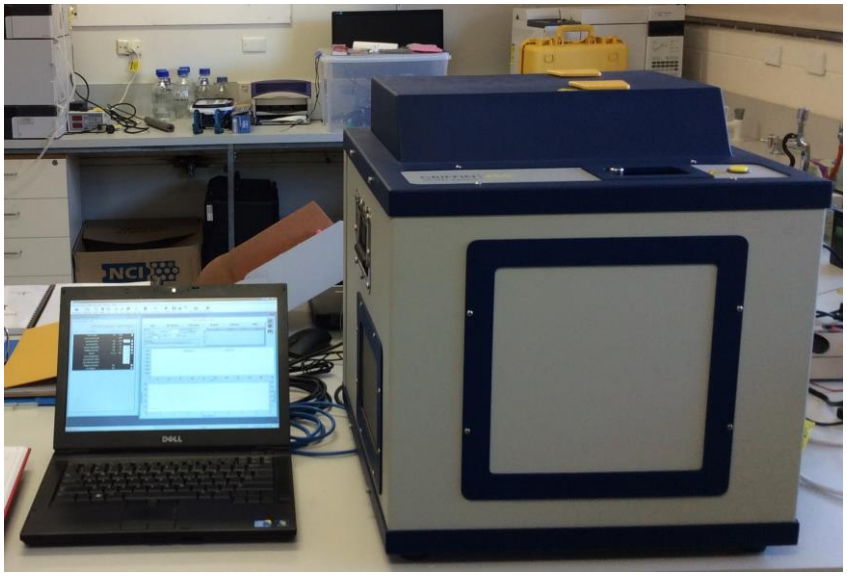

Figure 3-7: The Griffin 450™ Gas Chromatograph–Mass Spectrometer

3.3.1 Experimental

3.3.1.1 Reagents

Nitroaromatic explosives and peroxide explosive standards were chosen to perform the experiments. TNT, DNB, TNB, Tetryl, 3-NT, 2,4-DNT, 2-A-4,6-DNT, 4-A-2,6-DNT, and TATP were purchased from AccuStandard (New Haven, CT, USA) at a certified concentration of 1000 µg/mL in acetonitrile. A certified mix including the nitroaromatics and nitramines analytes of the EPA Method 8330B was purchased from Restek Corp© (Bellefonte, PA, USA) at a concentration of 1000 µg/mL in methanol. Analytical grade solvents (methanol, acetonitrile, and acetone) were purchased from ChemSupplies Pty Ltd (Gillman, SA. Australia). Sodium dodecyl sulphate (SDS) was obtained from Sigma-Aldrich (St. Louis, MO, USA) and sodium tetraborate from Honeywell Fluka (Switzerland). Ultrapure grade water (18.2 MΩ.cm^{-1}) was obtained from a Sartorius 611 water purification system. DNA 1000 Dye Concentrate® (blue) was obtained from Agilent Technologies©.

3.3.1.2 Instrument

A FLIR Systems (West Lafayette, IN) Griffin™ 450 Gas Chromatograph–Mass Spectrometer was used for all analyses in a controlled laboratory environment. The Griffin 450 is a semi-portable cylindrical ion-trap mass spectrometer (CIT-MS) [212], equipped with a low thermal mass GC. A CIT is a modified form of the quadrupole ion

trap (QIT) with the advantage of easier fabrication and miniaturization [27–29]. The CIT and QIT advantages when compared to a conventional ion trap mass spectrometer are the higher repetition rates and lower noise allowed by the fast mass analysis; the greater ion trap capacity; and more accurate intensity measurements and better ion mass accuracy [213].

The Griffin 450 GC-MS is built in an aluminium chassis equipped with shock and vibration isolators, with a depth of 48.8 cm, width of 48.8 cm, and length of 53.6 cm, which contains all of the instrument components. The instrument provides an operational method for the detection of explosives in bulk and trace samples. The method run time and data collection takes approximately 9 minutes per sample from the time of injection. The operational conditions of the GC-MS are shown in Table 3-2.

Table 3-2: GRIFFIN 450 GC-MS operational method for the detection of trace explosives

Chromatographic Parameters	
Column	15 m VB-5 MS 0.25 mm Inner diameter 0.25 µm film thickness.
injection method	Manual
injection temperature (°C)	175
Initial temperature (°C)	50 (hold 2 min)
Final Temperature (°C)	280
Ramp / Rate (°C/min)	30
Hold in final temperature (min)	0
Flow rate (mL/min)	1.1
Split Flow (%)	23.2
Volume injected (µL)	1
Mass Spectrometry Parameters	
Mode	EI
Tranfer line temperature (°C)	175
Electron energy (eV)	N/A
Scan mode	Full
Emission current (µA)	N/A
Mass scan rate (scan/sec)	5

The carrier gas and spectrometer buffer was helium, which had to be supplied by an external source. The instrument had two lateral hinges and a total weight of approximately 44 kg, allowing the instrument to be carried to the field by two people. The maximum

power consumption is 600 W, which is supplied by a 5-kW portable generator. The Griffin 450 GC-MS is controlled by a laptop computer with a Microsoft Windows operating system running Version 3.7.1 of the Griffin System Software package, which was used for the collection and analysis of all data related to this study.

3.3.1.3 Liquid Injections

Individual stock solutions of explosive standards (1000 ng/µL) were diluted to the desired concentration in methanol or acetonitrile depending on the solvent of the standard, while mixtures from different explosives combining 4 to 16 standards were diluted into methanol, as proved optimal in the literature [26]. The GC-MS analyses were performed by manually injecting 1 or 2 µL sample volumes into the instrument and using the method described in Table 3-2. Different explosives standards were analysed on randomly selected days.

The LOD and LOQ were determined based on the signal-to-noise ratio of 3:1 and 10:1, respectively for seven explosive standards individually tested. The experiments were performed by adding aliquots of the target standards to the samples in decreasing concentrations in triplicate. Concentrations were chosen in order to give the best response for each individual standard as it was noticed that the instrument was able to detect some explosives more easily than others.

3.3.1.4 Vapour Injections

According to the instrument's user manual, the Griffin 450 GC-MS includes a split/splitless injector that accepts liquid and gas samples, in addition to solid-phase microextraction (SPME) fibres [210]. This feature makes this instrument relatively unique and an excellent experimental tool for field deployment tests and vapour analysis. However, as will be discussed later in this chapter the instrument did not respond well to liquid injections and after numerous issues that could not be resolved, vapour tests were abandoned, hence no further details are provided here.

3.3.2 Results

3.3.2.1 Liquid Injections

Figure 3-8 shows the chromatographic separation of a mixture containing 14 explosives over time and temperature. The sample is a certified nitroaromatic and nitramines solution

in methanol. Two microlitres of the sample at 200 ng/µL were injected and analysed using the instrument method for trace explosive detection. The inlet was held at 175 °C and the oven temperature started at 50 °C (held for 2 minutes) and achieved a final temperature of 280 °C with a ramp rate of 30 °C.min^{-1}.

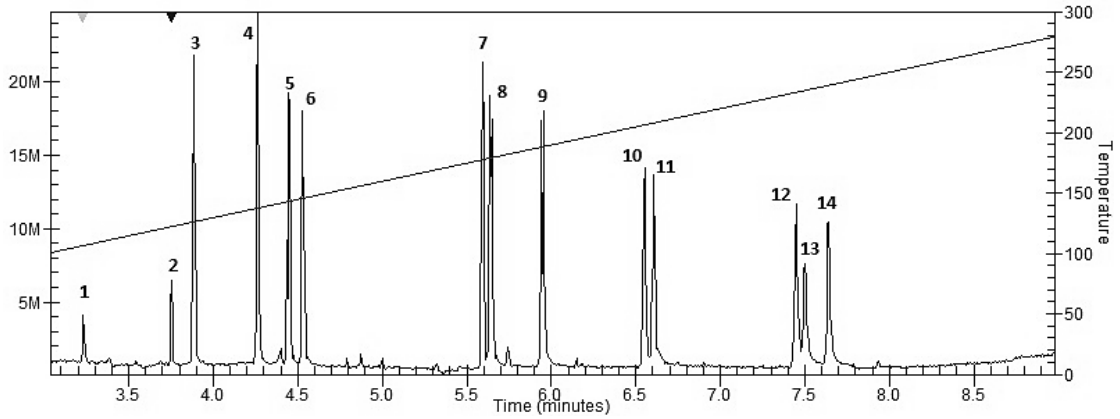

Figure 3-8: Chromatogram of a mixture of 14 explosives analysed using the Griffin 450™. The sample was diluted in methanol to the desired concentration of 200 ng/µL and an aliquot of 2 µL was injected into the instrument. Analyses were carried out using the instrument's method for the analyses of trace explosives. The non-confirmed identities for each peak are: 1) HMX; 2) RDX; 3) Nitroglycerine; 4) Dinitroaniline; 5) NB; 6) 2-A-4,6-DNT; 7) 2-NT; 8) 1,3-DNB; 9) 2,6-DNT, 10) TNT; 11) 2,4-DNT; 12) TETRYL; 13) PETN; 14) TNB.

One microliter of each of seven selected explosives (TNT, Tetryl, RDX, HMX, PETN, TATP, and NB) were individually injected in triplicates on three randomly selected days, in order to determine the limits of detection and quantification of the standards. Calibration curves were produced by selecting seven different concentration points for each explosive after the initial assessments to evaluate their sensitivity on the instrument. The linearity tests showed limits of detection and quantification lower than 1 ppm, as can be seen in Table 3-3. The achieved values were comparable to benchtop instruments and to other experiments conducted with the Griffin 450 GC-MS reported in the literature [212, 214]. However, validation tests confirmed that these LOD values were not achievable. For example, PETN was calculated to have a minimum detectable mass of 560 pg but experimentally never achieved a signal to noise ratio of 3:1 under 10 ng (injection of 1 µL of a 10 ng/µL sample).

Table 3-3: Limits of quantification found by linearity for the 7 target explosives chosen for the GRIFFIN 450 GC-MS experiments. 1 µL of each standard in different concentrations was injected individually into the instrument (n=3)

STANDARD	LOD (ng/µL)
TNT	0.41±1.5
PETN	0.56±3.5
TATP	1.15±1.2
TETRYL	0.35±1.4
RDX	0.50±3.8
NB	0.35±1.5
HMX	0.50±3.8

Unfortunately due to problems with the instrument discussed below, it was not possible to appropriately test other figures of merit such as repeatability, reproducibility, and intra and interday RSD, as these values varied considerably.

3.3.2.2 Instrument Issues

It is important to note that this was an imported instrument which was on loan to Western Sydney University for a series of validation tests. There was no close technical assistance or support. Moreover, the users were restricted from opening or changing any hardware settings on the instrument since it is military grade and should be validated for its current features.

Before commencing the initial liquid standard analysis, the instrument had a software issue that restricted the communication between the laptop and the instrument itself. This issue could not be remotely resolved and the laptop that accompanied the instrument had to be replaced by the supplier. The new laptop was also unable to connect to the instrument and could not be remotely resolved which resulted in the instrument and laptop being returned to the USA for repairs. After several months a new operative set arrived in Australia.

During the experimental trials it was noted that this instrument was not ready for field deployment. The instrument took over 30 minutes for start-up and equilibration and needed more than 12 minutes to perform sample analysis with added time for cooling down and warming up in between runs, limiting its use for real life scenarios that demand fast action and decision making. It was not easily portable as marketed but would be better described as transportable. Compared to existing handheld and portable instruments [15]

it features a large foot print (49 x 49 x 54 cm) and weight (44 kg), requiring handling by a two-person team. Moreover it requires external supplies of carrier gas and power.

This instrument operates with electron ionization (EI), which is the most commonly applied technique in mass spectrometry [215]. EI produces molecular ions from gas phase analytes [216]. The energy for ionizing and fragmenting gas phase analyte molecules is acquired by interaction with 70 eV electrons produced by a hot filament [215]. In order for an EI operating mass spectrometer to be functional the filament must be working. The efficiency of the EI is also related to the degree of interaction between sample molecules and energetic electrons, what can be increased by increasing the filament current reducing the lifetime of this item in order to achieve a higher sensitivity [215]. During the validation experiments the Griffin 450 indicated a failure in one of the filaments (Figure 3-9), which meant that it was not possible to continue the experiments with the current set up.

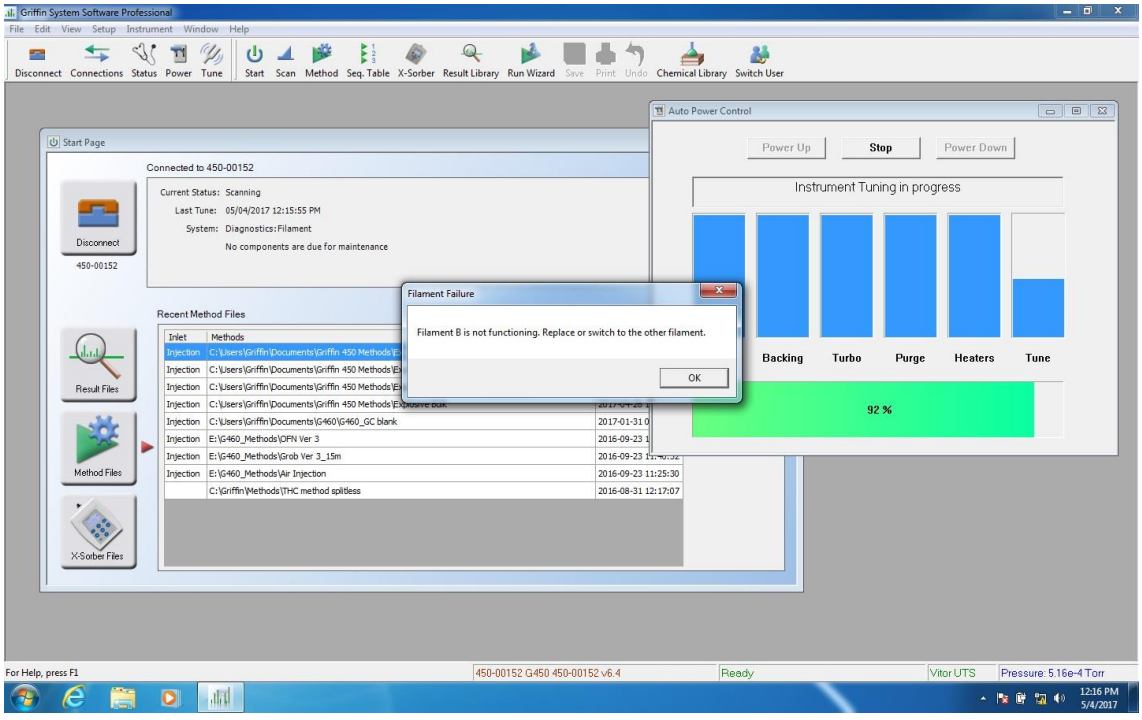

Figure 3-9: Screenshot taken from the first filament failure presented by the GRIFFIN 450™ GC-MS.

The Griffin 450 is equipped with two filaments and an easy click-and-play selection to switch between the two (Figure 3-10). After selecting the second filament, the instrument reported it as inoperative or faulty (Figure 3-11). The testing continued with the faulty filament. The new tests produced a variation of more than two orders of magnitude. For

example it was no longer possible to detect PETN under 50 ng/µL. As described previously, there was no local support for this instrument and it was not possible to change any of its hardware features.

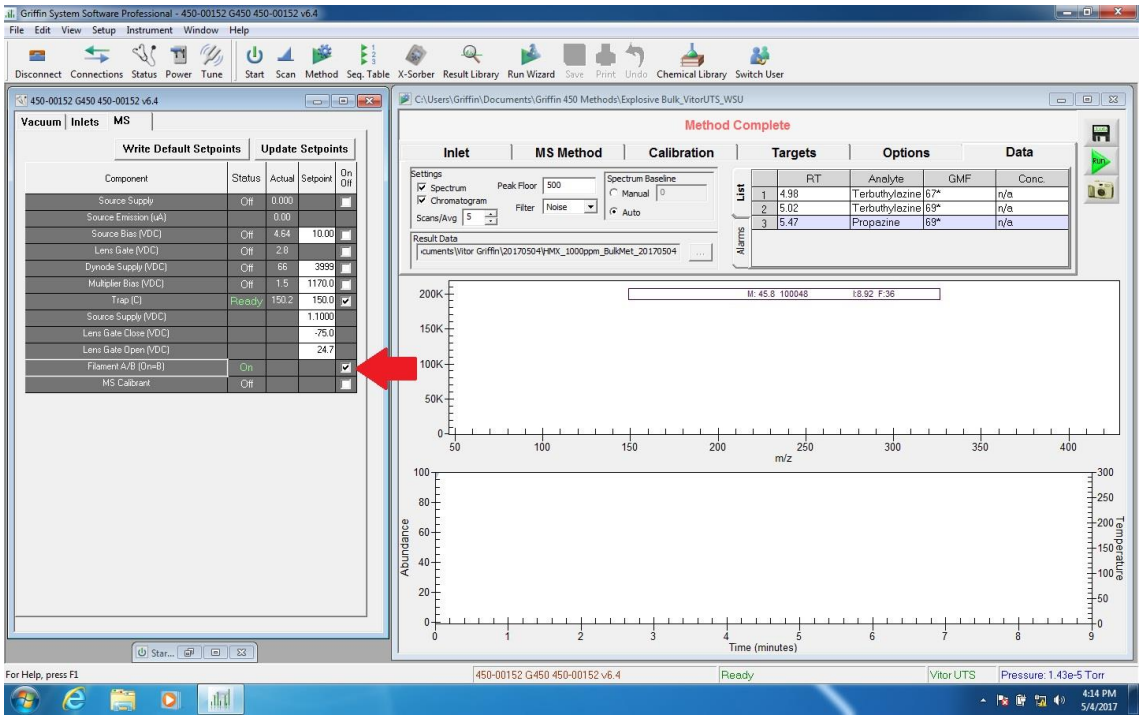

Figure 3-10: Screenshot of software feature that allowed the switch between filaments.

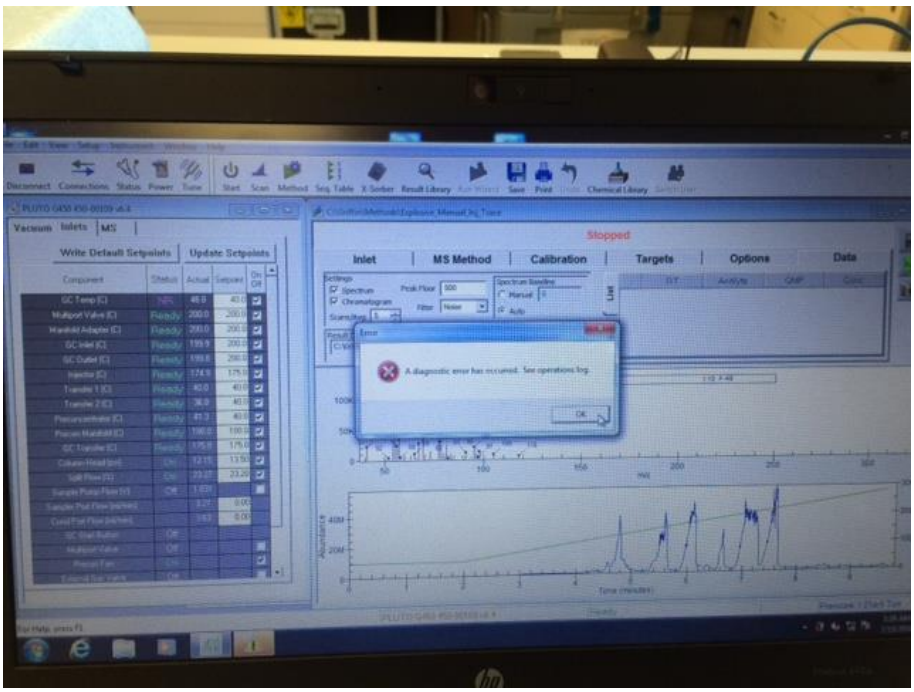

Figure 3-11: Photo taken from computer screen demonstrating issues after filament swap.

After the limited results achieved with this instrument and the long period taken to perform uncomplicated analysis, it was ultimately decided that the instrument should be returned to the supplier as it could not be fixed without major consultation. As the instrument had to be returned and it was no longer available for use in this study, the vapours analysis could not be performed and comparison between the Agilent Bioanalyzer 2100 and the Griffin 450 GC-MS was compromised. Without the vapour trials it was not possible to test the inlet for its capability to perform liquid and SPME analysis. It was also not possible to compare the instrument method and limits of detection for samples extracted from the headspace of explosive standards to other instruments. Although vapour analysis was planned as part of this study the series of issues faced with this instrument lead to subsequent testing being abandoned. The decision was made to focus on more reliable benchtop instruments for the detection of explosive VOCs produced by liquid standards and explosive samples.

3.4 CONCLUSIONS

Two semi-portable analytical instruments, the Agilent Bioanalyzer 2100 and the Griffin 450 GC-MS, were selected to perform tests on the screening of explosives from liquid and vapour samples. The main criteria were instrument availability, detection method, analysis time, and required consumables. These instruments represent two different mechanisms for the current issue of explosive detection in air, both offering versatility, a basis for method development and optimization, and promising platforms but with considerable need for improvements and future research.

3.4.1 Portability

Both instruments are marketed as portable, but the need for an external power supply in both cases and the need for an external source of carrier gas for the Griffin 450 GC-MS limits the portability of these instruments. According to Agilent Technologies Inc©, the Bioanalyzer 2100 dimensions are 162 mm (W) x 412 mm (D) x 290 mm (H) and the weight is 10 kg. The instrument can perform 12 sample analyses in 1800 seconds, demonstrating the instruments rapid analysis performance. However the instrument is not ruggedized, it only operates on temperatures ranging from 15 to 27 °C, and it requires an external power supply, limiting its field use.

The Griffin 450 GC-MS entered the market of portable solutions for chemical analysis of clandestine products in 2007, and although designed to be portable, the large footprint of this instrument (49 x 49 x 54 cm; 44 kg) requires handling by a two-person team. This platform is marketed as being ruggedized, having shock and vibration absorbers, being capable of working in temperature ranges from 5 to 40 °C and under conditions of high humidity (over 85 %) and dust, making it suitable for use in conditions that would not be conducive to most ordinary benchtop GC-MS. However the instrument has a limited mass scan range of 40 to 425 m/z, requires at least 30 minutes to power up and equilibrate, requires external supplies of carrier gas and power, and takes at least 12 minutes between analyses making it difficult to be deployed in situations where rapid in-field analysis are needed. This instrument has now been discontinued and a new version called Griffin 460^{TM} has been released.

3.4.2 Bioanalyzer Liquid and Vapour Analysis

The liquid and vapour analysis with the Agilent Bioanalyzer 2100 showed that this instrument is a promising technique for the detection of explosives residues. This method was able to detect and quantify vapour emitted from explosive standards after evaporation. The extraction process used minute amounts of standards and reagents and the use of a filter paper to collect explosive residues in the headspace provides an inexpensive approach. The explosives were detected through fluorescence quenching and separation occurred in under 20 seconds, representing a fast and accurate method for the detection of explosives in vapour samples. The Bioanalyzer device does not require pumps or gas for its operation and can analyse up to 12 samples in under 1800 seconds. The limits of detection are close to those reported in the literature and the insertion of the paper chad directly into the sample well represents a significantly simplified extraction process, which proved to be fast and reliable. Results show that a minimum temperature of 40 ºC is necessary to vaporize the compounds with the assistance of acetone or other organic solvents. Nonetheless, the optimized extraction process for this study has shown positive results as demonstrated by the LOD and recovery rates achieved.

Although all compounds were separated and detected, the analytes had to be identified by their retention time and mobility, since the instrument does not contain software for compound identification. It is important to note that this is a preliminary test that has demonstrated the potential and suitability of microchip electrophoresis analysis for the

detection of explosives in the headspace, but is a long way from being the answer for the issue of detection of explosive VOCs in real case scenarios. This experiment does not reflect true detection of concealed explosives in real scenarios as the paper chads are placed only centimetres away from the liquid standards, maximizing the amount of vapours extracted in the pre-concentration step. Heating the liquid standards also considerably raises their volatility facilitating the analysis and analyte detection but consideration must be given to sample degradation. The results promote the viability of this method in future portable instruments for field work, and it is highly recommended that further studies be conducted with this platform. However, the method was not deemed sufficiently advanced to assist in this study which aimed to identify the key volatile compounds in explosive vapours since this would require a chemical library for identification purposes and was not available (nor could it be created) at the time of study.

3.4.3 Griffin 450 GC-MS Liquid Analysis

The Griffin 450 GC-MS analysis proved that the instrument has limits of detection close to those achieved with benchtop instruments, but a validation test of the statistically relevant LOD values suggest that these numbers are not readily achievable. Although the system software incorporates AMDI, NIST, and a user defined compound library, the instrument is not capable of automatically identifying the detected compounds, as it is necessary for them to be tagged manually in the system. These findings contradict the NFSTC study [211], which found that the instrument was capable of detecting and identifying trace amounts of drugs, ignitable liquids, and explosives compounds. During the instrument tests, numerous hardware and software issues were identified, which did not allow for further tests to be conducted including the vapour analysis. The instrument does not present itself as a viable solution for the headspace analysis of explosive VOCs in its current form, further confirmed by the recent choice of the manufacturer to discontinue the instrument for a newer platform with the same method.

This chapter aimed to test and prove two different semi-portable methods for explosives detection, however both methods were not able to convincingly demonstrate detection and identification capabilities for in-field detection of VOCs. It became evident through the investigation of the CE-C^4D system, the Agilent Bioanalyzer 2100, and the Griffin 450 GC-MS that semi-portable systems are not sufficiently advanced for the detection of explosive VOCs which was the primary aim of this thesis, and so the decision was made

to revert to benchtop instrumentation to determine the optimal method for identifying key volatile compounds in explosives. The following studies therefore investigated SPME-GC-MS which is considered one of the gold standards, and SPME-GCxGC-TOFMS which offers several advantages over one dimensional GC-MS. After two years of trials, it was deemed more important to identify the compounds through enhanced sensitivity and selectivity, rather than focusing on the portable capability of the instrument(s).

Chapter 4: THE USE OF GC-MS AND GC×GC-TOFMS FOR THE PROFILING OF VOCs FROM EXPLOSIVE STANDARDS AND TRAINING AIDS

Chapter 4: THE USE OF GC-MS AND GC×GC-TOFMS FOR THE PROFILING OF VOCs FROM EXPLOSIVE STANDARDS AND TRAINING AIDS

4.1 INTRODUCTION

This chapter aims to describe an optimal benchtop method for identifying and chemically profiling key volatile organic compounds (VOC) present in the headspace of explosives. Certified explosive standard mixtures were used for the method development using traditional gas chromatographic (GC) separation methods and advanced two dimensional gas chromatography (GC×GC). Several training aids commonly used by the NSW Police Dog Unit (NSWPDU) during the training of explosive detection dogs (EDDs) were analysed and chemically characterised. Training aids are tools used to train detection dogs and can be represented by the real substance (e.g. drugs, explosives, blood etc.) or odorants, which mimic the odours of the real substance. The NSWPDU uses a variety of real explosives for the training of their EDDs, which were the samples targeted in this study. The goal of the study was to identify key VOCs present in these training aids in order to determine if these VOCs are the key components to which the dogs alert.

4.1.1 Headspace - Solid Phase MicroExtraction (HS-SPME)

HS-SPME is a widely used technique and has proven to be an important sample collection method for the headspace analysis of forensic specimens, due to the many advantages that this technique offers [217]. The use of HS-SPME provides sufficient pre-concentration for most explosives samples [34, 78, 105, 117, 218], which is a necessary step in the analysis of these compounds due to their low volatility [219]. Moreover, HS-SPME has been successful in previous studies when coupled to GC and GC×GC instruments for the analysis of plastic explosives, chemical warfare agents, explosive taggants, drugs, decomposition odours, and for the recovery of explosive residues from soil and water [118, 218, 220, 221].

4.1.2 Mass Spectrometry Detection

There are many forms of mass spectrometry (MS) detection including, quadrupole, ion trap, time-of-flight and tandem based techniques [1]. Two alternative detection methods

for GC analysis of explosives other than MS [222] are the electron capture detector (ECD) and thermal energy analyser (TEA) [223]. However, mass spectrometry offers a higher information content than ECD or TEA and the use of extracted ion chromatograms and selected ion monitoring (SIM) can provide added discrimination in data analysis [96]. It was also chosen in this study due to its availability and prevalence in forensic laboratories.

4.1.3 HS-SPME-GC-MS

GC-MS is a fundamental part of the analytical arsenal in analytical forensic laboratories and it is currently considered the gold standard technique for the detection of volatile and semi-volatile compounds [208]. GC is a reliable separation method, which retains compounds based on differences in volatility and solubility in the liquid and gaseous phases [224]. Gas chromatographic methods for the analysis of trace explosive and incendiary chemicals have recently been reviewed [26, 102, 225], and trace analysis of picogram quantities of explosives by injection of standard mixtures has previously been demonstrated [107]. The extremely low vapour pressures for many of the common explosives hinders the detection of these compounds directly through vapour samples [18], however, several studies were able to identify dominant compounds and profile VOCs from propellants, and plastic and nitroaromatic explosives [78, 107, 108] with the use of HS-SPME-GC-MS extraction methods.

4.1.4 HS-SPME-GC×GC-TOFMS

Compared to one dimensional (1D) GC analysis, comprehensive two dimensional (2D) gas chromatography (GC×GC) analysis delivers improved resolution and separation power due to increased peak capacity [130]. The GC×GC capability to separate complex mixtures containing hundreds or thousands of analytes makes it an ideal technique for the development of chromatography methods with sufficient resolution to investigate the volatile signatures present in the headspace of explosives [118]. HS-SPME-GC×GC-TOFMS extraction methods have been previously applied using fast conditions to resolve the volatile signature of explosives standards and samples in five minutes [118]. The high degree of resolution allows the separation of co-eluted analytes from nitroaromatic standards and resolves the complex profiles of commercial explosive samples.

4.2 HS-SPME-GC-MS ANALYSIS OF EXPLOSIVE TRAINING AIDS USING A CONVENTIONAL CHROMATOGRAPHY METHOD

Due to the issues encountered during portable GC-MS instrument tests (see section 3.3.2.2) it was not possible to perform the headspace analysis of explosive compounds. In order to progress to the second stage of this project a bench-top GC-MS was determined to be the most viable and logical alternative. HS-SPME-GC-MS for the analysis of explosives has been previously demonstrated [78, 79, 116, 119, 226, 227]. The HS-SPME-GC-MS analysis of high and low explosives was expected to provide an understanding of the VOCs that comprise the odour signature of explosive materials. These key VOCs will be subsequently used in field trials with EDDs during training to determine if they accurately represent the odour recognised by the dogs. The results of these trials will be used for the development of better training aids and protocols for EDDs by comparing the knowledge of explosive odour chemistry, the canine reaction to the chosen key VOCs and the effectiveness of available training aids. A better understanding of these key VOCs will also assist in improving future development of chemical detection methods.

4.2.1 Materials and Methods

4.2.1.1 Training Aids

Explosives and propellants used as training aids by the NSWPDU were sampled in order to carry out a non-target analysis of the VOCs present in the headspace of real explosive materials. This was important to identify the major VOCs for purchase of liquid standards for subsequent optimisation and development of a fast GC method (see Section 4.4). The NSWPDU approved sample collection of the training aids under supervision in a controlled environment. A list of the training aids sampled, including weight, primary compound described on the material data sheet and appearance can be seen in Table 4-1.

Table 4-1: List of real explosives used as training aids sampled for the VOC profiling experiments, including weight and major compound as described in the supplied MSDS

COMMERCIAL NAME	WEIGHT (g)	MAIN COMPOUNDS	APPEARANCE
Power Gel	0.111	Ammonium Nitrate	Industrialized Gel
Primer Sheet	0.209	PETN + DMDNB	Industrialized Gel
Black Powder	0.326	Potassium Nitrate	Granules
Smokeless Powder	0.248	Nitrocellulose + Nitroglycerin	Powder
Detonation Cord	0.176	PETN	Industrialized Cord
Composition B	0.709	RDX+TNT	Plastic Block
Pentolite	0.125	PETN+TNT	Plastic Block
PE-4	0.233	RDX	Industrialised Clay
ANFO	0.244	Ammonium nitrate + Fuel oil	Improvised Powder

4.2.1.2 VOC Collection Using SPME

A SPME holder, polydimethylsiloxane (PDMS) 100 μm SPME fibre, and PDMS-divinylbenzene (PDMS-DVB) 65 μm SPME fibre were purchased from Supelco (Bellefonte, PA, USA). All fibres were conditioned at 270 °C for ten minutes prior to extraction based on the manufacturer's recommendations. Fibre blanks (no extraction) for each of the fibres used were analysed prior to each sampling day aiming to avoid cross-contamination from sample to sample on a given day or across different days. PDMS was chosen for investigation due to the polyvalent trapping properties and the proven stability of this fibre for studies that investigated explosive detection and field sampling [34, 105, 117, 218]. PDMS-DVB was chosen due to its proven ability to yield optimum extraction of several nitroaromatic explosives and a nitramine, namely TNT, 2-NT, 3-NT, 4-NT, 2,6-DNT, and Tetryl, in addition to being reported in several studies for explosive extraction [105, 226].

4.2.1.3 HS-SPME Extraction of Explosive Training Aids

Each individual training aid was placed in an aluminium tin with pre-perforated aluminium lids, which allowed the insertion of the SPME needle (Figure 4-1). The headspace extraction was conducted for 30 minutes at room temperature as previously optimised in the literature [107]. Triplicates for each sample were collected with the use of three PDMS 100 μm and three PDMS-DVB 65 μm SPME fibres, which were compared to optimize the VOC extraction procedure. The extraction of the explosive samples was conducted in a safe and controlled environment at the NSWPDU. To assure the retention

of the extracted VOCs and to avoid contamination by different odours that could be present in the room [228], the fibres were individually wrapped in aluminium foil and inserted into individual screw cap Pyrex® culture tubes (Sigma–Aldrich, St. Louis, MO, USA) following the extraction. The tubes were sealed with Parafilm® supplied by Sigma–Aldrich (St. Louis, MO, USA), before being transported to the university laboratory and analysed by GC-MS.

