DEDICATION

This work is dedicated to my son

Teshan

who is suffering from Duchene Muscular Dystrophy

A Comparative Study of Solvent Extraction, Soxhlet Extraction, Steam Distillation, Headspace Analysis and Headspace Solid Phase Microextraction for the Extraction of Volatile Terpenoid Compounds in the Curry Leaf Plant (*Murraya koenigii*)

by

Hogantharanni Govender

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requirements for the degree of
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Faculty of Science and Agriculture,
University of KwaZulu-Natal,
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As the candidate's supervisors we have approved this thesis for submission.

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ABSTRACT

A comparative study was undertaken of different extraction methods for the isolation of volatile organic compounds from *Murraya koenigii* (curry leaf plant). The techniques studied included the traditional methods of extraction, namely, Soxhlet and solvent extraction as well as steam distillation. The solvent–free extraction techniques of headspace analysis and headspace solid phase micro-extraction (HS-SPME) were also investigated. In the evaluation of SPME, two different fibre coatings, poly(dimethylsiloxane) and poly(acrylate), were compared. Preliminary work to determine the effect of extraction parameters, such as extraction time, was carried out.

The volatile oils in the fresh leaves of *Murraya koenigii* were isolated by the above-mentioned extraction methods and analysed by gas chromatography-mass spectrometry. The main aroma contributing compounds were identified by comparison of their retention times with those of standards and their mass spectra with those of known compounds contained in the National Institute of Science and Technology Standard Reference Database 1A (NIST 98).

The essential oil contained mainly terpenes: monoterpene and sesquiterpene hydrocarbons. The constituents were identified and only the five selected analytes of interest, α-pinene, β-pinene, α-phellandrene, β-caryophyllene and α-caryophyllene were quantified in three of the methods, namely solvent extraction, soxhlet extraction and steam distillation.

From the quantitative determination of the compounds of interest, steam distillation favoured the extraction of β-caryophyllene. The solvent and Soxhlet extractions showed no significant differences between the quantities obtained for α- and β-caryophyllene. The steam distillation and Soxhlet methods showed similar quantities of α-caryophyllene extracted. The extraction of the monoterpenes, α-pinene, β-pinene, and α-phellandrene, was favoured by the Soxhlet method of extraction. Quantification was difficult with HS-SPME and headspace analysis.

Headspace analysis proved effective in the detection of the very volatile analytes. Headspace-SPME combined with GC-MS was found to be suitable for the identification of both monoterpenes and sesquiterpenes of *M. koenigii*.

From this study, solvent extraction and Soxhlet extraction were found to be superior to the other methods studied for the characterisation and quantitation of the volatile organic compounds in essential oils of *Murraya koenigii.*

PREFACE

The experimental work described in this thesis was conducted in the School of Chemistry, University of KwaZulu-Natal, Durban, under the supervision of Professor B.S. Martincigh and Professor A. Kindness.

These studies represent original work by the author and have not been submitted in any other form to another university. Where use was made of the work of others it has been duly acknowledged in the text.

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to the following people for their contributory role in this study and to whom I am indebted to:

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My late father for being an inspiration in my life. My mother, brothers and sister for their constant moral support and encouragement.

Finally, my husband Thagaraj and my sons Teshan and Sherwyn Chester for their love and sacrifice. I thank both Thagaraj and Sherwyn for helping to take care of Teshan in my absence and most importantly, to Teshan, for his patience and understanding.

FACULTY OF SCIENCE AND AGRICULTURE

DECLARATION 1 - PLAGIARISM

I, Hogantharanni Govender, declare that

1. The research reported in this thesis, except where otherwise indicated, is my original research.

2. This thesis has not been submitted for any degree or examination at any other university.

3. This thesis does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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   a. Their words have been re-written but the general information attributed to them has been referenced.
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5. This thesis does not contain text, graphics or tables copied and pasted from the Internet, unless specifically acknowledged, and the source being detailed in the thesis and in the References sections.

Signed

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ABBREVIATIONS

GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
HS–SPME	headspace solid phase microextraction
HSA	headspace analysis
MAHD	microwave-assisted hydrodistillation
MASD	microwave-assisted steam distillation
MS	mass spectrometry
NIST	National Institute of Standards and Technology
PA	polyacrylate
PDMS	polydimethylsiloxane
mg L^{-1}	parts per million
RT	room temperature
SBSE	stir bar sorptive extraction
SD	steam distillation
SFE	supercritical fluid extraction
SPE	solid phase extraction
SPME	solid phase microextraction
VOCs	volatile organic compounds

CHAPTER 1

INTRODUCTION

In this work different extraction methods for volatile organic compounds, namely terpenes, in *Murraya koenigii* (curry leaves) were studied. Headspace analysis, headspace solid phase microextraction, Soxhlet and solvent extraction, as well as steam distillation, were used to isolate terpenes from fresh curry leaves.

The use of *Murraya koenigii* (*M. koenigii*), containing terpene compounds, in flavouring foods has become common practice in South Africa. High temperature extraction of herbs is of interest, because extraction around 100 ºC is similar to the extraction of the aroma contributing compounds during the cooking process. Thus the identity (and possibly quantity) of the compounds extracted with steam distillation may closely resemble that which is present in a cooked meal (Yang *et al*., 2007).

The aim of this project was the comparison of the five extraction methods for the determination of volatile organic compounds. The different extraction methods were compared in order to determine the relative sensitivities, reproducibility and efficiency of these techniques for the analysis of volatile organic compounds in essential oils. Optimization of the extraction conditions was also investigated. The work involved identifying the methods' strengths and weaknesses by ensuring that the methods which were studied are reproducible, time effective and cost effective under the given conditions. This study also involved the identification and characterization of the aroma contributing components present in the essential oil of *M. koenigii* obtained from the different extraction techniques.

In general, a sample preparation method should have the following analytical performance characteristics: It should be efficient, selective, applicable to various compounds and matrices, allow for simple automated use and field analysis, easy to use, inexpensive, compatible with a large number of analytical instruments, fast, use a minimum amount of solvent or be solvent-free and have few steps (Steffen and

Pawlisyn, 1996).

This thesis consists of four chapters. Chapter 1 contains a general introduction and the purpose of the study. It also introduces the different techniques employed in this work. The techniques involved include headspace analysis (HSA), headspace solid phase microextraction (HS-SPME) with two different fibre coatings, polydimethylsiloxane (PDMS) and polyacrylate (PA); Soxhlet (SOX) and solvent extraction (SE), as well as steam distillation (SD). Chapter 2 gives details of the experimental procedures undertaken in this study. In Chapter 3 the results obtained from this study and their discussion is presented. Chapter 4 provides a conclusion of the work undertaken.

1.1 Curry leaf, *Murraya koenigii*

The genus *Murraya*, named after John Andrew Murray, which in turn belongs to the plant family *Rutaceace* (Bailey, 1958), consists of 11 species (Ranade *et al*., 2006). This genus of small trees is found throughout tropical and subtropical Asia from China and India to New Caledonia and North-eastern Australia (Li *et al*., 1988). It is also found in various areas throughout South Africa, for example, Phoenix, Chatsworth and Reservoir Hills. The curry leaf is a small tree with small white flowers (Figure 1.1) and spice-scented leaves (Figure 1.2) and fruit.

Figure 1.1 Curry leaf tree with small white flowers (http://www.plantoftheweek.org/week129.shtml).

Figure 1.2 Spice scented leaves
(http://www.healthy-lifestyle.most-effective-solution.com/wp-content/uploads/2007/04/curry-leaf.jpg).

1.1.1 Societal value

The curry leaf has been used in Asian-Indian cooking for its characteristic flavour and aroma (Fiebig *et al*., 1985). Greater interest has been generated in the use of the plant since its antioxidant and anti-carcinogenic potential has been reported (Palaniswamy *et al*., 2002).

This plant has important medicinal properties. The leaves have been shown to be effective for the control of dysentery, diarrhoea and vomiting (Rahman and Gray, 2005; Walde *et al*., 2006). The extracts of the bark and leaves have been utilised for poisonous animal bites (Aman, 1996). Fruit juice has been administered to patients with kidney conditions and the leaf extracts have been used effectively against fungi which cause ringworm (Walde e*t al*., 2006). *M. koenigii* has been used in Ayurveda (the traditional Indian herbal medicine system) in the West Indies and parts of Asia due to its hypoglycaemic activity (Bhattacharyya and Chakraborty, 1984). The plant has also been used in the system of indigenous medicine (Ranade *et al*., 2006). This species

has been included for the treatment of ailments such as rheumatism and for analgesia (Adesina *et al*., 1988). Malmuthuge *et al*. (2007) showed in their work that the use of *M. koenigii* decreased the blood cholesterol level of mice.

1.1.2 Phytochemistry

Indian workers have carried out a number of studies on the leaf composition and qualities of *M. koenigii* which have been reported in literature (MacLeod and Pieris, 1982; Paranagama *et al*., 2002). MacLeod and Pieris (1982) reported that a study undertaken earlier by Mitra obtained ca. 2.6 % of essential oil by means of steam distillation and Prakash and Natarajan (1974) identified the presence of caryophyllene (Figure 1.3), α-pinene (Figure 1.4) and β-pinene (Figure 1.5) in the volatile oil.

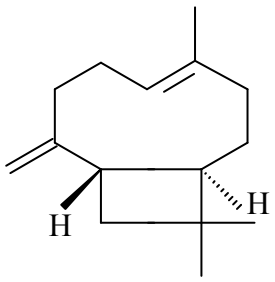


Figure 1.3 The structure of β- caryophyllene.

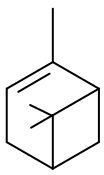


Figure 1.4 The structure of α-pinene.

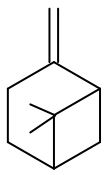


Figure 1.5 The structure of β-pinene.

According to Bhattacharyya and Chakraborty (1984), *M. koenigii* was known to be the richest source of carbazole alkaloids reported. Mukherjee *et al.* (1983) isolated mukonicine, a carbazole alkaloid, from the leaves of *M. koenigii*. Studies on other parts of the plant have been done mostly on trees growing in India (Reisch *et al*., 1994). In the root extract of the curry leaf plant, murrastifoline-F was found to exist as a 56:44 mixture in favour of the *M*-enantiomer (Bringmann *et al*., 2001).

Some physical properties of selected compounds investigated in this study, including the internal standard, dodecane are shown in Table 1.1.

Table 1.1 Some physical properties of selected compounds and the internal standard.

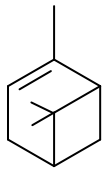
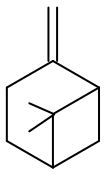
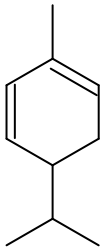
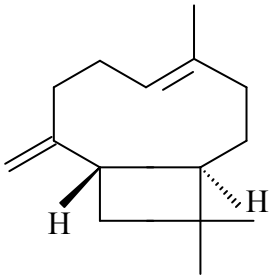
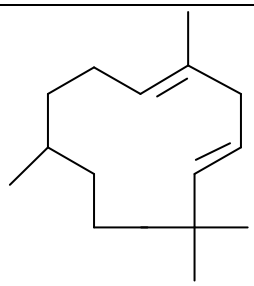
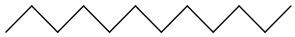
Compound	**Molar mass/g mol^{-1}**	**Boiling point/°C**	**Density/ g mL^{-1}**	**Structure**
α-pinene	136	154-156	0.858	
β-pinene	136	164-169	0.859	
α-phellandrene	136	171-174	0.840	
β-caryophyllene	204	262-264	0.902	

Table 1.1 Contd. Some physical properties of selected compounds and the internal standard.

Compound	Molar mass/g mol^{-1}	Boiling point/°C	Density/ g mL^{-1}	Structure
α-caryophyllene	204	266-268	0.889	
Dodecane	170	215-217	0.748	

1.1.3 Compounds that give rise to aroma

Compounds that contribute to the aroma of the *Murraya* species are found mainly in the volatile fraction designated the essential oils. The fresh leaves of *M. koenigii* containing these aroma compounds are an important ingredient in Indian food thereby imparting a flavour to the food. The essential oil composition is made up of mixtures of volatile substances: terpenes, sesquiterpenes and oxygenated derivatives which are usually present in low concentrations (Deng *et al*., 2006; Diaz-Maroto *et al*., 2002). The aroma is determined by those compounds which are released into the surrounding atmosphere, due to their vapour pressure. Thus, this gas phase composition can be characterized by using headspace gas chromatography. However, an important consideration is that the aroma producing compound can be present in concentrations near or well below the limit of gas chromatographic detection (Kolb, 1982).

Since the constituent compounds are volatile, the essential oils can be analysed by gas chromatography and gas chromatography-mass spectrometry (GC-MS). However,

since there is very little structural difference between the compounds, the mass spectra obtained from the GC-MS are similar, which makes identification of the compounds difficult (Oprean *et al*., 2001). Studies on the composition of the essential oils have been done which has led to the identification of the key aroma contributing components (Clery, 2006). Different blends of compounds are responsible for the production of an aroma, e.g. some common terpenes found in *M. koenigii* leaves are also found in dried fruits of *Xylopia aethiopica* and their aroma qualities, as reported in the work of Tairu *et al*. (1999), are listed in Table 1.2.

Table 1.2 Aroma qualities of some terpenes found in dried fruits of *Xylopia aethiopica* (Tairu *et al*., 1999).

Aroma Compound	**Aroma Quality**
α-thujene	sweet, terpeny
α-pinene	terpeny
camphene	spicy, fruity
β-pinene	terpeny
α-phellandrene	light minty
limonene	light, lemon-like
trans-β-ocimene	flowery
β-phellandrene	terpeny
α-farnesene	sweet, flowery
sabinene	terpeny

1.1.4 The biogenesis of terpenes in plants

The terpenes are the most diverse group of plant secondary products (King *et al*., 2004). Experimental studies have been performed on the biosynthesis within these organisms, and the structure and configuration of some major terpenes has been established (Bernfeld, 1967).

The terpenoids, which belong to a large and diverse group of natural products, are formed from the formation of C_5 (isoprene) units (Figure 1.6). Leopold Ruzicka proposed the *isoprene rule*: joining of isoprene units linked together in a head-to-tail manner (Sarker and Nahar, 2007). They are modified further by cyclization reactions and specific re-arrangements involving oxidation, reduction and hydroxylation.

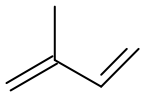


Figure 1.6 The structure of isoprene from which terpenoids are formed.

Although isoprene occurs naturally, in rubber as well as in plant and animal sources (Morrison and Boyd, 1987), it is the biochemically active isoprene units formed from acetate metabolism by way of mevalonic acid (MVA) and identified as the diphosphate (pyrophosphate) esters, dimethylallyl diphosphate (DMAPP) and isopentenyl diphosphate (IPP), that are involved in the formation of these compounds (Figure 1.7) (Dewick, 1997). Typical structures, shown in Figure 1.7, contain carbon skeletons, with the monoterpenes represented by C_{10} and the sesquiterpenes represented by C_{15} (Haagen-Smit, 1953).

