A STUDY OF THE POTENTIAL EVIDENTIAL VALUE OF PERFUMES, ANTIPERSPIRANTS AND DEODORANTS IN FORENSIC SCIENCE

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Abstract

Perfumes and other fragranced products are abundant in our environment and are therefore likely to be abundant in a crime scene environment. They have properties which make them ideally suited to chemical detection and analysis but are currently underutilised as a potential source of evidence and intelligence. This work provides evidence supporting the hypothesis that such products have the potential to be forensically useful when analysed using modern analytical instrumentation.

Gas Chromatography (GC), Fourier Transform Infrared Spectroscopy (FTIR) and High Performance Liquid Chromatography (HPLC) were each evaluated for their ability to distinguish between perfumes, deodorants and antiperspirants.
GC analysis proved to be straightforward and provided sufficient detail to distinguish between products using visual pattern matching and statistical tools such as principal component analysis. FTIR was also able to discriminate between products with some success but it was felt that HPLC produced results with insufficient product detail to distinguish between perfumes.

Using GC as the primary analytical technique, further experiments explored the most appropriate ways to store samples, recover liquid deposits from a crime scene and analyse a suspect or victim’s garments. It was also demonstrated that the change in composition of perfumes with evaporation follows a predictable pattern with forensically significant implications. This research has also established vital groundwork for future study into individual chemical profiles and lifestyle indicators.

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Contents

List of figures

List of Tables

1 Introduction

Perfumes, antiperspirants and deodorants are used on a daily basis by millions of people (Duckett, 2015, Libby, 2015). They are specifically marketed on the basis of their aroma, which is formulated to be easily detectable, distinctive and persistent. The fragrance mixture used to create the aroma of these products is a carefully-controlled blend of fifty to a hundred different volatile organic compounds (VOCs) chosen from a range of over 3,000 aroma chemicals available to the perfumer (IFRA, 2015b). Perfumes, antiperspirants and deodorants are formulated to release their odour gradually over 6-8 hours (Salvador and Chisvert, 2007, Sanchez-Prado *et al*., 2011b), with some antiperspirants now formulated to be effective and maintain their distinctive scent for up to 48 hours (Unilever, 2015).

The number of fragrances on the market is estimated to be over 16,000 (Edwards, 2015) and 1620 new perfumes were launched in 2014 alone (Perfumer&Flavorist, 2015). As fashions change, and new molecules are synthesised (Herman, 2005) there are regular and significant changes in the chemicals used in fragranced products. Each of these products is designed for and marketed to specific socio-economic groups (Burr, 2008), so the product worn by an individual can provide information about their age and economic status. Additionally, the number and variety of personal care products used in daily life results in a mixture of aroma chemicals and other chemicals being present on the skin and clothing, potentially providing an individualising chemical profile.

Although aroma chemicals have a wide range of solubilities, volatilities, thermal and pH stabilities (Da Costa and Eri, 2005), to be detected by the nose they must be volatile. This restricts their molecular weight to below 300 (Cheetham, 1991) which makes them ideal for analysis by Gas Chromatography (GC) (van Asten, 2002, Dewulf and Van Langenhove, 2002). The most highly-fragranced of the personal products are the perfumes and aftershaves, which consist almost entirely of volatile aroma compounds carried in a relatively simple alcohol solution and have proved relatively simple to analyse (Davidson, 2008). Antiperspirants and deodorants incorporate more non-volatile materials but the addition of modern headspace techniques such as Solid Phase MicroExtraction (SPME) facilitates analysis of the aroma chemicals without the interference of ingredients which are less amenable to GC analysis (Snow and Slack, 2002).

The combination of these attributes suggests that crime scene samples arising from such products would be ideally suited to chemical detection and analysis. Such samples could have the potential to provide intelligence and corroborative evidence to investigators, yet no comprehensive study in this area has been published to date and there is currently no process for their collection or examination. This work aims to rectify that shortfall.

1.1 Evidential Potential

It is the goal of the forensic scientist to obtain as much information from the crime scene as possible to aid the investigator in determining what happened, why, how and when. Physical evidence is an invaluable contribution to any investigation, offering scientifically sound and neutral proof of the facts being disclosed *(Prada et al.*, 2015 p. 3). The hypothesis underpinning this work is that the chemical evidence originating from perfumes, antiperspirants and deodorants worn by an individual has potential to provide valuable forensic information. This type of evidence has been overlooked by forensic scientists and police investigators but, together with human scent traces and other chemicals that form the human chemical profile, it is potentially of great evidential value.

For any material to have evidential potential it must be transferable and persistent – qualities which can be said to form the basis of all evidence (Houck and Siegel, 2010 p. 54). It is these qualities which result in the material being available, i.e. present in the environment, whether the 'environment' pertains to a person or a location. The transferability of aroma chemicals and other human scent chemicals can be demonstrated by our own awareness of the scent of clothing and by the tasks performed by scent dogs (Prada *et al*., 2015 p. 3). There are also numerous studies where pads have been use to collect odour from donors for later presentation to participants in odour perception studies and bioassays (Roberts *et al*., 2005, Havlicek and Lenochova, 2006, Mitro *et al*., 2012). The persistence of aroma chemicals is also well documented and Preti *et al.* stated that such chemicals could still be found in the axillary headspace of subjects 14 days after they were used (2006). Additionally, (Curran *et al*., 2005a) studied the persistence

of human scent on gauze pads using a simple weight-dissipation study and their results suggested that even after 84 days the pads retained some of the original sample. Aroma chemicals from personal care products have been detected in indoor air (Hollender *et al.*, 2002, Kubwabo *et al*., 2012) while phthalates and polycyclic musks are particularly persistent and have been found in waste-water and lakes (Simonich *et al.*, 2000, Lopez-Nogueroles *et al*., 2011) and even breast milk (Reiner *et al*., 2007, Yin *et al*., 2012). The frequency of occurrence in the environment is also important however, if a chemical is ubiquitous in an environment, it ceases to have evidential value at a crime scene: fortunately although perfumes, antiperspirants and deodorants are worn by the millions of people in the UK and around the world (IFSCC, 1998 p. 4, Duckett, 2015, Libby, 2015) the combination of chemicals that make up a specific fragrance formulation is only likely to be found near the source material, thus providing a forensic situation which has parallels to evidence types such as fibres evidence.

To have evidential potential perfumes, antiperspirants and deodorants must not only be present at the crime scene but they must be detectable and recoverable. Detection is potentially a problem with some forms of biological and chemical trace evidence as it is not only impractical to swab a large area but it is potentially destructive to other forms of evidence. Fortunately, when considering products deliberately formulated for their volatile aroma, both the human nose and some gas sensors can be used as a detectors. The evidence must then be collected in some manner, either physically recovered or recorded, so that it may be submitted for analysis, the objectives of which have been described as follows:

- To provide associative evidence by establishing whether any links exist between suspects, victims and / or crime scenes

- To corroborate or refute evidence from another source (e.g. eyewitness testimony)
- To provide intelligence to further an investigation
- To reveal whether an event occurred or to provide information on the likely sequence of events
- To establish the identity of an individual.

(Jackson and Jackson, 2004 p. 3)

In order to achieve these objectives the evidence should first be identified (e.g. as the residue of a perfume rather than of an ignitable liquid) and then classified (e.g. as Chanel No. 5) and, ideally, further classified until the evidence becomes 'individualising' i.e. specific to an individual or single common source (Bell, 2006 p. 2).

Criminal cases which have specifically involved fragranced products are not always well documented but aftershave has been used to mask the smell of decaying bodies (BBC, 2003) and as a fire accelerant (R v Gray and Others 1998). Perfume bottles have also been used to fashion explosive devices (Gibson and Ridley, 2004) and in circumstances such as these there is a clear benefit to being able to produce evidence identifying the brand of product and linking it to the perpetrator. Brand identification would also be extremely useful in sexual assault cases where the victim reports the smell of aftershave on the attacker (BBCNews, 2007a, 2007b) or at the scene of the crime (BBCNews, 2004). In many such cases the victim is unable to give a full description of her attacker and there may be few other investigative leads due to the opportunist nature of the attack. Such

information is rarely pursued because of the perceived lack of physical evidence, but in the case of serial rapist Paul Capener the overpowering smell of a readily identifiable brand of cologne led directly to his questioning by police and eventual conviction (Cowan, 2004). The concept of 'nosewitness identification' has recently been introduced by Alho *et al.* (2015) who reported that participants who witnessed a violent crime (albeit a video version) while inhaling a body odour scent attributed to the attacker were generally able to subsequently identify the attacker from a five person scent line-up.

Attention is also turning to the 'lifestyle indicators' that can produced from evidence and how a total chemical picture of an item could provide information on the habits of a suspect and offer useful intelligence leads to an investigation (Wilkinson *et al*., 2002). A number of research teams are now investigating the chemistry of latent fingermarks (e.g. Ricci *et al.*, 2007, Bailey *et al.*, 2012, Cadd *et al.*, 2015, Ferguson *et al*., 2012, Francese *et al*., 2013) and it is likely that a greater understanding of the persistence and transfer of chemicals from personal care products and the constituents of the human chemical profile will be an asset to such work.

The most prevalent form of forensic case work involving scent is, of course, carried out by scent detection dogs and when considering human scent from a living subject there are various approaches that are used, as listed in Table 1.1. Trailing, tracking and area searches are relatively non-controversial as they have been used for many years and, by their nature, they provide a degree of continuity of evidence in that an individual (the source of the scent) is ideally found at the conclusion of the task. Human scent identification or scent line-ups are used in

various parts of Europe and it has been calculated that scent evidence is comparable with bloodstain analysis, questioned documents, tool marks and hair analyses; and better than paint, glass or fibres (Schoon, 1998 p.73). In many countries, including the UK, scent dog evidence is only admitted as expert witness testimony (Curran *et al*., 2005a, Prada *et al*., 2015 p.33) but in the US the judge presiding over the case of California v. Salcido (2005) allowed human scent identification by canine to be admitted as forensic evidence. This ruling depended on the prosecution being able to show that the technique used the correct scientific procedures, the training and expertise of the dog-handler team is proven to be proficient, and the methods used by the dog handler are reliable *(Schoon et al*., 2009). To ensure that these conditions continue to be met, scent line-ups in the US are carried out by the Federal Bureau of Investigation's Human Scent Evidence Team rather than by individual police forces (Prada *et al*., 2015 p.10). A Scientific Working Group on Dog and Orthogonal Detector Guidelines (SWGDOG) has also been established to produce best practice guidelines for this work (SWGDOG, 2010) and to continue to add scientific rigour to these procedures. In the UK, however, scent line-ups are still considered controversial and police dog units do not train their dogs to work in this manner (Ellis, 2012, Newbury, 2014).

A lesson may be taken here from the work of Accelerant Detection Canines (ADCs). In the US during the 1980s and 1990s, undue weight was often given to the expert testimony of ADCs handlers during arson cases and this led to a number of miscarriages of justice and appeals (Katz and Midkiff, 1998). Concerns were raised about the fact that dogs may not alert consistently and whether handlers were honestly reporting alerts as well as the training and testing used to evaluate canine team capabilities (Kurz *et al*., 1994, 1996).

Table 1.1 - Tasks undertaken by human scent evidence canines

Trailing	The dog is given an object to scent (known as pre-scenting) and then tasked to determine if the same scent can be detected in an area and to follow the scent to the source (i.e. the person) or until the trail ends.
Tracking	The dog is not given a scented object to work from but is tasked to find a recent scent and follow it to its source.
Area searches	The dog is tasked to locate any human in the area. Used in wilderness areas, urban search and rescue and avalanche rescue.
Human scent identification (scent line-ups)	The dog is given an object to scent and then tasked to find another object with a 'matching' scent. This task is usually conducted under semi-controlled environmental conditions e.g. scent line-ups.
Location check	The dog is pre-scented and trained to alert if that scent is subsequently found at a location.
Article searches	The dog is tasked to locate articles which hold the scent of any human (e.g. articles which have been dropped)

Tasks are those involving the scent of living humans only. (Compiled from: Prada *et al*., 2015 p. 81, Mesloh *et al*., 2002, and Stockham *et al*., 2004).

Figure 1.1 – Scent Identification by Czech Republic Police dogs

(Pinc *et al*., 2011)

A great deal of work was conducted to evaluate the protocols being used by ADCs and the limits of detection that could be achieved by dogs, and by laboratory analysis, following which a position paper was produced by The Forensic Science Committee of the International Association of Arson Investigators (Chasteen *et al*., 1995). This paper concluded that ADCs were extremely valuable in the investigation of suspected arson cases but that canine alerts must be confirmed by laboratory analysis to be considered evidence. Additionally the committee specified that forensic laboratories should work in conjunction with dog handlers to document the training and proficiency of the canine (Chasteen *et al*., 1995). This approach of using the dog as a screening tool with results checked by chemical analysis has resulted in ADCs being considered a mainstay of modern arson investigation and it is recognised that a similar approach could be used for other forms of forensic evidence (Ramsey *et al*., 2009).

1.2 Perfumes, Antiperspirants and Deodorants

Humans have been using perfume since around 1500 BC when aromatic plant materials preserved in animal fats were used as odour control (IFSCC, 1998, Herman, 2005). With the advent of distillation techniques such as those documented by Herodotus in 425 BC, essential oils could be produced from a wider variety of materials and, by the 10-14th Century AD, the Arab techniques of distilling led to ethanol becoming the solvent of choice (Leffingwell, 2003). From around 1830 industrialisation and advances in chemistry made perfumes accessible to the middle class and as early as 1888 MUM® introduced a zinc oxide antimicrobial antiperspirant to the market (IFSCC, 1998, Pybus, 2006). The development of perfumes, antiperspirants and deodorants continued to evolve rapidly during the 20th century with chemists, including Nobel prize winners such as Wallach, von Baeyer, Ruzicka and Robinson, continually working on the identification and synthesis of ingredients for this new market (Pybus, 2006).

Today, the fragrance industry is dominated by six multinational companies: two Swiss based companies, Givaudan and Firmenich, the American International Flavors and Fragrances (IFF), Symrise in Germany, the Anglo-Dutch company Quest International (a member of the ICI group of companies) and the Japanese company Takasago (Burr, 2003). Together they account for over 70% of total global fragrance compound sales (Pybus, 2006 p.133) and those sales comprise both individual ingredients and completed fragrances (Sell, 2006 p.53). In many cases the names of the big fragrance companies are not seen on the packaging of a scented product and they act as ‘ghost writers’ selling the fragrance formulations to companies such as Unilever, Proctor & Gamble, L’Oreal and Estee Lauder who

dominate the branded personal care and household products market (Pybus, 2006 p.299, Libby, 2015, Extance, 2015).

Of the range of personal care products applied to the skin, perfumes, antiperspirants and deodorants are the most commonly used (IFSCC, 1998). While perfumes are the most heavily fragranced of personal care products, the percentage of perfume in antiperspirants and deodorants is also high (see Table 1.2). These products were specifically chosen for study because their primary purpose is to have a highly persistent fragrance and also because they are left on the skin rather than washed off.

Table 1.2 - Percentage of fragrance material in perfume and other personal care products

Product	Fragrance Material % by weight	Type
Perfume (fine fragrances)	3 - 40	Leave on
Antiperspirant-deodorant	0.50 – 10.00	
Soap	1.70	Wash off
Shower and bath gel	1.00	

From Beerling (2006 p.172)

Perfume is used as a generic term to refer to what the industry would call ‘fine fragrances’: perfumes, colognes, eau de toilette and aftershaves (Herman, 2005 p. 306). As shown in Table 1.3, within the fragrance industry the term ‘perfume’ specifically refers to an ethanol based product containing 15-40% (by weight) of fragrance material. The other fine fragrance products are similarly categorised by percentage of fragrance material with the prefix eau-de- indicating that water is

used as a further diluent. This use of ethanol and water as the carrier solvent gives rise to the generic term ‘hydroalcoholics’ which is also used to describe the fine fragrances (Herman, 2005 p. 306), although eau de cologne (usually just referred to as cologne) and aftershaves are not always considered to be ‘fine’ fragrances as they have a higher percentage of water than other formulations. In aftershaves, the alcohol content is also limited to 60-65% to reduce stinging on shaving cuts (Herman, 2005 p. 306).

Table 1.3 - Categories of fine fragrances

Category	Fragrance Material % by weight
Perfume	15-40
Eau de Parfum	10-30
Eau de Toilette	5-20
Eau de Cologne	3-5

Definitions vary but the table here gives an indication of the terms used. (Source: Libby, 2015)

The fragrance material used in these products is referred to by the industry as ‘jus’ and is a concentrated mixture of various fragrance ingredients. Although this fragrance material is largely made up of pure aroma chemicals, essential oils may also be incorporated into the formulation and (as described in Section 1.4) these natural extracts contain some non-odorous chemicals.

A fragrance mixture will contain 50 to 500 individual aroma chemicals depending on the target market and price range of the final product. The aroma chemicals are chosen to ensure there are a range of volatilities which are balanced to provide the

desired odour throughout the use of the product (Small, 2006 p.147). The most volatile aroma chemicals are called ‘top-notes’ and provide immediate aroma impact to the customer and last for around 15 minutes. There are also less volatile chemicals known as ’middle-notes’ (or modifiers) and ‘base’ or ‘heart’ notes which will last for hours (Pybus, 2006). Each ‘note’ is a characteristic odour provided by a single aroma chemical and mixtures of two or more notes with a unified theme (e.g. floral) are termed ‘accords’ (Herman, 2005 p. 306). Normally a ratio of 25% top-notes, 20% middle-notes and 55% base-notes is considered a well-balanced blend (Herman, 2005 p. 306). The aroma compounds for each set of notes will differ but the same accords are usually represented within each set of notes so that the overall fragrance maintains its character throughout the ‘dry-down’ period, e.g. so that the floral smell remains throughout the time on the skin (Small, 2006 p.147).

The manufacture of modern perfumes, antiperspirants and deodorants depends not only on the art of the perfumer but on the formulation scientist as these products are complex mixtures of solvent, fragrance material and other ingredients. The formulation of a simple Eau de Parfum is shown in Table 1.4 but the formulation of antiperspirants and deodorants is more complex (as shown in Table 1.5) and will include ‘actives’ such as antiperspirant drying agents, antimicrobials and moisturisers (Beerling, 2006 p.173).

Table 1.4 - Formulation of a typical eau de parfum

Ingredient	% by weight	Purpose
Alcohol DEB100	78.00	Solvent
Fragrance Material	12.00	Fragrance
Distilled water	8.50	Diluent
PPG-20 methyl glucose ether	1.00	Moisturising ingredient and fragrance fixative
Benzophenone-2	0.50	UV radiation absorber

(Beerling, 2006 p.170)

Table 1.5 - Formulation of the concentrate of a typical aerosol antiperspirant

Ingredient	% by weight	Purpose
Volatile silicone-fluid	48.65	Solvent
Activated aluminium chlorohydrate powder	36.00	Antiperspirant
Isopropyl myristate	10.00	Emollient [b]
Bentonite	3.65	Suspension aid [a]
Dimethiconal	0.50	Moisturiser and cloud suppressor
Fragrance material	1.20	Aroma

The concentrate makes up 25% of the volume of the aerosol with the remaining 75% being the propane-butane propellant.

From Beerling (2006 p.174) with additional information from [a] Schreiber (2014) and [b] Epstein (2014)

Antiperspirants and deodorants are often grouped together as ‘deo-colognes’ by the industry (IFSCC, 1998) and there is considerable overlap between the products. Antiperspirants are primarily formulated to reduce the amount of sweat produced by the eccrine glands (i.e. wetness) and the most common antiperspirant actives are the inorganic salts aluminium chlorohydrate (ACH) and aluminium-zirconium chlorohydrex-glycerine (AZG) (Jenner, 2006 p.257). These are both polymeric, loosely hydrated complexes of aluminium chloride which effectively block the eccrine sweat gland (IFSCC, 1998, Beerling, 2006 p.173, Small, 2006 p.150). This ‘occlusive plug’ is formed by the hydrolysed metallic cationic salt through the action of pH change caused when entering the eccrine duct (Quatrale, 1988). As discussed in Section 1.3, it is however, the interaction between bacteria and sweat that actually causes body malodour and deodorants may be formulated to counteract malodour in one or more of the following ways:

- Odour masking or disguise – using large amounts of fragrant materials (up to 10% of total formulation) to overwhelm the unwanted odour.
- Odour reduction or removal – achieved in a number of ways including using materials to adsorb, absorb, form complexes with or encapsulate glandular extractions or the products of microbial action.
- Odour prevention – inhibiting bacterial growth through the use of antimicrobials (particularly triclosan), enzyme inhibitors or antioxidants

(IFSCC, 1998, Herman, 2005 p.326)

In the United Kingdom fragranced antiperspirant products dominate the market (IFSCC, 1998) and are usually sold as ‘antiperspirant-deodorants’ while products without ‘active’ ingredients (e.g. antiperspirant action) may be sold as ‘body-sprays’ (Libby, 2015). In other parts of the world, underarm wetness is more

socially acceptable and deodorant products are more popular (IFSCC, 1998). In the fragrance and cosmetics industry antiperspirants and deodorants are formulated within the ‘personal wash’ division of a company rather than the fine fragrance division but the chemists and perfumers will collaborate to produce the fragrance formulation for the deo-colognes (Sell, 2006). These products are some of the most technologically sophisticated fragrances on the market as they now use fragrance encapsulation technologies and water-triggered release of fragrances to make extremely persistent fragrances and deodorants which activate with sweat (Gunaratne *et al.*, 2015, Duckett, 2015).

The complexity of the fragrance mixtures used in these products, together with the variety and increasing number of fragrance ingredients on the market, ensure that products have distinctly different chemical profiles from each other. It therefore follows that with appropriate chemical analysis perfumes, deodorants and antiperspirants should be distinguishable from each other and be potentially identifiable. Coupled with their forensically useful properties, particularly their persistence and transferability, this leads to the hypothesis that perfumes, deodorants and antiperspirants have evidential potential. Additionally, when combined with human scent and other endogenously produced compounds, the resultant chemical profile may be individualising.

1.3 Human 'Scent' and Chemical Profiles

In investigating the potential evidential value of fragranced products, we must consider the presence of other chemicals present on the human body, whether they will be observed in our samples and whether they may interact with the target aroma compounds. Research into to the human chemical profile has been ongoing since at least the 1960s when studies into axillary (underarm) odour and the means to counteract it became the subject of intense research by the personal care industry (Preti and Leyden, 2010). Early studies into the biological mechanisms associated with production of this odour and its chemical constituents revealed that although there are different types of glands in the epidermis with varied anatomical distribution (see Table 1.6 and Figure 1.2) the characteristic body odour is in fact due to the interaction of apocrine secretions with certain bacteria rather than the production of eccrine sweat (Shehadeh and Kligman, 1963a, 1963b, James *et al*., 2013, Costello *et al*., 2014), thus resulting in a heterogeneous mixture of metabolically derived (endogenous) and microbially transformed chemicals (Soini *et al*., 2006). Chemicals found on human skin may also be of exogenous origin (Gallagher *et al*., 2008). Some chemicals found on the skin are non-volatile while others are volatile but not necessarily odorous (Bernier *et al*., 1999). Additionally, the characteristic odour compounds of sweat (those with the strongest odour) are not necessarily the most abundant chemicals as some exhibit a low olfactory threshold and have a high odour impact at low concentrations (Preti *et al*., 2006, Zeng *et al*., 1991).

When investigating the use of chemical analysis to obtain forensic evidence there is some difficulty in finding appropriate terminology (see Table 1.7).

Table 1.6 - Human glandular excretions

Eccrine Sweat Glands	Apocrine Sweat Glands	Sebaceous Glands
Cover entire body. Higher densities in forehead, axilla, palms and soles of the feet	At base of hair follicles. Highest densities in axilla, areolae, periumbilical and genital areas	Face, scalp, upper trunk and pubic area
Produce sweat primarily for thermoregulation but also linked to nervous reflex	Produce sweat in response to stimuli other than heat	Produce sebum which spreads over skin surface and mixes
Composition varies across individuals, body locale and samplings of the same person	Produced in response to emotional stimuli including pain, fear, and anxiety. Activity starts with onset of puberty	Varies with age and diet. Excreted slowly so physiological changes may only effect composition after 6-9 days
99% water plus salts such as NaCl, potassium salts and HCO_3^- also urea, ammonia amino acids and proteins	Lipids, proteins steroids, fatty acids, squalene, triglycerides, ammonia and sugars	Fatty acids, squalene, triglycerides, wax esters and free sterols
Clear, odourless, colourless, acidic	Sterile odour free and weakly acidic but thicker and more viscous	Yellow viscous fluid

(Prada *et al*., 2015)

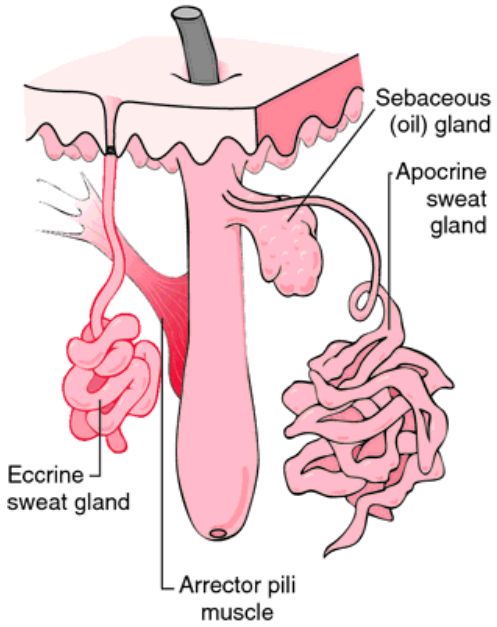


Figure 1.2 – Eccrine, Apocrine and Sebaceous glands

(Copstead, 1995 accessed via, Miller-Keane and O'Toole, 2015).

As described above, chemical abundance does not necessarily equate to odour and odour may not be the more important forensic evidence as this term pertains to a behavioural response to chemical stimulus. Consequently, the focus on human 'scent' by many authors (e.g. Prada, 2015) is somewhat limiting and it may be more appropriate to consider the odorous chemicals as a sub-set of what has been called the 'human chemical profile' (Wyatt, 2014).

Table 1.7 - Terminology of human 'scent' and chemical profiles

Terminology and originator	Comment
Odour fingerprint (Nicolaides, 1974)	Not appropriate in a forensic context, especially now that research is being conducted on the chemicals found in fingerprints.
Chemical fingerprint (Penn *et al*., 2007)	
Human Scent / Human Odour	The words odour and scent should not be used to refer to any study using purely analytical chemistry rather than bioassays or olfactrometry as without including these approaches a study cannot address the issue of 'scent' or 'odour'.
Odorprint (Barash *et al*., 2009)	
Volatome (Phillips *et al*., 2013),	Specific to the study of volatile metabolites and their biological significance and associated with metabolomics studies.
Signature mixture (Wyatt, 2014).	Used to describe the smell that may be recognised by others as belonging to an individual but with the clear understanding that this is a sub-set of the whole chemical profile. In this work a signature mixture is considered to include some primary constituents of an individual's chemical profile together with some of the more stable secondary and tertiary constituents.
Human chemical profile (Wyatt, 2014).	Used to describe the total chemical profile of an individual human. This term is considered the most appropriate for this study, as neither bioassays nor olfactometry will be conducted and it is not only volatile compounds which may be of interest.

An alternative and useful approach to categorising the constituents of the human chemical profile was introduced by (Curran *et al*., 2005b) and can be adapted for

use in this study. The various chemicals found on the surface of the human body can be classified as:

- Primary constituents – endogenous metabolically derived and microbially transformed compounds which are stable over time regardless of diet or environment.
- Secondary constituents - endogenous and exogenous compounds which change over time due factors such as diet, illness and environment.
- Tertiary constituents – exogenous compounds which are deliberately applied to the body e.g. perfumes, antiperspirants and deodorants.

Primary constituents are the most heavily researched area of the human chemical profile, partly because some of these chemicals are the sources of human malodour. Early investigations into axillary odour focussed on the presence of odorous steroids in the axillae particularly androstenol and androstenone (Labows *et al*., 1979, Labows *et al*., 1982, Gower and Ruparelia, 1993, Gower *et al*., 1994) but there is now organoleptic and analytical evidence that a mixture of C6–C11 normal, branched, hydroxy- and unsaturated acids constitute the dominant, characteristic axillary odour (Zeng *et al*., 1991, 1992, 1996a, Natsch *et al*., 2003). Following this, researchers also identified trace amounts of thio-alcohols in axillary sweat and these compounds have a low olfactory threshold meaning they provide intense odours at low concentrations (Natsch *et al*., 2004, Troccaz *et al*., 2004, Hasegawa *et al*., 2004).

Other research has focused not on the odours perceived by humans but on those chemicals which might serve as attractants for various disease vectors, particularly the Yellow Fever Mosquito (e.g. Qiu *et al*., 2011, Smallegange *et al*., 2005,

Verhulst *et al.*, 2011). A strong link was found between mosquito attraction and lactic acid in 1968 (Acree *et al*., 1968, Steib *et al.*, 2001) and subsequently Bernier's studies identified 346 compounds emmanating from human hands (Bernier *et al*., 1999, 2000). More recent research using purely chemical detection includes determination of VOCs which may be used to identify the presence of humans, thereby revealing victims of human smuggling or aiding search and rescue of earthquake victims (Giannoukos *et al*., 2014, Mochalski *et al*., 2014, Statheropoulos *et al.*, 2014).

Anthropological and ethnographical research has also been conducted to determine if male and female subjects have different primary constituents (Zeng *et al*., 1991, 1996b) and to investigate the individuality of chemical profiles. The uniqueness of human scent has been discussed in scientific literature since 1887 (Romanes, 1887). In the era of instrumental analysis, Nicolaides in Science Magazine (1974) suggested that the number and variety of human skin lipids resulted in a complexity which:

> "…*allows each individual to have a distinct odor or chemical fingerprint*."
>
> (Nicolaides, 1974)

There have been numerous behavioural studies purporting to show that humans produce an individualising scent that can be recognised by other humans (Hold and Schleidt, 1977, Schleidt, 1980, Cernoch and Porter, 1985, Porter *et al.*, 1985, Lord and Kasprzak, 1989) or by scent trained canines (Kalmus, 1955, Hepper, 1988, Stockham *et al*., 2004, Curran *et al*., 2005a). Only in the last two decades has there been a concerted effort to link these observational studies to chemical analyses and current research is focused on the individuality of human scent for

biometric identification security and forensic identification as well as anthropological and ethnographical purposes. This research is centred on demonstrating that the primary constituents of the human chemical profile are stable over time but vary between individuals i.e. that intra-individual variation is much less that inter-individual variation (Curran *et al*., 2005a, 2007, Penn *et al.*, 2007, Rodriguez-Lujan *et al*., 2013).

Evidence to support individuality of the chemical profile comes from studies on mammalian signalling where there is evidence that individual odour signatures are associated with the major histocompatibility complex (MHC), which in humans is known as the Human Leukocyte Antigen (HLA), and, as with other mammals, is highly polymorphic (Wyatt, 2014 p.280). HLA proteins are found in human sweat (Prada *et al*., 2015 p. 18) but the exact mechanism by which they influence odour profiles is still not clear and may be related to the skin bacteria (Penn, 2002, Restrepo *et al*., 2006). Supporting evidence for this comes from the work of Sommerville *et al*. (1994) who reported that the pattern of ‘sweat volatiles’ from two pairs of identical twins showed significantly higher match correlation than that of unrelated people. Curran then studied two unrelated males of the same age and noted some variability in the chromatographs obtained from the same person (mostly in the ratios of the compounds present) but reported more significant differences between the two individuals as well as the presence of differing compounds (Curran *et al*., 2005a).

Later studies increased the number of subjects and identified a number of compounds as potentially individualising as they were present in samples from all donors but with variable relative amounts produced by each donor (Curran *et al*.,

2005b, Natsch *et al*., 2006). All of these studies, however, are considered to have 'significant flaws' (Penn *et al*., 2007): they are limited in the number of individuals studied, the number of repeated samples and the identification of the chemical compounds. To overcome such issues, a study was conducted in 2005 by a multinational team comprising ethnologists, chemists and statisticians collecting and analysing samples from 197 individuals in an isolated village in Southern Austria. The team were rigorous in their sampling, storage and statistical analysis of the data and reported considerable individual-to-individual variation, relatively stable profiles from repeated samples from the same person and that many subjects had very distinctive GC–MS signatures, (Soini *et al*., 2006, Penn *et al*., 2007). Despite some expansive claims in the mainstream media (Gray, 2007) the peer reviewed work does admit that similarity indicators were more qualitative than quantitative (Penn *et al.*, 2007). These studies focussed on identifying primary constituents and therefore required that subjects use unscented soap for a period of days before sampling and avoid certain foods. Thanks to such work we now have a good understanding of some primary constituents but it is also clear that even the endogenous human chemical profile is exceedingly complex: Dormont published a review in 2013 which listed 400 VOCs emanating from the human body and in 2014 a review by de Lacy Costello *et al*. (2014) produced a list of 1840 chemical compounds, 532 from skin secretions alone (Dormont *et al*., 2013, Costello *et al*., 2014).

The definition of secondary constituents originally provided by Curran *et al.* (2005b) is rather vague and can be adapted to include any compounds which change over time but cannot be categorised as tertiary: thus including hormones (Preti *et al*., 2003), of which stress hormones may be of particular interest in a

forensic context (Hauser *et al.*, 2005, Eachus *et al.*, 2013) and disease biomarkers (e.g. Preti *et al*., 2008, Jiang *et al*., 2013, Calenic and Amann, 2014). There is also an indication that the endogenous chemical profile changes with age (Haze *et al*., 2001) and that certain chemicals are excreted following the consumption of particular foods (Havlicek and Lenochova, 2006). In some texts pheromones are included as constituents of the human chemical profile (Preti *et al*., 2003, Grammer *et al*., 2005, Kippenberger *et al*., 2012), however there is still a great deal of controversy surrounding this (Wysocki and Preti, 2004, Doty, 2010, Wyatt, 2015). To be classed as a pheromone a chemical must have evolved as a chemical signal between members of the same species, be characteristic of all members of a sub-class of that species (e.g. males or lactating females) and elicit a specific behavioural response (Wyatt, 2015). In some cases candidate molecules associated with behaviour such as kin-recognition have been reported as potential pheromones (Vaglio *et al*., 2009) when they are more likely part of an individual's signature mixture which has changed due to hormonal influences. There has been little reliable evidence of human pheromones thus far (Doty, 2010, Wyatt, 2015) but if they are present then they would be classed as secondary constituents.

Tertiary constituents have already been investigated for their evidential value and examples include chemicals present on human skin following the handling of ignitable liquids or explosives (e.g. Twibell *et al.*, 1982, Almirall *et al*., 2000, Muller *et al*., 2014). Additionally, other chemicals present due to the individual engaging in particular activities such as smoking tobacco or cannabis may have the potential to provide useful intelligence to an investigation (Benton *et al.*, 2010, Voss *et al.,* 2014). Perfumes, antiperspirants and deodorants are the exogenous sources with

the most concentrated aromas but other fragranced personal care and household products (soap, body lotion, washing powder, fabric conditioner etc.) will contribute to the overall chemical profile. To date there has been no published research on the distribution of such chemicals within a population or any attempt to identify products from skin or clothing samples or to use these chemicals as forensic evidence.

The studies described above highlight the complexity of the human chemical profile. While the investigation into the primary constituents is fascinating and valuable it does not realistically reflect the forensic environment. Secondary constituents may also prove extremely valuable in the forensic arena in providing intelligence regarding past or future actions and behaviours.

1.4 Ingredients of Perfumes, Deodorants and Antiperspirants

There are currently around 3000 chemicals in use as fragrance compounds (IFRA, 2015b) and in texts examples of these chemicals may be grouped by aroma, by chemical structure and by source (e.g. Sell, 2006, Rowe, 2005). Each of these methods of classification has benefits and together they help explain why some chemicals have come to be especially popular.

1.4.1 Classification of Aroma Chemicals by Sources

Aroma chemicals can be classified as natural, nature identical or synthetic.

1.4.1.1 Naturals

Natural compounds are those derived directly from natural sources such as flowers, wood, resin or even (historically) from animal sources. Some natural materials have a long history of use including cedar wood, myrrh resin and frankincense, while rose was used by the Romans and lavender favoured by the Normans (Pybus, 2006). Natural ingredients may consist of a single ‘character impact’ aroma chemical of high purity or a complex mixture of compounds such as those found in essential oils. The term ‘natural’ with regard to aroma chemicals is rather poorly defined legally, but the EU Directive on Food Flavourings (EC 1334/2008), which also applies to materials used to impart odour to food, allows the label ‘natural’ to be applied materials whose source is animal, vegetable or microbiological and where the resulting ingredient is prepared using ‘traditional’ food preparation processes (Sabisch and Smith, 2015). In the US the definition of the permitted production methods is somewhat broader and covers a wide range of isolation methods and extraction technologies (Rowe, 2011 p.11). Methods currently in use to produce ‘natural’ fragrance ingredients include all forms of

distillation and solvent extraction as well as the use of sub-critical and super-critical solvents and membrane technologies (Margetts, 2005 p.174). The most commonly used extraction techniques are physical pressure, solvent extraction and steam distillation (Rowe, 2011 p.20).

Of the natural fragrance ingredients, some of the best known are the essential oils. These expressed oils are produced when physical pressure alone is used to force oils out of the source material in a process known as cold pressing (Rowe, 2011 p.19). Essential oils are mixtures which may contain 300 or more chemicals including ‘character impact compounds’ which are instantly recognisable, other odorous compounds which contribute to the scent to a lesser extent, and compounds with no obvious odour which may act as natural fixatives (Clery, 2006 p.214). Although essential oils are present in botanical source material at just 1-2% of the dry weight of the harvested material (Sell, 2006 p. 40) they can still be highly profitable to extract. The most economically viable of the essential oils are the citrus oils of which ~15,000 tonnes / annum are produced. Many natural aroma chemicals are labour intensive to collect, however, and difficult to extract which, as shown in Table 1.8, affects the price (Jenner, 2006 p.261). Additionally, production may suffer from variations in local weather conditions, pests and disease as well as a host of other extrinsic factors (Lawrence, 2002). Despite this, demand for natural aroma compounds is increasing, in part due to a misconception that naturals are safer (Margetts, 2005 p.169). As well as a thriving market existing for pure essential oils (IBISWorld, 2015) many types of natural extracts are still used by perfumers in fine fragrance formulations as they are considered to add depth and character and to be luxurious (Sell, 2006 p. 40).

Table 1.8 - Price comparison for jasmine scent by source

Source	Product name	2006 Price (£ / kg)
Natural (7million flowers, handpicked, to produce 1kg of oil)	Jasmine natural absolute	3000-5000
Nature identical character impact chemicals	Jasmone	300-500
	Methyl jasmonate	
Synthetic cyclopentane derivatives	Dihydrojasmone	10-50
	Methyl dihydrojasmonate	

Compiled from Jenner (2006 p.261)

Natural extracts are much less likely to be used in other personal care products as some of their constituents are unstable in acidic, basic or oxidising environments. For example, jasmine oil cannot be used in soap because it contains a significant concentration of indole which causes soap to discolour and benzyl acetate which is not a character impact compound and which is hydrolysed at the high pH of soaps (Sell, 2006 p.45). For this reason the perfumer may just use the character impact compounds in the fragrance formulation, and in this case those chemicals can be extracted or synthesized.

1.4.1.2 Nature Identical

Many of the character impact compounds found in natural sources have been identified using techniques such as Gas Chromatography–Olfactometry, separated using preparative gas chromatography, and their structures elucidated using techniques such as Nuclear Magnetic Resonance spectroscopy (Lis-Balchin, 1995). These chemicals may then be synthesized and the resulting products are termed ‘nature identical’ compounds (Sell, 2006 p.128). While this process requires a significant initial investment of time and money the resulting synthesis

will often be much cheaper than extracting the chemical from its natural source and is likely to be of higher purity (Pybus, 2006 p.19). Synthetic chemicals benefit from stability of supply, barring major oil crises, and are largely unaffected by the factors which can cause large fluctuations in the price and quality of natural extracts (Pybus, 2006 p.135). The vast majority of synthetic compounds are produced by the six companies listed previously, some of which take advantage of ability to buy in feedstock as part of wider chemical industry (e.g. Quest is part of the ICI group of companies).

1.4.1.3 Synthetics

While perfumes and other hydroalcoholics are relatively benign chemical environments, deodorants, antiperspirants and other personal care products are much harsher and many of the aroma chemicals found in nature are not stable enough to be used in such products: vanillin for example discolours soap and turns purple in contact with iron (Herman, 2005 p. 75). Consequently, fragrance chemists have been keen to find chemical analogues which have the same odour properties as naturally derived chemicals but are easier and cheaper to synthesize and are stable in acid, alkaline or oxidising media (Sell, 2006 p.128). The 1960s saw a boom in the manufacture of aroma chemicals and alternatives were found for many of the most popular ingredients: Vanillin may now be substituted with ethyl vanillin and Ultrvanil® while Indole can be replaced by Indolal® (Sell, 2006 p.45). These synthetic aroma chemicals are made up of a wide variety of chemical species and in addition to the aroma analogues, molecules with entirely new odours have been designed and manufactured. The synthetics industry is highly competitive and multinational fragrance companies put tens of millions of dollars

into research and development (Pybus, 2006 p.133) with molecule development taking 3-5 years (Jenner, 2006 p.260). The most prized synthetics are high impact aroma compounds with low odour detection thresholds and good chemical stability: these products are held ‘captive’ by the originating company for some years and will only appear in formulations sold by that company (Jenner, 2006 p.261).

There is a final twist in the story of aroma chemicals and their origins: a number of aroma chemicals have enantiomers with different olfactory properties, but identical physical and chemical properties. While a synthetic process may produce a mixture of enantiomers and their resolution offer significant challenges for the synthetic fragrance chemist, natural sources (or bioprocesses) show a high degree of enantiomeric selectivity and may actually prove to be more cost effective (Sell, 2003 p.72, Margetts, 2005 p.171).

1.4.2 Classification of Aroma Chemicals by Structural Class

Aroma chemicals are volatile organic molecules with molecular weights generally between 100 and 300. Most of the several thousand aroma compounds available to the perfumer are hydrocarbons although the inclusion of nitrogen in aroma molecules is not uncommon (nitriles often smell somewhat metallic) and many tropical fruit notes are provided by molecules containing sulphur (Small, 2006 p.145). There are also a range of different chemical structures and functional groups represented and a detailed discussion of aroma chemicals by chemical class can be found in Rowe's *The Chemistry and Technology of Flavors and Fragrances* (Herman, 2005). A brief overview of some of the most commonly used aroma chemicals is given here with a focus on four chemical classes: alcohols, aldehydes, ketones and terpenoids. The heterocycles are discussed in the following section regarding musks. It should also be noted that although classification here is by class, many chemicals fall into more than one chemical class and are often closely related, with terpene backbones being especially common (Cheetham, 1991). For a comprehensive review of such chemicals Sell's *A Fragrant Introduction to Terpenoid Chemistry* is highly recommended (2003).

1.4.2.1 Alcohols

The saturated alkyl alcohols such as ethanol and dipropylene glycol (DPG) are used as solvents (Herman, 2005 p. 58) but many other classes of alcohols are found as aroma chemicals. Of the unsaturated alkyl alcohols the largest group are the terpenic alcohols which are discussed with the other terpenoids below, but also important is *cis*-3-hexanol (leaf alcohol) which is naturally produced when green vegetation is cut. This odour, characteristic of the fresh smell of cut grass, is

used to add freshness to a formulation (Herman, 2005 p. 57). Aromatic alcohols are also used in fragrances and benzyl alcohol with its mild sweet aroma is used in significant quantities as it also acts as a carrier solvent. Phenylethyl alcohol (PEA) is a particularly important aroma chemical with a sweet, simple rose-like fragrance: the synthesis of PEA is considered a milestone in the fragrance industry (Herman, 2005 p. 59) and it is an exceptionally rugged molecule used in many applications (Herman, 2005 p. 324). Phenol derivatives are generally well represented in the perfumer's portfolio with eugenol, a key component of clove oil, being very popular despite its status as a potential allergen (see below). There are also a number of more complex alcohols used in perfumery, including the synthetic sandalwood aroma chemicals (Herman, 2005 p. 58).

1.4.2.2 Aldehydes

The simple aliphatic aldehydes were some of the first synthetic molecules to be synthesised and methylnonylacetaldehyde (aldehyde C12 MNA) with its fatty 'animalic' character was famously used in Chanel No. 5 in 1921 (Sell, 2006 p.45). Today, one of the most significant of all aroma chemicals is Vanillin (guaiacol-4-carboxaldehyde) which in most listings is grouped with the aromatic aldehydes although it is also an alcohol (phenol derivative) (Rowe, 2011 p.5). Other important aromatic aldehydes include benzaldehyde and cinnamaldehyde both of which were first identified as character impact compounds in essential oils and have since been synthesised (Herman, 2005 p. 73). There are also many aroma chemicals which are considered cinnamaldehyde derivatives although they are usually synthesised from benzaldehyde with other aldehydes. One of the best known of these is α-hexylcinnamaldehyde, which has a sweet floral jasmine odour

and is cheap enough and stable enough to be used in a wide range of personal care and household products (Herman, 2005 p. 74). There are also a number of unsaturated aldehydes including the synthetic Lyral® which are much used for an odour known by perfumers as ‘muguet’, a floral fragrance typical of the flower lily-of-the-valley (Small, 2006 p.145).

1.4.2.3 Ketones

Although many of the odorous ketones are more often used in the flavour industry than in fragrances, aromatic ketones are well represented in the fragrance industry (Herman, 2005 p. 58). Cyclopentanone derivatives dihydrojasmone, *cis*-jasmone and methyldihydrojasmonate (MDJ) are especially important ingredients (Sell, 2006 p.128). Methyldihydrojasmonate (Figure 1.3) is of particular interest as the synthesis of this ingredient produces four stereoisomers with (+)-*cis*-methyl dihydrojasmonate providing the most intense jasmine odour (Davies, 2015, Leffingwell and Leffingwell, 2011).

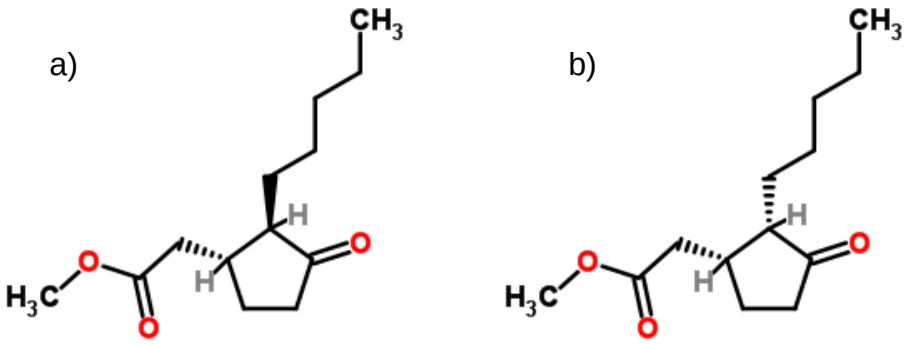


Figure 1.3 – Methyl dihydrojasmonate

a) Methyl dihydrojasmonate, (-)-*trans*- and b) Methyl dihydrojasmonate, (+)-*cis*- (RSC, 2015)

The percentage of each isomer varies according to the synthetic route used and although Hedione® is the best known MDJ product there are a number of others

on the market (Pybus, 2006 p. 19, Rowe, 2005 p. 80). This raises the possibility that the specific product used in a fragrance formulation could potentially be identified by the percentage of isomers.

1.4.2.4 Terpenoids

The terpenoids are a particularly important class of aroma chemicals and are by far the largest group of natural odorants used as modern fragrance ingredients (Sell, 2006 p. 54). As defined by Wallach in 1887, terpenoids are chemicals which have a carbon skeleton made up of units of isoprene (syn. 2-methylbuta-1,3-diene) and from this simple building block there are 1000s of different structures both natural and synthetic (Sell, 2003 p.1, Sell, 2006 p. 54). The term terpene strictly refers to terpenoid hydrocarbons originally found in turpentine, with the suffix –ene indicating the presence of olefinic bonds (Sell, 2003 p.2), however in the perfume industry it is often used when referring to mixtures containing monoterpenoid hydrocarbons and sometimes it is used to refer to any terpenoid (Sell, 2006 p. 54). Most important as aroma chemicals are the oxygenated terpenoids, with esters and alcohols of the monoterpenoids (2 isoprene units = 10 carbons) being particularly key ingredients, especially as they can be easily synthesized from turpentine and petrochemicals. With their low molecular weight monoterpenoids are volatile and consequently ‘odour effect’ need not be as great i.e. they can be detected by the nose at lower concentrations (Sell, 2006 p.128). The popular ingredients citronellol (floral, rose-like), citronellal (fresh) and linalool (Figure 1.4, sweet, floral), are all acyclic terpenoids. As with many aroma chemicals all three of these key ingredients are chiral and have enantiomers which vary considerably in odour and odour threshold.

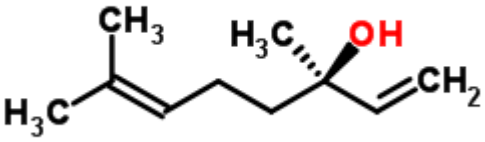


Figure 1.4 - (R)-(–)-Linalool

(RSC, 2015)

There are also a number of important cyclic monoterpenoids and one group is worth mentioning in more detail here: the ionones. Ionones are carotenoids and have odours which are characteristic of violets: two structural isomers α- and β-ionone account for over half of the material found in the headspace above *Violet odorata,* although they were actually produced synthetically about 50 years before this was confirmed (Sell, 2003 p.254). Methyl ionones are also 'indispensable' in perfumery and there are six positional isomers (Poucher *et al.*, 1991 p. 226). Although α-iso-methyl ionone is most commonly used, commercial products are often a mixture of isomers resulting in a signature blend produced under each specific trade name (Sell, 2003 p.255).

Figure 1.5 - α-Isomethyl Ionone

(RSC, 2015)

Sesquiterpenoids have a relatively high molecular weight and correspondingly lower vapour pressure which means that they need to have a low odour threshold to be useful. This reduced volatility does however make them slower to be lost and provides what perfumers call ‘tenacity’ and because of this they are often used as base-notes and fixatives in perfume. The woody smells are often due to these compounds and although some, such as nerolidol (Figure 1.6) are synthesized, many other sesquiterpenoids have complex cyclic structures makes them difficult to manufacture and it is still more economically viable for them to be extracted from natural sources (Sell, 2006 p.54).

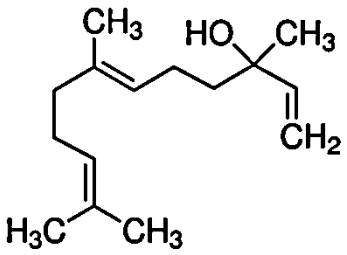


Figure 1.6 – (±)-*cis* Nerolidol

(Sigma-Aldrich, 2017)

Other terpenoids are less used in perfumery: a small number of hemiterpenoids and their esters (1 isoprene unit) are used, the most important of which are prenyl acetate and benzoate (Sell, 2006 p.63) and diterpenoids with their 20 carbon atoms and associated low volatility are unsuitable aroma chemicals but are sometimes used as solvents (Sell, 2006 p. 88).

1.4.3 Classification of Aroma Chemicals by Odour: Musks

Musks are a group of aroma chemicals which are most appropriately grouped by their very special animalic, sweet odour. Almost every perfume and most deodorants and antiperspirants on the market contain musk odorants and they hold a special place in perfumery as they are considered to:

> *"…refine, exalt, fix, balance perfumery compositions, have an erotic effect and bring volume and warmth"*
>
> (Kraft, 2005 p.144)

Musks are the largest molecules used in fragrance formulations and thus are the last to evaporate from the skin, which in turn delays the evaporation of volatile chemicals, an effect known as 'fixing' in the fragrance industry. This maintains the overall fragrance ('character') of the perfume for longer, which would otherwise be lost as each ingredient evaporated (Kraft, 2005 p.144).

1.4.3.1 Natural Animal Musks

Natural musks such as Tonquin musk from male musk deer *Moschus spp.* were extremely popular in the beginning of 19th Century when grains of the extracted material were worth twice their weight in gold (Kraft, 2005 p.144). In 1906, the biggest perfume manufacturer of the day, Scimmel and Co., isolated the character impact compound which was determined to be a ketone, $C_{16}H_{30}O$ which was named 'muscone' (Kraft, 2005 p.144). Later, in 1922, Ruzicka (working for the company that would later become Firmenich) established the structure as 3-methylcyclopentonedecan-1-one (Figure 1.7) and it has since been established that all animal musks are macrocyclic ketones (Kraft, 2005 p.145). Muscone's value is not simply due to its appealing smell but also its extremely low odour threshold, being detectable at concentrations of just 4.5 ng L^{-1} air (Kraft, 2005

p.164). The popularity of Tonquin musk inspired chemists to discover new of musk-like materials that had a comparable performance and, as extraction from animals resulted in a devastating reduction in their populations, the focus turned to other sources.

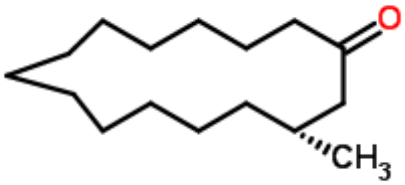


Figure 1.7 - (R)-(-)-Muscone

(RSC, 2015)

1.4.3.2 Plant Musks

In 1927 Kershbaum discovered a musk-like aroma was produced by 15-pentadecanolide, a constituent of angelica root oil. Now known as Thibetolide® or Exaltolide®, it and its enantiomers are still one of the most important musks with a 'typical' musk odour and an odour threshold of 2.1 ng L^{-1} air (Kraft, 2005 p.147). Like all plant musks it is a macrolide, i.e. a macrocyclic lactone and, although originally too expensive for consumer products, it and other macrolides (e.g. from Galburnum oil) are still popular today (Kraft, 2005 p.150).

1.4.3.3 Nitromusks

In 1888 when the renowned chemist A. Baur was working to develop explosives he discovered that a t-butylation of TNT produced a compound with a pleasing musk odour (Sell, 2006 p.46). He named the chemical Musk Baur and it sold for $500 per kilo (Kraft, 2005 p.145). Now known as Musk Toluene, this chemical and two other nitromusks, Musk Xylene and Musk Ketone, became very popular and in

1921 the famous Chanel No. 5 consisted of over 10% nitromusks, most of which was Musk Ketone (Kraft, 2005 p.150). Musk Ketone has an odour threshold of 0.1 ng L^{-1} in air, far exceeding that of Muscone (Kraft, 2005 p.164), however hazardous preparation, discolouration problems in consumer products, and phototoxicity concerns caused the decline of nitromusk production (Kraft, 2005 p.164). In 1994 industry organisations recommended they no longer be used (Sell, 2006 p. 98, SSNC, 2000) and their use is now restricted in Europe and the US (Rowe, 2011 p.14).

1.4.3.4 Polycyclic Musks (PCMs)

The first nitro-free aromatic musk introduced to perfumery was an acetyl indane discovered by Kurt Fuchs in 1951 (Kraft, 2005 p.152). Later named Phantolide® but known by toxicologists as AHMI (6-acetyl-1,1,2,3,3,5-hexamethylindan) this polycyclic arene proved to be very stable as did the PCM's that followed including Tonalide® (7-acetyl-1,1,3,4,4,6-hexamethyl-1,2,3,4-tetrahydronaphthalene syn. AHTN) and Galaxolide® (1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-*γ*-2-benzopyran syn. HHCB) (Sell, 2006 p. 98, Peck *et al*., 2006). These and other PCMs were used in high doses in both fine fragrances and other products from the late 1960s and in 1998 75% of musks were PCMs (Kraft, 2005 p.155). Unfortunately, the chemical stability of the PCMs meant they were also non-biodegradable and, together with their high octanol/water partition coefficients, this resulted in their bioaccumulation in fish and other marine organisms, human fat and breast milk (Peck *et al*., 2006, Yin *et al*., 2012). Nonetheless, for some years Galaxolide has been one of the most widely used musks with a production volume of 7000-8000 tonnes per annum (Kraft, 2005 p.154).

1.4.3.5 Synthetic Macrocycles

Throughout the 20th Century there were continual efforts to understand the structure of natural musks and to synthesise other macrocylic ketones and lactones (Kraft, 2005 p.155). Synthesis of these structures finally became commercially viable in the 1960s when ring expansion reactions based on cyclododecanone were discovered (Kraft, 2005 p.155). The most popular macrocyclic synthetic musks are Ethylene brassylate (syn. Musk T), Muscenone® and Nirvanolide® (Sell, 2006 p.123). Nirvanolide®, introduced in 2000 as a Givaudan captive, has an odour threshold of 0.1ng L^{-1} in air (Kraft, 2005 p.161).

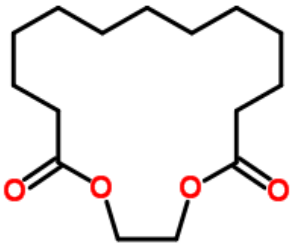


Figure 1.8 - Ethylene brassylate

(RSC, 2015)

1.4.3.6 New musk structures

Fragrance chemists continue to search for new chemical structures for musks and Helvetolide®, used in perfumes from 2000 onwards by Firmenich, is a cyclopentenyl ester (Kraft, 2005 p.164). A cyclopentanoate of Helvetolide also has a musk-like aroma and an odour threshold of 1.4 ng L^{-1} in air: this molecule has surprised perfumers: with a formula of $C_{20}H_{36}O_3$ and a molecular weight of 324.27, this may be the heaviest known odorous molecule (Kraft, 2005 p.164).