Figure 4-1: Training aid sampling scheme. The training aids were placed in an aluminium tin covered with a pre-perforated aluminium lid to allow the insertion of the SPME fibres. PDMS and PDMS/DVB fibres were used in triplicate for each sample. The fibre was exposed to the headspace for 30 minutes at room temperature, before being transported to the laboratory for analysis.

4.2.1.4 GC-MS Instrumental Parameters

The 1D GC-MS analysis was performed using a Trace 1300 Gas Chromatograph coupled to an ISQ QD Single Quadrupole Mass Spectrometer (ThermoFisher Scientific, Waltham, MA, United States).

The training aids were analysed with the use of a 25 m DB-5MS UI column from Agilent Technologies© with 0.25mm diameter and 0.25 µm film thickness. The injection port was held at 220 °C, with a 5 min SPME desorption. The oven program started at 40 °C for 5 min followed by a 10 °C.min^{-1} ramp to 280 °C, which was held for 1 min. The injection was conducted in splitless mode. The carrier gas was helium at 1.0 mL.min^{-1}. This method

was previously reported in the literature for the VOC profiling of high and low explosives [107] and was adapted for the instrumentation used.

4.2.1.5 Data Analysis and Interpretation

Data analysis was performed using the Chromeleon 7.2.8 Chromatography Data System (ThermoFisher Scientific, Waltham, MA, United States) and library matching was carried out using the NIST Mass Spectral Library (NIST 14). The MS was operated in electron ionization (EI) full scan mode from 50 to 500 amu with a 1 min solvent delay. Identified compounds were compared with literature reports and their abundance was recorded and used for further analysis.

4.2.2 Results

The samples were separated according to the material safety data sheets (MSD) provided, as follows: TNT based explosives, also called cast explosives, are commonly high explosives based on TNT [107]; plastic explosives, which generally involve nitramine or nitrate ester explosives, such as RDX, HMX and PETN [19]; tagged explosives, which were the samples listed as having 2,3-dimethyl-2,3dinitrobutane (DMDNB) in their composition; blasting agents, such as detonators and ammonium nitrate based explosives [9]; and propellants, which are predominantly powders and pyrotechnics [229].

The VOCs identified were also divided into groups: explosive compounds, which are the analytes exclusive to explosives such as 2,4-dinitrotoluene (DNT), 1,3-dinitrobenzene (DNB), TNT, PETN and RDX; taggants, which is the group composed of the four plastic explosive markers including DMDNB; and additives, which comprise all the compounds associated to explosives that can also be found in other substances and in the environment.

4.2.2.1 Headspace Analysis

The HS-SPME extraction and GC–MS analysis of the explosive training aids presented a complex matrix for all of the analysed samples. The headspace analysis showed some large unresolved hydrocarbon mixtures for several of the explosive training aids, as can be seen in Figure 4-2 shows the chromatograms of four different high and low explosives extracted with either PDMS or PDMS/DVB fibres as an example.

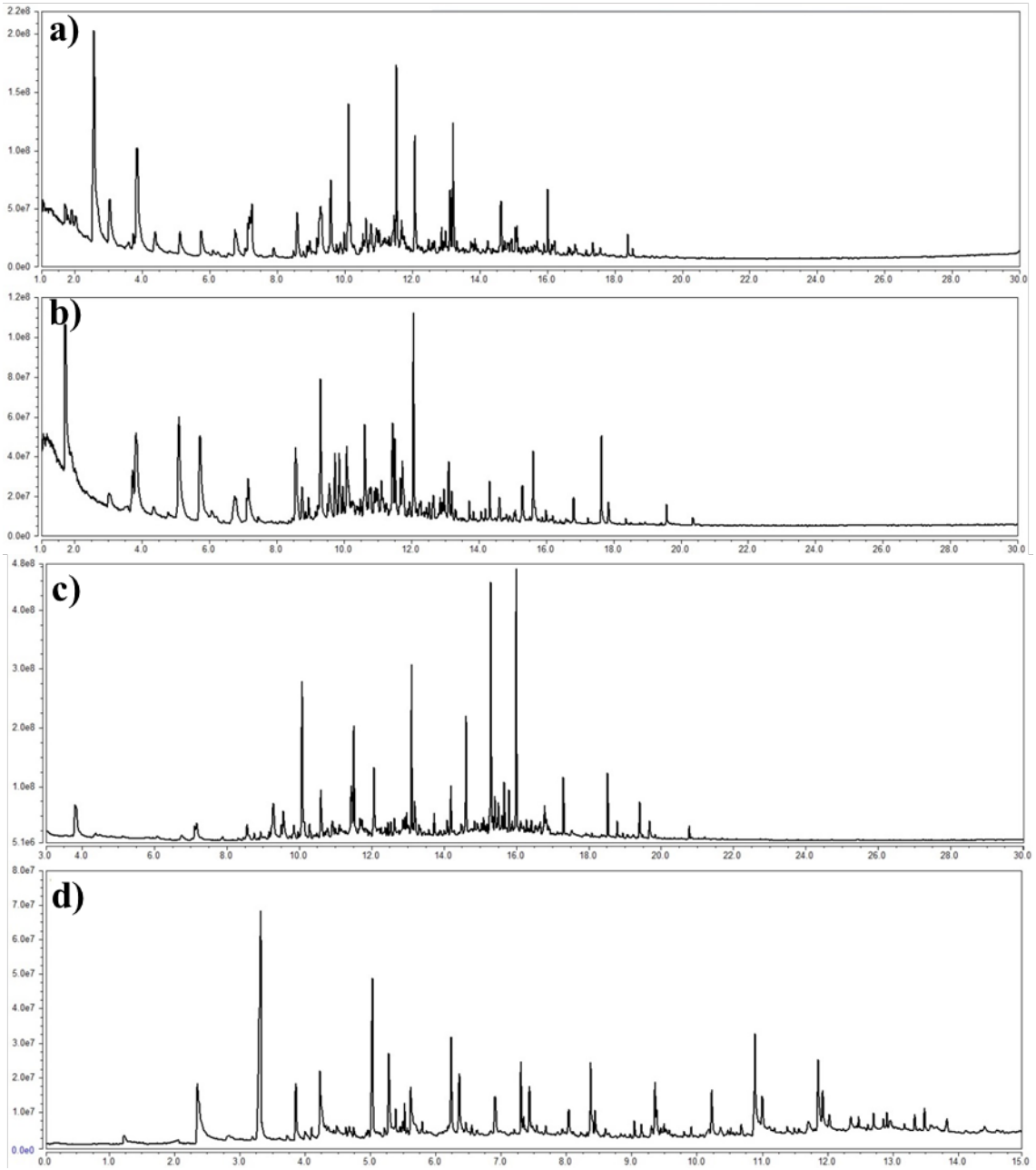

Figure 4-2: Chromatograms of VOCs from explosive training aids analysed by HS-SPME-GC-MS. The samples analysed and fibre used for extraction of each compound are: a) high explosive Detonation cord extracted with a PDMS/DVB fibre; b) high explosive Pentolite extracted with a PDMS/DVB fibre; c) high explosive Power gel extracted with a PDMS/DVB fibre; and d) low explosive Black powder extracted with a PDMS fibre.

The use of the library to search for an initial identification was challenging for these mixtures as many of the compounds co-eluted and were classed as an unidentified ester, alcohol, or acyclic alkane based on their chemical structure. This classification is used in the following sections for peak labelling and comparison.

4.2.2.2 Fibre Comparison

Previous studies have reported varying results for the optimal fibre chemistry to be used on headspace extraction of explosives. One study reported PDMS/DVB fibres to have the highest overall recovery rates compared to PDMS [105], while a second study reported that PDMS was the most sensitive fibre in comparison to PDMS/DVB for each extraction time investigated [218]. In the analysis conducted during this project, both PDMS and PDMS/DVB extractions yielded similar chromatograms. Figure 4-3 compares a high explosive extracted with (a) PDMS and (b) PDMS/DVB fibres, while Figure 4-4 compares a low explosive extracted with (a) PDMS and (b) PDMS/DVB fibres.

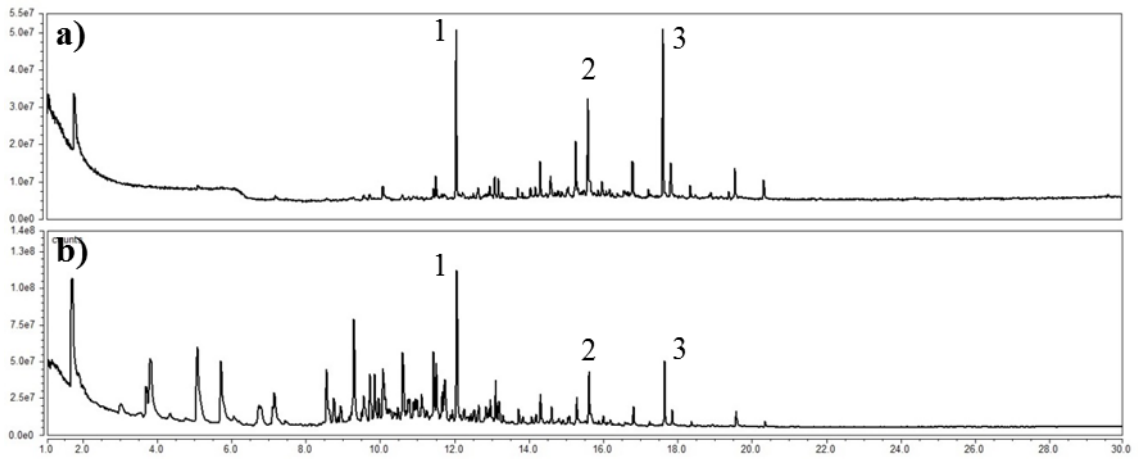

Figure 4-3: Comparison between a) PDMS and b) PDMS/DVB fibres for the HS-SPME-GC-MS analysis of a high explosive (Pentolite). Chromatograms were extracted from analysis made with the different fibres on the same day, with the same explosive training aid sample, and analysed using the same method. Splitless injection at 220 °C with 5 min fibre desorption. Key VOCs identified in the headspace: 1) 2-ethyl-hexanol; 2) Butylated hydroxytoluene; 3) 2,4-dinitrotoluene.

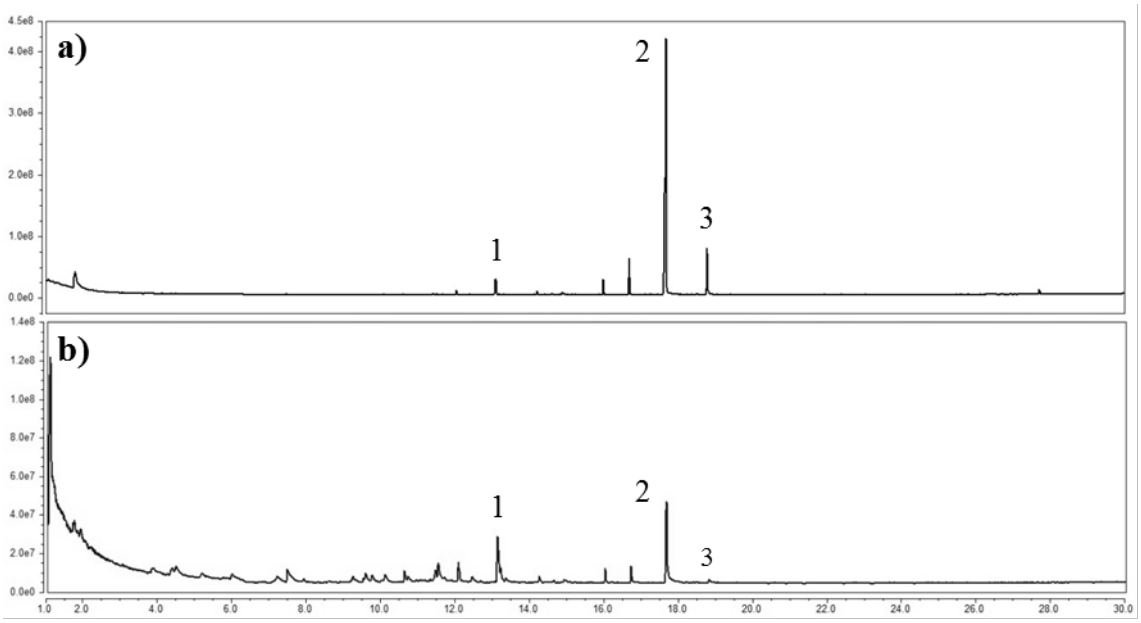

Figure 4-4: Comparison between a) PDMS and b) PDMS/DVB fibres for the HS-SPME-GC-MS analysis of a low explosive (Smokeless Powder). Chromatograms were extracted from analysis made with the different fibres on the same day, with the same explosive training aid sample, and analysed using the same method. Splitless injection at 220 °C with 5 min fibre desorption.
Key VOCs identified in the headspace: 1) Dodecane; 2) 2,4-dinitrotoluene; 3) Diphenylamine.

The compounds identified were comparable between fibres however, the PDMS fibres produced a better peak shape and better baseline separation with less noise interference. For that reason PDMS fibres were selected as the most appropriate extraction phase and were used in all subsequent analyses.

4.2.2.3 TNT Based Explosives

Two TNT based explosives, namely Composition B and Pentolite, were analysed from the training aids provided. Pentolite is a PETN and TNT mixture (shown in Figure 4-5 a), while Composition B is a mixture of RDX and TNT (shown in Figure 4-5 b).

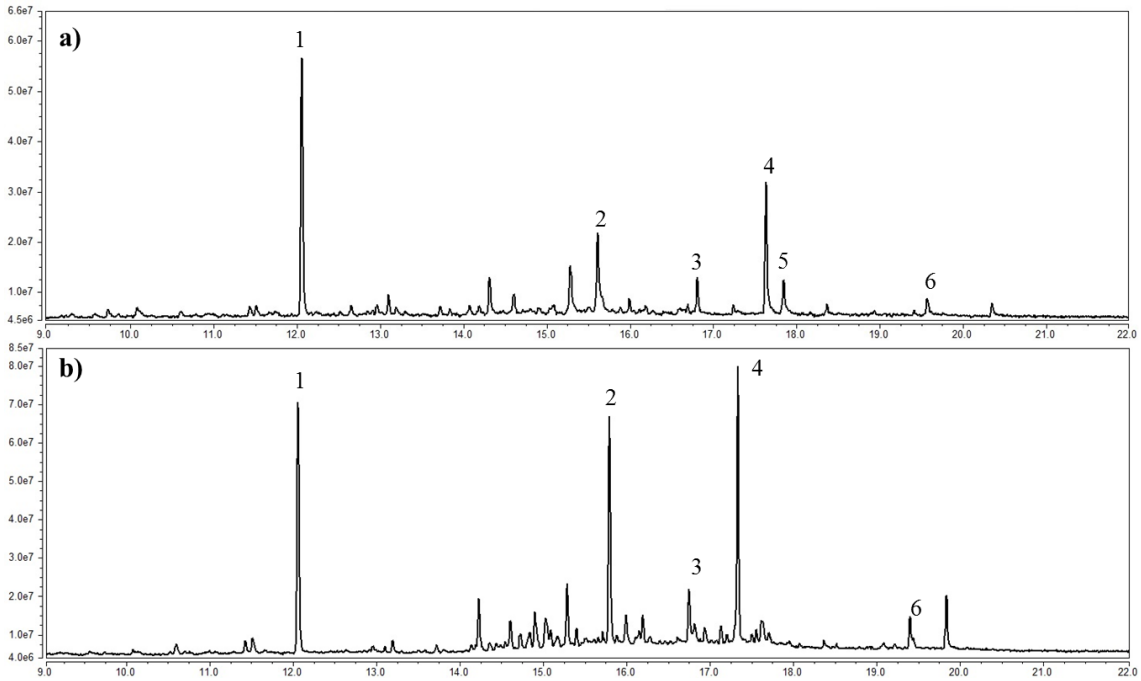

Figure 4-5: Chromatogram of VOCs detected in the headspace of cast explosives used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC-MS analysis. a) Represents the chromatogram of Pentolite, an explosive mainly composed of a mixture of TNT and PETN, and b) represents a chromatogram of Composition B, an explosive mixture of TNT and RDX. Key VOCs identified in the headspace: 1) 2-Ethyl-hexanol; 2) Butylated Hydroxytoluyene; 3) Naphtalene; 4) 2,4-Dinitrotoluene (DNT); 5) 1,3-Dinitrobenzene (DNB); and 6) 2,4,6-Tinitrotoluene (TNT) .

The chromatograms of the headspace analysis of both TNT based explosives demonstrated the presence of TNT, however neither of the other two parent compounds, i.e. RDX and PETN, were detected. Previous literature reports have demonstrated the challenge associated with the detection of RDX and PETN via air sampling of plastic explosives due to their very low vapour pressures [78, 107, 117]. One study reports that PETN and RDX could not be observed in the headspace of Booster and C-4 samples using SPME-GC-MS and SPME-GC-ECD [107]. A second study states that PETN and RDX were detected using SPME-IMS, however, the results could not be replicated [117]. The primary challenge for detecting these compounds is that RDX and PETN do not have sufficient vapour pressure to allow for routine headspace sampling [25].

Analysis of the chromatograms shows that no explosive taggants were detected in the headspace of either samples, however, several additives were found. Additives can include stabilizers, binders and plasticizers which are added to the composition of explosives to improve stability, burn properties, shelf life, to build the explosive casing, and to optimize safety and product performance [107]. Among the additives identified was cyclohexanone, which is a common recrystallization solvent used in RDX synthesis

[230]. Cyclohexanone has been reported as a common headspace component of PE-4 in previous publications [78, 154, 218, 231], and has been studied for its volatility and composition, including the evaluation of explosive detection dogs response to this compound [107, 218]. Due to its volatility cyclohexanone is reported to be more commonly observed in fresh samples [231]. Interestingly, none of the samples analysed in this study were fresh samples, as they had been stored and used for the training of canines, and therefore exposed to the environment, for more than one year. Although several studies have reported cyclohexanone for its known relation to RDX, this compound is primarily used in the production of nylon and is related to the manufacture of other synthetic substances [78]. Therefore, considering the high volatility of cyclohexanone it is likely that it did not originate from the RDX compound but is related to other polymers involved in the manufacture of these explosive samples. The other additives identified were the plasticizers: 2-ethyl-1-hexanol which is primarily used in the manufacture of the diethyl hexyl phthalate (DEHP) present in several plastics [78]; butylated hydroxytoluene which is a phenol derivative used as an antioxidant and present in several industrial synthesis [232]; and naphthalene which is a common precursor of resins.

Two explosive related compounds were identified, namely 2,4-dinitrotoluene (DNT) which was identified in both samples, and 1,3-dinitrobenzene (DNB) which was detected in the headspace of Pentolite. Both of these compounds were present at higher concentrations than the parent compound TNT although neither are reported in the MSD supplied for the explosive samples. Previous HS-SPME-GC-MS analysis of various explosive samples have reported DNT and TNT as the primary VOCs in the headspace of TNT samples [218]. DNT is used in explosives as an additional fuel source [231] and has been observed in the headspace of explosive samples [107, 117], while DNB has been reported to be less abundant [107] but still present individually and/or in combination with DNT and TNT in the headspace of cast explosives [231].

As one of the goals of this project was to identify key VOCs in the EDDs training aids, DNT and DNB were deemed promising VOCs for field trials with EDDs due to their high concentrations and volatility.

4.2.2.4 Tagged Explosive Sample

The only explosive sample analysed in this study that reported the inclusion of DMDNB in its composition, according to the MSDS supplied, was Primer sheet (Figure 4-6). DMDNB is one of four chemicals which can be added as a marker to plastic and sheet explosives according to the International Civil Aviation Organization (ICAO) under the 1991 Convention on the Marking of Plastic Explosives for the Purpose of Detection [162], and therefore is expected to be found on the headspace of most plastic explosive samples, including military and industrial explosives [117].

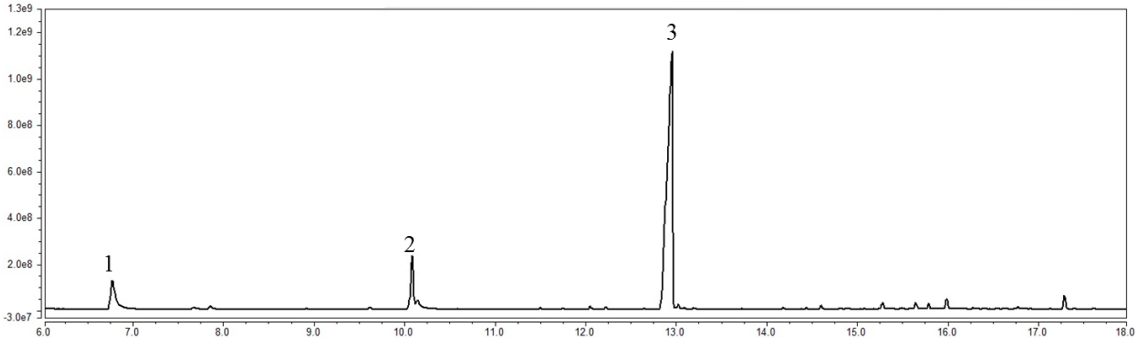

Figure 4-6: Chromatogram of VOCs detected in the headspace of a tagged explosive used as training aids for EDDs. Chromatogram achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC-MS analysis of a Primer Sheet sample, which is mainly composed of PETN and DMDNB. Key VOCs identified in the headspace: 1) Cyclohexanone; 2) 2-Ethyl-hexanol; 3) 2,3-Dimethyl-2,3-Dinitrobutane.

The analysis of the tagged explosive revealed the highly volatile explosive marker DMDNB, which is more than one million times more volatile than nitramines and nitrate esters [18]. The high volatility of DMDNB explains the large concentration of this analyte in the headspace of the explosive sample. The addition, interaction and permeation of DMDNB in explosives is well documented in the literature [78, 117, 233], including the evaluation of EDDs response to this compound [107]. SPME has been shown to be a very effective tool for the extraction of taggants from the headspace of explosive samples, even when the extraction times are short [117]. The efficiency of the SPME extraction of taggants is mainly due to the high vapour pressure of these compounds [117]. PETN, which is the other main component of Primer sheet, is a nitrate ester which has a vapour pressure one millions times lower than DMDNB [18]. No traces of PETN or its degradation products were detected in the headspace of the explosive sample analysed. No other explosive related compounds were detected. Only 2-ethyl-1-hexanol and cyclohexanone were detected in relative abundance in the explosive headspace. Although the explosive sample is manly composed of polyisobutylene, a synthetic rubber, no butyl compounds were detected in the headspace analysis. A literature report shows that when

the DMDNB taggant is present in the headspace of explosives, it competes with other VOCs, including butylated compounds [78]. These findings suggest that the other compounds may be present in the headspace of the Primer sheet explosive in low concentrations and are not readily detected due to competition with DMDNB or because they are present below the detection limits of GC-MS. DMDNB was therefore deemed a promising VOC for field trials with EDDs due to its high volatility and its ability to outcompete other VOCs in a mixture.

4.2.2.5 Plastic Explosives

Plastic explosives are also referred to as polymer bonded explosives due to their manufacture procedure in which the explosive compounds are bound together in a matrix using small quantities of a synthetic polymer [3]. Due to the high presence of polymers, several plasticizers and other compounds can be found in the headspace of these samples [107]. Figure 4-7 shows the chromatograms of the two plastic explosive samples analysed. PE-4 (a) is a RDX based explosive and Detonation cord (b) is a PETN based explosive.

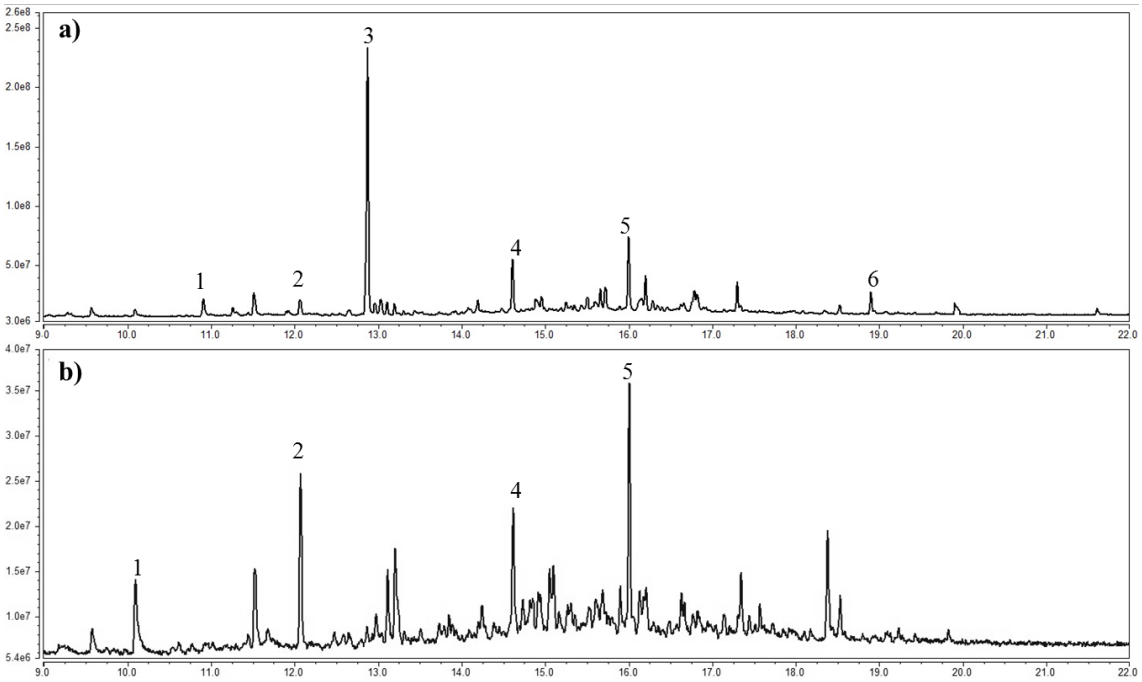

Figure 4-7: Chromatogram of VOCs detected in the headspace of plastic explosives used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC-MS analysis. a) Represents a chromatogram of PE-4, an explosive mainly composed of RDX, and b) represents a chromatogram of Detonation Cord, an explosive mainly composed of PETN. Key VOCs identified in the headspace: 1) Cyclohexanone; 2) 2-Ethyl-hexanol; 3) 2,3-Dimethyl-2,3-Dinitrobutaned; 4) Dodecane; 5) Unidentified ester compound; and 6) Benzophenone.

The analysis of PE-4 shows the presence of DMDNB in the headspace, demonstrating that this is also a tagged explosive. The headspace analysis of PE-4 also identified benzophenone. Benzophenones and other ketones are known block builders commonly found amongst polymer bonded explosives, often used as polymer binders [234] and in the packaging of polymers or its contents to prevent degradation [235].

The chromatogram for Detonation cord made it difficult to identify the many co-eluting peaks. It was not possible to identify conclusively the many organic compounds in the headspace of this sample. A previous study also reported difficulties in detecting and identifying VOCs in the headspace of Detonation cords. This study reported that even by increasing the SPME exposure time to 18 hours there were no successful identifications of any potential headspace components [231].

The HS-SPME-GC-MS analysis of both samples was not able to detect any traces of RDX or PETN in the headspace or any other explosive related compounds. Cyclohexanone was found in the headspace of both explosives. Other compounds common to both explosives included 2-ethyl-hexanol (common plasticizer), hydrocarbons such as decanes and dodecanes, and several unidentified esters. Hydrocarbons have been previously described in the literature as one of four most dominant peaks extracted and detected by GC-MS from plastic explosives [78]. Previous studies have also reported the presence of different esters, alcohols and ketones in the headspace of plastic explosives [78, 231]. Aliphatic esters and phthalates are the most common plasticizers for plastics or additives and are responsible for the malleability of PE-4 and other explosives [236].

4.2.2.6 Blasting Agents

The primary composition of blasting agents is ammonium nitrate, which cannot be easily identified by HS-SPME-GC-MS analysis [227]. Although HS-SPME coupled to GC-MS is an extremely useful technique for the recovery and identification of many explosive traces, there are no reports of its application to the analysis of the ammonium nitrate based explosives such as ANFO and Power Gel. The low molecular weight of ammonia and ammonium results in minimal retention on most common, low polarity phase columns, and necessitates data acquisition from a very low initial scan mass, causing SPME-GC-MS analysis of these compounds to be impractical [227]. Figure 4-8 shows the

chromatograms of Power gel and ANFO, two blasting agents used as training aids by NSWPDU. Both Power gel and ANFO are manly composed of ammonium nitrate with no other explosive parent compound or taggant described in their respective MSDS.

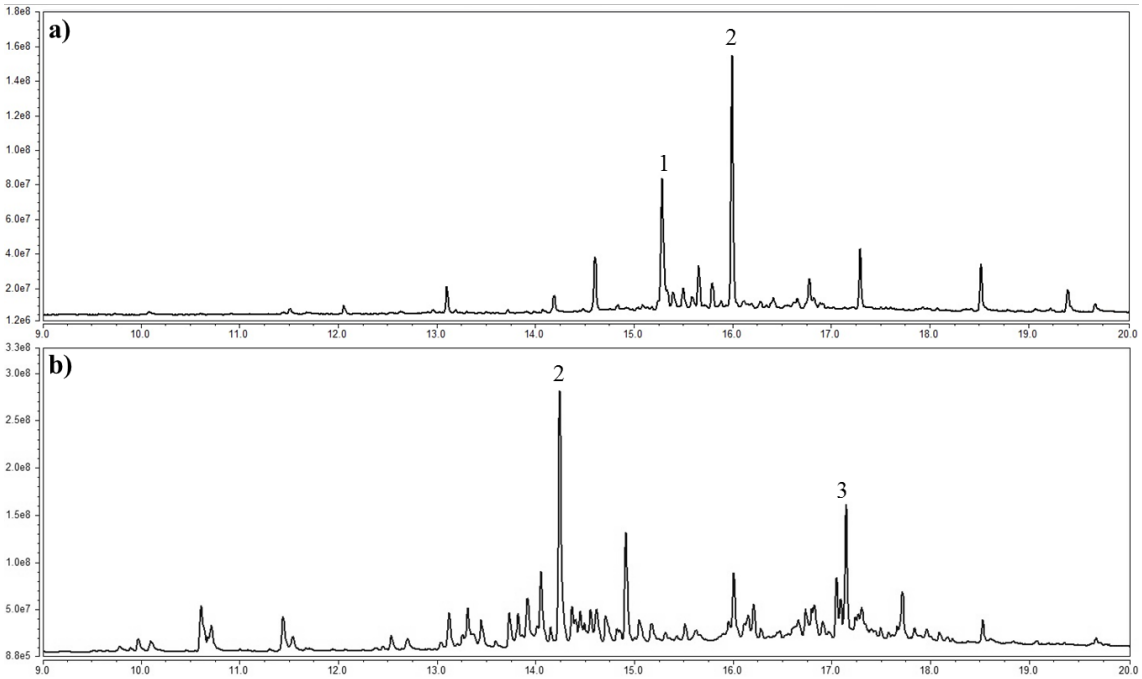

Figure 4-8: Chromatogram of VOCs detected in the headspace of blasting agents used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC-MS analysis. a) Represents a chromatogram of Power Gel and b) represents a chromatogram of ANFO. Both explosives are mainly composed of ammonium nitrate. Key VOCs identified in the headspace: 1) Isobutyl ester; 2) Dodecane; and 3) Heptadecane.

The complex matrices presented by these two samples hindered the identification of compounds in their headspace. Power gel is an industrialized block explosive used for mining and other industrial activities, and as such showed the presence of a plasticizer, namely isobutyl ester. ANFO is an improvised powder and was mostly comprised of acyclic alkanes such as dodecane and heptadecane.

4.2.2.7 Propellants (Powders)

Propellants are low explosives, which have low detonation velocities when compared with high explosives. These compounds' explosion is a deflagration, which is a very rapid combustion, rather than an actual detonation [19]. Applications of propellants are primarily related to the propulsion of ballistic projectiles such as bullets. Figure 4-9 shows the chromatograms achieved after HS-SPME-GC-MS analysis of Smokeless powder (a) and Black powder (b).

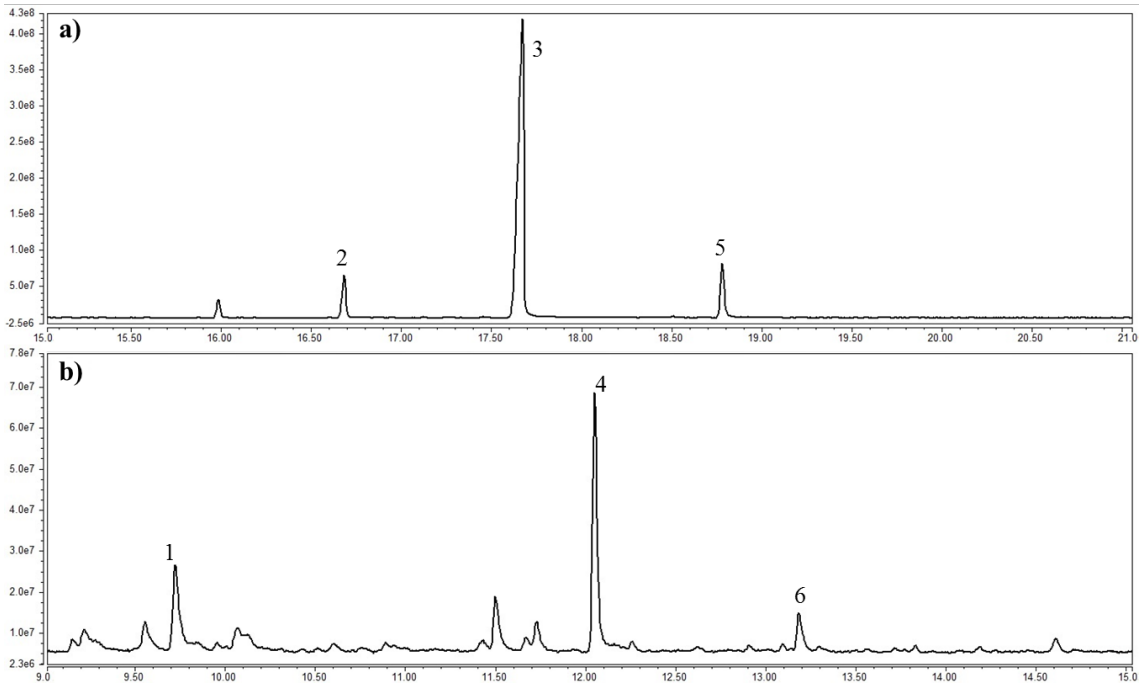

Figure 4-9: Chromatogram of VOCs detected in the headspace of propellants used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC-MS analysis. a) Represents a chromatogram of Smokeless Powder and b) represents a chromatogram of Black Powder. Key VOCs identified in the headspace: 1) Dichloro-benzene; 2) 1,3-Dinitrobenzene; 3) 2,4-Dinitrotoluene; 4) Unidentified ester compound; 5) Diphenylamine; and 6) Unidentified alcohol compound.