1.1.4.1 Biogenesis of monoterpenes

Ruzicka suggested a series of ionic mechanisms for the formation of cyclic monoterpenes from geraniol (or linalool or myrcene) or a radical mechanism for cyclization of the hydrocarbons ocimene or myrcene. The basis for these suggestions was the cyclization of an acyclic precursor to a cyclic form which, by re-arrangements, forms more complex cyclic terpenes. According to Gascoigne (Bernfeld, 1967), the bicyclic monoterpenes are likely to be formed in a single reaction from an acyclic prototype rather than from a monocyclic hydrocarbon. In Croteau *et al.* (1986), it was reported that relevant model systems for the biogenesis of the bicyclic monoterpenes have not, to date, been realised.

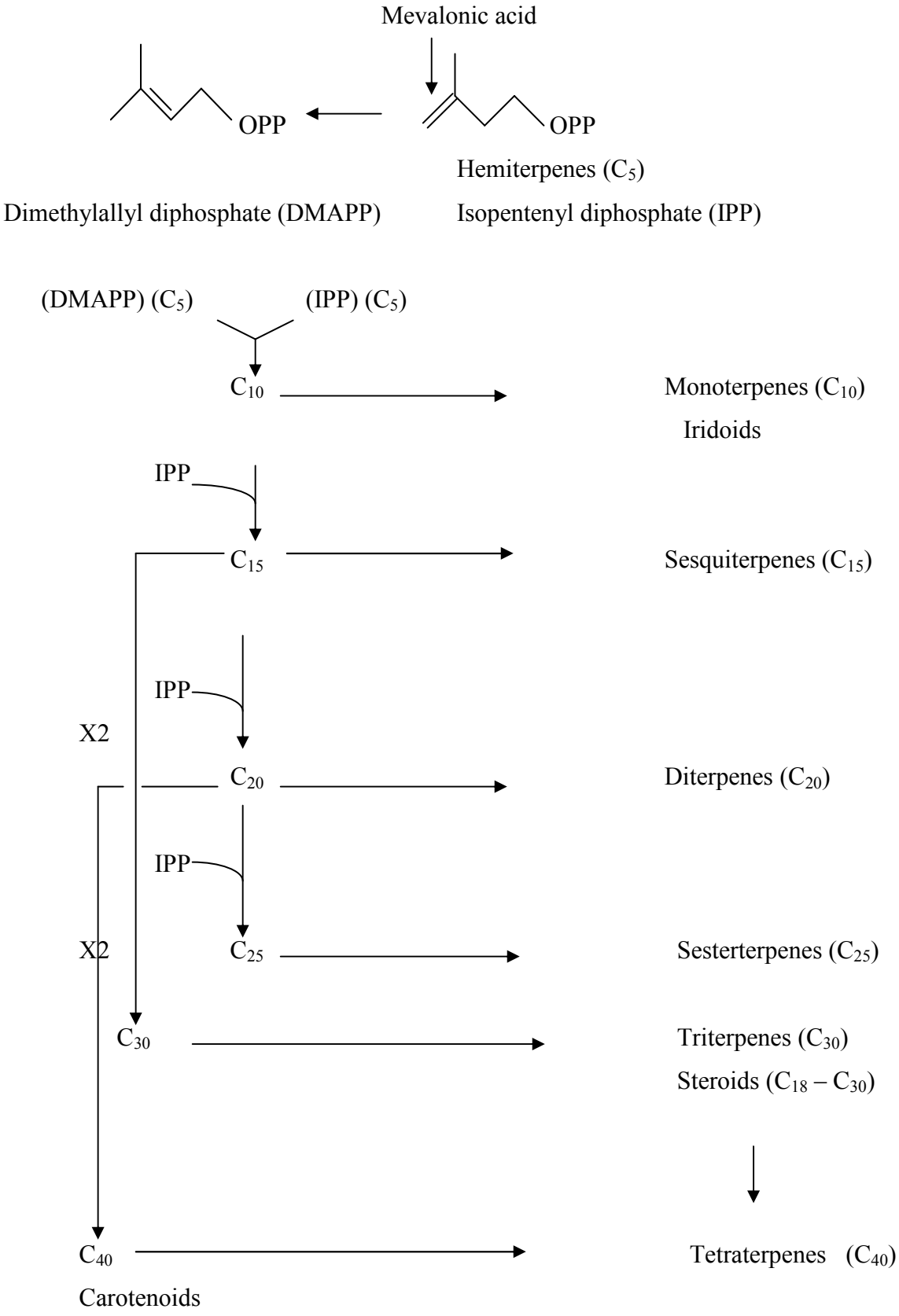


Figure 1.7 The mevalonate pathway (Dewick, 1997).

1.1.4.2 Biogenesis of sesquiterpenes

This group of compounds, may occur in acyclic, bicyclic and tricyclic forms and can be divided into three isopentane units connected head-to-tail (Bernfeld, 1967). One such example of these terpenes is gurjunene (Figure 1.8). Farnesol serves as a precursor for a large number of sesquiterpenes (Bernfeld, 1967).

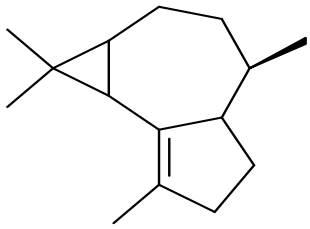


Figure 1.8 The structure of gurjunene.

1.2 The determination of volatile organic compounds in *M. koenigii*

A classical technique for the determination of essential oils and aromas from plants or spices utilises two steps: extraction (steam distillation, hydro-distillation, simultaneous distillation–extraction) and analysis (gas chromatography (GC) or GC-MS). The extraction step can last for up to several hours, while the analysis step is usually short (Chemat *et al*., 2006).

Various extraction techniques have been reported for the extraction of essential oils from curry leaves (Li *et al*., 1988; MacLeod and Peiris, 1982; Paranagama *et al*., 2002; Wong and Tie, 1993). In the study undertaken by MacLeod and Pieris (1982), the leaves (100 g) from Sri Lanka were chopped, mixed with 500 mL of water and extracted for four hours in a modified Likens and Nickerson distillation-extraction apparatus with trichlorofluoromethane as the extracting solvent. The main aroma contributing

constituents of *M. koenigii*, namely, β-caryophyllene, β-gurjunene, β-elemene, β-phellandrene and β-thujene, were identified by GC-MS.

The essential oil from *M. koenigii* found in Malaysia was extracted by means of a conventional steam distillation technique by Wong and Tie (1993), and analysed by GC-MS. In their work, fresh whole leaves were steam distilled for four hours. This was followed by extraction of the steam distillate with dichloromethane. After solvent removal, 0.23% (m/m) of light yellow oil with an aroma characteristic of the leaves was obtained. Sixty-two components were identified in the oil. The major constituents identified by GC-MS were α-pinene (17.5%), β-phellandrene (24.4%), β-caryophyllene (7.3%) and terpinen-4-ol (6.1%).

In the work carried out by Li *et al.* (1988), the samples from China were extracted by concurrent steam distillation-solvent extraction for three hours in a Likens and Nickerson apparatus with diethyl ether as the solvent. The oil was analysed by GC-MS and showed α-pinene to be the most abundant compound present.

Paranagama *et al.* (2002) isolated the volatile oil in the fresh leaves of *M. koenigii* grown in Sri Lanka. They used a method similar to that of MacLeod and Pieris (1982), described above, but used isopentane (20 mL) instead of trichlorofluoromethane to trap the volatiles. The extract was concentrated to 2.0 mL, dried over anhydrous sodium sulfate and evaporated to almost dryness. Individual constituents were identified by GC-MS. The terpenoid profile of the oil obtained from this study was reported to be different from the earlier study of MacLeod and Pieris (1982), which showed the presence of 16% monoterpenes and 80% sesquiterpenes. They found 44.6% monoterpene and 37.3% sesquiterpene hydrocarbons.

The monoterpene hydrocarbons have been widely studied whilst the study of the sesquiterpenes has been hindered by separation problems since most plants have complex matrices which produce compounds with similar mass spectra (Andersen and Falcone, 1969). Therefore, the need for successful separation prior to the analytical determination arises when dealing with complex mixtures (Morrison and Freiser, 1957).

Even though GC-MS can be used in the interpretation of the plant aroma profile, the problem of extraction and concentration of the aroma constituents, before GC analysis, has not been resolved (Guerrero *et al*., 2006).

1.3 Sample preparation

'The goal of any sample preparation step is to yield the target analytes in a form and concentration that can be readily analyzed' (Wang, 1997). The factors that should be taken into account to achieve this goal are determined by the nature of the sample, including its matrix, the information required, the time available for the analysis as well as qualitative and quantitative factors (Jennings and Rapp, 1983).

The main goal of sample preparation procedures is the isolation of the analytes (Romanik *et al*., 2007). Each step in the procedure can result in the loss of analyte, which becomes important when the amounts of isolated substances are present in small quantities. The procedure for the determination of compounds in plants includes three steps: the preliminary sample preparation, followed by the extraction of the target analytes and finally the analyte enrichment step.

The preliminary sample preparation requires collection and homogenization of the plant material. The collection of representative plant material is problematic due to variability of individual plants among a species or variety. The analyte enrichment stage involves the use of techniques such as steam distillation, solvent and Soxhlet extraction, as well as headspace and headspace microextraction.

Essential oils are a collection of volatile compounds that gives the distinct aroma. The objective of this study was to extract all the volatile compounds from the leaves of *M. koenigii*, without losing them, while at the same time eliminating all the non-volatile compounds. A large amount of research has been carried out on the study of volatile organic compounds from complex matrices.

According to Mamede and Pastore (2006), when the volatile fraction is to be studied, it is usually necessary to combine different extraction methods to obtain a total extraction profile of the volatile analytes, which would be a true representation of the sample aroma. Mamede and Pastore (2006) reported that several extraction methods, each with its own advantages and limitations, have been developed and used. However, as reported by Gomez and Witte (2001), many of these methods require long extraction periods and a sufficient quantity (in grams) of sample. Ortega-Heras *et al.* (2002) also found that different extraction methods were complementary for the characterisation of aroma composition.

1.4 Extraction techniques used in this study

The different techniques employed for the extraction of the volatile compounds in the essential oils of the leaves of *M. koenigii* are discussed in Sections 1.4.1 to 1.4.2.4.

1.4.1 Solvent extraction

Solvent extraction is an important part of an analysis and is widely used in the study of natural products. The method is used to isolate the analyte from any interferences that may be present with the analyte (Hargis, 1988). In this technique, the volatile analytes are extracted from an aqueous matrix into an organic solvent. The basic principle of this method involves the partitioning of the solute between an organic phase and an aqueous phase. Due to the immiscibility of the two phases, two layers are formed, with the denser layer settling at the bottom. After the extraction process, the solute is present in both phases (Harvey, 2000). The correct choice of solvent concentrates the analyte preferentially in one phase, thereby improving the extraction efficiency of the method.

The solvent used in this method of extraction needs to meet the following requirements:

i) It must have a low solubility in water.

ii) It has to have the chemical properties that will induce the analytes to have a greater affinity for the extracting solvent than water.

iii) The volatility of the extraction solvent must be taken into account if an additional concentration step is required (Grob, 1995a).

A disadvantage of this technique is that it is non-discriminatory, i.e. all compounds, the volatiles, semi-volatiles and non-volatiles, are extracted during the same extraction step. Also, during the evaporation step, loss of the highly volatile compounds can take place (Diaz-Maroto *et al*., 2002).

1.4.2 Soxhlet extraction

This is one of the traditional methods used for the isolation of metabolites from plant material. Analytes that have medium and low volatility (which may play a role for the aroma and quality of oil extracted from the plant material) are extracted with this technique (Romanik *et al*., 2007). The correct choice of solvent is important in order to obtain a good yield from the extraction as well as to prevent the loss of volatiles. The solvent used with this method is indicative of the polarity of the compounds extracted. This technique is a continuous extraction process and is described in Chapter 2, Section 2.4.2.

The extraction is usually carried out for a long period. The disadvantage of this technique is that, due to the long heating period, the analytes are exposed to high temperatures, which may lead to thermal degradation of some compounds (Grob, 1995a). The recovered sample is dilute and has to be concentrated further (Jennings and Shibamoto, 1980), by evaporation. It is during this step that loss of volatiles can take place.

1.4.3 Steam distillation

Steam distillation involves passing a steady flow of steam through the sample material to isolate water-insoluble volatile compounds (Mann and Saunders, 1960). It has been widely used to isolate volatile compounds, such as essential oils, from plants (Romanik *et al*., 2007). The oil that is obtained usually has an odour characteristic of the original plant (Strietwieser *et al*., 1992). However, this method of extraction has limitations. Due to the extraction being carried out at high temperature (100 ºC), thermal decomposition of the substances may occur (Romanik *et al*., 2007). The monoterpenes have also been reported to be vulnerable to chemical changes when steam distillation is used and loss of the compounds with high volatility can take place during the evaporation step (Diaz-Maroto *et al*., 2002).

1.4.4 Gas phase extraction

In this sample preparation method, the analytes are partitioned into the gas phase. During this process, the non-volatile compounds are eliminated, simplifying the analysis, e.g. preventing contamination of the gas chromatographic column (Pawliszyn, 1997). Despite this technique being less time-consuming and solvent free when compared to solvent extraction and steam distillation, it still has limitations, such as lack of sensitivity when compared to liquid phase extraction.

This sample preparation method is classified further into headspace and supercritical fluid extraction methods (discussed in Section 1.5.1). The headspace sampling method has been widely used for the analysis of volatile compounds (Kolb and Ettre, 2006).

1.4.4.1 Headspace analysis

This solvent free sample preparation method has shown potential for the development of enrichment techniques for the analysis of volatile organic compounds (Tolgyessy and

Hrivnak, 2006). Headspace analysis combined with GC is an indirect method used to determine the volatile components in the vapour phase which are in equilibrium with the sample to be analysed. It is employed mainly for the determination of trace components in samples which cannot be handled by conventional GC analysis. It is used for samples which undergo decomposition when the sample is vaporized and for samples which form dissociation products during sampling (Hachenberg and Schmidt, 1986).

The headspace concentration of a solute is influenced by several factors. The amount of component *i* in the headspace is determined by its partial pressure, p_i. The partial pressure itself depends on the following:

i) the vapour pressure of the pure component, *i* (p_{io}),
ii) the concentration of *i* (x_i), and
iii) the activity coefficient of *i* in the sample to be analysed (y_i), which is given by equation (1.1)

$$p_i = x_i y_i p_{io} \tag{1.1}$$

Since the peak area (F_i) gives a measure of the amount of component *i* in the headspace and $F_i = (p_i)$, the peak area is then given by:

$$F_i = f(x_i, y_i, p_{io}) \tag{1.2}$$

The concentration of the analyte in the headspace is affected by temperature and equilibration time. Since the vapour pressure is temperature dependant, the precision of temperature control becomes important. Also, the time required to reach the vapour pressure equilibrium, which is dependent on the nature of the sample, needs to be taken into account (Jennings and Rapp, 1983).

There are two methods of headspace analysis: static and dynamic.

i) **Static headspace analysis**

In this method, a small volume of the sample is injected into the GC, after it has reached equilibrium with its headspace.

At equilibrium, the partition coefficient, *K*, is given by the following equation:

$$K = \frac{C_s}{C_g} \tag{1.3}$$

where C_s is the concentration of the analyte in the sample phase and C_g is the concentration of the analyte in the gas phase.

The gas phase (headspace) lies above the sample phase which contains the compounds of interest. Since the method is not an exhaustive extraction, except for very volatile gases, it lacks a concentrating effect and therefore the sensitivity obtained is low. This technique has applications in the analysis of volatile organic compounds (VOCs) in food, beverage, clinical, and frequently in field analysis (Pawliszyn, 1997a).