1.4.4 Restricted Aroma Compounds

There are a number of aroma chemicals, both natural and synthetic, which have restrictions on their use. These restrictions have largely come about following the work of the Research Institute for Fragrance Materials (RIFM) which was formed in 1966 and which has an ongoing program to evaluate the safety of fragrance ingredients (RIFM, 2015). Fragrance materials are tested to determine whether they may cause an allergic reaction, irritation or sensitisation and if a reaction is present then dermatological testing is used to establish a 'no-effect' level. The results of these tests are reviewed by an expert panel and then passed on to the International Fragrance Association (IFRA) which holds information on odour thresholds and commercial usage: if the fragrance material can usefully be used at $1/10^{th}$ of the 'no-effect' level, then a restriction is set at that level, otherwise it is banned (Meakins, 2006 p.187). All results of the RIFM tests are published in Journal of Food and Chemical Toxicology and all decisions on restrictions are communicated by IFRA to their members (Meakins, 2006 p.185, RIFM, 2015, IFRA, 2015a). There are currently 186 substances which IFRA have either banned or restricted their members from using in fragrance products including chemicals which demonstrate phototoxicity effects (e.g. a number of coumarin derivatives), those with significant environmental impact (e.g. Musk Xylene) and two materials considered to be at risk of neurotoxicity: the polycyclic musk AETT (6-acetyl-7-ethyl-1,1,4,4-tetramethyltetralin) and the nitro musk, Musk Ambrette (Meakins, 2006 p.194, IFRA, 2015c).

In addition to the work by RIFM and IFRA, the European Union also monitors and regulates the safety of fragrance ingredients and other cosmetics and the manner in which they are manufactured and marketed. This regulation was originally

included as part of the Cosmetics Directive which was adopted in 1976 (76/768/EEC) and revised many times over the following years. In 2009 the EU Directive was replaced by EU Regulation 1223/2009 (Cosmetics Regulation) which came fully into force in 2013 (EC, 2015a). Annex II of the Regulation lists substances prohibited in cosmetic products, and Annex III lists substances which are subject to restrictions when used in cosmetic products, including the 26 fragrance materials shown in Table 1.9 which are considered to be potential allergenic substances (CosIng, 2015).

The 26 potential allergenic substances (PAS) are of particular note as the legislation regarding these substances resulted in a new transparency from the fragrance industry with regard to the ingredients used in perfumes. The list of PAS was compiled by the Scientific Committee for Consumer Products (SCCP) which identified 26 fragrance ingredients seen as the most common cause of sensitisation and contact dermatitis (Meakins, 2006 p.190). As a result of the report by the SCCP, the 7th Amendment to the EU Cosmetics Directive (Directive 2003/15/EC) required that if these ingredients are present in 'leave-on' cosmetic products above 0.001% (10 ppm) or 'rinse-off' above 0.01% (100 ppm) they must be listed on the label (Meakins, 2006 p.190). This EU Directive had a profound effect on the fragrance industry and for the first time methods for the analysis of fragrances were published outside of trade journals (Augusto *et al*., 2003, Rastogi *et al*., 2003). Additionally, in preparation for the 7th Amendment taking effect in 2005, IFRA created a working group to develop and publish analytical methods for their members to use in the determination of the PAS (Chaintreau *et al*., 2003).

Table 1.9 - Potential Allergenic Substances

Name
Amyl Cinnamal
Amylcinnamyl Alcohol
Anisyl Alcohol
Benzyl Alcohol
Benzyl Benzoate
Benzyl Cinnamate
Benzyl Salicylate
Cinnamal
Cinnamyl Alcohol
Citral
Citronellol
Coumarin
d-Limonene
Eugenol
Farnesol
Geraniol
Hexyl Cinnamal
Hydroxy-citronellol
Isoeugenol
Lilial® (2-(4-tert-buyylbenzyl)propionaldehyde)
Linalool
Lyral® (hydroxy-methylpentylcyclohexenecarboxal dehyde)
Methylheptin Carbonate
α-Isomethyl Ionone
Oak Moss extract Evernia Prunastri *
Treemoss extract Evernia Furfuracea *

(CosIng, 2015)

* Note that Oak Moss extract and Treemoss extract are a mixture of chemicals many of which are non-volatile (Rastogi *et al*., 2004).

By 2006 both IFRA and The European Cosmetic Toiletry and Perfume Association (COLIPA) had produced technical guidance documents for their members which were also widely available to those outside the industry (COLIPA, 2006, IFRA, 2006). The IFRA working group also continues to publish refinements and validation procedures for the determination of PAS (Chaintreau *et al.*, 2011) and many other research groups now routinely publish methods for the analysis of the PAS and other chemicals thought to be of potential risk such as musks, phthalates and parabens (e.g. Sanchez-Prado *et al.*, 2011b). Finally, it should be noted that the industry is currently awaiting the outcome of a public consultation on the addition of more substances to Annex III of the Cosmetics Regulation and, significantly for the fragrance industry, the prohibition of the PAS Lyral®, a previously popular ingredient which has now found to be unsafe. Atranol and chloroatranol which are components of the fragrance materials Oak Moss and Tree Moss are also being considered for prohibition (EC, 2015b).

1.4.5 Non-aroma Compounds

Perfumes, antiperspirants and deodorants also contain ingredients other than aroma chemicals and these ingredients may be significant in analysis. Perfumes and other fine fragrances are hydroalcoholics with the fragrance material carried in a solution of ethanol and water. Commercial ethanol is an azeotropic solution of 95-96% ethanol and 4-5% water and the source of the ethanol may be synthetic or natural (Herman, 2005 p. 306). Natural sources of ethanol include grain starch, sugar beet or molasses with each considered to impart a slightly different odour to the end product (Beerling, 2006 p.171). Legally a denaturant must also be added to the ethanol to discourage the ingestion of the product and in most cases the denaturant is the extremely bitter denatorium benzoate (syn. Bitrex) which, used at

concentrations of 10ppm, does not affect the odour of end product (Beerling, 2006 p.171). Small amounts (0.1 %) of marker compounds such as t-butanol may also be added to the alcohol base (Herman, 2005 p. 307).

For aerosol antiperspirants and other fragranced products dispensed by an aerosol spray, a propane-butane blend is used as a propellant gas and the product formulation must be anhydrous to keep the active ingredient dry and to avoid rusting the can. The powdered formulation is therefore suspended in a volatile silicone fluid, usually cyclomethicone, which is soluble in the propellant–suspension system and sufficiently volatile to evaporate from the skin surface (Beerling, 2006 p.173). Solubility may be a problem in volatile silicone-fluid based antiperspirants (Beerling, 2006 p.168) and in products containing natural oils which may contain waxes that are insoluble in alcohol as well as crystals or resins that may precipitate (Herman, 2005 p. 307). A commonly used solubiliser is polysorbate but this and other solubilisers are used sparingly if possible as they can adversely affect how the product feels on the skin (Herman, 2005 p. 307).

There are also various chemicals added to the formulations of both hydroalcoholics and other systems to enhance the stability of the fragrance. UV radiation absorbers are commonly added, particularly to extend the shelf-life of perfumes sold in glass bottles. A commonly used UV stabiliser is Benzophenone-2 (Herman, 2005 p. 306) but there is growing concern that some of the benzotriazole derivatives used for this purpose have adverse effects on aquatic organisms (Montesdeoca-Esponda *et al*., 2013). Antioxidants may also be added to stabilise aroma chemicals such as hexanal and other simple aliphatic alcohols and prevent rancidity. Common antioxidants include BHT (butylated hydroxytoluene) and BHA

(butylated hydroanisole) as well as citric, tartaric or oxalic acids and a combination of these ingredients have been found to work synergistically (Herman, 2005 p. 306).

The final group of non-aroma compounds that may be included in a formulation are the ‘Active Ingredients’. These are chemicals which act on the human skin in a specific and well-defined way and which, in some cases, have pharmacological potential (Council of Europe, 2008 p. 5). For antiperspirant-deodorants the most common and best-proven actives are the anti-microbial, Triclosan, and the drying agents aluminium chlorohydrate (ACH) and aluminium-zirconium chlorohydrex-glycerine (AZG) (Beerling, 2006 p.173, Small, 2006 p.150). ACH and AZG are based on $AlCl_3$ which was the compound used in the first antiperspirants in 1903 (IFSCC, 1998) but the low pH of $AlCl_3$ leads to skin irritation and it is no longer considered safe (Schreiber, 2014). There are actually a range of allowable compositions for ACH according to the Al/Cl atomic ratio (from $Al_2(OH)_4Cl_2$ to $Al_2(OH)_5Cl$) and for AZG according to the (Al+Zr)/Cl atomic ratios (from $Al_8Zr(OH)_{20}Cl_8$ to $Al_4Zr(OH)_{13}Cl_3$) (IFSCC, 1998). Both ACH and AZG are buffered to improve the pH (ACH has a pH ~ 4.0) and in the case of AZG, the amino-acid glycine acts as the buffering agent (Rosenberg and Fitzgerald, 1999, Schreiber, 2014 p.141).

Other actives include healing agents and moisturisers. Healing agents such as allantoin are considered particularly useful in aftershave preparations (Herman, 2005 p. 305) and moisturisers are often found in the deo-colognes. Polypropylene glycol methyl glucose ether (Glucone P20 ® ex Americhol) is a popular moisturising agent as it also has useful fragrance fixative properties (Beerling,

2006). Dimethiconal, a high molecular weight silicone gum is also uses in aerosol products as it provides a soft moisturised skin feel and is readily diluted in cyclomethicone (supplied as DC1501 ®, ex Dow Corning). It also helps prevent the formation of an aerosol cloud which can cause choking or sneezing if inhaled (Beerling, 2006). Other chemicals are added to aftershaves and related products to modify the feel of the product on the skin: such chemicals include water soluble emollient oils such as glycerine and polypropylene glycol (PPG) as well as menthol or its ester derivatives for cooling effect (Herman, 2005 p. 307).

1.4.6 Summary

In order to have evidential value perfumes, deodorants and antiperspirants must be identifiable: as the large number of aroma chemicals used in fragrance formulations ensures that each new product on the market is different from its competitors, the target chemicals for analysis in this research are the aroma chemicals which are far more diverse than the non-aroma compounds. There are thousands of single-odour aroma chemicals produced, some relatively common and used in a range of products while others are reserved for high-market products due to their limited availability and price (Herman, 2005). Additionally, while perfumes and other hydroalcoholics are relatively benign, deodorants and antiperspirants are much harsher: this affects the aroma compounds used in such products and consequently even products from the same line which may have very similar odours are likely to have different chemical formulations. As each global fragrance company manufactures a portfolio of synthetic ingredients to ensure the originality of their products new aroma chemicals are continually launched and some are kept as ‘captives’ with their use is restricted to the

originating company (Pybus, 2006). Finally, as can be seen from the review of the chemical classes of aroma chemicals, these ingredients are generally apolar and volatile, and are ideally suited to analysis by the instrumental methods described in Section 1.5.

1.5 Analytical / Instrumental Methods

1.5.1 Gas Chromatography (GC)

Gas Chromatography (GC) is the technique of choice for the analysis of volatile organic compounds (VOCs) (Dewulf and Van Langenhove, 2002) and is seen as the most important analytical technique for perfume formulation, quality control and competitor analysis (van Asten, 2002). Following the industry recognition of potential fragrance allergens, methods for the quantification of the 24 volatile aroma chemicals (i.e. not including oak moss and tree moss extracts) were published by the European Cosmetic Toiletry and Perfume Association (COLPA) and the International Fragrance Association (IFA) (COLIPA, 2006, IFRA, 2006). GC analysis has also been successfully used for analysis of fragrances in a number of other cosmetic products, including deodorants and antiperspirants (Rastogi, 1995, Rastogi *et al*., 1998) and home and personal care (HPC) products (Jones *et al*., 2009, Baier, 2005, Homem *et al*., 2013, Celeiro *et al*., 2015). In the field of human chemical profile studies, GC has also proved a useful technique and has been used by almost every researcher (see for example Penn *et al*., 2007, Bernier *et al.*, 1999, Pandey and Kim, 2011).

For successful analysis by GC there are various criteria that must be optimised and the first of these is the column material that is used for the stationary phase. For the analysis of the 24 volatile allergens, which have a range of polarities, both IFRA and COLIPA recommend a stationary phase which is apolar to medium polarity (COLIPA, 2006, IFRA, 2006). For an apolar stationary phase the 100% methylpolysiloxane (DB1 or equivalent) columns are used and for medium polarity IFA recommend the DB17 type column (50% phenyl, 50% dimethylpolysiloxane).

However, in the fragrance and flavour industry the slightly polar DB5 (or equivalent) columns (5% phenyl methylpolysiloxane) are considered the most suitable and have the advantage of having more standard Retention Index (RI) values listed for them than for any other phase, partly because RI values are more reproducible on these rugged columns (Costa *et al*., 2007). For many fragrance chemicals the DB5 columns also provide slightly better separation than the DB1 (Bazemore, 2011 p.58). When analysing samples of human chemical profile however, Bernier (1999) found that on a DB5 column, polar compounds such as lactic acid produced poor peak shapes and carboxylic acids also produced non-symmetrical fronted peaks and were prone to overloading the column. Bester (2009) also noted that DB5 columns are not able to separate all the diastereomers of musks such as Galaxolide® (HHCB) and that the more polar RTX columns (50% phenyl in dimethylsiloxane) were more successful in this respect.

Some of the most ‘aroma active’ chemicals used in fragrance formulations are those which are more oxygenated and therefore more polar, so for these chemicals the more polar wax columns such as PEG FFA (Polyethylene Glygol - Free Fatty Acid) provide best separation (Bazemore, 2011 p.58). Using a standard fragrance mixture at 500 ppm Oosdijk and Farrell (2007) showed good response and separation on a VF-WAXms column and Divisova *et al.* used a DB-WAX column for determination of allergens in fragranced cosmetic products (2015). Additionally, Bernier (1999) found that some of the more polar human skin emanations had a better peak shape and retention on the polar Carbowax column than on the DB1 or DB5 columns. Unfortunately, these wax columns are less rugged and retention times will vary between manufacturers (due to variations in

coating thickness), between methods (with different oven conditions) and even with repeated injections (Bazemore, 2011 p.58). The IFRA method (2006) recommends that carbowax-type phases should not be used due to their poor batch-to-batch reproducibility and warned that due to their instability these columns require daily calibration. Bernier also noted that the Carbowax column had limitations in terms of temperature range and cannot be heated to the temperatures necessary to elute the heavier fatty acids and other lipids without producing high levels of stationary phase bleed (Bernier *et al*., 1999).

Following selection of a stationary phase the next criterion usually considered is the GC oven temperature program. The aim is to start with a low initial temperature to provide good resolution of compounds with low boiling points, then increase (ramp) the temperature at a suitable rate to reduce retention times while still achieving good peak resolution. The final temperature should then be set either according to the temperature at which the last compound elutes or the maximum temperature of the column, with additional hold time if necessary (Grob and Barry, 2004, Sparkman *et al*., 2011). The permutations of these conditions are numerous and will not be discussed in detail here but generally start temperatures are between 40 and 100˚C, final temperatures between 200 and 280 ˚C and ramp rates are between 3 and 25 ˚C min^{-1} (e.g. Chaintreau *et al*., 2003, Sanchez-Prado *et al*., 2011a). Within the field of fragrance analysis however, there is a recognised problem regarding the separation of the chromatogram peaks, in part due to the large number of chemical compounds in most fragrance mixtures but also because many aroma chemicals have very similar structures and retention characteristics, especially the terpenoids (Chaintreau *et al.*, 2011). Another difficulty is that fragrance companies will preferentially add high sensory-impact

aroma chemicals at very low concentration (Bazemore, 2011). These factors often mean that the full detail of the sample composition cannot easily be resolved within a reasonable timescale (e.g. around 30 minutes).

There are three main approaches that can be used to address the issue of complex chromatograms: chromatographic resolution, detector selectivity and resolution and analytical resolution i.e. data processing (Semard *et al*., 2009 p.40) Where chromatographic resolution has been unsuccessful using a single column it is now possible to analyse the sample using multi-dimensional chromatography which uses two stationary phases of differing polarity. One way to achieve this is to switch the effluent of one column periodically into the flow of a second column, which is sometimes known as heart-cutting (David and Klee, 2009, Boehme and Baier, 2009). Another approach is to use two-dimensional GC (GCxGC) where the effluent of the first column passes into a modulator which causes the peaks to sharpen before they pass into a second, shorter column housed in a separate oven. The usual configuration is to have a 30 m non-polar column for the first dimension which produces separation by volatility. The second (2D) column is usually a short (1-2 m) polar column which provides orthogonal separation according to the polarity of the analytes (Semard *et al.*, 2009 p.20) and GCxGC is fast becoming one of the most popular GC techniques for the analysis of fragrances material (see for example Augusto *et al.*, 2010, Devos *et al*., 2012, Tranchida *et al*., 2013).

The second approach to address the matter of complex chromatograms is detector selectivity and resolution and here a multi-dimensional detector such as a mass spectrometer (MS) has some advantages over the flame ionisation detector

(FID). GC-MS is now common across the industry (Chaintreau *et al*., 2003) and one of the most useful techniques is to use the MS in Selected Ion Monitoring (SIM) mode whereby the instrument is scanning for individual masses with a concurrent increase in the number of scans per second (Marriott and Shellie 2002). This not only improves resolution of peaks with the target ions but can reduce or eliminate the noise of any unwanted peaks. Both IFRA and COLIPA recommend that when quantifying the 24 volatile fragrance allergens, a set of three ions is used for each target analyte, with quantification performed using just one of the ions (COLIPA, 2006, IFRA, 2006, Chaintreau *et al*., 2011). Further improvements in the resolving power of the mass spectrometer can be achieved through the use of Time-of-Flight (TOF) technology where the ions are separated along a flight path before reaching the detector. Because the detector does not have to scan across the mass range, the acquisition rates are much higher and are independent of the mass range, which can radically improve the peak resolution in a chromatogram (Fjeldsted, 2003, Binkley, 2010).

The third approach to solving the problem of complex chromatograms is analytical resolution whereby resolution is achieved through data processing approaches such as deconvolution reporting software (DRS). This type of software uses sophisticated algorithms to process data regarding peak shape and the isotopic patterns of over-lapping peaks to effectively separate the spectra co-eluting compounds and visually present the peaks as fully resolved (Inoue and Toyo'oko, 2015 p.671). This type of software is increasing supplied with TOF instruments and has been used very successfully for the analysis of fragrance samples (Luan *et al*., 2008, Jones *et al*., 2009, Augusto *et al*., 2010).

It can be seen from the overview provided above that there is a wide range of techniques within the field of capillary gas chromatography. While techniques such as GC-TOF-MS offer great potential it should also be noted that a reasonably simple GC-FID or GC-MS system still has a key role to play in this research. For the purposes of discriminating between perfumes (establishing class characteristics) it may be that a full resolution of GC peaks is unnecessary and a pattern analysis approach can be employed as it is for analysis of fire accelerants. There may also be key chemicals (e.g. certain aroma chemicals) which are particularly useful indicators for the identification of a product, or which are particularly persistent and transferable. If these marker chemicals can be identified then GC-MS may be sufficient to identify them within samples. It is certainly the case however that the success of any analysis is largely dependent on the sampling and sample preparation techniques employed.

1.5.2 Methods other than Gas Chromatography

1.5.2.1 Direct MS

Direct analysis MS, without a separation phase, can also be used for the detection of counterfeit perfumes and, in 2006, researchers from the ThoMSon Mass Spectrometry laboratory in Brazil proposed the use of Direct Infusion Electrospray Ionisation MS (ESI-MS) as a screening technique. The method involves mixing a few microliters of the perfume with a 1:1 methanol : water solvent with a little formic acid and directly infusing the solution into the ESI source using a syringe pump (Marques *et al*., 2006). The same team later proposed a related technique, Easy Ambient Sonic-spray Ionisation (EASI-MS) in which the perfume is sample first sprayed on to paper or glass and minute charged droplets of the same solvent used in ESI are sprayed onto the sample by a nitrogen stream which is then drawn into the EASI source (Haddad *et al*., 2008). A third MS technique, Neutral Desorption Extractive Electrospray Ionisation (ND-EESI-MS) has been proposed by researchers from the Zenobi laboratory and with their system the perfume sample is again sprayed onto a paper substrate but the nitrogen stream is used to desorb the analyte molecules directly – the molecules are then charged by infusing them into an electrospray of pure solvent (Chingin *et al*., 2008).

In all three techniques the more polar compounds in the mixture are ionised and a mass spectrum is produced which combines the molecular ions of all the ionised compounds plus the few fragments which are produced. EASI uses the softest of the ionisation techniques and will produce protonated, deprotonated or sodiated ions whereas ESI spectra will primarily consist of protonated molecules. Additionally, EASI and EESI are effectively preparation free and ESI only requires

minimal sample preparation and all the techniques are extremely fast. Each technique does however have its drawbacks and ESI in particular can suffer from "memory effects" i.e. carry-over as less volatile, high mass "sticky" components can persist in the instrument transfer lines (Chingin *et al*., 2008) which makes the technique unsuitable for analysing crèmes, lotions and other leave-on personal-care products. ND-EESI suffers less from ion-suppression effects but the N_2 flow is another variable to control and the technique does suffer somewhat from "chemical noise" (probably due to background levels of volatile compounds in the laboratory air). ND-EESI also preferentially recovers more volatile compounds, compared to the other two techniques, although ions with m/z values of up to 659 were detected (Chingin *et al*., 2008). Finally, in soft ionisation techniques such as EASI the lack of fragmentation may make the resulting profile less discriminating and high resolution MS and MS-MS would be needed to provide more detailed information about some of the individual chemical compounds (Haddad *et al*., 2008).

In 2009 in the field of human scent investigation, Martínez-Lozano & de la Mora (2009) investigated vapour species and suspended particles above a human hand using secondary electrospray ionization (SESI) coupled with atmospheric pressure mass spectrometry (API-MS). This approach used an electrospray source to create a cloud of charged drops which are mixed with gases drawn into the sample chamber, ionising polar species. A peak was seen for lactic acid (m/z 89 following collision-induced dissociation) and around 20 other compounds, mainly volatile fatty acids (VFAs), up to m/z 281 (assigned to oleic acid) with the Time-of-Flight (TOF) mass spectrometer providing sufficient resolution between the VFAs. However, there were some interferences from the laboratory air also being drawn

into the sample chamber and, in this communication, the results from only one subject's hand were reported (Martinez-Lozano and de la Mora, 2009). Rodriguez-Lujan *et al.* (2013) later improved upon this system by using a clean gas to avoid high backgrounds. They also reported that by replacing the electrospray with a corona they could use higher temperatures to eliminate the memory effects caused by vapour deposition. They used 13 subjects and were reportedly able to achieve a recognition rate of over 85%.

Research continued with papers published in 2013 by Tobolkina *et al*. trialling electrostatic-spray ionization (ESTASI) and the world-renowned Cooks team reporting on low-temperature plasma (LTP) ionisation MS as a semi-quantitative method for volatile compounds in commercial cleaning products The ESTASI instrumentation successfully sampled fragrances from the hand onto blotting paper while the Cooks team sampled from glass, paper and cloth with limits of detection at the low pictogram level. Campbell *et al*. were also able to achieve chemical imaging with spatial resolution better than 1 cm. Such ionisation techniques also seem to be becoming more flexible with Tobolkina *et al.* reporting success in analysing non-volatile compounds such as peptides and proteins and *Campbell et al*. coupling their LTP ioniser with ion-trap, quadrupole and Orbitrap mass-analysers. However, sample sizes were again small with only eight fragrances analysed by the Cooks team and six by Tobolkina *et al.* (Campbell *et al*., 2013, Tobolkina *et al*., 2013).

Another ionisation approach is to use precursor ions generated from gases rather than solvents. In selected ion flow tube mass spectrometry (SIFT-MS) precursor ions (e.g. H_3O^+, NO^+ and O_2^+) are formed in a microwave discharge through moist

air and selected to react with the gas phase analyte (Turner *et al*., 2008, Smith and Spanel, 2015). This technique was used in the determination of a number of volatile compounds from the skin of five subjects and to demonstrate a change in acetone emissions with blood glucose concentrations (Turner *et al*., 2008). SIFT-MS is currently being used by a number of research groups, primarily for breath analysis and may have potential to become a semi-portable technique for real-time analysis of VOCs (Smith and Spanel, 2015). Proton Transfer Reaction (PTR) with Selected Reagent Ionisation (SRI) also uses different precursor ions and Mochalski and Amman's research team have used this technique in NO^+ mode to monitor 12 preselected VOCs from volunteers enclosed in a body-plethysmography chamber (Mochalski *et al.*, 2014). Quantitative measurement was achieved for a range of chemical classes (aldehydes, ketones and one terpene) in near real-time to establish emission rates with the aim of developing detectors for search and rescue operations. PTR-MS and SIFT-MS are both highly-sensitive techniques but the precursor ions must be correctly selected to ensure that the enthalpy of formation of analyte ions is higher than that of the reactant ions used (Agapiou *et al*., 2015).

It is clear from the research described above that there is a continuing trend towards direct sampling from a substrate – this has been a promising concept for several years with Direct Analysis in Real Time (DART) and Matrix Assisted Laser Desorption/Ionization (MALDI) and is now much more of a commercial reality with Waters, Thermo, Agilent and others all now offering high resolution mass spectrometers with flexible ionisation source architecture (e.g. Waters, 2015). A range of ionisation sources are available including Atmospheric Solids Analysis Probes (ASAP), Electrospray Ionisation (ESI), Desorption Electrospray Ionisation

(DESI) and Atmospheric Pressure Chemical Ionisation (ACPI). This offers a wide range of ionisation options for compounds of different molecular weights and for polar and non-polar species.

Direct MS has been used in the flavour and fragrance industry for some years for the analysis of liquid samples and without the expense of the advanced ionisation sources described above. In many cases a GC MS system is simply modified by replacing the GC column with a much shorter (1 m) capillary column, termed a 'retention gap', or by keeping the GC oven at around 220°C. This reduces the run time to 2 and 4 minutes, respectively. This combined with advanced multivariate analysis software such as Agilent's ChemSensor allows samples to quickly be categorised (Marsili, 2011 p.155).

The direct MS techniques described above are very fast and may have an advantage when dealing with complex sample matrices (Chingin *et al.*, 2008), but the lack of a separation step may limit the usefulness of the methods for discriminating between samples. In each of the papers cited above MS spectra were produced which were considered by the authors to be discriminating however sample size in all the studies was rather small and RSD values (where given) were rather high, especially in the EESI study. The MS systems now on the market offer excellent resolution and mass accuracy (1 ppm or better) and incorporate technologies such as mass traps and collision cells to produce fragmentation patterns as well as molecular ions. These technologies coupled with structure prediction software and the searchable resources such as ChemSpider now facilitate compound identification and although there are still questions to be answered regarding the degree of discrimination possible for complex samples, it

would be interesting to see whether the level of discrimination was maintained across the hundreds of fine fragrances available on the market.

1.5.2.2 Electronic Noses

Electronic noses, or e-noses, have been described as cheap, portable devices which can quickly detect a wide range of gaseous substances (Roeck *et al*., 2008, Wilson, 2012 p. 454, Sliwinska *et al.*, 2014) with the promise of high sensitivity, selectivity and the provision of results in real time (Pearce, 2003, Gutierrez and Horrillo, 2014 p.95). Such devices would seem to be an ideal solution to the challenges of performing fast analytical procedures to produce accurate identifications and classifications of samples (Di Natale *et al*., 2000, Dirinck *et al*., 2009) and have been proposed as a replacement for human sensory panels in the fragrance industry (Branca *et al*., 2003, Arshak *et al*., 2004). E-nose systems have been trialled at Yves St Laurent (Carrasco *et al*., 1998) and at IFF (Branca *et al.*, 2003) but in both cases it took significant time and effort to achieve results that were comparable to GC-MS analysis. Cano *et al*. (2011) used a commercial e-nose to test an authentic perfume against counterfeits and reduced analysis time to 10 minutes per sample reporting that the system outperformed a panel group. The sample size was however very limited and the samples needed preparation before analysis. One feature of e-nose devices that has particularly divided opinion is the reporting of differences between samples without detailed analysis of the chemical composition. While seen as an advantage by some (Cano *et al*., 2011) many industry chemists are unconvinced of the value of this approach and would like to understand what the signals correspond to and what the instrument is really measuring (Dirinck *et al.*, 2009). Fragrance laboratories in particular are more likely to turn to direct MS methods (see above), which they feel are better suited to

their existing knowledge and which some now refer to as ‘MS-nose’ systems (Dirinck *et al.*, 2009, Marsili, 2011 p.157).

1.5.2.3 High Performance Liquid Chromatography (HPLC)

While GC has become the standard technique for analysis of aroma chemicals within the perfume industry, it does have limitations in terms of the analysis of involatile and thermally labile substances and some limited research has been done on the use of HPLC for the analysis of perfumes, antiperspirants and deodorants. The best known of study in this area is the work done by Villa *et al.* (2007) who quantified 24 fragrance allergens with a C18 column and a Diode Array Detector (DAD), although the low resolution of the LC column resulted in some peaks overlapping, particularly the aromatic alcohols, anisyl alcohol and benzyl alcohol. Researchers at Coventry University successfully adapted the method of Villa *et al*. for the determination of Eugenol, Geraniol, Linalool, Citronellol and Limonene and the method proved sensitive enough to use in transfer experiments involving these five chemicals (Lodge and Reid, 2013). More recently, Pérez-Outeiral *et al.* (2015) combined the method of Villa *et al*. (2007) with ultrasound-assisted emulsification microextraction to achieve limits of detection (LODs) from 0.001 to 0.154 μg mL^{-1} although with poor peak resolution and significant broadening of some late eluting peaks. HPLC has also been used for the analysis of other restricted fragrance materials including the various components of Oak Moss extract (e.g. Schulz and Albroscheit, 1989, Feige *et al.*, 1993, Yoshimura *et al*., 1994). The complexity of this fragrance ingredient (a mixture of at least 170 chemicals) often leads to co-elution which can be overcome to some extent by using HPLC interfaced with tandem mass spectrometry (Feige

et al., 1993, Hiserodt *et al*., 2000) and a method for the quantitative analysis of the sensitizers atranol and chloroatranol has now been successfully developed and trialled (Bossi *et al*., 2004, Rastogi *et al*., 2004).

There are also a number of HPLC methods for the quantification of selected furocoumarins (e.g. Frerot and Decorzant, 2004, Govindarajan *et al*., 2007, Dugo *et al*., 2009, Vogl *et al*., 2011) but the Analytical Working Group of the International Fragrance Association (IFRA) reported that these methods had poor LODs and suffered from interferences by other matrix constituents and recommended the development of an HPLC-MS method (Macmaster *et al*., 2012). This challenge was taken up by Corbi *et al.* who published a method using a high resolution-accurate mass Orbitrap mass spectrometer to achieve limits of quantification around 0.01–1 mg L^{-1} for seven furocoumarins and successfully applied the method to 52 commercially available fragrances (Corbi *et al.*, 2014).

Less volatile, non-fragrance components in cosmetics such as UV stabilisers, antimicrobials and other preservatives have all been analysed using HPLC and reviews have been published by Peck *et al.* (2006) and Ocaña-González *et al.* (2015). In terms of antimicrobials, triclosan (2,4,4-trichloro-2-hydroxydiphenyl ether) is one of the most commonly used in deodorants and is unsuitable for analysis by GC without derivatization (Piccoli *et al*., 2002). A number of HPLC methods have been reported for determination of triclosan (e.g. Scalia *et al*., 1994) but the method proposed by Picolli *et al.* (2002) has the advantage of a very simple sample preparation and essentially quantitative recoveries (100.2–102.4%). Organic acids and phenol derivatives are also used as preservatives along with

the alkyl esters of benzoic acid (parabens) which have come under increasing focus due to their potential endocrine disrupting effects (Ocana-Gonazlez *et al.*, 2015, Marengo *et al*., 2001). HPLC is the standard technique for the determination of parabens (see Ocana-Gonazlez *et al*., 2015 and references therein) and as preservatives are often in multicomponent mixtures, work is ongoing by a number of researchers to find methods that will work with a range of hydrophilicities and chemical properties (Lee *et al*., 2006, Baranowska *et al.*, 2014).

1.5.2.4 Fourier Transform InfraRed Spectroscopy (FTIR)

Until recently very little use has been made of FTIR for the analysis of perfumes, antiperspirants, deodorants, cosmetic products or human scent. Some researchers such as Le Dreau *et al.* (2009) have however used FTIR to elucidate the aging process of essential oils and cosmetic emulsions (Masmoudi *et al*., 2005, Gallarate *et al*., 2009). Schultz *et al.* did a significant amount of work on the characterisation of essential oils using FTIR (as well as NIR and Raman Spectroscopy) and reported successful discrimination between some oils with the aid of statistical tools including principal component analysis (e.g. Schulz *et al*., 2002, 2003, 2005, Baranska *et al*., 2006). Rohman (2009) also successfully used FTIR for the quantitative analysis of virgin coconut oil in cosmetic creams. In terms of single aroma chemicals, Chu *et al*. used FTIR to understand the thermal degradation of phenylacetaldehyde to benzaldehyde (Chu and Yaylayan, 2008) and Wang *et al.* (2014) reported quantitative analysis of nine suspected fragrance allergens in commercial essential oils. As with the work of Schultz *et al*. (2005), Wang *et al*. (2014) found the statistical treatments were key to getting the most out of the data, and used hierarchical cluster analysis and partial least squares

regression on the characteristic region for each of the suspected fragrance allergens (Wang *et al*., 2014).

Recently FTIR has also proved useful for the analysis of the interaction between odours and odour precursors on fabric: Velmurugan *et al*. (2014) investigated the antibacterial activity of silver nanoparticle-coated fabric against odour and Rathinamoorthy *et al*. (2014) investigated the effect of Terminalia chebula extract coating fabric on odour forming short chain fatty acids. Kayar (2015) also investigated the effects of perfume on both mechanical and colour properties of cotton fabrics.

All of the work describe above has made use of attenuated total reflectance–infrared (ATR–IR) spectroscopy and the minimal sample preparation required by this technique.

1.6 Sampling and Sample Preparation

One of the advantages of GC as an analytical technique is that samples may be presented in gas, liquid or (via pyrolysis) solid form but in all cases the goals are to maintain the integrity of the sample (Bernier *et al*., 1999), to ensure the sample is representative of the source material (Jennings and Filsoof, 1977) and where possible incorporate some form of pre-concentration (Soini *et al.*, 2006).

1.6.1 Liquid Samples

For sample preparation of positive controls of liquid perfumes, primary sources of literature were sought which directly related to the analysis of such products. Unfortunately, although perfume houses and personal-care product companies routinely used GC to analyse their own and their competitors' products for decades, historically very little information was in the public domain due to the highly competitive nature of the industry (van Asten, 2002). However, in the mid-1990s the industry came under increasing pressure to address growing concerns about 'cosmetic dependant allergic contact dermatitis', for which perfumes were considered to be one of the major causes (Rastogi, 1995), and accordingly analytical methods started to be published in variety of sources. Most sources agree that liquid perfumes such as Eau de Toilette and aftershaves can be directly injected on to the GC with no preparation (Bazemore, 2011) but that dilution with a solvent in ratios of 1:1, 1:2 or 1:5 will aid the quantification of more concentrated analytes (Rastogi, 1995). The most basic requirements of the diluent are that it should be available at high purity, be non-reactive and elute from the GC column before the target compounds (Chaintreau *et al*., 2003) and popular solvents for the dilution of off-the-shelf products are methanol (Rastogi, 1995) and ethanol (Ellendt

et al., 2001, Mondello *et al.*, 2007, 2008, Cicchetti *et al*., 2008). This seems reasonable considering that perfumes and aftershaves are sold in an alcohol base, either of denatured ethanol or methanol (see Section 1.2). It is also important that the solvent system for calibration mixtures be similar to that used for the analyte as extraction efficiency is always matrix-dependant (COLIPA, 2006) and, while off-the-shelf products are formulated with UV stabilisers and other compounds to ensure they are stable for some time at room temperature and at moderate light levels, individual perfume component reference standards have no such protection (Sell, 2006, Salvador and Chisvert, 2007). Both Rastogi (1995), and Ellendt *et al.* (2001) reported problems with the stability of individual perfume component reference standards with more than a 10% decrease in concentration when stored at 20 ˚C or 4 ˚C after 24-30 hours.

Stability problems were also recognised in the landmark paper authored by the Analytical Working Group of (IFRA) the International Fragrance Association whose authors assert that protic solvents such as ethanol are not suitable due to their reactivity and specifically highlight the potential formation of acetals from the reaction of aldehydes with ethanol (Chaintreau *et al*, 2003). They also felt that ethanol and other volatile solvents were unsuitable as they could evaporate from standard solutions thereby affecting the concentration. As alternatives they recommended the use of isooctane for lipophilic fragrances and *o*-fluorotoluene for hydrophilic fragrances and listed the advantages of these compounds as their early elution, purity, low volatility and non-reactivity (although Chaintreau *et al.* mention in a 2011 paper that the *o*-fluorotoluene must be *o*-cresol free). The 2003 paper went on to become the basis for IFRA's Analytical Procedure and it is of note that version 2 of that publication (IFRA, 2006) recommends the use of *o*-

fluorotoluene for both lipophilic and hydrophilic fragrances but neither this, nor a later paper from the IFRA working group (Chaintreau *et al*., 2011) mention using isooctane.

A variety of other solvents have been used in published research and acetone is a popular choice (Leijs *et al*., 2005, Baier, 2005, David and Klee, 2009), with Leijs and colleagues from the fragrance giant IFF routinely using it as a solvent in their quality control process as it was less likely to overload the GC-MS system. Leijs *et al.* reported excellent performance from their method and appear to have overcome any stability problems by making up reference standards from stock on a weekly basis. Methyl trimethylacetate (syn. methyl pivalte) was 'under evaluation' by the IFRA working group in (2006) and later recommended by them as an inert and non-volatile solvent (Chaintreau *et al.*, 2011) although it does not seem to have been used in any of the other literature reviewed. Dichloromethane is recommended by some researchers (David *et al.*, 2006) possibly because it can also be used to extract perfume components from a wide variety of other personal-care products and ethyl acetate was recommended by Sanchez-Prado *et al.* (2011b) presumably as they found it more effective for the dilution of the wider variety of compounds of interest to them, including musks, phthalates and preservatives.

As can be seen there is no single solvent which all researchers agree upon and in fact Chaintreau's own team at Firmenich tested ethanol, dichloromethane and acetonitrile against other experimental parameters for their effect on the variability of response factors and did not identify solvent as a significant influence (Cicchetti *et al.*, 2008). The European Cosmetic, Toiletry and Perfumery Association seem to

recognise this in their Technical Guidance Document (COLIPA, 2006), as they state that the solvents recommended by IFRA should be used or “others (based on own experience)”: although with the proviso that quality, purity and origin information should be provided for each solvent and standard solutions and additional stability data should be provided for the standards. COLIPA do however require that the solvent system for calibration mixtures be similar to that used for the analyte and recommend that a standard additions method is used to verify extraction ratios.

1.6.2 Sampling Garments

Detailed literature describing sampling odours from garments is very limited and even industry insiders acknowledge that very little research on the analytical techniques for analysing odours in textiles has been published (Munk *et al*., 2000). Fortunately, core texts describing general sampling techniques used in the fragrance industry are available. Texts such as Goodner and Rouseff's *Practical Analysis of Flavour and Fragrance Materials* (2011) provide useful direction as do texts edited by Marsili (2012), Rowe (2005) and Sell (2006).

In light of the forensic aspects of this work non-destructive techniques which would preserve other forms of evidence such as fibres and DNA are preferred. As discussed in Section 1.5, FTIR-ATR is well suited to such an approach, but for chromatographic analysis a sampling / sample preparation stage is required to isolate the compounds of interest from the garment matrix and to produce a sample in suitable form for introduction to the instrument. Unfortunately, with the notable exception of the work by Denawaka *et al*. (2014) described below, the few studies which have been conducted on textiles by perfumery researchers and scientists working on laundry detergents have generally relied on solvent extraction (Hasegawa *et al.*, 2004, Munk *et al.*, 2000, 2001, Kubota *et al*., 2012, Takeuchi *et al*., 2012, 2013). However, as VOCs partition into gas phase, a representative proportion of the compounds will be present in the headspace around the garment which can be sampled in a manner suitable for GC analysis. Accordingly, the focus here is on headspace sampling and consequently the targeting of VOCs for analysis. This is appropriate as such compounds are likely to be among the most individualising in fragranced products (see Section 1.4).

Headspace sampling techniques are used in a wide range of applications (de Koning *et al*., 2009) and they have been a staple technique in the analysis of food aromas and in the identification of fragrance compounds from natural sources (Snow and Slack, 2002, Augusto *et al*., 2003, David and Sandra, 2007). As discussed previously headspace sampling is less used when sampling garments, therefore information on sampling techniques that could be adapted to this application was sought from other fields. In a forensic context the most common reason for sampling VOCs from clothing is to determine the presence or absence of ignitable liquids used as fire accelerants in cases of suspected arson (Newman, 2004b). The challenges of recovering samples from fire debris generally have been well examined over the years, so a number of those studies are reported below. Sampling techniques used for the analysis of human 'scent' were also investigated and are considered in this section where they may be applicable to sampling garments. In addition to these main areas, literature was also reviewed from fields as diverse as environmental science, human decomposition studies and pathogenic vector research (e.g. mosquito attractants).

Various review papers were also consulted for examples of headspace techniques and Woolfenden's (2010) review of sorbent-based sampling methods for air monitoring provided some useful information as did the review by de Koning *et al*. (2009) of Modern Methods of Sample Preparation for GC Analysis. Unfortunately, more general reviews of sample preparation were less helpful and the review by Chen *et al*. (2008) hardly mentions these approaches while Smith's (2003) review primarily focuses on sampling from the headspace of liquid matrices. This seems surprising considering that Majors (2002) reported that analysts' demand for sampling liquid and solid samples was essentially the same, but when reviewers

do consider solid samples the focus is generally on aggressive fluid-phase partitioning methods (Raynie, 2004, de Koning *et al*., 2009) and other destructive techniques such as pyrolysis (Smith, 2003). Authors such as Zygmunt and Namiesnik (2001) also refer to this imbalance, noting that solid samples seem to be the 'analytically most troublesome'. It is clear, however, that there are a number of headspace extraction mechanisms which may be suitable for collecting VOCs from garments ranging from directly sampling the static headspace to sorptive methods such as Solid Phase MicroExtraction (SPME). Accordingly these and related techniques are described below and their relative merits considered.

1.6.2.1 Static headspace (SHS)

The quickest and easiest method of extracting a headspace sample is simply to use a gas-tight syringe to collect an aliquot from a static, contained headspace above the object of interest and this approach is known as Static Headspace Sampling (SHS). Providing the matrix and headspace are at equilibrium, the concentration of a component in the gas-phase will be proportional to its concentration in the matrix, and if conducted at ambient temperature, SHS is considered to provide a 'true representation' of the chemical compounds responsible for the aroma of the source material as it reflects the 'natural' headspace concentrations (Bazemore, 2011 p.24). Not surprisingly, this simple form of headspace sampling was for many years the primary tool for analysis of VOC's in environmental and flavour and fragrance analysis (Snow and Slack, 2002) and has also been applied successfully for many years to the forensic analysis of ignitable liquids used as fire accelerants (Pohl and Keller, 1980).

To ensure that there is sufficient amount of the headspace to analyse without the requiring an excessive amount of time to reach equilibrium, it is recommended that the source material should take up two-thirds of the sample container (Bazemore, 2011), although this may prove hard to estimate when sampling garments. Setting up a sampling environment with a large volume of headspace is also unnecessary as the maximum acceptable volume for a gas injection on a capillary column with a standard injection port is about 200 µL (Kolb, 2009 p. 235). This volume is dictated by the column type and capacity of the injection port and injection of higher volumes will cause the pressure and flow to increase resulting in peak broadening and reduced resolution (Jennings and Filsoof, 1977).

The main advantages of static headspace sampling are simplicity, speed (allowing for equilibration time and heating) and that once collected in the syringe, the sample can be transferred into a standard GC injection port with no additional equipment. A forensic benefit is that sampling is effectively non-destructive and non-exhaustive allowing repeat and inter-laboratory analyses. Snow and Slack (2002) contend that it is the most versatile of headspace techniques and particularly suitable for use by non-specialists, however, core texts describing sampling techniques used in the fragrance industry (Rowe, 2005, Sell, 2006, Goodner and Rouseff, 2011, Marsili, 2012) all indicate that the static headspace methods are being superseded by SPME.

The key drawback of SHS is limited sensitivity and it is most effective for samples where the analyte concentration is high-ppb or greater (Snow and Slack, 2002, Tobiszewski *et al.*, 2012 p.111). Additionally, if the vapour sample is too dilute, only the components with higher vapour pressures may be present in sufficient

concentration to produce an identifiable detector response (Jennings and Filsoof, 1977) and, in samples consisting of a mixture of high and low volatility compounds, high concentrations of the more volatile components may saturate the headspace and inhibit the recovery of components that are less volatile (ASTM International, 2012a). Reproducibility may also be poor and Goodner and Rouseff (2011) state that SHS is 'notorious' for poor precision and even when the recommended source material to headspace ratio is maintained there are problems with non-equilibrium conditions and serial extractions.

For applications requiring accuracy and precision modern SHS instrumentation includes some additional steps to improve recovery such as an incubation chamber to maintain the vial at a set temperature and heated air-tight syringes (Denawaka *et al*., 2014). Other systems replace the syringe with a heated transfer line and pressurize the sample vial above the capillary column head pressure thereby allowing the sample to remain relatively inert whilst still allowing for rapid sample transfer (Snow and Slack, 2002). However such systems are designed for small volumes of liquid samples and are not currently suitable for sampling entire garments.

Another approach is to improve the sensitivity of the instrumentation and this can be achieved using GC-MS in selected ion monitoring mode and by using advanced detectors such as TOF-MS (see Section 1.5). Some researchers are also exploring the use of static headspace-multicapillary column-gas chromatography-ion mobility spectrometry (SHS-MCC-GC-IMS). This instrument uses approximately 1000 short capillary columns to achieve rapid chromatographic separations which, combined with IMS, results in a compact, fast

and sensitive technique which has been used for clinical samples and to differentiate between olive oils (Garrido-Delgado *et al*., 2012, Juenger *et al.*, 2012). Of particular interest is the application of this technique to determine 32 volatile components potentially responsible for sock malodour (Denawaka *et al.*, 2014). This work reported detection limits of 0.1 ng for (E)-2-nonenal, however, sock samples were limited to 0.5 g of material which was placed in an incubated vial prior to automatic sampling - making this technique unsuitable for larger garments (Denawaka *et al.*, 2014).

The literature reviewed above makes it clear that while SHS is still widely used in some fields, the poor sensitivity of the technique means that, in the absence of specialised instrumentation, it is most useful as a screening technique. For sampling VOCs from traces of perfumes, antiperspirants and deodorants on clothing it is likely that the concentration levels are likely to be too low to use SHS and a sampling technique with some sort of pre-concentration would be more suitable (Snow and Slack, 2002).

1.6.2.2 Trapping on Adsorbent Materials

For sampling at low concentrations it is advantageous to collect the sample onto adsorbent material thereby incorporating a pre-concentration phase (Jennings and Filsoof, 1977, Woolfenden, 2010). The collection may be achieved through passive, active or dynamic sampling and each of these approaches is described below, however, the same adsorbent materials may be used in each case and the choice has a significant bearing on sample selectivity. There are a wide range of materials which can be used including silica and activated magnesium silicate

(Floricil) (Pert *et al*., 2006), but the most common choices are activated carbon and a range of adsorbent resins (Ayoko, 2004) and these are discussed below. Other materials which have been used as traps include glass beads and cotton pads and these are discussed in later sections as they are more limited in application.

Activated carbon has been used as a selective adsorbent in sample preparation since at least the 1960s (Jennings and Filsoof, 1977) and has the benefit of being cheap and plentiful (Newman, 2004b). Activated carbon has a large capacity and is considered particularly suitable for sampling ignitable liquids from fire debris as it has a low affinity for water (which would interfere with GC analysis) and a high affinity for hydrocarbons (Jennings and Filsoof, 1977, Bertsch and Ren, 2000) and this affinity also extends to most aroma compounds (Reineccius and Heath, 2006). Use of activated carbon has fallen out of favour with some analysts and the main reason is that the strong interaction of the hydrocarbons with the charcoal requires that solvent extraction be used for sample recovery, normally with hazardous solvents such as carbon disulfide or diethyl ether (Steffen and Pawliszyn, 1996, Ren and Bertsch, 1999, Cacho *et al*., 2014). However, Bertsch and Holzer (1988) have reported that micro-scale sampling using just 5 mg of charcoal can be used to achieve good results while using just a few microliters of solvent and Massey *et al*. (2002) have reported that other solvents may be suitable in some cases and that dichloromethane will give acceptable and consistent results for sampling of a range of ignitable liquids.

In the 1970s a variety of porous polymer adsorbents became popular (see for example Jennings *et al*., 1972, Zlatkis *et al*., 1973), both as GC column packing

material and for use in sampling devices. These materials continue to be widely used today, usually packed in glass or stainless steel tubes for use in both in passive adsorption mode and for active sampling (Pert *et al*., 2006, Borusiewicz and Zieba-Palus, 2007). Most of the porous polymers have a low affinity for water, ethanol and low molecular-weight permanent gases (all potential interferents) and they have the additional advantage that they are suitable for thermal desorption (TD), eliminating the use of the hazardous solvents (Jennings and Filsoof, 1977, Andrasko, 1983). These materials are sold under their trade names and fall into four main families: Poropak consisting of first generation materials such as styrene-divinylbenzene copolymers; Chromosorb with uniform rigid structures of polyaromatic cross-linked materials; Hayesep a second generation material with minimal bleed developed for chemical weapons sampling; and Tenax®, 'the world's favorite TD sorbent' (Pert *et al*., 2006, Markes International, 2013, Sigma-Aldrich, 2015).

Tenax® is a polymer resin based on 2,6-diphenylene-oxide and is the most thermally stable of the porous polymers, whilst having a low affinity for water, a fast desorption time and requiring no additional treatment before re-use (Russell, 1981, Jones, 1986, Borusiewicz and Zieba-Palus, 2007). Tenax® is suitable for the analysis of straight chain alkanes ('normal' or 'n' alkanes) with six to 30 carbon atoms (n-$C_{6/7}$ to ~n-C_{30}), and most other compounds with boiling points in the same range, including aromatics (Markes International, 2013). However, Tenax does have an affinity for non-polar, high-boiling point compounds, meaning that these may be extracted preferentially (Borusiewicz and Zieba-Palus, 2007). Additionally, in a widely cited study on peanut aroma, Buckholz *et al.* (1980) reported that there was significant breakthrough of some compounds and,

although it should be noted that this was using Tenax® GC which has now been replaced by the improved Tenax® TA, authors warn that the material has a low adsorption capacity, potentially resulting in loss of components and an unrepresentative sample as well as component separation on the trap (Kebbekus and Mitra, 1998 p.219, Reineccius and Heath, 2006 p.45).

Perhaps the next most widely used sorbent materials are the Graphitized Carbon Blacks which include Carbopack, Carbotrap and Carbograph. These are also non-specific and, depending on the material, are suitable for normal alkanes n-C_8 to n-C_{20} or n-C_6 to n-$C_{12/14}$ and compounds with boiling points in the same range (Markes International, 2013). Some sorbents in this family contain traces of metals which can react with monoterpenes (Markes International, 2013) and as these compounds are extremely prevalent as aroma compounds (Sell, 2003) these sorbents would not be suitable for analysis of perfumed products on garments. Graphitized Carbon sorbents range widely in retention strength but the strongest adsorbent materials are the carbonised molecular sieves which include Carboxen and Carbosieve. Both materials are suitable for extraction of very low molecular weight materials (in the ranges of n-C_2 to n-C_6 and n-C_2 to n-C_5 respectively) but their applications can be limited due to the various degrees of water retention exhibited by these materials (Markes International, 2013).

Each of the adsorbent materials described above vary in selectivity, capacity and desorption properties and, when evaluating porous polymers in 1976, Butler and Burke concluded that there was no ‘universally suitable’ sorbent, a situation which still holds true today as evidenced by the development of new materials such as Hayesep® used particularly for smaller molecules (Butler and Burke). To add to

the difficulty in selecting a suitable sorbent, much of the literature comparing and evaluating sorbents for particular applications may now be out of date as products have been refined and superseded, as have sampling systems and instruments. For example, Jennings and Filsoof (1977) compared static headspace sampling, with extraction onto Tenax GC and Porapak Q for a model system selected for range of functional groups and boiling points (78-192˚C) and their results show clear differences between each extraction technique; while Agelopoulos and Pickett (1998), using a similar mix and also comparing static headspace against Tenax TA and Porapak Q reported that there was no significant difference between the chemical profiles obtained with these techniques. Butler and Burke (1976) suggested that the adsorbent be selected for the analytical problem and today, suppliers not only offer a wide range of sorbent materials and mesh sizes but also suggest suitable combinations of adsorbents (Markes International, 2013).

One further approach to trapping is cold-trapping or cryo-cooling where the sorbent material is held at a temperature at which the analytes will condense or even freeze in the trap (Wampler, 2002). Cold-trapping on to glass wool and other materials with a large surface area has been used for many years as a simple enhancement to the dynamic headspace and such traps will retain almost any volatile component (Reineccius and Heath, 2006 p.44). When coupled with the purge-and-trap methods described below, even compounds with low molecular weights are collected, and the lack of selectivity of the cold trap provides retention of all the components of the original sample (Bazemore, 2011). However this lack of selectivity can be a disadvantage when collecting samples with a high water or alcohol content as these compounds may be detrimental to chromatographic analysis (Jennings and Filsoof, 1977).

Cryo-trapping is also used in modern Automated Thermal Desorption (ATD) instruments to re-collect the sample following desorption from the primary trap (usually a sorbent packed tube as described above). This secondary, pre-column, trap is then flash heated to ensure the analytes are transferred to the GC column as a sharp band (Snow and Slack, 2002, Pert *et al.*, 2006). The technique works well with Tenax as the pre-column trap as a 10°C reduction in temperature doubles the retention capacity of this material (Brown and Purnell, 1979). Another advantage of using Tenax is that the hydrophobic nature of the material reduces the risk of ice forming in the apparatus which would cause damage by expanding the joints (Bazemore, 2011). Modern instruments also include a 'dry-purge' stage prior to desorption of the sample to further reduce the risk of ice damage (PerkinElmer, 2015).

1.6.2.3 Passive (Diffusive) Sampling and Passive Headspace Concentration

Passive sampling uses the principles of mass transport where gaseous analytes will diffuse into the sampling area and be adsorbed on to the trapping material (the passive sampling media, PSM). The rate of diffusion is proportional to the diffusion coefficient for the analyte and the surface area of the adsorbent material as described by Fick's Law and can also be calculated by the equation below (Sunesson, 2007).

$$\text{The rate of diffusion, } J, = \frac{\text{mass adsorbed (g)}}{\text{time (s)}}$$

Equation 1 - The rate of diffusion (*J*)

Passive headspace sampling is routinely used in arson investigation where it is known as ‘passive headspace concentration’ (Pert *et al.*, 2006, Newman, 2004b, ASTM International, 2012b) but the approach differs to that used in environmental analysis as it is used to determine the VOCs associated with an item rather than the environment. The item to be sampled is placed in a container such as a metal can, glass jar or nylon bag (Stauffer *et al*., 2008) thereby creating a static headspace which is sampled by passive adsorption under equilibrium conditions. In the United States, the most common technique for sampling fire debris is passive headspace concentration using an activated charcoal strip (ACS) which is suspended in the (static) headspace of the sample and subsequently eluted with carbon disulphide (Pert *et al.*, 2006, Newman, 2004b, ASTM International, 2012b). As with SHS, composition of the headspace depends on the partition coefficients and related factors and, as with diffusive sampling described above, the gas phase molecules move around the headspace by diffusion and randomly collide with the ACS and thus become adsorbed (Newman, 2004b). Because of this mechanism, the amount of sample which may be recovered is again largely dependent on time, and standard practice is to sample for 16 hours (Harris and Wheeler, 2003, Baerncopf and Hutches, 2014) although Warnke *et al.* (2005) contend that extractions can be obtained in less than 5 hours. As described previously, the partition coefficients of target analytes are temperature dependent and therefore temperature is the other key factor in recovery levels (Pert *et al*., 2006). For fire debris sampling, temperatures above 60˚C are generally used to ensure the heavier compounds are recovered, including the higher boiling-point n-alkanes which can be used diagnostically to confirm identification of some ignitable liquids (Newman *et al.*, 1996). Passive headspace with ACS has also been used to sample clothing and shoes to determine if petrol is present in the environment for

certain workers (e.g. those using petrol lawnmowers). Petrol was fully profiled from the positive controls and sampling was conducted for 16 hours at room temperature (Coulson *et al.*, 2008).

Passive headspace concentration can also be used with trapping materials other than ACS and porous polymers have the advantage of thermal desorption and therefore automation (Jackowski, 1997, Pert *et al*., 2006). However, while Carbotrap has been successfully used for recovery of ignitable liquids, the use of Tenax in adsorption tubes was less successful with a poor recovery of high boiling point compounds even when temperatures increased to 120˚C (Borusiewicz *et al*., 2004, Borusiewicz and Zieba-Palus, 2007). While passive headspace concentration is favored by arson investigators in the US for its simplicity and nondestructive nature, the long sampling times and the use of toxic organic solvents have resulted in passive sampling with ACS falling out of favour with the UK forensic community (Waters and Palmer, 1993, Cacho *et al*., 2014) and techniques such as active (pumped) sampling which offer a shorter sampling time and greater pre-concentration have been found to be advantageous.