The analysis of the Smokeless powder yielded very few identifiable peaks. Three main compounds were identified, namely 1,3-dinitrobenzene (DNB), 2,4-dinitrotoluene (DNT) and diphenylamine. DNT was the most abundant compound detected, followed by the explosive related compound DNB and the additive diphenylamine. DNT has been previously described in the literature as a common additive for Smokeless powder, to reduce the initial burn rate and lower the burn temperature, increasing the powder burst [231, 237-240]. Previous studies have also detected the presence of DNB in the headspace of explosives [238-240]. Diphenylamine has been reported in several studies in the headspace of smokeless powder samples of different compositions [231, 237, 239, 241]. Diphenylamine is a stabiliser used to increase the shelf life of powders by removing nitric acid formed during decomposition of the nitrated energetics [238].

The analysis of the Black powder also yielded very few identifiable peaks. Dichlorobenzene was detected in the headspace of this sample. This compound can be originated from either: a derivative of benzene, which is one of the elementary petrochemicals and a constituent of black powders; or as a precursor of nitrobenzene

[242], which is normally added to propellants to increase their burning properties [239]. Other peaks may have represented an ester and alcohol compound but could not be identified with certainty. Black Powder is one of the oldest explosives and is used in various applications including gun propellant, blasting fuses, distress rockets and fireworks [231]. It is manly composed of potassium nitrate in a mechanical mixture of fuel, charcoal and sulphur [243]. The low molecular weight of potassium nitrate and charcoal hinders the HS-SPME-GC-MS analysis and results in minimal retention for most common low polarity phase columns, requiring data acquisition from a very low initial scan mass [227].

4.2.3 Summary

The analysis of high explosives covered TNT-based, plastic and tagged explosives, and blasting agents. TNT-based or cast explosives are commonly based around TNT and other aromatic nitrates; the other explosives are generally composed of nitramines or nitrate ester explosives such as RDX and PETN; and blasting agents contain ammonium nitrate as its primary compound. The low explosives analysis involved two powder samples: smokeless and black powder.

Following HS-SPME-GC-MS analysis, TNT was the only parent compound identified in the samples, while RDX and PETN were not identified. Identified additives included plasticizers such as 2-ethyl-1-hexanol; block builders such as benzophenone; stabilizers such as diphenylamine and the taggant normally added to all common plastic explosives 2,3-dimethyl-2,3-dinitrobutane (DMDNB). Results revealed the presence of 2,4-dinitrotoluene (DNT) in high abundance in the headspace along with 1,3-Dinitrobenzene (DNB). Overall the most commonly observed headspace VOCs across all samples were DNT, DMDNB and 2-Ethyl-hexanol. The high abundance of DNT and DNB in Smokeless powder (low explosive) along with their presence in the TNT-based high explosives, confirmed that they were promising VOCs for field trials with EDDs

4.3 EXPLOSIVE TRAINING AIDS STABILITY ANALYSIS

Some VOCs related to explosives are known to be more easily detected in fresh samples than aged ones, as demonstrated with cyclohexanone which is more commonly observed in fresh samples of C-4 [230]. When planning the development of a training aid for

detection dogs or studying the efficacy of the training aids currently in use, it is essential to evaluate how long the VOCs are identifiable in the headspace, and at what rate they degrade if at all. Identifying VOCs that are present for longer and remain stable over time was important for selecting key VOCs to trial with EDDs.

4.3.1 Materials and Methods

4.3.1.1 Samples and Extraction Process

The tested samples are described in section 4.2.1.1 and followed the same procedures for VOC extraction as described in section 4.2.1.2

4.3.1.2 Degradation Studies

The samples were analysed at three distinct timeframes over a one-year period, denoted as zero (first analysis), six months degradation (second analysis) and twelve months degradation (third analysis). These were arbitrary values since the training aids had all been aged for varying periods prior to these analyses. Each extraction was conducted in triplicate using PDMS and PDMS-DVB SPME fibres. This study aimed to evaluate whether degradation of the VOC profile occurred over time and whether they remained as optimal training aids for EDDs. Analytes Selected

The VOCs for this study were selected based on the most consistently observed analytes during the chemical profiling of the training aids with HS-SPME-GC-MS. Two or three analytes with highest abundance per sample were chosen, in a total of nine different VOCs. The analytes were chosen and divided into three groups: explosive related compounds, which are compounds exclusive to explosives; additives, which are compounds added to the samples in their manufacture process; and explosive taggant, which is the marker compulsorily added to explosives. The list of compounds can be seen in Table 4-2.

Table 4-2: List of compounds chosen for the degradation studies

EXPLOSIVE RELATED	ADDITIVE	TAGGANT
TNT DNT DNB	2-Ethyl-Hexanol Cyclohexane Decane Dichloro-Benzene Butyl Ester Diphenylamine	DMDNB

The total abundance (peak area) of the selected analytes was measured every time the analytes were identified per explosive sample. The extracted peak areas of the chosen compounds were averaged for both fibres (n=6) per extraction time (0, 6 and 12 months) and compared for each of the explosive samples. The relative standard deviation was calculated for each of the averaged totals.

4.3.2 Results and Discussion

The most abundant analytes identified via HS-SPME-GC-MS were used to determine if there was degradation of the training aids over the one year period studied. None of the explosives sample displayed notable decreases in any of the analysed compounds.

Figure 4-10 shows the averaged abundance for each analyte per sampling month across all explosive samples, including error bars determined as the highest standard deviation found for one explosive sample. The individualized explosive samples degradation studies can be found in Appendix A Figure A-1.

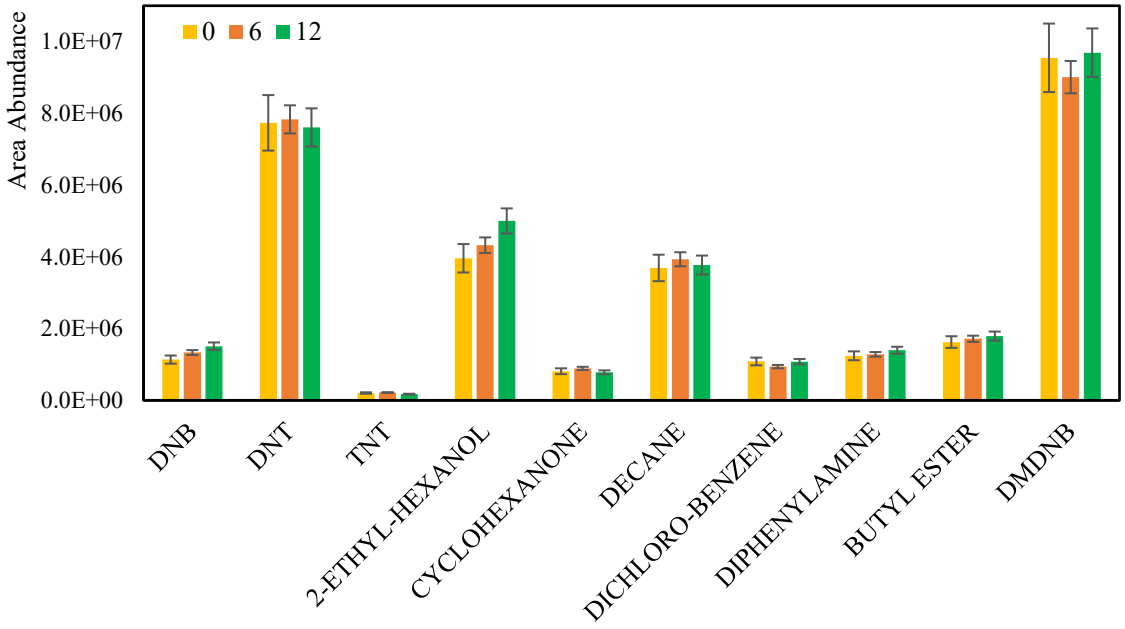

Figure 4-10: Average abundance for the selected compounds of interest from all training aids analysed demonstrating their degradation over time. Note: for chart appearance DMDNB and DNT values were reduced in two and one orders of magnitude respectively.

Figure 4-10 highlights that there was no evident degradation of any of the major compounds throughout the degradation study. The overall degradation of the VOCs was used as an indicator for the reliability of the chosen explosives as training aids. This result

suggests that the training aids have been stored appropriately and do not have a tendency to degrade rapidly. Industrial and military explosives, such as those analysed, contain a series of additives used to improve burning properties and shelf life, such as 2-ethyl-1-hexanol, diphenylamine, benzaldehyde and other benzoic compounds [107]. Plastic compounds and binders used to make the explosive blocks, such as phthalates and other organic compounds can also protect these explosives and propellants from environmental conditions and thus degradation [231].

Identifying the key VOCs in explosives must consider the degradation rate of the analytes. Analytes that do not show significant variations between fresh and aged samples are preferred for chemical and biological detection. The results of this study suggest that the VOCs chosen have low rates of degradation and therefore are suitable detection compounds.

4.3.3 Summary

The results show that there was no significant degradation for the most common compounds found in the headspace of the explosive samples tested by HS-SPME-GC-MS. This may indicate that these samples have been properly stored and are reliable to use as a training aid. However, this can also indicate that the chosen method is not comprehensively profiling the headspace of the explosive training aids, detecting only the most stable compounds and missing other important compounds that may undergo thermal degradation and not be detected using a lengthy conventional GC-MS method. A lengthy chromatography method has two main underlying issues for the detection of explosives in the headspace: the thermal degradation of compounds; and the time consumed for an analysis that requires real time responses. Fast GC provides many distinct advantages over other analytical methods such as the rapid throughput required in forensic scenarios, and the reduction in thermal degradation of compounds [244]. For this reason, it was investigated in the subsequent study using liquid standards (based on the identification of key compounds in this study) and subsequently applied to the more advanced GC×GC method.

4.4 HS-SPME-GC-MS ANALYSIS OF LIQUID STANDARDS USING A FAST CHROMATOGRAPHY METHOD

Fast GC is a term used to describe GC methods that operate using a reduced analysis time in comparison to conventional GC. These advantages are particularly important for the screening of explosives for security purposes, where a rapid and reliable analysis is paramount [245]. Fast GC is usually achieved by the combination of a short GC column, with a high flow and a rapid temperature ramp. This combination of factors decreases the elution temperature and retention time, contributing to less thermal degradation [244].

The HS-SPME-GC-MS for the training aids showed some large unresolved complex hydrocarbon mixtures and only one parent compound was identified (i.e. TNT). The conventional HS-SPME-GC-MS method used to analyse the samples may be hindering the separation of major components, due to the thermal instability of most compounds associated to explosives. To test this, a shorter method was applied to reduce the loss of thermally unstable compounds.

Initially it was necessary to determine whether a fast chromatography method could be applied with HS-SPME for the detection of explosives compounds and whether this method could identify compounds not readily detectable in the headspace of real explosives such as PETN and RDX. The first step to prove this concept was to introduce the fast gas chromatography method for the analysis of known liquid standards by HS-SPME-GC-MS.

4.4.1 Materials and Methods

4.4.1.1 Standards

Standards of nitrate explosives and a common taggant used in plastic explosives (Table 4-3) were purchased from AccuStandard (New Haven, CT, USA) at a certified concentration of 1000 µg/mL in acetonitrile. Methanol was purchased from ChemSupplies Pty Ltd (Gillman, SA. Australia). These standards were chosen following the findings described in section 4.2.3, and supplemented with literature reports for the parent compounds [78, 246]. Published studies have previously described these analytes as being associated with, added to or a parent compound of common explosives [9, 107].

Table 4-3: List of standards analysed by HS-SPME-GC-MS, grouped by use, including individual classification

STANDARD	CLASSIFICATION	USE
TNT	Nitroaromatic	Industrial and military explosives. Components of plastic explosives and boosters
TETRYL	Nitramine	
RDX	Nitramine	
PETN	Nitrate Ester	
DNB	Nitroaromatic	Precursors for explosives and propellants
DNT	Nitroaromatic	
DMDNB	Volatile Organic Compound	Detection taggant for plastic explosives

4.4.1.2 HS-SPME Extraction from Standards

The liquid standards were diluted to concentrations varying from 100 to 1000 ppm using methanol into 20 mL glass vials (Sigma, Bellefonte, PA, USA). After dilution the solutions were dried under nitrogen. The vials were covered by an aluminium cap with a silicone septa (Sigma, Bellefonte, PA, USA) which could be perforated by the SPME needle. A PDMS fibre (Sigma, Bellefonte, PA, USA) was exposed to the dried standards for 30 min at room temperature. A schematic of the explosive extraction process can be seen in Figure 4-11, adapted from [247]. Triplicates were collected from each concentration in order to use these values for linearity analysis. The fibre composition and analysis method were adapted from the literature [118], while the extraction procedure was developed in the laboratory and the extraction time optimized during the analysis.

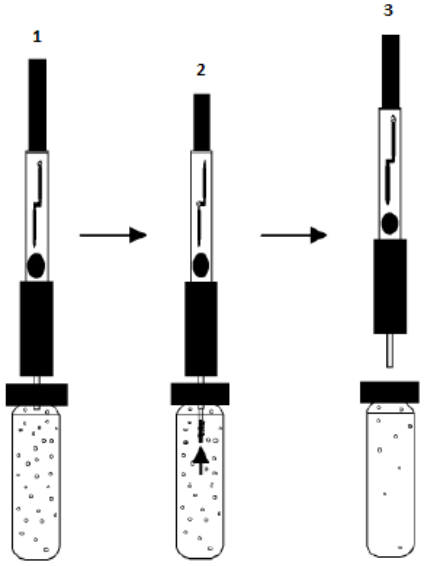

Figure 4-11: Schematic of SPMEextraction from dried explosive samples in 20 mL vials. 1) Silicone lid is perforated by SPME needle; 2) fibre is exposed to the headspace of the dried samples; and 3) fibre and needle are retracted after a 30 minute extraction time. Image adapted from Supelco, 1998 [247].

4.4.1.3 GC-MS Instrumental Parameters

The GC analysis was performed using a Trace 1300 Gas Chromatography coupled to an ISQ QD Single Quadrupole Mass Spectrometer (ThermoFisher Scientific, Waltham, MA, United States). The VOCs of dried standards were extracted using HS-SPME and analysed using a 6 metre semi-polar BPX-50 fused silica column with internal diameter of 0.25 mm and film thickness of 0.25 µm. Helium was chosen as the carrier gas and held to a 3 ml/min^{-1} constant flow. This flow rate and column length were chosen as appropriate for a fast chromatography method. Conventional chromatography methods generally use flow rates between 1 and 1.5 ml/min^{-1} and column lengths from 15 to 40 m. The higher carrier gas flow and shorter column was intended to lead to a faster transport of the analytes through the column to the detector. After extraction the SPME fibre was introduced into a 220 °C inlet for a 5 minute desorption. The injection was conducted in splitless mode. The oven was programmed to start at 50 °C, with a 2 minute hold and a ramp to a final temperature of 280 °C at a rate of 30 $°C/min^{-1}$.

4.4.1.4 Data Analysis and Interpretation

1D GC data analyses was performed using the Chromeleon 7.2.8 Chromatography Data System (ThermoFisher Scientific) and library matching was carried out using the NIST Mass Spectral Library (NIST 14). The MS was operated in electron ionization (EI) full

scan mode from 29 to 290 amu, with a 30 sec solvent delay. This mass range was chosen given the lowest and highest masses described for the standards being analysed.

The collected data was plotted and used to create calibration curves for each of the analytes. Calibration curves were constructed by SPME extraction of the dried explosive standard solutions at 100, 250, 500, 750 and 1000 ng/µL. The respective LOD's were calculated as 3.3 σ/ slope of the calibration curve, where σ is the slope deviation [175].

4.4.2 Results and Discussion

4.4.2.1 Extraction Time

A PDMS fibre was chosen to conduct the experiments following the previous findings described in section 4.2.2.1 and in the literature [118, 248]. Figure 4-12 shows the comparison between 15, 30 and 60 minutes extraction of DNT, DNB and DMDNB. The three compounds were dried and extracted individually at 800 ng/µL. This experiment showed that an exposure time of 30 minutes at room temperature provided optimal VOC extraction without compromising extraction time. Although 60 minutes produced an improved VOC extraction, it was not deemed sufficiently higher to warrant doubling the extraction time.

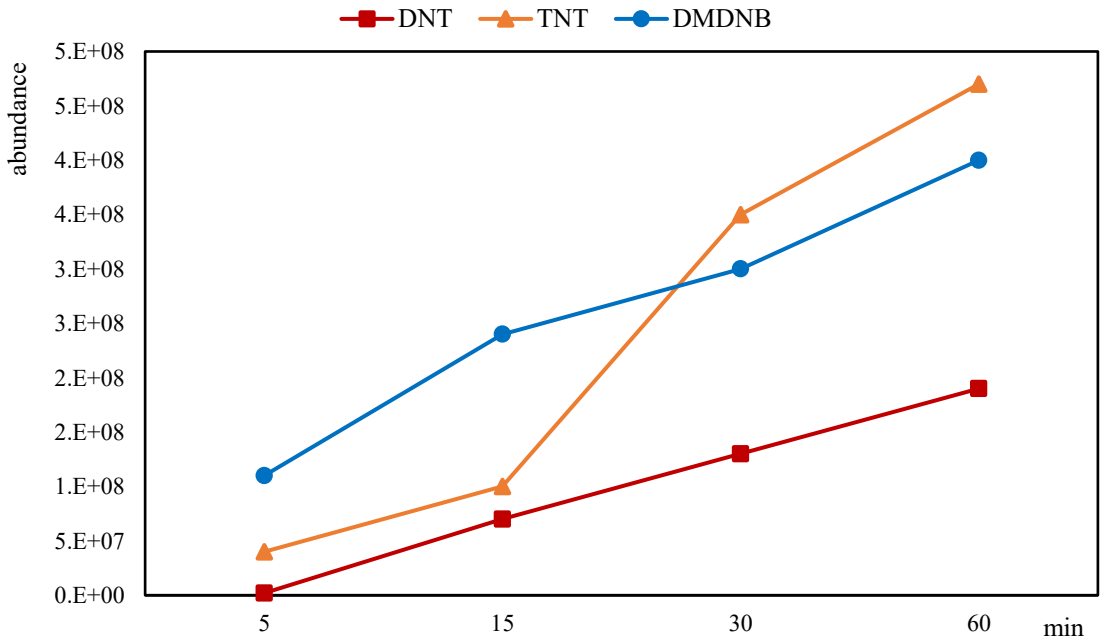

Figure 4-12: Effect of the SPME fibre exposure time on the extraction of TNT, DNT and DMDNB standards at 800 ng/µL. These three standards represent three of the most common VOCs present in the headspace of explosive training aids.

Headspace SPME sampling involves a three-way equilibrium between the concentrations of the target analytes in the sample, the sample headspace, and the SPME fibre surface [231]. Frequently the headspace collection is performed at non-equilibrium conditions, whereby the exposure time of the SPME fibre is insufficient to achieve equilibrium, however, detectable levels of analyte are observed. The concentration of analyte upon the fibre surface increases steadily, proportional to the length of exposure up to a certain maximum point of saturation. After saturation the concentration can be seen to reduce [247]. During HS-SPME smaller and lighter volatiles are seen to adsorb faster than the larger, less volatiles ones [249]. Therefore longer exposure times will favour heavier analytes, whereas shorter exposures will favour lighter elements of the headspace [250]. Previous studies with TNT and DNT revealed that maximum extraction was obtained after 120 minutes, after which analyte concentrations were observed to decline [231]. Another study has demonstrated that longer exposure times increases the extraction efficiency for DMDNB, showing an extracted amount six times higher at 180 minutes than the same extraction achieved with 5 minutes exposure [233]. However, both of these studies also chose thirty minutes as the optimal exposure time, as it was the lowest time that yielded an acceptable response [231, 233]. Examination of the peak shapes during GC data analysis reveal peak tailing at longer exposure times, as a result of overloading or slower thermal desorption.

4.4.2.2 Standard Analysis

The PDMS fibre satisfactorily extracted the seven explosive standards analysed. Figure 4-13 shows an extracted ion chromatogram (EIC) of a 1000 µg/mL standard mixture containing TNT, TETRYL, PETN, RDX, DNT, DNB, and DMDNB. Although published literature reports that direct immersion sampling of the SPME fibres in aqueous solutions can significantly increase the recovery of all target analytes when compared to headspace sampling [105, 251], this experiment showed that HS-SPME of dried standards can be used for the detection of these analytes.

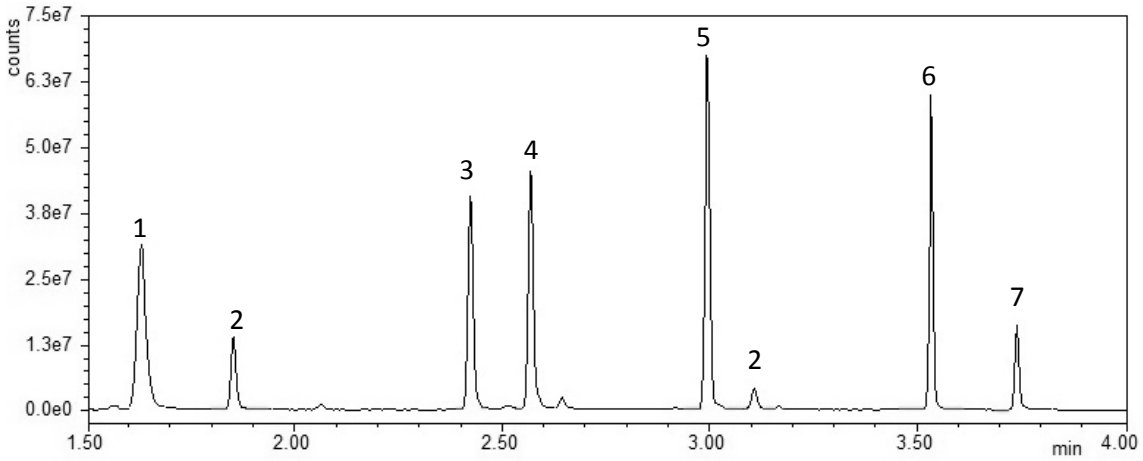

Figure 4-13: Extracted ion chromatogram of a mixture of seven explosive related compounds, extracted from the headspace of dried standards. Extraction was conducted with a PDMS fibre for 20 minutes at room temperature. Analysis was carried out by GC-MS. The confirmed analyte with its respective qualifier ion in order of elution is as follows: 1) DMDNB (57.1); 2) PETN (46); 3) DNB (75.1); 4) DNT (165.1); 5) TNT (210.1); 6) RDX (46); and 7) TETRYL (77.1).

The chromatogram of the fast chromatography method used for the HS-SPME-GC-MS analysis of standards was effective in the detection of PETN and RDX. However, PETN separated into two smaller peaks due to thermal degradation during the analysis. This behaviour has been reported previously [102, 252]. All nitroaromatic explosives were successfully separated and identified. Due to the higher volatility of DMDNB compared to the other compounds present in the mixture, it was expected that it would be the most abundant analyte in the mixture. However, its abundance was comparable to all other analytes in the mixture. Previously studies reported that the addition of other organic compounds and nitroaromatics to a mixture can supress the DMDNB response [233]. As noted by Furton et al., the recovery of explosives is highly influenced by several factors, such as extraction times, sampling mode, fibre chemistry, extraction temperature and analyte type [105].

4.4.2.3 Limits of Detection

Five solutions containing all of the explosive analytes in concentrations varying from 100 to 1000 µg/mL were prepared and analysed aiming to determine the limits of detection for the HS-SPME-GC-MS analysis of standards. The peak area was used for the linearity studies. The average response for the total ion abundance (counts/min^{-1}) of each analyte acquired (n=4) with the use of EI detection were plotted against the analyte concentration to obtain calibration curves and determine the linearity of the method by linear regression. The relative standard deviation was calculated from the averaged area of each concentration. The resulting linearities (R^2) and RSD (%) are provided in Table 4-4.

LODs, which represents the lowest analyte concentration in a sample that can be detected but not quantified, was calculated as 3.3σ/slope [253], and the resulting values are reported in Table 4-4. The LOD values ranged between 0.23 and 3.36 ng/µL. Most of the figures of merit values were in accordance with literature findings [105]. As described previously in the literature [96], the method used to calculate the LOD values has proven to generate considerably higher values than those based on an estimated signal-to-noise ratio of 3:1. PETN and DMDNB were the analytes that displayed the smallest and highest LOD values, respectively. The calibration curves for this study can be seen in Appendix B Figure B-1.

Table 4-4: Figures of merit for the liquid standards analysed with HS-SPME-GC-MS

COMPOUND	RSD (%)	R^2	LOD (ng/µL)
DMDNB	4.62	0.98	0.23
PETN	7.54	0.98	3.76
DNB	1.53	0.99	0.78
DNT	2.06	0.99	0.37
TNT	3.87	0.99	0.71
RDX	3.63	0.99	1.36
TETRYL	4.51	0.98	2.31

The difference in the abundance of the nitroaromatics (DNB, DNT and TNT) to the nitramines (RDX and TETRYL) and nitrate ester (PETN) in the mixture can be explained by the stability and volatility of these compounds [3]. Explosives by their very nature are thermally unstable, and the stability and low volatility of these compounds presents a fundamental problem for their analysis by GC, especially for explosives with very low vapour pressures such as PETN, RDX and TETRYL [18]. In general, nitroaromatics have higher vapour pressures and are more stable than nitramines and nitrate esters [119], and are consequently easier to detect by HS-SPME-GC-MS.

4.4.3 Summary

The analysis of nitrate explosives and an explosive taggant by HS-SPME-GC-MS using a fast chromatography method has shown to be efficient for the detection of these compounds in the headspace of dried liquid standards. The results prove fast chromatography to be a suitable method for the analysis of explosives and a promising method for the analysis of VOCs from the headspace of explosive samples. Despite

successfully separating seven standards rapidly, the issue of the complex matrixes observed in the headspace analysis of the training aids with GC-MS remains unresolved.

1D GC lacks the separation power needed to fully separate complex mixtures containing hundreds of compounds and chemically similar analytes. Due to the complexity observed in the explosives training aids using 1D GC, the fast method would improve the detection of the thermally unstable VOCs, however, it would further reduce the chromatographic space. Moreover the chemical similarity of many of the compounds resulted in co-elution of several analytes. Hence, an instrument with a higher separation capability such as the GC×GC would prove useful to discover the complex matrix of VOCs present in the headspace of explosives. Compared to 1D GC, 2D GC analysis provides improved resolution and separation power, rendering this method optimal for the development of fast chromatography methods with sufficient resolution to investigate the volatile signatures present in the headspace of explosives [118, 130].

4.5 HS-SPME-GC×GC-TOFMS ANALYSIS OF LIQUID STANDARDS USING A FAST CHROMATOGRAPHY METHOD

GC×GC methods are known for their separation power and resolution, even when fast chromatography methods are applied [118]. Due to the use of two GC columns with different selectivity connected via a modulator, which allows mixtures of analytes to be separated by two orthogonal mechanisms, GC×GC methods demonstrate much greater peak capacity than a 1D GC separation [245].

The first step of the HS-SPME-GC×GC-TOFMS experiment focused on comparing the recovery of explosives from dried liquid standards. This step aims to prove the viability of this instrument for the use of fast chromatography methods to chemically profile the VOCs present in the headspace of explosive training aids.

4.5.1 Materials and Methods

4.5.1.1 Standards

A certified mix including the nitroaromatics and nitramines analytes of the EPA Method 8330B [254] was purchased from Restek Corp© (Bellefonte, PA, USA) at a concentration of 1000 µg/mL in methanol. To determine the limits of detection of the standards by HS-SPME-GC×GC-TOFMS, the standard mixture was diluted into five solutions of 1000, 700, 500, 200, and 100 ng/µL in methanol. The solutions were diluted into 20 mL vials and the solvent was evaporated with a nitrogen flow at room temperature prior to extraction. The vials were sealed with aluminium lids and silicon septa, which allowed the insertion of the SPME needle.

4.5.1.2 HS-SPME Extraction from Dried Standards

The VOC extraction was performed with a PDMS fibre following the method previously described in section 4.4.1.2. PDMS fibres were acquired from Supelco (Bellefonte, PA, USA) and were first conditioned for 250 °C for 30 minutes following the supplier instructions [247]. Following, the fibres were conditioned at 270 °C for ten minutes prior to extraction each sampling day to avoid any sample carry over. The extraction was conducted in triplicates for 15 minutes per sample at room temperature. After the

extraction, the SPME fibres were inserted into a 220 °C inlet for a 5 minute desorption time, followed by GC×GC-TOFMS analysis.

4.5.1.3 GC×GC-TOFMS Instrumental Parameters

GC×GC-TOFMS analyses was performed with a 7890A GC System (Agilent Technologies, Palo alto, CA, USA) coupled with a Pegasus 4D GC×GC TOFMS (LECO Corporation, St Joseph, MI, USA).

A fast chromatography method for VOCs extraction using HS-SPME was optimized following the prior GC-MS study and literature reports [118]. The column combination used for the analysis was composed of a semi-polar BPX-50 fused silica column (6 m x 0.25 mm x 0.25 μm) in the first dimension, and a BPX-5 fused silica column (0.6 m x 0.25 mm x 0.25 μm) in the second dimension (SGE, Melbourne, VIC, Australia). The chosen carrier gas for the experiments was helium, with a constant flow rate of 3 mL/min^{-1}. The oven temperature program started at 50 °C, was held for 30 sec, and then increased to 180 °C at 50 $°C.min^{-1}$, followed by an increase to 270 °C at 70 $°C/min^{-1}$, where it was held for 1 min. The 2D oven temperature was offset +5 °C. The transfer line temperature was set at 270 °C and the TOFMS ion source was held at 250 °C. The modulation period (PM) applied was 1.5 s (hot pulse 0.5 s, cold jet 0.25 s) with an offset of +15 °C. The TOFMS detector operated in electron ionization mode at 70 eV, with a mass range of 40–450 amu, an acquisition frequency of 100 Hz, and a detector voltage of 1500 V.

4.5.1.4 Data Analysis and Interpretation

ChromaTOF® 4.50 (LECO Corporation) was used for the acquisition and processing of the data acquired using the TOFMS. Library matching was carried out using the NIST Mass Spectral Library (NIST 11) and the Wiley Registry of Mass Spectral Data (9th Edition) with a match threshold >700.

The collected data was plotted and used to create calibration curves for each of the analytes. Calibration curves were constructed by SPME extraction of the dried explosive standard solutions at 1000, 800, 600, 400, 200, and 100 ng/μL. The respective LOD's were calculated as 3.3σ/slope of the calibration curve, where σ is the slope deviation [175].

4.5.2 Results and Discussion

4.5.2.1 Standards Analysis

The fast chromatography method using GC×GC-TOFMS was successful in the detection of dried liquid standards. All 18 explosives (Table 4-5) present in the certified EPA Method 8330B standard (Restek®) were successfully separated and identified, as can be seen in Figure 4-14, ensuring the instrument can detect VOCs from nitroaromatics, nitramines and nitrate esters.

Table 4-5: List of all eighteen compounds present in the EPA Method 8330B grouped by explosive classification

COMPOUND		CLASSIFICATION
HMX RDX Tetryl		Nitramines
Nitroglycerin PETN		Nitrate Ester
Nitrobenzene 1,4-Dinitrobenzene 1,3-Dinitrobenzene 1,3,5-Trinitobenzene 3,5-Dinitroaniline 2-Amino-4,6-dinitrotoluene 4-Amino-2,6-dinitrotoluene	2,4,6-Trinitotoluene 2,4-Dinitrotoluene 2,6-Dinitrotoluene 2-Nitrotoluene 4-Nitrotoluene 3-Nitrotoluene	Nitroaromatic

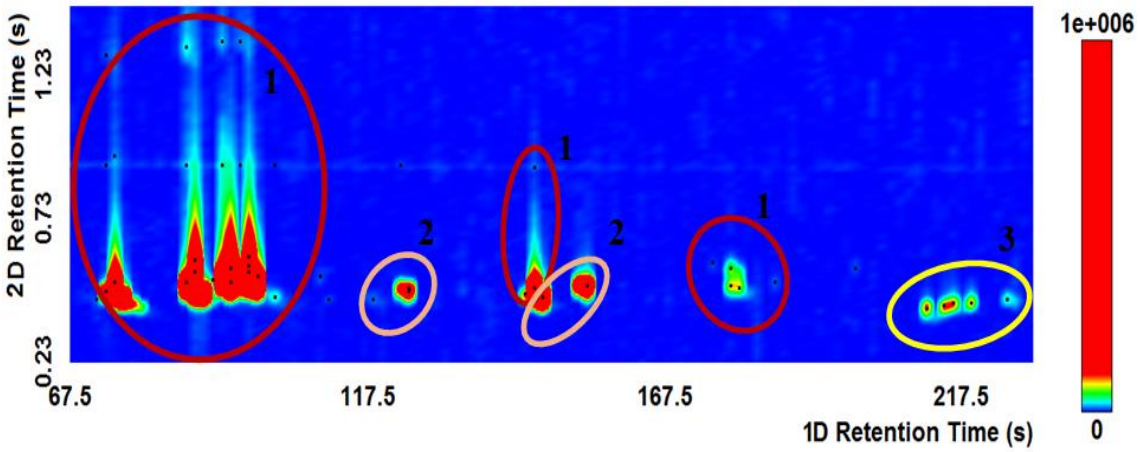

Figure 4-14: Two dimensional plot of HS-SPME GC×GC-TOFMS analysis of VOCs extracted from a certified mixture of nitroaromatics and nitramines explosives showing the grouping of compounds found: 1) Nitroaromatic compounds; 2) high volatility compounds; and 3) Nitramines and nitrate esters.