The prepared sample obtained from the headspace method must have the maximum concentration of volatile components present in the headspace, while at the same time, eliminating contaminants from other compounds in the sample matrix. By adjusting the temperature of the extraction process, the volatility of the analyte changes and this improves the transfer of volatile compounds from the sample material into the headspace of the flask. Equilibration times may vary due to the different physical characteristics of the sample matrix. This equilibration can be achieved quickly, if the flask is shaken or vibrated. By increasing the headspace sample size and temperature, the sensitivity of the method can be improved (Hachenberg and Schmidt, 1986).

ii) **Dynamic headspace**

This technique uses multiple processes. One such example is the purge and trap method. In the first step of this approach an inert carrier gas is bubbled through a liquid and removes the volatiles from the matrix. In the second step, the stripped volatiles are then collected quantitatively by using a cold or a sorbent trap. After adsorption onto a sorbent, the compounds are then desorbed by heating in the injection port of the GC. A disadvantage of this technique is carryover from a previous determination (Pillonel *et al*., 2002) which can lead to incorrect results.

1.4.4.2 Solid phase microextraction (SPME)

This technique, developed by Pawliszyn in 1989, has been used as an alternative to the dynamic headspace method as a sample pre-concentration technique before gas chromatographic analysis (Vichi *et al*., 2007). It was developed to overcome the limitations of solid phase extraction (SPE) and has been used for the analysis of polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pesticides and more recently for the analysis of a new group of organic pollutants, brominated flame retardants (Polo *et al*., 2006). This technique is environmentally-friendly because it is a solvent-free determination, i.e. it does not make use of any organic solvent (Guillen *et al*., 2004).

(For a more comprehensive study of this technique see Pawliszyn, 1997).

i) **Basic principles of SPME**

This method makes use of a simple apparatus, the SPME device, illustrated in Figure 1.9, for the extraction of the analytes from a sample. In this process, the coated fibre is exposed to the sample or its headspace. This is followed by partitioning of the analytes from the sample matrix into the fibre coating. Once equilibrium between the sample matrix and the fibre coating has been

established, the extraction is complete and the fibre containing the concentrated extract is thermally desorbed into an analytical instrument where separation and quantitation occurs (Pawliszyn, 1997b).

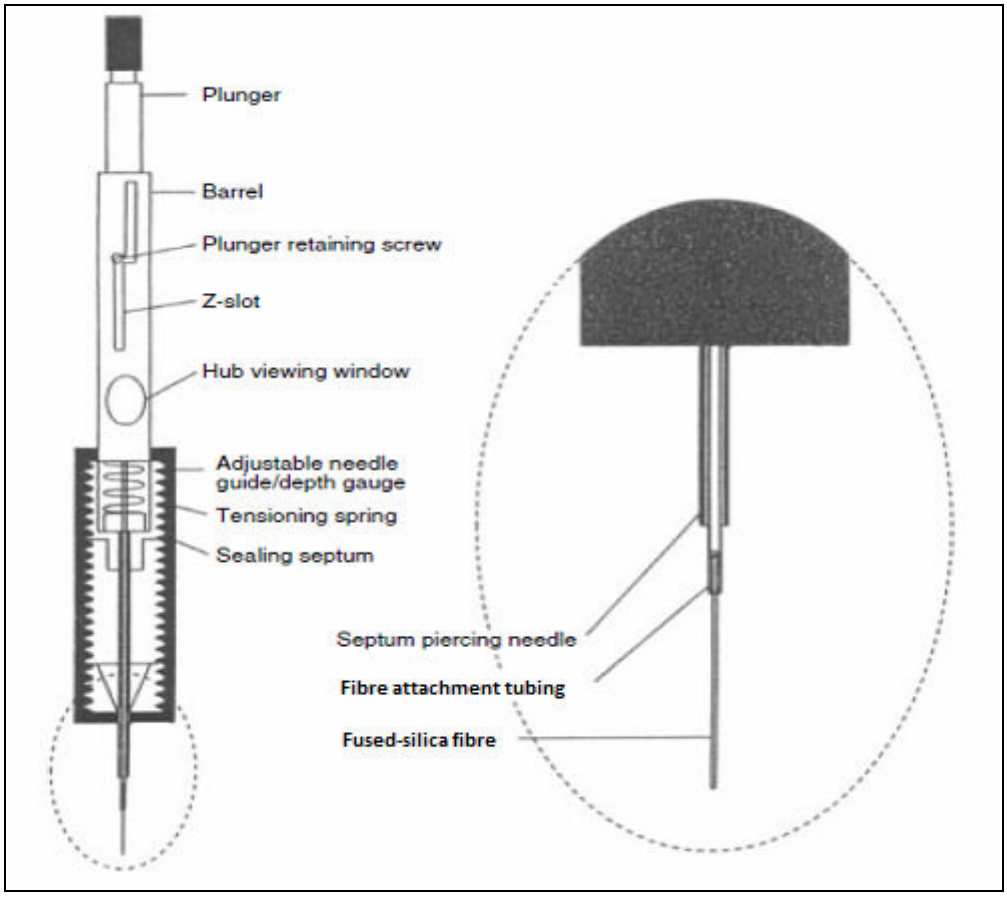


Figure 1.9 Schematic drawing of a solid phase microextraction device (Vas and Vekey, 2004).

The choice of the fibre coating can improve the selectivity of the analysis as a suitable stationary phase can be selected appropriate to the target analytes (Steffen and Pawliszyn, 1996). The selection of the appropriate fibre in an analysis is important since the type and quantity of compounds extracted from the sample is dependent on the properties of the fibre stationary phase and also on the film thickness. The extraction of the analyte into the fibre makes use of the principle of 'like dissolves in like' and there are specific coatings available for different applications (Garcia-Estabana *et al*., 2004).

Thus far, the most commonly used fibre coating for the analysis of nonpolar compounds is poly(dimethylsiloxane) with a thickness of 100 μm and for the analysis of polar compounds, polyacrylate with 85 μm thickness and carbowax/polyethylene glycol-divinylbenzene with a film thickness of 65 μm (Wang, 1997). The coating thickness of the fibre influences the quantity of the analyte extracted and the equilibration time is also affected which in turn is affected by temperature. However, by increasing the extraction temperature there is an an increase in the diffusion coefficient and a decrease in the distribution constant which results in more analyte being extracted and faster determinations (Pawliszyn, 1997c). An advantage of this method is that sampling, extraction and concentration can be performed in one step (Lompart *et al*., 1998).

ii) **SPME sampling**

There are three modes of SPME sampling: direct extraction, headspace SPME, and membrane-protected SPME as shown in Figure 1.10.

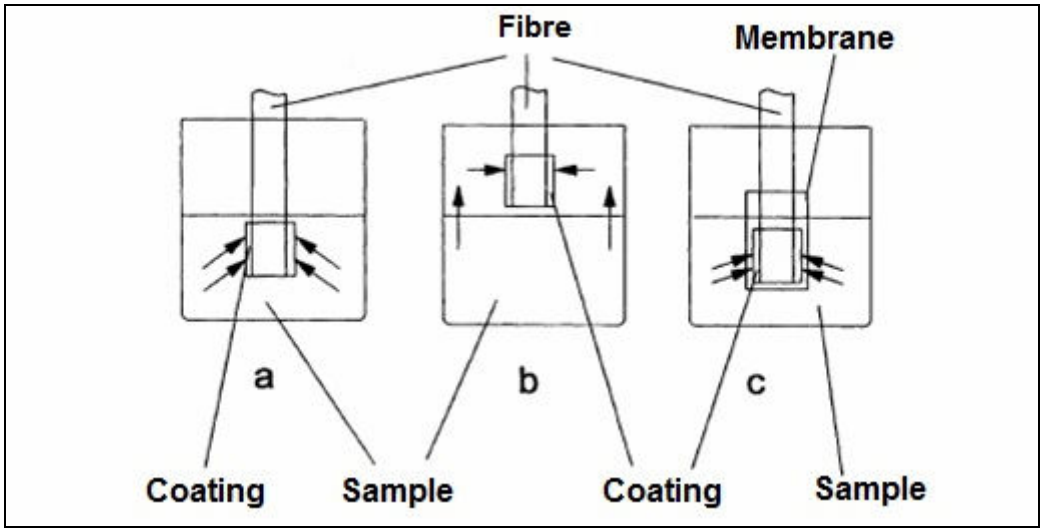


Figure 1.10 The three extraction modes of SPME: a) direct extraction, b) headspace SPME, (c) membrane-protected SPME (Pawlisyzn, 1997d).

Direct extraction

In this mode, there is direct insertion of the coated fibre into the gaseous or aqueous sample. This facilitates the transport of the analytes directly from the sample matrix into the extracting phase. Once equilibrium between the sample matrix and the fibre coating has been established, the extraction is complete and the partition coefficient can be defined as:

$$K_{fs} = \frac{C_f}{C_s} \tag{1.4}$$

where K_{fs} *i*s the partition coefficient, C_f is the equilibrium concentration of analyte in the fibre coating and C_s is the equilibrium concentration of analyte in the sample.

In the case of direct sampling, the mass of the analyte can be determined from equation (1.5) below:

$$n = \frac{K_{fs} V_f C_o V_s}{K_{fs} V_f + V_s} \tag{1.5}$$

where C_o *i*s the initial concentration of the analyte in the matrix and V_f and V_s are the volume of the coating and matrix, respectively.

Headspace mode

Since the analytes are transported through the air before they reach the coating, the fibre coating is protected from damage by high molar mass and other non-volatile interferences present. If the sample and headspace volume is kept constant, then the amount of analyte extracted into the coating using direct and headspace sampling are the same. This is due to the fact that the equilibrium

concentration is independent of fibre location in the sample/headspace system. If this condition is not satisfied, a significant difference between the direct and headspace methods exists only for very volatile analytes. This method makes provision for the modification of the sample matrix, such as change in pH (Pawliszyn, 1997d).

In the headspace mode, volatiles are extracted faster than semi-volatiles since they are at a higher concentration in the headspace. Equilibration times are shorter in headspace SPME than for direct extraction under similar conditions. Headspace SPME is applicable to medium to high volatiles and has applications in food and pharmaceuticals, environmental, as well as clinical and forensic studies. In the case of food quality control, HS-SPME was found to be the most common and easiest technique as reported by Bosch-Fuste *et al*. (2007).

Membrane-protected SPME

In this method of analysis, the fibre is is separated from the sample with a selective membrane, which allows the analytes to diffuse through. In this way, the fibre is protected by the membrane against damage, especially when dirty samples are used. However, the extraction is hindered as the analytes need to diffuse through the membrane before reaching the coating. This can be overcome by using thin membranes and increasing the extraction temperature. This method proves to be useful for the determination of analytes having low volatility.

iii) **Selection of an extraction mode**

When selecting an extraction mode the sample matrix, analyte volatility and its affinity for the matrix, need to be taken into consideration. Direct sampling can be done for clean matrices. The headspace should be selected for samples which

contain substances that can damage the fibre coating. As stated earlier, the quantity of analyte extracted into the coating from the same vial with the direct or headspace mode is the same as long as sample and gaseous headspace volumes are kept constant. The headspace is the preferred method for the high volatile analytes, because of faster equilibration times. The equilibration time of aqueous samples is determined by the effectiveness of the agitation technique (Pawliszyn, 1997d). For compounds with low volatility, the membrane-protected SPME method is found to be suitable.

1.5 Other extraction methods

A brief discussion on other extraction methods used for the isolation of volatile compounds, but not used in this work, follows in Sections 1.5.1 to 1.5.8.

1.5.1 Supercritical fluid extraction (SFE)

This method uses compressed carbon dioxide as an extracting phase to remove less volatile compounds at ambient temperature. In addition to CO_2 obtaining supercritical conditions readily, its properties, namely, non-toxic, non-flammable, chemically stable with no retention of solvent residue, makes it a suitable extracting phase. By regulating the pressure and temperature conditions, the dissolving power of supercritical fluids can be adjusted thus making SFE an alternative to conventional extraction procedures. The final composition of the extract will be affected by the extraction pressure and temperature range because the solubility of all the components in the fluid will depend on these parameters (Diaz-Maroto *et al*., 2002).

SFE can employ static, dynamic or static-dynamic modes of extraction. In the case of the **static** mode, a fixed amount of CO_2 is used to interact with the matrix in a sealed

vessel. When the **dynamic** extraction mode is used, a more exhaustive extraction occurs as fresh CO_2 is continuously pumped through the sample. The **static-dynamic** mode is used when CO_2 is required to diffuse through the matrix. The extractants are generally recovered in a cooled liquid solvent or by solid trapping and are analysed off-line via GC-MS or HPLC-MS (Jublot *et al*., 2004).

A supercritical fluid extraction of the volatile and semivolatile compounds from commercial cigarettes, undertaken by Xu and Zhang, (2004), showed that in addition to the working temperature of this technique being low, it uses less solvent and the extraction period is shorter. A disadvantage of CO_2 in SFE extraction is its low polarity which limits the dissolution of polar analytes. Although the compounds become difficult to extract, addition of polar modifiers such as methanol can overcome this (Cao *et al*., 2007). The elevated pressure as well as the high cost of the equipment used with this technique therefore disadvantages it as a method of choice.

1.5.2 Membrane extractions

This method of extraction consists of two processes: analytes from the sample matrix are extracted by the membrane material, and at the same time the stripping phase extracts the analytes from the membrane. In addition to volatile compounds, this method can also be used for the extraction of high molar mass compounds by using higher temperatures or micro-porous membranes with various pore diameters, and has been applied to the analysis of semi-volatile compounds by using a high pressure stripping gas (Pawlisyzn, 1997).

1.5.3 Sorbent extraction

This process involves using an adsorbent material, a sorbent, to extract and concentrate trace organic compounds from various matrices such as water, air and soil (Pawliszyn, 1997b).

1.5.4 Solid–phase extraction (SPE)

In this method, the compounds from an aqueous sample are extracted from a liquid phase, partitioned and/or adsorbed onto a stationary phase (sorbent). When a flat membrane (disk) is used, the liquid matrix is passed through the disk containing sorbent dispersed on a particulate support to extract analytes together with interfering compounds (Pawliszyn, 1997b). To allow extraction of different classes of compounds, the extracting phase is varied (Christian, 2000). For the reversed phase extraction of nonpolar or compounds with medium polarity such as caffeine, drugs and pesticides, C-18 (octadecylsilane) can be used.

The proper selection of solvent is dictated by the polarity of the target analyte of interest, i.e. whether it is polar or non-polar. This technique is used mainly for the extraction of semi-volatiles and non-volatiles from a liquid matrix. Hence it could not be used in this work since highly volatile components were extracted from a solid matrix.

1.5.5 Stir bar sorptive extraction

The mechanism of this technique is similar to SPME. To increase the rapid transfer of analytes to the polymer coating, a magnetic stir bar coated with polydimethylsiloxane (PDMS) is added to the sample. The analytes are desorbed in the GC injector once the extraction period is complete (Guerrero *et al*., 2006).

1.5.6 Microwave distillation-solid-phase microextraction

A study undertaken by Deng *et al*. (2006) on traditional Chinese medicines combined microwave distillation with SPME. The extraction, isolation and concentration of the oils was done in a single step, requiring little sample and no organic solvent and water.