1.6.2.4 Active (Pumped) Sampling

When using active sampling, the gaseous sample (from either an open environment or a confined headspace) is moved onto the sorbent trap, either by pumping (pushing) or by suction (pulling). The total volume is controlled and recorded, either by use of a marked suction device or by monitoring the flow rate and time of the pump used (Ayoko, 2004). The greater volume of sample collected onto the trap in a short time results in a very efficient technique but care

must be taken not to exceed the breakthrough volume of the sorbent. Recommended precautions include the inclusion of a second sorbent trap in series (which can be analyzed to determine if breakthrough has occurred) and the setting of ‘safe sampling volumes’ at <66% of the breakthrough volume (Ayoko, 2004). Smaller screening samples may also be taken to establish the concentration of analytes particularly when sampling from a headspace (ASTM, 2012).

As discussed previously, the trap material must be selected according to the chemical class and molecular weight of the target analytes and Tenax TA, Porapak Q and activated charcoal are all popular choices (Agelopoulos and Pickett, 1998). However Reineccius warns that even with a broadly selective trap such as Tenax, dynamic sampling rarely results in a chemical mixture that is representative of the odor due to biases of which volatiles enter the gas stream and which are trapped on the sorbent material (2006 p.45). A further consideration is the method for recovering the sample from the trap, and while thermal desorption is currently favored, solvent elution does not require any specialized equipment (Bertsch and Ren, 2000, Agelopoulos and Pickett, 1998).

In the UK, active sampling is the technique currently used for forensic analysis of ignitable liquids (Jones, 1986, Partridge, 2014, Markes International, 2014). Fire debris which is thought to contain traces of accelerants is sealed into nylon bags, heated to 125˚C and, following extraction of a screening sample using static headspace, a volume of up to 100 mL is extracted through a tube packed with around 70 mg of Tenax. The tube is then sealed and placed in an Automated Thermal Desorber (ATD) in which the analytes are desorbed from the Tenax at 225-250˚C, cryo-focused at -30˚C and transferred directly to the GC. This

approach is much faster than the passive sampling described above and sample collection can take place in less than 30 minutes. Thermal desorption and GC analysis takes another 30 minutes but this can be done as a batch overnight.

Active sampling is also widely used in environmental studies (Ayoko, 2004) which are of interest here because of the chemical classes examined. For example, Hollender *et al*. (2002) monitored indoor air for monoterpenes which are also prevalent in fragrance mixtures. Air was drawn through sorbent tubes at 10 ml min^{-1} for 1 hour with the parameters chosen to avoid exceeding the breakthrough volume (BTV) of the tube. A range of different sorbent materials were tested and Tenax GR (Tenax mixed with 23% graphitized carbon) produced the best results with detection limits of approximately 1 µg m^{-3} being achieved. In another study Rodriguez-Navas *et al*. monitored VOCs from municipal waste treatment plants, pumping over 3 L of air onto tubes packed with Tenax™ TA and Carboxen™ 1000 to determine that terpenes and aromatic compounds were the most prevalent type of the 93 compounds detected (Rodriguez-Navas *et al*., 2012)

The simplicity of active sampling has resulted in the development of two portable devices which have been used for forensic applications in the United States. The first is the Portable Arson Sampler which has been designed to sample debris at the fire scene (Almirall and Furton, 2004 p.86, Stauffer *et al.*, 2008 p.171). Debris is placed into a Teflon-lined chamber which is heated at 50-60˚C for at least 10 minutes and then the headspace gases are drawn through a tube packed with charcoal or polymer beads. Conner *et al.* (2006) felt that it efficiently collected volatile compounds from burned debris and that although lower volatility compounds were not collected as efficiently as the higher volatility compounds, an

identifiable pattern of compounds was still recovered from diesel fuel samples. However, the chromatograms published by Conner *et al*. are not completely convincing and other authors such as Stauffer *et al*. (2008 p.170) have yet to be convinced of the benefit of this device over sample recovery and analysis in the laboratory.

The second portable device of note is the Scent Transfer Unit, STU-100, developed by Tolhurst and Harris (1998) to collect 'scent' from an evidence item or area with a pump (Curran *et al*., 2005a, Eckenrode *et al.*, 2006). The sample is collected onto a sterile cotton gauze pad, a trapping medium specifically chosen so that it can then be made available to a trained canine to initiate a track or for scent-article matching without contamination or disruption of the evidence (Eckenrode *et al*., 2006, Big T LLC, 2015). Whilst the use of the device had proven successful in field trials with bloodhounds, the ability of the cotton pads to adsorb and desorb chemical compounds was evaluated using GC-MS by an extended team from the Federal Bureau of Investigation at Quantico and the Oak Ridge National Laboratory (Eckenrode *et al*., 2006). The results were disappointing, with only 15 chemicals out of the 39 VOCs used in a test mix being detectable and both recovery and release studies producing very variable results across samples (Eckenrode *et al.*, 2006).

While Eckenrode's team sampled a test mix using rate of 300 L min^{-1} a later study used the lowest airflow setting of the device, 86.04 L min^{-1}, which had been established as optimal during pilot studies (Prada *et al*., 2011). This second team working under the direction of Kenneth Furton and based at the International Forensic Research Institute at Florida International University also evaluated the

STU-100, with a focus on human hand odor and with consideration of the sorbent material. The team tested cotton, viscose rayon, polyester and wool and recovered only 20 compounds in total (compared to a total of 58 using contact sampling but with the same four textile materials). They also noted that while polyester performed well for recovery of carboxylic acids and wool for the recovery of aldehydes, cotton and rayon performed well across a range of chemical classes and produced the most reproducible results. It should be noted that overall, samples collected with the STU-100 produced rather poor and highly variable recoveries but also that samples were only exposed to the device for 1 minute (Prada *et al*., 2011).

The literature reviewed above makes it clear that active sampling is simple and quick, and the conditions are easily controlled but some caution should be observed: as with other trapping methods the concentration of the analytes must be suitable and the choice of trapping material is key. Also, where elevated temperatures are used there is the potential to degrade thermally labile compounds and for samples at higher concentrations a screening sample should be taken so that the pumped volume can be adjusted to avoid exceeding the breakthrough volume of either the sampling trap or the pre-column cryo-trap. Those techniques described above involving the headspace sampling of items placed in a bag or chamber would seem to be ideally suited to the sampling fragrance VOCs from garments but for very low concentration samples the dynamic headspace techniques described below will improve sensitivity.

1.6.2.5 Dynamic Headspace Sampling and Related Techniques

The active sampling methods described above move a discrete portion of a gaseous sample on to a trap but when sampling the headspace of an item there is also the option to collect the entirety of the gaseous sample. In Dynamic Headspace Sampling (DHS) the sample is placed in an 'entrainment chamber' and the headspace is purged: the gas is moved out of the chamber and replaced with an inert gas or purified air (Bazemore, 2011). Not only is the full volume of headspace gas collected but this continuous flow of gas over the sample changes the equilibrium between the headspace and the source material thus favoring further extraction into gas phase (Wampler, 2002, Smith, 2003, Gutierrez and Horrillo, 2014). The collected gas is subsequently trapped either in a simple cold trap or onto a sorbent material resulting in a very sensitive technique (Agelopoulos and Pickett, 1998, Bazemore, 2011). As with other trapping techniques, careful selection of the sorbent is needed and with selection of the sample temperature, specific analytes can be targeted for collection (Wampler, 2002). Other key factors are equilibrium time, extractor-gas flow rate and purge time but with careful attention to contaminants and instrument background, samples at ppb and even ppt level can be collected for analysis (Gutierrez and Horrillo, 2014).

In the field of chemical ecology DHS techniques are routinely used for the identification and quantification of fragrance compounds and volatile semiochemicals from living organisms placed into a glass bell or polyvinyl-acetate bag (Agelopoulos and Pickett, 1998). In these applications, purified air is used instead of an inert gas in order to keep the organism alive but this does incur a risk that certain compounds may be oxidized (Bazemore, 2011). An example of this approach is the work conducted by Vercammen *et al.* (2000) who allowed

flowering plants to equilibrate for 1 hour in a glass bell before sampling 100 ml min^{-1} for 10 minutes onto sample tubes packed with Tenax or PDMS which were thermally desorbed. This research team found that although Tenax is a popular sorbent some Tenax decomposition products were observed which could be mistaken for plant volatiles (benzaldehyde and acetophenone). Additionally, the results showed that PDMS packing had the highest recovery, especially of higher molecular weight compounds; the recoveries using DHS were comparable with extraction by SPME (discussed below). Using a similar approach, Smid *et al.* (2002), sampled Brussels sprouts plants, trapping the headspace at 500 mL min^{-1} for 5 hours onto 150 mg of Tenax. In this case the make-up air was thoroughly cleaned by molecular traps (4 Å), silica gel, Tenax traps and activated charcoal. Samples were eluted with pentane and evaporated to about 150 µL for GC injection. A subsequent study using this method was also conducted by Qiu *et al.* (2004) to determine which foot odours are attractant for *Anopheles gambiae,* the malaria mosquito, by performing dynamic headspace trapping of nylon stockings.

In the flavour and fragrance industry dynamic headspace sampling of shirt material was used by researchers from the Japanese cosmetics company Shiseido and fragrance company Takasago (Haze *et al*., 2001) during their research into the effect of aging on human body odour. The team asked subjects to wear a shirt for three days after which time a 20 x 30 cm piece of fabric was cut from the back of the shirt and sealed into a 10 L TEDLA bag. The make-up air used during the headspace sampling was deodorized with activated carbon and pumped in at a rate of 1.8 L min^{-1} and the resultant sample was trapped onto a Tenax-TA column. Headspace collection was carried out at 23˚C for 18 hours and there was no mention in the study of how these conditions were optimized or whether checks for

breakthrough from the trap were included. The analytes were also then eluted from the column with 10 ml of diethyl ether and although, following addition of an internal standard, the sample was evaporated to 100 µL to increase the concentration, this procedure may have resulted in the loss of some compounds. This is reflected in the results as just 22 compounds were reported, although 2-nonenal (considered a potential marker of aging) was detected at concentrations of less than 5 ppm (Haze *et al.*, 2001).

In forensic applications an exhaustive extraction is not always desirable (Pert *et al*., 2006) however DHS has been used for analysis of fire debris in the US where it is known as Dynamic Headspace Concentration (ASTM International, 2013). In the method recommended by the ASTM the specified adsorbent is either activated charcoal (with solvent elution) or Tenax (with thermal desorption) (ASTM International, 2013). The purging gas is typically purified air and a negative pressure approach is usual where a vacuum pump is attached to the tube which has been inserted into the sample chamber (Newman, 2004a). Sampling is conducted for 5 minutes at 90°C, which is a significant time saving over the passive headspace technique, but DHS is considered controversial because of its potentially destructive nature (Newman, 2004a, Lentini, 2012) . Modern ATD instrumentation does however offer a solution to this problem as samples can now be re-collected. When the sample is desorbed from the secondary, pre-column, trap the sample flow is normally split so as to avoid overloading the GC column. Normally the split effluent is vented but with instruments such as the PerkinElmer TurboMatrix 650 ATD, this portion of the sample can be re-directed back onto the primary Tenax tube or onto a fresh tube (Goodman, 2007).

The flavor and fragrance industry has historically used dynamic headspace methods in two distinct forms: DHS as described above and 'Purge and Trap' (P&T) where the gas flow passes through the sample (Bazemore, 2011, Gutierrez and Horrillo, 2014). This second technique is only appropriate for certain types of sample and is usually applied to liquid samples, although P&T has also been applied to soil samples (Snow and Slack, 2002 p. 615) and it may be possible to apply the technique to the sampling of garments.

DHS offers many of the same advantages of SHS: elimination of the solvent peak, analysis of just the volatiles, automation and easy sample preparation. Additionally, DHS provides increased sensitivity with analyses routinely detecting analytes at ppb concentrations and even ppt with careful attention to contaminants and instrument background (Wampler, 2002). Dynamic headspace methods are however, still less popular than passive equilibrium methods as they are inherently labor intensive and prone to contamination via the vacuum or gas supply; plus instrumentation is not cheap and is maintenance heavy (Waters and Palmer, 1993, Wampler, 2002). From a forensic point of view it should also be noted that DHS is an exhaustive, and therefore destructive, method of sampling which is not always desirable (Pert *et al.*, 2006).

1.6.2.6 Solid Phase MicroExtraction (SPME)

The sampling techniques described above are based on the use of adsorbent material but an alternative is sorptive extraction where the analytes are absorbed into a sorbent material (David and Sandra, 2007, Greenwood *et al*., 2007). Of this

group of techniques, the most popular is Solid Phase Microextraction (SPME) in which the sorbent takes the form of a coated fused silica fibre (Snow and Slack, 2002 p. 612). The most widely used sorbent fibre coating is polydimethylsiloxane (PDMS) (Bazemore, 2011 p. 58). In sorptive extraction the mechanism is equilibrium-based between the gaseous (or liquid) matrix and a sorbent phase, into which the solutes migrate (David and Sandra, 2007). In headspace-SPME (HS-SPME) this becomes a three-phase equilibrium between the sample, its vapours and the SPME fibre and the practical effect of this is that analytes are concentrated onto the fibre (Agelopoulos and Pickett, 1998). The procedure is extremely straightforward with the fibre being exposed in the headspace (or liquid sample) for around 10-30 minutes then retracted back into the holder (Pert *et al*., 2006). The fibre is then exposed in the heated injection chamber of any standard GC whereupon the analytes are thermally desorbed from the fibre, a process which can often be achieved in 30 seconds (though a 10 minute desorption will also clean the fibre). This process has proved effective at recovering a wide range of analytes from samples at concentrations of parts per billion or better (Snow and Slack, 2002 p. 612).

Various SPME fibre types are available providing some degree of selectivity and all are inert, can be rapidly desorbed at mild temperatures and have good thermal stability (David and Sandra, 2007). PDMS is particularly favored for its inertness and its efficiency in adsorbing polar compounds (Ayoko, 2004 p. 8, Pert *et al.*, 2006) although it has relatively low extraction efficiency for partially water soluble solutes (Arthur *et al*., 1992) and if extracting a polar compound from an aqueous matrix, competition can occur (David and Sandra, 2007). Of particular interest to the flavor and fragrance industry however, is that PDMS extraction capabilities can

be predicted based on octanol-water partition coefficients which have widespread application in the perfume industry (Perring, 2006 p. 208, Bazemore, 2011). For heavy polar compounds such as vanillin, a mixture of PDMS and divinylbenzene (PDMS-DVB) works well and for organic acids a carbowax-DVB mix is recommended (Augusto *et al.*, 2003, Divisova *et al.*, 2015) while Carboxen fibres may prove useful for sampling water-soluble alcohols from aqueous matrices (Pert *et al*., 2006). Fibres are also available with different thicknesses of the sorbent material, which changes the capacity and specificity in much the same way as the thickness of the stationary phase in a GC column: i.e. with thicker phases more efficient for volatile compounds, and thinner coatings favouring large hydrophobic molecules (Harris and Wheeler, 2003).

In the flavour and fragrance industry SPME is a very highly regarded technique (Marsili, 2012 p. v) especially suitable for qualitative and quantitative analysis of fragrance compounds released by solid and liquid samples (Augusto *et al.*, 2003). SPME is also considered an appealing alternative to DHS and as with other headspace approaches benefits from being a solvent-less technique allowing the detection of highly volatile compounds that would otherwise be blocked by the solvent peak (Bazemore, 2011 p. 58). Commentators such as Harmon (2002) consider SPME to approach the ideal of providing an analytical sample quickly, with minimal processing, and whose composition is as close as possible to that of the original chemical mixture within the matrix, although when used for headspace sampling it can preferentially recover low molecular weight compounds (Pert *et al.*, 2006).

SPME has been used for a wide range of applications from environmental to

biomedical (de Koning *et al.*, 2009) and been used to collect samples from the jungles of Madagascar to the International Space Station (Clery, 2002, Mookherjee *et al*., 2002). Of particular interest to this research is Stapleton and Dean‘s use of SPME for the characterisation of odour that can occur after a laundry wash cycle (Stapleton and Dean, 2013). A polyacrylic fibre was used to sample VOCs from various fabrics all sized 5 x 5 cm and although little information was provided on sample containers, a 15 minute equilibration time was followed by extraction at 50˚C for 10 minutes. Target compounds demonstrated very different limits of detection depending on the fabric used, but all the LODs were in the ng range and 0.4 ng for guaiacol on cotton (Stapleton and Dean, 2013). Chien *et al.* (2011) also used SPME to evaluate VOCs from clothing textiles that had been exposed to cigarette smoke. Again small rectangles of fabric were used (2 cm by 8 cm) and placed in headspace vials to equilibrate at 37 ˚C for 15 minutes before being sampled using a CW/DVB SPME fibre for 10 minutes. Nearly 70 different compounds were tentatively identified (as determined by NIST matches above 80) but LODs and actual concentrations are not given as the authors opted to quantify components using ‘toluene-reference concentration’ where toluene headspace standards were used from 20 ug ml^{-1} (Chien *et al*., 2011).

SPME has also been used semi-routinely as a screening test for forensic analysis of ignitable liquids (Steffen and Pawliszyn, 1996, Harris and Wheeler, 2003, Almirall and Furton, 2004 p.86). The ASTM guidelines make it clear that the sensitivity is not in doubt and state that a 0.1 µL spike of gasoline on a wipe inside a 1 gallon can is detectable using this technique (ASTM International, 2015). Steffen and Pawliszyn (1996) also consider SPME GC-FID an excellent screening technique for fire debris and recommend heating the sample in a sealed glass

container for 15 minutes at 95 ˚C then using selected ion chromatograms (SIC) at m/z 57 (for normal or branched alkane fragments) and m/z 120 (for alkyl substituted benzene fragments) to interferences from the fire debris (Steffen and Pawliszyn, 1996). It should be noted however, that Lloyd and Edmiston (2003) found that both PDMS and Carboxen/PDMS fibres preferentially extracted aliphatic and aromatic compounds from fire debris and that the compounds extracted varied depended on sampling temperature as well as fibre type (Lloyd and Edmiston, 2003). Sandercock's 2008 review of fire investigation and ignitable liquid residue analysis show that while SPME has been the subject of a number of research papers it is yet to become a common technique in many forensic laboratories around the world (Sandercock, 2008). While there was recognition that the SPME fibre assembly was portable, there have also been concerns raised about the fragility of the fibres, their limited lifespan and the inability to archive samples (see Pert *et al*., 2006, Sandercock, 2008 and references therein). Kwon, *et al*. (2003) also showed that highly volatile accelerants were better sampled using a Carbopack sampling tube than with a SPME fibre. However, Pert *et al.* (2006) also indicated that investigators may feel that HS-SPME is too sensitive for use with fire debris samples, and may detect previously undetectable levels of petroleum-derived compounds in samples, which may not necessarily be of accelerant origin, thus giving false positives (Pert *et al*., 2006).

Detractors of SPME find issue with the low recoveries of SPME sampling which are sometimes as low as 10 % (de Koning *et al.*, 2009) but it could be argued that if sufficient sample is collected to produce useful GC results then a less exhaustive extraction may be a benefit. Augusto *et al.* (2003) in their review of sample preparation for fragrances, noted the SPME sometimes required longer equilibrium

times for heavier compounds but other review authors concluded that SPME is faster than most other methods and simpler, because it does not require a special thermal desorption device (de Koning *et al.*, 2009). The same paper also asserts that if a detector such as MS in the SIM mode is used, LODs for both volatile and semi-volatile analytes typically are in the low-ng mL^{-1}, and sometimes in the ng L^{-1}, range (de Koning *et al.*, 2009). Ultimately though it is the ease of use that many analysts find most attractive about SPME and as noted by Snow and Slack (2002 p. 613) large amounts of sample information can be gained following relatively straightforward procedures.

1.6.3 Summary of Sampling

Jennings and Filsoof warn that regardless of which sampling method is used it is unlikely that a truly representative profile can be produced (Jennings and Filsoof), so the researcher must endeavour to find the sampling method that will be most suitable for the analytical technique and provide the most useful information. The goals of such sampling are that the sample should be representative of the whole and ideally should not contain compounds which might be considered interferents (Harmon, 2002 p. 75). Interferents might include unwanted compounds from the sample matrix or solvent residue and even impurities from the sampling technique, so the sample preparation technique must be chosen to minimise these issues, something for which the headspace techniques seem very well suited. Speed and ease of sampling are also considerations and, for this research, non-destructive sampling of VOCs from source material is a key requirement (David and Sandra, 2007).

1.7 Research Hypothesis and Aims

The hypothesis underlying the research that follows is that perfumes, antiperspirants and deodorants have characteristics which make them valuable as forensic evidence: they can be recovered from a crime scene, their products are distinguishable from each other and they are persistent and transferable. This hypothesis was used to set out five key aims of the research project:

A. **Distinguishing between products:** Investigate whether perfumes, antiperspirants and deodorants can be profiled and distinguished from each other efficiently by rapid chemical analysis.

B. **Storage:** Investigate how different storage conditions affect samples and their identification.

C. **Investigate how samples age:** Investigate whether perfumes, antiperspirants and deodorants age in a predictable manner.

D. **Recovery of Liquid Residues:** Explore whether traces of the products under study can be sampled and identified by chemical analysis through comparison with a positive control sample.

E. **Garment sampling:** Investigate whether samples can be collected from garments and identified by chemical analysis through comparison with a positive control sample.

2 Experimental

2.1 Samples and Reagents

For the standards, a range of 42 perfumed products were collected: perfumed body-sprays for women, deodorising body-sprays for men, perfumes for women and eau de Cologne for men. All products were available commercially and were either new (purchased or provided as a 'tester' from a high street chain) or part-used and donated. Details of all the samples used are in Appendix A. During various GC experiments, C_8-C_{22} alkane standards were also used, in hexane (Sigma-Aldrich, UK).

For sample blanks and to prepare dilute solutions of the perfumed products, HPLC grade methanol and ethanol were purchased from Fisher Scientific (Fisherbrand, Loughborough, UK). The same solvents were used in the swabbing experiments along with deionised water with a resistivity ≥18.2 MΩ cm^{-1} obtained by means of a PureLab Option Q water purification system from Veolia Water Technologies (High Wycombe, UK). For the FTIR evaluation standards were analysed of ethanol and methanol (source as above) and also of methylated spirits (Fisherbrand, Loughborough, UK).

For cleaning the desiccator used for sampling from garments, deionised water was used to prepare separate solutions of 2 M sodium hydroxide from analytical reagent grade sodium hydroxide and 10% nitric acid from concentrated (70%) nitric acid (analytical reagent grade). Dichloromethane and acetone were also used (both HPLC grade). All of the above chemicals were Fisherbrand, sourced

from Fisher Scientific, Loughborough, UK.

The HPLC mobile phase described in section 2.3.1.5 was made using HPLC grade acetonitrile (Fisherbrand, Loughborough, UK) and deionised water (resistivity ≥18.2 MΩ cm^{-1}) which was filtered under vacuum through 0.45 µm x 47 mm Nylon 66 membranes (Supelco, Bellefonte, PA, USA) or the equivalent Durapore® filters (Millipore, Ireland).

For evaporation of the samples in the sampling study PureLab nitrogen gas was used (Oxygen Free from BOC, Guilford, UK) and samples were evaporated under a flow of nitrogen at ambient temperature.

2.2 Apparatus

All general laboratory glassware (beakers, funnels, watch glasses etc.) were purchased from Fisher Scientific (Loughborough, UK). Autosampler vials of 2 mL and 0.3 mL volume in clear glass and brown glass together with the appropriate screw-top lids and septa were Chromacol™ from Thermo Scientific or Fisherbrand (Loughborough, UK). Other vials used included 4 mL screw-top vials and 6 mL headspace vials (both from Supelco, Bellefonte, PA, USA).

2.3 Method

2.3.1 Evaluation of Instrumental Methods

2.3.1.1 General Preparation of Liquid Samples

Laboratory samples were prepared for analysis from the range of collected perfumes, deodorants and antiperspirants. For the studies involving chromatographic analysis of liquid samples, hydroalcoholic products such as perfume and eau de Cologne (which included ethanol as a carrier alcohol) required minimal sample preparation: for products packaged in tester vials and packets, approximately 1 mL of each was simply transferred into a 2 mL autosampler vial. Hydroalcoholics in atomisers were sprayed into a glass funnel and collected into a 10ml glass vial with approximately 1 mL of each of these samples subsequently transferred into a GC vial. Aerosol products such as antiperspirants, deodorants and body-sprays were sprayed into 50 mL beakers and dissolved in 1mL of HPLC grade methanol (Fisherbrand) which was then pipetted into a GC vial. Funnels, beakers and 10 mL vials were rinsed with HPLC grade methanol before use. The full list of perfumes, deodorants and antiperspirants used for this stage is provided in Appendix A. The C_8-C_{20} alkane standard used during experiments on the GC-MS and the GC-FIDs was drawn up directly from the vial and manually injected at volumes of 0.1 µL. When used on the ATD-GC-FID, the 0.1 µL was dispensed directly into the ATD tube.

2.3.1.2 Gas Chromatography (GC)

Gas Chromatography was conducted on two Clarus 500 Gas Chromatographs with a Flame Ionisation Detector (GC-FID) and on a Clarus 500 Gas

Chromatograph fitted with a Clarus 500 Mass Spectrometer (GC-MS). The columns used on all instruments were low-polarity DB5 equivalents (5% Phenyl / 95% dimethylpolysiloxane) with a length of 30 m, diameter 0.32 mm and internal diameter 0.25 µm. Columns for the GC-FIDs were Zebron ZB-5MS (Phenomenex, Macclesfield, UK) and for the GC-MS, Supelco SLB™5-ms (Sigma-Aldrich, Gillingham, Dorset, UK). Helium was used as carrier gas for all the GC instruments: the GC-FIDs were pressure controlled at 8 psi (1.5 mL min^{-1} at 60°C as calculated using the Clarus Column Carrier Gas Calculator) while the GC-MS was flow controlled at 1.5 mL min^{-1}. System checks were routinely performed on all the GC instruments and these checks included blank runs without any injection on to the column ('column blanks') and solvent blanks using an appropriate solvent (methanol unless otherwise stated). In addition the GC-MS was tuned and calibrated on a monthly basis.

For liquid samples the autosamplers were set to dispense an injection volume of 1.0 µL and the injection temperature was 250°C. For the MS method a three minute solvent delay was included to avoid interference from the carrier alcohol, and for more concentrated samples, split injection (100 ml min^{-1}) was used. The detector temperature for the GC-FID was 250°C. For the MS the transfer line temperature was 300°C and the ionisation energy was 70 eV with the instrument scanning from 40 to 500 amu (scan time 0.2 s, interscan delay 0.05 s).

The GC temperature program used for the initial evaluation of instrumental methods was designated 'Perfumes' and is shown in Table 2.1. This temperature program was based on that developed for quantification of perfume allergens by the IFRA working group (Chaintreau *et al*., 2003) but, as the run time of the

original program was 45 minutes, the ramp rates were doubled to reduce the run time to 27 minutes. The original program was also specified for use on a DB5 column with dimensions 60 m x 0.25 mm x 0.25 µm with an initial flow of 1.7 mL min^{-1} while the column used was 30 m x 0.32 mm x 0.25 µm with an initial flow of 1.5 mL min^{-1}.

Table 2.1 - GC temperature program 'Perfumes'

Program Name	Start Temperature	Hold Time (minutes)	Ramp Rate 1	Ramp Rate 2	Total Run Time (minutes)
Chaintreau 2003	60°C	1	3°C min^{-1} to 150°C	6°C min^{-1} to 280°C	45.00
Perfumes	60°C	1	6°C min^{-1} to 150°C	12°C min^{-1} to 280°C	27.00

For the initial evaluation of GC-FID and GC-MS (results in Section 3.1.1) eleven products were analysed: six fine fragrances two body-sprays and three deo-colognes (details of which are in Appendix A).

2.3.1.3 Fourier Transform Infrared Spectroscopy (FTIR)

Analysis by FTIR was conducted using a Nicolet 380 Spectrometer with a Smart Orbit diamond crystal attenuated total reflectance (ATR) accessory and Omnic software. A background scan was collected at the start of each experiment and, where appropriate, before the collection of each sample spectrum. Each sample spectrum comprised 32 scans across the mid-infrared range of 4000 to 400 cm^{-1} with a 4 cm^{-1} resolution. Data spacing was 1.929 cm^{-1} and the format used for the data was % Reflectance. Between samples the stage was cleaned with deionised water.

Samples of the perfumed products and standards of methylated spirits, ethanol and methanol were dispensed on to the ATR crystal by various means: using the dipstick provided with the commercial sample (tester) bottle; sprayed directly onto the stage; dispensed directly from the roll-on applicator; or pipetted using an auto pipette in aliquots of 20 µL from the vials prepared for the analysis by Gas Chromatography. To determine the most appropriate sampling procedure two initial studies were conducted using the perfume *Hugo XX* (details in Appendix A). In both cases a background spectrum was collected at the start of the experiment with the plastic cap covering the ATR crystal, this background spectrum was then used as the background for the remainder of the experiment. To collect the sample spectrum, two drops of a sample of the perfume were placed on to the ATR crystal and covered with the plastic cap before the scans commenced. The sample was then left uncovered and allowed to evaporate for one minute, whereupon the plastic cap was replaced and another spectrum collected. To study the change in spectra due to evaporation over the short-term, the procedure was repeated until 10 spectra had been collected representing a total evaporation time of 10 minutes. To determine the effectiveness of the plastic cover to reduce evaporation, the cover was then left in place for a further 3 hours with spectra collected at intervals. For the aging study, the initial spectrum was collected and then the sample was left uncovered and allowed to evaporate for 30 minutes, whereupon the plastic cap was replaced and another spectrum collected. This procedure was repeated at intervals up to 4.5 hours.

The results of the analyses were compared and evaluated to establish that an evaporation time of two minutes was sufficient to produce a representative spectrum. For the remaining samples the following procedure was adopted: a

background sample was collected and the sample was then deposited on the ATR crystal. The sample was covered with the plastic cap to slow evaporation and a spectrum collected. The plastic cap was then removed and the sample exposed on the stage for a timed interval (usually one minute) so that the carrier alcohol could evaporate. The process was repeated and spectra collected at further timed intervals as needed.

In total 18 products were analysed by FTIR: ten perfumes, two body sprays, three aerosol antiperspirant-deodorants and two roll-on antiperspirant-deodorants. The spectra were collected at timed intervals as shown in Appendix B. Representative spectra, usually taken after two or three minutes evaporation time, were saved to a user defined library. Spectra taken at different times and / or later exposures were subsequently searched against all available libraries including the user defined library. In some cases it was desirable to view the region from 1700 cm^{-1} to 525 cm^{-1} in more detail: use of a method specifically targeting that region was trialled but found to be unsuitable as a separate background spectrum was required, so this was achieved by simply adjusting the viewed area of the collected spectra.

2.3.1.4 UV-Visible Spectroscopy (UV-Vis)

Prior to analysis by HPLC perfume samples Mania and Daisy were analysed by UV-Vis to determine the most suitable wavelengths. Both perfumes were diluted with HPLC grade methanol, first in a 1:1 ratio and then in a 1:10 ratio and placed in 1 cm^3 quartz vials. Each perfume was analysed using a Perkin Elmer Lambda 25 spectrometer with a scan from 800 nm to 200 nm at intervals of 1.0 nm and a scan speed of 480 nm min^{-1}.

2.3.1.5 High Performance Liquid Chromatography (HPLC)

Analysis was conducted using a Perkin Elmer 200 High Performance Liquid Chromatograph (HPLC), with dual channel Diode Array Detector (DAD) and a silica-based, 5 µm ODS (C18) Phenomenex Hyper column (120 Å, 250 x 4.60 mm). The method was based on that developed by Villa *et al.* (2007) for the simultaneous detection of the 24 listed perfume allergens in scented products and consisted of a gradient elution with a mobile phase of acetonitrile (MeCN) and water (H_2O). The eluent mixture was initially set at 50:50 organic : aqueous with an equilibration step of 0.5 minutes and a flow of 0.7 mL min^{-1}. Following the injection of the sample the flow was increased to 1.0 mL min^{-1} and a linear ramp was used to change concentration over the course of 15 minutes to 60% organic and 40% aqueous. After 15 minutes the ramp increased to produce a final concentration after 40 minutes runtime of 90% organic and 10% aqueous as shown in Table 2.2. Acquisition wavelengths were set at 210 and 280 nm and the reference wavelength at 400 nm. Perfume samples Mania and Daisy were analysed.

Table 2.2 - HPLC conditions

Duration (min)	Run Time (min)	Flow (mL/min)	MeCN (%)	H_2O (%)
0.5	*equilibration*	0.7	50	50
15	0 - 15	1.0	60	40
25	15 - 40	1.0	90	10

2.3.2 Evaluation of GC Methods

The evaluation of GC methods comprised two main stages the first of which was to investigate temperature programs to understand the effect of different start temperatures, hold times and temperature ramp rates with reference to the 'Perfumes' temperature program used in the evaluation of instrumental methods. The second stage was to further evaluate the 'Perfumes' temperature program, this time in comparison with other published temperature programs.

2.3.2.1 Temperature Programs - Variation of Temperatures and Times

Using both the GC-FID and the GC-MS, a range of temperature programs were trialled with variations on the method 'Perfumes' including changes in start temperature (40, 50 and 60°C) and initial hold time (1 minute or 5 minutes), the inclusion of a second (5 minute) hold at various temperatures (120,130,140 & 150°C), a variation in ramp rates (6, 10 and 12°C min^{-1}) and final temperature (200, 280 and 300°C). For this work, twenty variations on the temperature program were trialled and evaluated using a C_8-C_{20} alkane standard series and a number of different perfumes and deo-colognes, details of the experiments are provided in Appendix A. Temperature programs were evaluated using visual observations and a number of numerical approaches.

2.3.2.2 Comparison of 'Perfumes' with Published Programs

Temperature programs described by IFRA (2006) and Sanchez-Prado *et al.* (2011b) were also evaluated (named 'IFRA and 'SP001' respectively). The programs are shown in Table 2.3, although it should be noted that the temperature

program described by IFRA (2006) was for a non-polar DB1 column with a length of 60 m and an initial gas velocity of 50 cm sec^{-1} or a 20 m medium polar DB17 column at 60 cm sec^{-1}. This work was done on the GC-MS system described above with the slightly polar DB5 column. Both programs were evaluated using visual and numerical approaches using a C_8-C_{20} alkane standard and the perfumes *Daisy, Urban* and *Aphrodite*.

Table 2.3 – Temperature programs from IFRA and Sanchez-Prado *et al.*

Program Name	Start Temp.	Hold Time (mins.)	Ramp Rate 1	Ramp Rate 2	Ramp Rate 3	Total run Time (mins.)
IFRA	100°C	2	10°C/min to 280°C Hold 5 min	-	-	25.00
SP001	45°C	2	8°C/min to 100°C	20°C/min to 130°C Hold 3 min	25°C/min to 200°C	25.00

2.3.3 Distinguishing Between Products

Following analysis by Gas Chromatography, products were initially compared with each other on the basis of visual pattern matching and the identification of product ingredients by GC-MS peaks matched to NIST MS Search 2.0 library; specific masses as identified by the IFRA working group (Chaintreau *et al*., 2003) and Sanchez-Prado *et al.* (2011b); and retention indices. A more rigorous statistical approach was also employed using IBM SPSS Statistics 19 software to perform Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Discriminate Function Analysis (DFA).

2.3.4 Sampling Deposits of Perfumed Products by Swabbing

In developing a method for recovering deposits of perfumed products from surfaces, it was decided that the most appropriate approach would be to swab the sample from the surface. Two experiments were conducted, the first using solvent extraction to recover the sample from the swabs and the second using SPME. In each experiment the effectiveness of a dry swab compared to a swab wetted with ethanol or deionised water was evaluated. For both experiments the perfume used was Ysatis, a sample of which had previously been dispensed from an atomiser and collected in a vial.

To prepare a suitable surface for swabbing, watch glasses were soaked overnight in 10% HNO_3 and then left in a fume cupboard to dry for an hour and wiped dry if needed. For each experiment one watch glass was left as a control and the remaining samples were prepared by inverting the watch glasses to form a convex surface, and depositing 20 µL of the perfume on to the surface using an auto-pipette. All watch glasses were then put back in to a fume cupboard to dry. After one hour drying time a swab was used to collect the sample from the surface of the watch glass. For the dry swab protocol, the swab was used directly from the packet; for the ethanol swab, the swab was dipped into a beaker containing approximately 10 mL of ethanol; and for the deionised water, the swab was doused using a plastic bottle. In each case the flat side of the swab was used and wiped across whole surface of watch glass, the glass was then rotated 90˚ and re-swabbed with this procedure repeated twice more so that each watch glass was swabbed in all four directions.

For the solvent extraction experiment, the tip of each swab was placed into a 4 mL

screw-top vial and 1.0 mL of ethanol added using an auto-pipette. The sample was agitated for 10 minutes and the solution then drawn up and transferred into a 6 mL headspace vial with a plastic cap. This process was repeated until a total of five aliquots had been collected from each sample and combined in a series of 6 mL headspace vials. Each of the combined samples was then evaporated under nitrogen at 5 psi to dryness (over the course of approximately 1hr 45 minutes) with the evaporator positioned so that the outlet was 0.5 cm from the liquid. The samples were re-suspended by adding 300 µL of ethanol to the headspace vial and agitating gently. The extracted samples were transferred to 0.3 mL autosampler vials and loaded into the GC autosampler for analysis by GC-FID using the temperature program 'perfumes'.

For the SPME experiment the tip of each swab was then cut off and placed into a 2 mL glass autosampler vial and sealed with a slit septum and cap. A PDMS SPME fibre (conditioned and analysed to confirm that it was clean) was then pushed through the septum and exposed to the headspace surrounding the swab without touching the swab (see Figure 2.1). In each case the fibre was exposed to the headspace sample for 30 minutes and then retracted.

Figure 2.1 - SPME Sampling

To analyse the SPME samples the fibre was exposed in the GC injection port for at least 2.5 minutes and analysis was by GC-FID using the same instrument and temperature programme as the solvent extraction experiment but using a splitless injection.

2.3.5 Aging Study – Change in Products over Time

To study the behaviour of perfumes and deodorants over time both FTIR and GC were used. For the FTIR experiment two perfumes were used: *Hugo XX* and *Cassis Rose*. The spectra were collected using the standard procedure described above but for this experiment the evaporation time was increased with spectra collected at intervals up to 4.5 hours. For the GC experiment an initial study was conducted using perfume *Mariage* and then a longer study for which five perfumes were used: *Mariage, Ysatis, Daisy, Mania and Urban*. In each case 100 µL of perfume, previously prepared for use as a standard, was pipetted into a 2 mL GC vial. One vial of each perfume was used as a reference sample by adding 1 mL of HPLC grade methanol and sealing the vial immediately. The remaining vials were placed in a fume cupboard and after the specified length of time (from two hours to 16 days) the evaporation was halted by adding 1 mL of HPLC grade methanol and sealing the vial. Samples were collected at various intervals up to 16 days and additionally, samples at one day, two days and 16 days were collected in triplicate to check reproducibility. All samples were refrigerated at 4°C until analysis. The GC analysis was performed on GC-FID and GC-MS for the initial study and GC-FID alone for the longer study. GC conditions were as described in Section 2.3.1.2 and the GC temperature program designated ‘Perfumes’ was used.

2.3.6 Sampling from Garments

Items of clothing and also some items of bedding were collected from volunteers (see Appendix A for details) and prior to analysis were stored at room temperature in nylon bags with swan-neck ties. Samples were recovered from the headspace above the textiles using two methods: sampling onto Tenax ATD tubes and SPME. All samples were analysed by ATD-GC-FID (for the Tenax tubes) or GC-MS (for the SPME) and the GC temperature program 'Perfumes' was used for all samples and blanks.

For the analysis of headspace samples by ATD, all work was carried out using a Perkin Elmer 650 Automated Thermal Desorber (ATD) with a Tenax TA cold trap which was connected to a Clarus 500 Gas Chromatograph with a Flame Ionisation Detector (GC-FID). A 'Trap Clean' and a 'Trap Check' were performed at least once a week using the same ATD and GC methods described above. Thermal desorption tubes were stainless steel pre-packed with Tenax TA and were sourced from Perkin Elmer (Shelton, CT, USA). All tubes were individually numbered and a log kept of their use. New tubes were conditioned at 250°C with a helium flow of 10 mL min^{-1} for 30 minutes before use. When not in use, all tubes were stored in sealed nylon bags over activated carbon in a desiccator and tubes were checked by performing a blank run before use. Prior to thermal desorption the tubes received a dry purge of 1 minute at 50 mL min^{-1} followed by desorption at 250°C with a helium flow of 30 mL min^{-1} for 5 minutes. The inlet split was 35 mL min^{-1} and the trap hold 2 minutes at -30°C before ramping to 250°C at a rate of 99°C sec^{-1}. Valve and transfer temperatures were set to 225°C and an outlet split of 25 mL min^{-1} was used when transferring the sample onto the GC with a low-split of 25 mL min^{-1} (20:1) on the GC-FID inlet. In most cases a 'desorb and condition' ATD

method was used with a conditioning temperature of 250°C and a conditioning flow 10 mL min^{-1}. For this method a cycle time of 40 minutes was generally included in the ATD method. In some cases a desorb only method was used to facilitate the evaluation of the method, in which case a 10 minute conditioning method was used subsequently. The Tenax sorbent tubes were first tested using liquid samples, whereby 1 µL of sample was injected onto to the sampling end of a Tenax tube (previously conditioned and analysed to confirm it was clean). These trials were conducted with the C_8-C_{20} alkane standard and with samples of perfume. Blank samples were also analysed from the tubes and repeated blanks from a single tube compared.

Gas phase sampling of perfumes and garments by Solid Phase MicroExtraction (SPME) was conducted using a non-polar PDMS (polydimethylsiloxane), 100µm, non-bonded, SPME fibre from Supelco. New fibres were conditioned before use in the injection port of a GC for 30 minutes at a temperature of 250 ˚C and before each experiment a negative control of the fibre ('fibre blank') was analysed to ensure that the fibre was clean. The SPME fibres were exposed to the headspace of the sample for 30 minutes then retracted and immediately transferred to the injection port of the instrument (250°C) and exposed for five minutes. An exposure of this duration was sufficient to desorb the analytes of interest and blanks were analysed to confirm that this procedure resulted in a clean fibre for subsequent sampling.

Garment analysis initially involved sampling the headspace above the sample in a nylon bag under a number of conditions as shown in Table 2.4. Blank samples of nylon bags were also analysed.

Table 2.4 - Initial sampling experiments using nylon bags

Experiment	Conditions	SPME fibre exposure time	ATD sampling
1	Room temperature	1 hour 50 mins.	active sampling volume 100 mL
2	Room temperature	5 hours 30 mins.	passive sampling for 5 hour 30 mins.
3	Sample heated in nylon bag at 80°C for 45 mins.	1 hour 20 mins.	active sampling volume 100 mL
4	Sample heated in nylon bag at 130°C for 10 mins.	44 mins.	active sampling volume 100 mL

A further set of experiments was then conducted using a large vacuum desiccator which had been thoroughly washed and dried in an oven overnight at 100°C. The desiccator was sealed with silicon grease, stoppered and returned to an oven at 130°C for 10 minutes before blank samples were collected from the desiccator using both ATD tubes and SPME. The desiccator was then returned, un-stoppered, to the oven for a further two hours before use. Four textile samples were sampled using this method, in each case the sample was heated in the stoppered desiccator at 130°C for 10 mins, then the desiccator un-stoppered and a sample taken on to the Tenax tube by actively sampling a volume of 100 mL. The SPME fibre was then exposed to the headspace for 30 minutes. In between each sample the desiccator was cleaned using a series of solvent washes consisting of methanol, acetone, dichloromethane, 10% nitric acid, and 2M sodium hydroxide with a final rinse of deionised water before heating in the oven overnight. Prior to collecting each sample the desiccator was checked by collecting blank samples using ATD and SPME.

To establish the best method for sampling garments in the desiccator, experiments were conducted to determine whether it was more effective to sample the headspace with a Tenax tube first, followed by an SPME fibre, or whether SPME sampling should be conducted first and then the active sampling onto Tenax. A one-second spray of the deodorant-antiperspirant Lynx Twist was applied to a clean watch glass. This was then placed into the desiccator which had been heated to 130°C and sealed with aluminium foil and string in place of the usual stopper. The desiccator was then placed back in the oven at 130°C for 10 minutes, after which sampling took place. For the first experiment the SPME fibre was exposed to the headspace for 30 minutes and then withdrawn for analysis. The desiccator was immediately re-sealed with fresh foil and returned to the oven for 10 minutes to re-heat. After re-heating a Tenax tube was inserted, onto which a 60 mL headspace sample was drawn. For the second experiment the 60 mL sample was taken onto the Tenax tube first, the desiccator re-sealed with fresh foil and placed back into the oven for another 5 minutes to re-heat and the fibre exposed then exposed to the headspace for 30 minutes. Chromatograms were compared to each other and to those obtained by direct injection. Samples of two further products, Lynx Africa and Sure Cotton Ultra Dry were also analysed by this method. These products were chosen as they were used by the subjects whose garments were analysed.

2.3.7 Storage Study

To compare the effect of different storage conditions, 20 µL of perfume *Ysatis* (prepared as described previously) was pipetted into a series of 0.3 mL vials. Three samples were stored in normal light at room temperature (by a window), three in a dark cupboard at room temperature (in brown glass vials), three in a fridge (4°C) and three in a freezer (-12°C). After three weeks one vial from each group was taken from storage and after 20 minutes (to defrost the freezer sample) the sample was made ready for analysis by adding 0.3 mL of methanol. Samples were then analysed by GC-FID using the conditions described in Section 2.3.1.2 and the GC temperature program 'Perfumes' with a methanol blank analysed between every sample. Following analysis the septum for each vial was changed and the samples were returned to their original storage conditions. As the chromatographic response was quite low for all samples, the same vials were then re-analysed after another week, this time GC-MS was used with the solvent delay and splitless injection to obtain a better response. For both the GC-FID analysis and the GC-MS analysis one sample vial was analysed three times to check reproducibility.

A second set of vials was retrieved after four weeks storage and made ready for analysis by adding 0.3 mL of methanol. Analysis was by GC-MS and again one vial was analysed three times to check consistency. Unfortunately, the GC-MS system checks following this analysis revealed some contamination and the injection liner was changed. A repeat analysis was conducted on one sample following the maintenance. The third set of vials was retrieved after six weeks storage and were analysed by GC-FID.

3 Results and Discussion

3.1 Evaluation of Instrumental Methods

The aim of this first study was to investigate whether perfumes, antiperspirants and deodorants can be profiled and distinguished from each other efficiently by rapid chemical analysis. The first part of this investigation was to compare GC analysis with analysis by HPLC and FTIR and to evaluate each of these instrumental methods for their potential in being able to distinguish between a range of perfumes, antiperspirants and deodorants. The results of the evaluation of each instrumental method are below.

3.1.1 Gas Chromatography

3.1.1.1 Gas Chromatography with Flame Ionisation Detection (GC-FID)

Chromatograms for the 11 products initially analysed using GC-FID showed that the pattern of the peak distribution was different for each of the products and that each chromatogram could be visually distinguished from those of the other samples. This can be seen with the examples in Figure 3.1 below (and with all of the chromatograms in Appendix B). The first notable feature of the chromatograms is the overloaded peak eluting around two minutes indicating low-boiling point, unretained compound(s) which are expected to be from the solvent. For some fine fragrances this ‘solvent peak’ consisted of two unresolved peaks with a total base width of 0.2 minutes. Other fine fragrances showed a dominant overloaded peak of 0.1 minutes base width, with a small (5-11 mV) peak eluting just before and another peak (height 104-150 mV) eluting immediately afterwards. None of these peaks were completely resolved. The largest solvent peaks were in the body-sprays and deo-colognes which displayed a single overloaded peak with a base

width of 0.85 minutes. The exception was the body-spray *Aphrodite*, with a solvent peak base width of 0.2 minutes and a second smaller, unresolved, peak eluting immediately afterwards.

For all the samples the remaining run time of the chromatograms showed a range of strong peaks. Between 5 and 8 minutes, clusters of small, broad, unresolved peaks were observed which are characteristic compounds with a greater affinity to the column's stationary phase. On the somewhat polar DB5 column being used these are likely to be polar compounds, possibly amines or carboxylic acids (Agilent, 2012). After 8 minutes and until around 16 minutes the chromatograms tended to be dominated by a number of well separated peaks with strong responses, thereafter, the peaks became more tightly clustered and with greater responses (with the exception of the body-spray *Aphrodite*). Variations were seen between the types of products, as shown in Figure 3.2 (below) comparing the perfume *Daisy*, and a low-cost, body-spray, *Aphrodite*. The uppermost chromatogram Figure 3.2a, shows that *Daisy* displays a typical perfume formulation: lighter, more volatile molecules ('top-notes') eluting at the start of the program (after the solvent peak), followed by mid-weight, less volatile molecules ('heart-notes') and a final group of high molecular weight, low volatility, molecules ('base-notes'). In contrast the pattern of the chromatogram of *Aphrodite*, (Figure 3.2b), indicates a formulation consisting of a higher concentration of high-volatility chemicals.

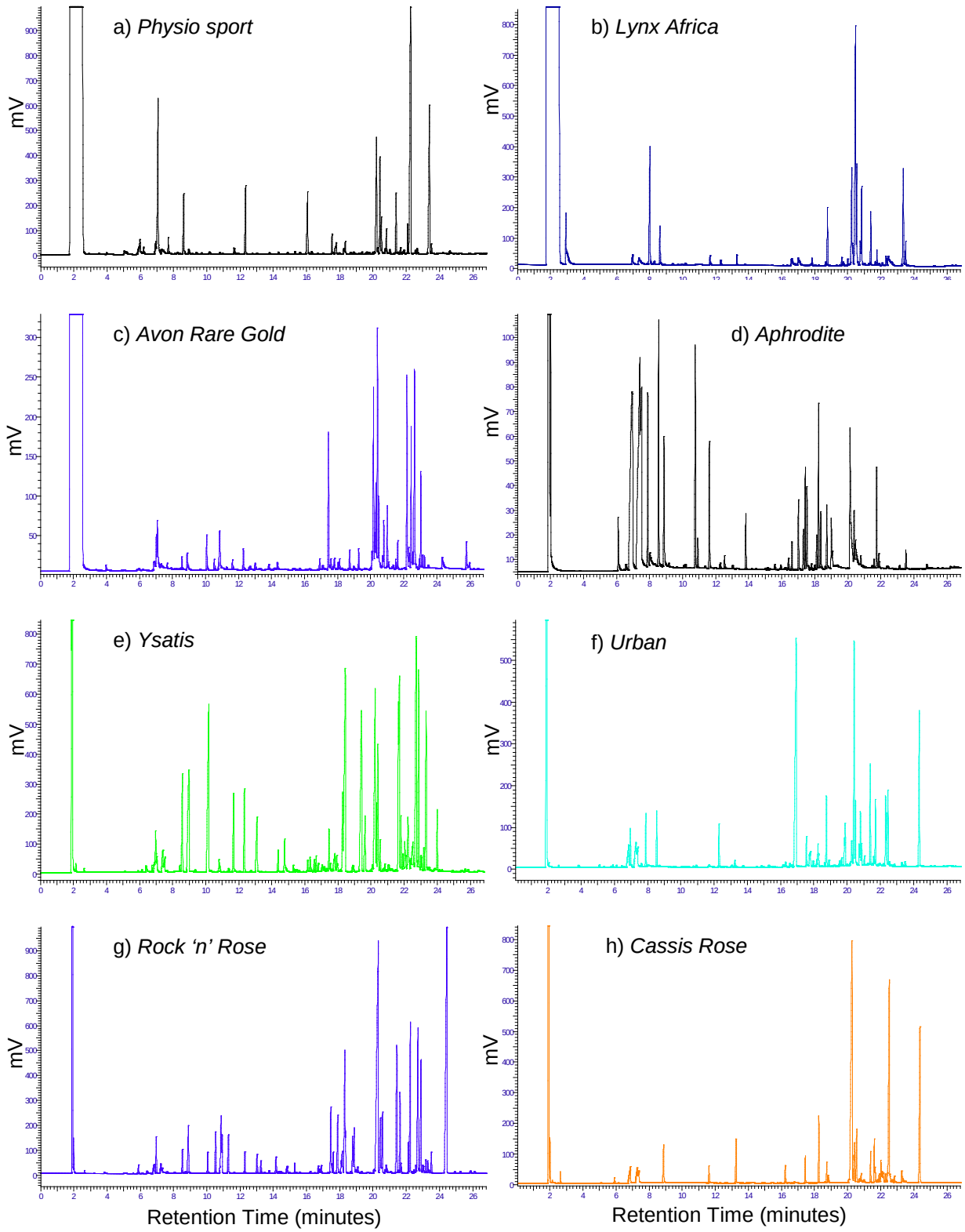


Figure 3.1 - Comparison of GC-FID chromatograms for eight products

The body sprays are *Physio Sport* (a), *Lynx Africa* (b), *Avon Rare Gold* (c), *Aphrodite* (d) and the perfumes are Ysatis (e), *Urban* (f), *Rock n Rose* (g) and *Cassis*, *Rose* (h)

The more volatile top-note aroma chemicals tend to have very low odour-detection thresholds (e.g. Linalool, MW 137, odour threshold 6 ppb), and are therefore usually included in the formulation at lower concentrations: thus the chromatogram for *Aphrodite* indicates that this body spray would provide the user with a very intense burst of fragrance on initial application (Sell, 2006). In contrast, the chemicals used as base-notes are less volatile and have a high odour-detection threshold and therefore must be included in the formulation at greater concentrations to be effective (Sell, 2006). The fine fragrance *Daisy*, shows a high concentration of heart and base notes and would therefore be considered by perfumers to be more 'tenacious' and provide an aroma for longer. For this region of the chromatogram, *Aphrodite* shows much lower concentrations of the less volatile base-notes and this is in keeping with the nature of this product being somewhat less persistent than a fine fragrance or a deodorizing-cologne.

Overall the GC-FID analysis was successful, the resulting chromatograms produced a range of peaks across the full duration of the temperature program and each chromatogram produced in excess of 100 integrated peaks which should aid in discriminating between products. In two main respects, however, the quality of the chromatograms was not ideal: the initial overloaded solvent peak and the poor resolution of peaks in the later stages of the chromatogram. For the solvent peaks the key consideration is whether they assist or hinder the use of GC to distinguish between the perfumed products. For the fine fragrances the solvent peak is expected to consist primarily of an ethanol component of the denatured alcohol carrier while for the deodorising aerosols the carrier solvent may also include the isopropyl myristate-cyclomethicone system (Beerling, 2006 p. 172).

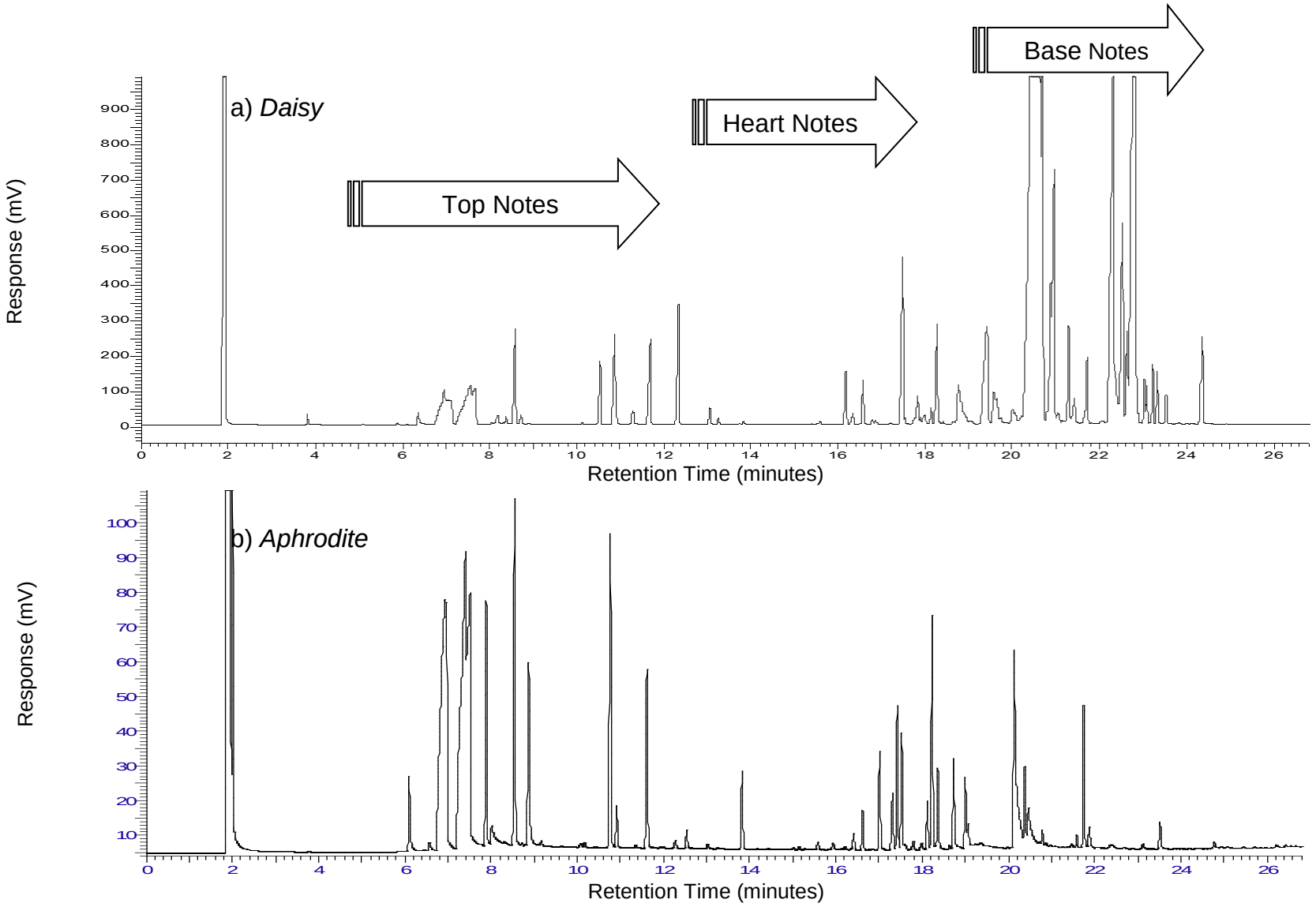


Figure 3.2 - Comparison of GC-FID chromatograms for a) the fine fragrance *Daisy* and b) the low cost body spray *Aphrodite*

While the solvent peaks differences seen in these results may potentially be discriminating it is unlikely that the volatile carrier solvent would still be present in the types of samples (residues) likely to be found at a crime scene: therefore at this stage in the research a more pressing concern was whether the overloaded solvent peaks are likely to obscure smaller peaks (although again any chemicals eluting in the earliest stage of the chromatogram are likely to be of low molecular weight and therefore too volatile to be useful aroma chemicals for a perfume formulation). To support this hypothesis it was observed that, for the majority of the samples (n = 10) for several minutes after the elution of the solvent peak, no other peaks eluted with a peak height above 10 mV.