The entire run took 300 seconds from sample injection to completion. The desired analytes were separated between 70 and 220 seconds and can be seen in three separated groups. Group 1 shows the elution of all nitroaromatic compounds. These compounds have similar chemical structures and molecular weight, normally eluting together in 1D methods [255]. The second column resolution on the GC×GC method was able to separate all 12 nitroaromatic analytes present in the mixture. Group 2 is composed of the high volatility compounds nitroglycerin, aniline, toluidine, methyl nitrate and ethylene glycol dinitrate (EGDN). All the compounds in this group except nitroglyerin are originated from reactions occurring with the other explosives present in the mixture [3] and were not part of the original standard mixture. It was expected that due to their volatility and molecular weight these compounds would appear earlier [256], however this was not observed using the current method. Group 3 corresponds to nitramines and nitrate esters. These analytes are heavier and have lowest volatility compared to the other compounds in the sample [18]. Therefore their elution at the end of the run was expected. It is important to note that the analytes with the higher abundance inside this group are PETN and RDX, which are normally hard to detect in the headspace of liquid standards [96] and explosive samples [78, 107], by conventional GC-MS. Although studies with GC-ECD have been published with the detection of PETN and RDX in the headspace [255], the method described in this study proved to be optimal in the recovery of these two compounds. The findings of this analysis represents the first time 23 explosive VOCs have been successfully extracted from the headspace and separated by a HS-SPME-GC method.

Although the thermal degradation of compounds was reduced by the fast chromatography method, there were still occurrences of thermal degradation exhibited due to multiple peaks detected for analytes such as PETN and dinitrotoluenes. This demonstrates that when training aids are exposed to an EDD, the compounds present in the headspace may not be only the ones listed in the sample MSDS. Environmental impact and aging of the samples are factors that may influence the reduction or transformation of the analytes present [3]. Some of these compounds are not able to be profiled by 1D GC analysis and 2D GC presents a promising platform for the chemical profiling of the VOCs present in the headspace of explosive training aids, to determine those compounds to which EDDs are alerting.

4.5.2.2 Limits of Detection

Linearity responses were achieved by plotting the average peak area of each compound acquired against the concentration of the solution. The resulting linearity (R^2), RSD (%) and LODs of the eighteen explosives in the EPA Method 8330B standard are provided in Table 4-6. A previous study described the reproducibility for TNT, PETN and RDX analysed separately, with no other parameter studied for these standards [118]. Therefore, this represents the first time that LODs of nitro containing explosives have been determined by HS-SPME-GC×GC-TOFMS. The eighteen target explosives were separated and identified in one run. The use of a fast chromatography method on a 2D GC instrument proved ideal for the separation and detection of mixtures containing several isomers with low parts-per-billion (ppb) limits of detection. The calibration curves for this study can be seen in Appendix B Figure B-2.

Table 4-6: Figures of merit for the standards analysed with HS-SPME-GC×GC-TOFMS

COMPOUND	RSD (%)	R^2	LOD (ng/µL)
HMX	3.65	0.99	0.23
RDX	2.73	0.99	0.56
PETN	1.87	0.99	1.48
1,3,5-Trinitobenzene	1.25	0.99	0.04
1,4-Dinitrobenzene	1.49	0.99	0.28
1,3-Dinitrobenzene	3.27	0.99	0.27
3,5-Dinitroaniline	3.91	0.99	0.05
Nitrobenzene	3.71	0.99	0.08
Nitroglycerin	1.61	0.99	0.03
Tetryl	1.85	0.98	0.94
2,4,6-Trinitotoluene	1.42	0.99	0.90
2-Amino-4,6-dinitrotoluene	3.78	0.99	0.85
4-Amino-2,6-dinitrotoluene	3.84	0.99	0.63
2,4-Dinitrotoluene	2.06	0.99	0.69
2,6-Dinitrotoluene	3.32	0.98	0.24
2-Nitrotoluene	3.81	0.99	0.71
4-Nitrotoluene	2.92	0.99	0.76
3-Nitrotoluene	1.21	0.98	0.91

As expected the limits of detection achieved with GC×GC-TOFMS analysis were considerably lower than the values observed with GC-MS for HS-SPME analysis of the explosive standards (Section 4.4.2.3). Compared to 1D GC, GC×GC provided improved resolution and separation power, resulting from the increased peak capacity [132]. GC×GC has been successfully used for the analysis of complex environmental,

petrochemical, and biological samples [257]. Compounds that co-elute in the 1D GC can be separated in the 2D GC due to the additional selectivity provided by the 2D column [132]. In addition, the 2D separation removes interfering chemical signals, decreasing noise and thus improving sensitivity. Furthermore, the chromatographic peaks were compressed into highly focused pulses, as a result of zone compression occurring at the modulator, enhancing peak detectability.

4.5.3 Summary

This method was applied as a proof-of-concept for the detection of nitrate explosives. A total of eighteen explosives were separated. GC×GC demonstrated to be superior over GC-MS as it facilitated a more comprehensive approach to the analysis of VOCs from the complex explosive matrix and represents a promising platform for the chemical profiling of real explosive samples as outlined below.

4.6 HS-SPME-GC×GC-TOFMS ANALYSIS OF EXPLOSIVE TRAINING AIDS USING A FAST CHROMATOGRAPHY METHOD

Due to the initial success of the fast chromatography method for the analysis of explosive standards using GC×GC-TOFMS, it was selected as the most suitable method for chemical profiling of VOCs from explosive training aids. The aim of this section is to determine the key VOCs in real explosive samples using the highly developed and optimised method. This will dictate the key VOCs to be presented to EDDs in the following field trials (see Chapter 5).

4.6.1 Materials and Methods

4.6.1.1 Samples and Extraction

The samples and extraction method utilized in this section are described in sections 4.2.1.1 and 4.2.1.3 respectively, with the exception of the following. All HS-SPME extractions were performed with a 100 μm PDMS fibre only; and only eight explosive training aids were sampled, as for this experiment ammonium nitrate was not available.

4.6.1.2 GC×GC-TOFMS Instrumental Parameters

The instrumentational parameters were the same used for the analysis of dried liquid standards by HS-SPME-GC×GC-TOFMS (section 4.5.1.3).

4.6.1.3 Data Analysis

ChromaTOF® (version 4.51.6.0; LECO) was used for data processing. The baseline was automatically smoothed by the software with an 80% offset. The 1D peak width was set at 20 s while the 2D peak width was set at 0.1 s. The minimum signal-to-noise ratio (S/N) for the base peak and sub-peaks was set at 250 and 20, respectively. A minimum similarity match >700 to the 2011 National Institute of Standards and Technology (NIST) mass spectral library database was used for initial identification. The Statistical Compare software feature in ChromaTOF® was used for peak alignment.

Samples were input into Statistical Compare to facilitate data visualization by multivariate analysis. All acquired samples were input into a single file and separated into eight classes, one for each individual explosive sample (n = 8 classes with n = 3 samples within each class). For this data analysis approach, analytes were only retained if found in 3 samples out of the 24 total samples or if found in 30% of the samples within a class. This approach was used to investigate the generalization between samples and further determine the key VOCs for each of the explosive training aids.

A Fisher ratio (i.e. the ratio of between-class variance to within class variance) was also calculated for each analyte using the Statistical Compare software feature. In the case where an analyte was absent from a class or only detected in a single sample in a class, the within-class variance could not be calculated (or was equal to 0) and a value of undefined was given for the Fisher ratio. Analytes with higher Fisher ratio values (or those labelled as 'undefined') indicated compounds that statistically differed in abundance between the defined classes. Fisher ratio filtering was performed based on its success in previous applications for identifying class distinguishing compounds [258]. Compounds with Fisher ratios above the critical value (F_{crit} = 2.69), which includes those labelled as 'undefined', were exported as a *.csv file and imported into Microsoft Excel for the manual removal of chromatographic artefacts (i.e. column bleed and siloxanes). The F_{crit} value is computed based on three approach-dependent criteria: the number of classes in the analysis, the degrees of freedom for each class and the significance level chosen (α = 0.05). One challenge presented with the use of this approach was that it

normally consider phthalates as artefacts [259], while phthalates are important compounds for this project due to the composition of plastic explosives [78]. The *F*-distribution was used to calculate F_{crit} for each aligned Statistical Compare compound list. Principal component analysis (PCA) was carried out in The UnscramblerX® (version 10.3; CAMO Software, Oslo, Norway). Data pre-processing steps performed in The UnscramblerX® prior to PCA included mean centring, variance scaling and unit vector normalization. These pre-treatment steps have been previously demonstrated for multivariate VOC analyses [259, 260].

4.6.2 Results and Discussion

The results were separated according to the samples main composition as follows: TNT based explosives, whose major components are TNT and RDX (Composition B), and TNT and PETN (Pentolite); tagged explosives, which is a PETN based explosive and the only sample described as having the plastic explosive marker DMDNB in its composition; plastic or polymer-bonded explosives, whose major components are RDX (PE-4) and PETN (Detonation cord); blasting agent, whose main component is ammonium nitrate (Power Gel); and propellants or powders, which are the two low explosives (Smokeless powder and Black powder) that were available for sampling. The VOCs identified were also divided into three groups, namely: 1) explosive compounds, which are compounds exclusive to explosives such as DNT, DNB, TNT, NG and others; 2) taggants, which is the group composed of the four plastic explosives markers, DMDNB, EGDN and both nitrotoluenes; and 3) additives, which comprise all the compounds associated to explosives that can also be found in other substances and in the environment.

4.6.2.1 TNT Based Explosives

Figure 4-15 shows the two dimensional chromatograms of the two TNT based explosives tested, Pentolite and Composition B. Pentolite (a) is a mixture of equal parts of TNT and PETN, and Composition B (b) is composed of RDX and TNT, with RDX being the more prominent explosive in the mixture.

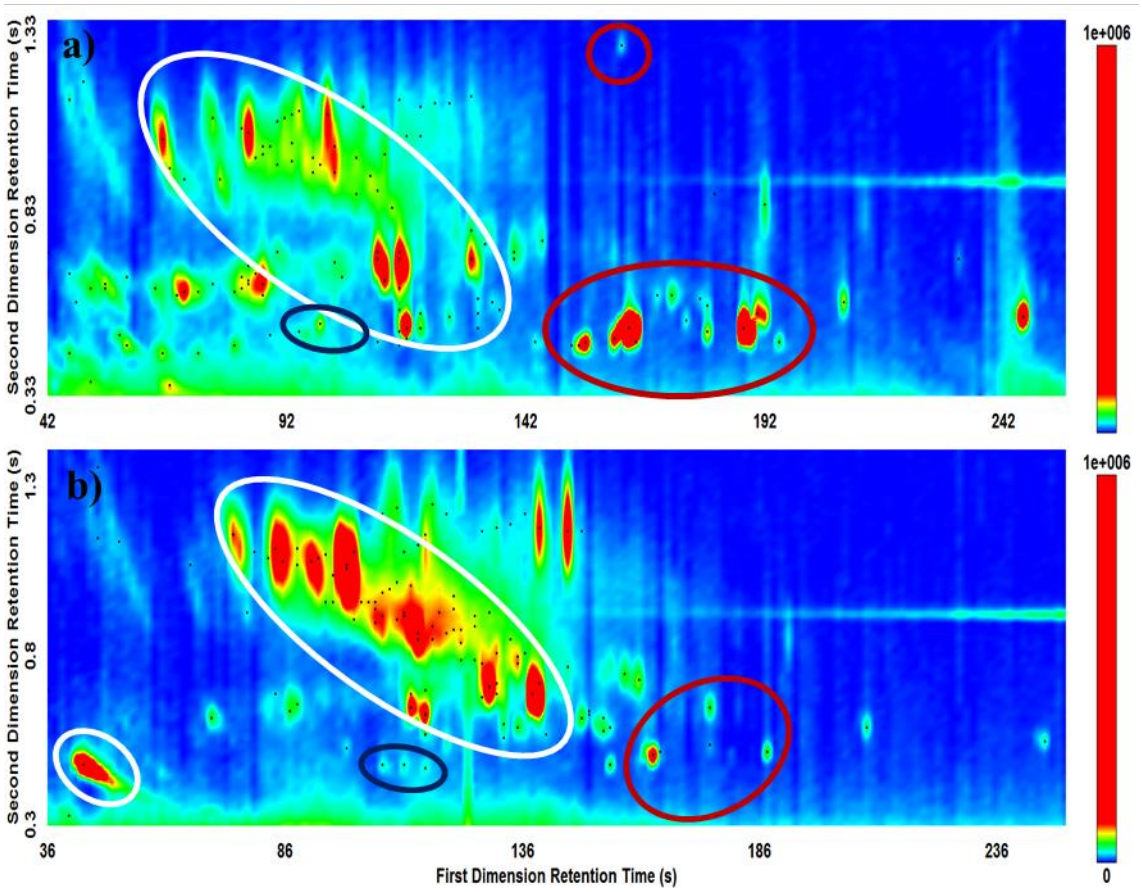

Figure 4-15: Two dimensional chromatogram of VOCs detected in the headspace of TNT based explosives used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC×GC-TOFMS analysis. a) Represents a chromatogram of Pentolite, an explosive mainly composed of a mixture of TNT and PETN, and b) represents a chromatogram of Composition B, an explosive mixture of TNT and RDX. The white ellipses represent additives; the blue ellipses represent the explosive taggants; and the red ellipses represent the explosive compounds detected in the headspace.

The HS-SPME-GC×GC-TOFMS analysis of TNT-based explosives showed the presence of several new plasticizers and additives. In addition to 2-ethyl-hexanol, benzophenone and naphthalene which were identified using the GC-MS method (section 4.2.2), the method was able to detect: phthalates, phenols, benzaldehyde, benzene, benzonitrile, benzothiazole, biphenyl, and dibutyl phthalate. These compounds were not detected with the GC-MS analysis (section 4.2.2.3). The 2D analysis highlights that the additives comprise a majority of the compounds detected in the headspace of Composition B and Pentolite, as can be seen by the area marked by the white circles. Phthalates, esters and phenol derivatives have been reported previously in the literature [78, 231]. Biphenyl, or phenylbenzene, is used in the production of emulsifiers and plastics [261]. Benzonitrile is a solvent and a versatile precursor to many derivatives and resins, known to react with biphenyl-containing compounds [262]. All the newly identified additives as well as those

previously identified are commonly used in plasticizers and therefore commonly present in the headspace of plastic and cast explosives [78, 231]. Benzothiazole is composed of benzene and thiazole, which is a compound that contains both sulphur and nitrogen. This is an indicator that this compound may be a product of the interaction of chemicals present in the headspace when heated and analysed. Benzothiazole is an aromatic compound also found in commercial products and in nature [85]. Cyclohexanone was detected in high abundance in the headspace of Composition B only. This compound eluted early in the chromatogram (within 40 seconds of the first dimension) in Figure 4-16b circled in white. Composition B differs from Pentolite as it contains RDX which suggests that cyclohexane may be present as a precursor of RDX [230].

The blue circles on both chromatograms represent a range of taggants. DMDNB was detected in low abundance in the headspace of both Pentolite and Composition B, while nitrotoluene, which is also a plastic explosive marker [162] was detected in the headspace of Composition B only. The majority of explosives are tagged with different concentrations of explosives markers explaining the huge variation in abundance between samples.

The explosive related compounds detected in the headspace, represented by the red circles, were different isomers of dinitrotoluenes (3,5-, 1,4-, 2,3-, and 2,4-dinitrotoluenes). Pentolite showed a higher abundance and higher number of explosive related compounds compared to Composition B. The presentation of different isomers to EDDs during field trials is a possible way to discover whether the canines can alert to varying explosive training aids, however it is first important to identify the key VOCs. Neither RDX nor PETN were detected in the headspace, confirming that the parent compound is not the most volatile and is unlikely to be the VOCs to which EDDs are alerting [263].

4.6.2.2 Tagged Explosive Sample

The analysis of the tagged explosive Primer sheet revealed a high abundance of DMDNB in Figure 4-16 marked in blue. No other taggants were detected.

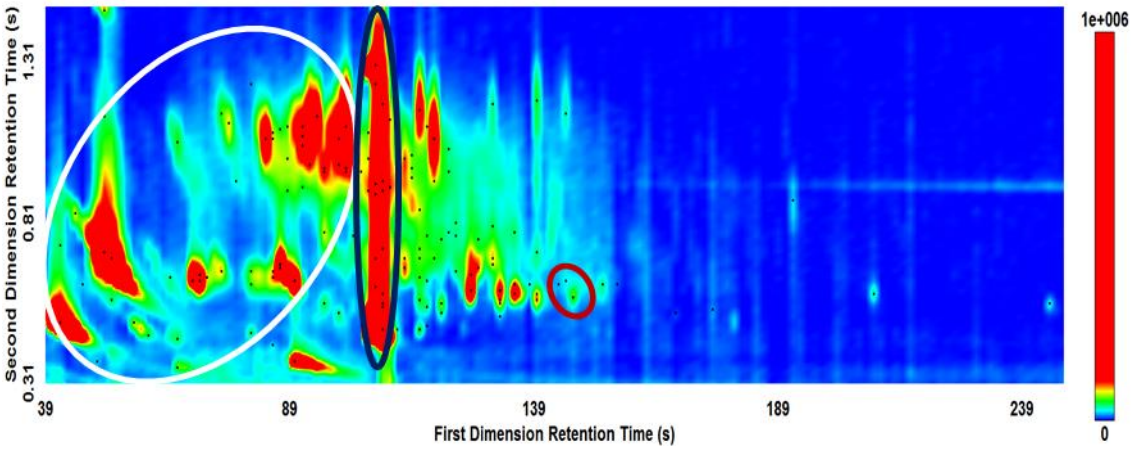

Figure 4-16: Two dimensional chromatogram of VOCs detected in the headspace of a tagged explosive used as a training aid for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC×GC-TOFMS analysis of a Primer Sheet sample, which is mainly composed of PETN and DMDNB. The white ellipses represent additives; the blue ellipses represent the explosive taggants; and the red ellipses represent the explosive compounds detected in the headspace.

Compared to the 1D analysis, the 2D analysis was able to identify several additives in the headspace of primer sheet, as demonstrated by the white circle in Figure 4-17. In addition to 2-ethyl-1-hexanol and cyclohexanone described previously, the 2D GC analysis detected: benzonitrile, benzothiazole, and benzaldehyde which are common solvents; benzophenone and acetophenone, which is a resin precursor [264]; and the esters, phthalate, DEHP and dibutyl phthalate, commonly used as plasticizers [78]. No traces of PETN or its degradation products were found, however, the 2D GC analysis revealed traces of 3,5-dinitrotoluene in this sample headspace, following the same trend of analytes detected in the headspace of the TNT-based explosives (section 4.6.2.1).

4.6.2.3 Plastic Explosives

The analysis of the plastic explosives PE-4 (Figure 4-17a) and Detonation cord (Figure 4-17b) demonstrated a complex matrix of compounds.

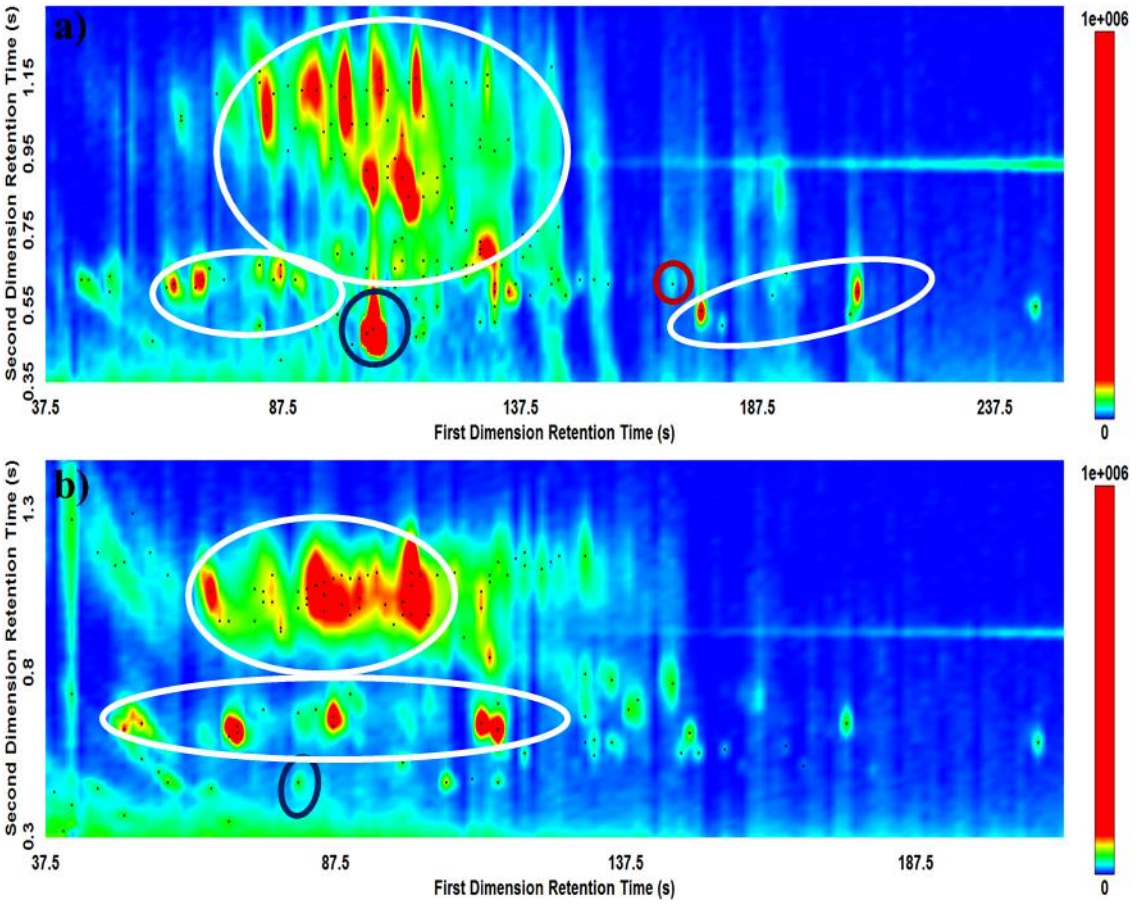

Figure 4-17: Two dimensional chromatogram of VOCs detected in the headspace of plastic explosives used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC×GC-TOFMS analysis. a) Represents a chromatogram of PE-4, an explosive mainly composed of RDX, and b) represents a chromatogram of Detonation Cord, an explosive mainly composed of PETN. The white ellipses represent additives; the blue ellipses represent the explosive taggants; and the red ellipses represent the explosive compounds found in the headspace.

Plasticizers and additives composed the majority of the compounds found in the headspace of these samples [107]. Plasticizers are additives that increase the plasticity or decrease the viscosity of a material, giving it a malleable appearance [78]. This group is manly composed by esters such as phthalates and naphthalene [78]. Both PE-4 and Detonation cord are polymer bonded explosives, meaning that other than the respective parent compounds (i.e. RDX and PETN) these samples are composed of plastics and derivatives [78]. Dibutyl phthalate, phthalate, benzophenone, 2-ethyl-hexanol and DEHP were again identified showing a consistency in the detection of these compounds across all explosives analysed thus far. However, cyclohexanone was not detected in the headspace of either sample. PE-4 is a RDX containing explosive and it was therefore expected to contain cyclohexanone in its headspace. Similar to the GC-MS method, a large number of hydrocarbons were detected in the headspace of Detonation cord (around

87-120s in the first dimension and 1 s in the second dimension), demonstrating the difficulty to analyse this type of sample even when powerful separation methods are used.

DMDNB was identified in the headspace of both samples, with remarkably higher abundance in the PE-4 sample. Studies need to be conducted to verify whether the variable abundance of this compound in different explosive training aids is due to variable concentrations in the sample manufacturing process or some other undetermined reason, which can greatly impact the training of EDDs [265]. Explosive related compounds were only detected in the headspace of PE-4. PETN and RDX were not detected. Dinitrotoluenes (2,4- and 1,4-) were once again the only explosive related VOCs identified. Their consistency and abundance across the samples, confirms that dinitrotoluenes are promising VOCs for the EDD field trials.

4.6.2.4 Blasting Agents

The chromatogram resulting from the analysis of the only blasting agent available i.e. Power gel, by HS-SPME-GC×GC-TOFMS is shown in Figure 4-18.

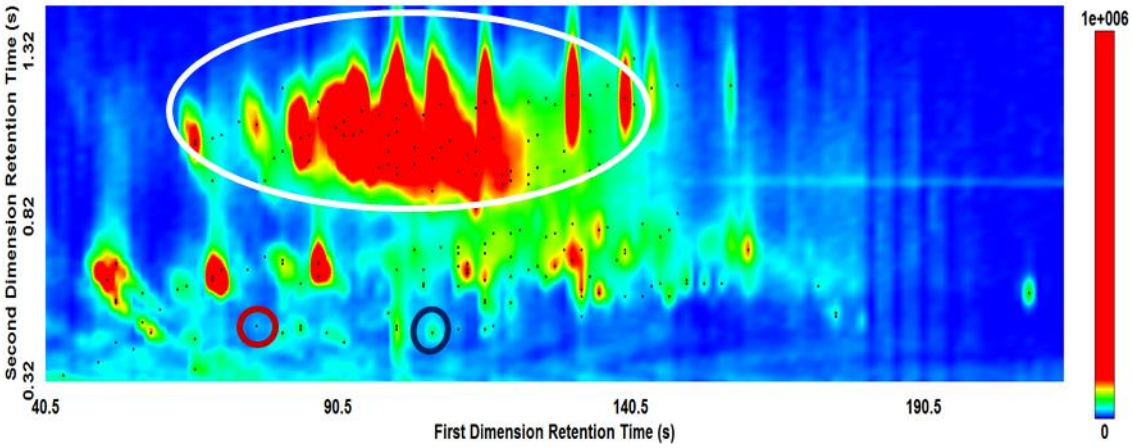

Figure 4-18: Two dimensional chromatogram of VOCs detected in the headspace of a blasting agent used as a training aid for EDDs. Chromatogram achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC×GC-TOFMS analysis of a sample of Power Gel, which is mainly composed of ammonium nitrate. The white ellipses represent additives; the blue ellipses represent the explosive taggants; and the red ellipses represent the explosive compounds detected in the headspace.

The primary composition of Power gel is ammonium nitrate, which due to its inorganic structure is not suited to SPME extraction and chromatography analysis [227]. The results showed minimal organic compounds compared to the other explosive training aids analysed. In addition to the commonly detected esters, ketones and butyl ester compounds, benzoic acid and benzoates were also detected. Benzoic acid is a nitrogen

binding agent that uses ammonium ion binding activity as its mechanism of action. Benzoic acid is a volatile compound that has been previously described in the headspace of explosives together and separated from butyl esters [231]. Nitrobenzene (Figure 4-19, red circle) was also detected in the Power gel. Nitrobenzene is known to be used as a stabilizer for explosives [107]. In addition DMDNB was detected in the explosive training aid (Figure 4-19, blue circle). The variation in the abundance of this taggant may indicate cross-contamination [106] and further analysis is necessary to confirm the source of this explosive marker.

4.6.2.5 Propellants (Powders)

The analysis of propellants was challenging even with the 2D GC method. The primary components are not conducive to chromatographic analysis but other VOCs were detected. Figure 4-19 compares Smokeless and Black powders analysed in this study.

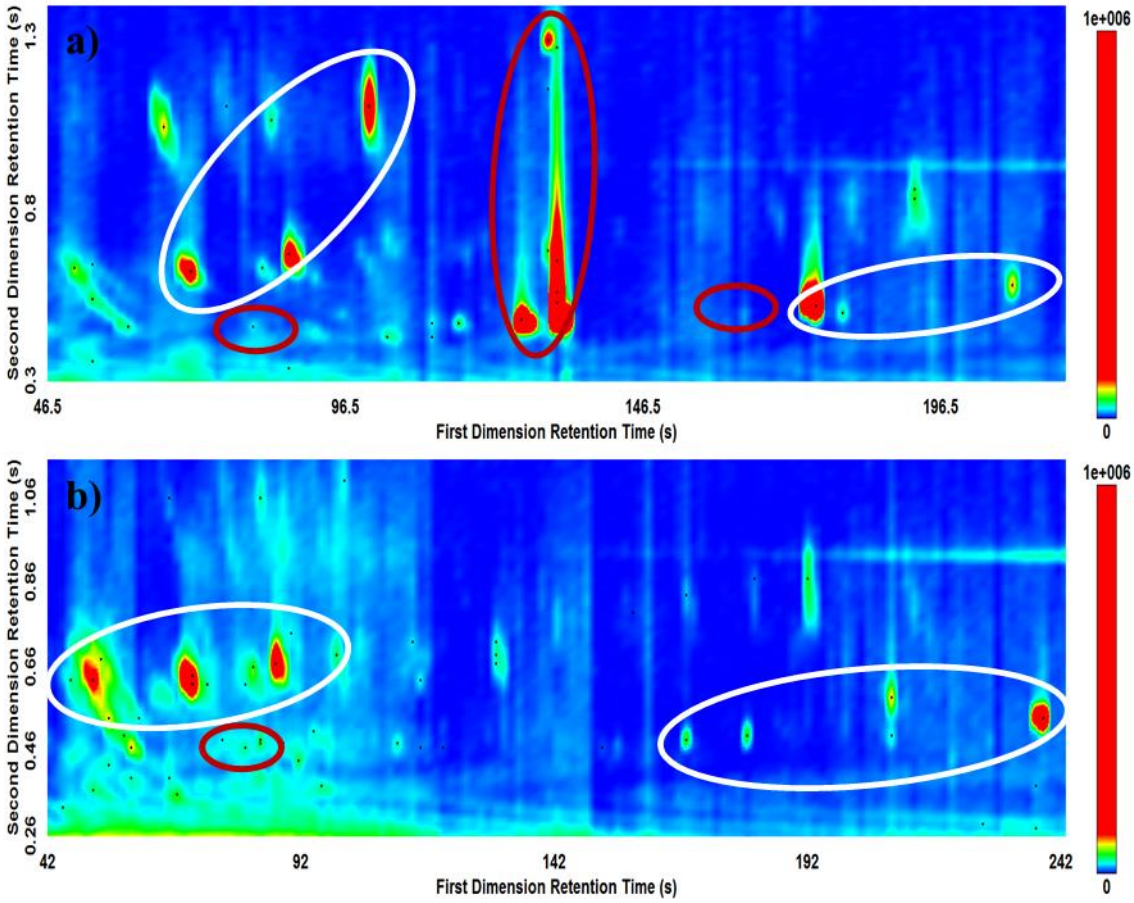

Figure 4-19: Two dimensional chromatogram of VOCs detected in the headspace of propellants used as training aids for EDDs. Chromatograms achieved after HS-SPME extraction with a 100 µm PDMS fibre and GC×GC-TOFMS analysis. a) Represents a chromatogram of Smokeless powder, and b) represents a chromatogram of Black powder. The white ellipses represent additives; the blue ellipses represent the explosive taggants; and the red ellipses represent the explosive compounds detected in the headspace.

The smokeless powder was confirmed to be a double base powder due to the detection of dinitroglycerin [239], shown by the red ellipse at the right of Figure 4-19 a). Nitrobenzene, a common stabilizer used to improve burn properties [107], and EGDN were also detected in the headspace of the Smokeless powder (Figure 4-19 a). Nitrobenzene has been marked with a red circle in the chromatogram, around 87 seconds and is the second time that it has been detected in the headspace of these training aids, the first being in the Power gel analysis. The red circle in the centre of the chromatogram marks the elution of EGDN with nitro and dinitroglycerins. EGDN is treated as a taggant for high explosives as it is a plastic explosive marker according to ICAO [162]. However, as nitroglycerin is the main component of smokeless powders [239] it is treated here as an explosive compound. The third red circle on the smokeless powder chromatogram is 2,4-DNT, usually found in the headspace of smokeless powders as it is commonly used as a deterrent [231, 239]. The additives detected included: diphenylamine (white circle) which is a volatile compound used to improve shelf life and burn properties of powders and has been used previously on EDD trials [107]; phthalates used to soften the powder granules and reduce the need for solvents [231]; and ethyl centralite which is a deterrent used to coat the powder, reducing the initial bum rate, and lowering the bum temperature [238]. These additives are often more volatile than the explosive compounds and represent important VOCs for understanding EDDs behaviour.

The black powder analysis detected numerous acyclic alkanes (Figure 4-19b, white circle). Black powder is manly composed of potassium nitrate which is not conducive to SPME extraction or chromatography analysis. However, the 2D GC analysis was able to identify nitrobenzene in the headspace of this sample. This highlights that nitrobenzene was an additive to the two powders as well as the blasting agent [231]. Propellants are not composed by main charges, such as TNT, PETN and RDX [9]. Nitrobenzene is added to improve burn properties of all three samples and is more volatile compared to the other explosive related compounds identified [231]. The study of nitrobenzene can aid in understanding how EDDs alert to ammonium and potassium nitrate based samples.