1.5.7 Microwave-assisted solvent extraction

The application of microwave heating for the isolation of essential oils from plant material has generated interest. An advantage of this technique is the reduction of extraction time and reduced use of organic solvent (Deng *et al*., 2006).

1.5.8 Microwave accelerated steam distillation (MASD)

The extraction of the essential oil with this technique is a one step process which uses microwaves and steam distillation (SD). During this process, the essential oil is released from plant material and is then evaporated by steam. The extracted oils can be analyzed using GC-MS. Although the extraction temperature for SD and MASD is the same, 100 ºC, the yields obtained after a shorter extraction period are comparable to the yields obtained using SD (longer extraction time), thus indicating the rapidity of MASD (Chemat *et al*., 2006).

1.6 Gas chromatography

The mixture to be separated and analysed in this work is the essential oils from the fresh leaves of *M. koenigii*. Two criteria which are extremely important in any analysis are that the data must be accurate and precise and be obtained within the shortest period of time (Grob, 1995b). Since the components present in the essential oils cover a wide range of volatiles, the use of gas chromatography (GC) combined with a suitable

detector, such as a mass spectrometer, as an analytical technique would be appropriate. This combined GC-MS analytical technique has been used to obtain both qualitative and quantitative information and has been widely used in the analysis of volatile compounds.

1.6.1 The gas chromatographic system

In gas chromatography, a sample is vaporised and the components are separated as a result of partitioning that takes place between the mobile gas phase and the stationary phase. The mobile phase is referred to as the carrier gas. The stationary phase can be either a liquid or solid, packed in the column. The vaporised sample is injected into the column and the compounds are eluted with the mobile phase. A schematic illustration of a gas chromatograph is shown in Figure 1.11.

There are two types of gas chromatography: gas-liquid chromatography (GLC), commonly called gas chromatography and gas-solid chromatography (GSC) but only GLC and its aspects relevant to this study will be discussed.

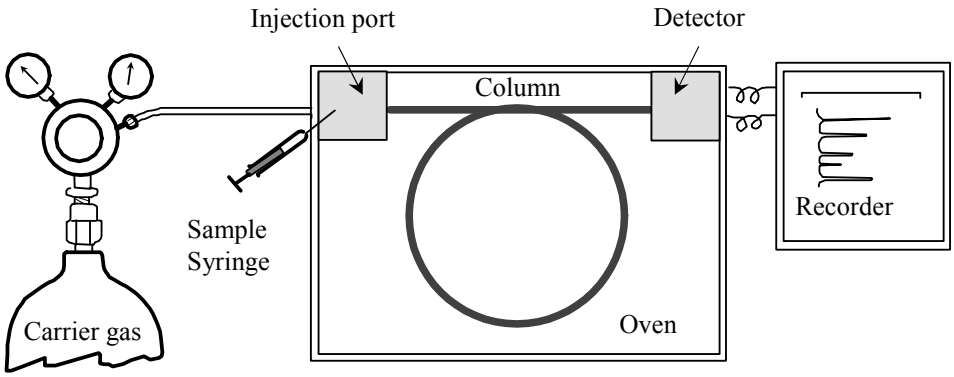


Figure 1.11 A schematic diagram of a gas chromatographic instrument and its components (Kindness, Practical Manual, Chem 340, University of KwaZulu-Natal, 2009).

1.6.1.1 Carrier gas

The most commonly used mobile-phase is helium for GC-MS, although argon, nitrogen and hydrogen can be used as well. Nitrogen, which is less expensive is generally used for GC. The carrier gas should be inert to prevent interaction with the sample. It should also be readily available, pure and inexpensive.

1.6.1.2 Sample injection

The sample size must be appropriate when injected as a "plug" of vapour. If too much sample is injected or it is injected slowly, band broadening can occur. With the aid of a microsyringe, the sample is injected through a rubber septum into a heated port situated at the head of the column.

To ensure that the sample is vaporised completely, the injector temperature is set at 50 ºC higher than the least boiling component. To prevent the sample from overloading the column, i.e by injecting a large sample size which can hinder column performance, capillary split and splitless injectors can be used. Once the sample is injected, it is mixed with the carrier gas in the injection chamber and the gas is purged. In the case of split injection, only a small amount of the sample is transported by the carrier gas and enters the column. By means of the splitless mode, a larger amount of the sample enters the column (http://en.wikipedia.org/wiki/Gas-liquid_chromatography).

1.6.1.3 Types of columns

There are two basic types of columns which have been used generally in gas chromatography: packed and capillary (open tubular) columns. The packed columns are made from glass or metal tubing and are 2 to 3 m in length. They are densely packed with a solid support which is coated with a thin layer of the stationary liquid phase. There are two types of capillary columns: wall-coated open tubular (WCOT),

constructed earlier of stainless steel, copper or plastic and later glass, with a thin layer of the stationary phase and support-coated open tubular (SCOT) columns, where the inner surface is lined with a thin film of support material such as diatomaceous earth onto which the stationary phase is absorbed. Currently, the most widely used capillary columns are the fused-silica open tubular (FSOT) columns. These columns are much thinner and give a better separation. Also, the separation of the FSOT column is greater than that of the WCOT, SCOT and the packed column. The FSOT column is a new type of WCOT column. In the WCOT column the walls are coated with a liquid stationary phase and in the FSOT column the walls, which are much thinner, are coated with polyimide. The FSOT column has a fused silca tube and a chemically bonded stationary phase which gives it added strength and flexibility (http://teaching.shu.ac.uk/hwb/chemistry/tutorials/chrom/gaschrm.htm).

The most common liquid stationary phase used for the separation of nonpolar phases, hydrocarbons and polynuclear aromatics is polydimethyl siloxane whilst 50% cyanopropyl-polydimethyl siloxane is used for the separation of polyunsaturated fatty acids, free acids and alcohols (Skoog *et al*., 2004). The separation of compounds is based on the boiling points of the different components. Compounds with low boiling points (the high volatile compounds) will pass through the column faster than the compounds with high boiling points (the compounds of low volatility) (http://orgchem.colorado.edu/hndbksupport/GC/GC.html).

1.6.1.4 Oven

Since the column temperature plays a key role in obtaining a good separation, the column is placed inside a thermostated oven. The boiling points of the solute govern the choice of the temperature program. Therefore, the temperature of the oven is set below that of the lowest boiling solute and thereafter increased uniformly (Harvey, 2000).

1.6.1.5 Detectors

In the selection of the suitable detector, several requirements need to be met. Some of these are:

i) The detector should display adequate sensitivity. Detectors which are currently available lie in the 10^{-8} to 10^{-15} g solute/s sensitivity range.

ii) It should afford good stability and reproducibility.

iii) A linear response should be obtained for solutes which are present over several orders of magnitude.

iv) It should have a similar response towards all solutes which are present, or a predictable and selective response towards one or more of the solutes belonging to the same class.

No single detector satisfies all these criteria. Although there are different types of detectors available, only two detectors will be discussed in this work: these are the flame ionization detector (discussed briefly) and the mass spectrometer (discussed in Section 1.7).

Flame ionization detector

This is the most widely used detector for gas chromatographic analysis. With this detector, the effluent from the column is pyrolyzed in an air/hydrogen flame. Organic compounds produce ions and electrons during this process and detection involves monitoring the current produced during the collection of these ions and electrons. A voltage applied between the burner tip and a collector electrode serves to collect the ions and electrons and the resulting current is then measured (McNair and Bonelli, 1968).

The use of the flame ionisation detector for the identification of the volatiles from the retention times of the compounds has long been in existence. However, this detector is limited in its use, as the sample is destroyed and no futher detection of the ions can be

done. A disadvantage of this detector is that it is not selective, i.e it responds to almost all organic compounds, producing peaks but does not tell us the nature of the compound. The identification of the compounds is based on the comparison of retention times with known compounds.

The greatest value of the retention information would serve as a complimentary criterion for the accurate identification of the compound. When the GC-MS produces similar mass spectra for the structurally related compounds, positive identification can be difficult. Thus, the retention times of the compounds can be used together with the mass spectra to positively identify the unknown compound. The sesquiterpene hydrocarbon is a good example of this. Once the compound has been identified as a sesquiterpene hydrocarbon, using the precise retention times from the use of an authentic sample, a more accurate identification of the sesquiterpene can be done (Jennings and Shibamoto, 1980).

1.7 Gas chromatography-mass spectrometry analysis

Gas chromatography when combined with mass spectrometry (GC-MS) is a powerful tool for the separation and structural elucidation of components of volatile mixtures (Jennings and Shibamoto, 1980). The mass spectrometer is a sensitive, universal detector and when combined with a gas chromatograph has been widely used for the analysis of foods, petroleum products and pharmaceuticals products.

1.7.1 Brief overview of the gas chromatograph-mass spectrometer

The essential features of a gas chromatograph-mass spectrometer are presented in Figure 1.12 and only certain aspects relevant to this study are discussed briefly in Sections 1.7.2 to 1.7.5.1.

After the sample is injected into the capillary GC and separated, the effluent from the GC enters the inlet of a quadrupole mass spectrometer. Once the sample is ionized in the ion source, the ions are propelled out of the chamber towards an exit slit by the application of a low positive potential. On exiting the ion chamber, the ions are accelerated through a high potential and passed into the analyser for separation according to their mass-to-charge ratio. A dynode electron multiplier is used to detect the ions. The amplified signals from the electron multiplier are passed to a computer which evaluates the incoming data and prints out the required information (Hoffmann *et al*., 1996).

The analysis of the data can be performed in different ways. In one approach, the ion abundances in each spectrum can be summed and plotted as a chart called the total ion chromatogram, with the ion current on the y-axis versus time on the x-axis. In another approach, a single ion can be selected and monitored and this is called selected ion-monitoring (Skoog *et al*., 2004). In this latter mode the mass spectrometer becomes a very selective detector and the chromatograms are greatly simplified.

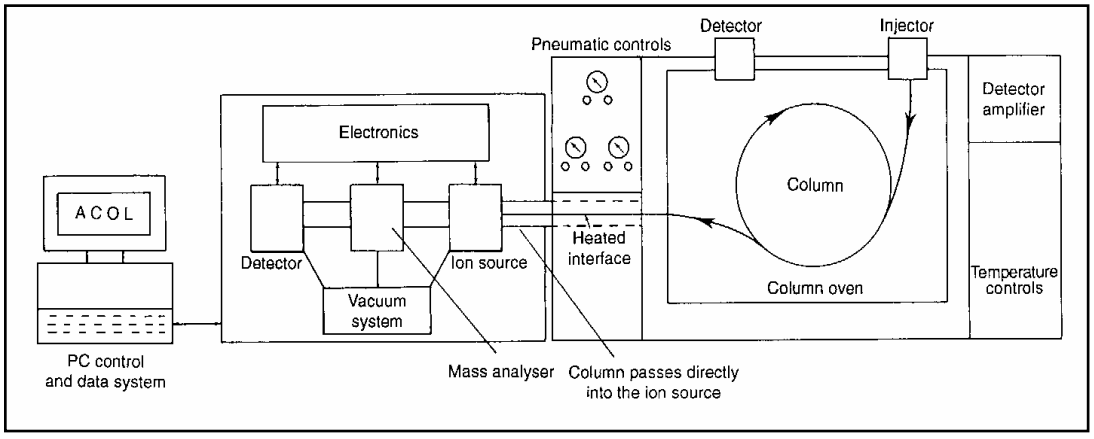


Figure 1.12 Schematic diagram of a typical capillary column gas chromatograph-mass spectrometer (Fowlis 1998).

1.7.2 Inlets

Sample introduction into the mass spectrometer is dependent on its phase (whether it is a gas, liquid, solid or solution) and the ionisation technique used. The effluent from the column of a gas chromatograph passes directly into the source of a mass spectrometer (Henderson, 2005b).

1.7.3 Ion sources

Detection using a mass spectrometer can be significantly affected by both the amount and the chemical nature of the compound. This is due, in part, to the ionization potential of the specific compound. The selected method of ionization depends on the type of analysis which is needed as well as on the type of compound. There are a number of methods for ionizing compounds, the commonest is electron impact (EI) (Johnstone, 1972).

The method used to ionize a substance affects the mass spectrum. The mass spectrum shows the *molecular ion* ($M^{+\bullet}$) resulting from the ionization of a molecule as well as the most intense peak in the mass spectrum, the base peak, which is assigned an intensity of 100% (Carey, 2007).

Although there are several ion sources available, only the two sources commonly used with GC-MS will be discussed briefly. These are the electron impact ion source, which was developed first and which is used commonly in most organic analyses, and the chemical ionization (CI) source.

1.7.3.1 Electron impact ion source

After the sample is injected into the capillary GC, the effluent enters the ion source where the molecules are ionized. The ion source consists of a heated filament giving off electrons which are accelerated towards the anode and collide with the gaseous analyte

molecules (Hoffmann *et al*., 1996). Once the sample is ionized in the ion source, the ions are propelled out of the chamber towards an exit slit by the application of a low positive potential. On exiting the ion chamber, the ions are accelerated through a high potential and passed into the analyzer. Some of the advantages of the use of this ionization source include its stability, lack of contamination problems and relatively high sensitivity, which makes it a popular choice (Chapman, 1993). However, the shortcomings of this ionization are that the sample must be thermally volatile and the molecular ion may be weak or not present for many compounds (Henderson, 2005a).

1.7.3.2 Chemical ionization source

In electron ionisation mass spectra, the abundance of the molecular ion is frequently low. Thus structural identification becomes difficult due to the lack of molecular weight information. This problem can be overcome by using a softer ionization technique called chemical ionization (CI) which is more suited for the analysis of polar compounds (http://www.cee.vt.edu/ewr/environmental/teach/smprimer/ms/ms.html). In chemical ionization mass spectrometry, the sample molecules react with reagent ions from the reagent gas, e.g. methane, to produce an analyte ion through the transfer of a charged species (which is usually a proton) between reactants. At low pressure, methane is ionised and the molecular ion (resulting from the ionisation of the molecule) is formed, $CH_4^{+\bullet}$. At higher pressures collision of these molecular ions with other methane molecules take place to form carbonium ions, CH_5^+. If a substance M is present, it will collide with the carbonium ions to form a pseudomolecular ion, MH^+ through the transfer of a proton, H^+. In this process the ions do not fragment as easily as during the electron impact mode. The spectrum produced is simpler with a few fragment ions (Henderson, 2005a), which makes it good for producing the molecular ion, however, the problem is that very few fragments are produced and therefore accurate identification of the compound can be problematic. The electron impact spectrum run on the same compound will have to be obtained to provide complementary information.

1.7.4 Mass analyzer

After the ions have been produced, separation according to their mass has to take place. There are several types of mass analyzer units available to separate the ions according to their mass-to-charge ratio. These include a sector field mass analyzer, the time-of-flight analyzer, as well as the quadrupole mass filter (Figure 1.13) and the ion trap (Pare and Yaylayan, 1997). The quadrupole analyzer, invented by W. Paul, consists of four cylindrical rods and only ions with a selected mass to charge ratio go between the rods. The ions are separated according to their *m/z* ratio.

Since the mass spectrometer used in this work was equipped with a quadrupole mass analyzer, this mass filter will be emphasised. In a quadrupole analyzer a voltage is applied between the adjacent rods. There is an electrical connection between the opposite rods. Once the ions have been injected within the filter with a small voltage, they start to oscillate within the electric field. The ions with the masses which fall within the stable oscillating region will continue on the same path within the rods and reach the detector. The ions with the masses which are present in the unstable oscillation region are lost on the rod assembly. This is how mass separation takes place in a quadrupole analyzer (Chapman, 1993). In the case of the ion trap instrument, all the ions present inside the trap are expelled according to their mass (Hoffmann *et al*., 1996).