The poor resolution of some peaks in the later stages of the chromatogram (particularly after 17 minutes) was noted in all of the samples which were analysed. This is likely to be partly due to the increased ramp rate of the temperature program (the ramp rate changes from 3 ˚C min^{-1} to 6 ˚C min^{-1} at 16 minutes) but may also be a result of aroma chemicals in the samples having similar structures and retention characteristics: a known problem especially with terpenoids (Chaintreau *et al*., 2011). It was also observed that for some products (including *Daisy* Figure 3.2a), the concentration of some aroma chemicals caused the peaks to exceed the 1000 mV limit set on the data capture, which could be avoided by reducing the injection volume or increasing the split at the injector (Restek, 2013). Despite these difficulties, the reproducibility of the chromatograms was good overall: for five repeats of the perfume *Ysatis* the maximum variation in retention times across 220 reported peaks was 0.02 minutes and the average %RSD of the peak areas was 9% with a maximum %RSD of 6% across the top twenty peaks).

3.1.1.2 Gas Chromatography with Mass Spectrometry (GC-MS)

Chromatograms produced by GC-MS for the 11 perfumed products were generally similar to those produced by GC-FID, as can be seen in Figure 3.3 and Figure 3.4, chromatograms for the same perfume (*Rock 'n' Rose*) on the GC-MS (top) and the GC-FID (below). The most apparent difference between the two chromatograms was due to the incorporation of a three minute solvent delay on the MS. This delay ensured that the solvent peaks did not overload the detector and enabled the chromatogram to be rescaled to the next highest peak, in this case that at 22.20 minutes, but with the loss of the potentially discriminatory information provided by the solvent. Another, less obvious, difference between the chromatograms is a shift in retention times due to the variation in carrier gas control between the two instruments: pressure control on the GC-FID versus flow-control on the GC-MS. This produces a shift in retention times which increases with temperature so that the peaks at the end of the GC-MS chromatogram elute approximately two minutes later than those for the equivalent GC-FID chromatogram. Despite these differences however, the pattern of the peaks in the chromatograms were easily compared between instruments. This can be demonstrated by comparing the two chromatograms head-to-tail as in Figure 3.4. The scale here for the GC-FID trace has been adjusted to 100% and the x-axis (time) adjusted to compare the pattern of each chromatogram rather than absolute retention times. The solvent peak has also been removed from the GC-FID trace. In this way it can be seen that majority of the peaks can be matched between the two instruments and there are minimal differences in the responses of the different detectors to the various chemicals in these samples.

a)

b)

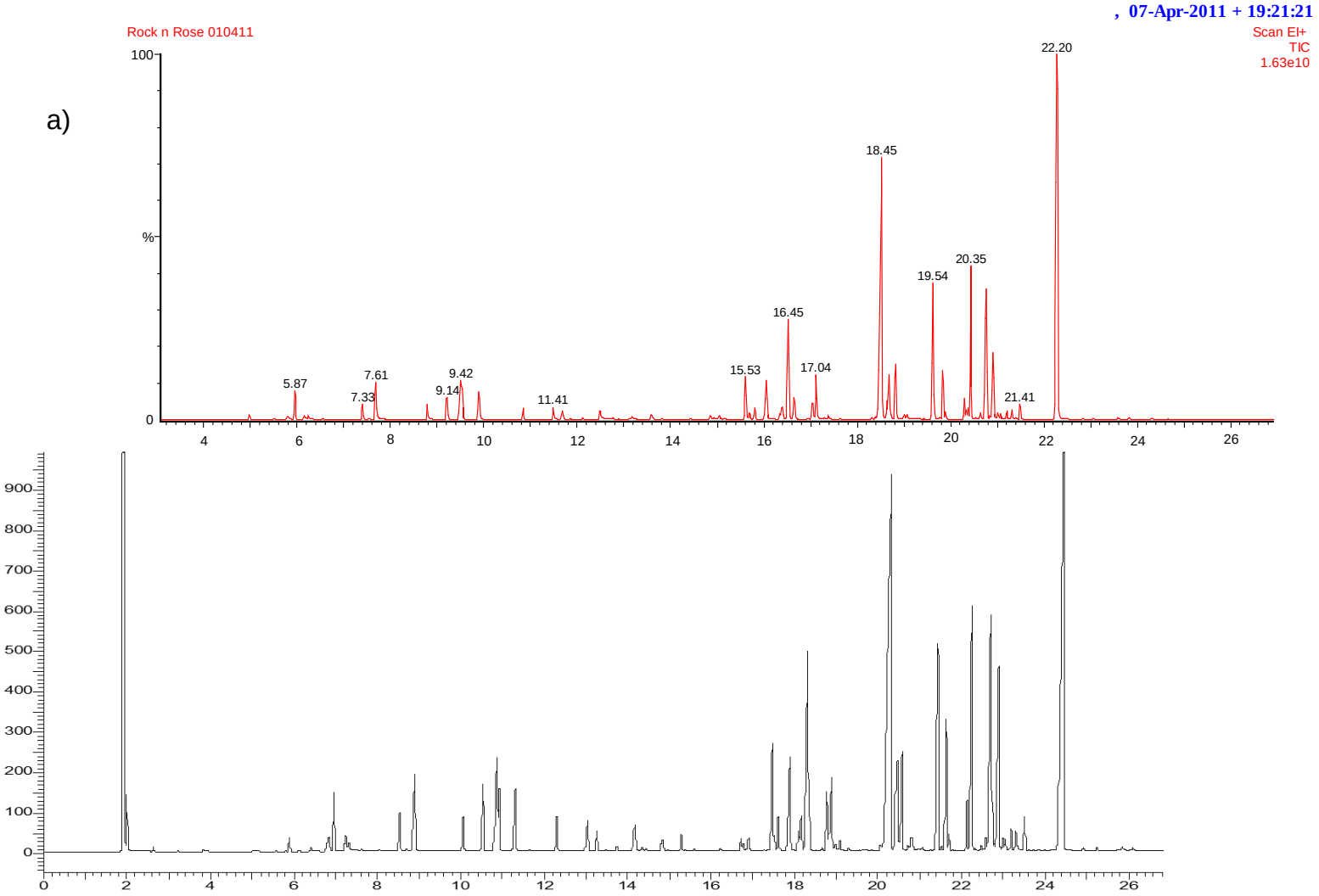


Figure 3.3 - Comparison of GC-MS (top) and GC-FID (bottom) chromatograms for perfume *Rock 'n' Rose*

NOTE: the GCMS chromatogram starts at 3 minutes due to the solvent delay.

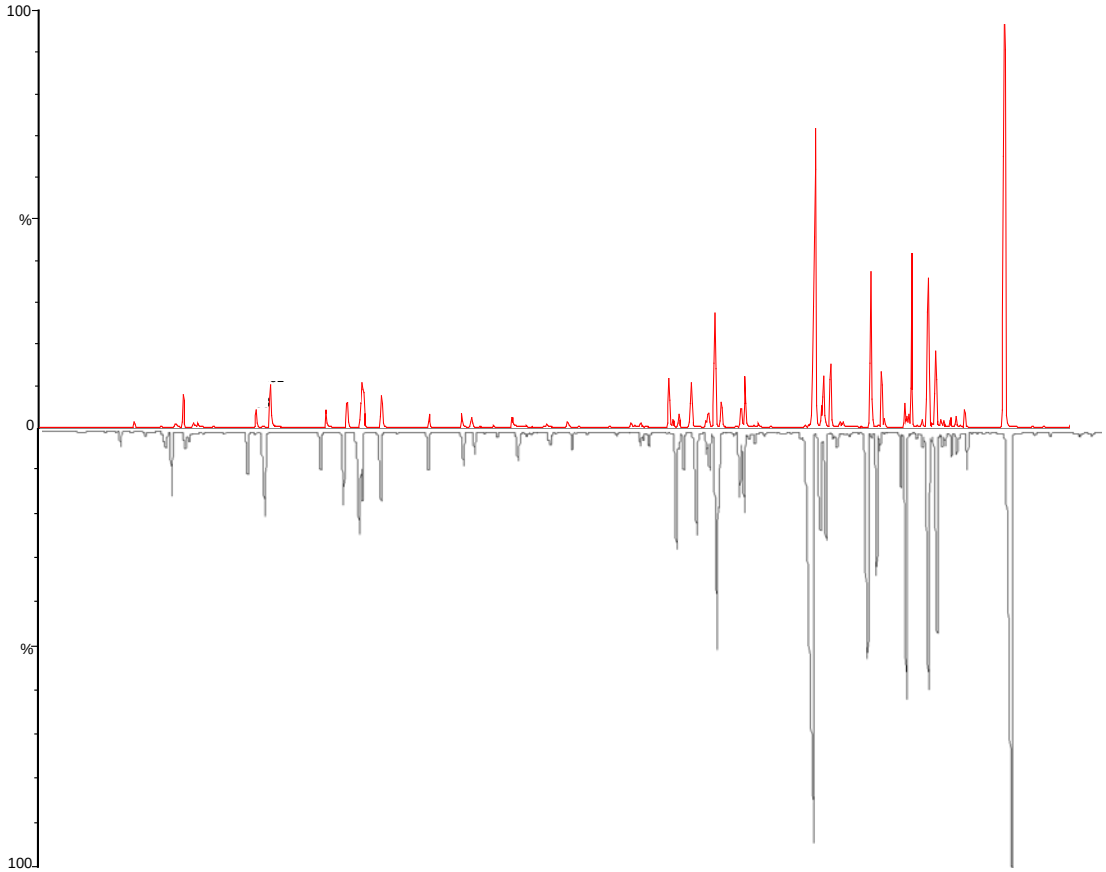


Figure 3.4 - Comparison of GC-MS (top in red) and GC-FID (bottom in black) chromatograms for perfume *Rock 'n' Rose*

NOTE: The scale here for the GC-FID trace has been adjusted to 100% and x-axis (time) compressed to compare the pattern of each chromatogram rather than absolute retention times. The solvent peak has also been removed from the GC-FID trace.

Analysis by GC-MS offers a significant advantage over GC-FID in that MS fragmentation patterns can be compared against a standard reference library (in this case NIST 2.0). Such comparisons were produced for the top-ten peaks in each perfume (as calculated by peak area) and those chemicals reported as matches are listed in Table 3.1, Table 3.2 and Table 3.3. The list of chemicals has been presented in separate tables which can be considered to separate the early-eluting 'top-notes' (retention times between 5 and 12 minutes) from the 'heart-notes (retention times between 15 and 20 minutes) and the 'base-notes' (retention times between 20 and 26 minutes). In order to assess the accuracy of the identification, the match and reverse match scores (out of 1000) and the probability percentage for each identification was considered and, using CAS numbers, chemicals were checked with lists of known aroma chemicals (TheGoodScentsCompany, 2015, Tadimety, 2016, Firmenich, 2016, Symrise, 2016). The tables below are those chemicals (n=41) which appeared in the top-ten peaks lists and had match scores above 800 which is considered to be a 'good' match using the NIST guidelines (NIST, 2008 p. 25). All identifications, however, must be considered indicative as it was not possible to run comparisons with reference standards due to the range of aroma chemicals needed. The results of this exercise confirmed the chromatogram results in that each perfumed product could clearly be distinguished from every other by the different list of chemicals produced for the top-ten peaks. The results also demonstrated the wide range of chemicals used in perfumed products with over 50 different chemicals reported by NIST Software.

Three non-aroma chemicals used as solvents were listed in the NIST reports:

dipropylene glycol, diethyl phthalate and isopropyl myristate. The earliest eluting of these is dipropylene glycol (DPG), a polar solvent widely used in the fragrance and personal care product industry which was a mixture of three isomers (Dow, 2017). The three isomers have different retention times (5.7, 6.1 and 6.3 minutes) each identified as ‘di(propylene glycol)’ or ‘dipropylene glycol’ by the NIST library. One or more of the DPG isomers frequently occurred in the NIST top-ten lists and a review of the chromatograms showed that DPG peaks reliably presented as three peaks: an early eluting single peak shortly followed by a pair of poorly separated peaks. All three peaks were non-Gaussian and asymmetrical with significant tailing which is likely to be due to the polarity of the chemicals and associated affinity to the DB5 stationary phase of the column. The second solvent to be identified by NIST was diethyl phthalate (DEP), a less polar solvent and particularly used as a diluent for musks such as Galaxolide (REACH, 2008). DEP is also valued in perfumery for its tendency to ‘fix’ other aroma chemicals in the perfume system: it has a greater affinity than alcohol for less polar chemicals such limonene, thus decreasing their activity coefficients and causing slower evaporation (Perring, 2006 p. 205). Although the use of phthalates has become controversial, DEP has been determined to have no harmful effects at the levels used in perfume formulations and its use is still supported by IFRA (IFRA, 2005, ACC, 2016). The third solvent was isopropyl myristate (IPM), a synthetic oil used to dilute oily fragrance ingredients (e.g. citrus oils) and which also prevents waxes forming during the formulation stage (Beerling, 2006 p. 172, Ruskin, 2015). IPM is also used to aid absorption and as an emollient (Beerling, 2006 p. 173-5, Epstein, 2014). One further non-aroma compound was listed in the top-ten report, benzophenone-1 which is used as an UV absorber to prevent UV degradation of

the fragrance and extend the shelf-life of the product (Beerling, 2006 p. 171).

The remaining chemicals listed in the tables below are used primarily as aroma chemicals although some may also act as solvents and fixers. Seven of the chemicals identified by NIST are potential allergenic substances (PAS): limonene (n=2), linalool (n=4), alpha-isomethylionone (n=3), Lilial® (n=5), benzyl benzoate (n=1), hexyl cinnamal (n=4) and benzyl salicylate (n=2). In some cases these chemicals appeared on the list of ingredients for a product but were not identified in the top-ten peaks and other PASs have not yet been identified despite being listed on labels e.g. citronellol, citral (as neral or geranial), geraniol, cinnamyl alcohol, amyl cinnamal and Lyral®. Limonene is of interest because despite the closeness of the match between the spectra (above 860 in all cases), the probability of the match was always listed below 40%. This indicates that there are a number of similar compounds in the NIST library and in the 'hit list' generated for the spectrum in (NIST, 2008 p. 25). For limonene ((+)-4-isopropenyl-1-methylcyclohexene) the low probability match is likely a result of the size and structure of the molecule limiting the number of different fragments in the mass spectrum combined with the high number of other chemicals in the NIST library with similar structures. An example of a related chemical is (R)-isocarvestrene (5-Isopropenyl-1-methyl-1-cyclohexene) which was in the NIST top-ten reports of two of the products and which had a retention time in the same window as limonene (between 5.8 and 6.0 minutes). In both cases where (R)-isocarvestrene was listed limonene was given as the second most likely match on the hit-list with a match value of nearly 900/1000. This highlights the difficulty of identifying aroma chemicals in complex mixtures as, even with reference standards, a conclusive

identification for these peaks may only be possible using multi-dimensional chromatography (see for example Augusto *et al*., 2010, Devos *et al*., 2012, Tranchida *et al*., 2013).

Other aroma chemicals which are of note but which are not PASs include methyl dihydrojasmonate (MDJ) which was identified by NIST as present in all but one of the perfumed products. MDJ often presented with two peaks, one around 18.4 minutes and another around 18.7 minutes and, as discussed in Section 1.4.2.3, the synthesis of MDJ results in a mixture of isomers which varies according to the synthetic route used (Rowe, 2005 p. 80, Pybus, 2006 p. 19). MDJ is considered one of the most important fragrance ingredients because it also can be used as a solvent, blending, fixing and even enhancing other ingredients (Sell, 2006 p.128). Also identified by NIST were a number of musk chemicals with all of the products except *Avon Rare Gold* having at least one musk listed in the top-ten peaks. The polycyclic musk Galaxolide was the most commonly identified (n=7) with the macrocyclic musks ethylene brassylate (syn. Musk T) and ethylene dodecanoate also being reported. One perfume (*Urban)* had the nitromusk Musk Ketone listed which was surprising as this chemical has some restrictions placed on its use in terms of the allowable concentration in fragrances and the percentage of Musk Xylene allowed as an impurity (CosIng, 2004, IFRA, 2015c).

Table 3.1 – Chemicals reported by the NIST library search for peaks with retention times between 5 and 12 minutes

Name	Lynx Vice	Avon Rare Gold	Lynx Africa	Physio Sport	Aphro-dite	Urban	Daisy	Mania	Ysatis	Cassis Rose	Rock 'n Rose
Beta-terpinene				✓							
Di(propylene glycol)	✓				✓		✓				
(R)-isocarvestrene				✓				✓			
Limonene		✓									✓
Dipropylene glycol	✓				✓	✓					
Dihydromyrcenol	✓				✓	✓					
Linalool					✓	✓		✓	✓		
Linalyl anthranilate				✓							
Phenylethyl Alcohol					✓				✓	✓	✓
Acetic acid benzyl ester		✓							✓		
Alpha-terpineol					✓						
2-Octen-1-ol, 3,7-dimethyl									✓		
(R)-(+)-beta-citronellol					✓						
Linalyl acetate						✓	✓	✓	✓		
Alpha-ocimene				✓					✓		
Muguet carbinol										✓	

Note: Chemicals have only been reported where the NIST library match included a CAS number and a match above 800.

Table 3.2 – Chemicals reported by the NIST library search for peaks with retention times between 15 and 20 minutes

Name	Lynx Vice	Avon Rare Gold	Lynx Africa	Physio Sport	Aphro-dite	Urban	Daisy	Mania	Ysatis	Cassis Rose	Rock 'n Rose
Diisopropyl adipate						✓					
Alpha-isomethylionone							✓	✓		✓	✓
Beta-ionone	✓							✓			
Phenyl Hexyl Acetate									✓		✓
Lilial®	✓				✓			✓		✓	✓
Ocean propanal								✓			✓
Amyl salicylate					✓				✓		
Diethyl phthalate (DEP)							✓		✓		
Ketone patchouli						✓			✓		
Methyl dihydrojasmonate	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Beta-methyl ionone	✓	✓	✓			✓	✓	✓		✓	
cis-3-hexenyl salicylate											✓
Benzyl benzoate									✓		
Hexyl Cinnamal			✓	✓		✓					✓

Note: Chemicals have only been reported where the NIST library match included a CAS number and a match above 800.

Table 3.3 – Chemicals reported by the NIST library search for peaks with retention times between 20 and 26 minutes

Name	Lynx Vice	Avon Rare Gold	Lynx Africa	Physio Sport	Aphro-dite	Urban	Daisy	Mania	Ysatis	Cassis Rose	Rock 'n Rose
Oxacycloheptadec-8-en-2-one						✓			✓		
Cyclopentadecanone, 2-hydroxy		✓					✓			✓	
Isopropyl myristate		✓		✓			✓	✓			✓
5-cyclohexadecan-1-one								✓			
Galaxolide	✓				✓	✓	✓	✓	✓	✓	
Benzyl salicylate		✓									✓
Ethylene dodecanoate			✓	✓							
Musk ketone (MK)						✓					
Ethylene brassylate (Musk T)	✓					✓	✓			✓	✓
Benzophenone-1						✓					
Diisooctyl adipate					✓						

Note: Chemicals have only been reported where the NIST library match included a CAS number and a match above 800.

Analysis of the data in Table 3.1, Table 3.2 and Table 3.3 showed that all of the products had at least one chemical in common with another product and some had seven compounds in common. The perfumes *Cassis Rose* and *Rock 'n' Rose,* which are discussed in Section 3.1.2.4 have five top-ten compounds in common: phenylethyl alcohol, alpha isomethyl ionone, Lilial, MDJ and ethylene brassylate (Musk T).

3.1.1.3 Evaluation of GC-FID and GC-MS

Both GC-FID and GC-MS showed excellent potential for distinguishing between products. For this initial investigation both techniques produced chromatograms which showed clear differences between each product and GC-MS provided additional information regarding the ingredients of each. Sample preparation was straightforward for the hydroalcoholics and aerosols and each analysis took approximately 30 minutes with the use of an autosampler reducing the actual time required by the operator to just a few minutes at the beginning and end of the analysis. The quality of the chromatograms was acceptable but further optimisation of the temperature program could improve peak resolution in the latter stages. It should also be noted that roll-on deodorants were not sampled. For future work, given the many hundreds of different perfumed products on the market, identification of individual products using pattern analysis is unlikely to be suitable and, even using GC-MS, identification of the chemicals used in each perfumed product may not be feasible, therefore a statistical approach is likely to be more robust.

3.1.2 FTIR Spectroscopy

3.1.2.1 Initial Study - FTIR Analysis of Perfume Hugo XX

The first study used the perfume *Hugo XX* with spectra collected at different time intervals to determine the most appropriate sampling procedure. The initial spectrum recorded for the perfume *Hugo XX,* taken immediately after the perfume was dispensed onto the crystal stage, is shown in Figure 3.5. Apart from the carbon dioxide peak at 2360 cm^{-1} (Chalmers *et al*., 2012) the spectrum is typical for an alcohol with a broad peak at 3333 cm^{-1} which is in the absorption range for O-H bond stretching for alcohols and which demonstrates the peak broadening caused by extensive hydrogen bonding (Housecroft and Constable, 2006 p. 408). To confirm this there is a strong peak present at 1045 cm^{-1} which is within the 1075-1000 cm^{-1} range indicative of the absorption from the asymmetric stretching of a primary alcohol C-O bond (Colthup *et al.*, 1990 p. 333).

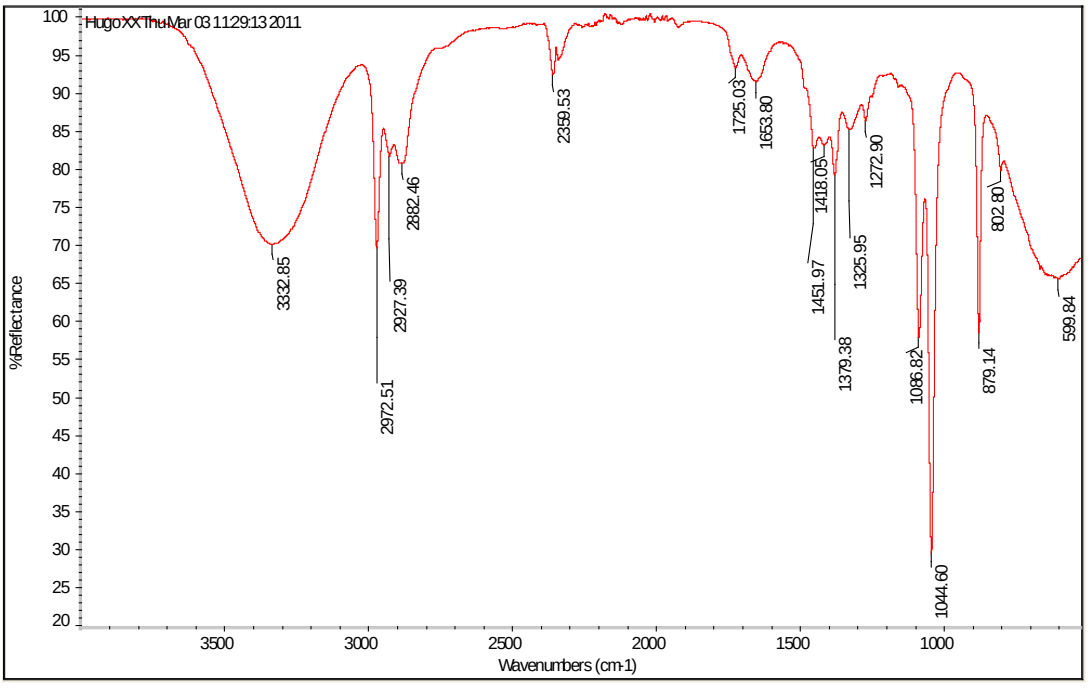


Figure 3.5 – Spectrum for *Hugo XX* taken immediately after the perfume was dispensed onto the crystal stage

Investigation of reference spectra indicated that the alcohol which provided the best match was ethanol (NIST, 2017) and for confirmation spectra were collected for ethanol, methanol and Industrial Methylated Spirits (IMS aka denatured alcohol) and compared with the spectrum of *Hugo* XX. The results are shown in Figure 3.6. While the *Hugo XX* spectrum had features in common with all three of the alcohol standards, a library search listed IMS as the top match at 99.09% and ethanol second at 98.84%. Methanol was not in the top 10 matches suggested by the library. As IMS (denatured alcohol) is a listed ingredient of *Hugo XX* and is often used as a carrier solvent in perfumes it seemed reasonable to conclude that the *Hugo XX* spectrum is dominated by IMS carrier solvent which is obscuring peaks from other ingredients, however, further confirmation of this conclusion was sought.

In the UK, there are various formulations (grades) of IMS and the grade used for the standard sample was Industrial Denatured Alcohol (IDA) which is generally a mixture of 95% ethanol with 5% methanol, although other 'marker' chemicals may be substituted for the methanol including tert-butanol at not less than 0.1 % (HMRC, 2017). The grade used in the perfume industry is typically a Trade Standard Denatured Alcohol TSDA 1 (formerly known as Alcohol DEB100) which consists of 99.9 % ethanol and 0.1 % tert-butanol with the addition of 10ppm Bitrex (denatorium benzoate) which used to make denatured alcohol unpalatable (Moffat, 2011, Mills, 2014).

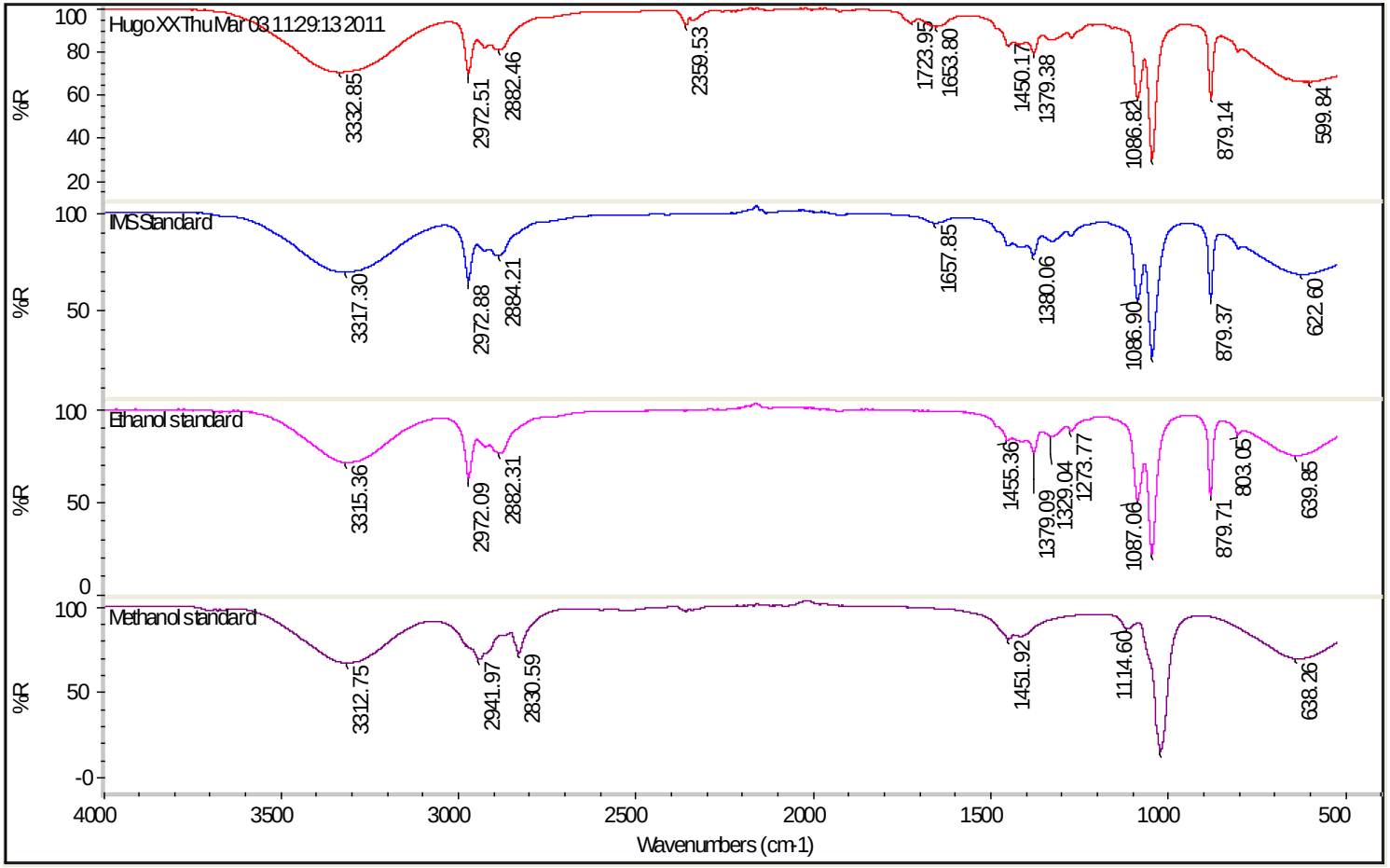


Figure 3.6 – Comparison of unevaporated *Hugo XX* with selected alcohol standards

The principal peaks for tert-butanol are given as 3038, 2875, 2564, 2385, 1630, 1606, 1501, 1022 cm^{-1} (ChemicalBook, 2016) none of which are apparent in the *Hugo XX* spectrum. For denatorium benzoate, principal peaks are given in reference texts at wavenumbers 1550, 1596, 1680, 719, 704, 757 cm^{-1} (Moffat, 2011) and the structure (shown in Figure 3.7) also contains a number of aromatic rings which would be expected to produce absorptions between 1600-1400 cm^{-1} but once again none of these peaks are evident in the *Hugo XX* spectrum.

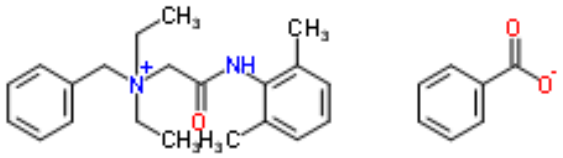


Figure 3.7 - Denatorium benzoate (Bitrex)
Source: ChemSpider

Using a different approach, it was noted that (as can be seen in Figure 3.6) only the IMS standard has the additional peak at 1658 cm^{-1} to compare with the *Hugo XX* peak at 1654 cm^{-1}. This peak falls in the range for many types of bonds including alkene C=C stretches and the C=O vibration of amides however it does not seem to be consistent with any of the listed ingredients in IMS. It was eventually concluded that this peak in both *Hugo XX* and the IMS is most likely a O-H bending peak from adsorbed water (Colthup *et al.*, 1990 p. 394). One must conclude that, while IMS produced the closest match, any additional ingredients in IMS are at much lower concentration and the associated peaks masked.

With regard to the suitability of the spectrum for the analysis of perfumed products, there is strong evidence that the spectrum collected for *Hugo XX* is dominated by

the absorptions from ethanol. It also seems clear from the 99.09% match of IMS with the unevaporated *Hugo XX* sample that the spectrum does not show any peaks which could be attributed to aroma chemicals. Accordingly, to remove the interference from the ethanol, the sample was left uncovered on the ATR stage for 30 minutes allowing the carrier alcohol to evaporate. The resulting spectrum is shown in Figure 3.8 and the full peak list is given in Appendix B. It can be seen that the previously dominant O-H peak is greatly reduced revealing a far more detailed spectrum. Interpretation of this spectrum is considered in Section 3.1.2.3 but at this stage it is sufficient to say that the quality and detail of the spectrum was considered suitable for the intended research. No significant improvement in the quality of the spectra was seen using intervals of 3 to 10 minutes (Figure 3.9) and a second study using the perfume *Hugo XX* whereby spectra were collected at 30 minute intervals up to 4.5 hours, also showed that, at this stage, there was little benefit in further evaporation (Figure 3.10).

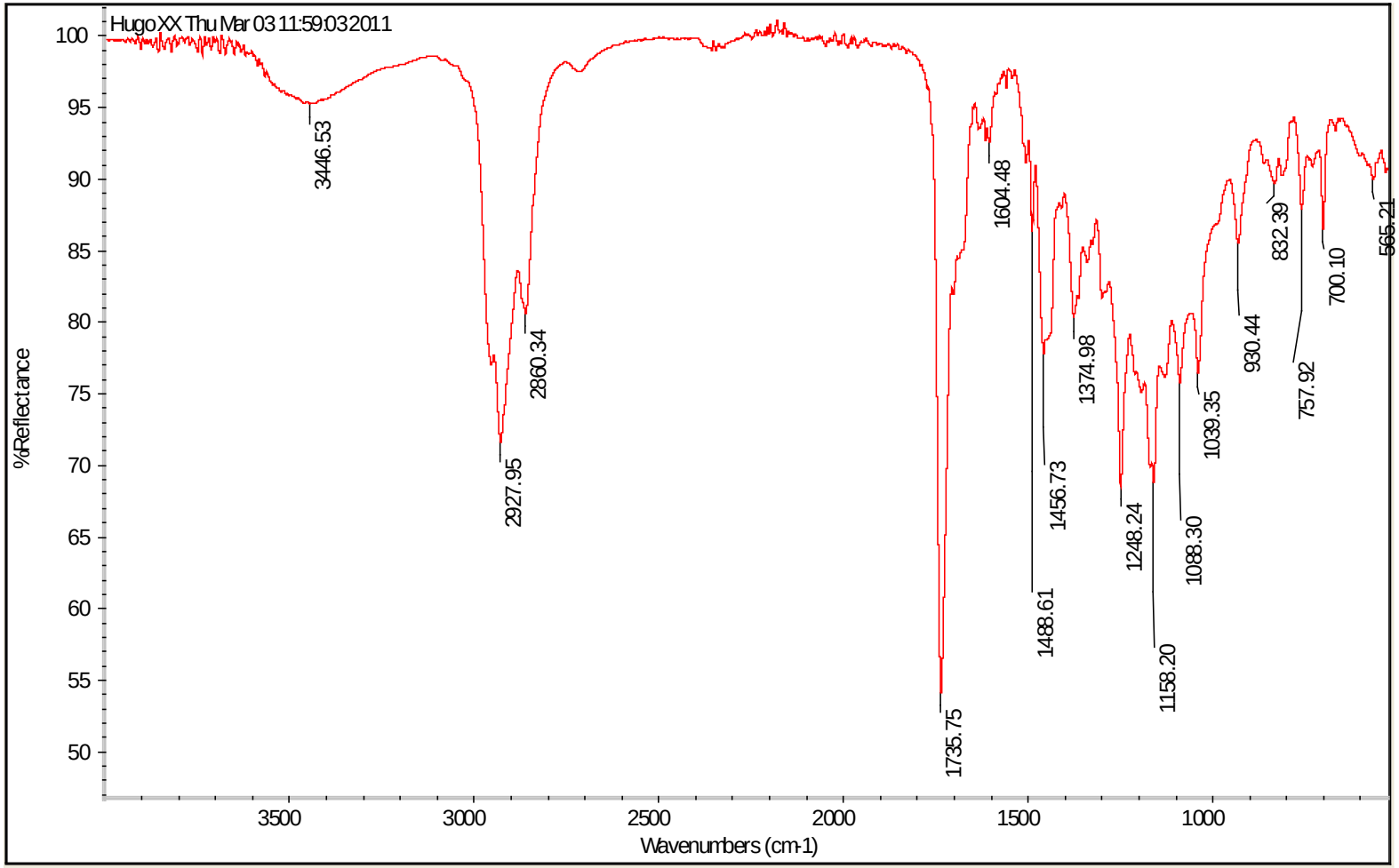


Figure 3.8 – Spectrum taken for *Hugo XX* after 30 minutes evaporation time

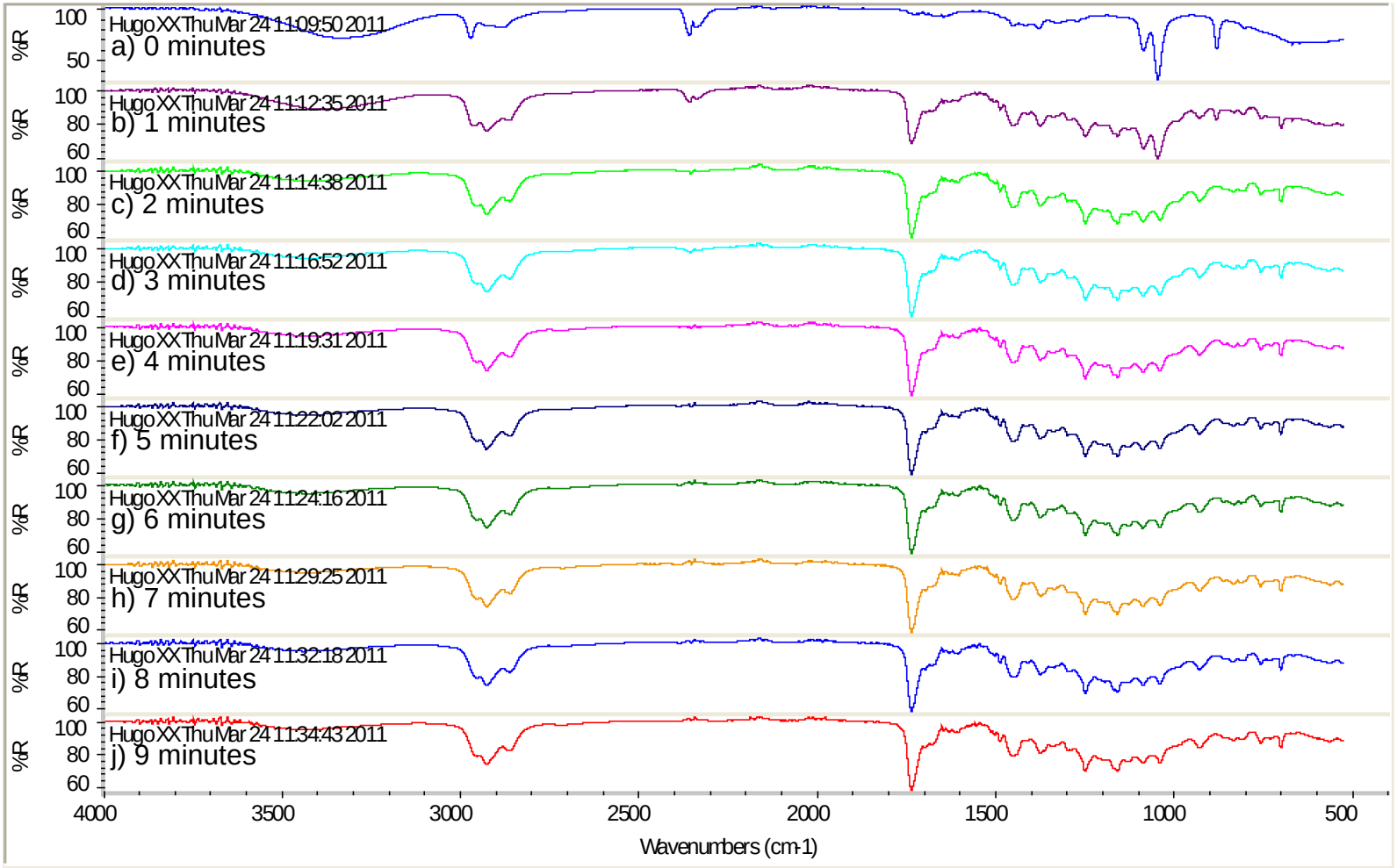


Figure 3.9 - Hugo XX 0-9 minutes

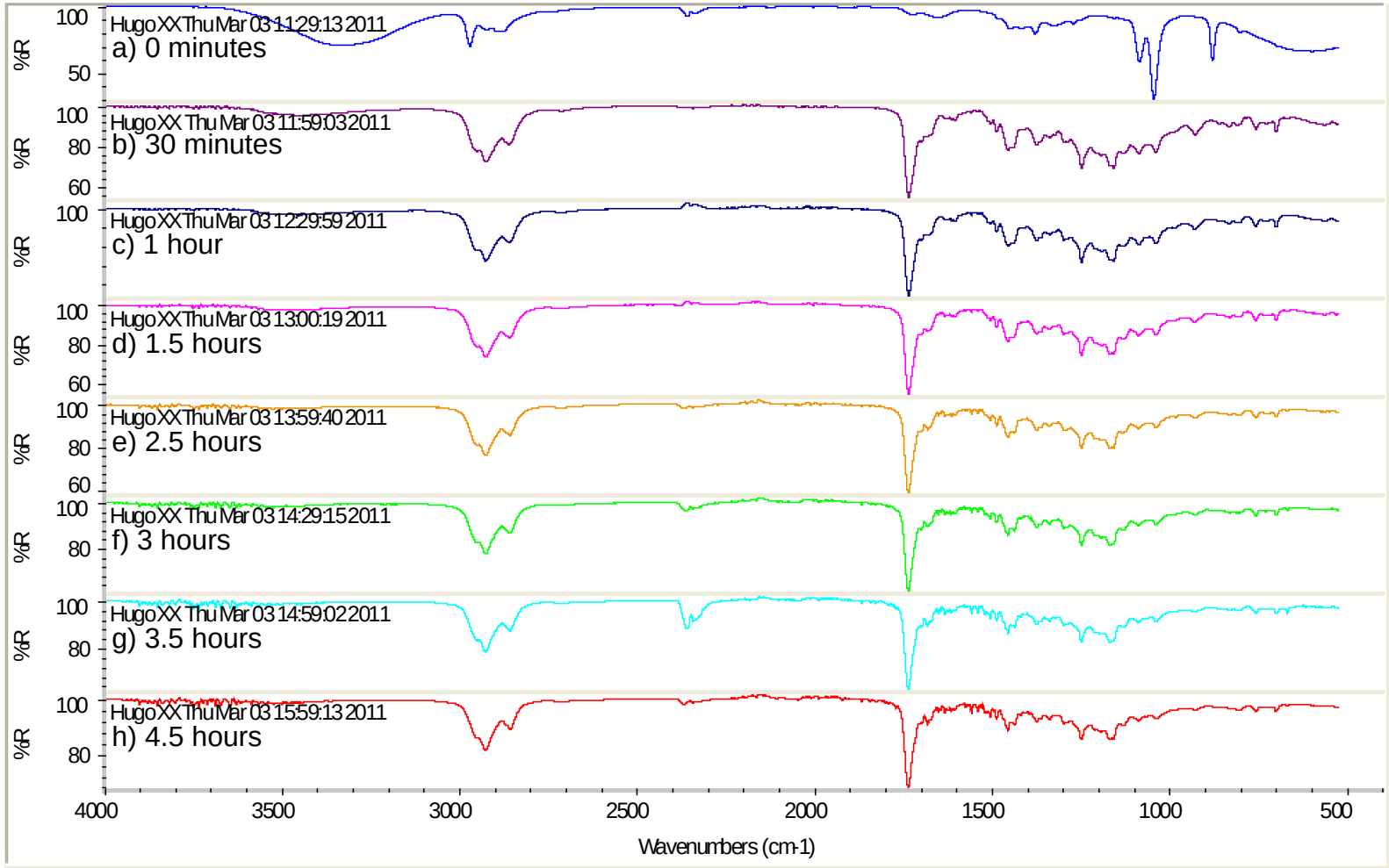


Figure 3.10 – FTIR Spectra for *Hugo XX* from 0 minutes to 4.5 hours

3.1.2.2 FTIR of Perfumes, Deodorants and Antiperspirants

For the next stage of the study, a further 16 products were analysed: nine additional perfumes, two body sprays, three aerosol antiperspirant-deodorants and two roll-on antiperspirant-deodorants. Without evaporation all the hydroalcoholic perfumes initially produced spectra that were indistinguishable from that produced by *Hugo XX* but after two minutes evaporation on the ATR stage the spectra appeared more individualising as seen from the examples in Figure 3.11. The spectra for the body sprays and aerosol antiperspirant-deodorants prior to evaporation also showed a broad O-H peak and a check of the ingredients lists showed that that denatured alcohol was used as a carrier solvent. As with the hydroalcoholics the spectra showed more discrimination after evaporation of 2 minutes (see Figure 3.11 and Figure 3.12). For all of the hydroalcoholics and aerosol products the evaporated spectra showed the same triple peaks between 2960 and 2860 cm^{-1} and the very intense peak around 1735 cm^{-1} (the interpretation of which are considered in Section 3.1.2.3). While the spectra for all these products initially appear very similar, an examination of the fingerprint region below 1300 cm^{-1} clearly shows there are differences (Figure 3.13). In contrast the spectra for the roll-on antiperspirant-deodorants showed very little change over time as can be seen for *Tea Tree Floral* in Figure 3.14. Water is listed as the primary solvent on the labels of these products and a comparison with the spectrum for water showed a high percentage match for these products (e.g. 81.48% match for *Tea Tree Floral*). All the products were allowed to evaporate for at least two minutes. The results of the subsequent library matching exercise are shown in Section 3.1.2.4.

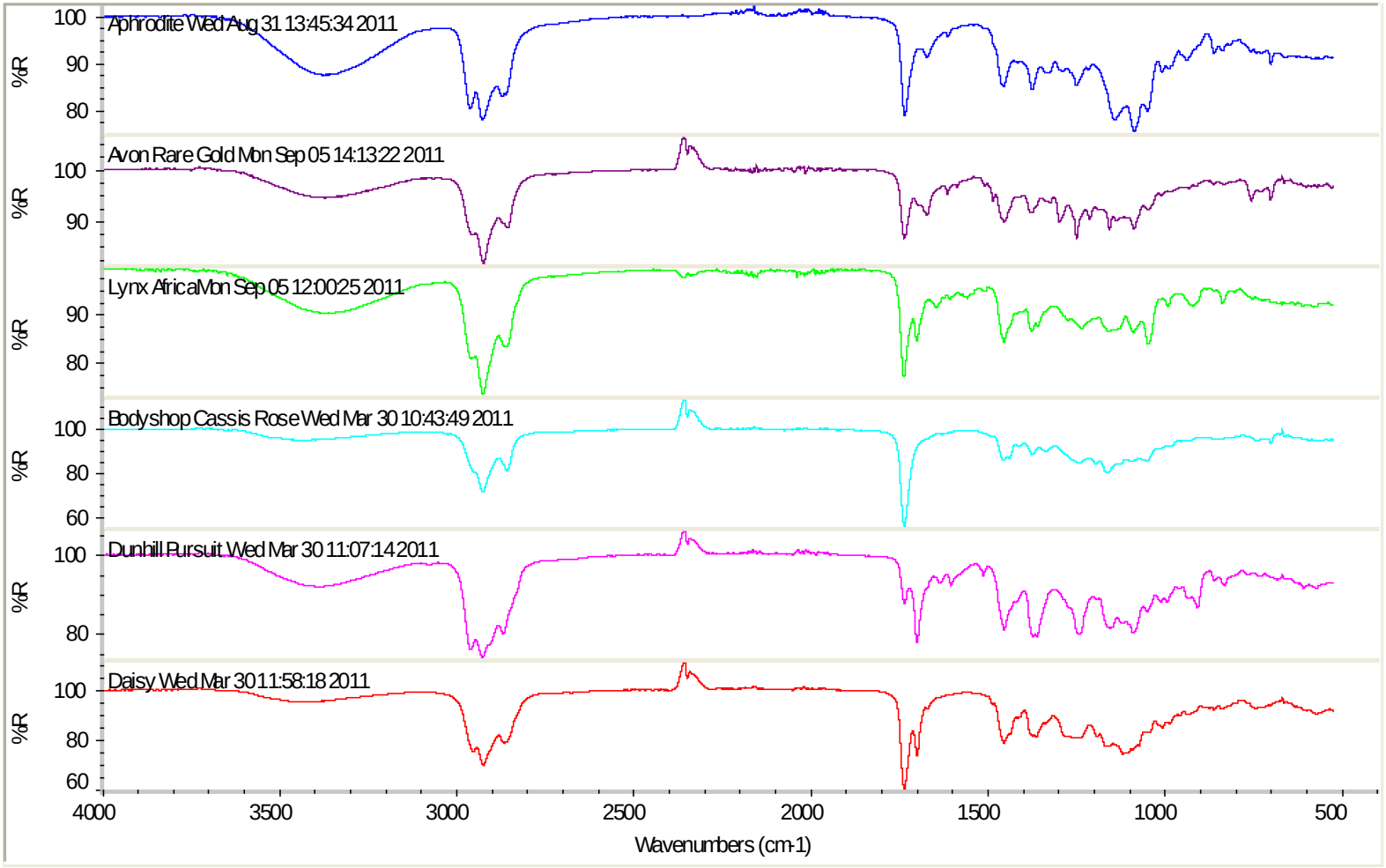


Figure 3.11 – FTIR spectra taken after two minutes for six perfumes and aerosol products

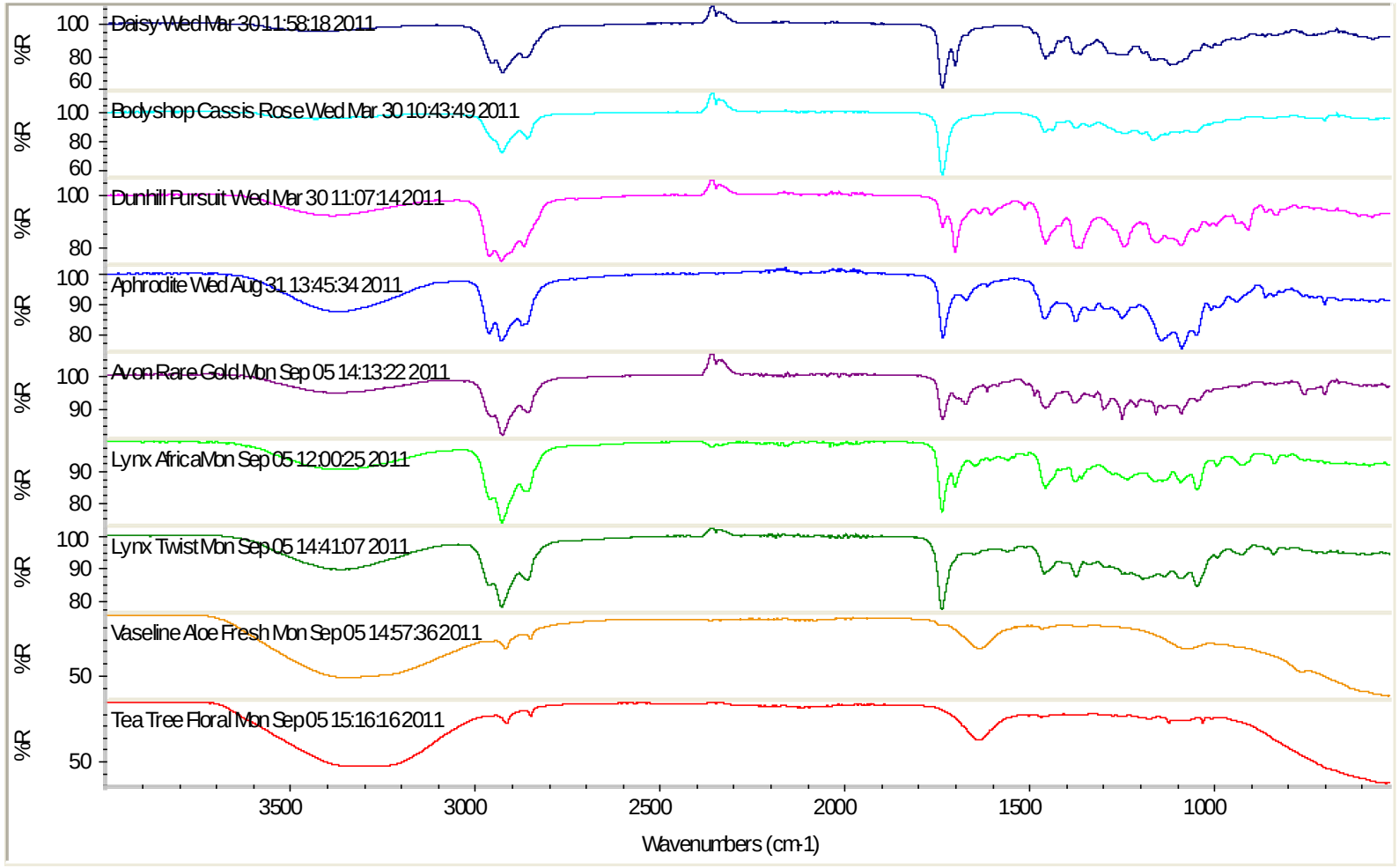


Figure 3.12 - FTIR spectra taken after two minutes for a range of perfumes, aerosol products and roll-ons

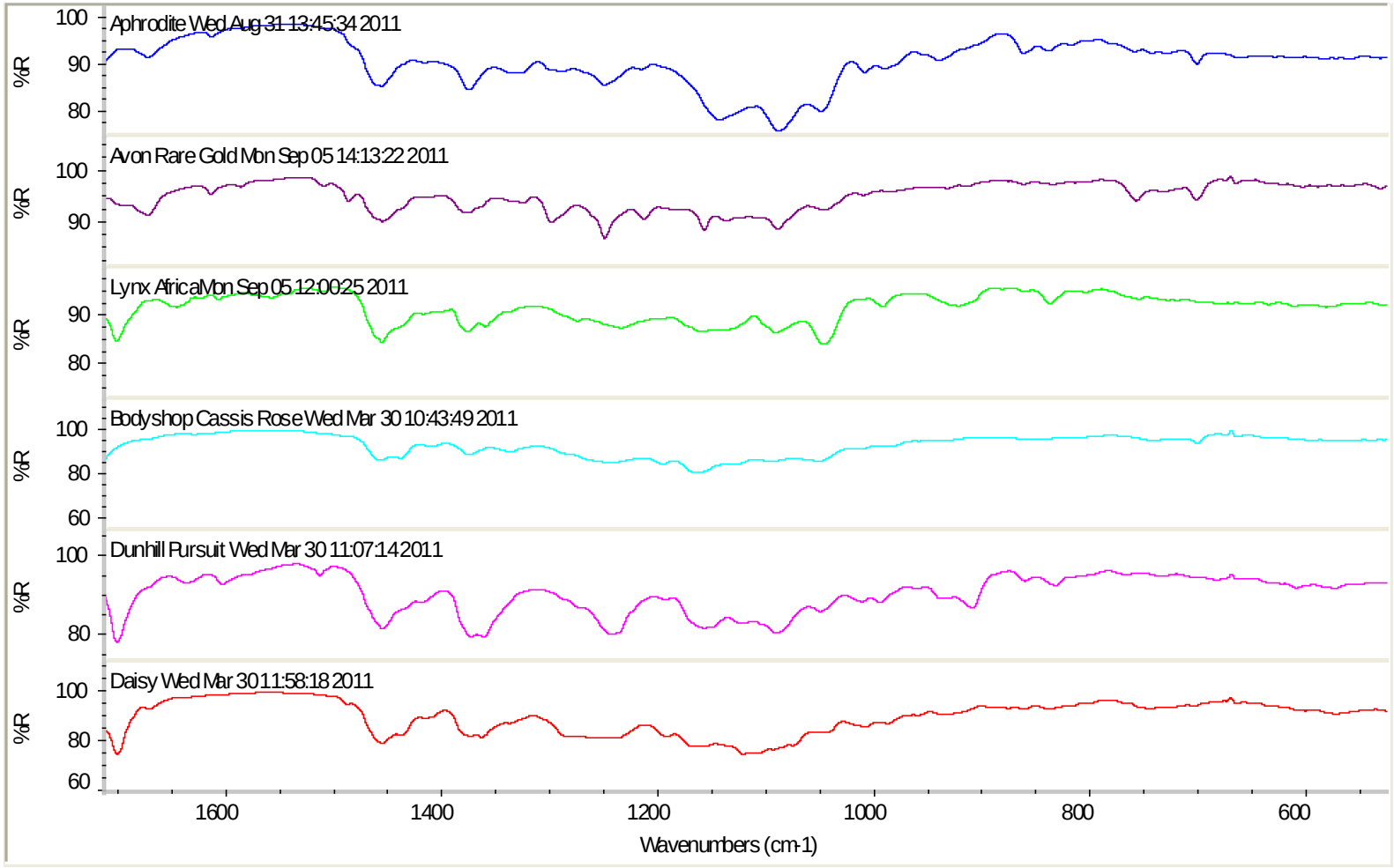


Figure 3.13 - Comparison of spectra for 1700 – 500 cm-1 only for six products (three body-spays and three perfumes)

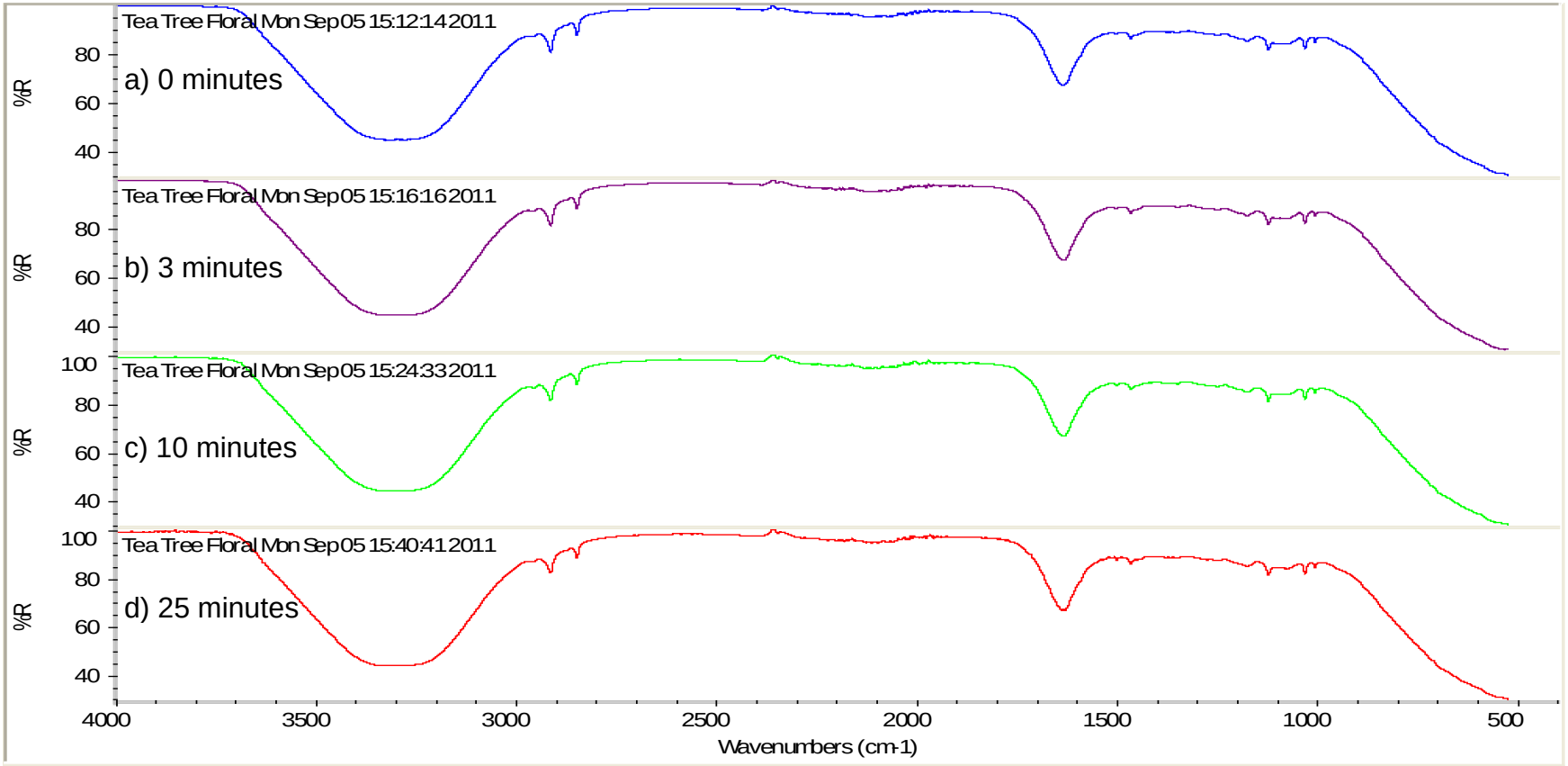


Figure 3.14 – Comparison of spectra for the roll-on antiperspirant-deodorant *Tea Tree Floral* for evaporation times of 0 to 25 minutes

3.1.2.3 Identification of Perfume by Spectral Interpretation

The first approach taken to identify and distinguish between individual perfumes, antiperspirants and deodorants was spectral interpretation. To test the practicality of this approach the spectrum for *Hugo XX* was subjected to a systematic analysis of its composition using group frequencies. The approach initially adopted was that recommended by Smith (1999 p. 27), first noting the presence or absence of the primary bands, then using the secondary bands to confirm the functional groups and combining this with other knowledge of the sample. The primary bands are shown in Table 3.4 together with the closest peaks from the spectrum.