4.6.3 Comparison of VOCs Identified

Several compounds that were not identified in the headspace of the training aids using 1D GC-MS analysis were able to be separated and identified with the 2D GC method. Noteworthy are the Power gel and Black powder samples, which could not be profiled by

conventional GC-MS but were successfully analysed by HS-SPME-GC×GC-TOFMS. The 2D analysis of these two samples and the Smokeless powder confirmed the presence of nitrobenzene in the headspace of ammonium and potassium nitrate based explosives. This is an interesting compound due to its use and volatility [266] and has been previously described in the literature as an additive to smokeless powders [107]. The analysis of Smokeless powder also demonstrated the presence of 2,4-dinitrotoluene. Dinitrotoluene (DNT) and its different isomers were the most consistent explosive related compound detected in the explosive training aids. They were detected in all commercial explosives with the exception of Detonation cord, and were therefore considered a key VOC to be presented to EDDs for the next stage of this project.

DMDNB was also detected in most samples. Six out of the eight explosive training aids contained this compound at varying concentrations. It is still not clear whether the low concentration in some samples are due to different concentrations added during sample manufacture or whether it has originated elsewhere. Due to its volatility and consistent identification in the explosive samples, DMDNB was also chosen as one of the key VOCs for subsequent EDD trials. The other mandatory taggants for plastic explosives were not as consistently detected as DMDNB, thus were not selected as key VOCs. Nitrotoluene was detected only in the headspace of Composition B, and EGDN was detected only in the headspace of Smokeless powder, to which it is not added as a taggant but a part of the main composition of this explosive.

Several additives were recurring in the explosive samples, as can be seen in Table 4-7, which outlines the most consistent VOCs per group and their detection per sample. Additives are often more volatile than explosive compounds and certain compounds such as 2-ethyl-hexanol, phthalates and benzophenone are found in almost all explosive samples due to their use [107]. Some compounds, such as cyclohexanone, are difficult to classify as to whether it originates from explosives or additives. However, even though these compounds are volatile and consistently present in explosive samples, they are not exclusive to explosives, being present in different materials and substances, and often found naturally occurring in the environment [78]. This may lead to confusion during the training of EDDs since they should represent distractor odours and hence they were not included as key VOCs in the EDD trials.

Table 4-7: List of compounds identified per explosive analysed by HS-SPME-GC×GC-TOFMS

	Comp. B	***Pentolite***	***Primer Sheet***	***PE-4***	***Det. Cord.***	***Power Gel***	***Smokeless Powder***	***Black Powder***
EXPLOSIVE COMPOUND								
1,3-Dinitrobenzene	✓	✓					✓	
1,4-Dinitrotoluene		✓		✓				
2,3-Dinitrotoluene		✓						
2,4-Dinitrotoluene	✓	✓		✓			✓	
2,4,6-Trinitrotoluene	✓	✓						
3,5-dinitrotoluene	✓	✓	✓					
Dinitroglycerin							✓	
Nitrobenzene						✓	✓	✓
Nitroglycerin							✓	
Nitromethane							✓	
Glycerine							✓	✓
ADDITIVES								
2-Ethyl-1-hexanol	✓	✓	✓	✓	✓	✓	✓	✓
Acetophenone			✓	✓	✓	✓	✓	✓
Benzaldehyde	✓	✓	✓	✓	✓	✓	✓	✓
Benzene	✓	✓						
Benzonitrile	✓	✓	✓		✓	✓		✓
Benzophenone	✓	✓	✓	✓	✓	✓		✓
Benzothiazole	✓	✓	✓	✓	✓	✓	✓	✓
Biphenyl	✓	✓		✓	✓			
Biphenylene	✓							
Butanone			✓				✓	✓
Butyl benzoate			✓					
Butyl octanol		✓						
Butylated hydroxytoluene	✓			✓	✓			
Cyclohexanone	✓		✓	✓	✓			✓
DEHP			✓	✓	✓			
Dibutyl phthalate	✓	✓	✓	✓	✓	✓	✓	✓
Diethyl benzene		✓			✓			
Diethyl methyl		✓			✓			
Diethyl phthalate				✓	✓			
Dimethyl naphthalene	✓							
Diphenylamine			✓			✓	✓	
Ethyl methyl	✓				✓			✓
Ethyl methyl benzene				✓	✓			

Naphthalene	✓	✓		✓	✓	✓	
Phenol	✓			✓	✓		
TAGGANTS							
2,3-Dimethyl-2,3-Dinitrobutane	✓	✓	✓	✓	✓	✓	
Ethyl Glycol Dinitrate							✓
Mononitrotoluene	✓						

4.6.4 Principle Component Analysis (PCA)

To evaluate the differences between the explosive samples based on the volatile signature, clustering multivariate techniques (i.e. PCA) were conducted on the resulting data (Figure 4-20). The compounds used in the PCA were defined as statistically significant by an F-crit value calculated to be 2.69. This threshold reduced the data matrix and defined the statistically significant compounds. The corresponding correlation loading can be found in Appendix C. Figure C-1 shows the full correlation loading and Figure C-2 show the correlation plot zoomed per quart for a better view of the scores.

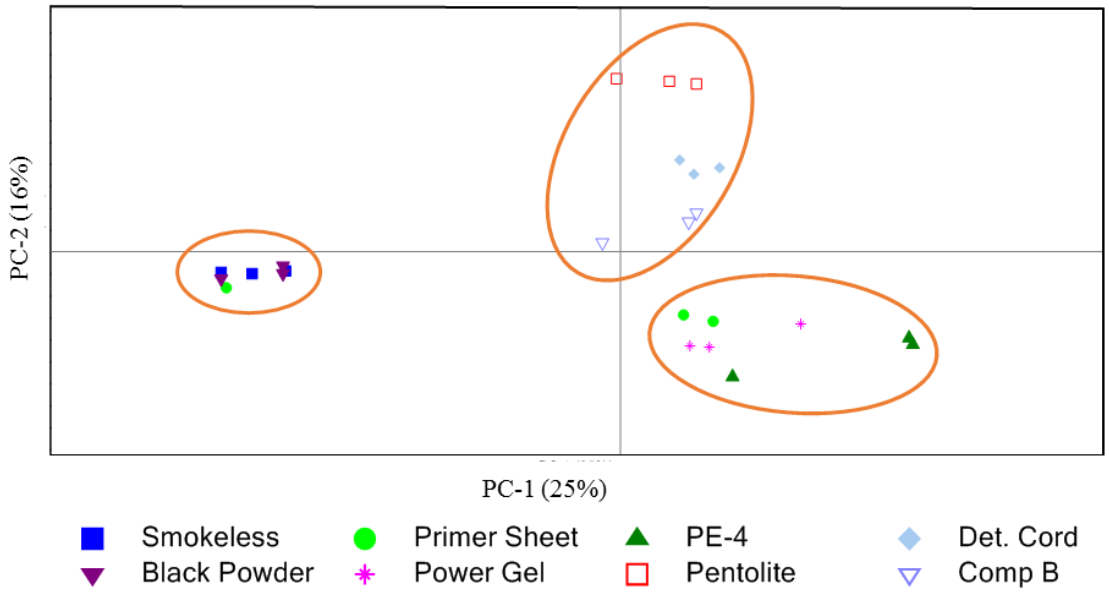

Figure 4-20: Principal component analysis using pre-processed GC×GC-TOFMS peak area data for explosive training aid samples using compounds detected with Statistical Compare.

The propellants, Black and Smokeless powders were clustered to the left of the plot (Figure 4-20). Potassium nitrate is a main component in both of these samples. Black and smokeless powders are also the only two low explosives sampled in this project and their

composition and appearance differentiate them from all other samples present. Their separation from the other samples is therefore explained by their chemical composition. Pentolite, Detonation cord and Composition B were clustered in the top right corner of Figure 4-20. These three high explosives are manly composed of plastics, such as polypropylene, due to their manufacture process and presentation. The chromatograms presented in sections 4.6.2.1 and 4.62.3 show the high abundance of hard plastics and hydrocarbons in the headspace of these three samples. Primer sheet, Power gel and PE-4 were grouped together in the bottom right of the plot (Figure 4-20). The parent components and composition of these three high explosives is not the same, however, they are all malleable explosives. These samples have texture similar to modelling clay and can be moulded to a desired shape and attached to walls or other superficies [3]. Malleable explosives are normally metastable, which means they need a detonator or blasting cap to explode. This finding indicates that the grouping of these samples was achieved by their appearance and consistency instead of their primary composition.

The study of the correlation loadings and bi-plot (shown in Appendix C. Figure C-3) illustrates the VOCs that are driving the groupings and separations. The separation of propellants (black and smokeless powders) from the other explosive training aids was based on the presence of nitroglycerin, EGDN and diphenylamine, while butanone was the most influential compound for the grouping of these two powders. Notably, the only other sample to contain butanone in the headspace was the Primer sheet. This may explain why one of the Primer sheet samples was singularly grouped with the powders, distinct from the other two Primer sheet samples and the other malleable explosives. The hard plastic explosives cluster (Pentolite, Detonation cord and Composition B) were classified by the presence of benzenes, esters and hydrocarbons. Phthalates made up the majority of the compounds present in the Pentolite samples and caused the separation of this sample from the others. Detonation cord and Composition B comprised resin precursors and benzenes as the most abundant VOCs in the headspace. These three samples have a plastic appearance, enclosed by a hard polymer casing or thick plastic film. Therefore the VOC grouping is compatible with these samples appearance and composition. Detonation cord is a blasting agent that was wrapped in a thick plastic film and the Composition B training aid was wrapped in hard plastic casing, therefore numerous hydrocarbons and resin precursors were present in the VOC profile.

The proximity of the two high explosives groups were due to phenols such as butylated hydroxytoluene and other butylated compounds. The malleable explosives group (Primer sheet, Power gel and PE-4), were grouped mainly by the presence of naphthalenes. Naphthalenes are the plasticizers responsible for the malleability of plastics and are present in the headspace of most other high explosives (see section 4.6.2.3). The abundance of naphthalenes and its derivatives in the headspace of the malleable explosives, however, was much higher than that found in the headspace of the other explosive samples, driving the grouping of Primer sheet, Power gel and PE-4.

4.6.5 Summary

The use of fast chromatography conditions combined with the separation power of 2D GC has proven to be ideal for the analysis of explosive samples extracted by HS-SPME. The method efficiently provided a detailed analysis of the complex VOC profiles of explosive training aids which was found to include taggants, plasticizers, and explosive related compounds. Most explosives consisted of multicomponent mixtures. It was particularly difficult to differentiate PETN containing explosives, which were the samples that demonstrated the least amount of explosive related compounds. The additives comprised the majority of the VOCs identified in the headspace of all samples. The use of multivariate statistical analysis aided in the exploration of potential VOCs for the separation of the complex mixtures associated with the explosives training aids. The PCA analysis aided in the separation of training aids by grouping them based on singular compounds and/or abundance of commonly appearing compounds. Samples such as the propellants could be separated by compounds such as butanone, nitroglycerin, diphenylamine and EGDN. Other samples, such as PE-4, Power gel and Primer sheet could be separated by phthalates and its derivatives, which although are common headspace compounds for plastic explosives were much more abundant in this compounds.

4.7 CONCLUSION

This project aimed to profile the headspace of explosive training aids used by the NSWPDU. By chemically profiling the headspace of real explosive samples it was possible to define the key VOCs to be used in field trials with explosive detection dogs and for subsequent development of chemical detection methods.

The low vapour pressure and thermal instability of explosive compounds presented a major challenge for the HS-SPME-GC-MS analysis of explosive training aids and VOC profiling. This method was not able to discover the complete range of VOCs present in the headspace of explosives. This experiment demonstrated the limitation of long chromatographic runs for unstable thermal compounds, which has been reported previously [245]. Moreover due to the complexity of the headspace matrices, the VOC mixtures exceeded the separation capacity achieved with GC-MS.

Aiming to facilitate the separation of explosives VOCs and reduce the effect of the temperature on thermal degradation of compounds, a fast chromatography method was created and a proof of concept was performed with both GC-MS and GC×GC-TOFMS analysis following HS-SPME extraction of dried explosive liquid standards. This method allowed a preliminary identification to be obtained for the nitro explosive compounds present in the samples. The fast chromatography conditions were successful in the separation and detection of a range of explosive compounds and limits of detection were determined for the standards analysed. The development of a fast chromatographic method decreased the elution time and temperature, reducing the degradation effects on the explosive samples. Once detection of liquid standards by HS-SPME was achieved, the investigation of the headspace of explosive training aids was revisited.

A 2D GC instrument was chosen to perform the headspace profiling of explosive training aids due to its enhanced separation capacity and resolution [132]. The resolution power of the GC×GC technique combined with the fast chromatography method allowed for the separation of the VOCs of different samples and provided valuable information about the headspace of explosive samples in less than five minutes. Although the low vapour pressure of explosives lead to low concentrations of volatiles hindering the detection of these samples in the headspace [18], the cryo-focusing effect of the modulation process used in GC×GC assisted to improve the detection of these compounds [118]. In

comparison to the 1D experiment, the 2D GC analysis resulted in a much wider range of compounds being detected and identified.

The HS-SPME-GC×GC-TOFMS analysis of explosive training aids showed complex mixtures of VOCs in the headspace. The most commonly identified VOCs were additives such as 2-ethyl-hexanol, phthalates, benzoic compounds, esters and ketones. Some additives have already been tested with EDDs in field trials [107] without a validated result. Due to their use in different substances and natural occurrence additives were not considered ideal for EDD trials. However the role of these compounds to increase or differentiate other odours when combined in the mixture was taken into consideration and should be analysed further in subsequent research.

Explosives markers were identified in the headspace of several samples, predominantly DMDNB. Due to its use and volatility DMDNB was chosen as a key VOC to be used on the next stage. Although PETN and RDX were not detected in the headspace of the explosive samples and TNT was found in only two samples and in low concentration, several nitro aromatic explosives were detected. Nitrobenzenes, dinitrobenzenes, dinitrotoluenes and its isomers were detected in the headspace of almost all samples with the exception of Detonation cord. For its use, volatility and consistency across samples, DNT and DNB were selected as key VOCs for the next stage of this project.

Based on the headspace analysis of explosive samples three target odours were selected to be presented to EDDs in field trials (DNT, DNB and DMDNB). These trials will provide additional information to define the key VOCs in explosive vapours. The trials will aim to qualify and quantify the alert of explosive detection dogs to target explosive compounds, and to confirm their use for future development of comparable chemical detection methods.

Chapter 5: EXPLOSIVE DETECTION DOGS' RESPONSE TO VOCS IDENTIFIED IN THE HEADSPACE OF EXPLOSIVE TRAINING AIDS

Chapter 5: EXPLOSIVE DETECTION DOGS' RESPONSE TO VOCS IDENTIFIED IN THE HEADSPACE OF EXPLOSIVE TRAINING AIDS

5.1 INTRODUCTION

Due to the complex nature of explosives a wide variety of samples need to be used to properly train explosive detection dogs, ranging from half a dozen to upwards of twenty samples depending on the training agency and deployment locations [107]. Moreover, the challenges in handling, storing and exposing animals to live explosives is justification for the development of mimic odours that could be certified and dependable. Non-hazardous training aids are commercially available but there are limited types available and they have had limited testing of their effectiveness under field conditions in double-blind studies [267].

5.1.1 Goals

The research described in this chapter aimed to test the response of EDDs to the key VOCs found via HS-SPME-GC-MS and HS-SPME-GC×GC-TOFMS (as described in sections 4.2 and 4.6). The key VOCs chosen were the most abundant analytes in the headspace of explosive training aids that were exclusive to explosive compounds.

The primary goal was to identify significant compounds in the headspace of real explosives using chemical detection and to confirm or refute their biological detection by EDDs. A secondary aim was to compare the canines' selectivity and reliability with the results of the HS-SPME-GC×GC-TOFMS analysis. GC×GC-TOFMS was the most sensitive and reliable method used during this project for the identification of explosive VOCs. The use of fast chromatography conditions allowed for the separation and identification of eighteen compounds from explosive liquid standards in under 5 minutes with ppb limits of detection. HS-SPME extraction is a validated technique for the recovery of vapours and yielded reliable results for the analysis of the EDDs training aids. A 15 minute exposure time was deemed a satisfactory pre-concentration for the recovery of these explosive compounds. The assessment of EDDs response to the key VOCs will

allow a comparison of their selectivity and sensitivity to the analytical method employed. It will assist in determining whether the most abundant VOCs produced by the range of training aids are in fact the compounds to which the dogs alert.

5.1.2 Previous research

Some studies report that the sensitivity of certain benchtop instruments and technologies are comparable to that of the canines [107], however EDDs still hold several advantages over instruments, such as providing a fast and reliable response, their ability to access multiple areas and be easily deployed in different scenarios [231]. To date, canines are still considered the gold standard for field detection of explosives [2]. The amount of explosive vapours available to a trained canine depends on the amount of material, container volume, explosive vapour pressure, and temperature of the explosive [109]. Canines have been reported to detect compounds in the parts-per-billion (ppb) range at atmospheric pressure conditions [2]. There are currently several theories about what is responsible for the canines' high sensitivity to explosives [107]. Although research suggests that dogs do not rely on vapour signatures from the pure compound, but rather a combination of odours from solvents and synthetic remnants from the manufacturing process and degradation [263], this project aims to investigate the EDD response to nanogram (ng) amounts of explosive related compounds to determine whether dogs know or can learn how to alert to singular compounds. The study will present varying ppm concentration of liquid standards to specially trained canines and qualitatively assess their response. The exclusion of solvents, synthetic remnants from additives, and manufacturing process and degradation compounds will eliminate the natural and multiple use compounds, which can be found in several applications besides explosives and are naturally occurring in the environment.

Previous studies have already demonstrated EDD response to additives and degradation products of explosives [107, 109]. One study used three liquid nitroalkanes, namely: nitromethane, nitroethane and 1-nitropropane, to simulate the odours found in containers. Trials were conducted with exposure amounts of 1 or 10 mL of the given compounds [109]. Another study exposed the EDDs to 0.1 and 10 mg of 2-Ethyl-hexanol, diphenylamine and cyclohexanone on two different substrates, assessing the dogs for: 'no alert'; 'interest'; or 'alert' [107]. The same study presented the dogs with three explosive

compounds, namely DNT, DNB and TNT utilizing the same set up, at 0.1 and 10 mg amounts of these compounds [107].

The study herein involved seven canines across a total of 24 trials. The EDDs were exposed to volumes varying from 10 ng to 100 μg of DNT, DNB, and DMDNB. The responses were assessed in four different categories, taking into consideration not only the alerts or lack of response to the target odours but also to controls and distractor odours. This test was designed to be as extensive and complete as possible, using the selected target VOCs.

5.1.3 Canine Detection of Explosives

The concept of odour availability can be controversial in the canine community because the quantity of explosive VOCs available for the canines during testing is easily measured, while the degree of confinement and the amount of detectable vapour in real case scenarios is not [109]. The extremely low vapour pressures of explosives hinder their detection by EDDs and instruments, for this reason the high volatility compound DMDNB and three other taggants were chosen as explosive markers [117].

It is unknown how exactly canines alert to explosives, but there are currently three theories: 1) canines alert to the parent compound of explosives regardless of their volatility (e.g. TNT); 2) canines alert to the more volatile compounds that are present in explosives independent of whether they are exclusive to explosives or not (e.g. 2-Ethyl-Hexanol); or 3) canines alert to a mix of characteristic volatiles including the parent compound and non-explosive compounds [268]. In this study, it was decided to test the second theory since, if shown to be valid, it could identify a set of VOCs common to a range of explosives which could be adapted for future training aids. This also allowed a direct comparison between the chemical and biological detection methods to determine whether commercially-available instrumentation is sufficiently sensitive for proving one or more of these theories.

To date, there is currently little scientific information available to aid in the optimal selection of training aids for EDDs [107]. In order to determine how and if these specially trained canines can alert to singular compounds, trials were carried out to measure the EDDs response to different concentrations of dried liquid standards of compounds detected in the headspace of the explosives used for their training. The trials tested the hypothesis that these canines' specificity to compounds can lead to the development of optimal training aids by studying explosive VOC signature compounds.

5.2 MATERIALS AND METHODS

The VOC profiles of explosive training aids were identified with the use of gas chromatography methods and compared to preliminary results of active odour profiles of explosives reported in the literature [79, 218, 231, 269]. Having confirmed the most abundant compounds in the headspace of explosives, field trials were conducted with the NSW Police Dog Unit (NSWPDU), the same law enforcement agency which provided

the explosives used as training aids for the GC-MS and GC×GC-TOFMS analysis. A certificate for "Animal-Research: Animal Care and Ethics" was granted to the main author of this project on August 2015 by the University of Technology Sydney (E17/3196) and a refresher training course was completed on July 2017 (E17/5358) to carry out experiments with the EDDs.

5.2.1 Standards

Liquid standards of 2,4-Dinitrotoluene (DNT), 1,3-Dinitrobenzene (DNB), and 2,3-Dimethyl-2,3-Dinitrobutane (DMDNB) were chosen as the target odours and purchased from AccuStandard (New Haven, CT, USA) at a certified concentration of 1000 µg/mL in acetonitrile. The odours chosen were the most abundant compounds found in the headspace of explosive training aids exclusive to explosive samples, following the rationale described previously.

Solutions varying from 1 to 1000 ng/µL of each of the three standards were prepared using acetonitrile purchased from ChemSupplies Pty Ltd (Gillman, SA. Australia). The prepared solutions were used to prepare the target odour for presentation during the EDDs regular maintenance training. Aliquots of 10 µL of solutions varying from 1 to 1000 ng/µL were pipetted onto two different substrates (further explained in Section 5.2.2) and left to dry overnight prior to the trials. Aliquots of 10 µL of acetonitrile were also dried overnight on the same two substrates to create control samples. Acetonitrile was chosen as the optimal solvent based upon its suitability to dissolve every explosive [231], and for the fact that most purchased explosive standards come in acetonitrile solution. The overnight drying allowed for the acetonitrile solvent to evaporate, creating what was estimated to be sample concentrations of 0.01 to 10 µg. These concentrations were chosen by evaluating the vapour pressure of the given compounds and assuming that trace explosives vapours are generally at concentrations of at least two orders of magnitude below the saturated vapour concentration in the environment. This resulted in the sample concentrations being significantly lower than sample concentrations described in previous researches [107, 109, 231]. The first trial involved exposing the dogs to concentrations of 0.1, 1 and 10 µg during three different trial runs. As the trials continued the concentrations presented to the EDDs was decreased reaching the lowest concentration of 0.01 µg. Most trial days involved presenting concentrations of 0.01, 0.1, 1 and 100 µg, aiming for consistency across the trials.

5.2.2 Choice of Substrate

Aluminium tins of 250 mL were used to prepare the samples following the training protocol of the NSWPDU. The 10 µL aliquots of standards were pipetted straight into the tins creating the first trial substrate (non-porous). To create an alternative substrate comparable to previous literature reports [107], 90 mm grade nº 1 Whatman™ filter paper sheets supplied by Sigma-Aldrich (St. Louis, MO, USA) were chosen as a second substrate (porous). Filter paper was chosen as it is a commonly used porous substrate and has been previously described in the literature for EDD trials [107].The filter paper was placed inside the aluminium tins, 10 µL of solution was then pipetted onto the paper filter and left to dry overnight, creating the second trial substrate. Comparison between the two surface types would determine if there was any difference in retention of the odour and its subsequent availability to the dogs during training.

5.2.3 Explosive Detection Dog Teams

Observational studies of dog trials were conducted at the NSWPDU training centre and followed their standard procedures in order to provide a baseline understanding of the canines' capabilities and responses. Seven dog and handler teams were assigned for the field trials, however due to operational commitments of the teams it was not possible to have all teams present for every training session. Therefore, a minimum of three teams was chosen as necessary for the trial to be conducted. The trials followed the maintenance training schedule, generally occurring once a week over no longer than a 5 hour period. This time frame allowed for three to four trials to be performed with all attending canines per trial day. The trials were conducted by the handlers and monitored by the training coordinator, ensuring the well-being of the animals at all times. Information about each team was provided by the unit supervisor and the canines' details are shown in Table 5-1.

Table 5-1: Summary of the EDD teams that participated in the trials and their background

TEAM	AGE	BREED	GENDER	EXPERIENCE (as at September 2018)
1	5 years 10 months	English Springer Spaniel	Male	4 years 11 months
2	5 years 10 months	English Springer Spaniel	Male	3 years 5 months
3	8 years 4 months	English Springer Spaniel	Male	5 years 11 months
4	5 years 4 months	Labrador	Male	3 years 6 months
5	5 years 4 months	Labrador	Female	3 years 4 months
6	2 years 9 months	Labrador	Female	11 months
7	1 years 8 months	Border Collie	Male	1 month and 1 day

5.2.4 Experimental Design

The observational studies served to establish a baseline of the canines' capabilities and achieve a better understanding of the VOCs to which they alert. This experimental design has been used previously for the study of blood and cadaver detection dogs with the NSWPDU as detailed in [270, 271]. Aliquots of 10 µL of each target compound (DNT, DNB, and DMDNB) were separately pipetted into the substrates (tin or filter paper) and left to dry overnight, creating six samples per desired concentration (varying from 1 to 1000 ng/µL). The filter paper samples were placed into the aluminium tins. All tins were covered with aluminium lids, which were perforated on the day of the trial prior to exposure to the EDDs.

Figure 5-1 demonstrates how the samples were prepared. Figure 5-1a shows a filter paper containing 10 µL of DNT, or DNB, or DMDNB standard solution in a concentration that varied from 1 to 1000 ng/µL. The filter paper was placed inside an aluminium tin and left to dry overnight retaining an expected sample concentration of 0.01 to 10 µg. Concomitantly, 10 µL of DNT, or DNB, or DMDNB standard solution in a concentration that varied from 1 to 1000 ng/µL was added directly into an aluminium tin (Figure 5-1 b) and left to dry overnight in order to evaporate the organic solvent. The tins were covered by aluminium lids, which were swapped for perforated lids during the training (Figure 5-1c). All covered tins were placed into a carton and transported to NSWPDU training centre for each training session. The aluminium tins were utilised to replicate the scenting

environment EDDs are exposed to during their training, following the NSWPDU protocols. All tins were stored at room temperature until the time of the trials.

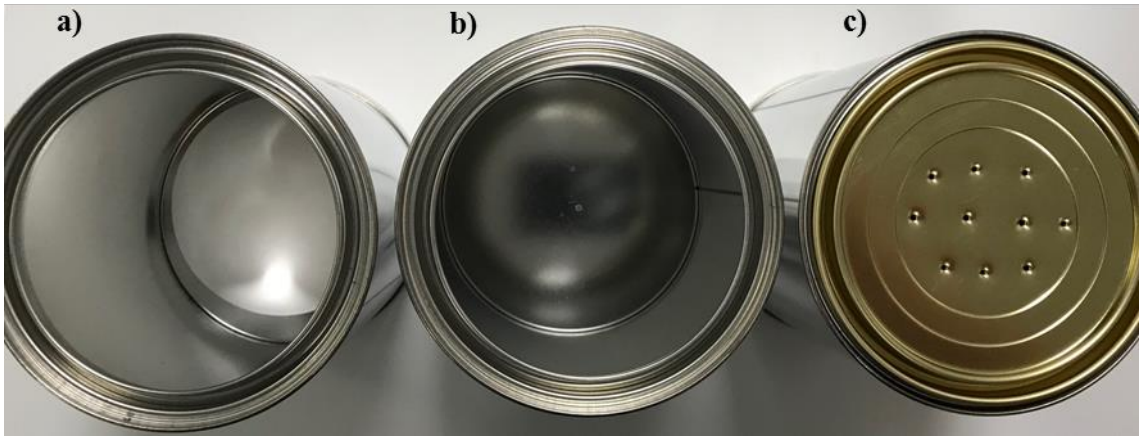

Figure 5-1: Aluminium tins used to present the samples during the EDD trials**;** a) 10 µL of liquid standard on filter paper placed in the aluminium tin and dried overnight; b) 10 µL of liquid standard placed directly in the aluminium tin and dried overnight; and c) tin covered with perforated lid at the commencement of each trial.

Control samples were created by adding 10 µL of acetonitrile (99%) to a filter paper sheet, which was placed inside a 250 mL aluminium tin, or by placing 10 µL of acetonitrile (99%) directly into the aluminium tin. Both samples were left to dry overnight the day prior to each trial. An empty aluminium tin and an aluminium tin containing a blank filter paper were also added to the trials as blanks. Other blank tins were filled with distractor odours such as dog food, toys, gloves, pipette tips, tap water, and chalk.

Training sessions were conducted inside an enclosed shed containing a standard scent line-up of cinder blocks in a U-shaped formation with 16 spaces in each row (Figure 5-2a). Each of the cinder blocks contained a set of two 250 mL aluminium tins, marked with their respective number (1-48) on the floor with chalk. The tins containing the target odours, control or blanks were covered with perforated lids to conceal the contents from the handlers and dogs but to allow the odours to disperse from the tin (Figure 5-2b).

Figure 5-2: Scent line-up and sample placement; a) shows the room configuration and b) the perforated aluminium tins contained within each cinder block. Figures adapted with permission from Rust, 2018 [270].

Prior to each trial, the room was closed to handlers and their dogs while the researchers placed the target odours, controls and distractors within the cinder blocks. The experiment was conducted as a single blind study based on the standard training protocols used by the NSWPDU, where the teams were not aware of the position of the target odour. In each

trial set, there were six tins containing target odours (hot tins), four controls and 38 distractors (cold tins). The detection canines would search across the cinder blocks, with the handlers ensuring that all blocks received a sniff directly above it. The hot tins were composed of two samples of DNT, two samples of DNB, and two samples of DMDNB, in each case one sample was dried on filter paper and the second sample was dried in the tin. The cold tins were composed of four controls: two samples of acetonitrile, one dried on filter paper and a second dried in the tin, a blank filter paper, and a blank tin. All remaining tins were filled with distractor odours. These distractor odours represented odours that might be associated with the preparation of the samples used in this study (e.g. pipette tips, gloves) or might otherwise generate interest from the dog (e.g. dog food, toys).

The position of the target odours, controls and distractor odours were randomly placed using a random number generator restricting the number of hot tins by a maximum of two per row. Each trial involved the dogs searching the U-shaped formation from tins 1-48 in order. The placement of the tins was kept constant for all dogs per trial set. Each dog and handler team would run the same set once only. Three to four trial runs per dog were performed per trial day. While the tins would remain in the same place until all dogs had completed that trial set, the cinder block that covered the samples would be replaced every time a dog alerted to it. The lids of all tins were also wiped between dog searches. An example of this set-up after randomisation is shown in Figure 5-3.

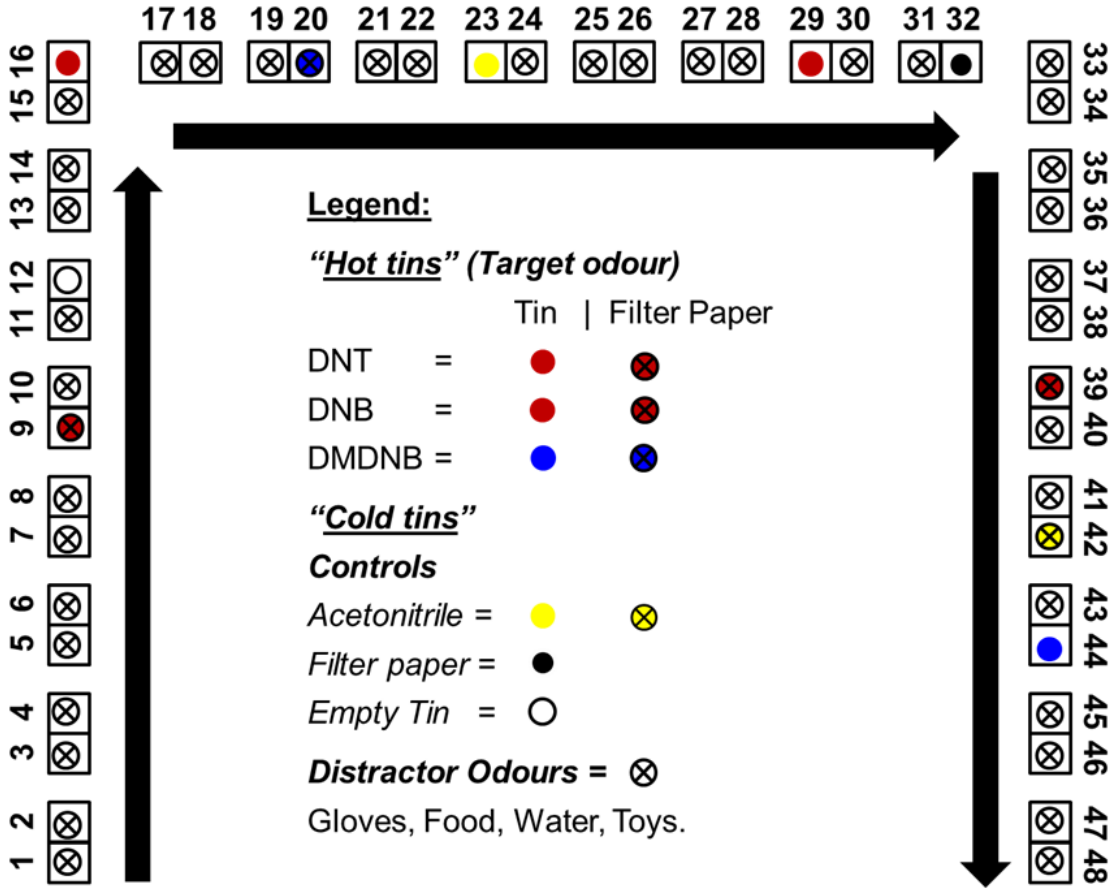

Figure 5-3: Example of one scent line-up showing the randomised placement of hot and cold tins for the dog trial studies with direction of search also shown.