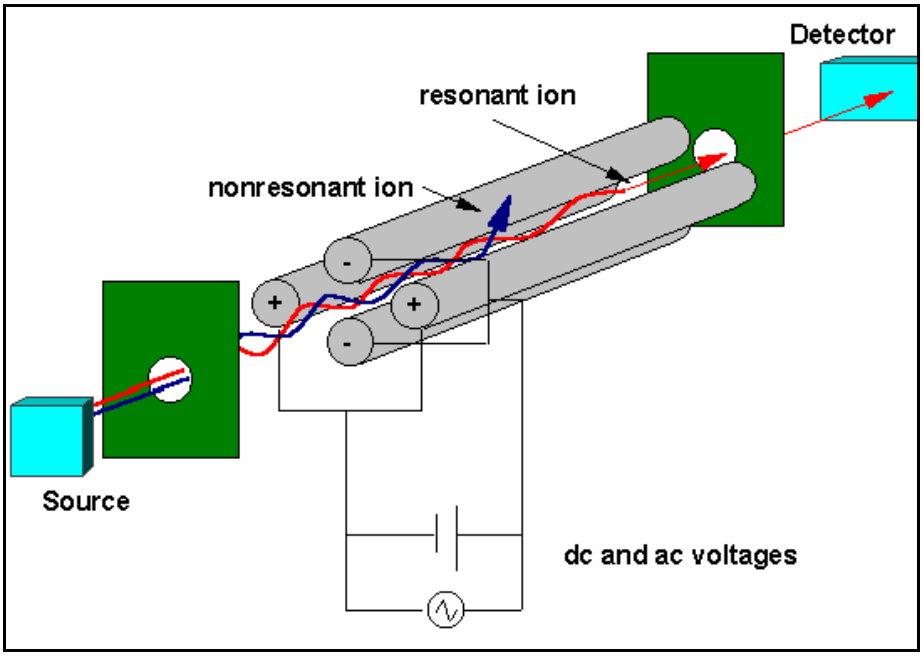


Figure 1.13 A quadrupole mass analyser consisting of the source, the rods and the detector (http://ael.gsfc.nasa.gov/saturnGCMSMass.shtml).

1.7.5 Ion detectors

The most widely used detector, the electron multiplier, which was used in this study to detect the ions which were produced, will be discussed.

1.7.5.1 Electron multipliers

After the separation in the analyzer, the ion currents with different intensities reach the detector. An electron multiplier used to detect the energetic ions causes the emission of several secondary particles when a positive or negative ion reaches the plate, also known as the conversion dynode. Thereafter, these secondary particles pass into the continuous-dynode electron multiplier. They then strike the cathode, dislodging electrons during the collision. As they pass further into the electron multiplier, more electrons are produced, thereby amplifying the signal. The amplified signals from the

electron multiplier are passed to a computer which evaluates the incoming data and prints out the required information. Once a mass spectrum is obtained, the problem arises of meaningful interpretation of the recorded data which consists of a large number of peaks of varying intensities. The following section (Section 1.8) will discuss this aspect for the compounds of interest in this work.

1.8 Mass spectra of terpenes

The mass spectra obtained for some of the compounds (the isomers) in the essential oils in this study were very similar which made positive identification difficult. Therefore, a brief discussion follows on the fragmentation of an ion, the factors influencing it and the fragmentation patterns of representative terpenes.

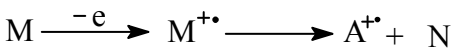
The interpretation of the mass spectrum can be problematic (Biemann, 1962). The spectra contained in the mass spectral library may have been obtained by different methods of ionization. Spectra obtained by chemical ionization are not suitable for matching the unknown spectrum with the spectra in the library due to variation in experimental conditions. Therefore, a standard of the compound of interest has to be used so that the mass spectrum of the unknown compound can be compared with that of the known standard (Johnstone, 1972).

1.8.1 Fragmentation

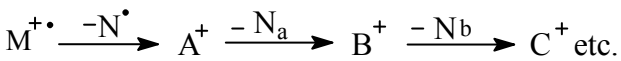
A *molecular ion* ($M^{+\bullet}$) resulting from the ionization of a molecule may contain sufficient internal energy to fragment by ejection of a neutral particle (N) with the formation of a *fragment ion* ($A^{+\bullet}$ or A^{+}). A neutral molecule gives a radical-cation as the molecular ion, and the fragment ion may be a cation or a radical-cation. The ejected neutral particle (N) may be a radical or neutral molecule.

$$M \xrightarrow{-e} M^{+\bullet} \longrightarrow A^{+} + N^{\bullet}$$

or



Further decomposition may occur, if the fragment ion (e.g. A^+) has sufficient internal energy. New fragment ions (B^+, C^+, etc.) are then formed, until there is insufficient internal energy for further reaction to take place.



Such a series of decompositions contained in a mass spectrum is a *fragmentation pathway*. The molecular ion ($M^{+\bullet}$) and any of the fragment ions (A^+, B^+, C^+, etc.) may decompose by more than one pathway. The different fragmentation pathways together comprise a *fragmentation pattern* characteristic of the compound which is being investigated. A fragmentation pattern consisting of only one pathway will produce a simple spectrum. On the other hand, if the fragmentation pattern contains more than one pathway, a complex spectrum is produced. The extent of fragmentation depends on the amount of internal energy imparted to the molecular ion ($M^{+\bullet}$), its structure, as well as the time allowed between ion formation and detection. As a result, the mass spectrum obtained is due to the appearance of the fragmentation pattern at specified energies and times (Rose and Johnstone, 1982).

1.8.1.1 Factors influencing the fragmentation of an ion

Fragmentation of the molecular ion takes place in the ion source. The molecule is bombarded with a high energy (70 eV), in order to ionize it. The energy transferred to the molecule is sufficient to break a bond or more than one bond. In the mass spectrum that is produced, numerous peaks are present (Biemann, 1962). Some peaks are intense, whereas others are weak or barely visible. The preferential formation of ions is due to three main factors:

i) the molecule's tendency to break some of the bonds rather than others,
ii) the stability of the fragmentation products (which predominates), and
iii) the relative spatial arrangement of the atoms (Frigerio, 1974).

1.8.2 The mass fragmentation patterns

The mass fragmentation patterns of terpenes have been widely studied. The mass spectra of only those terpenes relevant to this study are discussed briefly in this Section and in Chapter 3, Sections 3.10 to 3.10.6. The fragmentation patterns for representative terpenes are presented here and suggested fragmentation patterns are shown in Chapter 3, Schemes 1 and 2.

1.8.2.1 Acyclic terpenes

The formation of terpenes is based upon the polymerization of an isoprene unit. Isoprene has an abundant parent molecular ion, with the base peak corresponding to the loss of a single hydrogen atom. Dimerization of isoprene leads to the monoterpenes. These may be acyclic, monocyclic or possess two rings. The acyclic members representative of the series are myrcene and allo-ocimene. Although myrcene and allo-ocimene have the same molar mass of 136, they differ structurally, and the mass spectra shown in Figures 1.14 and 1.15 respectively, also differ. However, a closer examination of the mass spectra still needs to be done for positive identification.

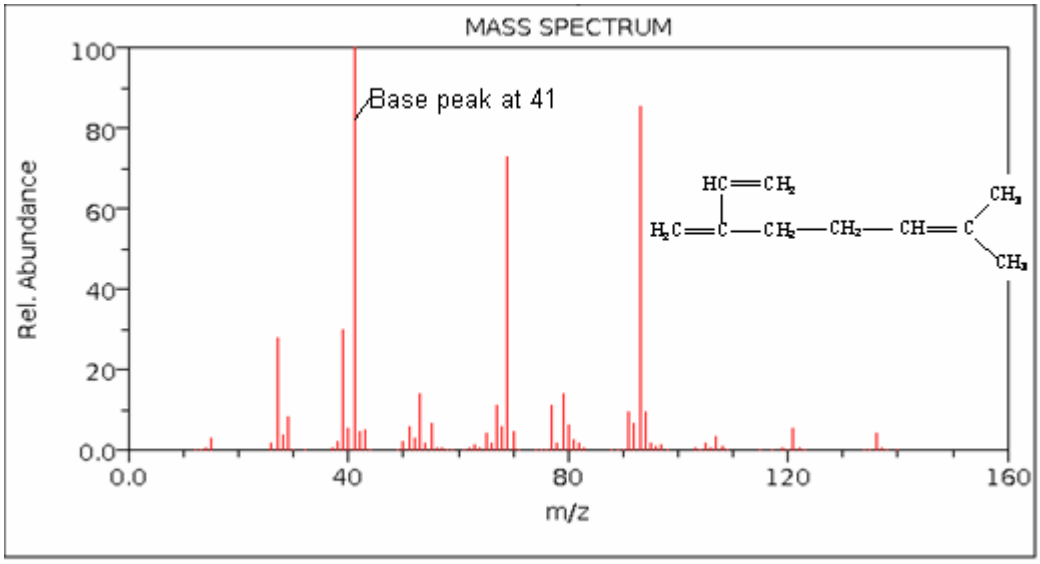


Figure 1.14 Mass spectrum of myrcene (http://webbook.nist.gov, date accessed: 16/09/2003).

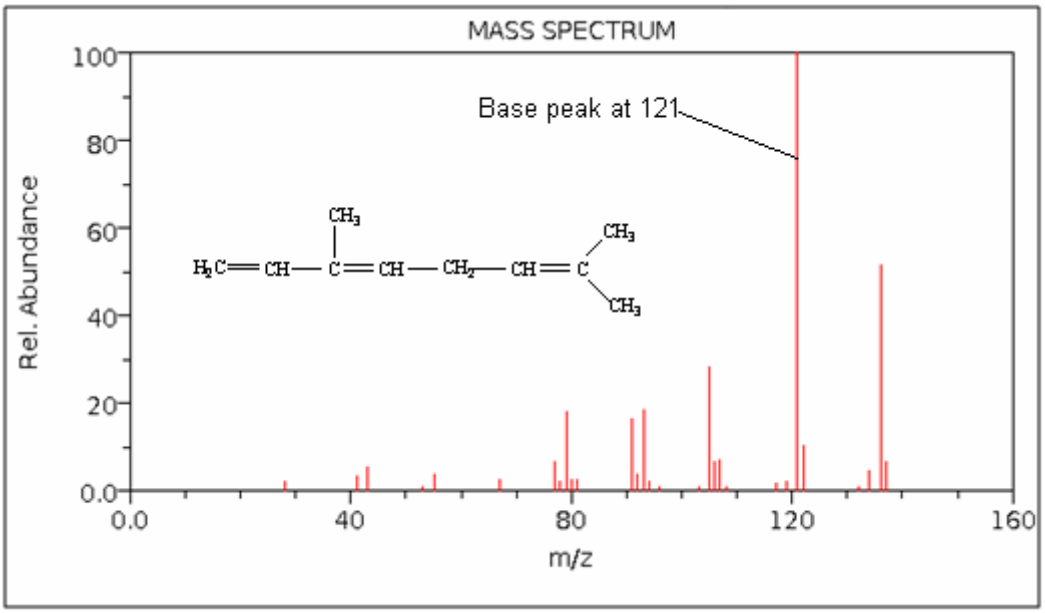


Figure 1.15 Mass spectrum of allo-ocimene (http://webbook.nist.gov, date accessed: 16/09/2003).

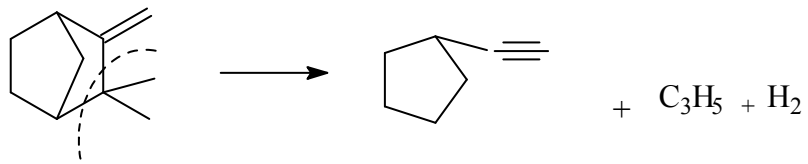
Close examination of the strucutures of both compounds show that they contain three double bonds and each terminates in an isopropenyl group. Myrcene has a single bond

which is doubly allylic. This feature correlates well with the low abundance (8%) of the parent molecular ion 136 in Figure 1.14. This is absent in allo-ocimene and thus the molecular ion 136^+, shown in Figure 1.15, is more abundant (51%). Myrcene undergoes decomposition more readily than other compounds in its group (Reed, 1966).

The loss of a methyl group in allo-ocimene, results in a base peak at *m/z* = 121 (M-15). The three methyl groups present are attached vinylically to double bonds and even in such circumstances a methyl can be lost (Ryhage and von Sydow, 1963). In comparison, the base peak in myrcene corresponds to the ion *m/z* = 41 (Figure 1.14). However, this is not readily derived without re-arrangement or at least extensive bond migration.

1.8.2.2 Cyclic terpenes

A series of cyclic monoterpenes including camphene, the isomeric pinenes, and the menthadienes, one of which α-1, 8(9)-p-menthadiene known as *d*-limonene, have been studied. The base peak 93^+ in the case of the pinenes, and camphene (Figure 1.16), is clearly the loss of the di-substituted bridge carbon. Thus, for camphene the sequence (Reed, 1966) is shown in Figure 1.16.

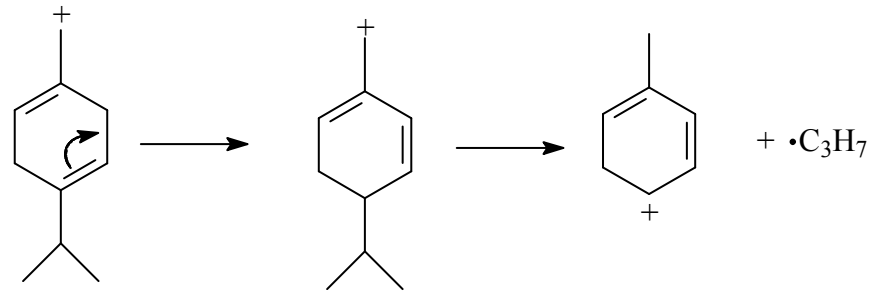


Camphene *m/z* 93

Figure 1.16 Fragmentation of camphene.

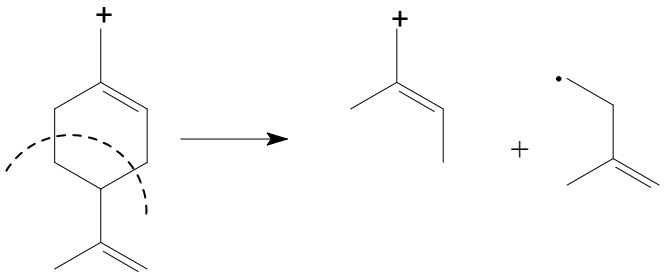
In the menthadienes, there is no bridge across the ring and the corresponding base peak is *m/z* = 93. The loss of the isopropyl group cannot be clearly explained. In the case of 1,4-*p*-menthadiene, it is assumed that double bond migration occurs. A conjugated double bond system will be more stable than a non-conjugated system (Reed, 1966).

The sequence is, therefore (Reed, 1966)



1,4-*p*-menthadiene conjugated system *m/z* 93 isopropyl

In the case of *d*-limonene, the rupture of two of the allylic bonds takes place via the fission process. Two isoprene molecules are formed, one of which carries the positive charge (Biemann, 1962).



d-limonene C_5H_8 (*m/z* 68) C_5H_8 (*m/z* 68)

In the mass spectrum of limonene, a strong peak representing the formation of the base peak at 68 (shown in Figure 1.17) is observed.