Table 3.4 – Primary bands in *Hugo XX* spectrum taken after 30 minutes evaporation time

Position (cm^{-1})	Functional group	Peak position(cm^{-1})
3500-3200	O-H or N-H	3447
3200-2800	C-H	2860 2928 2954
2250-2000	C≡N C≡C	Not identified
1800-1600	C=O	1604 1614 1635 1736
<1000	C=C, Benzene rings	Many peaks

Adapted from (Smith, 1999 p. 27)

For the first primary band at 3500-3200 cm^{-1} the evaporated *Hugo* XX spectrum shows a peak at 3447 cm^{-1} which could be attributed to either an O-H or an N-H bond stretch. The peak in this spectrum (compared the that for the unevaporated perfume) is shifted and much weaker and if it is from alcohol O-H bonds then

much less hydrogen bonding is taking place (Colthup *et al*., 1990 p. 332). To confirm the presence of alcohol(s) there are peaks at 1039, 1088 and 1129 cm^{-1} which all occur within the range for the stretching of the C-O bond for various alcohols (Colthup *et al.*, 1990 p. 332). It should be noted there are many aroma chemicals with alcohol groups, including the PASs linalool, benzyl salicylate, citronellol, benzyl alcohol and farnesol all of which are listed as ingredients in *Hugo XX*. A further consideration for this first primary band, is that the peak at 3447 cm^{-1} is due to an amine or amide N-H stretch, but these are usually doublets (Colthup *et al*., 1990 p. 338) and although some aroma molecules do have nitrogen they are more often present as nitriles which have absorption band around 2250 cm^{-1} but for which no peaks are seen in this spectrum (Colthup *et al.*, 1990, Burr, 2003). Nonetheless as N-H stretch peaks are typically narrower and weaker than an O-H peak it is possible that such a peak is present but obscured.

The second of the primary bands is that for the C-H stretches with absorptions occurring at different wavenumbers for the three hybridisations of carbon: *sp* around 3300, sp^2 3100-3010 and sp^3 2950-2850 cm^{-1} (Housecroft and Constable, 2006 p. 408). In the *Hugo XX* spectrum there are three peaks in this area (2954, 2928 and 2860 cm^{-1}) which all fall within the absorption range of saturated sp^3 alkyl C-H bonds. The presence of such bonds is not surprising as they are present in almost all organic compounds and, as discussed in Section 1.4, popular aroma compounds such as terpenoids are made up of isoprene units which contain C-H bonds. The presence of alkyl groups can be confirmed by -C-H bending peaks between 1350 and 1480 cm^{-1} and it can be seen from the spectrum in Figure 3.8 that there are significant peaks at 1456 and 1374 cm^{-1}. Given the wide range of

species used as aroma chemicals it is notable that C-H absorptions from alkene (sp^2) =C-H bonds (stretch absorptions expected at 3010-3100 cm^{-1}) and from aromatic (sp^2) =C-H bonds (expected at 3000-3100 cm^{-1}) are not seen. This is particularly interesting as benzyl alcohol, is given as a listed ingredient in *Hugo XX*. The aromatic =C-H bonds do however usually appear as a number of weak absorptions (Volland, 1999) and the aroma compounds are often present at very low concentrations, so it is possible that these are simply obscured by the more dominant alkyl absorptions. Confirmation of the presence of aromatic compounds would be provided by the presence of medium to strong absorptions in the region 1650-1450 cm^{-1} (Volland, 1999) and there are certainly a significant number of peaks within this region. Smith also suggests that to confirm the presence of benzene rings one should look for bands between 1600-1400 cm^{-1} and 800-600 cm^{-1} (Smith, 1999). Unfortunately, while there are a number of peaks in these regions, the group frequencies for substituted benzene rings are one of the most complicated groups to interpret (Colthup *et al.*, 1990 p. 261). Aldehyde C-H stretches are also not seen: they would be present at 2850 and 2750 cm^{-1} as two medium intensity peaks on the right hand shoulder of the alkyl C-H's (Hanson, 2017). The presence of the aldehydes can however be confirmed by looking for the associated carbonyl C=O stretch peak around 1740-1720 cm^{-1} and there is a strong peak at 1735 cm^{-1} which is discussed in more detail below.

The next primary band is between 2250-2000 cm^{-1} for the C≡N and C≡C stretches but for the evaporated *Hugo XX* sample there were no peaks identified in this area.

For the primary band between 1800 and 1600 cm^{-1} which is typical for C=O stretches there are a number of small peaks and also the most intense peak on this spectrum at 1736 cm^{-1}. This falls within the range for aldehydes (~1740-1720 cm^{-1}) with the higher wavenumber indicating a greater polarisation of the bond due to the higher δ^+ from the carbon (Housecroft and Constable, 2006 p. 405). As discussed above, confirmation of the presence of aldehydes would normally be found in the presence of the two C-H stretches between 2820-2850 and 2720-2750 cm^{-1} (Hanson, 2017) but these are not apparent. The peak at 1736 cm^{-1} is also on the edge of the range for ester C=O stretches (listed as ~1750-1735 cm^{-1} in Housecroft and Constable p. 405). Confirmation for the peak being produced by an ester would be the presence of two bands or more of C-O stretches between 1000-1300 cm^{-1} (Hanson, 2017) and there are a number of peaks within this range. Both esters and aldehydes are popular types of aroma chemicals. Ketones are also popular aroma chemicals but if it is present the characteristic strong C=O stretch near 1715 cm^{-1} (Colthup *et al*., 1990 p. 295) may be obscured by the intense peak at 1736 cm^{-1}. The remaining three peaks listed within this band for this spectrum are at 1604.48, 1614 and 1635 cm^{-1} which is the carbonyl C=O stretch range for more mesomeric compounds including carboxylic salts (Colthup *et al*., 1990 p. 390). Alkene C=C bonds also absorb at 1680-1600 (Colthup *et al.*, 1990 p. 390) and terpene derivatives are popular aroma chemicals (Sell, 2003 p.2) but the complexity of the spectrum makes it difficult to confidently assign the confirmatory strong C-H bending absorption around 675-1000 cm^{-1}. This is also the area within which benzene, C=C bonds produce medium to strong absorptions (in the region 1650-1450 cm^{-1}) although the CH stretch band is much weaker than in alkenes (Volland, 1999).

The last of the primary bands listed by Smith is for the area below 1000 cm^{-1} which Smith considers useful as an indicator of the presence of benzene rings (Smith, 1999 p. 27). There are strong peaks at 1087 and 1045 cm^{-1} which are within the band typical of Ar-H in-plane deformations but these are normally weak bands (Vogel and Furniss, 1978 p. 1274). The presence of aromatic compounds may be confirmed by bands between 1600-1400 cm^{-1} and 800-600 cm^{-1} (Hanson, 2017) and there are a number of peaks within both these bands. Many aroma chemicals (including 17 of the 24 volatile PASs) have at least one benzene ring structure including benzyl alcohol and benzyl salicylate which are listed ingredients for *Hugo XX*.

This attempt at a systematic analysis of the composition of *Hugo XX* using group frequencies has shown that in a complex mixture such as a perfume few of the structures can be determined with any certainty. The numerous absorptions in the 'fingerprint region' below 1300 cm^{-1} are especially difficult to assign to individual vibrational modes of particular aroma chemical molecules and Housecroft and Constable suggest that a more effective approach is to compare the spectra with reference standards (2006 p. 402).

3.1.2.4 Identification of Perfumed Product by Library Matching

The second approach taken to identify and distinguish between individual perfumes, antiperspirants and deodorants was library matching using the OMNIC software. To determine the best spectrum to use as a reference spectrum preliminary experiments were conducted with two perfumes, *Hugo XX* and *Bodyshop Cassis Rose,* results from which indicated that while the spectrum for each perfume continued to change over the evaporation time, the pattern observed at two or three minutes was representative of the spectra for that perfume for 30 minutes or more. At least one spectrum taken after two or three minutes evaporation was loaded into the library for each of the 17 perfumed products used in the part of the study (see Appendix B for details). Additional spectra were then collected across a range of dates and times and searched against this library and the general libraries provided with the software. Out of the 17 perfumed products the software successfully identified 16 with at least an 87% match (see Table 3.5). Some samples which had been evaporated for 30 minutes were also correctly matched as were the roll-on deodorant-antiperspirants, although the sample size was smaller for these products with just two roll-ons being analysed.

The incorrectly identified product was the perfume, *Rock 'n Rose*, which was identified as *Bodyshop Cassis Rose* (89%) with the correct identification as the second match (87%). Additional spectra were collected and the experiment repeated with similar results despite the spectra appearing to show clear differences (see Figure 3.15). As discussed in Section 3.1.1.2 analysis of GC-MS results indicated the two perfumes do have some chemicals in common.

Table 3.5 – Library matches for 17 products

Sample		Match		Match %	Correct match
Perfume	**Age (mins)**	**Matched with**	**Age (mins)**		
Hugo XX	3	Hugo XX	2	98.18	✓
Hugo XX	30	Hugo XX	2	93.77	✓
Cassis Rose	30	Cassis Rose	2	87.32	✓
Manifique	2	Manifique	2	97.63	✓
Rock ‘n’ Rose	2	Cassis Rose	2	89.38	×
		Rock 'n Rose	2	87.12	✓
Rock ‘n’ Rose	3	Cassis Rose	2	89.13	×
		Rock 'n Rose	2	86.89	✓
Pursuit	2	Pursuit	2	97.63	✓
Pursuit	7	Pursuit	2	92.43	✓
Essence de Femme	2	Essence de Femme	2	89.54	✓
Urban	2	Urban	2	96.86	✓
Ysatis	2	Ysatis	2	97.05	✓
Mania	2	Mania	2	97.80	✓
Daisy	2	Daisy	2	90.88	✓
Lynx Africa	5	Lynx Africa	3	97.23	✓
Avon Rare Gold	8	Avon Rare Gold	3	94.12	✓
Lynx Vice	5	Lynx Vice	3	94.43	✓
Lynx Twist	5	Lynx Twist	3	95.84	✓
Vaseline Aloe Fresh	10	Vaseline Aloe Fresh	3	91.48	✓
Tea Tree Floral	10	Tea Tree Floral	3	97.52	✓
Aphrodite	20	Aphrodite	3	96.52	✓

Note: where age of sample perfume and matching perfume are the same, samples were taken on different dates and details are given in Appendix B.

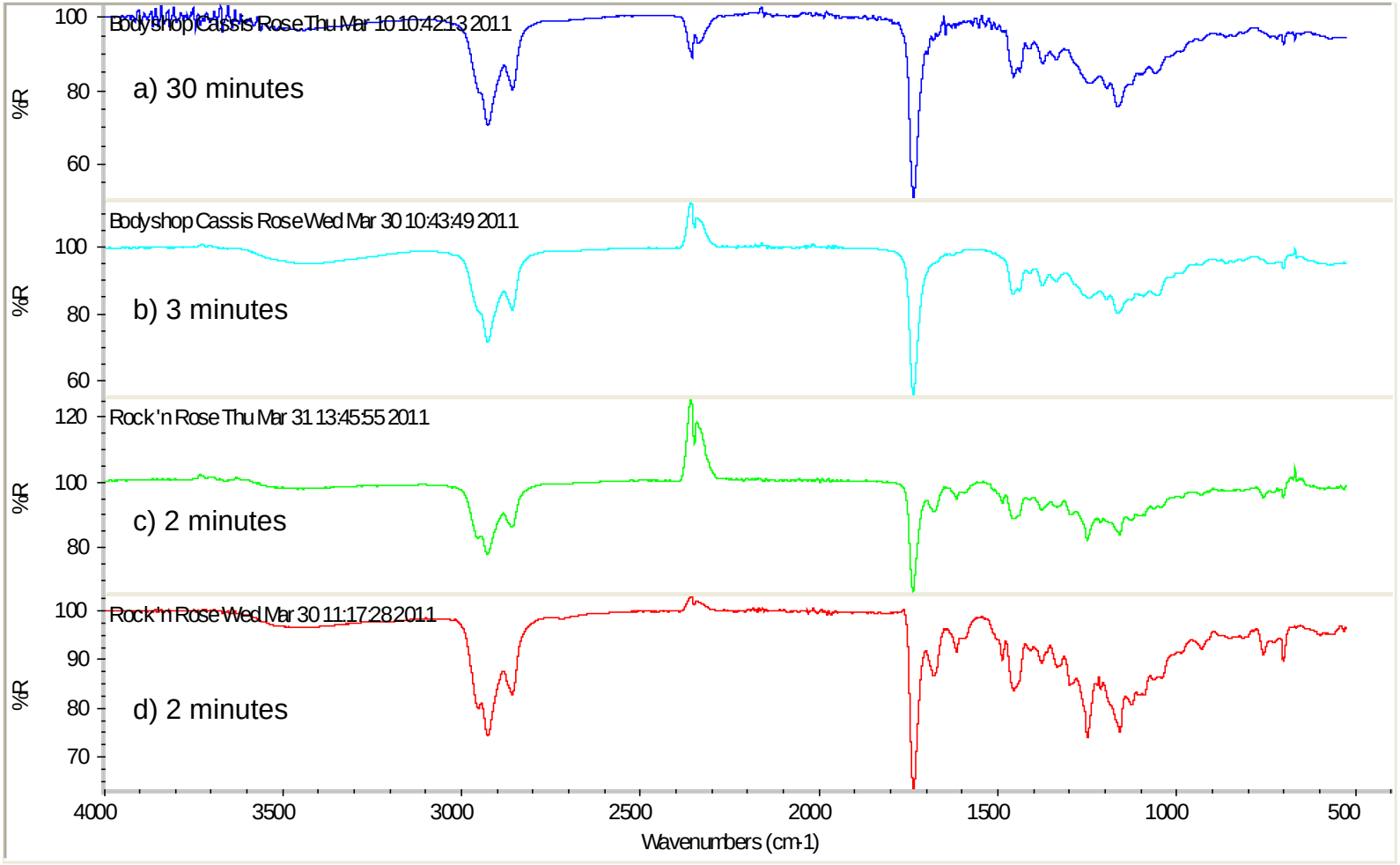


Figure 3.15 – Comparison of Rock 'n' Rose and Cassis Rose

3.1.2.5 Identification of Perfumed Product by Listed Aroma Chemicals

The final approach taken with FTIR to identify and distinguish between individual perfumes, antiperspirants and deodorants was to attempt the identification of particular ingredients. As discussed previously the complexity of the mixtures for each perfumed product made it difficult to assign peaks to vibrational modes in particular aroma chemicals, Wang *et al*. (2014) did however apply FTIR to the quantitative analysis of suspected fragrance allergens identify what they considered to be characteristic wavenumber regions for these chemicals (Wang *et al*., 2014). Of the compounds examined by Wang *et al*. (214), five are compounds which are considered Potential Allergenic Substances by the SCCP (see Section 1.4.4) and therefore must be listed on the label of perfumed products. These chemicals and the characteristic wavenumber region assigned by Wang *et al.* (2014) are given in Table 3.6. Four perfumed products were used to assess the potential of this approach: two body sprays and two perfumes.

Table 3.6 – Characteristic FTIR regions for selected aroma chemicals

Aroma Chemical	Cinnamyl alcohol	Eugenol	Citral	Geraniol	Amyl cinnamal
Characteristic Region (cm^{-1})	**732–734**	**792–795**	**1193 – 1194**	**1375 – 1378**	**1623– 1624**
Avon Rare Gold	Listed 731	N.L.	N.L.	Listed 1378	Listed
Cassis Rose	N.L.	N.L.	Listed 1195	Listed 1374	Listed
Hugo XX	N.L.	N.L.	Listed 1193	N.L. 1375	N.L.
Aphrodite	N.L.	N.L.	N.L.	N.L. 1375	N.L.

'Listed' denotes that this chemical was listed on the packaging; 'N.L.' denotes that this chemical was not listed on the packaging;. The wavenumbers given are the nearest within 1 cm^{-1} to the characteristic region as listed by (Wang *et al*., 2014).

As can be seen in Table 3.6 the identification of selected aroma chemicals was not very successful. The body spray *Avon Rare Gold,* for example, had three of the target chemicals as listed ingredients: cinnamyl alcohol, geraniol and amyl cinnamal but while peaks were identified just outside the expected region for cinnamyl alcohol and within the characteristic region for geraniol, the spectrum did not have a peak with a wavenumber close to the characteristic region for amyl cinnamal. Another problem was seen with the body spray Aphrodite which did not have any of these ingredients listed but which did show a peak within the characteristic region for geraniol. The remaining two perfumes had similar problems and of the four aroma chemicals investigated here, three, cinnamyl alcohol, citral and eugenol showed a consistent relationship between the ingredient listing and the presence of a peak but geraniol and amyl cinnamal seemed unreliable.

3.1.2.6 Evaluation

Analysis of liquid perfumed products by FTIR is quick and easy and shows potential for comparison of samples and their presumptive identification through the use of library matching software, this was however, shown to be prone to error, even with the somewhat limited sample size used here. Due to the complexity of the samples this study also indicates that it is unlikely that FTIR could be used to determine the ingredients of a perfumed product by spectral interpretation or by assigning characteristic wavenumbers to key aroma chemicals.

3.1.3 High Performance Liquid Chromatography (HPLC) with UV-Visible Spectroscopy (UV-Vis)

3.1.3.1 Analysis by UV-Vis Spectroscopy

Initially perfume samples *Mania* and *Daisy* were analysed by UV-Vis to determine the most suitable wavelengths for detection. The results for the 1:1 mix of perfume and methanol were poor with both samples producing absorptions above 2.0 for wavelengths from 200 nm to around 400 nm and thereafter a very low response. When diluted further (to a 1:10 ratio of perfume to methanol) there appeared to be a particularly strong response at 210 and 280 nm so these were chosen as the detector wavelengths for HPLC. For both perfumes the absorbance was at a minimum at 400 nm so this was chosen as a reference wavelength.

3.1.3.2 Analysis by HPLC

Results for HPLC analysis of perfume *Mania* using a detection wavelength of 210 nm are shown in Figure 3.16 below. There is a strong response with the peak for one, early eluting (unretained) compound reaching a height of 1000 mV and another, better retained compound (retention time 28 minutes) producing a peak height above 700 mV. There are also at least another 16 peaks but many of these are not fully resolved. The results for the same sample using a detection wavelength of 280 nm are shown in Figure 3.17 and show a much lower response overall with one, unretained compound, producing a peak at height around 550 mV and another better retained compound (retention time again at 28 minutes) producing a peak height at around 100 mV. There were also fewer peaks detected at this wavelength. As can be seen from the overlaid chromatograms in Figure 3.18, many of the peaks detected at 280 nm are also detected at 210 nm.

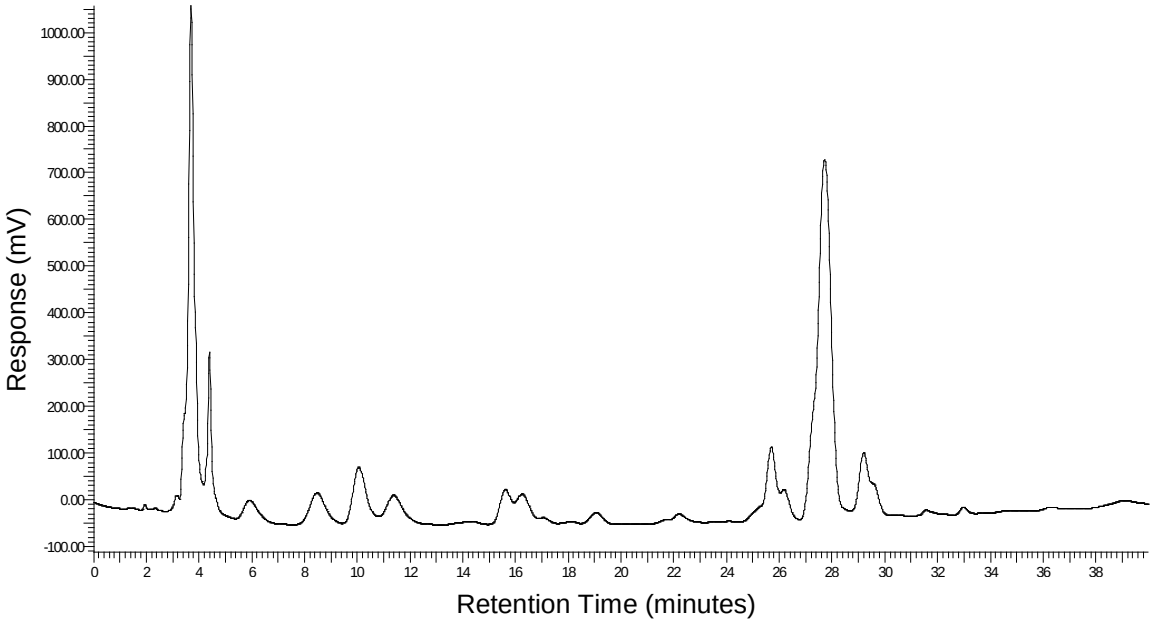


Figure 3.16 - Chromatogram for perfume *Mania* at 210 nm

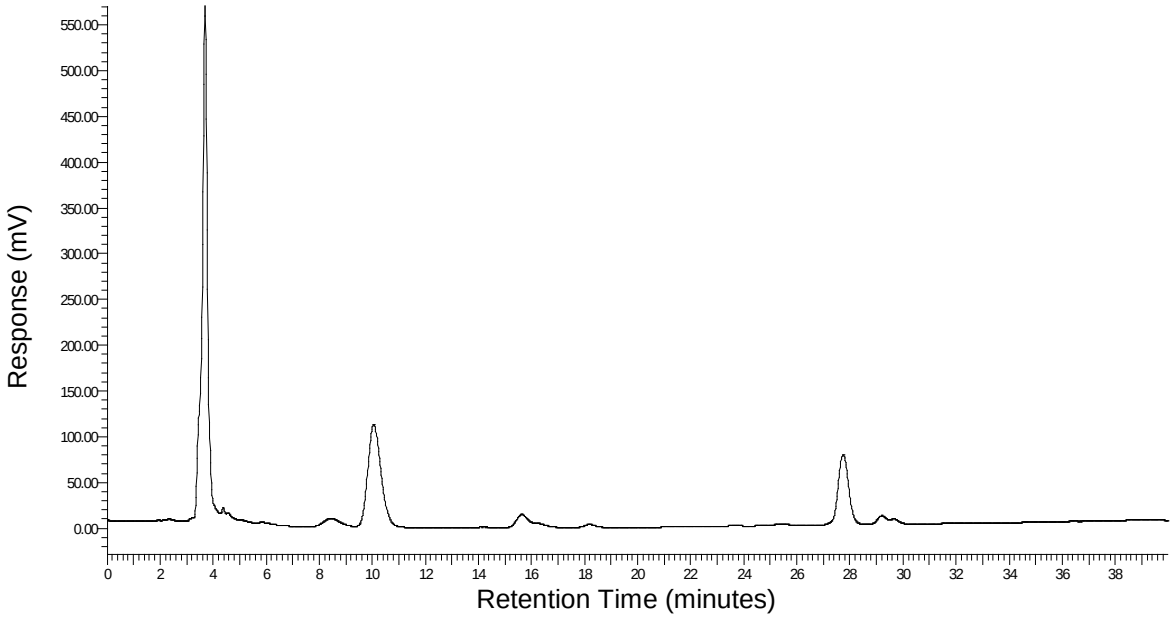


Figure 3.17 - Chromatogram for perfume *Mania* at 280 nm

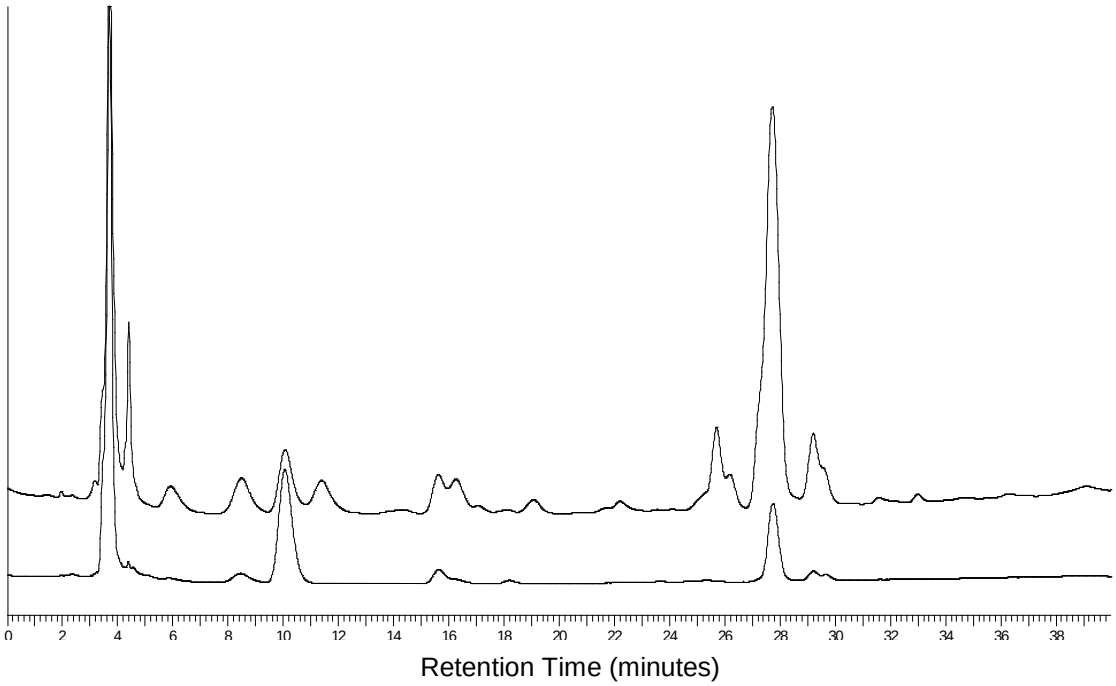


Figure 3.18 – Overlay of chromatograms for perfume *Mania* at 210 nm (top) and 280 nm (below) rescaled and offset for comparison

Results for perfume *Daisy* using the same detection wavelengths are shown in Figure 3.19 and Figure 3.20. The chromatograms were generally disappointing with only few peaks at either wavelength. A significant peak was seen at around 26 minutes for both wavelengths and may be the same compound as that in perfume *Mania*. Once again the chromatogram at 210 nm was more detailed and showed a better response for the peaks compared to detection at 280 nm. The most significant feature in both chromatograms consisted of a large non-Gaussian peak indicating the presence of an unresolved complex mixture or a compound which is very strongly absorbing across both UV-Vis wavelengths.

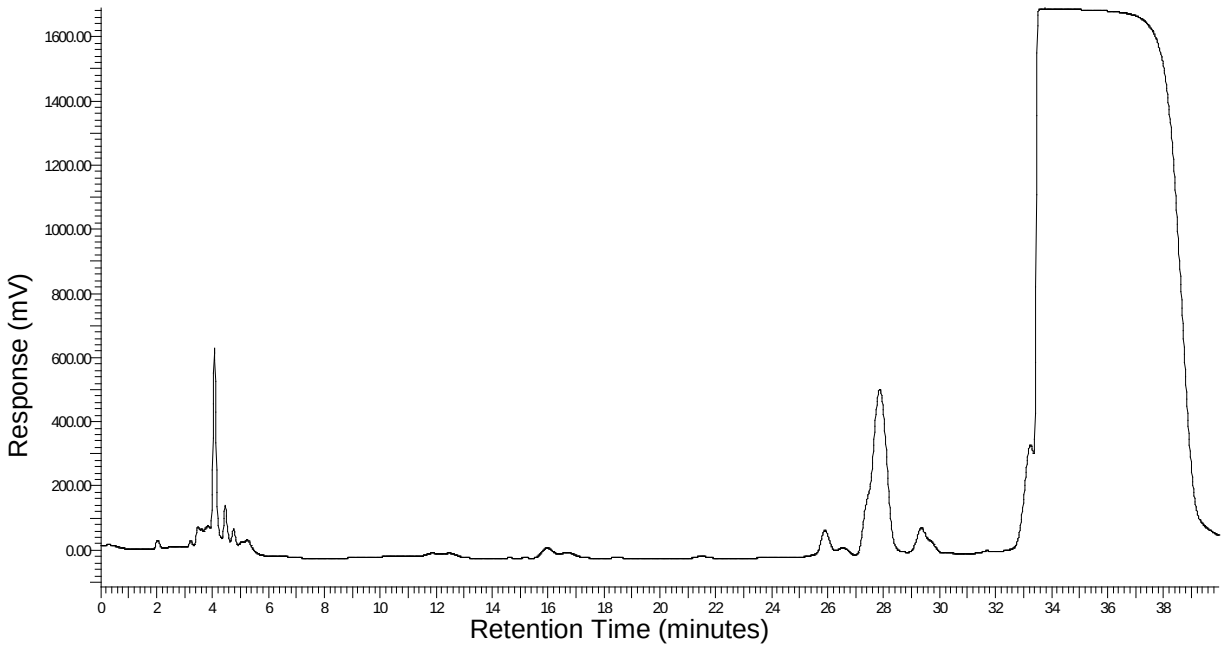


Figure 3.19 - Chromatogram for perfume *Daisy* showing the response at 210nm

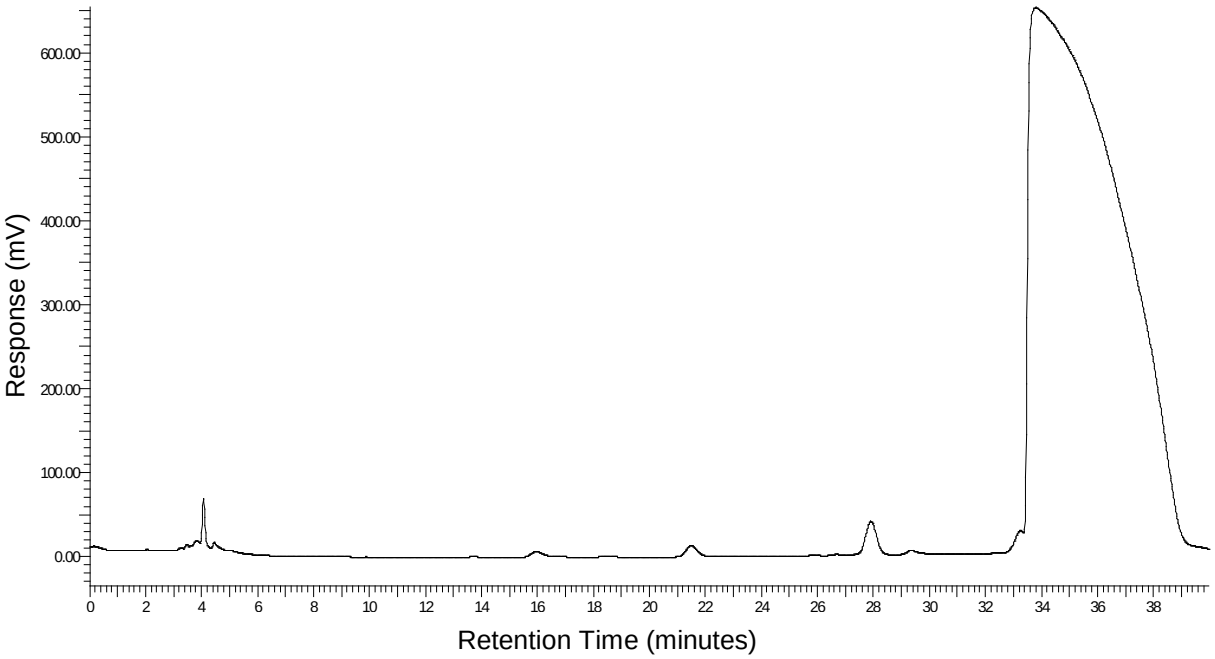


Figure 3.20 - Chromatogram for perfume *Daisy* showing the response at 280nm

Considering the results for the two perfumes it is first noted that more peaks and a better magnitude of detector response was achieved using a detection wavelength of 210 nm. Many aroma chemicals have chromophores which absorb strongly at this wavelength: ketones, aldehydes, carboxylic acids, esters and lactones all exhibit a similar absorption profile with a strong absorption band corresponding to the $\pi \rightarrow \pi^*$ transition starting at around 190 nm but the bathochromic shift caused by the conjugation seen in many aroma chemicals should mean that the longer wavelengths of 210 nm would be suitable for detection of these species (Vogel and Furniss, 1978 p. 1060). Polynuclear aromatic compounds (with fused benzene rings) are also popular aroma chemicals with naphthalene exhibiting strong absorbance at 220 nm and again, extending the number of benzene rings produces a bathochromic shift to longer wavelength and increase in intensity (Christian, 2004 p. 467).

The detection wavelength of 280 nm had no obvious benefit for the two perfumes analysed. Although the chromophores listed above all experience $n \rightarrow \pi^*$ transitions which absorb around 280 nm , these are much weaker absorbance bands (Vogel and Furniss, 1978 p. 1060) and few peaks were seen despite the likely presence of auxochromes (hydroxy and methoxy groups are common in aroma molecules) which should enhance absorption and (Christian, 2004 p. 466). It is recognised that other wavelengths may be appropriate for other ingredients and is noted that Villa *et al.* (2007) employed an additional acquisition wavelength at 254 nm. Some of the most commonly used aroma chemicals, however, are terpenoids and while some sesquiterpene species have aldehydes and ketone chromophores, many others do not have chromophores for the UV region, or

exhibit only weak absorptions between 116 and 254 nm (Simpan, 2005). An example of such a compound is limonene which has two isolated double bonds and shows no significant absorbance above 207 nm (Berger and Sicker, 2009 p. 363). The use of shorter wavelength may be possible and, close to 200 or 190 nm almost all organic analytes absorb light and the detector can be considered almost universal (Kok, 1998 p. 149) however most solvents also absorb at these wavelengths and acetonitrile and water (the mobile phases used here) both have a cut-off point of 190 nm (Snyder *et al.*, 1997 p. 722).

It was also noted that the peak around 26 minutes has features which are consistent between both samples and this could be the same chemical compound in both perfumes. Villa *et al.* (2007) identify a peak at 26.28 ± 0.12 minutes as benzyl salicylate but this would need to be confirmed by comparison with a standard and at this stage of the study peak identification was not the primary objective. Finally, although the chromatograms for *Daisy* and *Mania* are very different, and while detection at 210 nm appears to produce a potentially useful chromatogram, given that a perfume of this type would be expected to have 50 or more ingredients, the relatively small number of peaks and their poor resolution indicate that these chromatograms may not be suitably individualising.

3.1.3.3 Evaluation

These initial investigations indicated that the usefulness of HPLC with UV-Vis detection to discriminate between the hundreds of different perfumes, antiperspirants and deodorants may be limited and, unless coupled with MS detection, the technique is better suited to the analysis of specific target analytes.

3.1.4 Conclusion on Evaluation of Instrumental Methods

Methods were evaluated on the basis of:

- Speed
- Ease of use
- Ability to discriminate between products
- Ability to produce additional discriminatory information

FTIR proved to be a very easy and rapid form of analysis for liquid perfumes with sample preparation and analysis time totalling less than five minutes. While the library search facility is very appealing the level of discrimination was not 100 % and, for the complex mixtures being studied, the lack of a separative aspect to this technique and resultant lack of identification of ingredients limits the usefulness of FTIR in this study.

HPLC analysis was moderately easy to conduct with minimal sample preparation and a run time of 30 minutes per sample (plus additional instrument set-up time). While HPLC has the benefits of being a separative technique analysis, only a small number of peaks were produced for each perfume which would limit the level of discrimination possible. Using UV-Vis as the detection method identification for individual ingredients would only be possible using reference standards which is considered impractical given the thousands of aroma compounds in use.

GC analysis was also moderately easy with sample preparation and run time equivalent to that of HPLC but with a shorter instrument set up time (no need to filter eluent or flush columns). The chromatograms produced with both FID and

MS detectors contained a large number of peaks and proved discriminating, although this data analysis was more time consuming than the library search on the FTIR. When this separative technique was coupled with MS it had the additional benefit of being able to provide a tentative identification of some key ingredients.

Having evaluated the three instrumental techniques for their potential in being able to distinguish between a range of perfumes, antiperspirants and deodorants it was determined that, while FTIR offered some potential and should be used as an additional technique to investigate whether aged perfumes can be identified (see Section 3.5), GC-FID and GC-MS offered the best potential and the majority of the remaining research was conducted using this technique. Accordingly the next phase of the research was to address some of the concerns raised during the GC evaluation with regard to optimisation of the temperature program and the identification of ingredients.

3.2 Evaluation of GC Methods

Having determined that GC was the most promising instrumental technique, the next stage was to ensure that the GC methods would produce reliable results. Due to the complexity of the samples involved, this required the use of novel approaches to evaluate the suitability of GC temperature programs and to assess requirements for the identification of key ingredients.

3.2.1 GC Temperature Programs - Variation of Temperatures and Times

The first objective was to understand the effect of different start temperatures, holds and temperature ramp rates. These parameters were evaluated visually and then numerically, including comparison of peak heights and areas and calculating the area-to-height ratio for selected peaks. Results of this evaluation showed that the effect of using different start temperatures was particularly significant.

3.2.1.1 Visual Observations of Chromatogram Quality

The result of changing the start temperature can be seen in Figure 3.21 which shows GC-FID chromatograms for the perfume *Daisy* using start temperatures of 40, 50 and 60°C for otherwise identical temperature programs. It can be seen that increasing the start temperature causes a decrease in response for peaks across the chromatogram but resolution was improved, particularly for the early eluting peaks. An example of this is seen with feature ‘A’ which consists of a number of co-eluting peaks (tentatively identified as the mix of isomers of the highly polar solvent dipropylene glycol) which become better resolved as the start temperature increased.

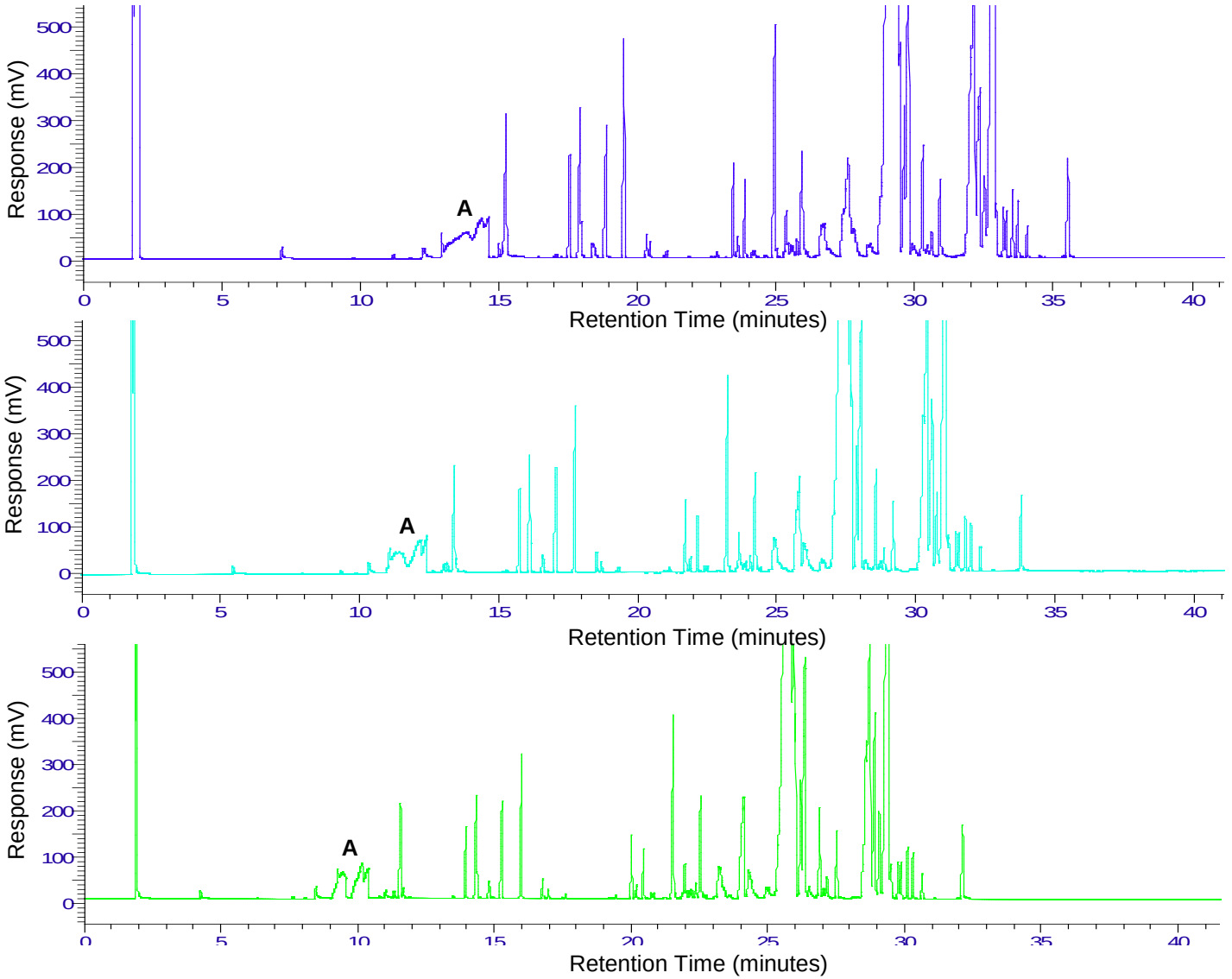


Figure 3.21 – Chromatograms for perfume *Daisy* using initial temperatures of 40°C (top), 50°C (middle) and 60°C (bottom)

While literature sources generally advise that a low initial temperature should improve the resolution of early-eluting peaks (Grob and Barry, 2004, Sparkman *et al*., 2011), the boiling point for DPG is 232˚C (Dow, 2017). and, if the first peak eluting after DPG in Figure 3.21 is linalool (as presumptively identified) with a boiling point of 198°C (Pubchem, 2017), then a temperature of around 150˚C should offer the most phase transitions and therefore the best resolution (Giddings, 1962 p. 13, Lundanes *et al*., 2014). For the temperature programs used above, the elution temperature of these two chemicals range between 88-100˚C for the 40˚C start temperature, to 108-120˚C for the 60˚C start temperature. It seems that, in the case of perfume analysis, the majority of aroma chemicals have boiling points which are too high for a lower start temperature to be beneficial and a 60°C start temperature was considered acceptable, although results indicate that a higher start temperature may be possible.

The effect of changing the initial hold time of a temperature program was, in contrast, relatively minor. Using a program with an initial temperature of 60°C it was observed that, for some peaks, increasing the initial hold from one to five minutes did improve resolution slightly but also reduced peak height. Literature sources suggest that increasing initial hold time may improve the resolution of early peaks (Grob and Barry, 2004, Sparkman *et al*., 2011) but it seems that this has limited benefit for the samples of perfumed products, where the earlier, lower boiling point peaks are less numerous than the later eluting ingredients.

The effect of incorporating a hold later in the temperature program was also limited: a five minute hold at 150°C produced somewhat better resolution of the

later peaks, but neither shorter holds of one minute nor holds at lower temperatures were observed to be especially effective. Incorporating two five minute holds, one at 150°C and 180°C did however result in a better separation of the final group of peaks for one perfume, *Mariage*. These five minute holds did however add to the overall run-time of the program, which was not desirable.

Increasing the temperature ramp rate would potentially have benefits in terms of shorter run times. For the analysis of perfumed products results of trials showed that, when using a single ramp rate of 6, 10 or 12°C per minute, the higher ramp rate did indeed cause a decrease in the resolution of earlier peaks but most peaks were still adequately resolved. The separation of later peaks initially appeared to be slightly improved but a closer examination of the chromatograms revealed that although the later eluting peaks in the chromatogram produced by the temperature program with a 12°C ramp appeared to be better resolved, this was actually due to peaks co-eluting, i.e. where two peaks (or a split peak) was present on the chromatogram produced by a slower ramp rate, only one was produced at the faster ramp rate. Although a ‘cleaner’ chromatogram may have some benefits for statistical analysis of peak area or height, this lack of separation would hinder peak identification by MS as the unresolved peak would include ions from more than one chemical.

3.2.1.2 Numerical Evaluation of Chromatogram Quality

The recommended approach for evaluating chromatogram quality is to quantify the separation between two peaks by calculating the resolution factor, R_s (Equation 2) and, if the peaks are identical Gaussian curves, a resolution factor of 1.5 would indicated that they are fully baseline resolved and the valley height does not exceed 0.02% of peak height (Rouessac and Rouessac, 2000 p. 16). It is generally considered that a resolution of 1.0 (2.3% overlap in peaks of equal width) is considered the minimum to allow accurate quantification with a resolution of 0.6 needed to discern a valley and enable the peak to be correctly integrated (Christian, 2004 p. 569).

Equation 2 - Resolution factor

$$R_s = 2 \times [(t_{R2} - t_{R1}) / (w_{b1} + w_{b2})]$$

Where t_{R2} and t_{R1} are the retention times of the peaks and $w_{b1} + w_{b2}$ are the peak widths at the base.

It should be noted that other versions of the equation use the width at half the peak height (Rouessac and Rouessac, 2000).

For the three start temperatures of 40, 50 and 60°C, resolution was calculated for four selected peaks as shown in Figure 3.22. Peaks 1 to 4 were chosen because of their ease of identification in the pattern and were subsequently tentatively identified by GC-MS (using the NIST library) as linalool, linalyl acetate, alpha-isomethylionone and ethylene brassylate respectively. For peak 1, the resolution improved by 32% for the first increase in start temperature and then by 33%, improving from R_s = 0.37 to R_s = 0.80, although as shown by these values the peak was not completely resolved even at 60°C.

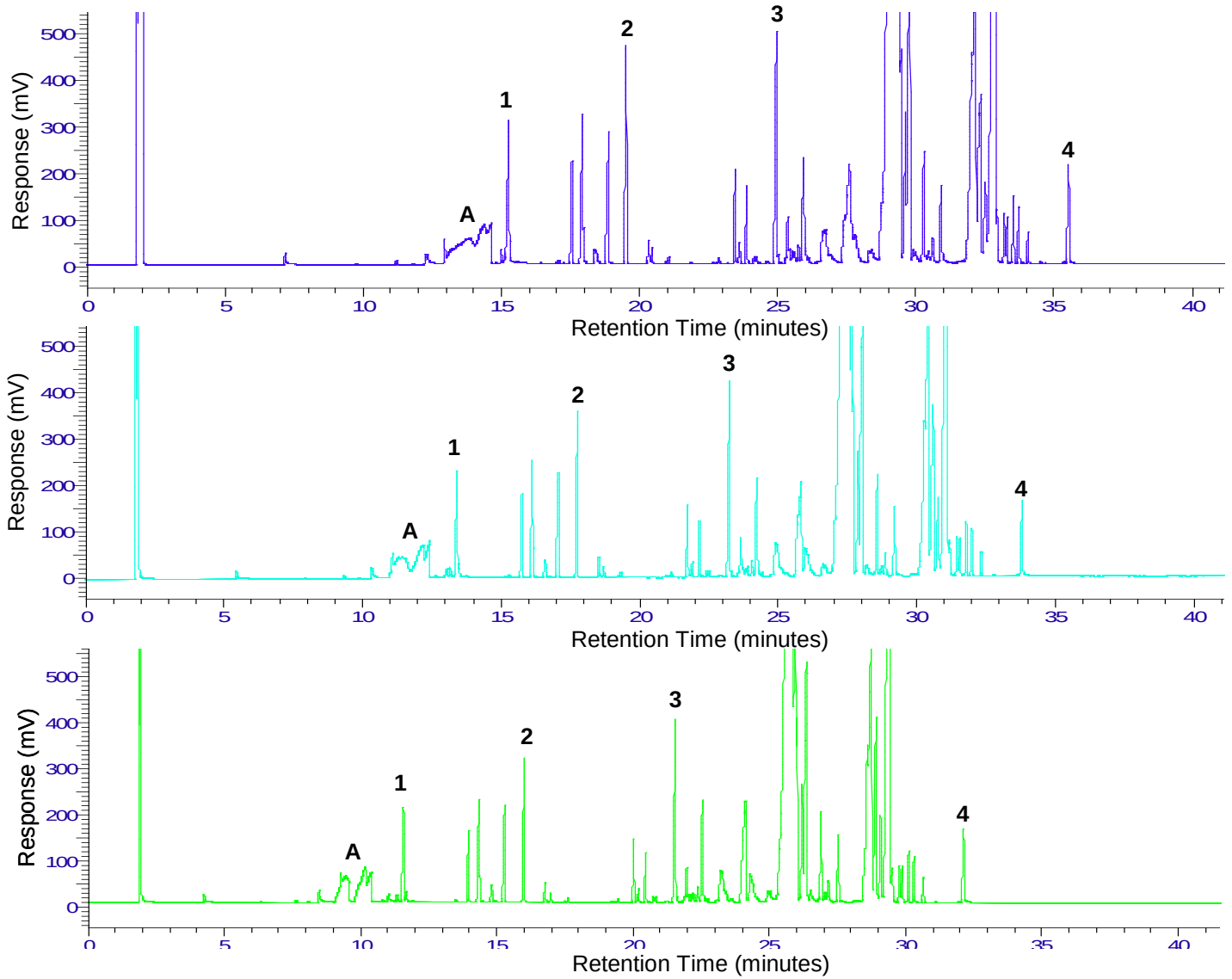


Figure 3.22 – Chromatograms for perfume *Daisy* using initial temperatures of 40°C (top), 50°C (middle) and 60°C (bottom)

For peaks 2 and 3, the resolution improved by 18% and 19% respectively for the first increase in start temperature and then by 10% and 7% respectively. The resolution of peak 2 was above 1.5 even with the 40°C start temperature, while for peak 3 it improved from R_s = 0.43 to R_s = 0.57. Peak 4 resolution values fluctuated around R_s = 1.15 with changes of -2% and 4%.

Calculation of resolution did not, however appear to be particularly useful as visual observations of resolution for these four peaks was much quicker and seemed more informative. Additionally, for distinguishing between complex samples (such as the perfumed products under study here) the aim was not to devise a temperature program optimized for the quantification of specific chemicals, therefore determining the resolution of individual peaks is unnecessary. Additionally, given the number of chemicals in the sample mixture, calculating resolution for each pair of peaks would be extremely time consuming. Accordingly, one of the objectives of this study is to explore novel approaches to assessing the quality and suitability of a temperature program for the analysis of these highly complex mixtures. One such approach was to compare peak heights and areas and calculate the area-to-height ratio. It was hypothesised that as a smaller area-to-height ratio would indicate a narrower peak, this could be used as a quick and simple proxy for how well peaks were resolved. A further benefit of this method is that the peak height and area are both available on the standard report produced with chromatograms on the Perkin Elmer TotalChrom system, whereas peak width is not reported. To test whether this approach was suitable the area-to-height ratio was calculated for selected peaks as shown in Figure 3.22 for the three start temperatures of 40, 50 and 60°C. Ratios were compared with visual observations

and values of *R* calculated from manually measuring peak widths on the chromatograms. Relative peak areas and heights were also examined for pairs of peaks to confirm that the results were due to the effect of start temperature rather than injection volume.

Comparisons of peak heights and peak areas for the four exemplar peaks can be seen in Figure 3.23 and Figure 3.24. The results confirmed the visual observations in that, for peaks 1 to 3, an increase in start temperature resulted in a decrease of peak area and peak height with the effect demonstrably greater for the earlier eluting peaks: for peak 1 the peak area at 60°C was 49% of that at 40°C compared to peak 3 for which the peak area at 60°C was 69% of that at 40°C. The effect of the start temperature on peak height for these peaks was less than for peak area: peak 1 at 60°C was still 68% of the height at 40°C and peak 3 was 80%. For peak 4 eluting at the end of the chromatogram, the peak height and area were smaller at 50°C than at 40°C (for peak area 78% and for peak height 76%), but there was no further reduction in peak height when the start temperature was increased from 50 to 60°C and there was, in fact, a small increase of the peak area.

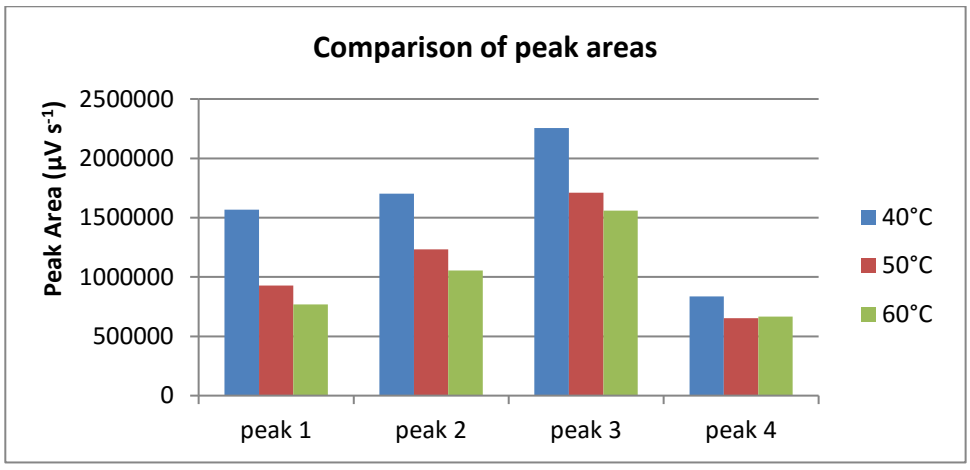


Figure 3.23 – Comparison of peak areas for four peaks

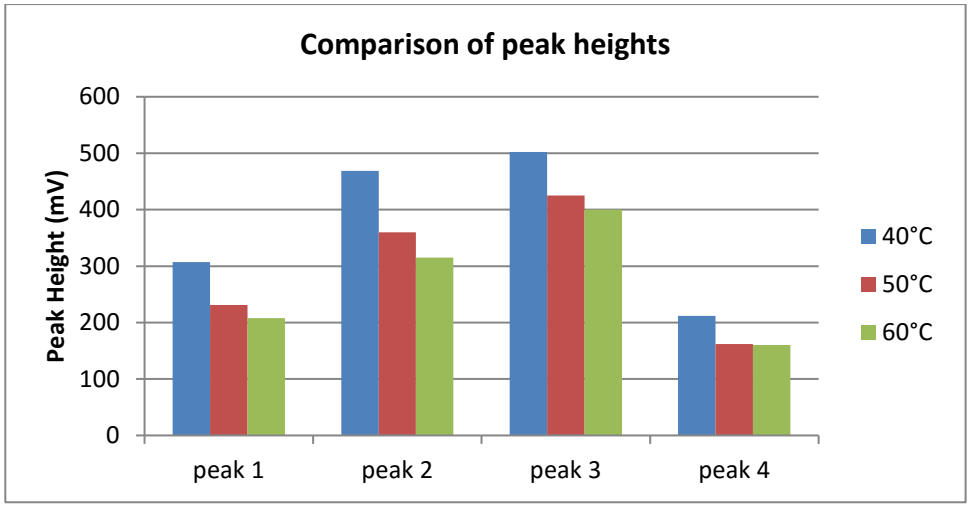


Figure 3.24 - Comparison of peak heights for four peaks

Calculations of the area-to-height ratio for the same peaks provided an assessment of the extent to which peaks become narrowed or broadened with changes in start temperature. As can be seen in Figure 3.25, for peaks 1 to 3 the ratio decreased as the start temperature increased, confirming that the peaks were becoming narrower. The peak narrowing effect was most marked for peak 1 which became 21% more narrow between 40 and 50°C and narrowed by another 8% between 50 and 60°C. Peaks 2 and 3 narrowed by 6 and 10% respectively between 40 and 50°C and then by 2 and 3%.For peak 4, the area-to-height ratio increased by 2% with each increase in start temperature showing that the peak slightly became broader. Comparing the results of the area-to-height ratio calculations with the resolution calculations it was noted that both approaches revealed the same trends for peaks 2, 3 and 4. For peak 1 however, the resolution calculations showed a similar improvement with each increase in start temperature while the area-to-height ratio calculations showed that the increase in start temperature 50 to 60°C was more significant. Visual observations showed that this was due to the peak area for peak 1 at the 40°C start temperature comprising of

three peaks which began to be resolved as the start temperature increased.