All tins were handled by the researchers attending the experiments, who were wearing gloves to avoid contamination of different odours on the tins. All tins and lids were stored in the same place aiming for a standard scent across all tins. Gloves were changed between handling tins within a single set.

The dog search was performed on-leash and as the canines scented across the experimental blocks, they would show changes in behaviour which varied from sit-and-stare, feet-tapping, tail-wagging, lay-and-bark, sit-and-bark, or nosing the block. A positive alert was considered as a complete stop in front of the cinder block containing the tin they identified as the target odour and pointing their nose to the location of the scent. Every time the dog correctly alerted to a target odour (classed as hot tins) the handler would reward the canine with their toy and play. If the canine alerted to a distractor odour (classed as cold tins), did not alert to a hot tin, or displayed a change in behaviour but the handler did not deem it to be a positive alert, no action was taken by the handler and the search would be resumed. The trials utilised a single blind protocol

whereby the handler was unaware of the location of the target or distractor odours and received the confirmation of a correct alert from the researcher. Although these training protocols may differ from international standards (e.g. double blind training), they are the standard to which this police service accredits their canines and for this reason, the protocol was followed without adaptation. It was important that the training followed the NSWPDU standard procedures so as not to influence the outcomes.

5.2.5 Data Analysis

Observational data was collected and assembled in Microsoft Excel to produce qualitative graphs for comparison purposes. Trends and variations in response were analysed. Significance of variation tests were applied whenever possible for the data collected.

The dogs' response were analysed taking into consideration not only the hot tins, but also the cold tins and lack of response. For observational purposes of these trials four types of responses were recorded: 1) true positive alert, described every time a dog alerted to a target odour; 2) true negative alert, described every time a dog did not alert to a control or distractor odour; 3) false positive alert, described every time a dog alerted to a control or distractor odour; and 4) false negative alert, described every time a dog did not alert to a target odour. These responses are summarised below in Table 5-2 for reference. Behavioural changes and partial alerts, such as pausing over tins, head-flicks or attempting to go back to previous tins by each dog were identified and noted for future training improvements.

Table 5-2: Summary of the responses observed by EDDs during trials

RESPONSE	TARGET ODOUR	DOG ALERT
True Positive	Present	Yes
True Negative	Absent	No
False Positive	Absent	Yes
False Negative	Present	No

5.3 RESULTS AND DISCUSSION

Six dog trial days took place over non-consecutive weeks with the use of seven EDD teams at the NSWPDU training centre. The teams were presented with three dried

standards of DNT, DNB and DMDNB. Studies conducted during this project (section 4.4.2) and previous studies in the literature showed that DNT is a common compound found in the headspace, while DNB is less commonly reported [79, 107] even though it was identified as one of the abundant VOCs in the range of explosive training aids. DMDNB was found in six out of the eight explosive training aids tested (Section 4.5.2). Following the project rationale these standards were chosen as they represented the most abundant VOCs in the headspace of the explosive training aids that were exclusive to explosives (see Section 4.6.3.1). The standards were dried onto a filter paper or directly into an aluminium tin producing six samples of target odours among 48 total samples, which included controls and distractors. The official training aids used for these dogs are either real explosives or synthetic training aids, intended to mimic the scent of real explosives in a non-hazardous way. Only real explosives were analysed in this study.

5.3.1 Target Odour Analysis

When evaluating the percentage of correct response rates for the target odours it is evident that the dogs' positive alerts increased as training progressed. As can be seen in Figure 5-4, the overall true positive alert rate per trial day increased considerably on the final training days. The overall analysis considered the positive alerts of all canines participating in the trial compared to the number of target odours presented per trial run.

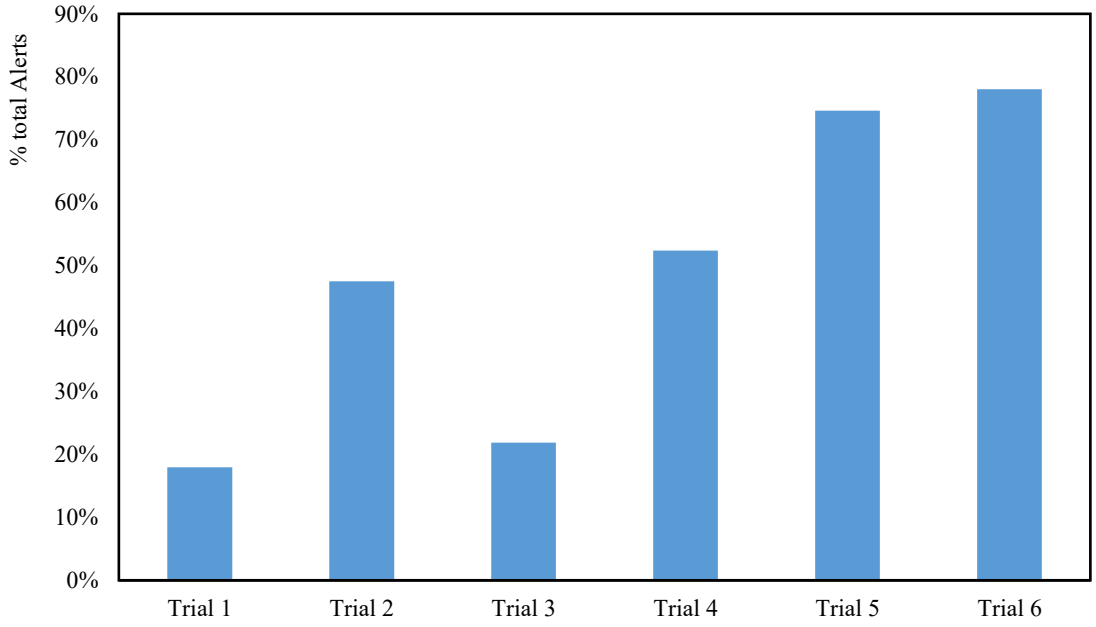

Figure 5-4: Overall percentage of true positive alerts recorded for explosive detection dogs when exposed to key VOCs found in the headspace of explosive training aids.

Initial trials involved presenting the teams with concentrations varying from 1 to 10 µg during three different runs. In the first run, none of the attending dogs alerted to 1 µg of any of the target odours. The concentration was increased to 10 µg for the following two runs, which elicited responses from half of the dogs present. The second trial involved presenting the teams with concentrations of 2, 4, 8 and 6 µg in this order. All concentrations elicited responses from at least one of the canines present. Following discussion with the training coordinator and aiming for consistency across the trials, from the third trial onwards all dogs were presented with concentrations of 0.01, 0.1, 1 and 10 µg in random order. The responses varied considerably between canines, concentrations and samples as outlined further in this section.

The results of this study demonstrate that DNT yielded the highest number of true positive alerts, receiving more than fifty percent of the total alerts, when compared to DNB and DMDNB. Figure 5-5 shows the overall increase in the true positive alerts per target odour per day.

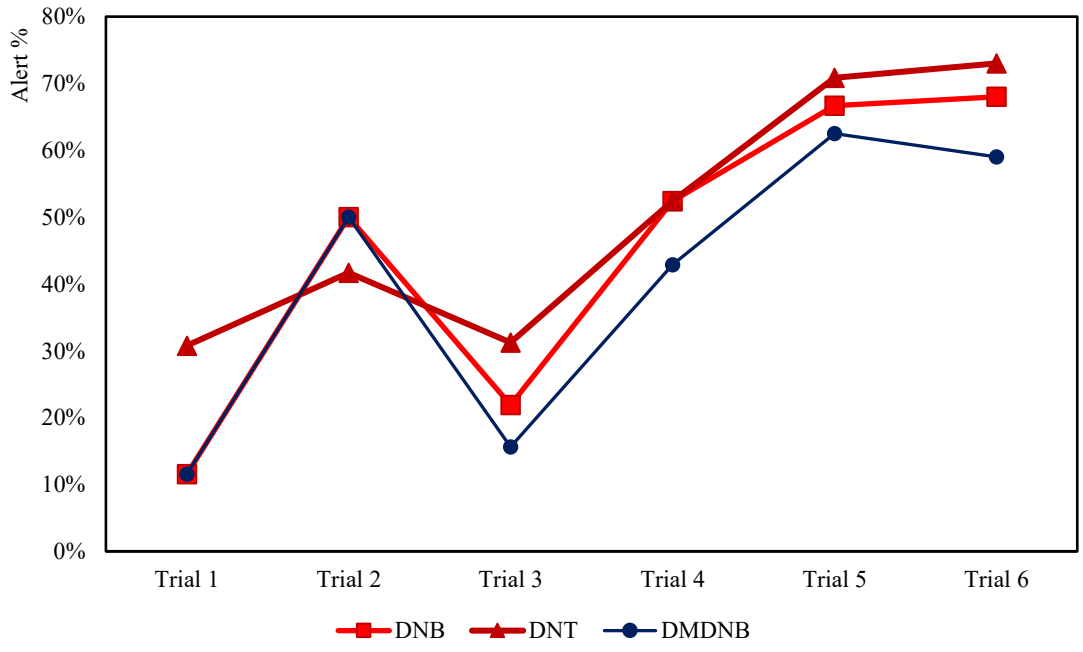

Figure 5-5: Overall true positive response by EDDs when exposed to the three target odours across the six trials.

All target odours produced a constant increase in the true positive alert rate over the six trials. The second trial demonstrated a steep increase, explained by the concentrations presented on that day and the group of canines attending, which were more experienced than those present during the following trials. The results show that although the target

odour concentration has decreased considerably from trial 1 and 2 (between 1 and 10 µg) to trial 3 onwards (between 0.01 and 10 µg), the overall alert to all the target odours has increased with training exposure. It is interesting to note that although DMDNB has the highest volatility of the selected target odours [18], is commonly used as a marker for explosive samples [272] and was found in six out of the eight explosive training aids tested (see section 4.5.2), it is the VOC that elicited the fewest correct responses compared to the other target odours. This result confirms that DMDNB should not be used solely as a training aid (which has anecdotally been suggested to some police services) or if used, that canines should be trained more consistently with DMDNB samples to maximize their efficiency at detecting this compound. These results corroborate previous literature findings of EDDs [107, 231] which have shown a higher positive alert rate to DNT compared to DNB [107]. A second study with nine EDDs showed no alerts for DMDNB, while four out of the nine canines alerted to DNT. However it is important to note that although Figure 5-5 suggests a higher rate of true positive alerts for DNT, a t-test applied with the results obtained from the target odours did not show a significant difference between the overall alert to the three target compounds (p value = 0.6385).

The number of positive alerts per target odour based on the total number of positive alerts was used to draw a comparison between the target odours (Figure 5-6). Comparing only positive alerts, this figure more clearly demonstrates that the canines' alerts are comparable to all three target odours.

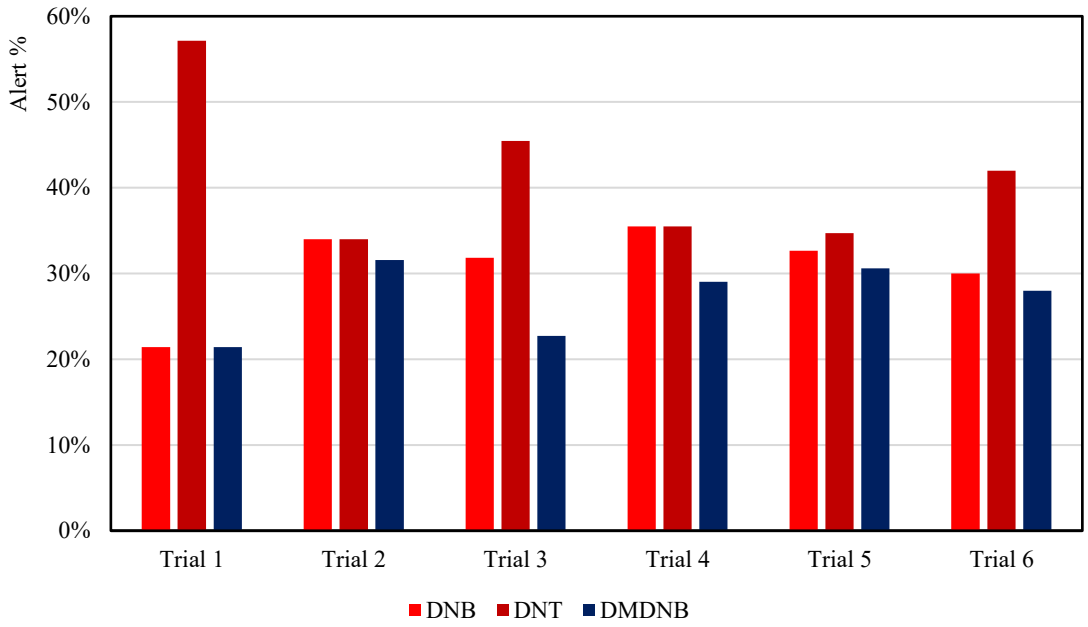

Figure 5-6: Percentage of true positive alerts per sample considering only true positive alerts recorded for each of the three target odours presented to EDDs over six trials.

The initial low number of true positive alerts suggests that the canines were not familiar with these VOCs as a single compound odour but learnt to recognise the compounds with training exposure. This suggests that the compounds chosen for this experiment are not the key VOCs recognised by the EDDs for the detection of explosives, or if they are, are more likely recognised as a ratio of VOCs rather than single compounds. Although preliminary results demonstrate that EDDs can be trained on these compounds, a wider range of VOCs would be more adequate for a future work.

5.3.2 Detection Limits

Given that the concentrations between 1 and 2 µg were presented only during the second trial, this set of trials was excluded from the concentration comparison. Figure 5-7 highlights the total alerts given per concentration per trial day. During the first trial, the teams were exposed twice to 10 µg of the target odours, and the same method was repeated during the third trial. In both trials, the alert rate to 10 µg more than doubled during the second run. During the fifth trial, the teams were exposed twice to 1 µg and showed the same response to that concentration during both runs.

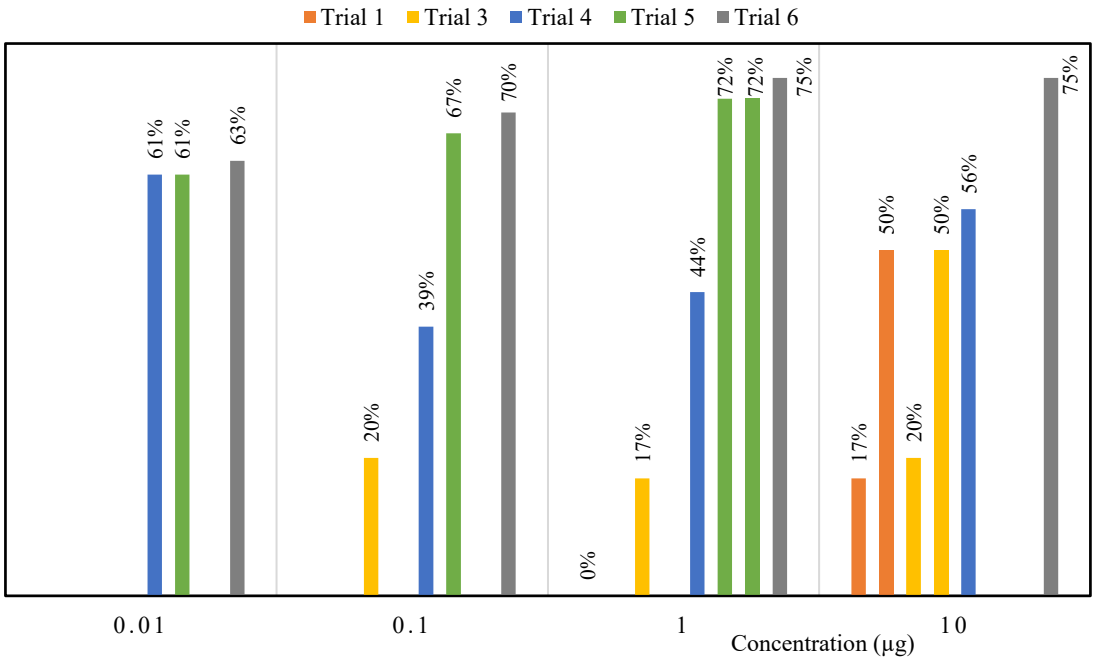

Figure 5-7: A comparison between true positive alerts recorded per concentration per trial day. Note: missing bars indicate that concentration set was not presented to the dogs on that day.

The percentage of correct alerts recorded for each exposure of the target odours demonstrated a clear improvement in efficacy and consistency over time. The lowest concentration (0.01 µg) was only introduced during the last three trials and showed a consistent true positive alert rate for the three trials. By comparing the results of the 6th and final trial (grey), it is also evident that the total positive alerts to all concentrations of the target odours became more consistent with training exposure.

When considering all target odours presented to the canines there was no clear preference for any concentration value. Figure 5-8 shows a comparison between the three target odours by analysing the total true positive alerts yielded per compound based on the total number of samples presented per concentration.

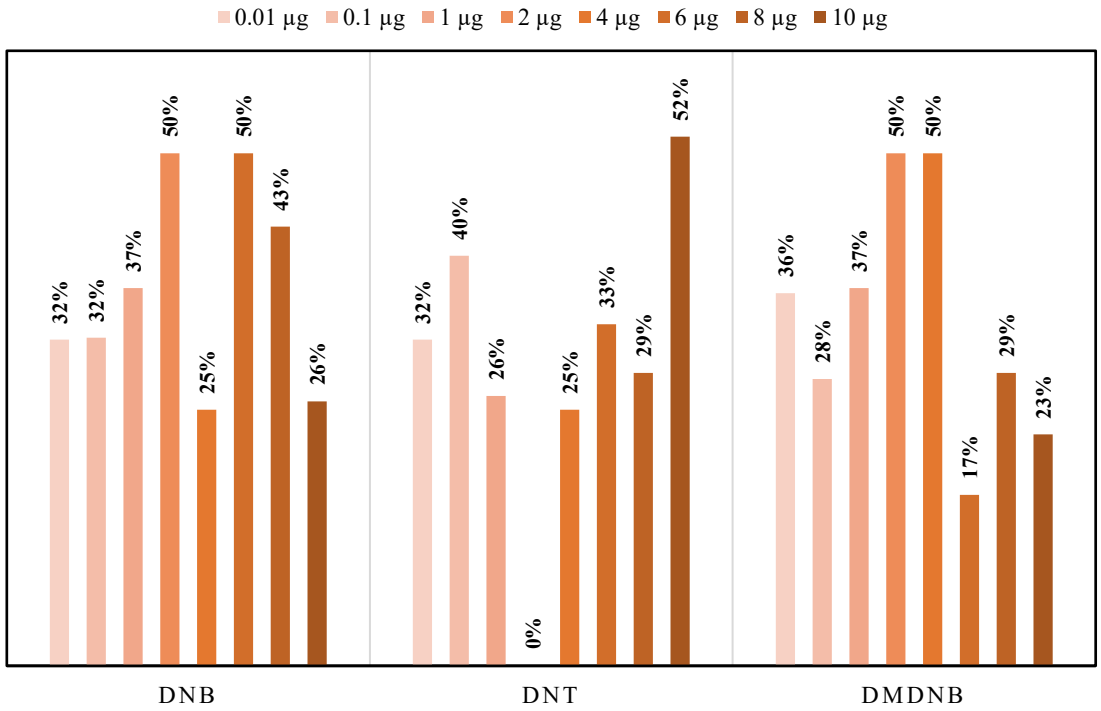

Figure 5-8: A breakdown of true positive alerts yielded per concentration of each target based on the total number of samples presented for each concentration.

Considering the range of concentrations chosen for this study, low concentrations (below 1 µg) yielded a comparable percentage of responses to all target odours. The middle range of concentrations (between 2 and 6 µg) showed an improved detection for DNB and DMDNB but not for DNT. However the highest concentration of DNT (10 µg) also produced the highest percentage of true positive alerts, although not statistically significant. The dogs' responses to different concentrations could suggest that there is a difference in permeability for VOCs and therefore a difference in how they are recognised by EDDs depending on the concentration presented. However given that there was no significant difference between the alerts amongst the target odours (p value = 0.6385) this can also mean a simple response variation by the canines. A previous study that aimed to validate short odour discrimination tests for scent detection dogs [273] found that the presentation of high or low concentrations of target odours, considered "Easy" or "Difficult" targets should not affect the independent target detection rate (general linear model, $P > 0.05$).

5.3.3 Substrate

The results of the substrate comparison demonstrate that target odours left to dry directly in the aluminium tins, without the use of filter paper yielded the highest number of true

positive alerts, for all three target odours (Figure 5-9). Acetonitrile was the solvent used to dilute the liquid standards and was also dried straight into tins and onto filter papers to generate control samples. There was no difference between the substrates containing the acetonitrile control samples as the same number of alerts was recorded for both aluminium tin and filter paper. A blank filter paper deposited into an aluminium tin and a clean aluminium tin were used to generate two blank samples. The blank filter paper elicited more than twice the number of alerts than the blank tin. Importantly, clean aluminium tins have always been presented to these canines according to their training procedure, while this was the first time a filter paper, either blank or containing different odours was introduced into their training.

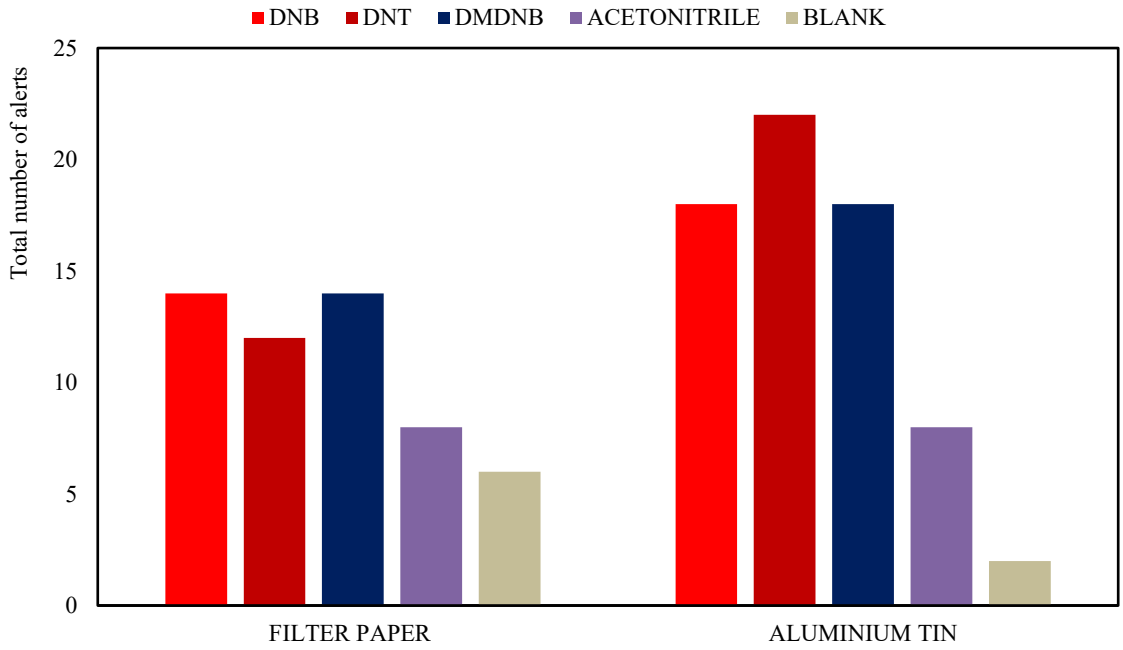

Figure 5-9: Total number of alerts for target odour and controls based on substrate.

This finding is in contrast to previous literature reports that describe porous substrates as aiding in the retention of the target odour [231]. The comparison between the total numbers of alerts elicited for all three compounds over both substrates showed that aluminium tins yield the highest number of true positive alerts for all three target odours. The higher number of alerts to the blank filter paper could have resulted from curiosity or guessing as this was an unfamiliar scent. Although for the human nose a filter paper may appear odourless it is clear that the dog olfactory system, which can detect three to four orders of magnitude lower than the human nose [164] is able to tell the difference between a blank tin with and without a sample of filter paper. Although the target odours

yield a higher response on the non-porous substrate, the control samples prepared by adding acetonitrile to both substrates showed the same number of false positive alerts. The results suggest that filter paper is not ideal as a background substrate for training aids and other porous (e.g. cotton gauze, sponge, etc.) and non-porous (e.g. ceramic, wood, etc.) substrates should be tested.

5.3.4 Dog Responses

The increase in true positive alerts by the canines to the target odours over time, considering all concentrations, indicates that they are learning which VOCs will yield a reward. A comparison between true positives and false negatives is a good indicator of this learning curve (Figure 5-10). False positives were scored every time one of the canines alerted to a control or blank sample or a distractor odour, which were represented by all the other tins in the trial that did not contain target odours. Out of the forty eight samples in each trial, six were target odours (DNT, DNB, DMDNB on two substrates each), four were controls (acetonitrile dried on two substrates, and blank substrates), and thirty eight were distractors such as gloves, tap water, food, toys, pipette tips and other odours associated with the preparation of the target odours.

The analysis of true positive and false positive alerts shows evidence of a possible learning curve by the canines, suggesting that these animals can rapidly adapt and learn to select target odours in their training protocol. The number of true positive alerts generally increased across the six trials, while the false negative alerts slowly increased from trials 2 to 5 but then started to show a decline. These results suggests that the dogs were becoming conditioned to a single compound (e.g. DNT or DNB) rather than a mixture of compounds as would typically be present in the real explosive. Although these VOCs are present in high abundance in the headspace of explosive training aids (see section 4.5.2) this curve shows that they are not the only VOCs used by the canines. Unfortunately, the trials did not continue past trial 6 to determine whether the decline in false positives continued with training exposure.

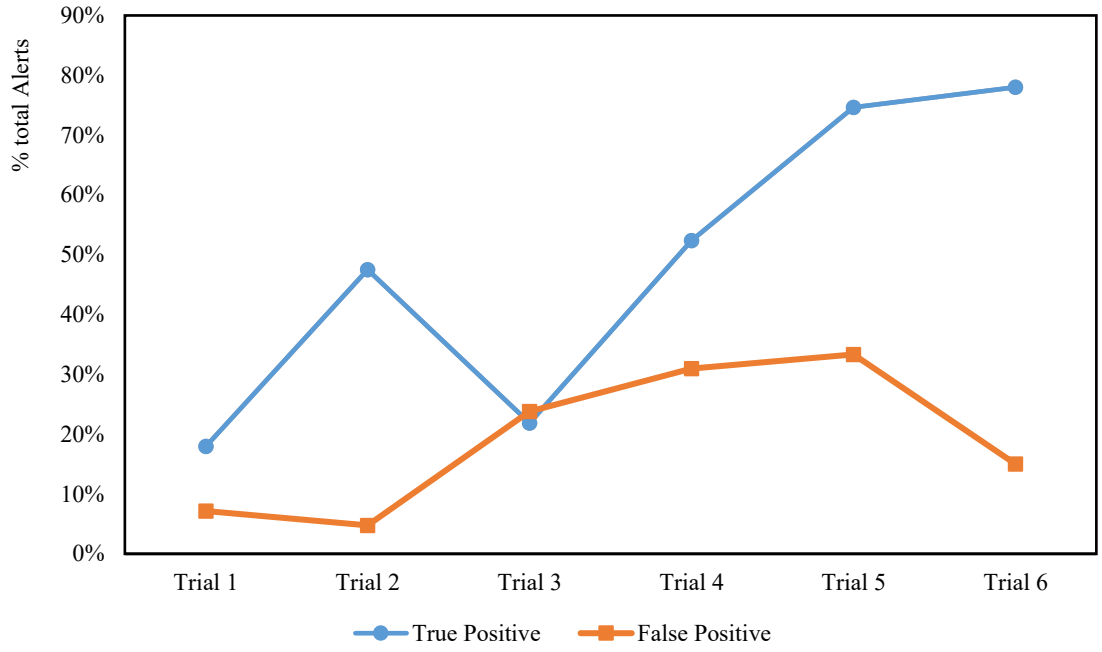

Figure 5-10: Percentage of true positive versus false positive alerts recorded for EDDs in the course of the six trials when exposed to dried samples of DNT, DNB and DMDNB, plus controls, blanks and distractors.

Evaluating the false positive responses in more detail (Figure 5-11), an increase in the alert rate for distractors, blank filter paper and acetonitrile control samples is evident, especially during the fourth and fifth trial. The control samples were prepared in order to analyse whether the dog was alerting to the target odour or to another unfamiliar scent in the training line-up, namely the acetonitrile solvent or the filter paper. The higher false positive alerts on the filter paper and acetonitrile confirm that at times the dogs were simply alerting to the new odour, since aluminium tins are already used in their training protocol.

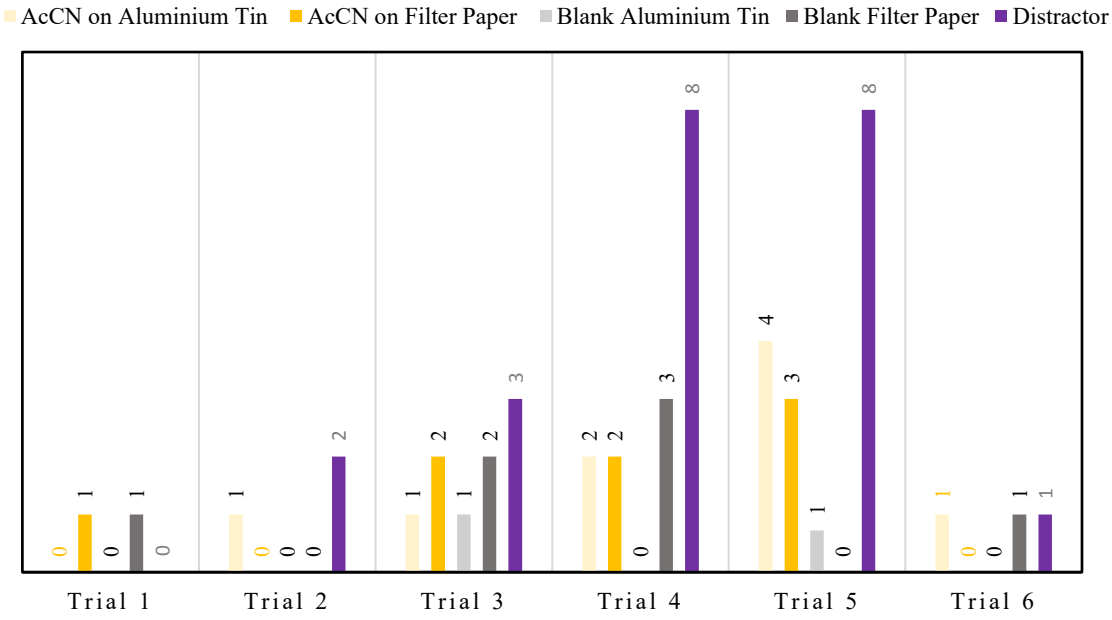

Figure 5-11: Total number of false positive alerts recorded for EDDs per trial when exposed to control samples.

The surprisingly high alert to distractor odours during the fourth and fifth trials were produced by the same dogs that yielded the highest true positive alert rates overall. Many of the false positive alerts by these two dogs on both days were to tin positions where the target odour had previously been. Although it is possible that there was residual scent at these locations, it is also possible that the dogs were attempting to alert at the same position whereby they were previously rewarded.

This highlights the importance of incorporating multiple control and blank sample odours into the training protocols to ensure that any associated background odours are recognised and differentiated, improving their selectivity to the target odour. Although an average false positive alert rate of 4 to 12 % is relatively low, it can mean that each dog is not selecting the specific target odour but rather the most volatile (or distinct) odour within the tin. Since the training sessions were conducted using a scent line-up in a confined room with tins in close proximity to each other, this could be a potential limitation for training protocols and that future studies should incorporate outdoor training scenarios where target samples can be placed over a larger distance without the potential for cross-contamination.

5.3.5 Dog Characteristics

Previous studies have suggested several factors that may influence positive alerts by EDDs, such as age, experience, breed, and previous type of training [79, 107, 153, 274]. In this study the attendance frequency of the canines had an obvious impact on their alert rate, as indicated by the learning curve presented previously. Table 5-3 shows a comparison between all canines that attended the trials according to their deployment experience, breed, gender and trial attendance. Type of training is an important characteristic to consider. EDDs can be trained with either real explosives or synthetic training aids or both, and this can alter the VOC mixture to which these canines are conditioned [107]. However type of training was not considered for comparison due to the fact that all canines present in this study had contact with both types of training aids at some stage in their training prior to these trials.

Table 5-3: Comparison between all canines according to the factors that may influence their response to the target odours

TEAM	ATTENDANCE (trials)	BREED	GENDER	EXPERIENCE (as at September 2018)
1	4	English Springer Spaniel	Male	59 months
2	6	English Springer Spaniel	Male	41 months
3	1	English Springer Spaniel	Male	71 months
4	2	Labrador	Male	42 months
5	4	Labrador	Female	40 months
6	4	Labrador	Female	11 months
7	2	Border Collie	Male	1 month

In order to compare experience, the teams were categorised into three groups depending on the dog's operational years of service. The first and youngest group incorporated two teams with 24 months or less of working experience; the second group was composed of mid-experienced canines, which incorporated three teams with at least 25 and a maximum of 48 months of deployment service; the third and most experienced group was represented by canines that had a least 49 months of operational service. Figure 5-12 shows the overall alert rate of true positives and false positives yielded by the EDDs per group, considering only the samples presented to these canines.