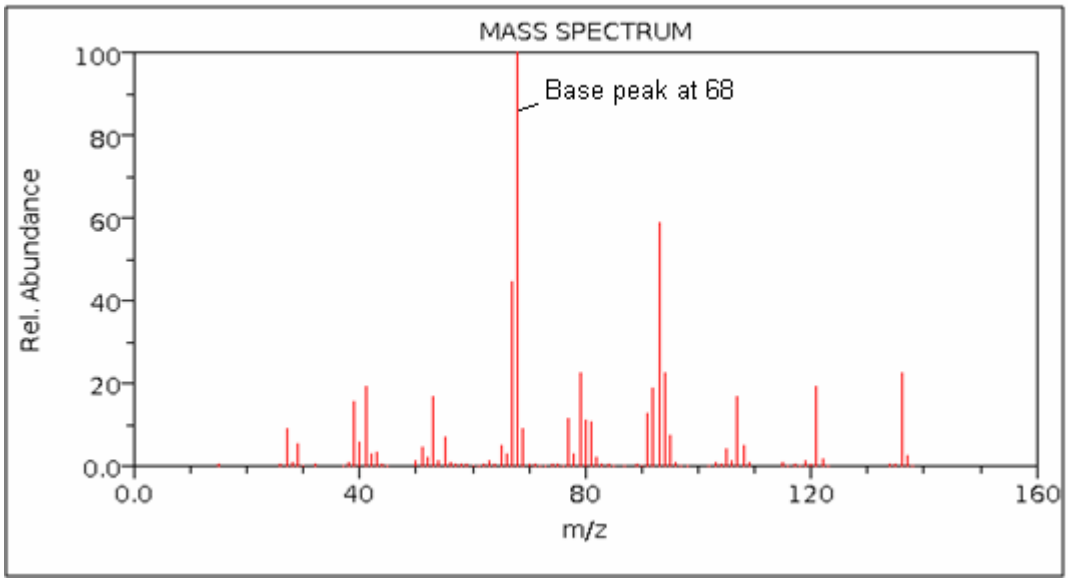


Figure 1.17 Mass spectrum of limonene (http://webbook.nist.gov).

The principal ions in the group together with the relative abundance are shown in Table 1.2. From this table four compounds, α-pinene, β-pinene, camphene and α-fenchene, have a base peak at 93. This would make complete identification extremely difficult.

To overcome this difficulty, the selected ion monitoring (SIM) mode, which is more sensitive than the full scan mode, should be used. In this mode, the mass spectrometer obtains data for selected masses unique to that compound.

Table 1.2 The principal mass spectral ion fragments and the relative abundance of the cyclic terpenes (Reed, 1966).

m/z	**Compound (% Abundance)**						
	camphene	**α-pinene**	**β-pinene**	**α-fenchen**	**d-limonene**	**p-methene**	**pinane**
27	44.1	21.1	31.4	44.0	32.46	36.28	58.72
29	14.7	9.44	10.9	15.8	12.68	15.25	34.11
39	51.4	23.7	33.2	49.0	44.29	31.60	59.17
40	10.4	-	-	-	12.02	-	9.75
41	58.6	23.2	63.9	58.9	34.68	42.24	100.0
43	-	-	-	-	-	11.79	16.51
53	15.3	10.5	14.0	21.4	28.23	18.27	24.29
55	-	-	-	-	-	25.71	86.24
67	33.7	-	-	-	40.19	40.19	49.35
68	24.5	-	-	-	100.0	55.06	33.72
69	-	-	46.7	-	-	16.81	49.29
77	23.0	22.1	18.3	30.9	15.87	11.12	-
79	37.5	17.7	19.9	62.6	25.18	14.98	10.50
80	12.2	9.81	10.4	47.0	10.18	-	-
81	-	-	-	27.7	10.54	26.22	44.90
82	-	-	-	-	-	14.96	57.24
83	-	-	-	-	-	-	53.88
91	21.8	21.2	13.2	26.2	14.52	-	-
92	-	29.7	-	16.1	16.33	-	-
93	100.0	100.0	100.0	100.0	53.36	-	-
94	16.7	-	13.5	30.3	18.77	10.19	-
95	21.9	-	-	-	-	100.0	74.42
96	-	-	-	-	-	16.48	29.13
107	29.2	-	-	25.3	14.48	-	-
121	62.6	13.2	-	39.1	16.69	-	-
123	-	-	-	-	-	13.52	19.96
136	14.2	80.5	7.01	23.7	19.35	-	-
137	1.5	0.85	0.76	2.58	2.16	-	-
138	-	-	-	-	-	26.73	4.52
139	-	-	-	-	-	3.46	0.65

1.8.2.3 Sesquiterpenes

This $C_{15}H_{24}$ group includes acyclic, monocyclic, bicyclic and tricyclic compounds. Little information of their cracking patterns is known.

1.8.3 Identification of compounds by using the mass spectral library

Prediction of the mass spectrum of most molecules (except for simple molecules) from first principles is difficult. This is because of the complex processes that take place during the dissociation of the molecule. For example, dissociation of complex ions can occur through a series of consecutive and competitive pathways. Also, rearrangement of the ions can result, making it difficult to assign the fragment ion to a distinct structural unit in the original molecule. Therefore, the mass spectra of unidentified compounds are compared to the mass spectra of known compounds which are contained in a reference library.

In the evaluation of each spectrum, during comparison, the assigned name, structural drawing and the spectrum should be consistent. In addition, the most characteristic peaks for the molecular structure must be present and an in-depth knowledge of established rules of fragmentation is required (Ausloos *et al*., 1999). The mass spectral reference library, however, cannot be used as a unique and absolute criterion for the identification of chromatographic peaks. This is due mainly to the vast number of mass spectra contained in the library which have been recorded under different conditions and which can lead to incorrect results (Oprean *et al*., 2001).

For unequivical identification of the compound, the 'unknown' spectrum is compared with the spectrum of the known compound. If the mass spectrum of the unknown is identical to the standard, then it is highly likely that the substances are identical (Leathard and Shurlock, 1970). The most reliable reference spectrum is that which is produced from the same mass spectrometer under the same operating conditions. Although some isomers give closely related spectra, those spectra that are identical with

respect to both mass and relative abundance data will in most cases demand identical molecular structures, except in the case of optical isomers (McLafferty, 1966).

In addition, spectra containing errors can be corrected. Some categories of errors are:

i) **Peaks due to impurities:** These peaks result from foreign compounds. One such example is that of "column bleed" from compounds previously determined in the mass spectrometer. A base-line subtraction should be done in this case.

ii) **Transcription errors:** One or more peaks may be displaced from their expected position. These errors can be commonly found in old mass spectra before computerized mass spectrometers were developed. Any ambiguity in the spectra can be corrected.

iii) **Spurious peaks:** Such peaks arise because of instrument noise and are simply disregarded (Ausloos *et al*., 1999). These peaks can contribute to the making of false identifications (Stein, 2005).

1.9 The advantages of using GC-MS

The GC-MS analysis used in this study can be readily adaptable to most laboratories performing volatile and semi-volatile analyses. Unique hydrocarbon distributions can be critically evaluated by using mass spectral analysis in order to determine the nature of the compounds present. Furthermore, the availability of the full-scan GC-MS data allows one to perform a mass spectral analysis and to evaluate unusual (or differing) hydrocarbon distributions in terms of unknown and tentatively identified compounds. The availability of the GC-MS data coupled with a competent mass spectral analysis can provide enough information to discern the nature of the organic material in the sample.

1.10 Outline of this project

The different sample preparation techniques according to the extracting phases employed in this work are shown in Figure 1.18. For the traditional methods of solvent and Soxhlet extraction, the solvent choice, as well as different extraction periods were studied to determine the optimium extraction conditions. In the case of the HSA and HS-SPME, extraction temperature, extraction time, desorption time and fibre coating were investigated to determine the optimium extraction conditions of volatile organic compounds. According to my knowledge, there has been no reported literature for the headspace and HS-SPME analysis of the essential oil in *M. Koenigii* thus far. A comparison of all the methods, including steam distillation was undertaken to identify the shortcomings and advantages of each technique.

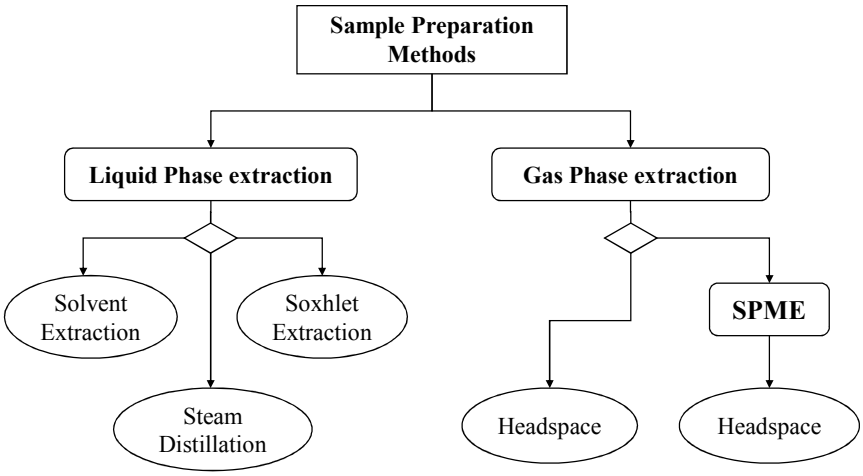


Figure 1.18 Extraction methods employed in this study.

The experimental procedures undertaken in this work are presented in Chapter 2 and Chapter 3 provides a discussion of the results obtained from the various experiments.

CHAPTER 2

EXPERIMENTAL

This chapter details the experimental procedures of the different extraction techniques that were used to extract the volatile components from the fresh leaves of *M. koenigii*. It includes details of the subsequent GC-MS analysis of these extracts.

2.1 Materials and equipment

The materials and equipment used for the various experimental procedures are listed in Appendix A.

2.2 Sample collection

The leaves of *M. koenigii* were collected from a garden in the Shannon Drive area of Reservoir Hills, Durban, South Africa. The tree from which the leaves were cut was identified by Emeritus Professor H. Baijnath of the School of Biological and Conservation Sciences, University of KwaZulu-Natal (Westville Campus).

2.3 Preliminary work

Different variables with major and minor effects have been found to affect plant growth. Some of these factors are environmental factors such as temperature and rainfall (seasonal variation), insufficent water or nutrients, plant diseases (Jackson, 1986) and soil conditions. Therefore differences occur when the final complex plant extract is analysed.

However, in this work certain fundamental aspects needed to be studied first before any studies on reproducibility could be performed. Therefore, preliminary work had to be done in order to ascertain the broad parameters used in extraction techniques and to obtain a reasonable starting point.

2.3.1 Choice of fresh or frozen leaves

An initial study was undertaken to determine whether there was any difference in the amount and proportion of volatile components between fresh and frozen leaves. This initial work was carried out by means of HS-SPME with a 15 minute equilibration period and the SPME fibre with the PDMS coating.

Prior to the extraction process, fresh as well as frozen leaves (~25 g) were milled to an average size of approximately 1 mm by means of a blender, in order to increase the surface area and thereby increase the extraction efficiency. The leaves were taken from the same tree. The sample was milled for the same time period (15 mins), weighed accurately and the mass recorded.

Five compounds, α-pinene, β-pinene, α-phellandrene, α-caryophyllene and β-caryophyllene, were tentatively identified and selected for the initial studies. The results of these preliminary studies are discussed in Chapter 3, Section 3.2, showed that extraction yields were greater for the highly volatile compounds (the monoterpenes) at a temperature of 40 ºC, when frozen leaves were used, and a greater yield was obtained for the sesquiterpenes when fresh leaves were used. Consequently, for the reason stated in Section 3.2, and since most studies on *M. koenigii* have been performed on fresh leaves (MacLeod and Pieris, 1982; Paranagama, *et al*., 2002; Wong and Tie, 1993), all analyses in this work were performed on fresh leaf samples. Also, due to the widespread interest in the analysis of volatiles released from food whilst fresh (Pare and Yaylayn, 1997), the essential oils in this work were extracted from fresh leaves. The study of the frozen leaves could lend itself to future work by examining the effect of freezing on the stability of the compounds.

2.3.2 Solvent choice for solvent and Soxhlet extraction

Although there are many factors that affect the yield from solvent and Soxhlet extraction methods, the two most important factors, extraction time and solvent choice, were evaluated in this work. The most suitable solvent to be used was investigated in a preliminary study and the extraction time was investigated in subsequent work.

Three solvents, namely, hexane, dichloromethane and ethyl acetate, were investigated for their ability to extract the aroma compounds, the terpenes, from the fresh leaves of *M. koenigii*. These results are discussed in Chapter 3, Section 3.2.2. The results showed that the largest yields for the five selected analytes were obtained when dichloromethane was used. In addition, Barra *et al*. (2007) reported in their work on the flavour components from French beans (*Phaseous vulgaris* L.), that it is the most suitable solvent for the extraction of a large class of flavour compounds.

2.3.3 Headspace analysis and headspace solid phase microextraction

Since sampling conditions could affect extraction efficiencies, the following parameters were investigated in a preliminary study: equilibration time for both headspace analysis and HS-SPME, and the fibre desorption time for HS-SPME. The adsorption capacity of the compounds with the two different fibre coatings: poly (dimethylsiloxane) (PDMS) and poly (acrylate) (PA), was investigated at room temperature. The headspace technique (no fibre present) was also investigated in this study.

2.3.3.1 Equilibration time

In the case of SPME, the fibre with the PDMS coating was exposed to the headspace of the fresh sample at room temperature for the following equilibration times: 15 and 70 minutes. From the results obtained, shown in Chapter 3, Section 3.2.3.1, it was observed that nothing was gained after the 15 minute equilibration period, since the

headspace extraction of analytes is an equilibrium technique and not an exhaustive extraction method. Fifteen minutes appears to be long enough for equilibrium to take place. Therefore, this 15 minute equilibration period was chosen for all the extractions in the headspace mode. Bichi *et al.* (2007) reported that non-equilibrium conditions are usually selected for the analysis of complex mixtures, and particularly for natural products.

2.3.3.2 Desorption time

Different fibre desorption times from 30 seconds to 5 minutes were investigated. A desorption time of 5 minutes (discussed in Section 3.2.3.2, page 75), which was also used by Flores *et al.* (2006) in their investigation of volatile compounds in food, was found to be sufficient for the quantitative desorption of all the analytes studied in this work.

2.3.3.3 Fibre coating

According to Hamm *et al*. (2003), for a matrix with a large number of compounds, a competition exists for the active sites on the SPME fibre coating. A preliminary investigation was therefore undertaken to examine which type of fibre would provide the most efficient sorption of the target analytes. Two different types of fibre coatings were investigated: PDMS with 100 μm thickness and PA with 85 μm thickness coating. The stationary phase thickness and the distribution constant determines the amount of analyte that can be adsorbed onto the fibre (Povolo and Contarini, 2003).

Standard mixtures of nine compounds as well as individual standards of some of the compounds were analysed. It needs to be pointed out that the concentrations of these compounds analysed differed from the concentrations of the components present in the oil and compounds other than those analysed were also present in the oil. The results of the selected compounds of interest are reported in Chapter 3, Table 3.10, page 81.