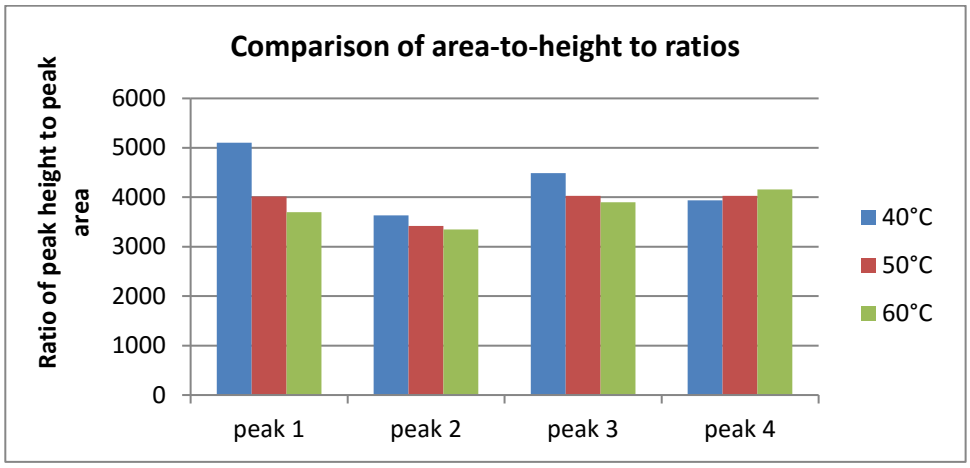


Figure 3.25 - Comparison of peak height to area ratios for four peaks

Nonetheless, calculation of area-to-height ratios provided a very effective means of assessing chromatograms and while visual observation showed that increased start temperature improved resolution for some early eluting peaks such as those identified as the polar DPG isomers, it was much harder to visually determine the effect on peaks throughout the chromatogram. Numerical analysis of the data enabled this effect to be quantified and showed that as the start temperature was increased, the area-to-height ratios of peaks in both the early and middle part of the chromatogram were reduced, indicating that these peaks became narrower, thus improving resolution, while the effect on the latest eluting peak was negligible.

A further useful metric was comparison of the number of peaks integrated by the chromatographic software as this is a measure of the number of peaks above the report's signal-to-noise threshold. For the 40°C start temperature 212 peaks were reported, for 50°C, 186 peaks (a 12% reduction) and for 60°C, 180 peaks (a further 3% reduction). This confirms the visual observation that peaks were of smaller magnitude at higher start temperatures so, while peaks became better

resolved, some fine detail of the chromatogram may be lost.

3.2.1.3 Application of Findings to the 'Perfumes' Temperature Program

Having explored the effect of different start temperatures, holds and temperature ramp rates the findings were viewed in light of the 'Perfumes' temperature program which had been devised for this research and which contained a 6°C per minute ramp until 150°C followed by a ramp of 12°C to the final temperature. When using the DB5 column the majority of interactions are dispersive and therefore dependent on boiling point, and although the slight polarity of the column allows for dipole interactions and acid-base interactions, the temperature program is vital to achieving good separation of peaks (Barnes *et al*., 2013). The 'Perfumes' temperature program offered a good compromise, producing a reasonable resolution, and whilst it did not suit the body-spray *Aphrodite,* which contained many ingredients which eluted early in the program, for general use it is not practicable for the temperature program to be tailored to one product.

It was also recognised that GC separations can be complex when the mixture being analysed contains so many chemicals and certain diastereomers will not be separated using a DB5 column (Bester, 2009). Additionally, the temperature program parameters affect each other (Rouessac and Rouessac, 2000 p. 19) so a limit must be set on the time spent optimising a temperature program for this type of sample. As with the analysis of complex fire accelerant samples, complete separation of all peaks is unlikely to feasible but a pattern matching approach is expected to still be successful (Smith, 1983). For this approach to work, however, the retention times should be reproducible across repeat analyses of a sample and

results did show that for five repeats of *Ysatis* analysed by GC-FID using the 'Perfumes' temperature program showed an average %RSD of retention time of 0.02 % across 118 peaks (equating to an average of 0.007 minutes). Maximum variation in retention time was 0.024 minutes. It was therefore decided that the 'Perfumes' temperature program was suitable for analysis of perfumed material. The next stage was to compare 'Perfumes' with two previously published programs to ensure it produced comparable results.

3.2.2 Evaluation of 'Perfumes' and Comparison with Published Programs

Results from the GC-MS analysis of three perfumed products and the C8-C20 alkane standard using the 'Perfumes' temperature program were compared with those recorded for published programs recommended by IFRA (2006) and Sanchez-Prado *et al.* (2011b) (named 'IFRA and 'SP001' respectively). Results were evaluated using the following approaches:

- visual observation of chromatograms quality
- numerical evaluation of peak height and area
- numerical evaluation of peak integration and library matching
- the relationship between retention time and carbon number

3.2.2.1 Visual Observations of Chromatogram Quality

The alkane standard results were initially most useful for comparing temperature programs and chromatograms for each can be seen in Figure 3.26. The increased ramp rate for 'Perfumes' and 'SP001' temperature programs can be seen by the closer spacing of the later peaks (Figure 3.26a and Figure 3.26c) compared to the more consistent spacing of the peaks during the 'IFRA' temperature program (Figure 3.26b). Both the 'IFRA' and 'SP001' programs also produced better relative responses (% peak height) for the early eluting peaks. For the 'Perfumes' program and the 'SP001' program, which had start temperatures of 60°C and 45°C respectively, the first peak to elute (after the solvent delay) was nonane but 'IFRA' had a higher (100°C) initial temperature and the first peak recorded was undecane.

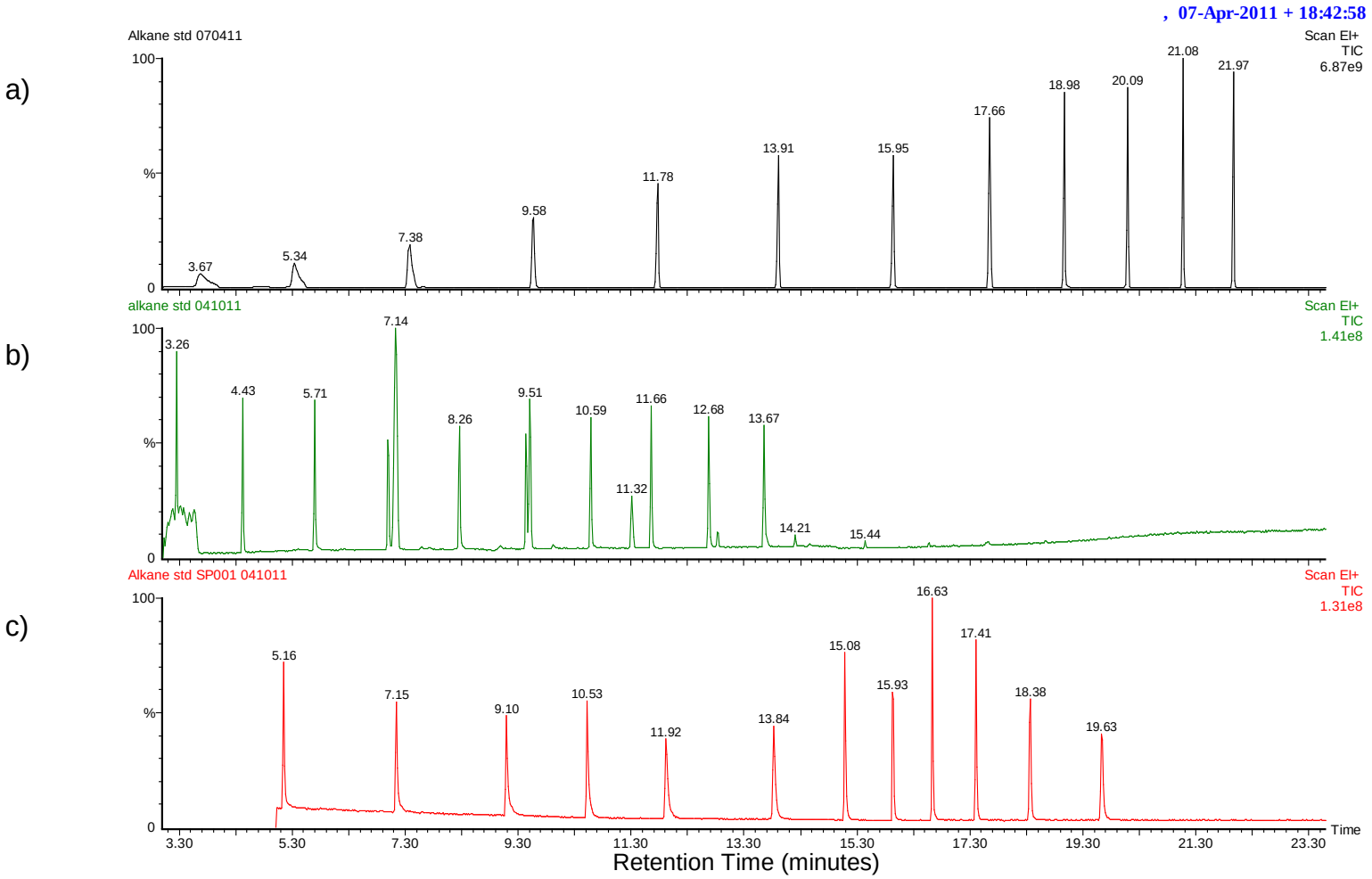


Figure 3.26 - Comparison of temperature programs: a) Perfumes, b) IFRA and c) SP001 for C_8-C_{20} alkane standard

While analysis of the alkane standard was a useful starting point, the results from the analysis of perfumed products was more able to reveal the limitations of each program. An example can be seen by comparing the chromatograms for *Daisy,* which can be seen in Figure 3.27. The first key difference is the effect of the start temperature and for the ‘SP001’ program the first peaks to elute are the distinctive polar peaks of DPG with retention times of 7.5 – 8.1 minutes. For the ‘Perfumes’ program again the DPG peaks are also first to elute but earlier, at around 5 – 6 minutes. The ‘IFRA’ program did not show the DPG peaks at all on the GC-MS analysis (it is assumed that they were eluted with the solvent during the solvent delay) and the first peak to elute with the ‘IFRA’ program was Linalool. In terms of peak separation, the DPG peaks were slightly better separated using ‘SP001’ than using ‘Perfumes’, but the remaining top-notes and heart-notes were slightly better separated using ‘Perfumes’, possibly due the relatively low ramp rate of 6°C/minute. In this temperature range ‘SP001’, which had two ramp rates of 8°C/min (to 100°C) and 20°C/min (to 130°C), still performed better than ‘IFRA’ with a single ramp rate of 10°C/min. None of the temperature programs produced particularly good separation of the base-notes although generally ‘IFRA’ was slightly better than the other two programs: this was likely due to the slightly lower ramp rate in ‘IFRA’ while the ramp rate for ‘Perfumes’ increases to 12°C/min and 25°C/min for ‘SP001’. The final point of note is that the relative height of the final peak (ethylene brassylate) was best for ‘Perfumes’ and worst for ‘IFRA’.

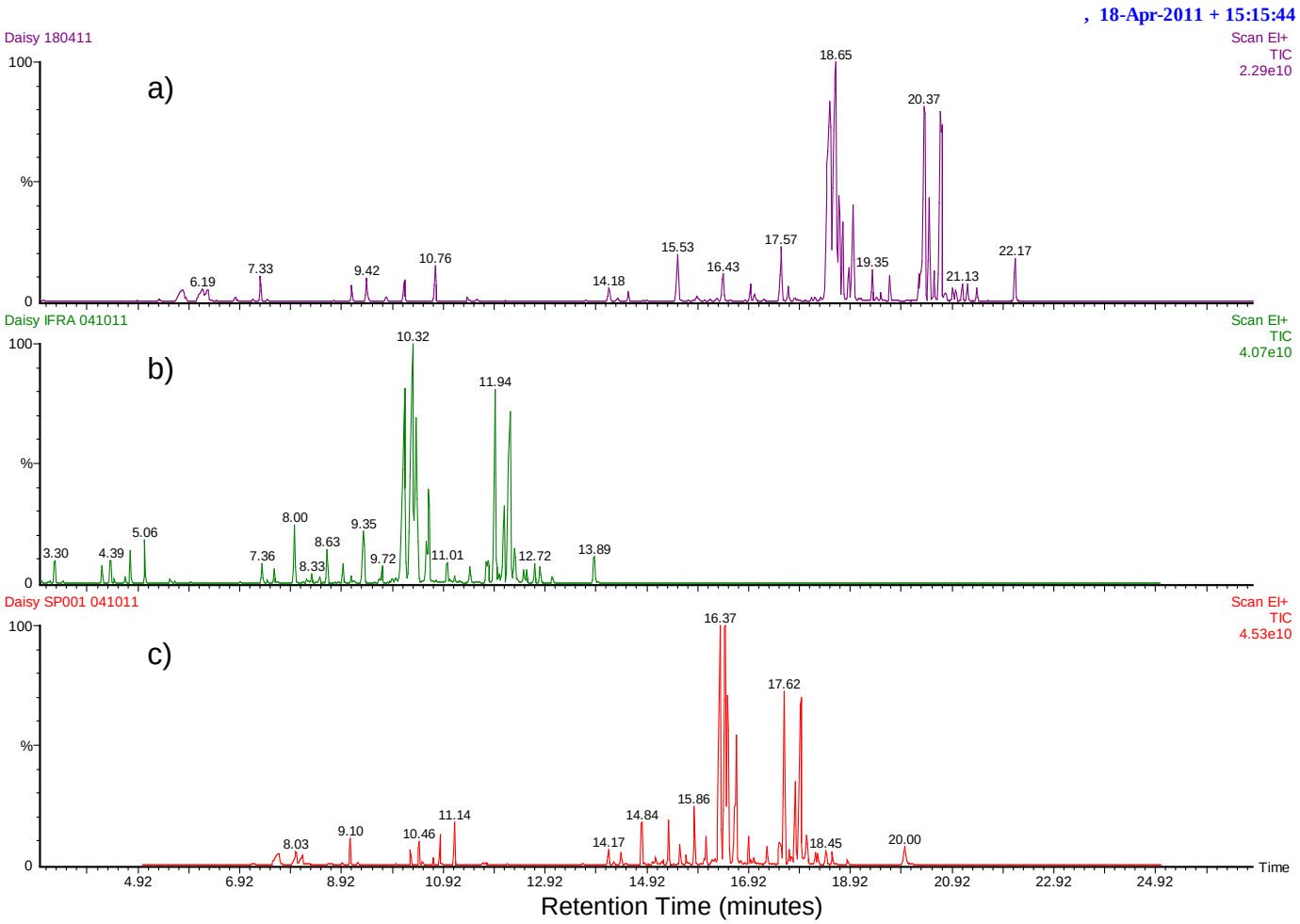


Figure 3.27 - Comparison of temperature programs: a) 'Perfumes', b) IFRA and c) SP001 for perfume Daisy

Interim conclusions from this phase of the evaluation were that the ‘Perfumes’ temperature program performed satisfactorily. The ‘SP001’ program showed better response on the early peaks but later peaks were not as well separated as with the ‘Perfumes’ program. The ‘IFRA’ program produced slightly better resolution for later eluting peaks but had no other significant advantages. Results also showed that the ‘IFRA’ program was generally unsuitable for the analysis of chemicals with boiling points lower than Linalool, although it should be noted that it is used here with a slightly polar (DB5) column but was developed specifically for use with a polar (DB1) or semi-polar (DB17) column on which polar chemicals would be more strongly retained (IFRA, 2006).

3.2.2.2 Numerical Evaluation of Chromatogram Quality Using Peak Heights and Areas

Looking at perfume *Daisy* and using the same four peaks as described in the previous section (linalool, linalyl acetate, alpha-isomethylionone and ethylene brassylate), it was seen that the greatest peak areas were produced by the ‘SP001’ program (see Figure 3.28). For the three later peaks, ‘Perfumes’ produced the lowest peak areas, however for the earliest eluting, Linalool, the ‘IFRA’ program, with its high start temperature, produced a very low peak area. In terms of peak heights, for the three later eluting peaks ‘IFRA’ produced the highest peaks but again produced the lowest peak for linalool. The peak heights produced by ‘Perfumes’ were generally lower than those from ‘SP001’ (see Figure 3.29) except in the case of the latest eluting peak, ethylene brassylate where ‘SP001’ produced the lowest peak. When the peak area to height ratio is examined (see Figure 3.30) the data reveals that the ‘Perfumes’ temperature program produces

wider peaks than ‘IFRA’ and ‘SP001’ and, according to the results found previously (Section 3.2.1.2) the peaks would therefore be expected to be wider and less resolved. The variation in peak area to height ratio is around 20% RSD for peaks 1-3 and 30% for peak 4. In summary, in respect of the magnitude and resolution of the chromatographic peaks for perfume *Daisy*, the ‘Perfumes’ temperature program performed poorly compared to the two published programs.

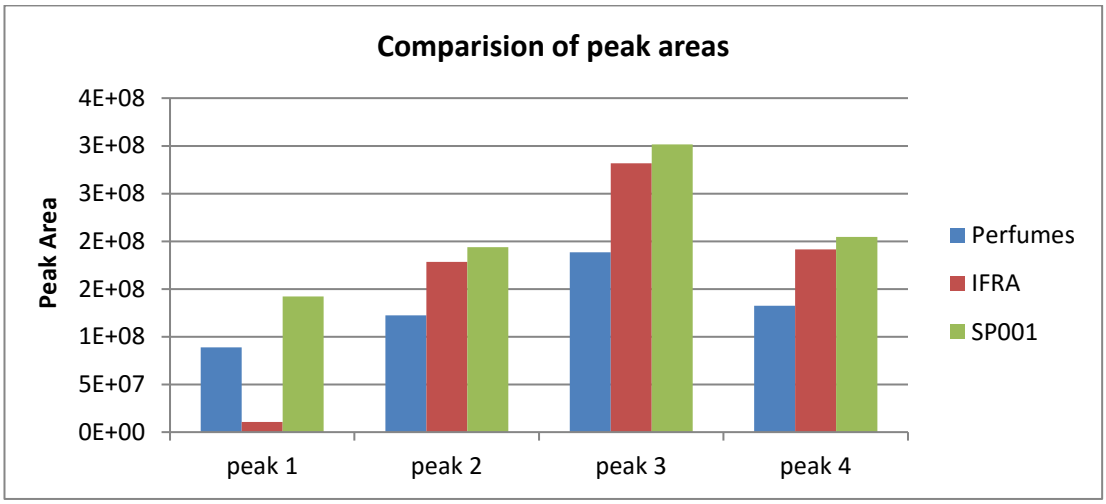


Figure 3.28 – Comparison of peak areas for four peaks

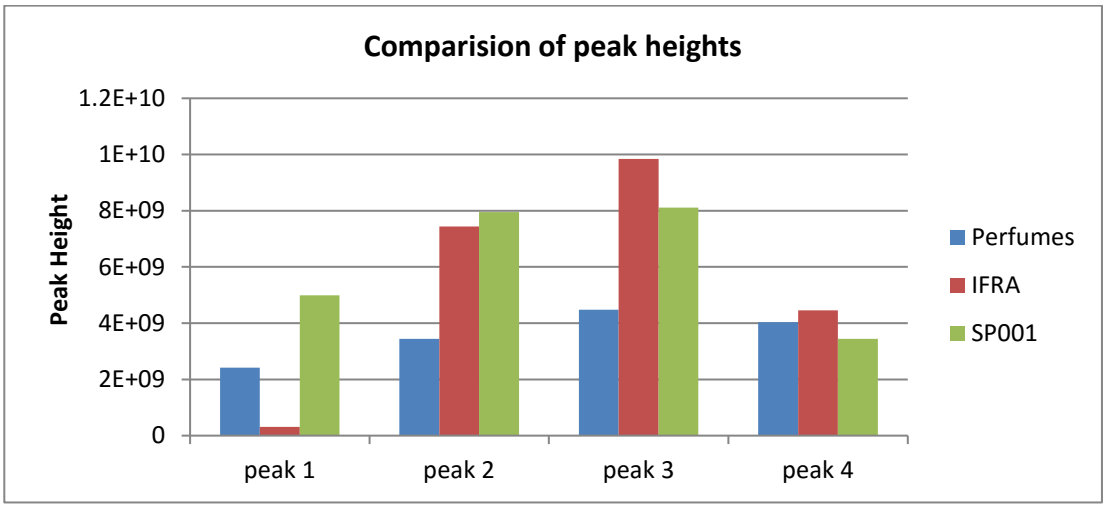


Figure 3.29 - Comparison of peak heights for four peaks

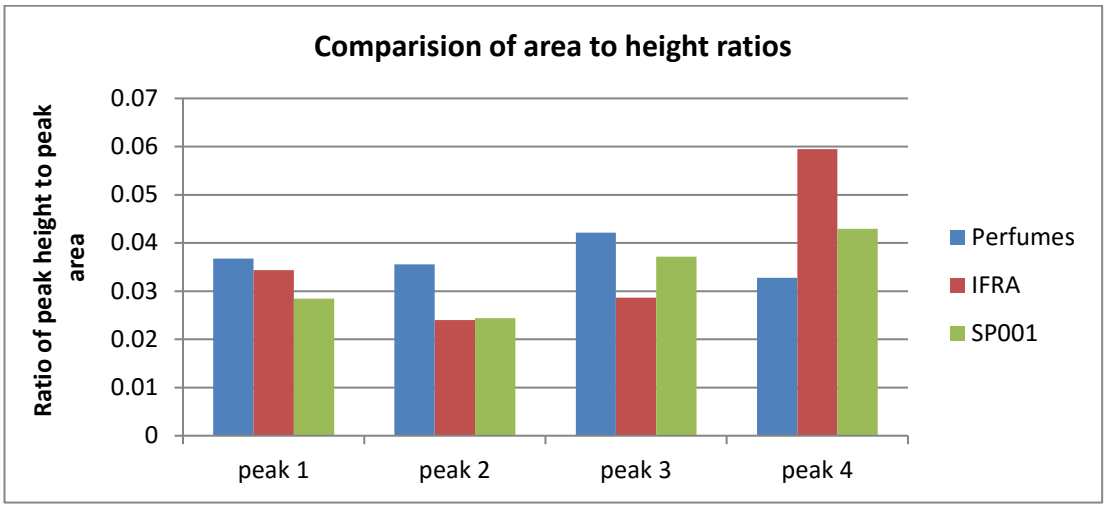


Figure 3.30 - Comparison of peak height to area ratios for four peaks

3.2.2.3 Numerical Evaluation of Peak integration and Library Matching

The results of this evaluation show that the temperature program 'Perfumes' performed as well as the two published programs with regard to the number of peaks with a signal-to-noise ratio above 2000 and also for the number of these peaks being reported by NIST as a good match (i.e. with a match value of over 800). The results are summarized in Table 3.7 and show that none of the three temperature programs was best for all three perfumes. For the perfumes *Urban* and *Aphrodite* the temperature program 'SP001' resulted in more peaks, which indicates better signal-to-noise ratios, but the 'Perfumes' temperature program, produced better match statistics for the peaks reported in *Aphrodite*. The IFRA program resulted in more peaks being reported for *Daisy* but produced the lowest number of 'hits' for the other two perfumes. The 'Perfumes' program produced match statistics for each perfume that were as good as or better than any of the other programs.

Table 3.7 – Number of peaks matched to the NIST library and degree of match for three perfumes for temperature programs 'Perfumes', 'IFRA' and 'SP001'

Perfume	Temperature program		
	Perfumes	**IFRA**	**SP001**
Daisy	16/20 (11 over 800)	18/20 (11 over 800)	14/20 (10 over 800)
Urban	15/20 (10 over 800)	14/20 (9 over 800)	16/20 (10 over 800)
Aphrodite	12/20 (11 over 800)	9/20 (9 over 800)	16/20 (9 over 800)

Further evaluation was conducted comparing the match statistics for specific peaks across perfumes. The results are shown in Table 3.8 and show that, in terms of spectra match score (out of 1000) and library match probabilities, the temperature program 'Perfumes' performed best and 'SP001' the worst. It should however be noted that the difference between the programs was generally small with the average 4% Relative Standard Deviation for the match score of the peaks. The most variation was seen for linalyl acetate, Lilial and β–isomethyl ionone which had a 7-8 % RSD in match score. There was a greater variation in % probability of match (%RSD = 13%) but again linalyl acetate, Lilial and β–isomethyl ionone had the greatest variation with 21-27% RSD. Further examination of the chromatograms and spectra revealed that for *Aphrodite* the peak identified as of linalyl acetate using the 'Perfumes' temperature program was identified as the closely related linalyl anthranilate when using the other temperature programs and showed a slightly different retention time to linalyl acetate (± 0.01 minutes).

Table 3.8 - Comparision of confidence of identification of peaks for three perfumes

Compound identified by NIST	Perfumes						IFRA						SP001					
	Urban		Daisy		Aphrodite		Urban		Daisy		Aphrodite		Urban		Daisy		Aphrodite	
	Match	%	Match	%	Match	%	Match	%	Match	%	Match	%	Match	%	Match	%	Match	%
Linalool	908 908	73	915 915	75	912 912	75	900 900	60	893 895	54	905 906	66	901 902	67	906 906	69	895 896	64
(R)-(+)-beta-citronellol	N.P.D.		915 916	50	949 951	59	N.P.D.		936 943	56	929 936	55	N.P.D.		944 948	60	917 927	48
Linalyl acetate	923 924	36	942 942	43	763 854	22	920 920	32	911 911	28	R.P.D		909 909	28	925 925	39	R.P.D	
α-isomethyl ionone	N.P.D.		932 936	72	934 938	73	N.P.D.		929 934	73	895 905	66			925 929	70	819 847	56
Lilial	903 907	80	933 937	83	927 931	83	768 836	36	917 922	78	920 923	78	784 840	43	922 926	79	920 924	79
β–isomethyl ionone	780 797	23	771 789	21	733 764	25	751 771	14	760 781	16	718 747	20	N.P.D.		758 779	15	612 651	17
Hexyl cinnamal	915 915	88	863 863	89	N.P.D.		892 894	81	867 868	90	763 777	72	889 892	82	833 835	84	746 742	62
Cyclopentadecanone, 2-hydroxy	N.P.D.		935 943	64	N.P.D.		888 900	53	932 943	62	N.P.D.		876 884	52	928 942	60	N.P.D.	
Ethylene brassylate (Musk T)	943 949	95	944 951	95	N.P.D.		921 926	94	927 934	93	N.P.D.		927 933	93	916 924	93	N.P.D.	

Note: Match is the score for the spectrum match out of 1000 with the first number scoring the forward match and the second for a reverse match. N.P.D. denotes that no peak was detected at the appropriate retention time; R.P.D. denotes that a related peak was detected (see text above).

The results also showed that the 'IFRA' temperature program performed poorly for Lilial which is one of the PASs the program was designed to quantify and, in perfume *Aphrodite,* two peaks were reported as Lilial, the first at 8.632 minutes (the same retention time as in the other two perfumes) and a second at 8.139 minutes. Although the second peak had much lower match statistics (616/655 68% c.f. 920/923 78%) this shows the importance of using retention times as a means of confirming peak identification. It should be noted however that the 'IFRA' program was developed for use with Single Ion Monitoring mode with the specific objective of quantifying Potential Allergenic Substances (Chaintreau *et al*., 2003, IFRA, 2006). Limonene was not reported at all by the 'IFRA' temperature in any of the perfumes which is most likely due to the high start temperature of 100°C. Previous examination of alkane standards analysed using this program showed that first alkane peak recorded was that of undecane which eluted as a distorted peak at 3.26 minutes, just after the 3 minute solvent delay. As the retention index for Limonene is 1033 (b.p. 176°C) this chemical would not be seen on the chromatogram. In contrast, the temperature program 'SP001' from Sanchez-Prado *et al.* (2011b) increased the reporting of Limonene in *Urban* as a top 20 peak (most likely due to the lower start temperature of 45°C). .

Table 3.9 - Comparision of confidence of identification of Limonene in Urban

Peak identified by NIST	**Perfumes**		**IFRA**		**SP001**	
	Urban		Urban		Urban	
	Match	%	Match	%	Match	%
Limonene	903 903	21	Not reported		913 914	24

3.2.2.4 The Relationship Between Retention Time and Carbon Number

The system of Retention Indices (RIs) is widely used in the fragrance industry (among others) as a tool to identify chemicals and to enable data from one laboratory to be used in another (Babushok, 2015). The original RI system devised by Kováts (1958), takes advantage of the fact that, when a series of alkanes is injected on to a column held at a constant temperature, the number of carbon atoms present in each alkane has a linear relationship to the logarithm of the retention time (Rouessac and Rouessac, 2000 p. 39). Subsequently, Van den Dool and Kratz (1963) showed that for a linear temperature programme (i.e. with a single ramp rate) the elution temperature and carbon number show a linear relationship and, as the elution temperature and retention time are usually highly correlated the RI can be calculated using the formula given in Equation 3, where RT is retention time and 'n' is the number of carbon atoms in the preceding alkane (Costa *et al*., 2007).

$$RI = 100n + 100\left\{\frac{RT_{(\text{peak of interest})} - RT_{(\text{preceding n-alkane})}}{RT_{(\text{following n-alkane})} - RT_{(\text{preceding n-alkane})}}\right\}$$

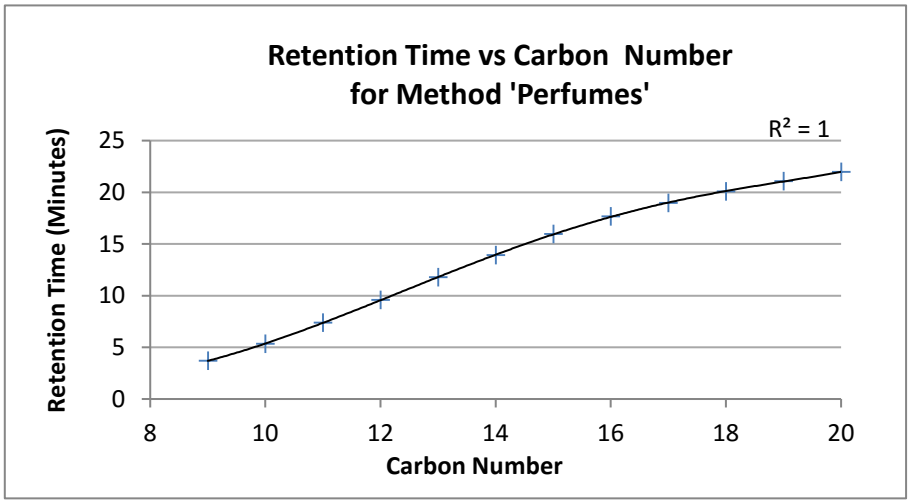
Equation 3 - Retention Index (RI)

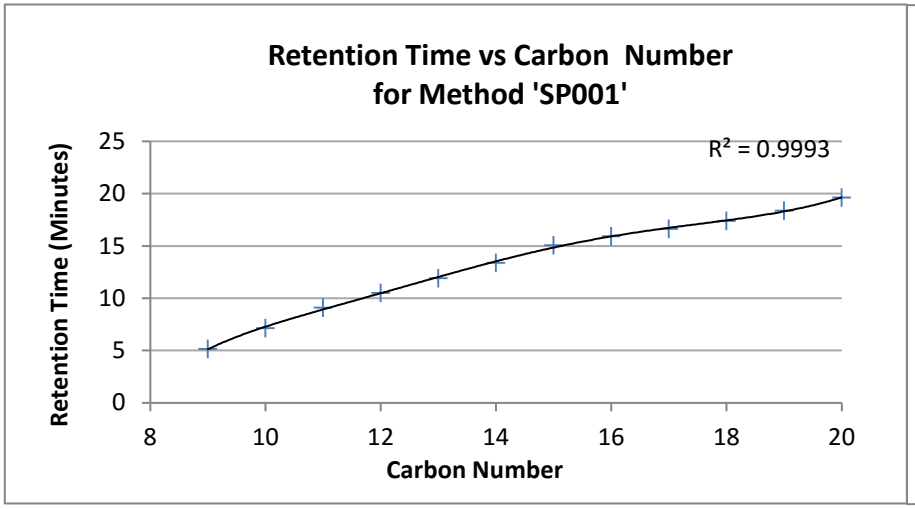
(Van Den Dool and Kratz, 1963)

The resulting calculation is often referred to as a Linear Retention Index (LRI) (Hübschmann, 2009 p. 309) or Linear Temperature Program Index (LTPRI) (Setkova *et al*., 2007). The Van den Dool and Kratz equation has also been successfully used to calculate RIs from temperature programs with varied ramp rates by authors such as Bianchi *et al.* (2007) and Strehmel *et al.* (2008).

Experimentation showed that when using a temperature program the relationship between the retention time and the carbon number could be expressed by a polynomial equation. This raised the possibility of evaluating temperature programs by comparing the polynomial equations: it is hypothesised that the temperature program with the best fitting trendline would produce more reliable RI calculations which would allow better identification of individual chemicals and provide the best comparison for results across different instruments, which would be particularly useful when access to GC-FID instruments is more available than for GC-MS.

Figure 3.31 shows retention times against carbon number for the C_8-C_{20} alkane standard for the temperature programs ‘Perfumes’, ‘IFRA’ and ‘SP001’.
The results show that the both the ‘Perfumes’ and ‘IFRA’ temperature programs had trendlines with an R^2 value of 1 when using a fourth order polynomial trendline. For ‘SP001’ sixth order polynomial trendlines (the highest available in Excel) were applied but even this failed to achieve an R^2 value of 1, probably because of the number of different ramp rates and holds. These results suggest that Retention Indices (RI) of individual peaks would be less accurate for the ‘SP001’ program. To check this, a brief examination was conducted of the RIs for two chemicals, Linalool and hexyl cinnamal, which were found in *Urban.* Results are shown in Table 3.10 and RI values calculated using Equation 3.





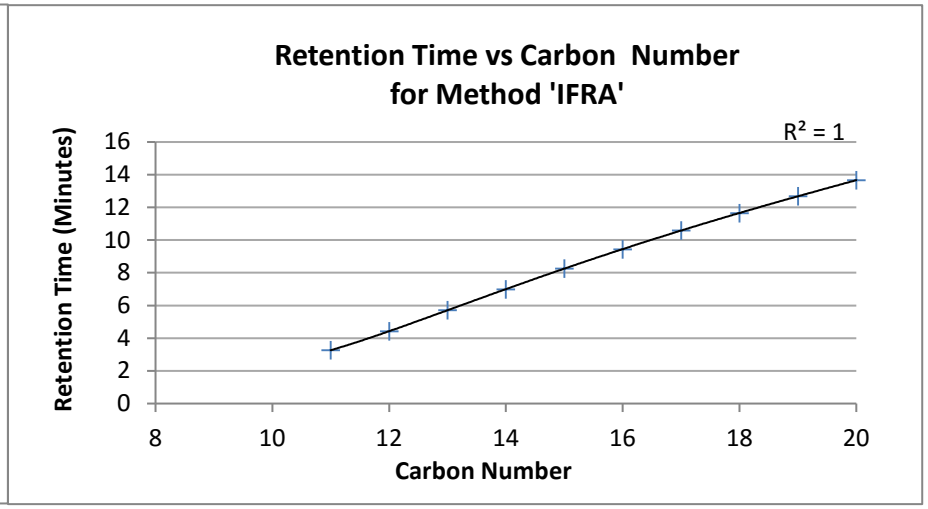


Figure 3.31 - Graphs showing retention time vs. carbon number for C_8-C_{20} alkanes for different GC methods

(adjusted retention times have not been used as all methods were tested on the same GC-MS instrument)

Table 3.10 – Comparison of Retention Indices using temperature programs 'Perfumes', 'IFRA' and 'SP001'

Chemical	Published RI	RI for each Temperature Program		
		Perfumes	IFRA	SP001
Linalool	1101	1098	1102	1100
Hexyl Cinnamal	1749	1749	1751	1750

Published values for Linalool were taken from Mondello *et al*. (2008) and for hexyl cinnamal from Alissandrakis *et al*. (2005) and both were derived from GC–MS analysis carried out on a column with the same chemistry, length and internal diameter as the column used in this research. The table shows that there was no significant difference in the accuracy of RIs between the programs. There are however issues with the reliability of RIs and a further assessment of their use is conducted in Section 3.3.2.3 below.

3.2.2.5 Summary of Evaluation of GC Methods

The work carried out on initial temperature programs showed that using the 60°C start temperature peaks were slightly smaller but the resolution of early eluting peaks was much improved with little detriment to later eluting peaks. As resolution was deemed potentially more useful for discriminating between perfumes it was considered that a 60°C start temperature was the best option.

Attempts to establish the best ramp rates and hold times were complicated by the inter-related nature of temperature program parameters but did provide reassurance that the 'Perfumes' temperature program did not require significant modification.

During this stage of the work it was also noted that many of the approaches for validating a temperature program are based on the assumption that a small number of specific chemicals are to be quantified (Chaintreau *et al*., 2011), however this is not a requirement of the current research and other approaches were explored. Numerical evaluation in terms of total number of peaks reported, or number of peaks reported above a known signal-to-noise ratio, proved a quick and easy means of establishing the relative response of different temperature programs. The area-to-height ratio also proved to be very useful in assessing the narrowness of peaks and therefore informing one regarding the resolution of peaks.

The ‘Perfumes’ temperature program was also compared to the published programs designated ‘IFRA’ and ‘SP001’ using both alkane standards and perfume samples. Visual observations were used along with numerical evaluation, which was now expanded to include evaluation based on the relationship between retention time and carbon number, retention indices and the match statistics of some common peaks. In summary, the ‘Perfumes’ program performed adequately: peak amplitude and separation for perfume *Daisy* was worse than the other temperature programs and affected the number of peaks reported above the signal-to-noise ratio of 2000, GC-MS match statistics were however better with peaks being reported with more confidence. The ‘Perfumes’ program also performed as well as the other programs in terms of accuracy of RIs. It was also noted that across the range of perfumed products, no single temperature programme suited every product.

3.3 Distinguishing Between Products

The objective for this part of the study was to show that the 'Perfumes' GC method was successfully able to discriminate one perfumed product from another. Three approaches were used:

- visual pattern matching
- identification of product ingredients by peak identification
- statistical analysis

3.3.1 Visual Pattern Matching

As reported in Section 3.1.1.1, chromatograms for the 11 products had visibly different peak distributions. In the context of fire accelerant analysis the use of GC pattern matching is 'almost universal' (Smith, 1983) but this purely visual processing can only be successful when there are a limited number of likely products (or distillation fractions in the case of ignitable liquids). As there are over 16,000 (Edwards, 2015) fine fragrance products alone, additional data processing is needed as human processing would not recall all the possible variations. Visual pattern matching has revealed useful information: of the 15 products analysed, the body-sprays and deo-colognes showed differences in the solvent peaks and with the exception of *Aphrodite*, the chromatograms were much less complex, as seen in Figure 3.32. There were also indications of differences between products targeted at men and women: one men's fine fragrance was analysed (*Urban*) and three out of the four body sprays analysed were for men and all of these products showed fewer top and heart notes than the products for women. It should however be noted that the sample size is not large enough to draw firm conclusions in this regard.

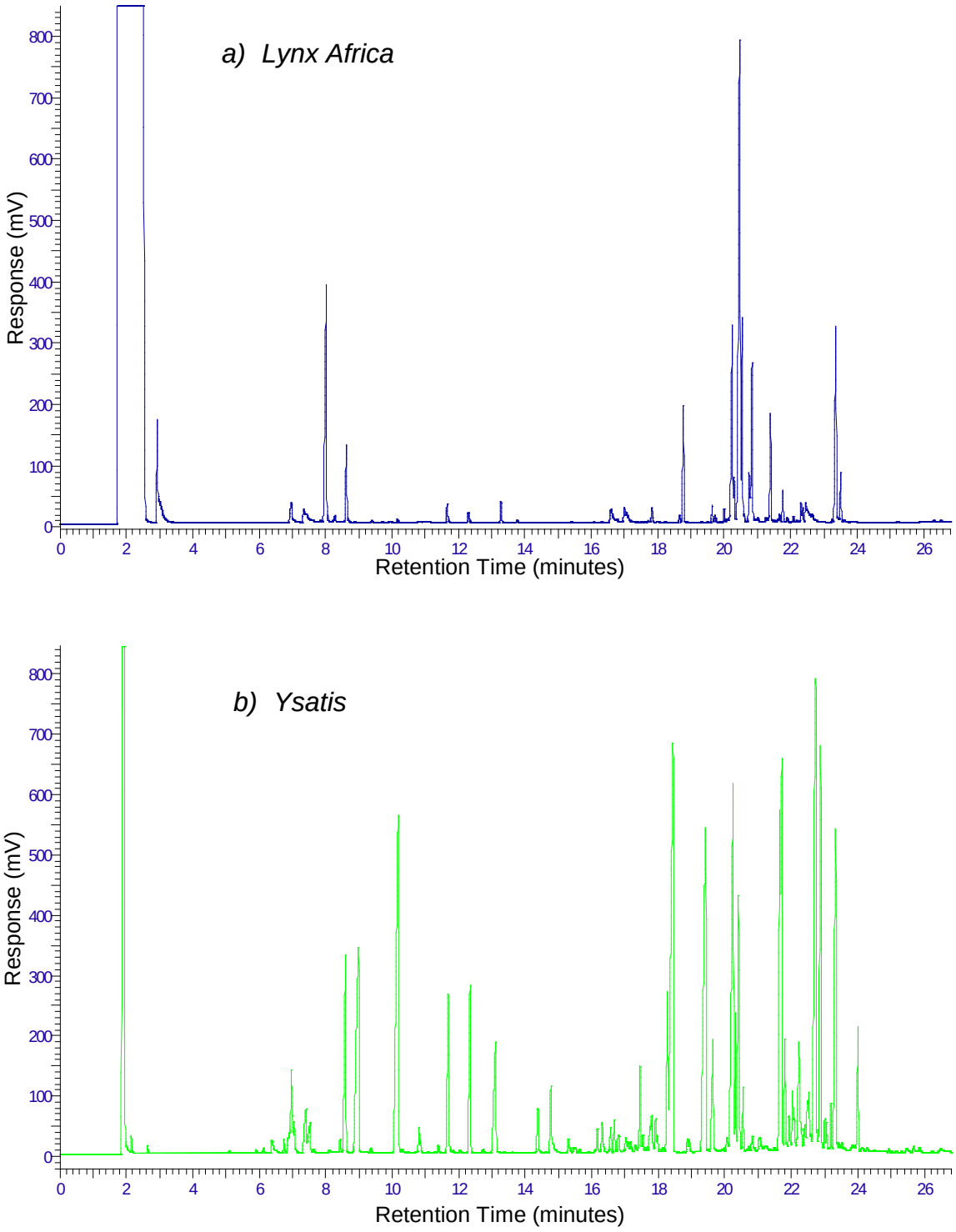


Figure 3.32 – Comparison of deodorant *Lynx Africa* with fine fragrance *Ysatis*

3.3.2 Identification of Product Ingredients by Peak Identification

Results from the initial evaluation study (Section 3.1.1.2) showed that the GC-MS chromatogram for each perfume, antiperspirant or deodorant produced a different list of peaks in the NIST library match report. All such identifications were considered presumptive and this subsequent phase sought to understand whether peaks could practicably be identified with more certainty and whether peak identification, and therefore identification of product ingredients, was a valid approach to distinguishing between perfumed products. In order to achieve this, three areas were investigated:

1. Presumptive identification of all ingredients in a perfumed product - to determine whether all the ingredients in a perfumed product can be presumptively identified using GC-MS analysis and NIST library matching.
2. Identification of a subset of key chemicals using specific masses - to determine whether a subset of key chemicals can be identified using information from literature and by reprocessing the GC-MS chromatograms to display specific masses.
3. Use retention indices - to determine whether it is possible to use these to support the identification of a chemical.

3.3.2.1 Presumptive Identification of All Ingredients in a Perfumed Product

Results from the initial examination of the perfume *Ysatis* analysed using the 'Perfumes' temperature program showed that in the report of the top twenty peaks there were only thirteen peaks for which the NIST library produced a 'good' match of above 800 (NIST, 2008 p. 25). Three further peaks in the top-twenty had matches above 700 and were reported with CAS numbers, but, of these, only one peak was subsequently identified as being sold commercially for use in perfumed products: the peak at 21.87 minutes in (Figure 3.33) which was matched to Musk Ketone (TheGoodScentsCompany, 2015). The same approach was used for six further perfumed products with similar results. In an attempt to produce better data, *Ysatis* and seven other products were then re-analysed using a temperature program with a single ramp rate of 3˚C/minute. While this temperature program did produce better separation of most peaks, a review of the top-twenty for *Ysatis* revealed that again only thirteen peaks produced a 'good' match of above 800. Additionally, for a number of peaks, a different match was proposed by the NIST library than previously. Again, comparable results were seen when the same investigation was performed for the other perfumed products. Finally, an attempt was made to work through a chromatogram for the perfume *Ysatis* with the aim of identifying as many peaks as possible. Using the temperature program with the single ramp rate of 3˚C/minute the number of distinct peaks in the chromatogram was counted at 77 and of these less than 50% could be assigned a 'good' match using NIST. This demonstrates that it is not practical to identify all the ingredients in a perfumed product for use in discriminating between products.

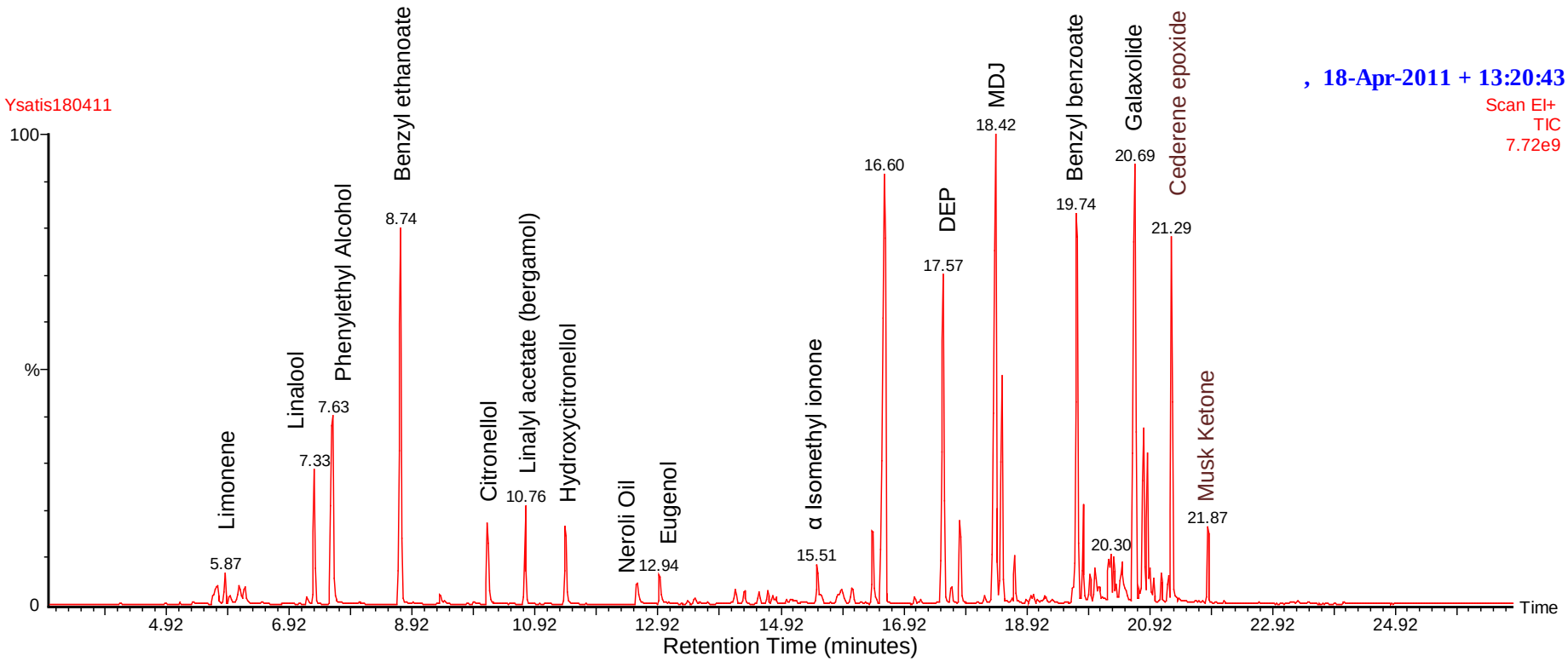


Figure 3.33 – NIST 2.0 identification of selected peaks for perfume *Ysatis*

3.3.2.2 Identification of a Subset of Key Chemicals Using Specific Masses

The chemicals most discussed in the literature are the PASs and musks: Sanchez-Prado *et al.* (2011b) published a list of specific ions used for reprocessing chromatograms to identify 52 such cosmetic ingredients while the IFRA working group (2006) used Selected Ion Monitoring to identify the 24 PAS chemicals. To establish whether the use of specific ions would be practical for distinguishing between products, Total Ion Chromatograms (TICs) collected using the 'Perfumes' temperature program for the perfume *Urban* were reprocessed using the masses suggested by the authors above, thereby producing Reconstructed Ion Chromatograms (RICs). Results showed that in some cases this approach did aid identification and improve visualisation of a peak: an example is shown in Figure 3.34 where the ions at m/z 216, 215 and 129, from the IFRA working group (2006), were used to identify hexyl cinnamal (a listed allergen). Overall it was felt however, that the usefulness of this approach was somewhat limited and, for the time it took, produced little additional data. For example, the perfume *Urban* had eight PASs listed as ingredients and of these two, hexyl cinnamal and linalool, were already identified with good matches in the TIC top-twenty peak list. Two further PASs, limonene and Lilial, had only appeared in *Urban*'s top-twenty peak list when using the temperature program 'SP001' devised by Sanchez-Prado *et al*. (2011b), and visualisation of these peaks using the 'Perfumes' temperature program was greatly improved by using the selected ions recommended by either Sanchez-Prado *et al.* (2011b) or the IFRA working group (2006), although the masses recommended by the IFRA working group resulted in better visualisation for limonene. The remaining four PASs listed as ingredients in *Urban* had not previously been identified as top-twenty peaks using any of the temperature programs but neither

were they identified using masses of selected ions. It is possible that these chemicals were present below the limit of detection for TIC analysis or that the perfume manufacturer only listed them as ingredients as a precaution.

In summary, in most cases those compounds for which the masses of selected ions were provided were already identified from the Total Ion Chromatogram (TIC) using the standard peak search but the RICs provided better visualization of the peak and would be useful if quantification was required. Using specific masses was, however, time consuming and did not appear to be practical for distinguishing between products. Better sensitivity may have been possible by using Selected Ion Monitoring (SIM) and this approach is recommended for the identification and quantification of specific target compounds, particularly in fragranced products which contain a large number of ingredients, many of which have closely eluting isomers or related impurities and where the NIST software may struggle to identify some novel chemicals (David and Klee, 2009, Chaintreau *et al.*, 2011). It should also be noted that even using SIM, some aroma compounds will experience strong fragmentation if the electron energy of the MS is too high and will produce spectra with nonspecific, low mass ions which are not readily assigned to a matching compound (David and Klee, 2009).

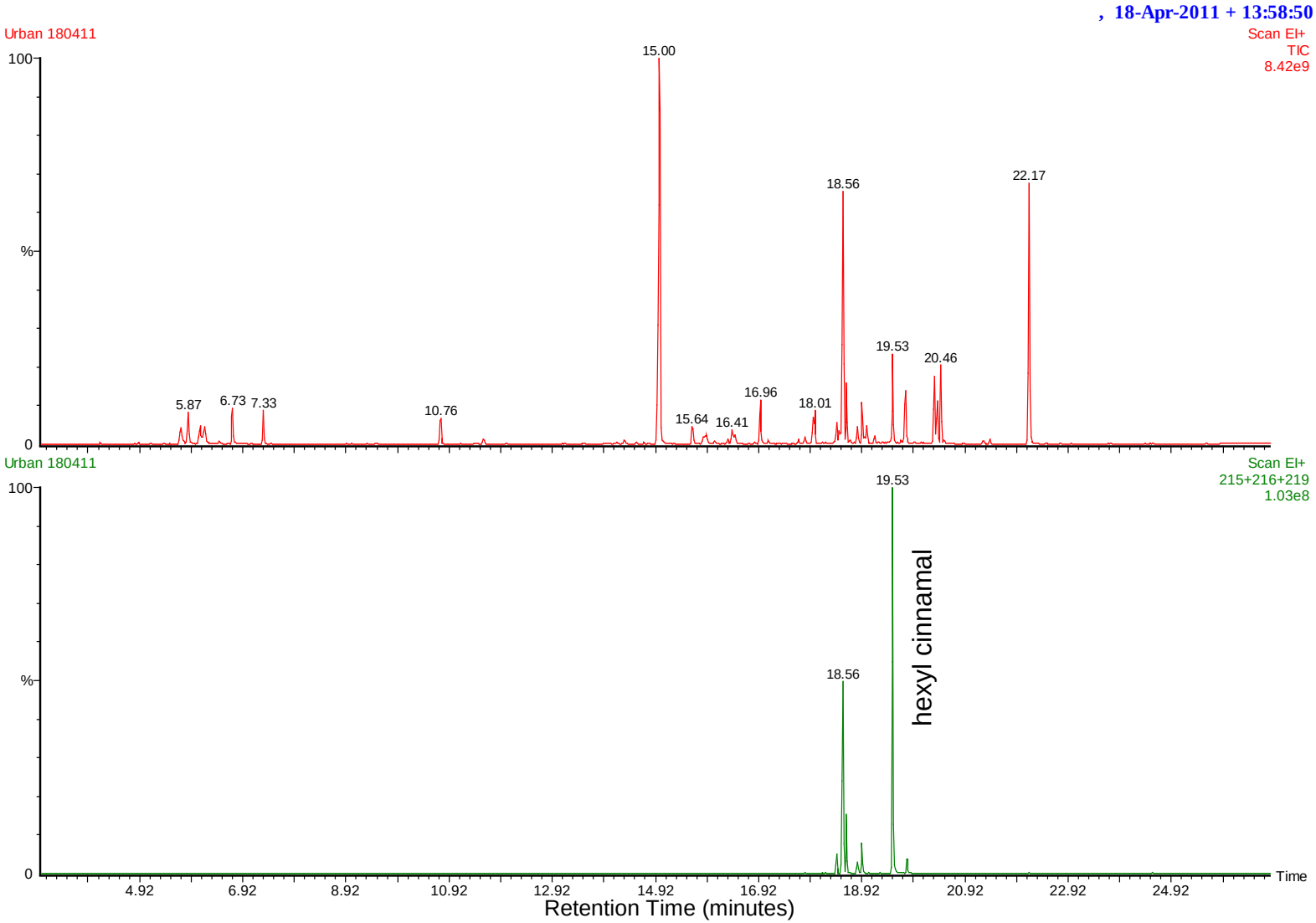


Figure 3.34 - Use of selected ions to identify hexyl cinnamal in perfume *Urban*.

3.3.2.3 Using Retention Indices (RIs)

To establish whether it was possible to use retention indices to support the identification of an ingredient, RIs for a range of chemicals presumptively identified by GC-MS were initially calculated using the Van den Dool and Kratz relationship (Equation 3) and examples of the results are shown in Table 3.11.