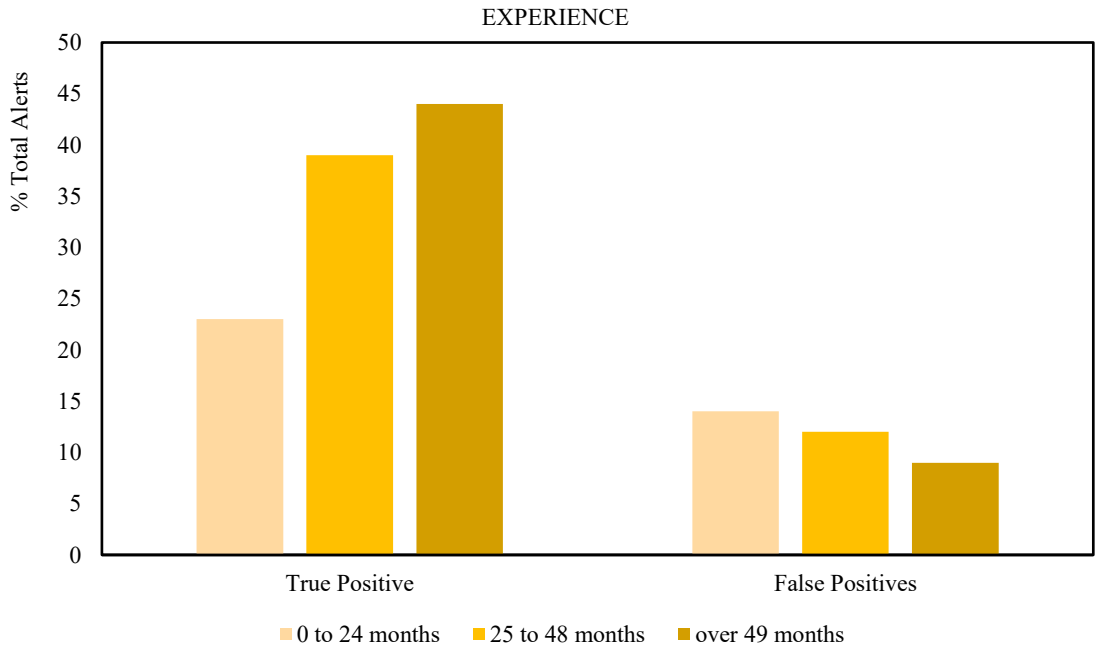

Figure 5-12: Percentage of true positive and false positive alerts recorded for the three target odours by EDDs divided by working experience into three groups.

The results demonstrate that canines with more field experience are more susceptible to true positives and less susceptible to false positives. Experienced EDDs are less likely to alert to new odours because of the diversity of their training exposure and deployment experience, therefore they are more adaptable to changes in the core VOC profile. It was also observed that the more experienced dogs would follow the line up more methodically than the younger dogs. The younger canines were more susceptive to show playful behaviour, to become distracted and frustrated, leading to a low true positive rate and highest false positive rate when compared to the other two groups.

For comparison of breeds, the teams were again separated into three groups. As shown in Figure 5-13 English Springer Spaniels produced the highest true positive alert rate. This group was composed of three canines, two of which represented the most experienced group. The Border Collie group had only one individual, which was the youngest and least experienced dog in the group. This may explain why this dog has the fewest true positives and highest false positives. The third group was the Labradors. This group was represented by young canines with a medium level of experience. Although this group did not have the highest true positive alert rate, it was the most constant group with the best ratio of true positives and false positives.

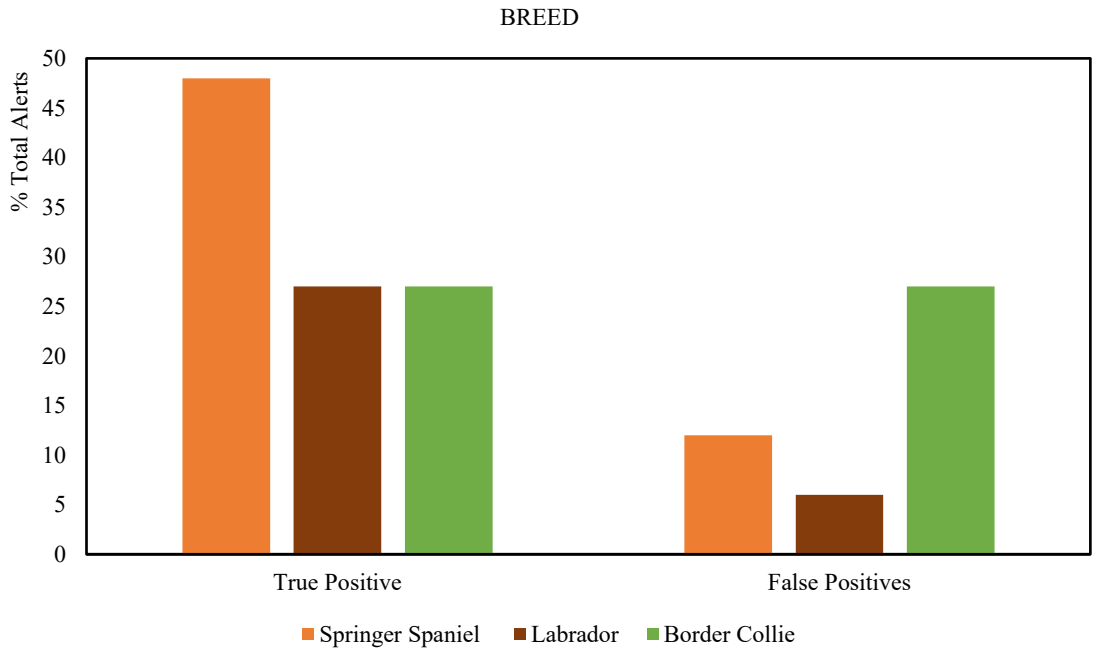

Figure 5-13: Percentage of true positive and false positive alerts recorded for the three target odours by EDDs divided into three groups by breed.

The teams were also compared by gender. The female group was composed of only two individuals, both Labradors in the young and mid-level experience age group. This is evident in the comparison between the true and false positive alert rates between the two groups, as can be seen in Figure 5-14.

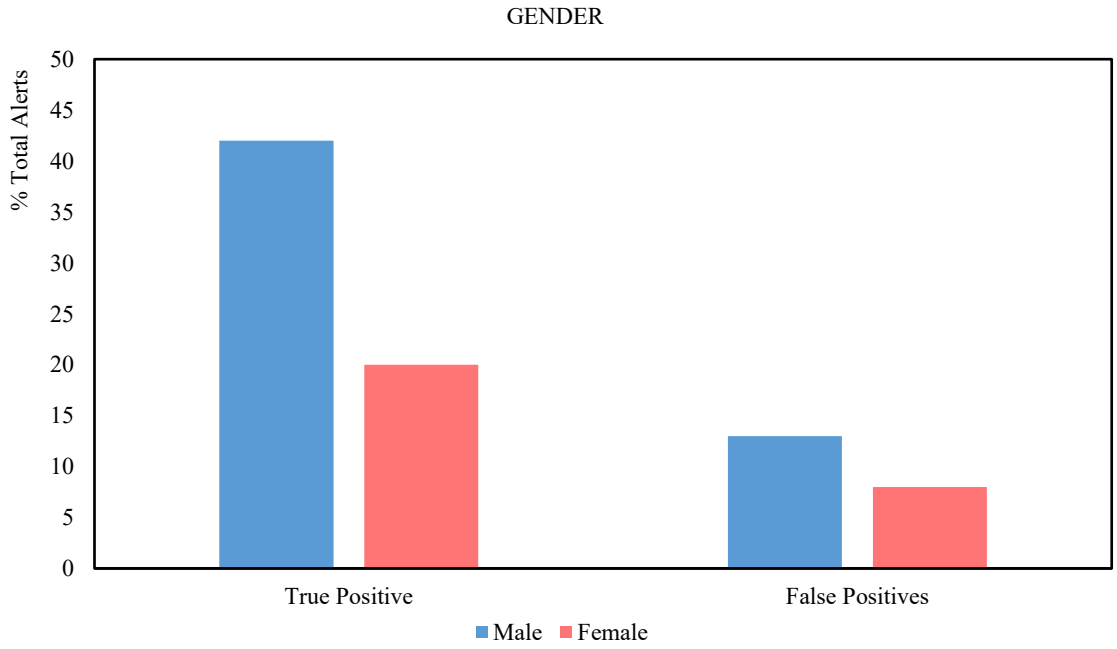

Figure 5-14: Percentage of true positive and false positive alerts recorded for the three target odours by EDDs divided into two groups based on gender.

However, this comparison was not meaningful for this study, since the male group included the most experienced canines with the exception of the Border Collie. Even though Labradors had the best ratio of true and false positive alert rates and the female group was composed of Labradors only, the Labrador that produced the best rate amongst the three was the most experienced male.

Ultimately the experience of the dog/handler team and the attendance at the trials appeared to have the greatest impact on true positive alerts. Figure 5-15 compares the teams that attended fewer than 3 trials with the teams that attended 3 or more trials. As expected following the learning curve described previously in this chapter, the canines that attended more trials demonstrated a higher percentage of true positive alerts.

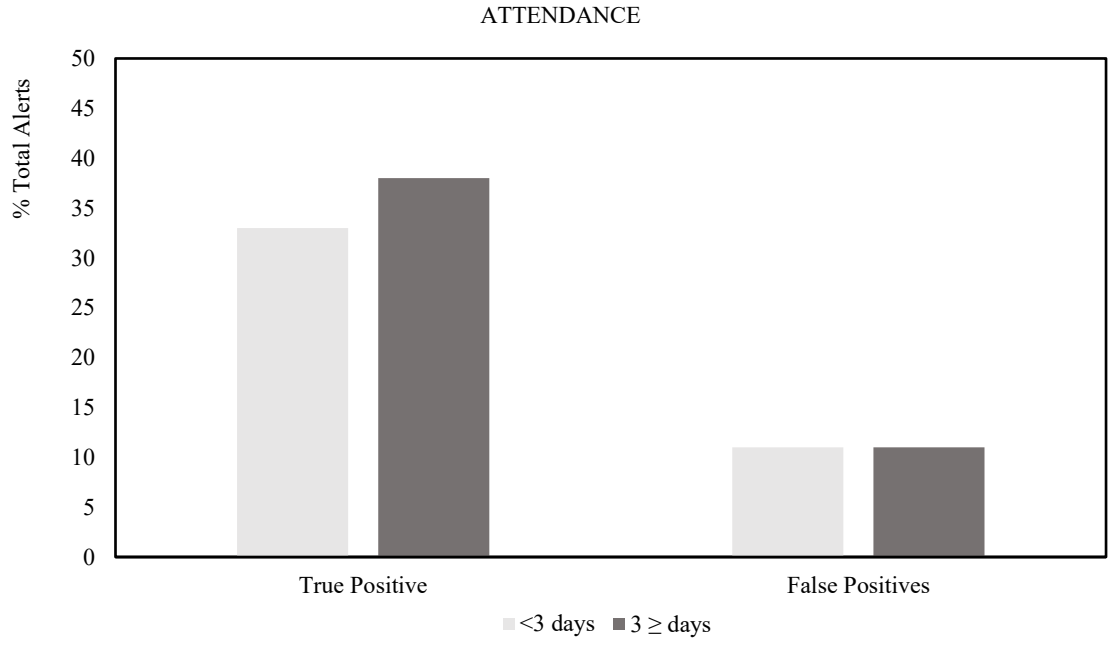

Figure 5-15: Percentage of true positive and false positive alerts recorded for the three target odours by EDDs based on attendance at trials.

The learning curve demonstrated by the dogs suggests that as they are presented with a more diverse range of unfamiliar scents, they can better associate and recognise the target odour. The teams that attended the fewest number of trials included a male Labrador which was the most consistent canine of the experiment, the Border Collie which was the least experienced canine in the experiment, and the older English Springer Spaniel which was the most experienced canine in the experiment. The group dynamic provides an

explanation for the results observed. While the most experienced and the most constant canines produced a good average for the true positive alerts, the least experienced dog had a poor average in contrast yielding more false positive alerts. The group with the highest attendance rate had more time to learn the target odours and alerted to the lowest concentration levels. Notably, both groups demonstrated the same number of false positive alerts.

Others factors that may influence the canines' alert rate are behaviour related. It has been reported in the literature that dogs are susceptible to boredom and frustration [164]. To avoid this behaviour, after successive runs with no alerts from the canines, real explosives were added to the scent line-up to trigger alerts so that the dogs were rewarded correctly. Each time a real explosive was included in the scent line-up all canines correctly alerted. It has also been documented that scent-detection dogs can be affected by their handlers beliefs and that each canine possesses a unique temperament that can influence their scenting abilities [275, 276]. It is therefore important to highlight that there is a large number of potential external influences that can affect these canines' performances, and future experiments should be conducted for a longer period with a larger number of EDD teams to help minimise these effects and build a clearer picture of their capabilities.

5.4 CONCLUSIONS

Explosive detection dogs have been widely regarded as the gold standard tool for explosive odour detection [2]. However there are currently insufficient studies that scientifically investigate the sensitivity and selectivity of EDDs. This study aimed to investigate the sensitivity and selectivity of EDDs to different concentrations of key VOCs found in the headspace of explosive training aids. This investigation had the goal to identify and confirm the key VOCs to which the dogs alerts and to compare the dogs' efficacy with highly sensitive analytical instrumentation (e.g. GC×GC-TOFMS).

5.4.1 Target Odour Analysis

Introducing single standards in the EDDs training procedure proved to be initially challenging for the dogs, however they appeared to be conditioned to the target odours as the training progressed. During this study DNT was the compound that elicited the highest number of positive responses, although it was clearly dependent on concentration. DNT is also a dominant VOC in powders, cast and plastic explosives. The results also highlighted the inefficiency of the mandatory plastic explosive taggant DMDNB, corroborating previous reports in the literature [231]. Although DMDNB can be a million times more volatile than most plastic explosives [18] it was the compound which yielded the lowest overall true positive alert rate from the canines. It may be advisable to add this sample during the dogs' training, as well as ratios of VOCs in low concentrations to enhance the canines' performance.

The results show that the target VOCs selected were not readily recognised by the dogs even though they have high positive alert rates to real explosives in their normal training procedures. Hence, the individual compound approach is not recommended for training EDDs and further research is required to accurately identify the key VOCs or ratio of VOCs that the dogs detect in explosive headspace. Future research should be conducted over an extended period of time with a broader range of VOCs, but it must also be considered than currently-available chemical detection methods are not optimal for discovering these VOCs.

5.4.2 Detection Limits

The canines' response to concentrations between 0.01 µg and 10 µg proved to be efficient. The EDDs produced a consistent overall response of over 60 % for 0.01 µg and over 70 % for all other concentrations presented during training. As the training progressed, the alert rate for the canines increased together with their sensitivity. When comparing the number of alerts with the concentrations presented between the first and last trials, it is evident why EDDs are regarded as the gold standard for explosive vapour detection. While the first trial yielded zero alerts to 1 µg and a low rate of alerts for 10 µg of odour, by the last trial all canines displayed an alert rate of over 61% for 0.01 µg of the target odours, while searching forty eight samples in under ninety seconds (disregarding the play time for reward).

Compared to highly sensitive bench top instruments such as GC×GC-TOFMS, the dogs' limit of detection for individual compounds in the headspace is not the same as the low ppb levels demonstrated by this instrument. However it is important to note that the application of a GC×GC-TOFMS instrument for field analysis is far from realistic due to its size and operation. Moreover a lengthy pre-concentration process with the use of HS-SPME extraction has to be conducted to perform the analysis. The minimum total time of analysis per sample achieved for this technique in this project was twenty one minutes (15 min pre concentration plus 6 minutes analysis). In comparison, the canines could learn to detect from 10 µg down to 10 ng in just six days for singular compounds. This comparison can only be made with single VOC standards, which initially were challenging for the dogs. If it was possible to measure the concentration of real explosive VOCs in real life scenarios, the EDD limit of detection would likely be far superior to most available analytical instrumentation.

5.4.3 Substrate

An aluminium tin and a 90 mm grade 1 paper filter were chosen as non-porous and porous substrates, respectively. The aluminium tin was already used in the training process of EDDs by the NSWPDU and for that reason was chosen as the non-porous substrate. Filter paper has been reported before in the literature and is a commonly used porous substrate [107], hence its inclusion in the trials. Aluminium tin proved to be a more efficient substrate as it yielded an overall higher rate of true positive alerts while the blank filter paper yield a higher number of false positive alerts. This demonstrates that the filter paper

may supress the target odours and also may promote the response of the dogs to an unfamiliar scent.

5.4.4 Dog Responses

The analysis of the true and false positive responses during the trials suggest a learning curve to the introduced target odours. The first two trials resulted in very low rates of positive alerts overall whereas the subsequent trials displayed an increase in the true positive alerts culminating in the highest values during the last trial. The false positive alerts followed a similar trend, a low initial rate followed by an increase with time. However the false positive alert rate reached its peak during the fourth trial and started to decline thereafter. More trials are necessary to evaluate if the decrease was an anomaly or whether the dogs showed a consistent reduction in false positive alerts with additional exposure to the odours.

The results achieved during the EDD trials and GC×GC-TOF-MS analysis indicate that a better choice of training aids may be needed. The canines may not need to be trained on as many plasticized explosives as typically employed in canine training programs, as these compounds have similar headspace odour signatures with a high abundance of plasticizers and DNT. Compounds such as diphenylamine and 2-ethyl-1-hexanol, have been previously studied in the literature [107, 231]. These compounds are common additives to plastic explosives and are two orders of magnitude more volatile than the common parent compound used in these samples (i.e. RDX and PETN) [107]. Due to their volatility and common use, both diphenylamine and 2-Ethyl-hexanol are readily found in the headspace of explosives and may present an important indicator for dog alerts. The results suggest that identifying one or several target VOCs that adequately represent the hundreds of explosives compounds and mixtures is unlikely. Therefore, incorporating real explosives with the range of VOCs related to explosives (plasticizers, binders and stabilizers) during training will assist in generalising these canines to the correct odours and improve their chance of success in operational scenarios. As shown in a previous study it is important to vary the odour on which canines are trained [277], and further research should incorporate additives as target compounds in more extensive trials.

5.4.5 Dog characteristics

Different factors were taken into consideration when assessing the dogs' responses, such as previous work experience, breed, gender and attendance at the trials. It was evident that no factor can be evaluated separately as multiple factors are present concomitantly. The results show that more experienced canines can adapt easier to unfamiliar or unrecognized scents, they are more prone to focus on their work and less susceptible to distractions and frustration, leading to higher true positive responses. The younger canines were more playful and more prone to distractions (personal observations) leading to a higher number of false positive alerts. The gender comparison was not considered meaningful due to the small female sample size and experience of the male dogs. English Springer Spaniels were the breed with the highest success rate but was also the group that had the most experienced canines and the highest attendance rate. Attendance rate proved to be an important factor, especially when concentration values were assessed. Canines' that attended more trials had higher true positive alert rates overall and showed improved sensitivity.

This study was conducted over a short period of time and it was not possible to compare long term increases for younger and more experienced canines. A longer study would be beneficial to reveal further trends in the findings and should include more EDD teams and different odour samples aiming to identify the priority VOCs of both high and low explosives. Trials should also be conducted in an open environment scenario to better assess the dogs' responses in a real life event with different distractor odours and varying environmental conditions.

A recent review stated that despite the broadly promising results of detection dogs they present several variations in response and effectiveness that can be challenging in operational deployment. Those challenges expose a need for further research to establish the effective limits of a dog's performance [278]. Although the results of this study did not identify the key VOCs to which the dogs alert, their mobility and independent thinking combined with their speed and sensitivity still places them as the preferred method for real-time detection of explosives. The shortcomings of EDDs can be diminished through ongoing training and additional research, such as identifying the variants of explosive odours that will yield optimal effectiveness for EDD training.

Chapter 6: CONCLUSIONS

Chapter 6: CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE WORK

This project focused on the identification of the key VOCs present in the headspace of explosives using both chemical and biological detection methods. Discovering these compounds and improving real time detection methods for explosives are imperative given the constant threat of terrorism and the presence of risk areas such as clandestine laboratories and demilitarized areas.

Chapter 2 proposed the development of a novel remote-controlled detection system for explosives with an original platform, which to the best of our knowledge has not been researched before for this purpose. The analysis of explosives with microchip-CE and an oscilometric detector although promising, was unable to detect neutral molecules such as explosives and thus, was not deemed suitable for the aims of this study. Future research may be conducted with this platform for the analysis of post blast residues as this detector has already being successfully used with this intent and the proposed separation technique is known to be suited for ions analysis.

Chapter 3 evaluated and compared a promising new technique for the detection of explosive residues in vapour samples and an already established technique used for field deployment and real-life scenarios. The promising technique was a LOC instrument which comprised of a microchip-CE separation method combined with UV detection system. A simple method for the pre-concentration of explosive vapours was developed using a 2 mL glass vial and a 0.5 m filter paper chad. This method was able to detect and quantify vapour emitted from explosive standards after evaporation with detection limits up to 6 ng and recovery rates up to 75 %. The explosives were detected through fluorescence quenching and separation occurred in under 20 seconds. Although the use of a filter paper to collect explosives residues in the headspace provides an inexpensive approach for the detection of explosives, it did not approximate a realistic scenario in terms of screening air for explosive VOCs. Future developments should focus on creating a platform that eliminates the need for the pre-concentration step and can constantly perform sampling of air and direct analysis. The next steps should focus on improving the detection limits of the instrument, optimizing the extraction procedure, determining the system capabilities and extracting air samples from real explosives.

The established platform investigated in this study was a military grade transportable GC-MS. This instrument was tested for its marketed capabilities and successfully separated and detected seven explosives from different classes with limits of detection at the ppb level. Although it performed as expected for liquid injections, the series of problems which occurred following the first analysis made it impossible to continue with vapour analysis and field trials. The instrument is marketed as 'portable', however its portability is not practical as it needs at least two people to transport, and requires both an external power and gas source. Multiple software failures and time consumed during equilibration and start up, added to the overall difficulty to troubleshoot simple issues. As a result, it was concluded that this particular instrument is not ready for deployment as advertised. It was later noted that the GRIFFIN 450 ™ was discontinued. Future work should investigate the newly released version of this instrument when available to test its capabilities against those that are marketed.

Chapter 4 investigated the VOCs present in the headspace of liquid standards of explosives and real explosives used as training aids by law enforcement agencies. Two published methods known for their separation capabilities and limits of detection were coupled to HS-SPME extraction to achieve a full chemical profiling of the key VOCs.

HS-SPME-GC-MS proved to be a robust method; achieving the separation of seven explosive related compounds with proven ppb levels of detection. However, the use of HS-SPME-GC×GC-TOFMS led to the separation of eighteen explosive related compounds producing an order of magnitude lower than that achieved by the one dimensional GC analysis. When both techniques were used for the chemical profiling of VOCs from real explosives it was evident that the second dimension of the GC×GC analysis demonstrates a powerful tool for the separation of complex mixtures. The two-dimensional GC method successfully detected and identified several compounds which were not detected using 1D GC-MS; including the separation of nitroaromatics of similar molecular structure which were potentially co-eluting in the previous method. The most abundant compounds found overall were taggants and plasticizers, however since this project focused on the profiling of compounds exclusive to explosives in order to better understand the VOCs to which EDDs alert, the analytes chosen for the next study were the three most abundant compounds exclusive to the samples analysed. These compounds were consistent for both GC-MS and GC×GC-TOFMS.

Chapter 5 reported on the experiments conducted with EDDs from the NSWPDU, in which three dried chemical standards (DNT, DNB and DMDNB), representative of the most abundant compounds exclusive to explosives found in the headspace of EDD training aids, were presented to the dogs. The experiment demonstrated a learning curve by the canines over the course of the trials, initially showing a very poor positive alert rate at the commencement of the trials but increasing with exposure and time. As the trial progressed, the true positive alert rates increased. The false positive alert rates also increased but then declined during the final two trials. Collectively, these findings suggest that the canines were learning the target odour with increased exposure. The results demonstrated that EDDs tend to alert more to DNT when compared to the other two compounds. Although no significant difference was found, the overall alert rate for DNT was the highest observed, followed by DNB and DMDNB. As the trials progressed the disparity between the response per chemical became smaller, another indication that the canines were learning to select the target odours. The canines were exposed to concentrations of 0.01, 0.1, 1 and 10 μg of the target odours. Comparing the responses given in the last trial, when the EDDs were more familiar to the target odours, the highest and smallest concentration had an overall difference of only 10 %, indicating that the canines can consistently detect to concentrations lower than 0.01 μg. The substrate employed to dry the target odours (aluminium tin – non-porous, and filter paper – porous) did not significantly affect the alert rates. However the overall response was higher for odours dried directly into the aluminium tin, while and the blank filter paper elicited several false positive alerts. These findings indicate that filter paper is not an appropriate substrate for field trials. Several individual factors were assessed with experience and attendance having the greatest influence on the dogs' alert rate. The learning curve presented by the canines, the time required to search all 48 samples per trial and the consistency in alerting to concentrations of 10 ng indicate that to date no analytical instrument is on par to the canines speed, sensitivity and selectivity. Future research should focus on including different compounds found in the headspace, not only those exclusive to explosives as well as different ratios of compounds to test the theory that dogs alert to a mix of parent and non-parent compounds. Additionally, increasing the length of the trials is a necessary requirement to determine whether the learning curve is acquired and used in field scenarios or only demonstrated during controlled training sessions when the dog is being rewarded. These future directions will build a sound

foundation for the development of accurate training aids and will assist in the validation of canines for court representation.

In conclusion, the results from these studies have identified variations in the detectable VOC profile present in the headspace of explosives through chemical detection. These variations raised questions about which VOCs should be targeted when developing a fast screening method for portable or benchtop instrument and whether they represent the same VOCs that are detected by EDDs. The link between the chemical and biological detection tools remains intact as it is increasingly evident that these tools must complement each other. However, considerable research still needs to be carried out to better understand both detection systems. While many obstacles were encountered throughout this study, ultimately the research described in this thesis provides a robust framework for a better understanding of explosives VOCs and the types of detection methods that may be more or less suitable for their analysis. Based on these findings, the GC×GC-TOFMS system is recommended as the most advanced and suitable chemical detection method for subsequent characterisation of explosive VOCs, while the EDDs still represent the most advanced and suitable biological detection method for rapid screening and explosive detection.

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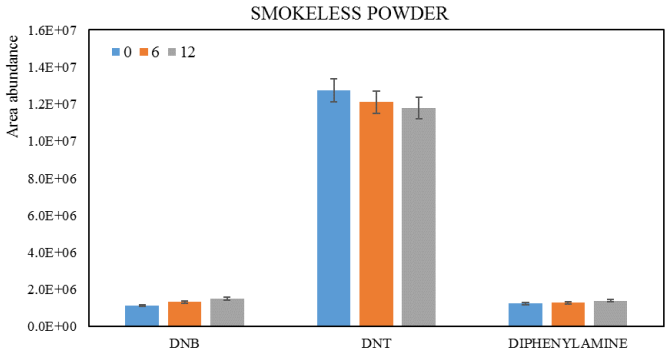
APPENDICES

Appendix A:

Supporting information for the degradation studies presented at Section 4.3.2.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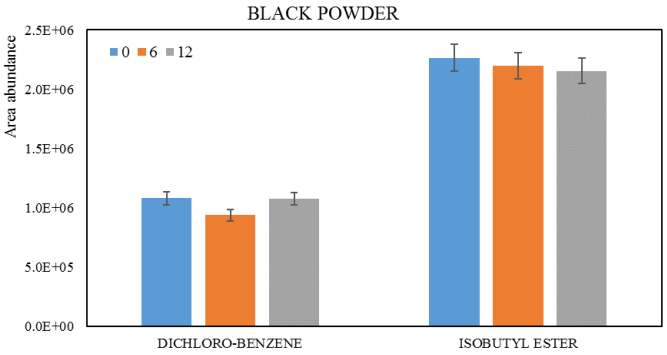
DNB
DNT
DIPHENYLAMINE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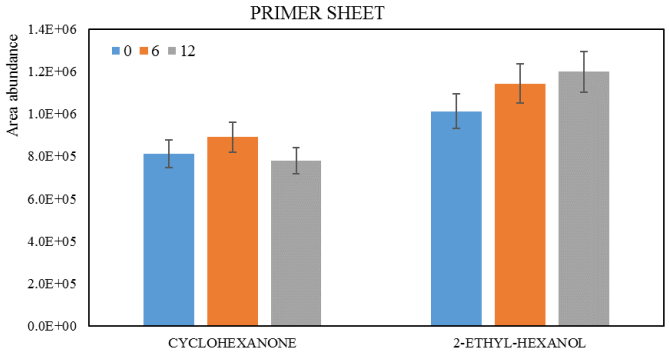
ISOBUTYL ESTER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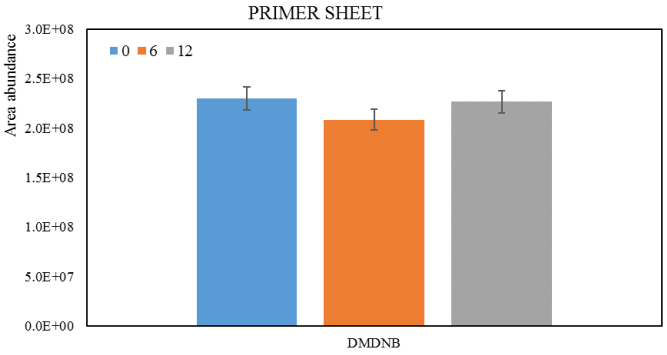
2-ETHYL-HEXANOL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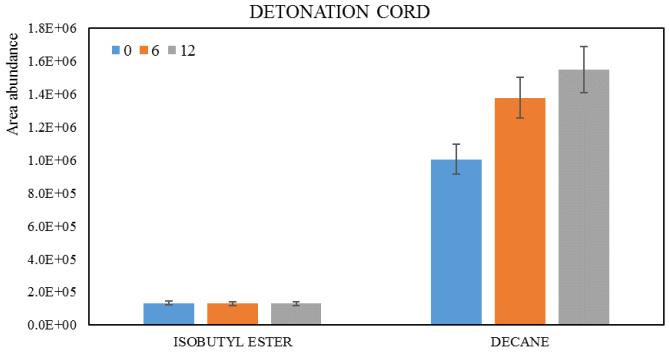
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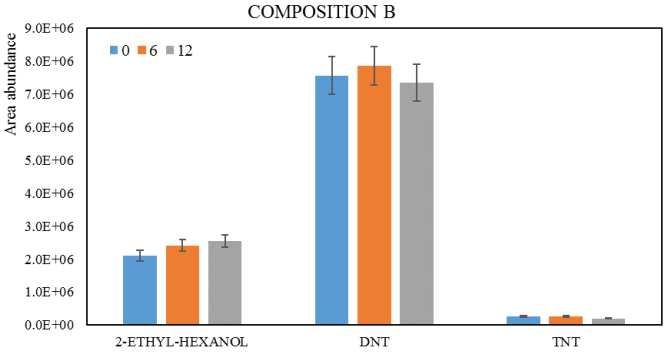
ISOBUTYL ESTER
DECANE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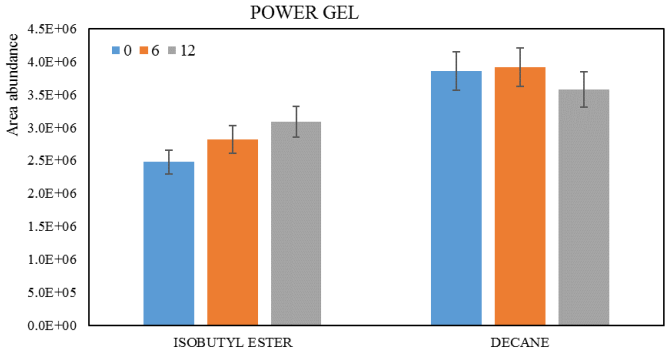
DNT
TNT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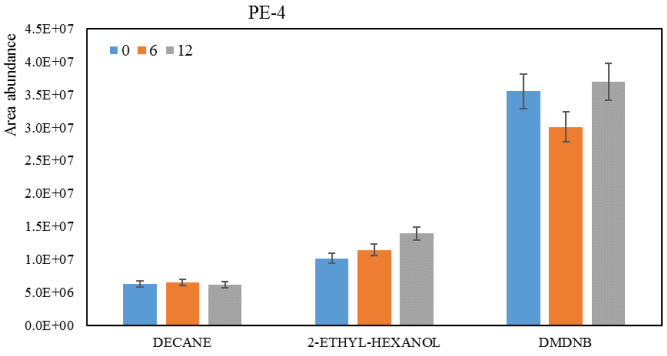
ISOBUTYL ESTER
DECANE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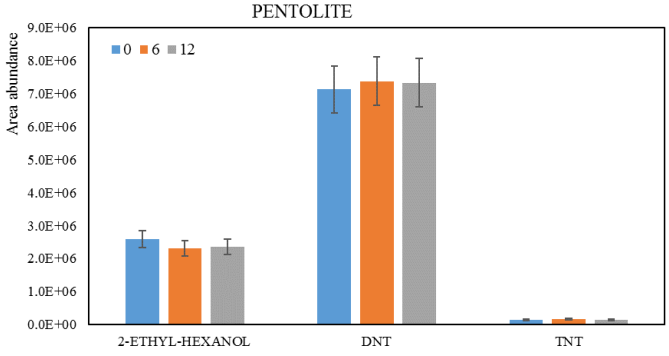
DECANE
2-ETHYL-HEXANOL
DMDNB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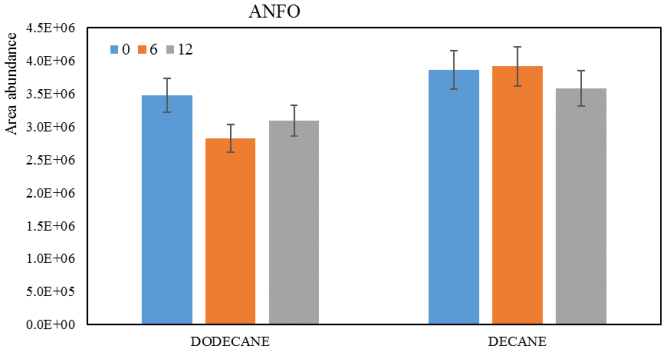
2-ETHYL-HEXANOL
DNT
TNT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1.0E+06
5.0E+05
0.0E+00
DODECANE
DECANE

Figure A-1: Individualized degradation study for all nine explosive training aids analysed with HS-SPME-GC-MS. The two or three most abundant VOCs per sample that have been consistently observed through the one year analysis were chosen and compared for their degradation over time. HS-SPME extraction conducted in triplicates with one PDMS and one PDMS/DVB fibres at room temperature for 30 minutes. GC separation was conducted with a 25 m x DB-5MS UI column with 0.25 mm diameter and 0.25 µm film thickness. Inlet was held at 220 °C for a 5 min desorption. The oven program started at 40 °C for 5 min followed by a 10 °C.min-1 ramp to 280 °C for 1 min. The injection was conducted in splitless mode. The carrier gas was helium at 1.0 mL.min-1.