Results showed that a larger amount was extracted for the individual standards and a smaller amount for the compounds present in the combined mixture for the monoterpenes for both the fibre coatings. In comparison, a larger amount of the sesquiterpenes was extracted for the combined standard mixture than when the compounds were present on their own. However, the amount of the selected hydrocarbons extracted was larger when the PDMS fibre coating was used and smaller when the PA coating was used. Further discussion of these results can be found in Chapter 3, Section 3.2.3.3, page 76.

2.4 Extraction and isolation of the volatile oils

The experimental procedures for the various techniques can be found in Sections 2.4.2 through to 2.4.5.3.

2.4.1 Extraction time

In both the solvent and Soxhlet extractions, three separate replicate samples were used. The different time periods studied were: 24, 48 and 72 hours. These results can be found in Chapter 3, Section 3.3.1.

A sequential Soxhlet extraction with dichloromethane was conducted to extract the volatile compounds. After the first 24 hour extraction, solvent was removed and a second 300 mL aliquot of dichloromethane was added to the same 24 hour sample. The results showed that the sesquiterpenes, β-caryophyllene, α-caryophyllene, β-selinene and valencene were still being extracted even after the further 24 hour extraction period was over, i.e. after 48 hours. A third extraction on the same 24 hour period sample indicated that the extraction process was complete, and no further compounds were extracted after 48 hours. Analyses of the results also showed that the optimium extraction period for the five selected compounds, was found to be 48 hours. The same extraction periods were used for solvent extraction.

2.4.2 Procedure for solvent extraction

The extraction of the organic components was carried by adding 300 mL of dichloromethane to 25 g milled leaves (weighed accurately) contained in a 500 mL Erlenmeyer flask. The flask was then stoppered and placed on a mechanical shaker for extraction of the analytes by agitating the milled leaves. The extraction process was conducted at room temperature. The extracts obtained were dried with anhydrous Na_2SO_4. This was followed by reducing the volume of the extracts with a rotary evaporator and transferring them to a 5 mL volumetric flask.

2.4.3 Procedure for Soxhlet extraction

Soxhlet extraction was conducted according to the standard method (Furniss *et al*., 1989) with similar apparatus as illustrated in Figure 2.1. The thimble (Advantec 30 x 100 mm) was first extracted with dichloromethane and dried. Subsequently, a 25 g (accurately weighed) sample of milled leaves was placed in the thimble. Extraction of the leaves was carried out by using 600 mL of dichloromethane. During this process the solvent is vaporised and condenses on the solid sample contained in the thimble and the soluble compounds are extracted. When the liquid level rises to the top of the extractor, it is siphoned back into the flask. This process occurrs continously for the required extraction period. After the extraction period, the samples were treated in a similar manner as described in Section 2.4.2.

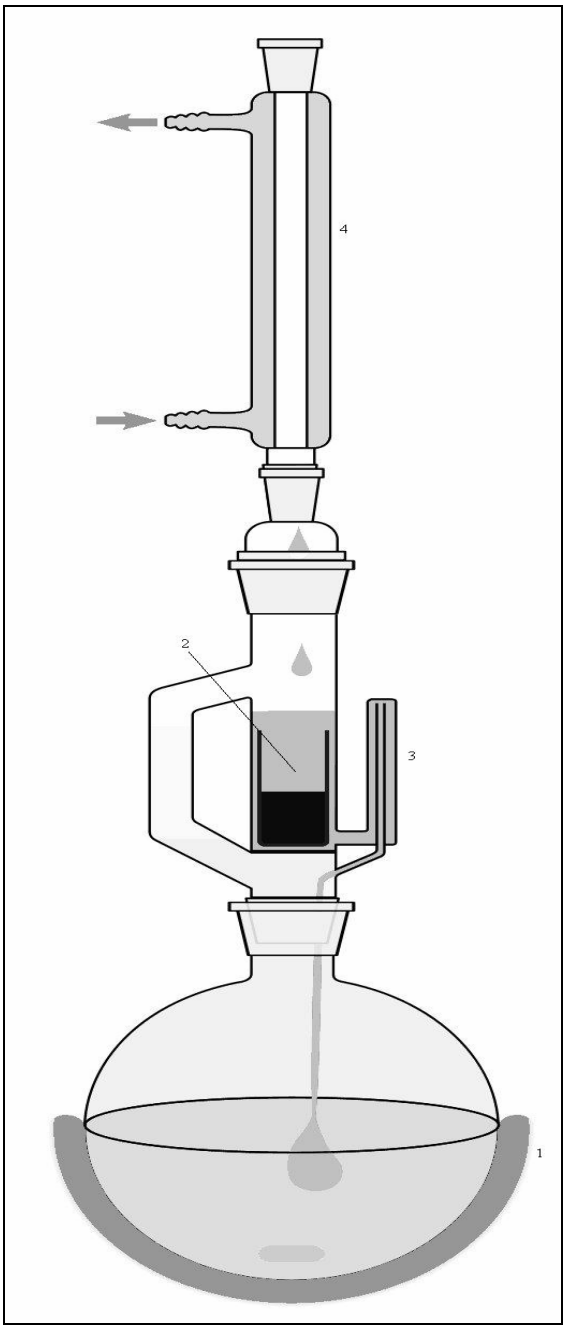


Figure 2.1 A schematic diagram of the Soxhlet apparatus, 1: round-bottom flask, 2: solid sample contained in the thimble, 3: siphon, 4: condensor (http://en.wikipedia.org/wiki/Soxhlet_extractor).

2.4.4 Steam distillation

A 25 g sample of accurately weighed milled leaves, done in triplicate, was placed in 300 mL water in a 500 mL round-bottom flask and a steady flow of steam was blown in from a steam generator via a glass inlet tube. The material to be steam-distilled was then heated, by means of a heating mantle, and the vapour (containing the volatile organic compounds mixed with steam) was passed through a condenser and collected in the receiver flask (Mann and Saunders, 1960). The duration of the steam distillation process was 3 hours. The condensate (approximately 500 mL) was divided into two fractions. Each 250 mL fraction was extracted with 3 x 50 mL aliquots of dichloromethane. The combined extracts were dried with anhydrous Na_2SO_4 and evaporated to dryness with a rotary evaporator. The isolated oils were weighed and the masses obtained are recorded in Table 3.16 which can be found in Chapter 3, Section 3.4.

In order for the samples to be injected into the gas chromatograph they must be present at an appropriate concentration level. When the concentration level of the analyte is too low, a good signal cannot be obtained whilst a high concentration level will cause the separation to degrade. To overcome these problems, the oil (with individual masses of 0.43, 0.50 and 0.59 g) was transferred to separate volumetric flasks and brought up to the 2 ml mark with dichloromethane so that the sample was be present at the appropriate concentration thereby rendering the analysis possible.

2.4.5 Vapour enrichment procedure

For extraction of compounds in the vapour phase, for both the headspace analysis and HS-SPME, a sample of fresh milled leaves (~25 g) was placed in a 500 mL round-bottom flask. The flask was sealed with a polytetrafluoroethylene septum and evacuated for 30 seconds. The evacuated flask was then immersed in an oil bath at the extraction temperatures: room temperature (~22), 40, 60 and 80 ºC, selected for the

experiment. Triplicate analysis was done on three separate samples for all the headspace extractions.

2.4.5.1 Extraction temperature

To determine the effect of temperature and the most suitable extraction temperature, the following temperatures were used for both headspace analysis and HS-SPME: room temperature (~22), 40, 60 and 80 ºC. A discussion of these results can be found in Chapter 3, Section 3.5.5.1.

2.4.5.2 Headspace analysis procedure

In the case of headspace analysis, a vapour phase sample was injected into a gas chromatograph after a 15 min equilibration time at the various temperatures. The syringe used to transfer the sample was flushed with air after each injection to prevent sample carryover from inside the syringe.

2.4.5.3 HS-SPME analytical procedure

Prior to usage, the SPME fibre was conditioned according to the manufacturer's instructions. The PDMS fibre was inserted for 60 min in the GC injection port at 250 ºC while the PA fibre was inserted in the injection port at 300 ºC for 120 min (Teixeira *et al*., 2007). After the conditioning process, the fibre was desorbed by inserting into the GC injection port at 250 ºC to ensure that the fibre was clean (Pena-Alvarez *et al*., 2006). This conditioning process was only performed when the fibres were used for the first time. Further conditioning after each sample was not required as the fibre was desorbed for five minutes after each run to eliminate sample carryover from one run to the other and at the same time preventing distortion of the results obtained.

The clean fibre was then immersed into the headspace of the flask containing the ground sample (similar to the experimental set-up in Figure 2.2) and the flask was then placed in a bath containing Julabo oil. After 15 minutes at the different temperatures studied, the fibre was retracted and removed. The analytes were immediately thermally desorbed by inserting the fibre into the GC injection port for 5 minutes. The injections were carried out in the split mode with a ratio of 1:75. Blank runs were carried out before each injection to avoid sample carryover. The precision of the HS-SPME method was also investigated. For this investigation, triplicate extractions were performed at room temperature ~22 ºC, 40 ºC and 60 ºC. The peak areas of the compounds in the *M. koenigii* leaves were used to calculate their relative standard deviation (RSD) values, to express the method precision.

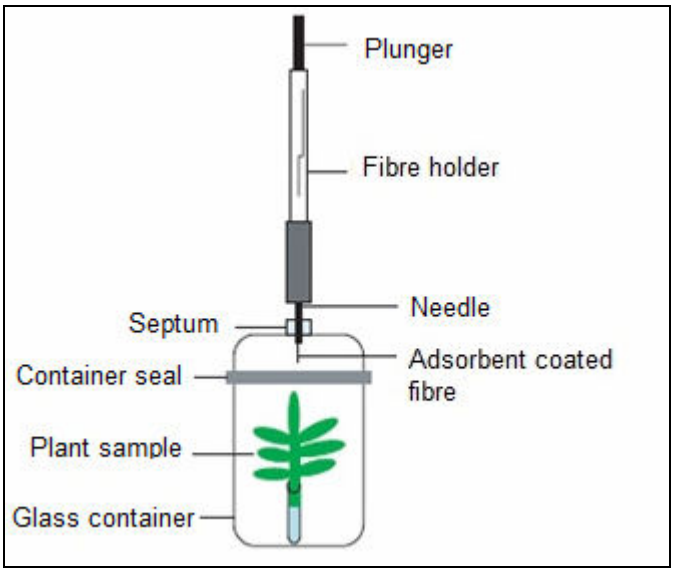


Figure 2.2 Headspace sampling with a SPME device (Tholl *et al*., 2006).

2.5 GC-MS parameters

The analyses of the compounds in *M. koenigii* were carried out on an Agilent 6890 series gas chromatograph, a model 5973 mass selective detector and a G1701CA MSD Productivity Chemstation Software data system. The GC column was a non-polar DB-5 (methyl phenyl siloxane) capillary column, manufactured by Agilent JW Scientific, with a film thickness of 0.25 µm, a length of 30 m and an internal diameter of 0.25 mm.

The oven temperature was run isothermally at 100 ^{o}C for the first 10 minutes, followed by an increase of 20 ^{o}C min^{-1} to 200 ^{o}C for the next 5 minutes and thereafter remaining at 200 ^{o}C at a helium flow rate 0.7 cm^{3} min^{-1}. Mass spectra were obtained at 70 eV ionization energy with the electron impact mode, using total ion current monitoring over the 35 to 550 *m/z* scan range. In this investigation, the sample was injected in the split mode with a ratio of 1:75. Rana *et al.* (2004) also used the split mode for their work on the volatile oil of *M. koenigii* leaves. The split mode is usually selected when the analytes are present at a high concentration and to prevent column overload. All samples were injected manually in the GC injection port at a temperature of 250 ^{o}C and the volumes employed are reported in the section which follows.

2.5.1 GC-MS sampling technique

Since different extraction methods were used, different sample introduction techniques for the liquid and vapour phase with an appropriate sample size were used and this is mentioned below.

2.5.1.1 Liquid phase

Liquid phase samples were transferred to the GC by using 1 μL injections with the aid of a Hamliton syringe.

2.5.1.2 Vapour phase

The split mode with a ratio of 1:75 was used for headspace analysis and HS-SPME.

Headspace analysis

Headspace vapour phase samples at the various temperatures investigated were removed with the aid of a 1 mL SGE gas-tight syringe. A volume of 0.1 mL was taken. The syringe was not pre-heated.

Headspace solid phase microextraction

For the solid phase microextraction technique, a Supelco TM solid phase microextraction device with different fibre coatings, PDMS and PA, was used. The fibres were desorbed for 5 mins in the injection port of the GC at a temperature of 250 ^{o}C.

2.5.2 Quantitative determinations

Quantitative analysis was performed for the steam distillation, Soxhlet extraction and solvent extraction methods only. Quantification of the headspace methods was not performed, since according to Tholl *et al*. (2006), quantification by SPME can be both difficult and impractical when dealing with compounds present with a broad range of volatility. Further discussion on this can be found in Chapter 3, Section 3.10. The concentrations of five selected aroma compounds: α-pinene, β-pinene, α-phellandrene, β-caryophyllene and α- caryophyllene, were determined in this study. These compounds were chosen since earlier work done on the leaves of *M. Koenigii* by Prakash and Natarajan (1974), MacLeod and Pieris (1982), and Paranagama *et al*. (2002) identified these compounds as the main aroma-contributing compounds. Also, preliminary work performed here confirmed this.

Working solutions of the standards in the concentration range of 3.36 mg L^{-1} to 1.80×10^{4} mg L^{-1} were prepared in dichloromethane directly from the pure compounds. The internal standard was prepared from a standard stock solution. The internal standard dodecane was used by Kalua *et al*. (2006) in their study of the volatile profile

of olive oil and was used in this work for the quantitative determination of the volatile components.

The same internal standard was used to check both the extraction and analytical efficiency. The extraction and analytical efficiency were investigated in the following ways respectively:

a) By adding the internal standard at the start of the extraction stage to account for any physical and chemical losses in the amount of the terpenes during the extraction process (Biermann and McGinnis, 1989).

b) By addition of the internal standard at the end of the extraction period, i.e. just before injection. The addition of the internal standard after the extraction process does not account for any losses during the extraction process but was added to serve as a point of reference for peak area measurements so that any variations in both the injection technique and the volume injected can be cancelled out since both the the internal standard and the analyte will be affected by the same variations (Kenkel, 2003).

The results of the quantitative determinations are presented in Chapter 3, Section 3.9.1.

2.5.3 Data analysis

In this work, the data was analysed from the total ion chromatograms which were produced. This method was selected because a large range of compounds were present in the *M. koenigii* leaves. The mass spectra of these compounds were closely related and showed that most of the compounds had a common base peak at *m/z* 93. The selected ion monitoring (SIM) mode could not be used, since this is more selective as only ions of certain masses are recorded and not the entire spectrum as compared to the total ion chromatogram which shows many components of a mixture (Herbet and Johnstone, 2003).

2.5.4 Component identification

For the analysis of the volatile compounds, spectra were obtained with the electron impact mode since these spectra contain more fragment ions which can be useful for the structural characterisation of the compounds. Also, under the electron impact conditions, due to a lack of selectivity, a wide range of compounds can be studied, (Chapman, 1993) which made it suitable for this analysis.