Table 3.11 – Comparison of RIs for seven chemicals

Chemical	Published LRI	Experimental RI	Difference
Limonene	1033	1026	- 7
Linalool	1101	1098	- 3
2-phenylethyl alcohol	1118	1111	- 7
Linalyl acetate	1259	1254	- 5
α- isomethylionone	1473	1479	+ 6
Lilial	1535	1528	- 7
hexyl cinnamal	1746	1749	+ 3

Published values were taken from the average Linear Retention Indices (LRIs) values held on the data repositories Flavornet (Acree and Am, 2017) and Pherobase (El-Sayed, 2016) or, if not available from those sites, from Mondello *et al*. (2008). All published RIs were derived from GC–MS analysis carried out on a column with the same chemistry and dimensions as the column used in this research. It should however be noted that, in most cases, a range of RI values were listed on the data repositories, for example, for linalool published RIs varied between 1098 (Choi, 2003, Adams, 2007) and 1112 (El-Sayed *et al.*, 2005) which is a range of 14 units. On the Flavornet database the most commonly quoted

values were 1100 and 1101, with the latter value being quoted by studies in the fragrance industry specifically (Jordan *et al.*, 2002, Mondello *et al.*, 2008). As can be seen from Table 3.11, the experimental RI values differed by up to 7 units and were biased both positively and negatively. The practice in contemporary fragrance analysis, is to accept values which are within ±5 units of the literature values (Costa *et al.*, 2007, Scandinaro *et al*., 2010) so, using that criterion, only three out of the seven chemicals listed in the table can be said to have been confirmed. Other literature sources however, suggest that a value within ±10 units is acceptable for supporting the MS identification (Bianchi *et al*., 2007) and even ±20 units is acceptable to Smith *et al.* (1977). The International Organization of the Flavor Industry take a different approach and recommend a threshold of 1% (IOFI, 1991) which means the thresholds would range from ±10 for limonene and ±17 for hexyl cinnamal, in which case all the experimental results fall well within acceptable limits.

A further investigation sought to establish whether specific ingredients could be identified if the product had been analysed by GC-FID. Chromatographic data from analysis by GC-MS and GC-FID was compared for the perfume *Ysatis* and results showed that the RIs for peaks eluting during the first half of the analysis, with values of up to 1550, varied by between two and ten units (less than 1%). For later peaks (RI's up to 1965) the values varied by between ten and thirty units which equated to 1.5% as a maximum. Examination of the chromatograms and consideration of the differences in the two chromatographic systems indicated that this may be due to the differences caused by the GC-MS system configured for constant flow analysis rather than the constant pressure mode used for GC-FID.

Several authors recommend that flow is kept constant (Bianchi *et al.*, 2007, Miyagawa *et al*., 2011) as changes in flow rate alter the effective steepness of the temperature ramp (Barnes *et al.*, 2013) and complex polycyclic organic compounds (such as the naphthalene derivatives which are popular aroma chemicals) are particularly sensitive to temperature differences (Zenkevich *et al*., 2009). Even relatively minor differences in the chromatographic system can alter RI values as retention mechanisms are sensitive to small variations in column stationary phase polarity and dimensions (Zenkevich *et al.*, 2009), as well as heating rate and inlet temperature (Lee and Taylor, 1982).

In terms of the usefulness of retention indices to support the identification of specific ingredients, although it might appear that the wide variation in reference values would make this impractical, the use of RIs to confirm identifications is standard in the fragrance industry (Bazemore, 2011 p. 56). RIs are particularly used to filter MS library hits for isomers and especially for diastereoisomers which produce fragmentation patterns which are very similar with mass spectra which are therefore 'approximately identical' (Liberto *et al*., 2008, Wei *et al*., 2014, Babushok, 2015). This is particularly problematic with the highly isomeric terpenoids, of which there are more than 400 isomers just in the sesquiterpene group (Costa *et al*., 2007, Zenkevich *et al*., 2009). Using RIs to distinguish between library search hits with similar match scores is considered to significantly increase reliability in compound identification (Costa *et al*., 2007) and, as RI values are based on different chemical properties of the analyte than MS identification, they are also considered to strengthen the identification by providing orthogonality (Liberto *et al*., 2008). The quality of the available reference collections must also

be considered when using RIS and (Babushok, 2015) warns that retention data may be of differing quality. Although Pherobase, Flavornet and the NIST Webbook (El-Sayed, 2016, Acree and Am, 2017, NIST, 2017) contain referenced values with a clear description of the chromatographic conditions, they do have a limited number of compounds and the data has not been verified other than by peer-review. Barnes *et al.* (2013) goes further opining that many retention databases are 'notoriously unreliable' and the RIs therein should, at most, play only a minor role in compound identification. The results do show, however, that to some extent RIs do help to identify ingredients in perfumed products by increasing confidence in the identification of some peaks in MS chromatograms. For GC-FID chromatograms RIs may also aid identification but it is recommended that these identifications are checked by another method.

3.3.2.4 Summary

Results have shown that identification of product ingredients, in order to distinguish between perfumed products is not straightforward. Presumptive identification of all the ingredients in a perfumed product is not possible due to the complexity of the mixture, the isomeric nature of many aroma chemicals and the rapid of development of synthetic ingredients. The identification of a subset of key chemicals using specific masses is time consuming and there is little evidence that this approach offers a significant advantage. Retention indices have been shown to, in some cases, support the identification of a chemical, particularly where it has already been matched to a reference structure in an MS library, but again, in terms of an aid to distinguish between products this is an inefficient approach.

3.3.3 Statistical Analysis

While visual pattern matching and identification of product ingredients were both partially successful, a more rigorous statistical approach was also employed using IBM SPSS Statistics 19 software. The three statistical tests employed were Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA) and Discriminate Function Analysis (DFA). Each test was performed using a previously collected dataset for nine perfumes analysed by GC-FID (Davidson, 2008).

PCA analysis was performed using retention times that were collected for the top twenty peaks, listed in order of decreasing peak area. The analysis was set-up to produce two factors to allow a biplot to be constructed, thus reducing the dimensionality of the data. The biplot is presented in Figure 3.35 and confirms that the retention times are sufficiently different for the perfumes to be widely scattered on the plot. Each perfume had each been analysed three times and it should be noted that data points for each product were so tightly grouped that, on the main plot, they appear as a single point clearly showing good reproducibility of results for each perfume. The insert provided with Figure 3.35 shows the three repeat samples for the perfume Live Lopez. It is also notable that the plot shows relationships between certain perfumes, for example the data points for the two Armani fragrances, Unforgivable and Code Armani are closest to each other and indeed they have nine of their top twenty peaks in common (retention times ± 0.03 minutes). The Youth Dew and Faubourg data points are also close to each other and they have 12 of their top twenty peaks in common (with retention times ± 0.02 minutes) despite being described as having very different odour profiles (Lauder, 2017, Hermes, 2017).

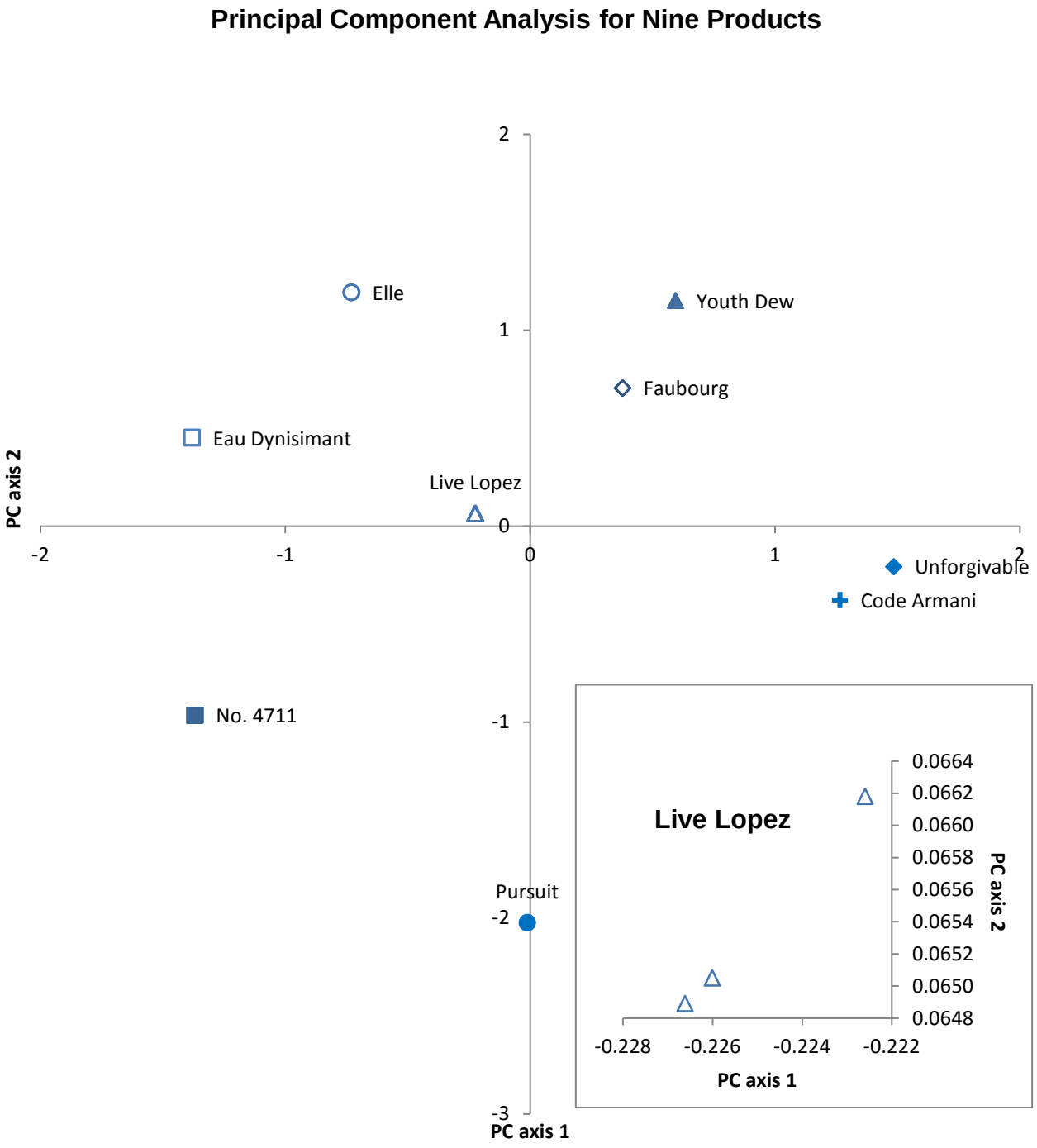


Figure 3.35 – Principal component analysis of the top 20 peaks of nine perfumes

NOTE: PCA was performed on the retention times of the top twenty peaks. Three datum points are included for each perfume and the insert shows magnification of the resamples for perfume *Live Lopez.*

Three products most targeted at a youthful, female demographic, *Elle*, *Eau Dynanismant* and *Live Lopez* are also clearly separated from those of the other products and the two fragrances for men, *Pursuit* and *No.4711*, also show some similarities.

HCA was performed on the same set of data and the resulting dendrogram shown in Figure 3.36 demonstrates that the repeat analyses for perfume are clustered with a minimal Euclidean distance. The same groupings of perfumes are seen as for PCA. Finally, DFA was performed on the data set and results showed that 100.0% of original grouped cases were correctly classified and that 100.0% of cross-validated grouped cases were correctly classified. The full table of results is provided in Appendix B.

As an additional test, PCA analysis was conducted using peak areas of four pre-selected peaks with retention times of 9.2, 13.1, 21.3, 23.5 minutes (for this analysis the identity of the peaks was not explored). Two approaches were used for normalising the peaks, firstly, taking the ratio of each peak with the highest of the four peaks within that sample, secondly, taking the ratio consistently to the peak at 21.3 minutes (which appeared in all the samples). Again biplots were created to see how well samples from the same perfume were grouped compared to samples from different perfumes but this approach proved much less successful than using the retention times of the top-twenty peaks.

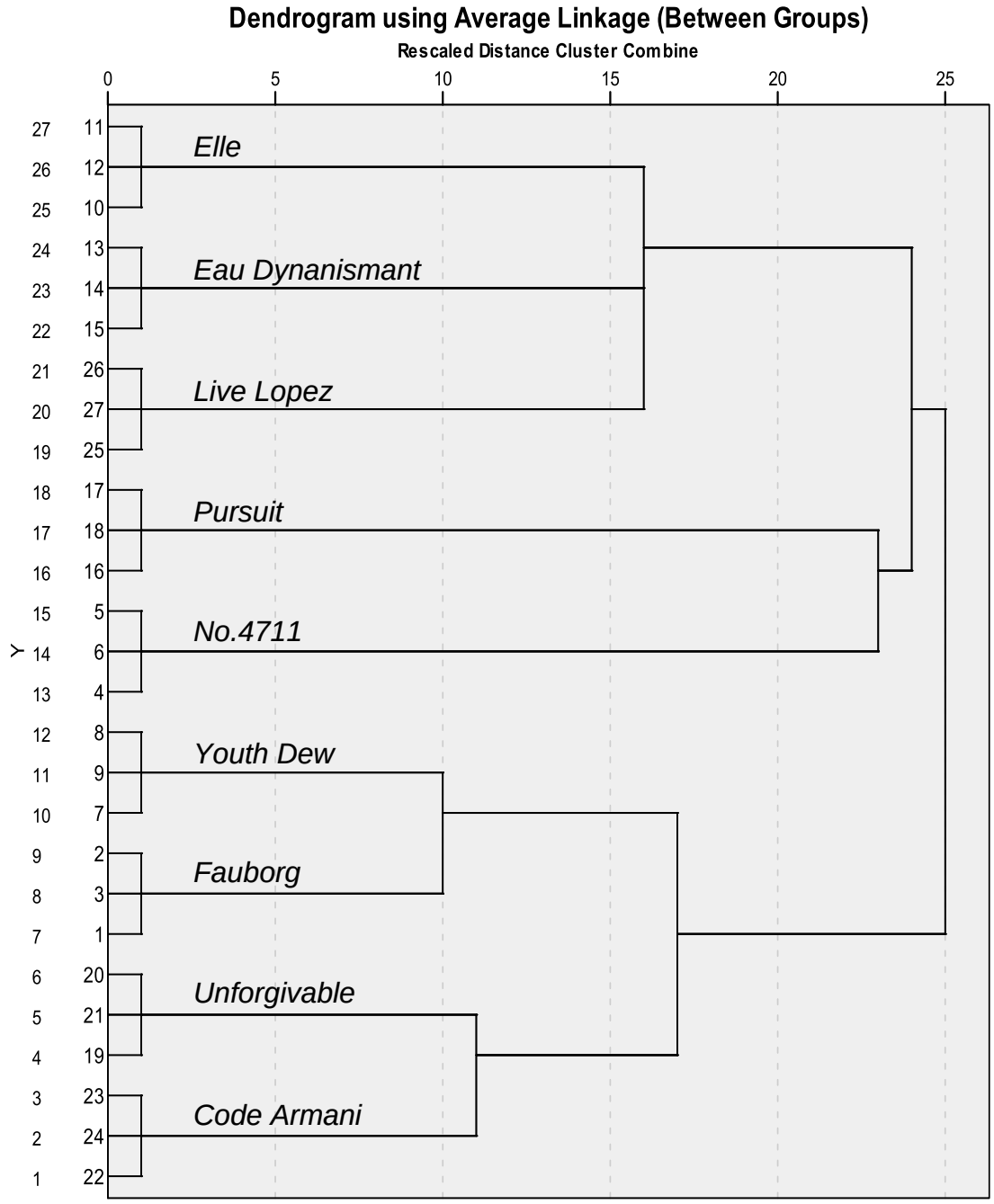


Figure 3.36 - Dendrogram for nine perfumes (product names added)

Forbes and Perrault (2014) assert that PCA is particularly useful where there is significant variability in the dataset is and a high number of variables, thus it is well suited to test whether perfumes can be discriminated from one another. PCA has been used for brand-dependent ‘fingerprinting’ of other complex products such as wines (Dall'Asta *et al*., 2011) though it should be noted that some authors warn that reduction of the GC data can lead to ‘anomalously good’ predictions (Dixon *et al*., 2007 p. 162). Unfortunately, the statistical tool used here was SPSS which limits number of variables which can be entered so some form of pre-selection was required. The ‘peak picking’ approach used by Penn *et al*. (2007) whereby peaks are chosen where they are common to all or most samples, was not successful (at least not when using only four peaks) but the use of the top twenty retention times produced a useful analysis.

3.4 Storage Study

The results for the examination of how storage conditions affect samples and their identification showed that, for the six weeks of the study, the samples were generally very stable if stored in the dark at room temperature or in the fridge but were less stable when stored in the freezer or at room temperature in the light (see Figure 3.37). Comparisons of the number and size of peaks for samples analysed by GC-FID after three and six weeks are shown in Table 3.12 and Table 3.13 and show that the sample stored in the dark at room temperature produced the chromatograms with the greatest peak heights. After three weeks the sample stored in the light at room temperature and the sample stored in the fridge had peaks of similar magnitude but the peak heights of the freezer sample were lower. After six weeks samples in these three storage conditions showed similar peak heights. The samples stored in the dark at room temperature, in the fridge and in the freezer all retained a range of chemicals with peaks eluting across the chromatogram. The chromatograms for the sample stored in the light showed that some of the earlier peaks were proportionally lower but more peaks were recorded than for the other samples: it is likely that additional peaks are due to the oxidation of some of the terpenes in the mixture (Rudback *et al*., 2013) or the formation of hemiacetal compounds through the reaction of alcohols and aldehydes which is common as perfumes age (Herman, 2005).

In summary, it is recommended that perfume samples are stored out of direct sunlight and, for best results, are refrigerated, however, manufacturers demand a long shelf-life from their products for commercial reasons and aroma chemicals in a hydroalcoholic solution are particularly stable (Herman, 2005).

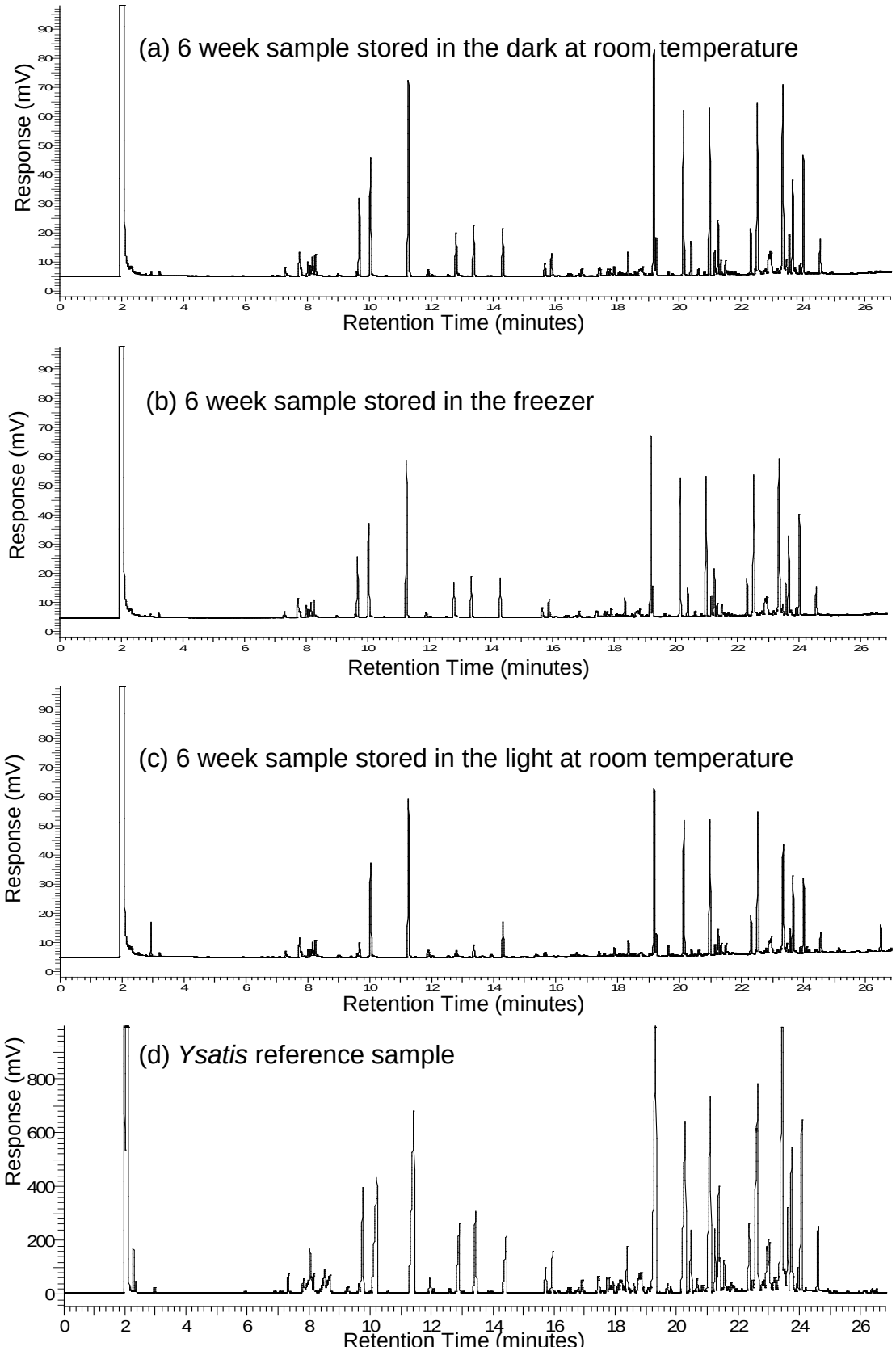


Figure 3.37 – Comparison of storage conditions

Table 3.12 – Comparison of storage methods (3 weeks)

Storage Method	No. peaks before 14.3 minutes *	No. peaks after 14.3 minutes	No. peaks with height > 40 mV *	Greatest peak height (mV) *
Dark	12	151	8	83
Light	12	176	6	74
Fridge	12	151	6	73
Freezer	11	146	6	54

* Not including solvent peaks between 2 and 3 minutes.

Table 3.13 – Comparison of storage methods (6 weeks)

Storage Method	No. peaks before 14.3 minutes *	No. peaks after 14.3 minutes	No. peaks with height > 40 mV *	Greatest peak height (mV) *
Dark	13	39	8	78
Light	14	52	3	57
Fridge	13	34	6	59
Freezer	14	36	6	62

* Not including solvent peaks between 2 and 3 minutes.

3.5 Aging Study

The aim of this work was to determine whether perfumes, antiperspirants and deodorants age in a predictable manner. Results following analysis by both FTIR and GC are discussed below.

3.5.1 Investigating Aging Using Gas Chromatography

Results of the analysis of samples of four perfumes aged over a period of 384 hours showed that the peak areas for more volatile chemicals decreased across the aging period while peak areas for other, less volatile chemicals, remained relatively stable. An example of this can be seen with perfume *Mania* in Figure 3.38.

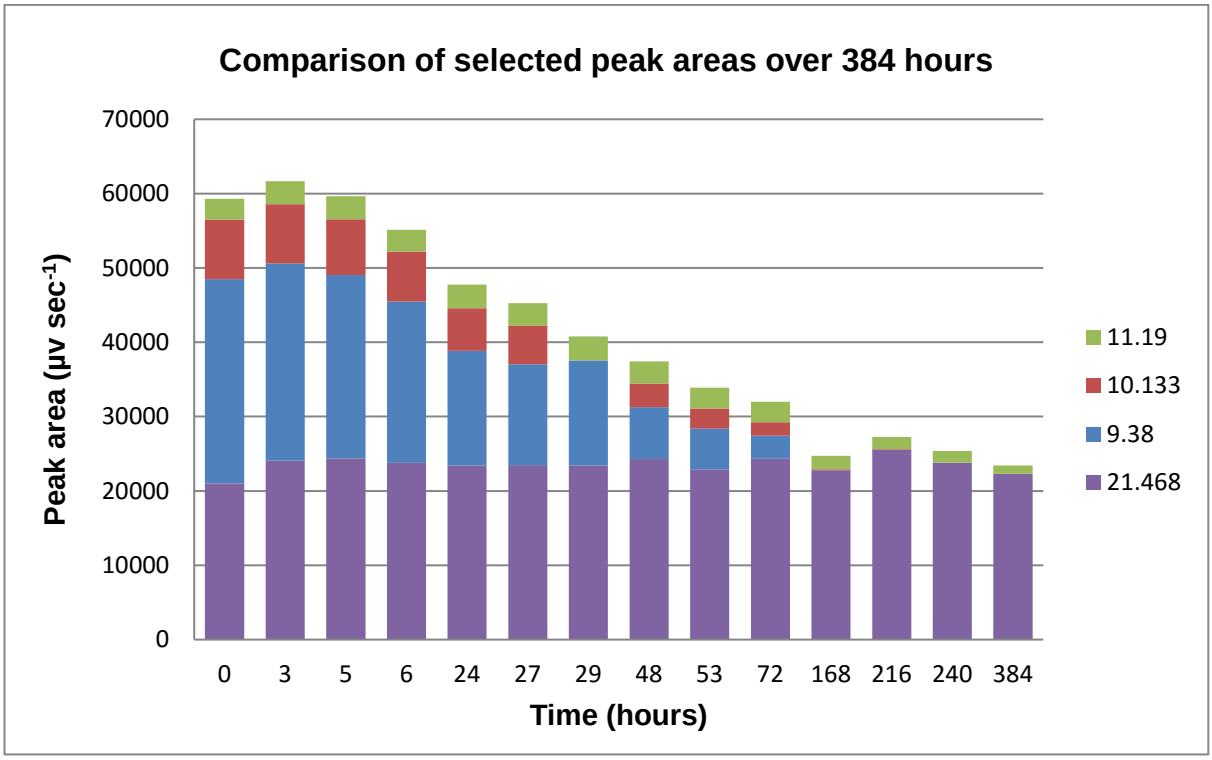


Figure 3.38 - Comparison of peak areas over 384 hours for perfume *Mania*

Repeatability of results was acceptable with peak areas for repeats at 24, 48 and 384 hours producing average % RSD values of less than 20% with most variability shown in the 24 hour samples. It was considered however, that using peak area ratios would offset the variability in the samples and could be used to demonstrate the predictability of perfume aging. Results are shown in Figure 3.39 for the first 72 hours of aging for the perfume *Mania* using the ratio of peaks with retention times of 9.830 minutes and 10.133 minutes to the peak at 21.468 minutes (average retention times, %RSD = <0.5 %). The peak at 9.830 minutes was no longer detected in *Mania* after 72 hours and the peak at 10.133 minutes was not detected after 216 hours so for samples aged for up to 384 hours (16 days) the ratio was used between the peak at 11.190 minutes to that at 1.468 minutes, as shown in Figure 3.40.

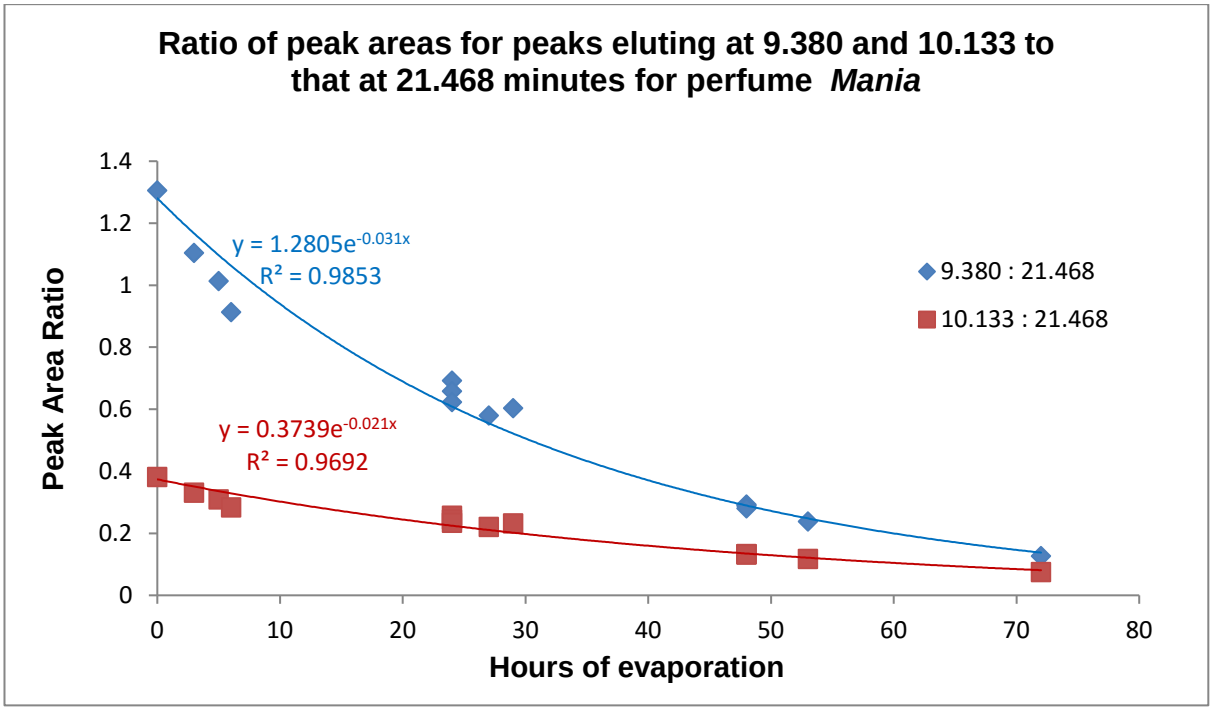


Figure 3.39 - 72 hour evaporation pattern

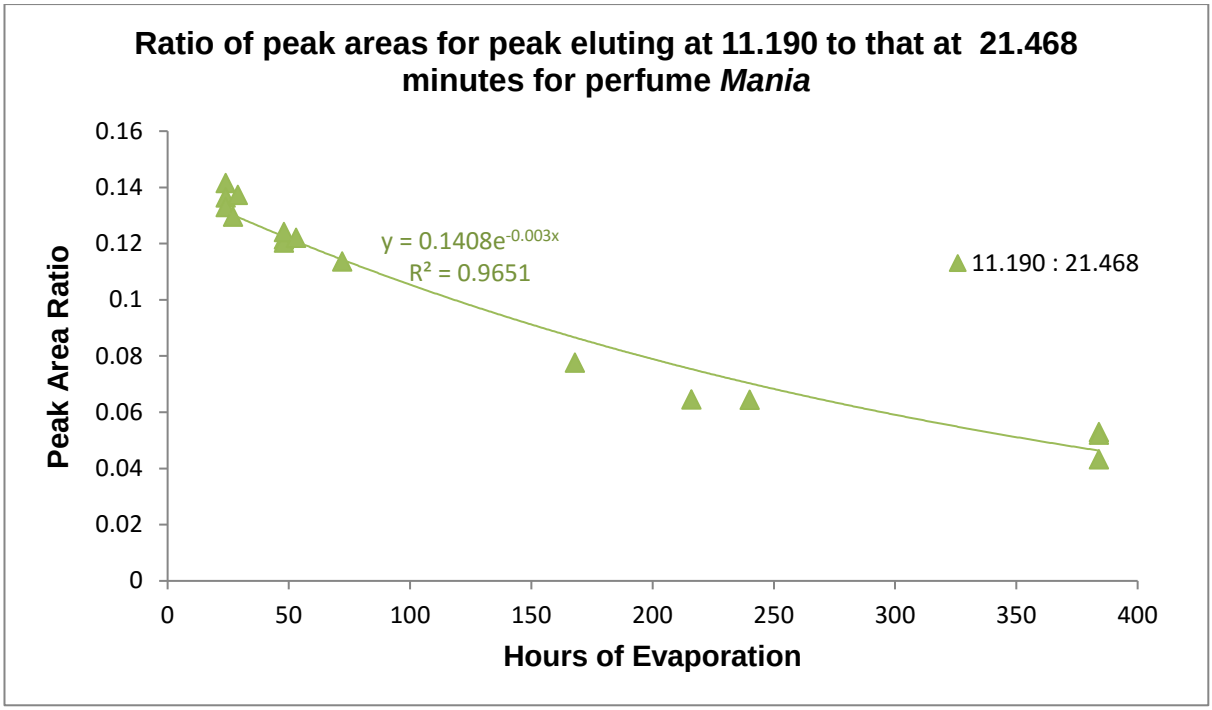


Figure 3.40 - 16 day (384 hour) evaporation pattern

The fit of the exponential trend lines indicate that these four peaks could be used to determine the age of the perfume up to 384 hours (16 days). When the same approach was used for the other three perfumes however, it became apparent that each perfume has different ingredients and different peaks must be used for each. Exponential curves were produced for the remaining perfumes (e.g. for *Ysatis* using the ratio of peaks at 12.00 and 24.00 minutes produced a curve $y = 9.1435e^{-0.004x}$ with $R^2 = 0.9526$) and demonstrated that, in the controlled conditions of this experiment, perfumes do evaporate predictably. In order to demonstrate that the predictable evaporation could potentially be used to determine the age of a recovered sample however, a multidimensional approach was considered likely to be more successful, specifically one using principal component analysis (PCA). For the analysis of perfume *Ysatis* all the peak areas across the entire chromatogram were used (n=81) and Figure 3.41, shows the resulting bi-plot.

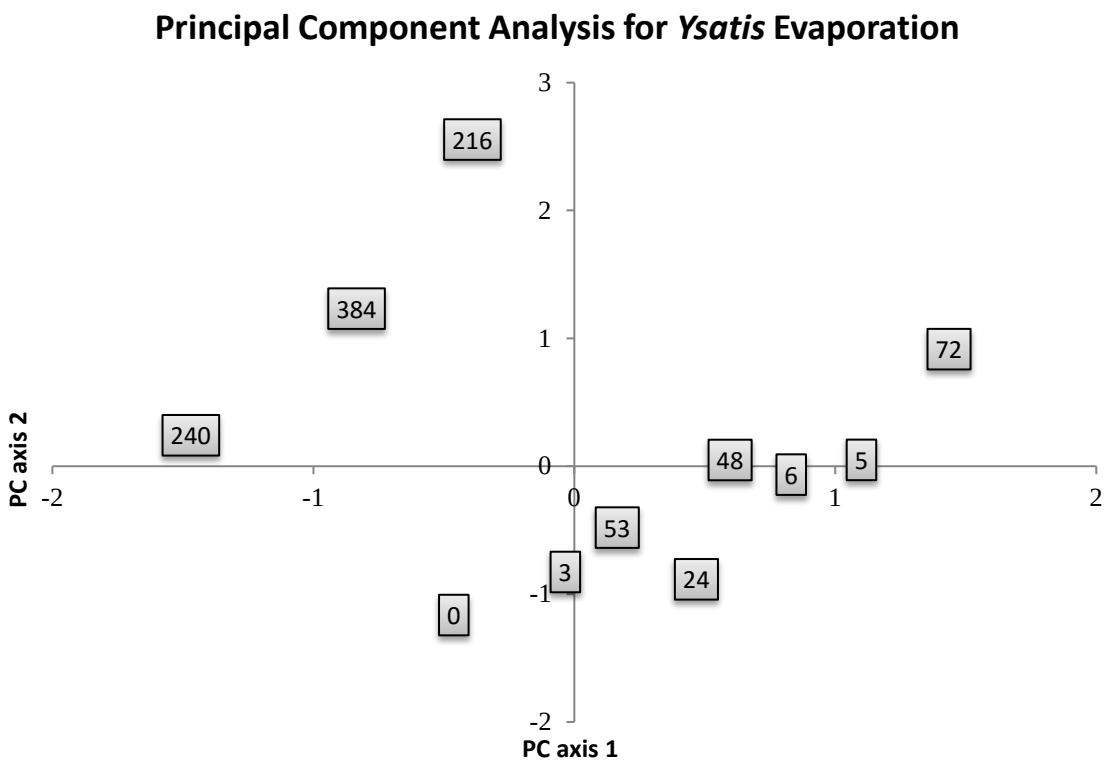


Figure 3.41 - PCA for all consistent peaks across all time periods (data labels refer to the number of hours of evaporation).

This analysis demonstrates that samples from each period are clearly different and that samples with less than 10 days (240 hours) are clearly differentiated from those that are older.

3.5.2 Investigating Aging Using Fourier Transform InfraRed Spectroscopy (FTIR)

As discussed in Section 3.1.2.1 when spectra for the perfume *Hugo XX* were collected at 30 minute intervals up to 4.5 hours (Figure 3.10), there was little further evaporation after 3 minutes. This raised the possibility of using FTIR to identify aged samples of perfumes, antiperspirants and deodorants and to evaluate the time since deposition. Table 3.14 (below) shows the results of library matching for samples which had been left to evaporate for extended periods. It can be seen that the two perfumes, *Cassis Rose* and *Hugo XX* produced correct matches after two and 4.5 hours respectively but the body sprays *Aphrodite* and *Lynx Africa* did not. As can be seen in Figure 3.42 the spectra for *Lynx Africa* showed significant degradation after two hours. In aerosols the highly volatile propellant increases the surface area of the droplets (Sell, 2006, p175) so it is possible that a greater proportion of the volatile compounds were lost during the sampling process. After 4.5 hours the background spectrum expired and reliable spectra could not be collected so older samples were not analysed.

Table 3.14 – Library matches for 3 aged products

Sample		Match		Match %	Correct match
Perfume	**Age**	**Matched with**	**Age**		
Cassis Rose	2 hours	Cassis Rose	3 mins	81.61	✓
Hugo XX	4.5 hours	Hugo XX	3 mins	60.95	✓
Aphrodite	2hrs 20 mins	Cassis Rose	3 mins	79.82	×
Lynx Africa	2 hours	Lynx Africa	3 mins	52.51	✓

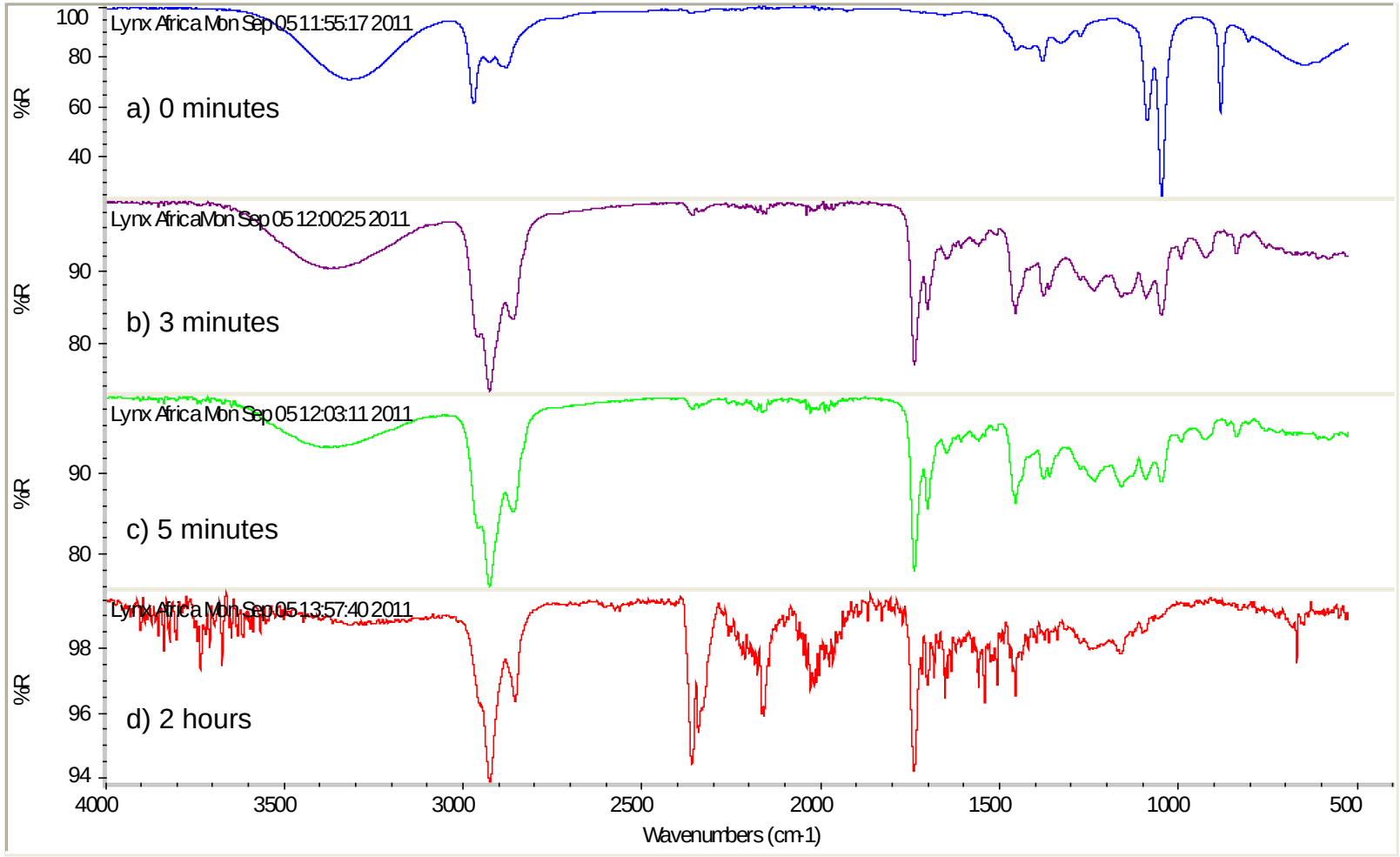


Figure 3.42 - Spectra for Lynx Africa for evaporation times ranging from 0 minutes to 2 hours

3.5.3 Further Discussion and Summary

Perfumed products are formulated to balance volatility (to allow the fragrance to be perceived by the wearer) and persistence (which perfume companies term 'substantivity'). As seen in the experimental results, some chemicals evaporate more quickly than others and this is due to differences in molecular weight, boiling point and, most significantly, vapour pressure (Perring, 2006 p. 201). Table 3.15 shows some representative physical properties of some perfume ingredients.

Table 3.15 – Representative physical properties of perfume ingredients

Ingredient	RMM	Boiling point at ca. 760 mmHg (˚C)	Vapour pressure at 25˚C (mmHg)	sp ($MPa^{0.5}$)	Log *P*
Benzaldehyde	106.1	178	1.10	21.9	1.50
Limonene	136.2	178	1.40	16.5	4.46
Methyl butanoate	102.1	102	30.2	18.4	1.18
2-phenylethanol	122.2	218	0.11	23.7	1.36

From (Perring, 2006 p. 201). Where log P = common log of octanol-water partition coefficient (Rekker, 1977) and sp = Hildebrand solubility parameter as calculated according to Hoy (Barton, 1985).

The two other parameters in the table, the logarithm of the octanol-water partition coefficient, Log *P*, and the Hildebrand solubility parameter, sp, are used by perfumers to classify aroma chemicals according to physical behaviour producing data on Quantitative Property-Activity Relationship (QPARs) for in-house databases (Perring, 2006 p. 201). Log *P*, provides a measure of the relative hydrophilicity of a chemical (Rekker, 1977) and may be plotted against boiling point to identify aroma chemicals that are persistent (Perring, 2006 p. 201). The

Hildebrand solubility parameter (Equation 4) represents the amount of energy needed to disrupt molecular cohesion (Barton, 1985) and is of particular note as molecules with similar sp values will interact which will modify evaporation behaviour (Perring, 2006 p. 211).

Equation 4 - Hildebrand solubility parameter

$$\text{Solubility parameter, } sp = [\ (\Delta H - RT) / V\ ]^{0.5}$$

Where ΔH is the molar enthalpy of vaporisation, R is the gas constant, T is absolute temperature and V is molar volume

The results of the experiments show that perfumes age in a predictable manner and using statistical tools it should be possible to determine the time since deposition of a perfume. The evaporation rate will however, be highly dependent on temperature, light, air movement and humidity and further experimentation would be required to develop a predictive model which would be suitable for a range of environmental conditions. Additionally, as solubility parameter is related to viscosity, surface adhesion and miscibility, evaporation will depend on the molecular environment and the substrate (Perring, 2006 p. 211) so it is important to note that these experimental results are likely to vary for samples in different containers or on surfaces.

3.6 Recovery of Liquid Residues

The aim of this phase was to explore whether traces of the products under study may be sampled and identified by chemical analysis through comparison with a positive control sample. The use of swabbing to recover samples from surfaces was found to be simple and reasonably effective and two methods of recovering the sample from the swab for GC analysis each had benefits.

3.6.1 Solvent Extraction of Swabs

Chromatograms are shown in Figure 3.43 for samples of perfume *Ysatis* which had been swabbed from the test surface with a dry swab (a), a cotton swab moistened with ethanol (b) and a swab moistened with water (c). In each case the sample was extracted from the swab using portions of ethanol. A control sample of the perfume is also provided for comparison (d). In all of the chromatograms the first significant peak was around 14.3 minutes (previously identified as hydroxycitronellol) with earlier peaks either absent or producing a very low response. All three extraction methods displayed a good range of peaks across later retention times. Unfortunately, a number of peaks were also seen in the swabbed samples after 25 minutes which were not in the Ysatis control sample and these peaks were also found in the negative control. Table 3.16 provides a numerical comparison of the solvent extraction methods and shows that the deionised water swab performed best with the greatest number of peaks and the greatest intensity of peaks. The dry swab performed reasonably well with the ethanol swab performing worst. The chromatograms for all of the solvent extracted swabs showed good comparison to the later portion of the chromatogram for the liquid sample of the perfume (*Ysatis*). Later peaks also seem to maintain the same ratios as the liquid sample.

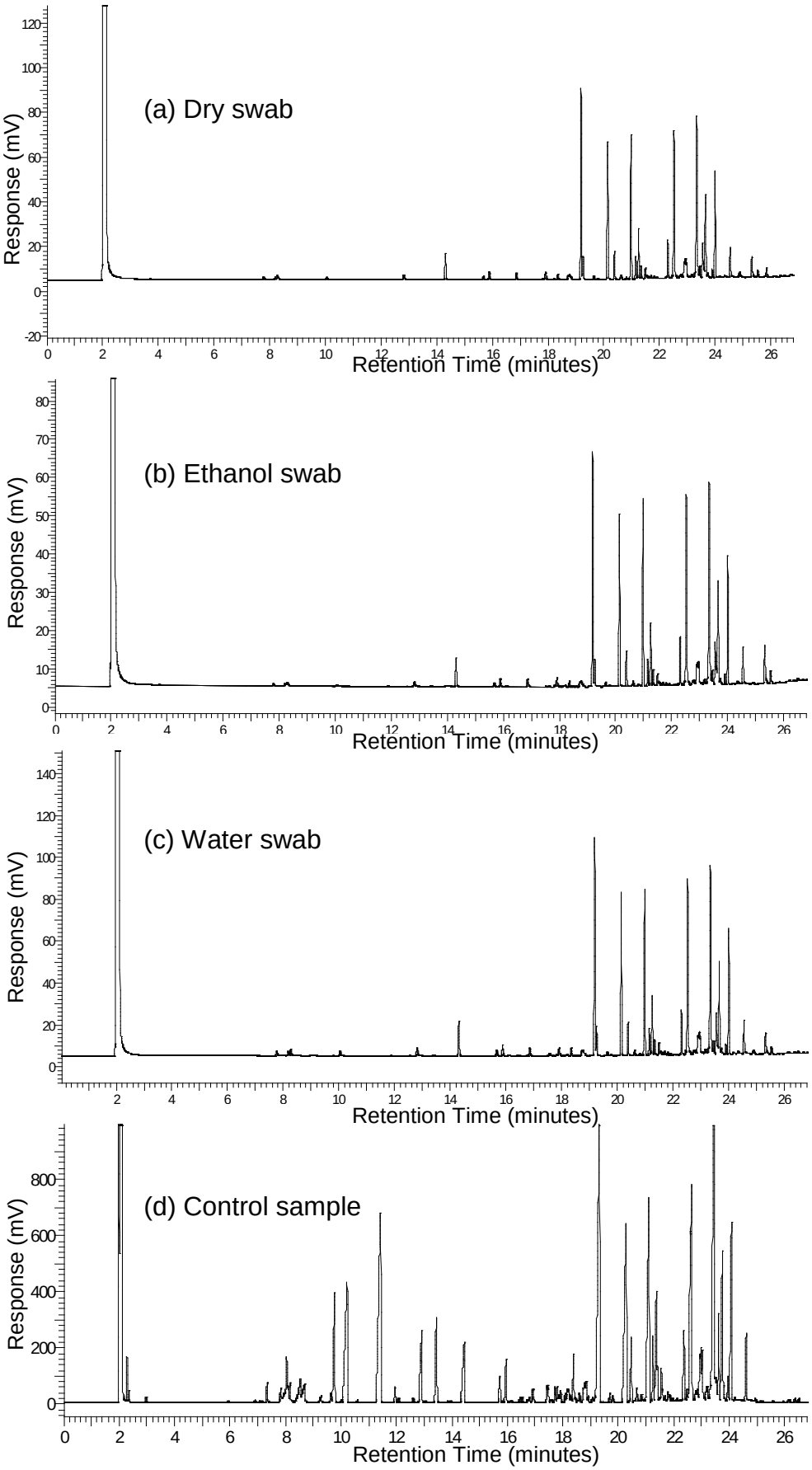


Figure 3.43 – Chromatograms of samples recovered with swabs and solvent extraction for perfume *Ysatis*

Table 3.16 – Comparison of swab methods (solvent extraction)

Swab	No. peaks before 14.3 minutes *	No. peaks after 14.3 minutes **	No. peaks with height > 40 mV	Greatest peak height (mV) *
Dry	2	27	7	86
Ethanol	1	25	5	53
Water	5	32	7	104

* Not including peaks at 1.9 and 2.0 minutes which are attributed to the ethanol used in the solvent extraction.
** Not including peaks which were also found in the negative control.

3.6.2 Solid Phase MicroExtraction (SPME) of Swabs

The chromatograms for the swabs that were sampled using SPME showed more variation (Figure 3.44). As in the previous experiment, the perfume *Ysatis* had been swabbed from the test surface with a dry swab (a), a cotton swab moistened with ethanol (b) and a swab moistened with water (c). A control sample of the perfume is also provided for comparison (d). In this experiment the sample was extracted from each swab using SPME and Table 3.17 provides a numerical comparison of the SPME from each swab. It can be seen that dry swabbing and swabbing with water produced a strong response for peaks between 9.5 and 14 minutes. Dry swabbing produced peaks with the greatest peak height but the early peaks were disproportionally higher than the later peaks. The chromatogram for the ethanol swab was dominated by the ethanol peak and otherwise showed a poor response across all retention times with increasing baseline interference after 20 minutes.

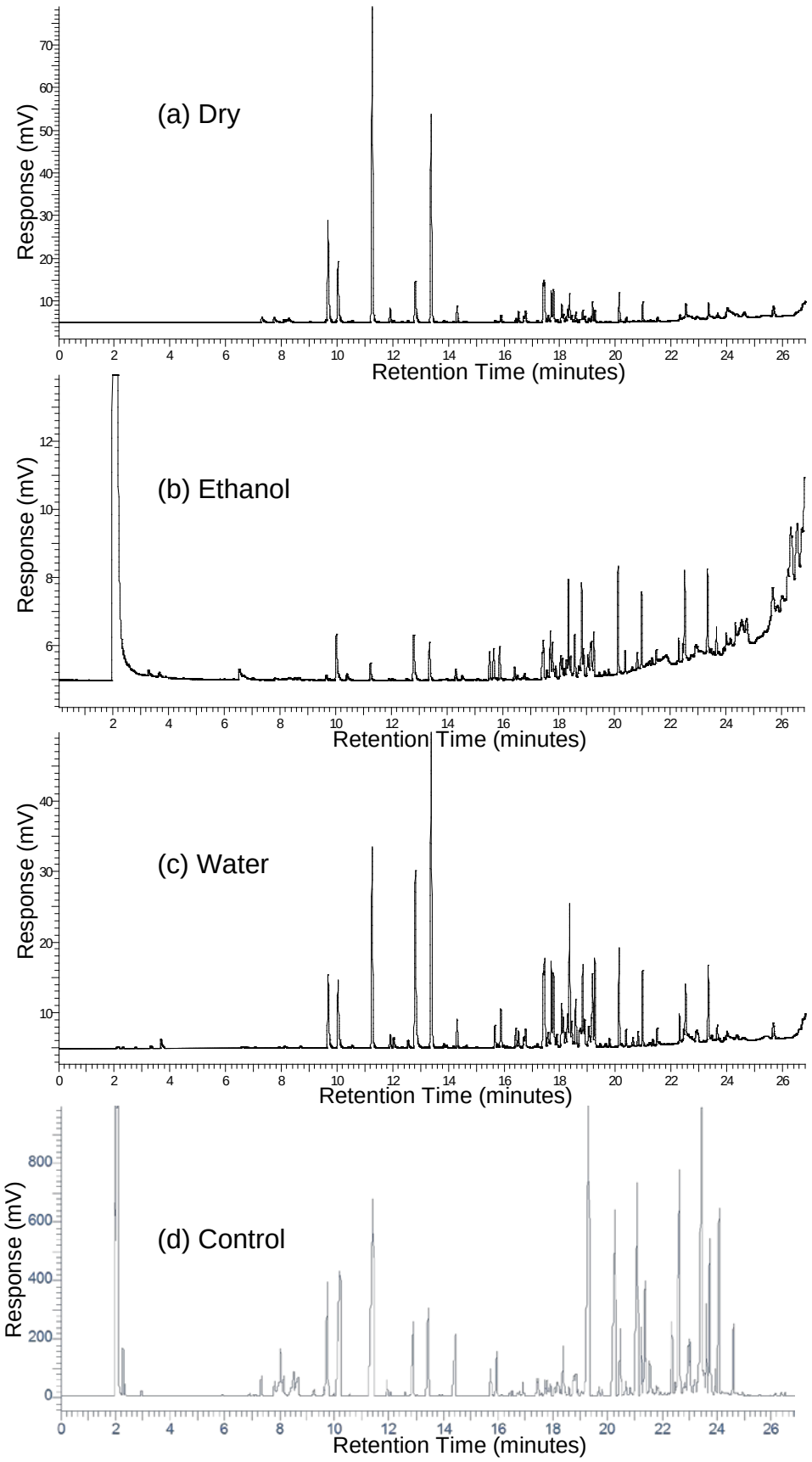


Figure 3.44 - Chromatograms of samples recovered with swabs and SPME for perfume *Ysatis*

Table 3.17 - Comparison of swab methods (SPME)

Swab	No. peaks before 14.3 minutes *	No. peaks after 14.3 minutes **	No. peaks with height > 40 mV	Greatest peak height (mV) *
Dry	10	25	2	74
Ethanol	2	13	0	3
Water	8	36	1	45

* Not including peaks at 2.0 minutes which is attributed to the ethanol used in the swabbing.
** Not including peaks after 25 minutes as these were also found in the negative control.

3.6.3 Further Discussion and Summary

The fact that both the dry swabs and the water saturated swabs performed well highlights the variety of chemicals in perfumes: aldehydes, esters and alcohols have some polarity and will therefore be soluble but other ingredients are non-polar (Herman, 2005). One parameter used by perfumers to understand the physical-activity relationship of aroma chemicals is log *P* the logarithm of the octanol-water partition coefficient, which provides an indication of the hydrophobicity of a chemical (Sangster, 1989). Ingredients such as 2-phenylethanol (PEA) which have low log *P* values (1.52) will be more easily recovered by the water soaked swab, while those such as limonene (log *P* 4.46) and the musks (log *P* 4.5 – 6.5) are hydrophobic and will be better recovered by the dry swab (Small, 2006 p. 148, Perring, 2006 p. 209). The polarity will also affect the degree to which chemicals can be extracted from the swab by SPME or solvent extraction: for example a chemical such as limonene which has been recovered by a water soaked swab will not interact strongly with the water molecules and will partition into the gas phase more easily ready to be absorbed by the SPME fibre (Perring, 2006 p. 206).

Overall, it is clear that 20 µL deposits of perfume left on a glass surface for 1 hour can be recovered. Results indicated that the best approach would be to moisten a swab with deionised water but a dry swab will also recover the sample well. Ethanol should not be used as it seems to facilitate the evaporation of lighter chemicals from the sample and, when extracting with SPME, dominates the headspace. The sample should then be extracted from the swab using ethanol but a prior extraction by SPME may provide a useful, and complimentary, chromatographic profile.

Note however, the drying time used in this experiment was only one hour which is not representative of a crime scene deposit. Future experiments, where samples are swabbed after a longer drying time would also need to account for the evaporation of the perfumed product (as investigated in Section 3.5). Additionally, the results of the swabs taken from a glass surface do not guarantee that the same effect will be observed for swabs taken from other surfaces. The persistence of a perfume (known as the ‘substantivity’ in the fragrance industry) varies with substrate (Perring, 2006 p. 204). Substantivity is especially complicated on skin as although vaporisation is increased by the warmth of the skin, the epidermis is moderately porous and hair proteins provide additional binding sites (Small, 2006 p. 149). Skin secretions also interact with perfume ingredients, for example, skin oils dissolve the lipophilic compounds such as the musks which are therefore more strongly retained on the skin (Perring, 2006 p. 210).

3.7 Sampling from Clothing

The results from the various trials conducted during this phase of the work showed that both ATD and SPME have potential as sampling techniques but, if passively sampling at room temperature the sorbent material (SPME fibre or Tenax packed tube) needed to be exposed for a significant length of time (e.g. 4 hours). For quicker SPME sampling, with the fibre exposed to the sample headspace for 30 minutes, prior heating of the sample to 80˚C produced a slightly improved response over room temperature sampling. An acceptable response was however were only achieved by pre-heating the sample to 130˚C. To avoid damaging the garments, the heating time was kept to 10 minutes which provided a satisfactory response. Active sampling using the Tenax packed tube followed the same trend but it should be noted that the maximum sample volumes extracted were 100 mL and a larger headspace sample may improve results.

When heating the samples to 130˚C it was observed that the nylon bags, which in the UK are standard forensic packaging for evidence to be analysed for VOCs, produced chemicals which interfered with the trace level samples being collected. Tests also showed that the chemicals released from the bags varied (see Figure 3.45) and so could not be easily offset. The current recommended crime scene packaging in the UK for garments generally is a paper bag but unfortunately these also produced VOCs on heating. To overcome these issues a desiccator was used as a sampling chamber with paraffin wax to produce a suitable seal, and after the first heating cycle, no interference was apparent. As seen in Figure 3.46 and Figure 3.47 chromatograms collected using this method were representative of liquid samples.