Appendix B:

Supporting information for the limits of detection presented on Section 4.4.2.3 and Section 4.5.2.2.

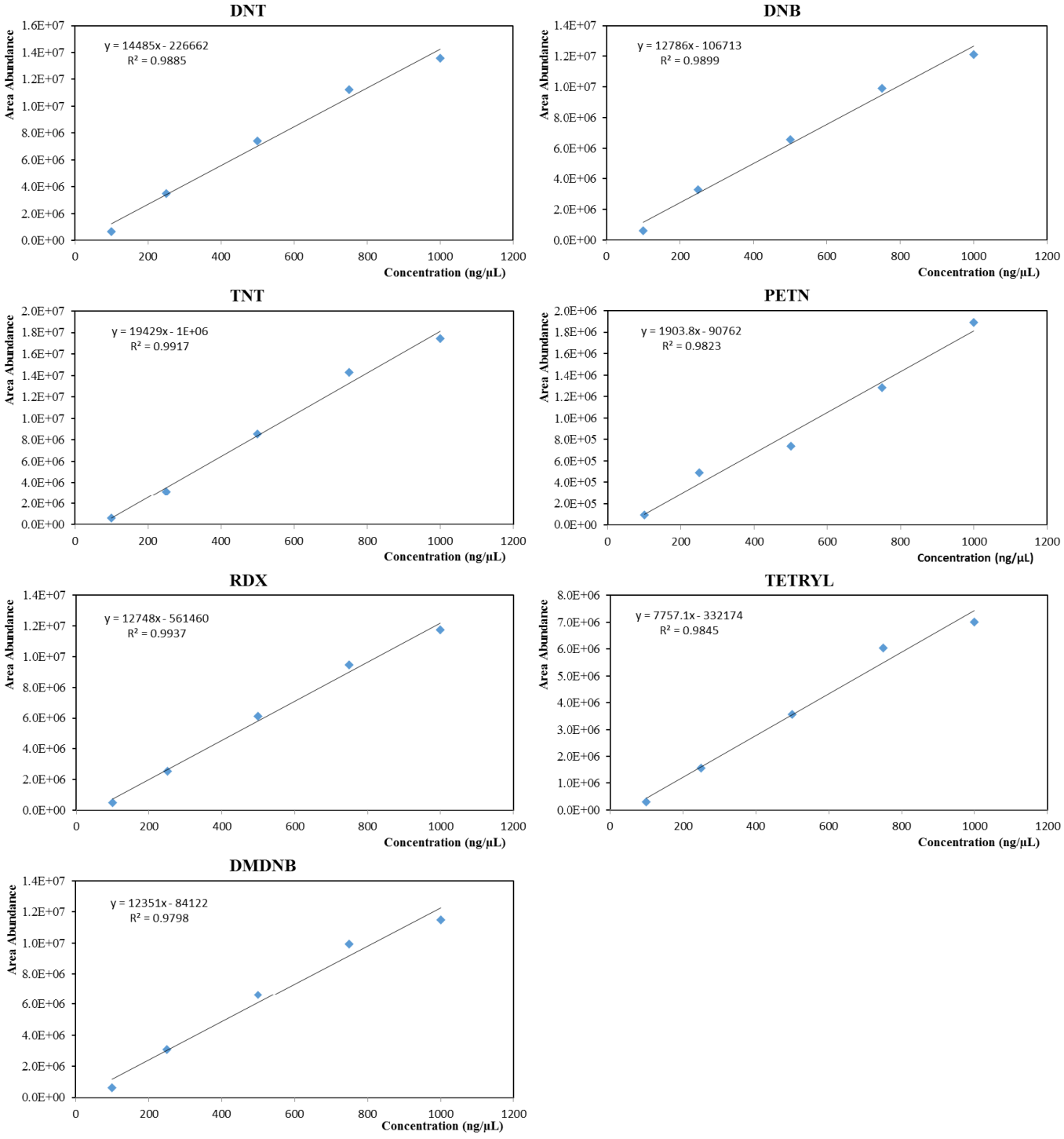
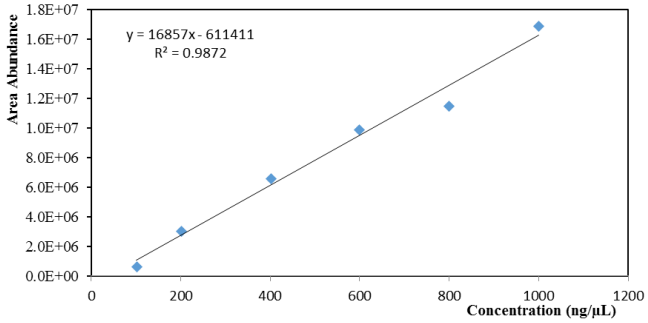
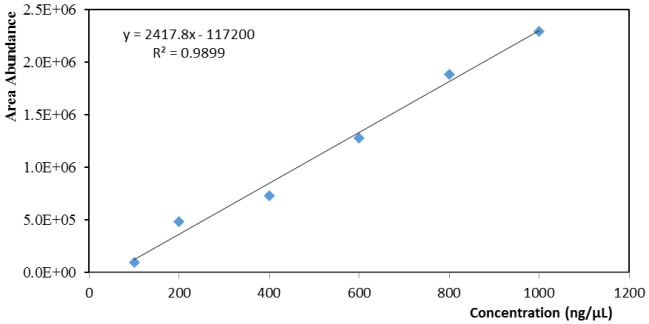


Figure B-1: Calibration curves calculated for the HS-SPME-GC-MS analysis of seven dried liquid standards. Triplicate measurements of five points at 100, 250, 500, 750 and 1000 ppm were taken with the use of a PDMS 100 µm fibre for 30 min at room temperature. Analysis performed under fast chromatography conditions.

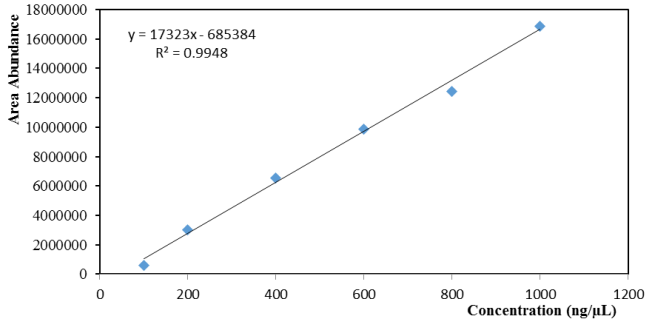
NITROGLYCERIN



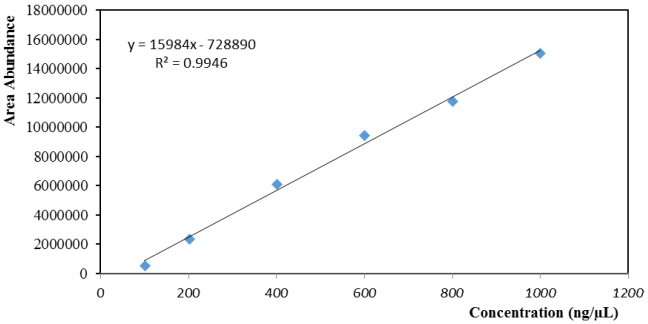
PETN



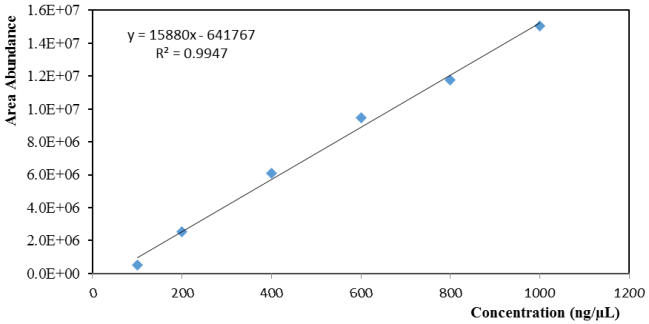
NITROBENZENE



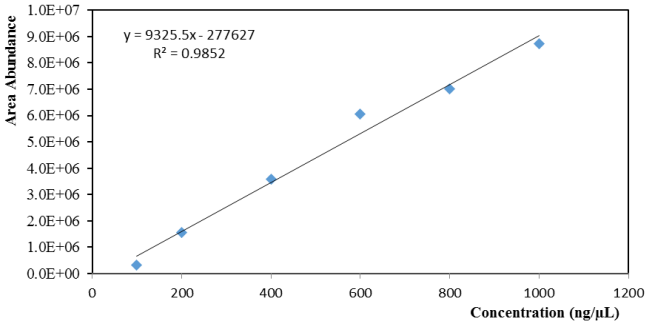
HMX



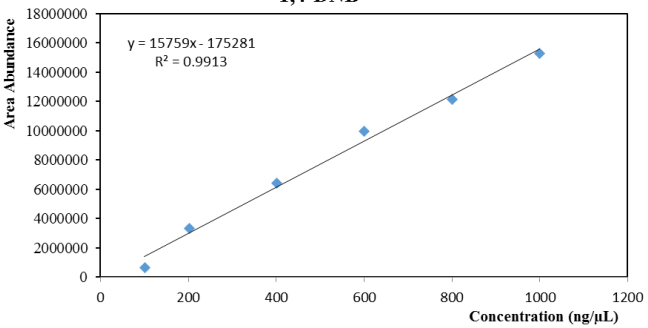
RDX



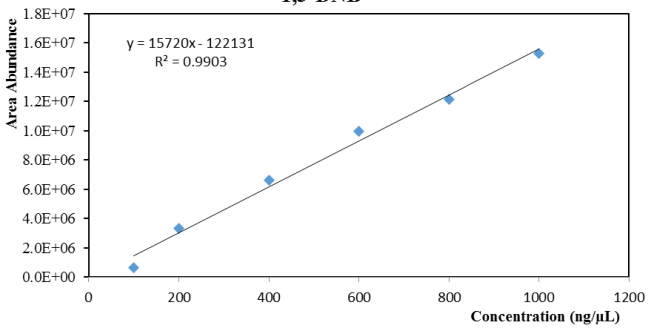
TETRYL



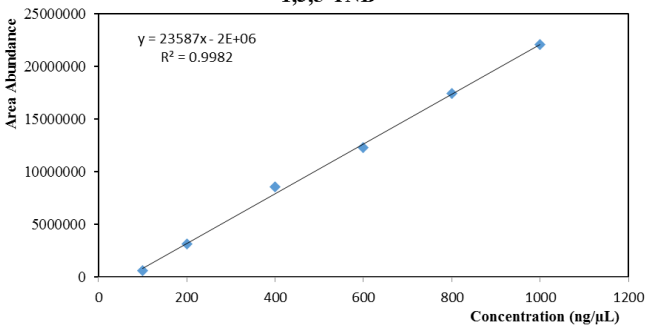
1,4-DNB

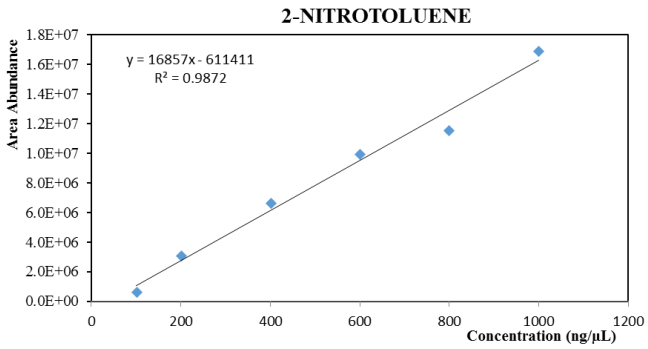


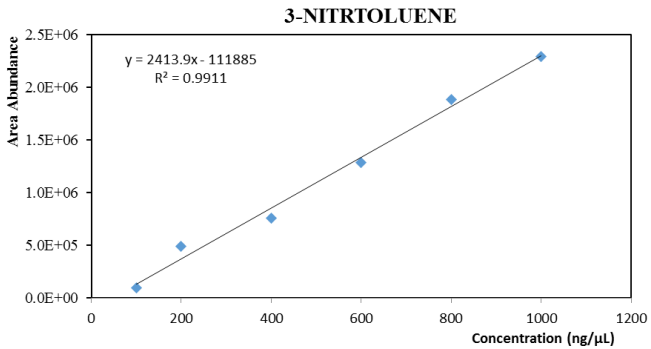
1,3-DNB

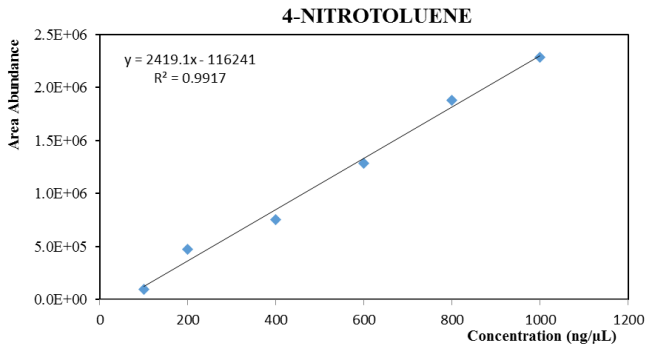


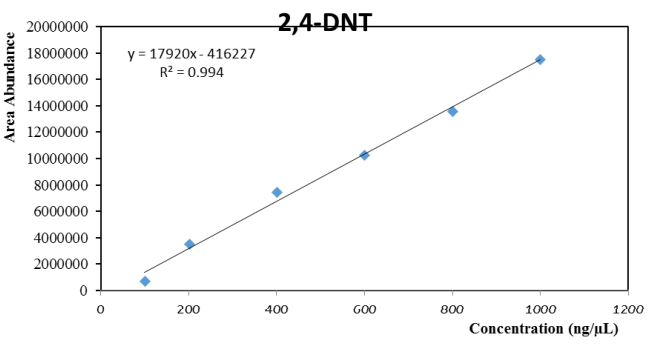
1,3,5-TNB

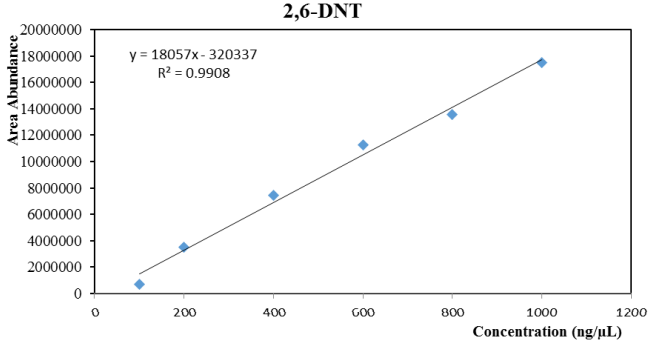


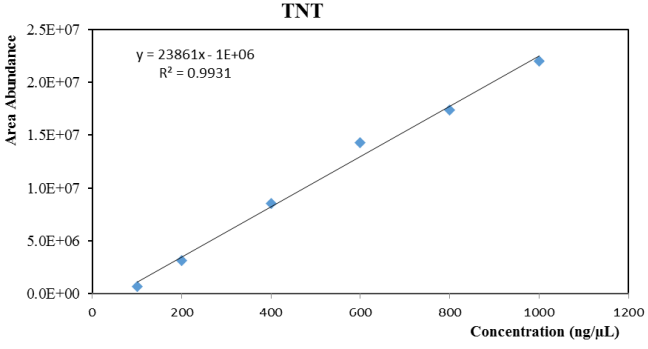

2-NITROTOLUENE
y = 16857x - 611411
R² = 0.9872
Area Abundance
Concentration (ng/µL)

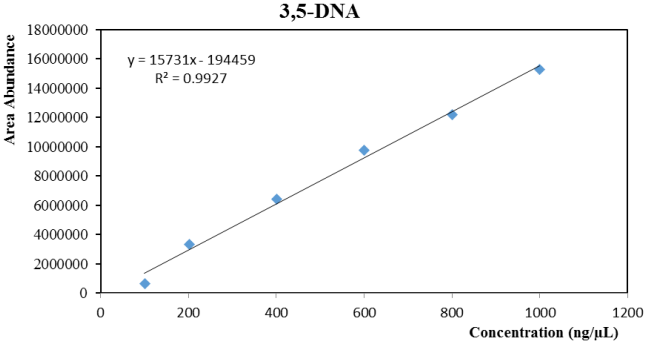

3-NITRTOLUENE
y = 2413.9x - 111885
R² = 0.9911
Area Abundance
Concentration (ng/µL)

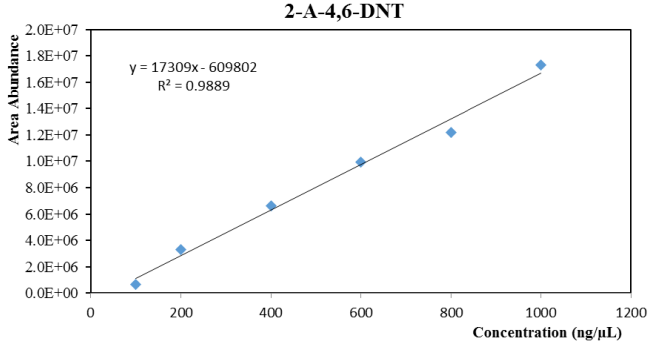

4-NITROTOLUENE
y = 2419.1x - 116241
R² = 0.9917
Area Abundance
Concentration (ng/µL)


2,4-DNT
y = 17920x - 416227
R² = 0.994
Area Abundance
Concentration (ng/µL)


2,6-DNT
y = 18057x - 320337
R² = 0.9908
Area Abundance
Concentration (ng/µL)


TNT
y = 23861x - 1E+06
R² = 0.9931
Area Abundance
Concentration (ng/µL)


3,5-DNA
y = 15731x - 194459
R² = 0.9927
Area Abundance
Concentration (ng/µL)


2-A-4,6-DNT
y = 17309x - 609802
R² = 0.9889
Area Abundance
Concentration (ng/µL)

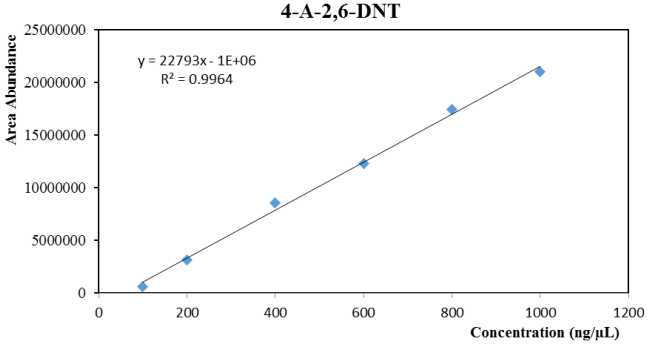

4-A-2,6-DNT
y = 22793x - 1E+06
R² = 0.9964
Area Abundance
Concentration (ng/µL)

Figure B-2: Calibration curves calculated for the HS-SPME-GC×GC-TOFMS analysis of one liquid standards containing all the explosives for the EPA Method 8330B. Triplicate measurements of six points at 100, 200, 400, 600, 800 and 1000 ppm were taken with the use of a PDMS 100 μm fibre for 30 min at room temperature. Analysis performed under fast chromatography conditions.

Appendix C:

Supporting information for the PCA analysis presented on Section 4.6.4.

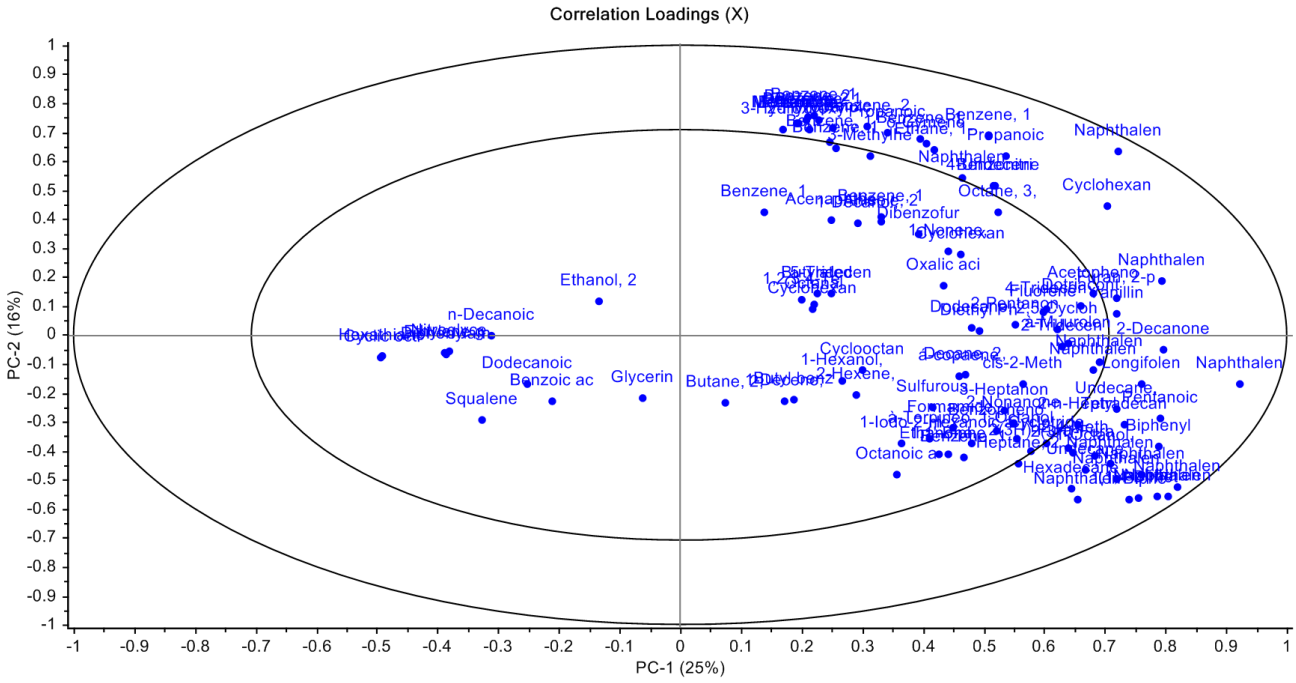
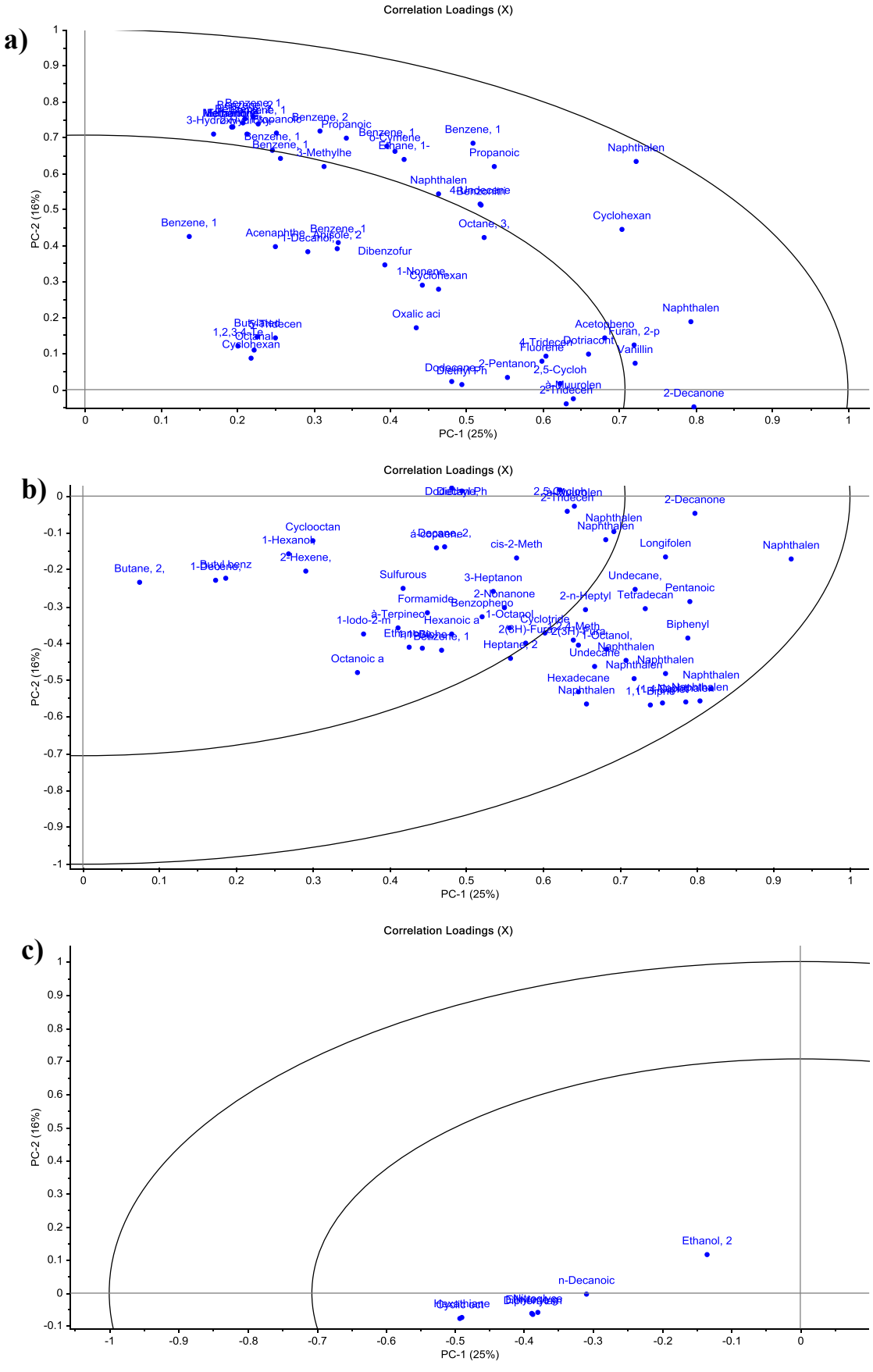


Figure C-1: Full view of the correlation loading plot achieved for the PCA analysis of eight explosive training aids analysed by HS-SPME-GC×GC-TOFMS using fast chromatography conditions. The matched scores were defined as statistically significant by an F-crit value calculated to be 2.69.


a)
Correlation Loadings (X)
PC-2 (16%)
PC-1 (25%)
Benzene, 1
Propanoic
Naphthalen
Cyclohexan
Octane, 3,
Dibenzofur
1-Nonene
Oxalic aci
Acetophene
Furan, 2-p
Vanillin
2-Decanone
b)
Correlation Loadings (X)
PC-2 (16%)
PC-1 (25%)
2-Decanone
Naphthalen
Cyclooctan
1-Hexanol
2-Hexene,
cis-2-Meth
Longifolen
Butane, 2,
Sulfurous
3-Heptanon
Undecane,
Pentanoic
Formamide
2-Nonanone
2-n-Heptyl
Tetradecan
Benzopheno
1-Octanol
Hexanoic a
Biphenyl
Heptane, 2
Undecane
Octanoic a
Hexadecane
c)
Correlation Loadings (X)
PC-2 (16%)
PC-1 (25%)
Ethanol, 2
n-Decanoic

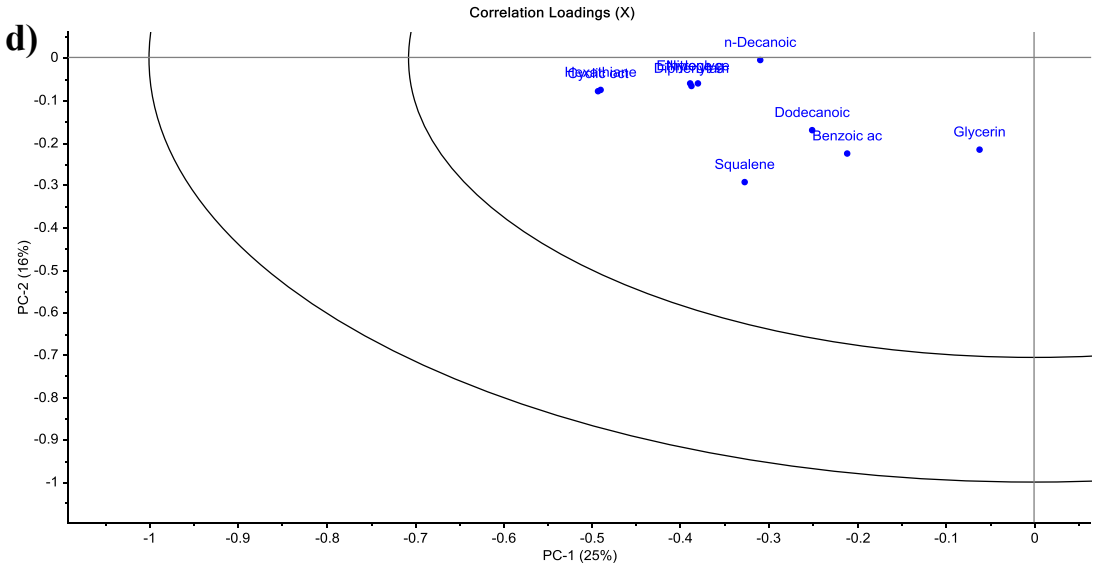


Figure C-2: Correlation plot of the PCA analysis of eight explosive training aids analysed by HS-SPME-GC×GC-TOFMS using fast chromatography conditions. The matched scores were defined as statistically significant by an F-crit value calculated to be 2.69. The plot was zoomed per quart for a better view of the score. a) shows the zoomed section for the top right quart of the correlation loading plot; b) shows the zoomed section for the bottom right quart of the correlation loading plot; c) shows the zoomed section of the top left quart of the correlation loading plot; d) shows the zoomed section of the bottom left quart of the correlation loading plot.

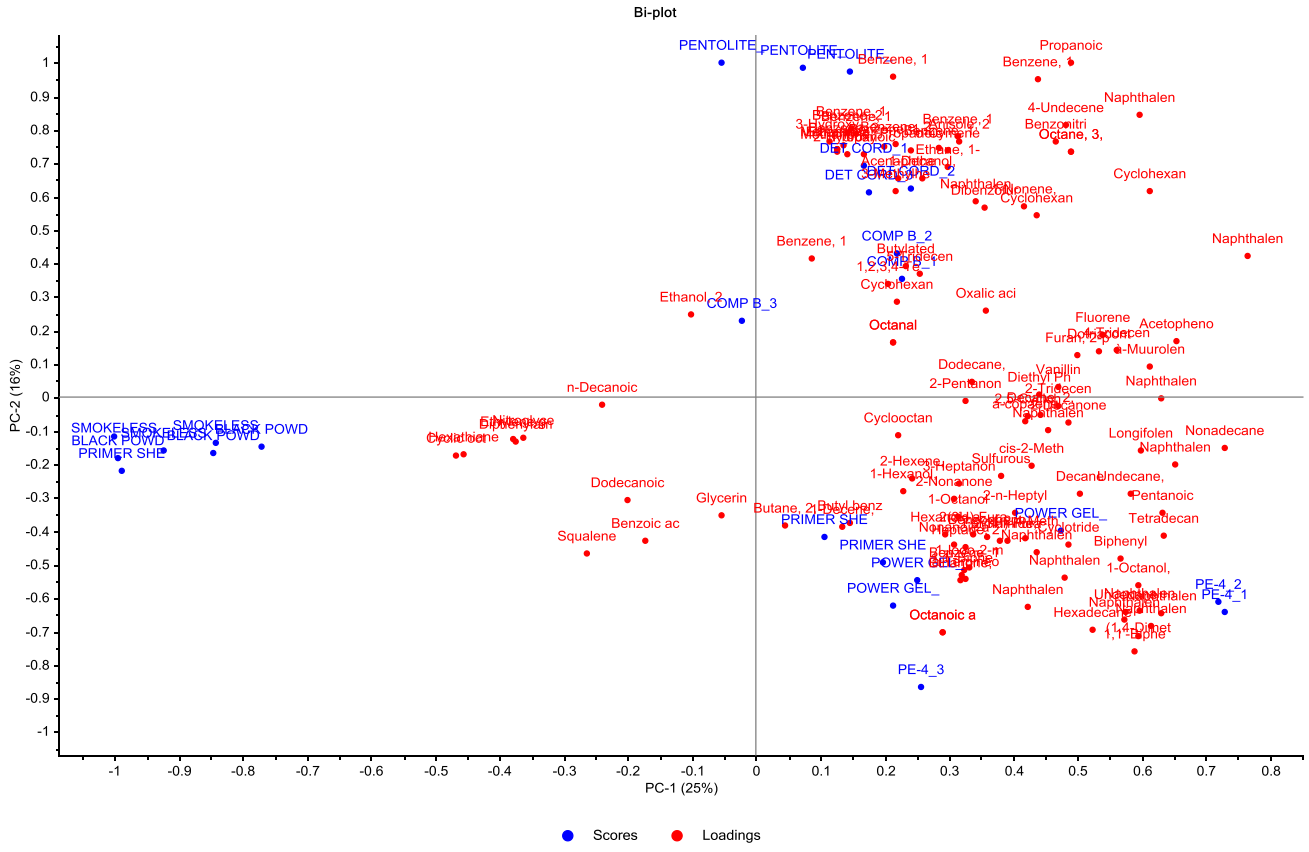


Figure C-3: Presentation of the Bi-plot achieved after the PCA analysis of eight explosive training aids analysed by HS-SPME-GC×GC-TOFMS using fast chromatography conditions. The plots shows the between scores and samples. The matched scores were defined as statistically significant by an F-crit value calculated to be 2.69.