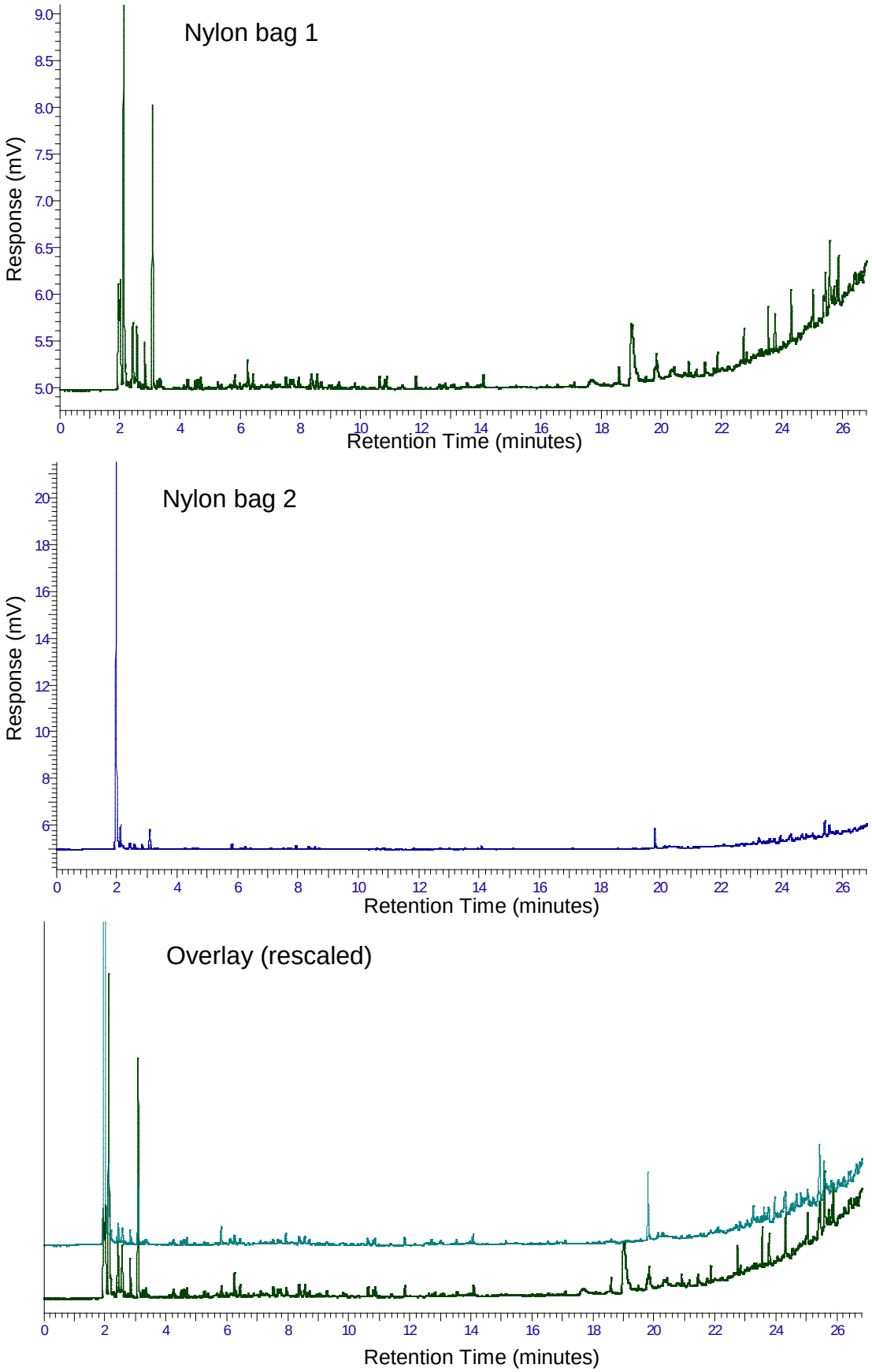


Figure 3.45 – Interference from nylon bags

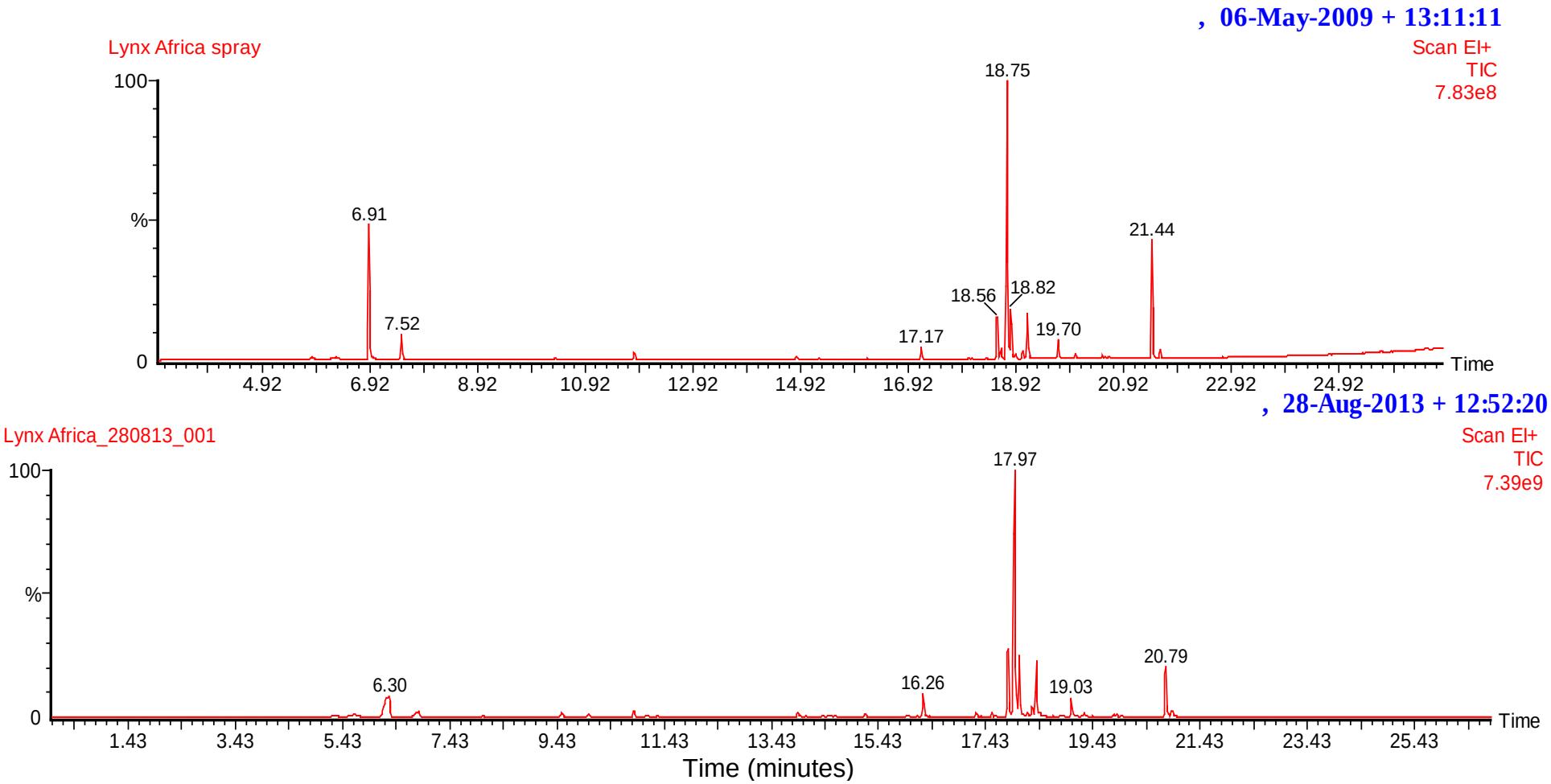


Figure 3.46 – GC-MS results for *Lynx Africa*

Liquid sample (top) compared to headspace sample (bottom) collected from desiccator sampling vessel using SPME. Note that the chromatogram for the liquid sample starts at 3 minutes due to the solvent delay. Retention times differ due to system changes between samples.

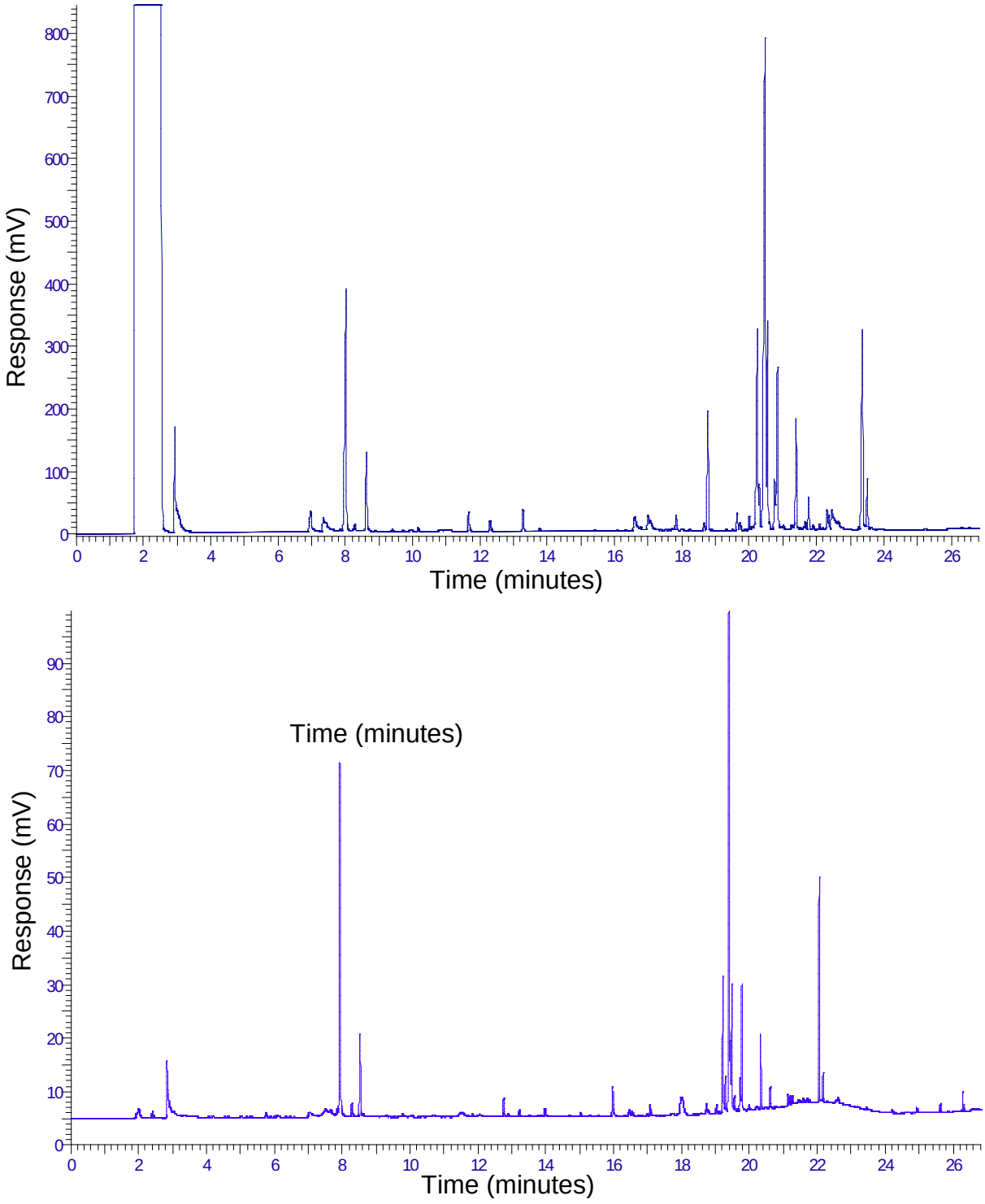


Figure 3.47 – GC-FID results for *Lynx Africa*

Liquid sample (top) compared to headspace sample (bottom) collected from desiccator sampling vessel using active sampling and analysed using ATD-GC-FID.

The results of subsequent trials showed that for strongly scented items the desiccator required cleaning with 2M sodium hydroxide to avoid carryover of persistent esters such as ethylene brassylate. The use of aluminium foil to seal the desiccator allowed a good seal to be achieved and for the headspace to be first sampled by SPME or ATD and then reheated and re-sampled using the second technique: Figure 3.48 and Figure 3.49 show comparisons. This was beneficial as the two techniques were complementary with some earlier eluting compounds seen to give a stronger response on the ATD chromatograms, and the SPME chromatograms showing better resolution on some peaks. SPME sampling also had the advantage that the sample collected could be analysed by GC-MS allowing chemicals to be tentatively identified. The order of sampling was also investigated and results indicated that when the Tenax tube was used before SPME the ATD sample was more concentrated. When the Tenax tube was used after SPME sampling (following a re-heating of the desiccator) the ATD sample was not only less concentrated but the relative intensity of ethylene brassylate (the last significant peak) was disproportionally lower. This was surprising as it was expected that earlier eluting, lower boiling point, chemicals would have been lost during SPME sampling and later eluting, higher boiling point, chemicals would have become relatively more concentrated. In contrast, when sampling using SPME, both the recovered sample size and the relative proportions of peaks seemed much less affected by order of sampling, presumably because the volume of sorbent material, and therefore the mass of sample collected is lower. One other potential issue seen with ATD sampling was that the retention times varied more than would normally be expected for GC analysis, even for samples collected in the same week.

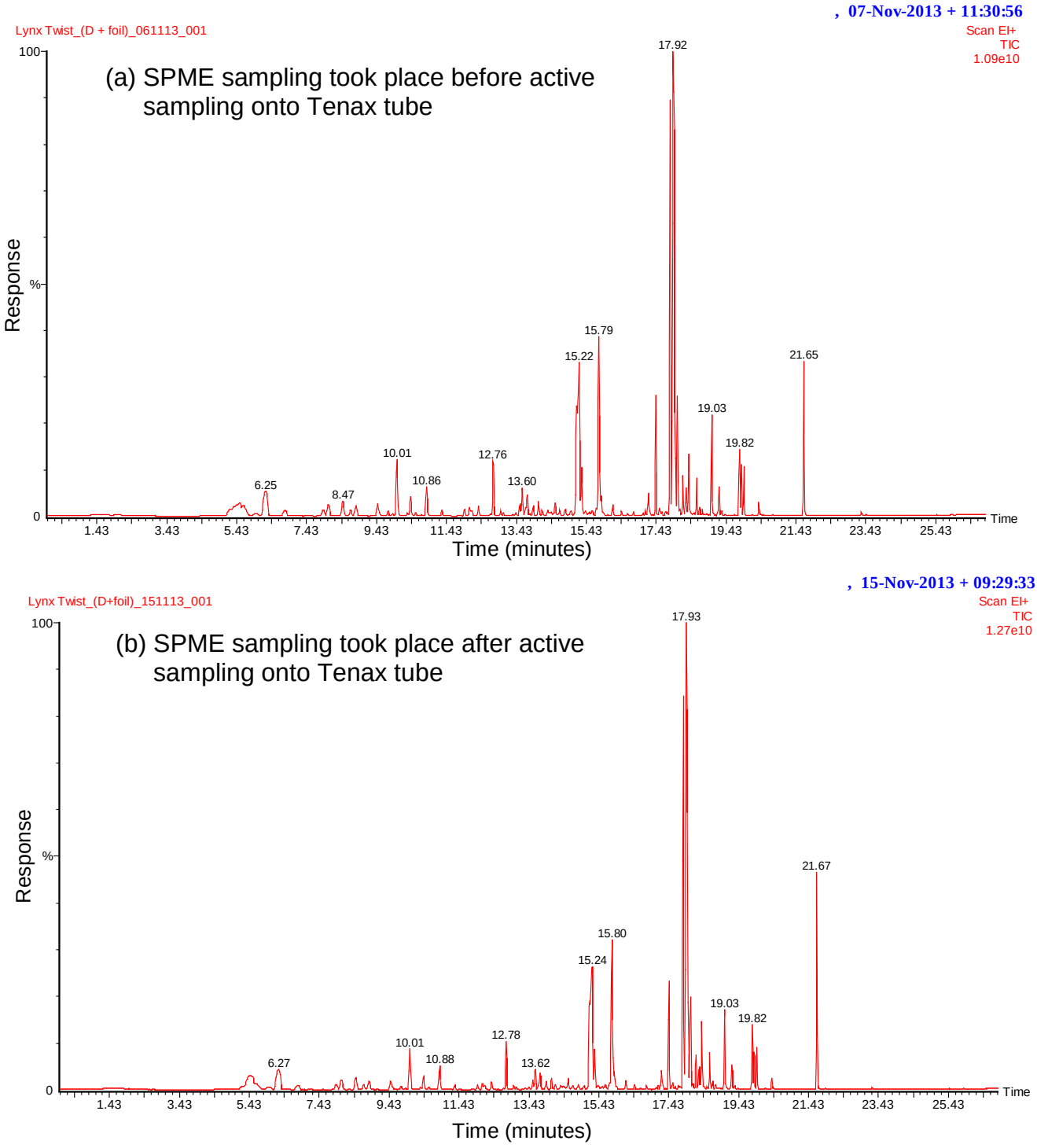


Figure 3.48 – Comparison of sampling order for SPME-GC-MS

The top chromatogram shows the GC-MS response for Lynx Twist when the sample was recovered using SPME first and then active sampling onto Tenax tube followed by ATD. The bottom chromatogram shows the GC-MS response when the sample was recovered using active sampling onto Tenax tube first then sampled by SPME.

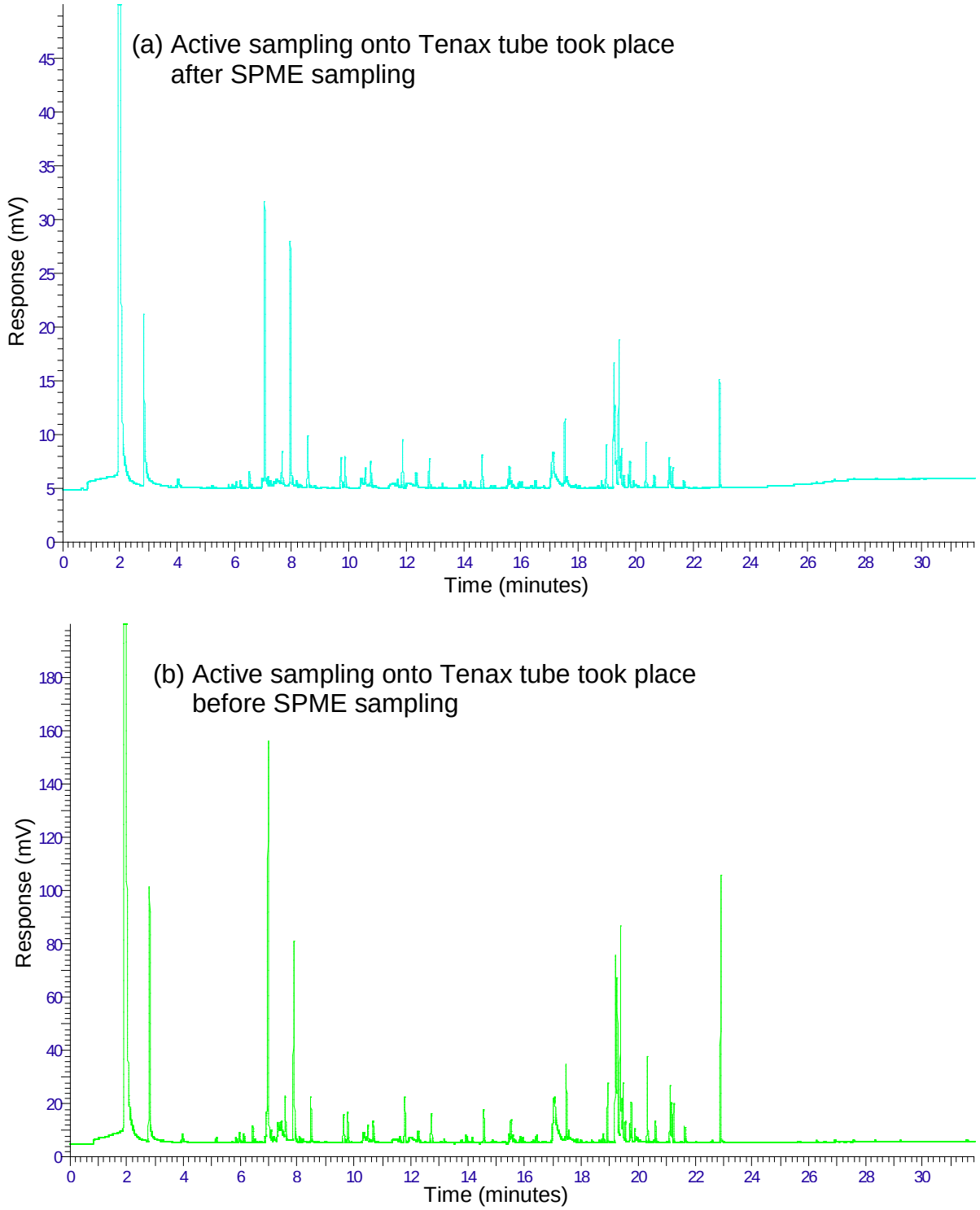


Figure 3.49 – Comparison of sampling order for ATD-GC-FID

The top chromatogram shows the ATD response when the sample was recovered using SPME first and then active sampling onto Tenax tube followed by ATD. The bottom chromatogram shows the ATD response when the sample was recovered using active sampling onto Tenax tube first.

The results of trials conducted of sampling from worn garments showed encouraging results, unfortunately these trials were conducted with garments heated in nylon bags and cannot be considered conclusive for the reasons discussed previously. Three key hypotheses were, however, supported by the results of these trials: first that chromatograms of garments worn by the same subject showed similarities; secondly that garments worn by different individuals showed different chromatographic patterns; and thirdly that chromatograms of garments showed traces of the deodorant or perfume worn by the individual (Figure 3.50).

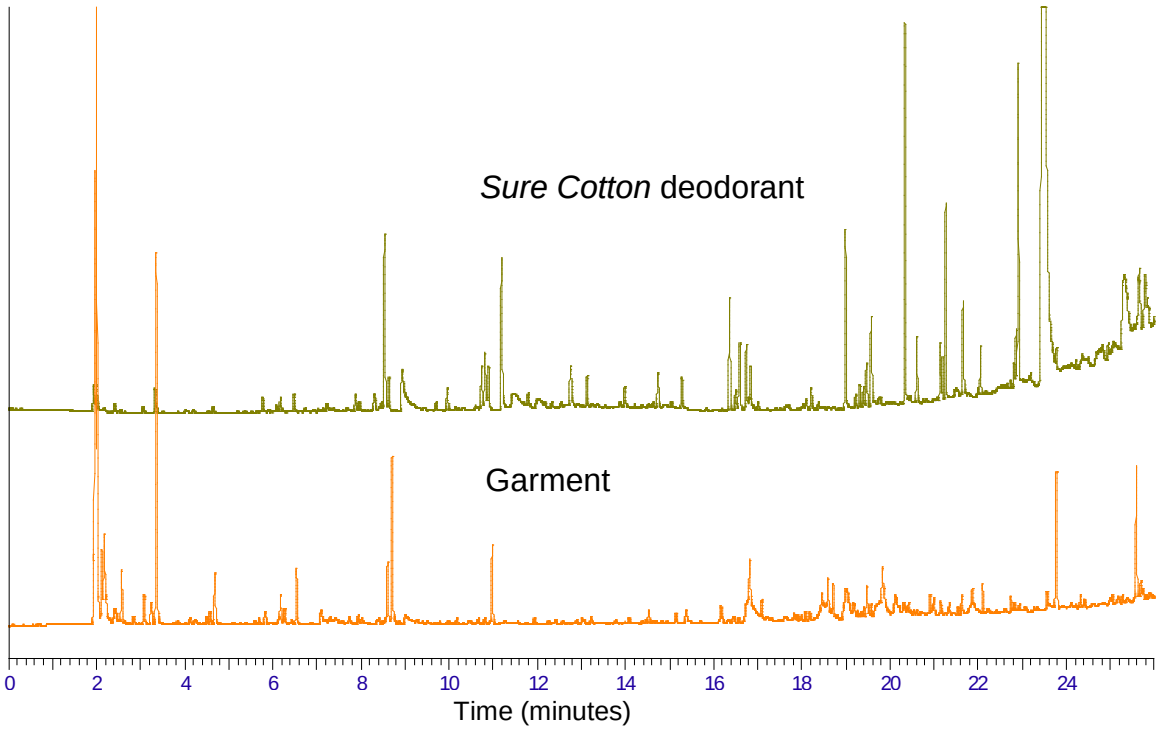


Figure 3.50 – ATD-GC-FID chromatograms for a worn garment and *Sure Cotton*

NOTE: Chromatograms have been offset for clarity - *Sure Cotton* deodorant (top, in green) had a maximum scale of 10 mV, the garment (bottom, in orange) had a maximum scale of 21 mV.

3.7.1 Discussion and Summary

Sampling from clothing using these instrumental approaches clearly has challenges and, for chemicals to be present in the headspace at detectable levels, the sample must be heated without damaging the article and without introducing interferences from the vessel. Heating the garment to a temperature of 130˚C for 10 minutes produced acceptable ATD results and although Borusiewicz *et al.* (2007) warn that at higher temperatures the higher molecular weight, nonpolar compounds tend to be preferentially adsorbed by Tenax, this effect did not seem to be replicated. The heating temperature also needs to optimised to achieve the best SPME results as, while higher temperatures may increase the concentration of chemicals in the headspace, the fibre will preferentially absorb the less volatile chemicals (Smith, 2003 p. 19) and this would account for the higher proportion of late-eluting peaks seen in the SPME-GC-MS chromatograms. The PDMS fibre used in these experiments has also been reported to have lower affinity for some highly volatile aroma chemicals including limonene and linalool (Divisova *et al*., 2015). David and Sandra (2007) however, assert that it is the polarity of the analyte which has the greater effect and apolar chemicals with a high log *P* value, such as limonene, are extracted proportionally while polar chemicals such as benzyl alcohol (log *P* = 1.1) experience a relatively decreased recovery at higher concentrations. So although Divisova *et al.* (2015) suggest the use of a fibre coated with a carbowax-PDMS blend for the extraction of fragrance allergens, the resulting sample may not be representative of the original product which would hamper identification.

While results showed that heating the sample is a necessary part of the method,

they also showed that nylon bags were not suitable for containing the sample while heating. The use of bags has been an area of study in the forensic analysis of fire debris with the risk of interferents vying with the benefits of low cost and ease of transport (Kocisko, 2001, Newman, 2004b). While glass containers have a higher leak rate than bags and are not suitable for long term storage (Williams and Sigman, 2007) they are considered least likely to produce interference (Hudson *et al*., 2009) which accords with the results of this study. The primary disadvantage of using the glass desiccator is the fixed size, as using a large desiccator to sample a small garment would cause a dilution of the sample VOCs in the headspace (Wampler, 2002), although for quantification, the fixed size is an advantage. Securing the sampling port with aluminium foil was also shown to assist sampling and results indicate that active sampling on to Tenax tubes (for later ATD) followed by SPME sampling will produce useful and complimentary data. It was also noted that the ATD-GC-FID method may suffer from excessive variations in retention times so an additional recommendation would be to analyse an alkane standard during every analytical session to allow the use of retention indices to compare results.

In terms of being able to identify a perfumed product present on a garment, experiments have shown that this may be possible given a standard for comparison, but will otherwise be extremely difficult. Over the period of time that the product has been worn, some aroma chemicals, particularly those of lower molecular mass, will have evaporated (Small, 2006 p. 148) while others, such as limonene and linalool may have oxidised forming hydroperoxides (Rudback *et al*., 2013). Additionally when analysing garments which have been worn, the

chemicals available in the headspace are, of course, not limited to aroma chemicals from perfumes, deodorants and antiperspirants. Other aroma chemicals are likely to be present from personal care products, laundry detergents and conditioners (Beerling, 2006 p.168) as well as alkanes from petroleum jelly used in skin care lotion (Jones *et al.,* 2009). Other environmental exogenous compounds may include hydrocarbons and aromatics from sources such as cigarette smoke (Chien *et al*., 2011). Chemicals produced endogenously by the wearer will also transfer to the garment, including odorous chemicals produced by skin bacteria and organic acids (Bernier *et al.*, 1999, Bartels, 2011).

Some fabrics will retain odour for longer which may increase the concentration of some chemicals on the garment but reduce the headspace concentration when sampling: for example the hydrophobicity of polyester has been shown to inhibit the removal of odorants when washing clothes (Munk *et al*., 2001). In contrast cotton is hydrophilic (Stapleton and Dean, 2013) and a greater range of functional groups may be recovered from cotton compared to polyester from which mainly acidic species are likely to be recovered (Prada *et al*., 2011). Considering these fabric characteristics in terms of Log *P*, also reveals that aroma chemicals such as limonene, with a low molecular weight but a high value of Log *P*, is likely to be more strongly retained on polyester (Small, 2006 p.148). The extent of these influences on the recovery and identification of a perfume sample is an area to be explored further but while fabric type plays a part, the critical factors are the molecular weight of the individual chemicals and vapour pressure, which, following Raoult's Law is, in turn, dependent on the proportion of each chemical in the fragrance mixture (Perring, 2006 p. 210, Herman, 2005 p. 316).

4 Conclusions

The hypothesis underlying the research was that perfumes, antiperspirants and deodorants have the characteristics which make them valuable as forensic evidence, namely that they can be recovered from a crime scene, that products are distinguishable from each other and that they are persistent and transferable. This hypothesis was used to set five key aims of the research project each of which has been achieved. The first aim was to investigate whether perfumes, antiperspirants and deodorants can be profiled and distinguished from each other efficiently by rapid chemical analysis. Of the instrumental methods tested, gas chromatography (GC) proved the most useful with a demonstrated ability to distinguish between products using principal component analysis of retention data, with additional identification of key compounds by mass spectra. The results of the HPLC analysis were disappointing but not entirely unexpected as the focus was on volatile aroma compounds, nonetheless this technique may prove useful for future research. By contrast, the results of the FTIR analysis were quite promising, with simple preparation, quick analysis and some degree of product identification (albeit with a limited sample set).

Having identified the key instrumental techniques the next stage was to investigate how different storage conditions affect samples and their identification. The results of the GC analysis showed that aroma chemicals in a hydroalcoholic solution are highly stable particularly if stored out of direct sunlight and refrigerated. The stability and persistence of the perfumed samples was further explored in an investigation into whether perfumes, antiperspirants and deodorants age in a

predictable manner. The results of this study demonstrated that each perfume exhibited a distinct and predictable aging profile, however, it is recognised that a practical application of this attribute will require a great deal more experimentation to understand the effect of the many possible variables.

The next stage of the research investigated the potential to recover liquid samples from a crime scene and demonstrated that 20 µL deposits of perfume left on a glass surface for 1 hour can be recovered with a dry or water soaked swab. Subsequent extraction using ethanol and with SPME produced chromatograms that were highly representative of the original product. It should be noted however that longer time trials would be more realistic and would have to account for the aging of the perfumed product and that the results were limited to a single substrate type.

The final phase was designed to demonstrate that perfumes, deodorants and antiperspirants are transferrable and such samples can be recovered and identified. The results of this stage confirmed that transference does occur but indicated that while the sample may show similarities to a specific perfumed product, a conclusive identification is likely to be more challenging. Of great interest however, was the indication that a garment will show an individualising chemical profile.

5 Further Work

In order to realise the potential of the research conducted thus far the methods used herein must be adapted to use in an operational context and the primary focus of any further work would be sufficient validation of the methods to allow the use of the results in a legal context. Such work would be quantitative and necessitate the use of internal standards so that limits of detection, recovery rates and reproducibility could be determined with stated levels of statistical certainty. Swabbing methods and extraction conditions for both SPME and ATD also need to be optimised and one aim of any future research would be to develop sampling methods which could be easily adopted by Scenes of Crime Officers and Custody Sergeants and extraction methods which would not interfere with other forensic evidence.

One exciting area of further work is investigating the individuality of human chemical profiles which has great potential as a biometric marker and also as a rich source of intelligence regarding an individual's lifestyle (Prada, 2015, p102; Valussi and Brereton in Gray 2007). Such work would necessitate the development of improved methods for sampling the skin of individuals in a custody suite setting and identification of significant exogenous chemicals to identify lifestyle indicators and other individualising information such as: age (Haze *et al*., 2001), sex (Zeng *et al*., 1996b, Penn *et al*., 2007), diet (Havlicek and Lenochova, 2006, Mebazaa *et al*., 2011); medical conditions (Preti and Leyden, 2002) and even level of anxiety (Ackerl *et al*., 2002, Hauser *et al*., 2005). This would be a novel application of the work cited above as only Hauser *et al.* (2005) appear to have applied their findings in a forensic context. This work would include

development of an understanding of whether body odour chemicals will confound the forensic identification of specific personal care products or provide additional opportunities in terms of identifying individuals i.e. does the application of personal care products add to the individuality of a person's chemical profile or mute it?

The results from the aging study look particularly promising and more work should be done to investigate the effect of temperature, humidity and light intensity on such samples as there is obvious forensic potential in being able to provide a good estimate of the time since a fragrance product was applied. Finally, investigation of properties such as persistence and transfer should be a part of any future work on fragranced products. Ultimately fragrance evidence should be able to: provide intelligence about individuals; match articles with individuals (with a stated level of probability); provide an estimated time since an activity took place; and provide an estimate of the intensity of an activity (e.g. contact). To support this, samples would need to be identifiable, recoverable, transferable, and persistent and, ideally, to behave predictably over time.

The importance of the continuation of this work originates from extensive literature searches and discussions with representatives from the National Police Improvement Agency (Mallinson, 2011) and the Home Office Centre for Applied Science and Technology (Bleay, 2011) as well as perfumers (Dallimore, 2011), forensic scientists (Partridge, 2011) and retired police officers (Raper, 2011), which indicate that the ability to collect and use such samples as evidence or intelligence would be a novel and useful addition to the forensic 'toolbox'.

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A STUDY OF THE POTENTIAL EVIDENTIAL VALUE OF PERFUMES, ANTIPERSPIRANTS AND DEODORANTS IN FORENSIC SCIENCE

ALISON ROSS DAVIDSON

APPENDIX A

January 2017

List of perfumes used

Designation and name	Type (if known)	Company (if known)
Acqua di Gio	Eau de toilette	Giorgio Armani
CKin2U	Eau de toilette	Calvin Klein
Mariage	Eau de parfum	Givenchy
Ralph Rocks	Eau de toilette	Ralph Lauren
Youth Dew	Eau de parfum	Estee lauder
Elle	Eau de parfum	Emporio Armani
Pursuit	Eau de toilette	Dunhill
Unforgivable	Eau de toilette	Sean John
Code	Eau de parfum	Armani
Live	Eau de parfum	Jennifer Lopez
Essence de Femme	Eau de parfum	Hugo Boss
Rock n Rose	Eau de parfum	Valentino
Game	Eau de toilette	Davidoff
Floris		
Isabella	Eau de parfum	Isabella Rosselini
Cassis Rose	Eau de toilette	Body Shop
Eau Dynanisant		Clarins
Unspoken	Eau de cologne	Avon
Faubourg		St Honore
No. 4711	Eau de cologne	Blue and Gold
Mania	Eau de parfum	Giorgio Armani
L'Aimant	Parfum de toilette	Coty
Sun, Moon, Stars	Eau de toilette	Largerfeld
Jonjo	Eau de parfum	Liberty
Beautiful (7ml)	Esprit de parfum	Christian Dior
Poison	Eau de cologne	Christian Dior
Miss Dior	Eau de parfum	Givenchy
Ysatis	Eau de parfum	Giorgio Armani
Pleasures		
Beautiful	Eau de parfum	Estee Lauder
Urban for Men		
Daisy (pop art ed)		
Aphrodite	Body spray	Co-op
Lynx, Vice	Body spray deodorant	Lynx
Lynx, Africa	Body spray deodorant	Lynx
Avon Rare Gold	Body spray	Avon
Physio Sport	Body spray	
Manifique		
Hugo XX		
Lynx Twist	Body spray deodorant	
Vaseline Aloe Vera	Roll on deodorant	
Tea Tree Floral	Roll on deodorant	
DNA The Fragrance		

Perfumes used in initial evaluation

Rock n Rose

Cassis Rose

Mania

Ysatis

Urban for Men

Daisy (pop art ed)

Aphrodite

Lynx, Vice

Lynx, Africa

Avon Rare Gold

Physio Sport

Temperature Programs

No.	Program Name		Start Temp.	Hold Time (minutes)	Ramp Rate 1	Ramp Rate 2	Final Temp.	Total Time (minutes)	Date	Perfumes
1	Perfumes		60°C	1	6°C/min to 150°C	12°C/min to 280°C	280°C	27.00	Various	all
2	Perfumes x1		60°C	5	6°C/min to 150°C	12°C/min to 280°C	280°C	30.83	13.10.09	Avon Rare Gold Mariage
3	Perfumes x2		60°C	1	6°C/min to 150°C Hold 1 min	12°C/min to 280°C	280°C	27.83	13.10.09	Avon Rare Gold Mariage
4	Perfumes x3		60°C	1	6°C/min to 280°C	-	280°C	37.67	13.10.09	Avon Rare Gold Mariage
	Perfumes split 6								28.06.12	C8-C20 AStd Ysatis
5	Perfumes x4		60°C	1	12°C/min to 280°C	-	280°C	19.33	14.10.09 19.10.09	Avon Rare Gold Mariage
	Perfumes split 12								28.06.12	C8-C20 AStd Ysatis
6	Perfumes x5		60°C	1	6°C/min to 150°C Hold 5 min	12°C/min to 280°C	280°C	31.83	14.10.09 19.10.09	Avon Rare Gold Mariage
7	Perfumes x6		60°C	1	6°C/min to 150°C Hold 5 min	12°C/min to 280°C Hold 5 min	280°C	36.83	14.10.09	Avon Rare Gold Mariage
8	Perfumes x7		60°C	1	6°C/min to 150°C Hold 5 min	12°C/min to 200°C Hold 5 min	200°C	27.83	14.10.09	Avon Rare Gold Mariage
	Blank		50°C	1	10°C/min to 300°C Hold 5 min	-	300°C	31.00		

No.	Program Name	Start Temp.	Hold Time (minutes)	Ramp Rate 1	Ramp Rate 2	Ramp Rate 3	Final Temp.	Total Run Time (minutes)	Date	Perfumes
9	Perfumes x8	60°C	1	6°C/min to 150°C Hold 5 min	12°C/min to 200°C Hold 5 min	12°C/min to 280°C	280°C	36.83	19.10.09	Mariage
10	Perfumes x9	60°C	1	10°C/min to 280°C	-	-	280°C	23.00	19.10.09	Mariage
	Perfumes split 10								28.06.12	C8-C20 AStd Ysatis
11	Perfumes x10	60°C	1	10°C/min to 150°C Hold 5 min	10°C/min to 180°C Hold 5 min	10°C/min to 280°C	280°C	33.00	19.10.09	Mariage
12	Perfumes x11	40°C	5	6°C/min to 150°C Hold 5 min	6°C/min to 180°C Hold 5 min	6°C/min to 280°C	280°C	55.00	07.04.11	C8-C20 AStd Mariage Cassis Rose Rock 'n' Rose

No.	Program Name	Start Temp.	Hold Time (minutes)	Ramp Rate 1	Ramp Rate 2	Ramp Rate 3	Final Temp.	Total Run Time (minutes)	Date	Perfumes
13	Perfumes z1	40°C	5	6°C/min to 300°C	-	-	300°C	48.33	18.4.11	Daisy
14	Perfumes z2	50°C	5	6°C/min to 300°C	-	-	300°C	46.67	18.4.11	Daisy
15	Perfumes z3	60°C	5	6°C/min to 300°C	-	-	300°C	45.00	18.4.11	Daisy
16	Perfumes z4	60°C	1	6°C/min to 300°C	-	-	300°C	41.00	19.4.11	Daisy
17	Perfumes z5	60°C	1	6°C/min to 120°C Hold 5 min	6°C/min to 300°C	-	300°C	46.00	19.4.11	Daisy
18	Perfumes z6	60°C	1	6°C/min to 130°C Hold 5 min	6°C/min to 300°C	-	300°C		19.4.11	Daisy
19	Perfumes z7	60°C	1	6°C/min to 140°C Hold 5 min	6°C/min to 300°C	-	300°C		19.4.11	Daisy
20	Perfumes z8	60°C	1	6°C/min to 150°C Hold 5 min	6°C/min to 300°C	-	300°C		19.4.11	Daisy

No.	Program Name	Start Temp.	Hold Time (minutes)	Ramp Rate 1	Ramp Rate 2	Ramp Rate 3	Final Temp.	Total Run Time (mins)	Date	Perfumes
21	IFRA	100°C	2min	10°C/min to 280°C Hold 5 min	-	-	280°C	25.00	4.10.11	C8-C20 AStd Daisy Urban Aphrodite
22	SP001	45°C	2min	8°C/min to 100°C	20°C/min to 130°C Hold 3 min	25°C/min to 200°C	200°C	25.00	4.10.11	C8-C20 AStd Daisy Urban Aphrodite
23	Perfumes split 3 (After Adams)	60°C	????	3°C/min to 246°C			246°C	62.00	28.08.13	Daisy Urban Aphrodite Cassis Rose Rock 'n' Rose
									05.09.13	Ysatis Lynx Africa DNA The Fragrance

FTIR

Study 1 - Hugo XX – 0 to 4.5 hours

	Hugo XX Eau de Toilette, woman, Hugo, Hugo Boss. Sample vial UK:95746517	Filenames: Hugo XX Thur Mar 03 11-59-03 2011.spa etc.	Evaporation Time
03.03.11	Sample no.	Time Sample Taken	
	1	11.29	0
	2	11.59	30 mins
	3	12.29	1 hr
	4	13.00	1.5 hr
	5	13.59	2.5
	6	14.29	3 hr
	7	14.59	3.5
	8	15.59	4.5

Study 21 - Hugo XX – 0 to 30 minutes

Hugo XX Eau de Toilette, woman, Hugo, Hugo Boss. Sample vial UK:95746517		Filenames: Hugo XX Thur Mar 24 11-09-50 2011.spa etc.	Evaporation Time
	Sample no.	Time Sample Taken	
24.03.11	1	11.09	0
	2	11.12	2
	3	11.14	3
	4	11.16	4
	5	11.19	5
	6	11.22	6
	7	11.24	7
	8	11.29	8
	9	11.32	9
	10	11.34	10

Items of clothing and bedding used in garment sampling

Donor	Type	Fabric (if known)	Designation
Subject 1 (F)	polo	cotton mix	Garment 1
	purple jumper	Wool mix	Garment 2
	blue jumper	Cotton mix	Garment 3
	Stripy polo	Cotton mix	Garment 4
	Black jumper	wool mix	Garment 5
Subject 2 (M)	T shirt	Cotton mix	Garment 6
Family group 1	pillowcase	cotton	Garment 7
Subject 3 (F)	White T shirt	cotton	Garment 8
Subject 4 (M)	Black T shiry	cotton	Garment 8

A STUDY OF THE POTENTIAL EVIDENTIAL VALUE OF PERFUMES, ANTIPERSPIRANTS AND DEODORANTS IN FORENSIC SCIENCE

ALISON ROSS DAVIDSON

APPENDIX B

January 2017

Chromatograms for the 11 products used in the initial study

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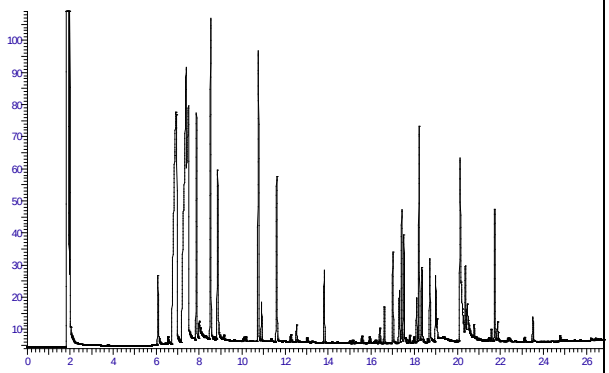
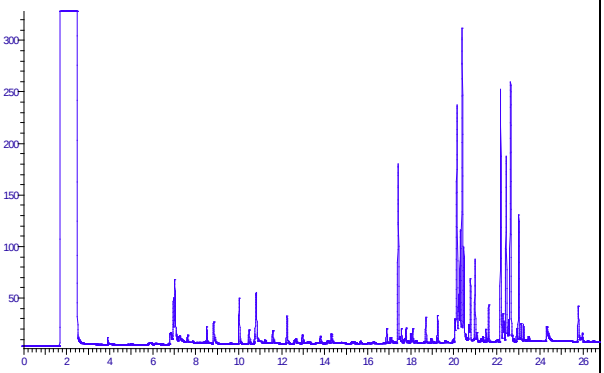


Avon RG sp 140411.raw



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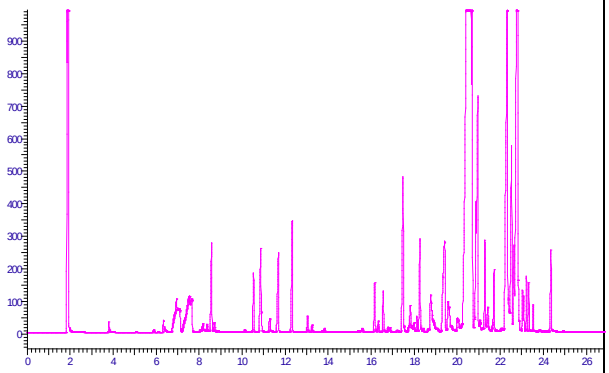

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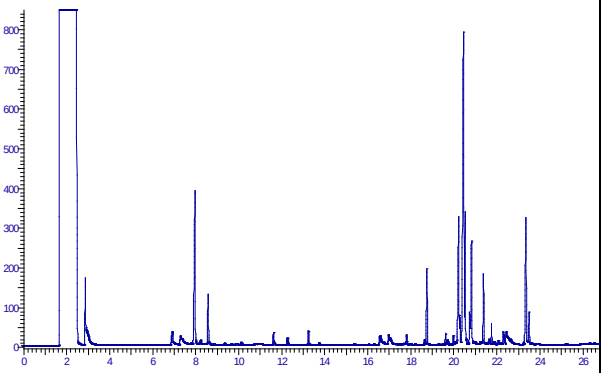

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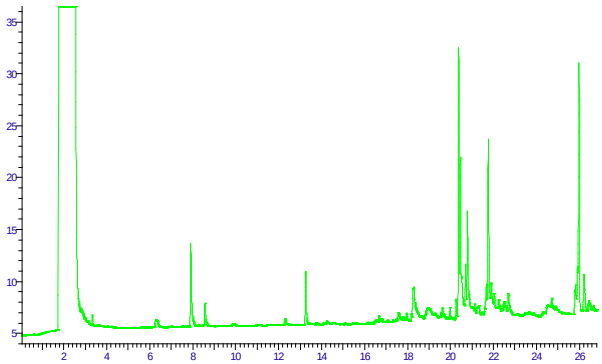

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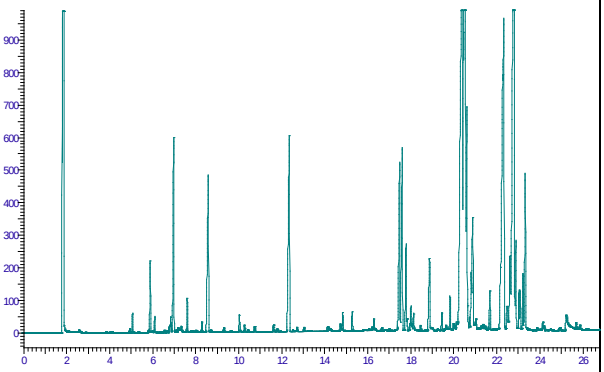
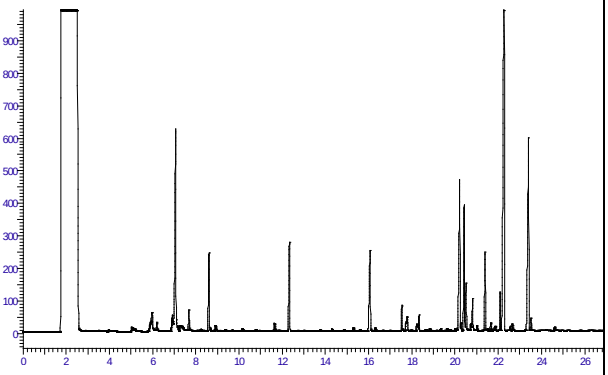
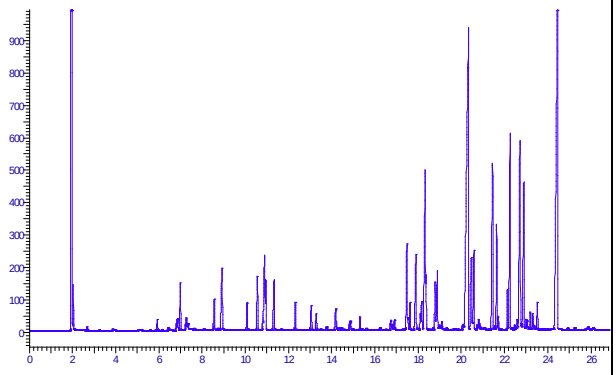


physio sport sp 140411.raw



rock n rose ns 140411.raw



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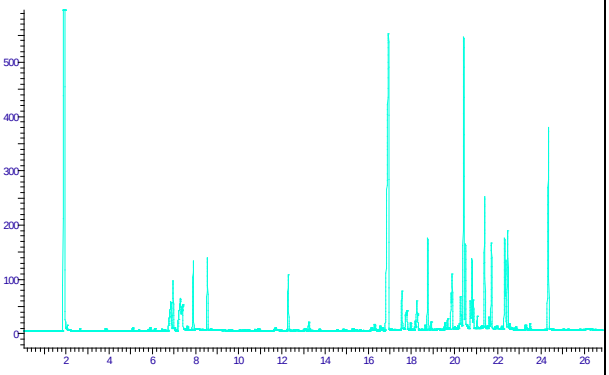

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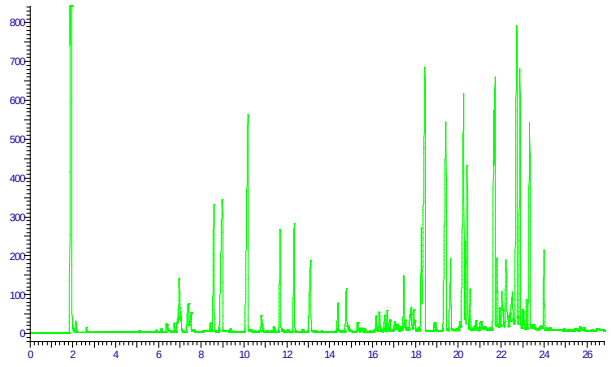

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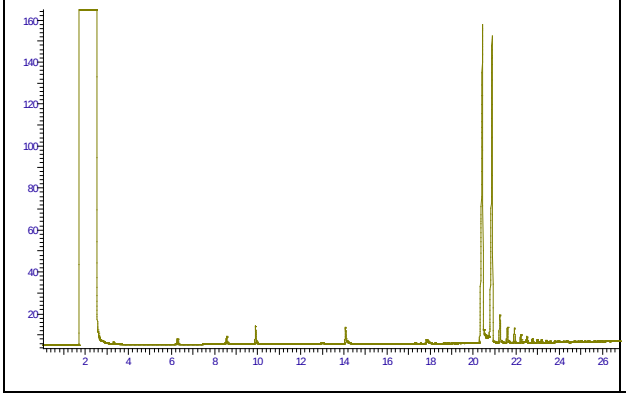
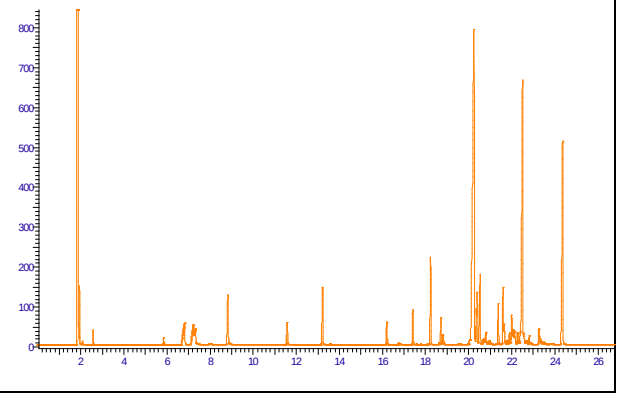


cassis rose ns 140411.raw



Peak list for spectrum of Hugo XX after 30 minutes evaporation time

Find Peaks - Hugo XX Thu Mar 03 11:59
Thu Mar 10 12:02:48 2011 (GMT+00:00)
FIND PEAKS:
Spectrum: **Hugo XX Thu Mar 03 11:59**:03 2011
Region: 4000.00 400.00
Absolute threshold: 96.578
Sensitivity: 70
Peak list:

Position	Intensity
565.21	89.905
667.50	93.256
700.10	86.390
729.21	90.775
757.92	87.771
811.82	90.134
832.39	89.539
930.44	85.365
1039.35	76.323
1088.30	75.582
1128.93	76.031
1158.20	68.569
1193.28	74.927
1248.24	68.380
1299.04	81.505
1339.16	84.047
1374.98	80.195
1456.73	77.605
1488.61	86.222
1505.34	90.919
1558.74	96.188
1604.48	92.470
1614.33	92.629
1634.82	93.321
1735.75	53.938
2860.34	80.516
2927.95	71.489
2953.63	76.901
3446.53	95.137

Spectra list for 17 perfumed products used in the part of the study

Product	Date	Time & note
Hugo XX	various	various
Cassis Rose	various	various
Pursuit	30.03.11	0 1 2
Essence de Femme	30.03.11	0 1 2
Rock n Rose	30.03.11	0 1 2
Mania	30.03.11	0 1 2
Ysatis	30.03.11	0 1 2
Urban for Men	30.03.11	0 1 2
Daisy (pop art ed)	30.03.11	0 1 2
Manifique	30.03.11	0 1 2
Aphrodite	31.08.11	0
		1
		2
		3
		4
		5
		10
		15
		20
		2hrs 20
Lynx, Vice	05.09.11	0
		3 (saved)
		5 (searched)

Product	Date	Time & note
Lynx, Africa	05.09.11	0 min
		3 (saved to library)
		5 (searched)
		2 hrs (poor quality)
Avon Rare Gold	05.09.11	0
		1
		3 (saved to library)
		8 (searched)
Lynx Twist	05.09.11	0
		3 saved
		5 searched
Vaseline Aloe Fresh	05.09.11	0
		1
		3 saved
		10 searched
Tea Tree Floral	05.09.11	0
		3 saved
		10 searched
		25

Discriminate Function Analysis (DFA)

Classification Results[b,c]

		VAR00022	Predicted Group Membership									Total
			1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	
Original	Count	1.00	3	0	0	0	0	0	0	0	0	3
		2.00	0	3	0	0	0	0	0	0	0	3
		3.00	0	0	3	0	0	0	0	0	0	3
		4.00	0	0	0	3	0	0	0	0	0	3
		5.00	0	0	0	0	3	0	0	0	0	3
		6.00	0	0	0	0	0	3	0	0	0	3
		7.00	0	0	0	0	0	0	3	0	0	3
		8.00	0	0	0	0	0	0	0	3	0	3
		9.00	0	0	0	0	0	0	0	0	3	3
	%	1.00	100.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
		2.00	.0	100.0	.0	.0	.0	.0	.0	.0	.0	100.0
		3.00	.0	.0	100.0	.0	.0	.0	.0	.0	.0	100.0
		4.00	.0	.0	.0	100.0	.0	.0	.0	.0	.0	100.0
		5.00	.0	.0	.0	.0	100.0	.0	.0	.0	.0	100.0
		6.00	.0	.0	.0	.0	.0	100.0	.0	.0	.0	100.0
		7.00	.0	.0	.0	.0	.0	.0	100.0	.0	.0	100.0
		8.00	.0	.0	.0	.0	.0	.0	.0	100.0	.0	100.0
		9.00	.0	.0	.0	.0	.0	.0	.0	.0	100.0	100.0

Classification Results[b,c]

		VAR00022	Predicted Group Membership									Total
			1.00	2.00	3.00	4.00	5.00	6.00	7.00	8.00	9.00	
Cross-validated[a]	Count	1.00	3	0	0	0	0	0	0	0	0	3
		2.00	0	3	0	0	0	0	0	0	0	3
		3.00	0	0	3	0	0	0	0	0	0	3
		4.00	0	0	0	3	0	0	0	0	0	3
		5.00	0	0	0	0	3	0	0	0	0	3
		6.00	0	0	0	0	0	3	0	0	0	3
		7.00	0	0	0	0	0	0	3	0	0	3
		8.00	0	0	0	0	0	0	0	3	0	3
		9.00	0	0	0	0	0	0	0	0	3	3
	%	1.00	100.0	.0	.0	.0	.0	.0	.0	.0	.0	100.0
		2.00	.0	100.0	.0	.0	.0	.0	.0	.0	.0	100.0
		3.00	.0	.0	100.0	.0	.0	.0	.0	.0	.0	100.0
		4.00	.0	.0	.0	100.0	.0	.0	.0	.0	.0	100.0
		5.00	.0	.0	.0	.0	100.0	.0	.0	.0	.0	100.0
		6.00	.0	.0	.0	.0	.0	100.0	.0	.0	.0	100.0
		7.00	.0	.0	.0	.0	.0	.0	100.0	.0	.0	100.0
		8.00	.0	.0	.0	.0	.0	.0	.0	100.0	.0	100.0
		9.00	.0	.0	.0	.0	.0	.0	.0	.0	100.0	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 100.0% of original grouped cases correctly classified.

c. 100.0% of cross-validated grouped cases correctly classified.