Identification of the unknown compounds was made by comparison with their retention times and mass spectra with those of the pure standards available. Further comparison was done with the mass spectra of known compounds contained in the National Institute of Science and Technology Standard Reference Database 1A (NIST 98). After the chromatogram was obtained (Figure 2.3 (a)), the selected peak of interest was represented on the screen together with the mass spectrum. Figure 2.3 shows the chromatogram for the comparison of the mass spectrum of the unknown compound (b) with the known spectrum (c) contained in the NIST library. Also, identification of the compounds was performed by visual interpretation of the fragmentation patterns of the compounds, i.e. by examining the peak intensities.

Identification of some of the main aroma-contributing compounds was also done with a comparison based on the retention times of the standard compounds (α-pinene, β-pinene, α-phellandrene, α-caryophyllene and β-caryophyllene) with the unknowns, run under the same experimental conditions, similar to the procedure used by Flores *et al.* (2006) in their work.

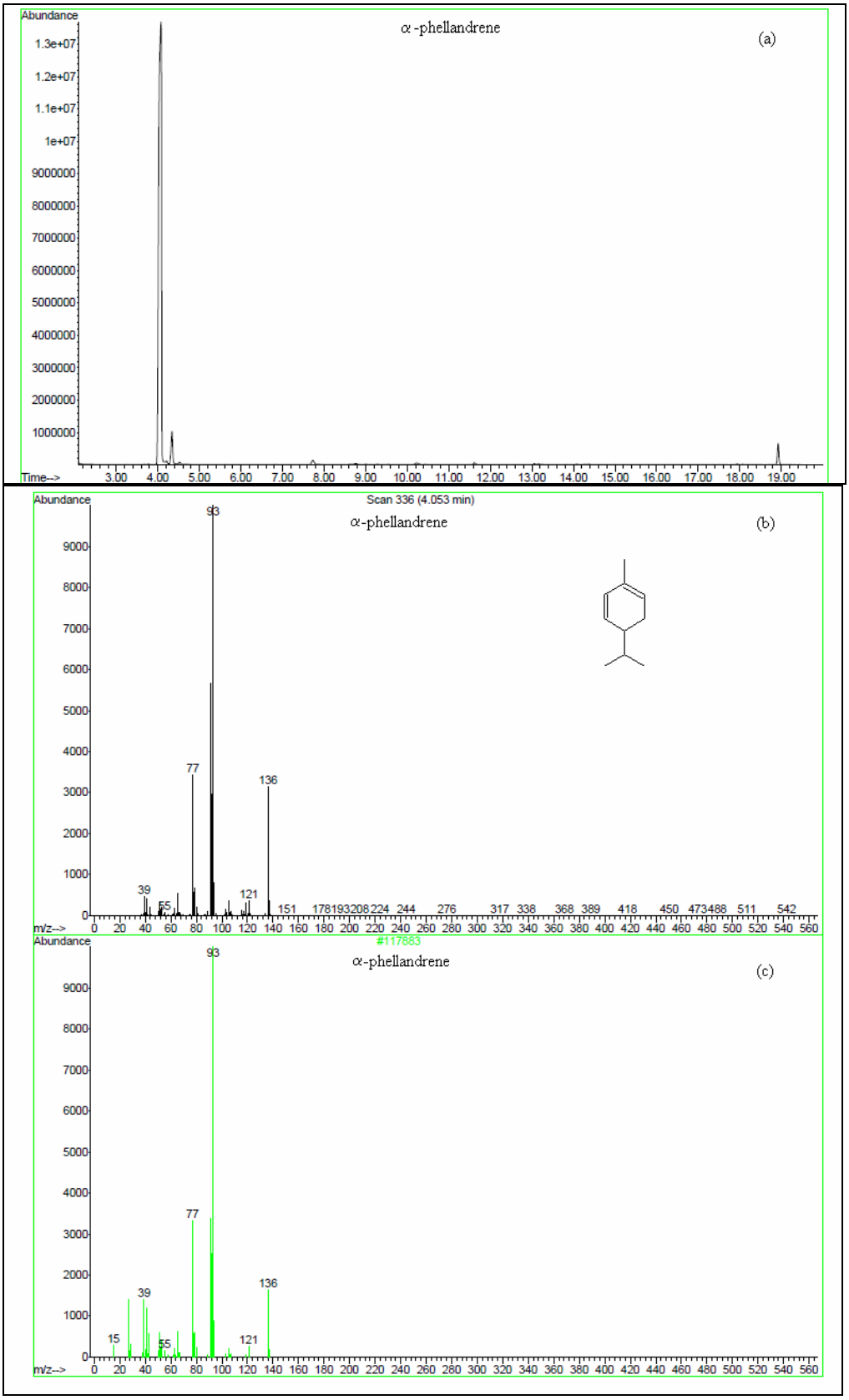


Figure 2.3 Library search results for α-phellandrene: (a) chromatogram (b) mass spectrum (c) mass spectrum contained in the NIST library.

2.6 Summary of conditions

Tables 2.1 and 2.2 provide a summary of the variables investigated with the various extraction techniques and the GC-MS analysis conditions used in this work respectively.

Table 2.1 A summary of the variables studied for the different extraction techniques.

Variables	**Extraction Method**			
	Solvent extraction	**Soxhlet extraction**	**Steam distillation**	**Headspace analysis HS-SPME (PDMS and PA coating)**
solvent choice	dichloromethane	dichloromethane		solvent–free analysis
extraction time/hrs	24, 48, 72	24, 48, 72	3	0.25
extraction temperature/^{o}C	room temperature ~22	40	100	room temperature (~22), 40, 60, 80

Table 2.2 Summary of the GC-MS analysis conditions.

Variables	**GC-MS Analysis Conditions**
stationary phase	5% diphenyl, 95% methylpolysiloxane (DB-5)
mobile phase	helium
injector temperature	250 ^{o}C
column temperature	isothermally at 100 ^{o}C for 10 mins, followed by an increase of 20 ^{o}C min^{-1} to 200 ^{o}C for the next 5 mins and thereafter remaining at 200 ^{o}C
helium flow rate	0.7 cm^{3} min^{-1}
scan range	35 to 550 *m/z*
ionization energy	70 eV
detector temperature	280 ^{o}C
mode	Electron impact

The results of this experimental work are presented and discussed in Chapter 3.

CHAPTER 3

RESULTS AND DISCUSSION

In this chapter the results obtained from the different extraction methods as well as a discussion of the results are presented.

3.1 Organisation of results

The results are organised into different sections, namely, calibration data, calibration curves, and chromatograms with their corresponding mass spectra and raw data. The calibration data and the curves can be found in Section 3.9. A comparison of the mass spectra of the standard compounds and those obtained in the mass spectral library associated with the software of the GC-MS can be found in Appendix C. The fragments in the mass spectra of some monoterpenes detected in *M. koenigii* are summarized in Table 3.25 (Section 3.6.1). Representative chromatograms of the essential oil analysis for the different methods adopted are in Appendix D and the raw data in Appendix E. The area percent reports can be found in Appendix F.

3.2 Preliminary study

An initial investigation was undertaken for the extraction of the analytes from fresh and frozen *M. koenigii* leaves. This experiment was performed on a single analysis at 40 ºC. The volatile components present consisted mainly of monoterpenes and sesquiterpenes.

The study on the fresh leaves of *M. koengii* for headspace-SPME (with PDMS fibre coating), showed greater extraction yields for the less volatile compounds (sesquiterpenes) compared to the highly volatile compounds (monoterpenes). In comparison, the amount of the volatile compounds extracted from the frozen leaves was found to be greater for the monoterpenes than the sesquiterpenes.

A representative overlay chromatogram showing the differences in the amounts of the compounds extracted (40 ºC) from both the fresh leaves and frozen leaves is shown in Figure 3.1. The area percent reports can be found in Appendix F, Tables F1 and F2. The run time for this chromatogram was 70 minutes with the earlier GC method. In the earlier GC method, the oven temperature was run isothermally at 100 ^{o}C for the first 40 minutes, followed by an increase of 10 ^{o}C min^{-1} to 200 ^{o}C for the next 10 minutes and thereafter remaining at 200 ^{o}C. After all the preliminary work revealed which components were present, the GC method was modified (refer to Section 2.5) so that all the compounds could be separated in 20 minutes.

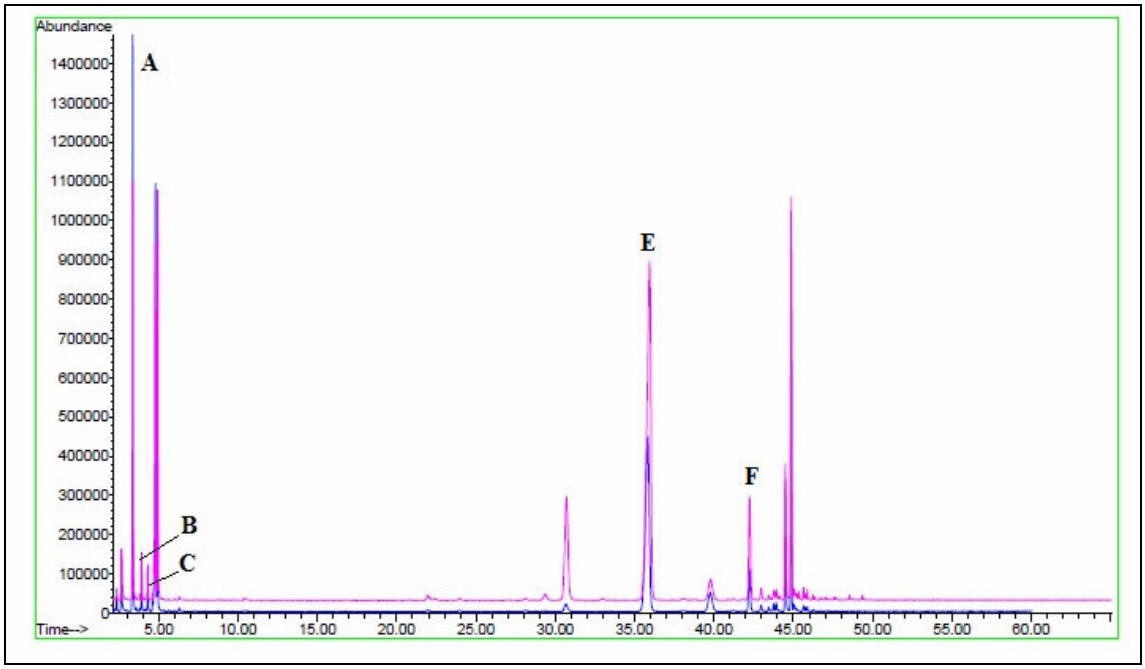


Figure 3.1 An overlay chromatogram for the essential oil obtained from the fresh (—) and frozen (—) leaves at 40 ºC for the HS-SPME (PDMS fibre coating) extraction.

This preliminary study indicated that it is preferable to extract the less volatile compounds from fresh leaves and the highly volatile compounds from frozen leaves. Since the low volatile compound, the sesquiterpene β-caryophyllene, was found to be present in a larger amount in the fresh leaves, it can be concluded that it was the main aroma contributing compound and therefore all other work was performed on the fresh

leaves of *M. koenigii*. Also, there was no way of knowing the stability of the compounds on freezing, so it was decided to use fresh leaves. It has been reported by Stashenko *et al*. (2004) that the freshness of a plant can affect the volatile profile, more especially the headspace fraction.

3.2.1 Identification of the volatile components in *M. koenigii*

The compounds in the essential oil of *M. koenigii* were identified from GC-MS analysis. Identification of the target analytes was performed by comparing the retention time and mass spectrum of each component in the sample with those of standard compounds and the mass spectra contained in the NIST Library run under similar conditions. However, where standards were unavailable, identification was done by comparison of the mass spectrum of the component with the mass spectra contained in the NIST library, as well as applying some basic knowledge of mass spectral fragmentations. Some compounds had very similar spectra which made the library search difficult to positively identify the peaks and thus a tentative identification was done. The identification process has been explained in Chapter 2, Section 2.5.4. Some of the compounds identified in the essential oil from the leaves were α-pinene, β-pinene, α-phellandrene, β-caryophyllene and α-caryophyllene.

For convenience, the selected compounds of interest on the chromatograms are labelled with an alphabetical letter (A to F) as indicated in Table 3.1. Compound D refers to the internal standard, dodecane, which was not added to the above sample and therefore is not seen in the chromatogram in Figure 3.2. A more comprehensive list of compounds obtained from the combined extraction methods is shown in Table 3.29.

Table 3.1 Peak labels for compounds of interest.

Compound	**Alphabet**
α-pinene	A
β-pinene	B
α-phellandrene	C
dodecane (internal standard)	D
β-caryophyllene	E
α-caryophyllene	F

A representative chromatogram of the oil obtained from the steam distillation method for the five selected compounds is shown in Figure 3.2.

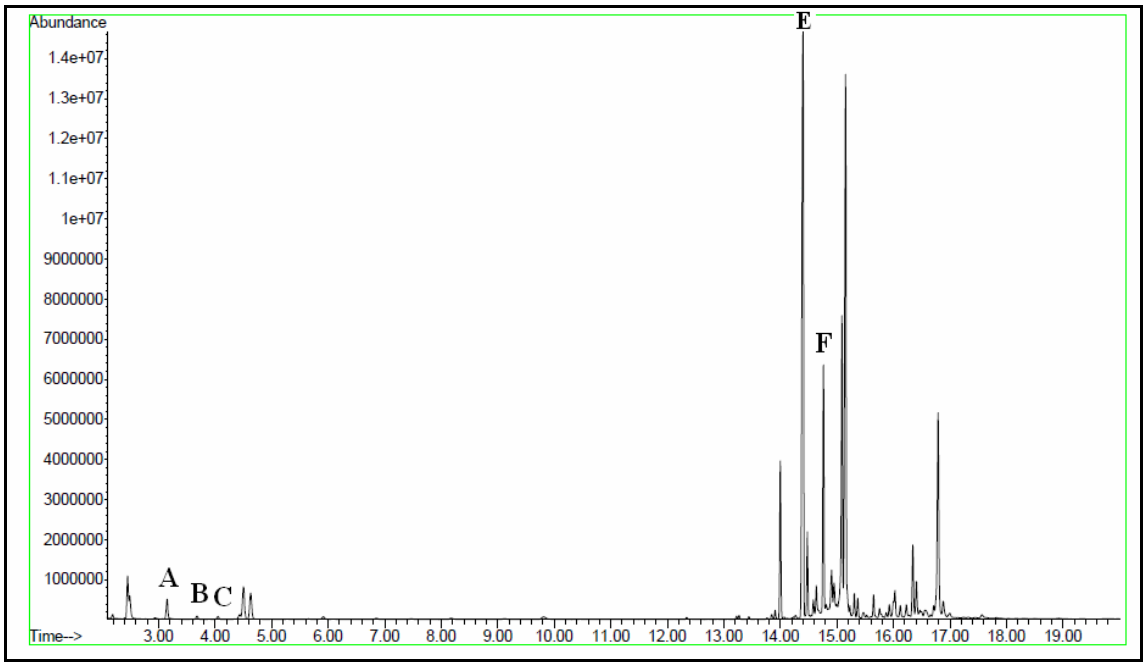


Figure 3.2 Total ion chromatogram of the oil obtained from the steam distillation method.

3.2.2 Choice of solvent for solvent and Soxhlet extractions

The choice of the extracting solvent is important for the extraction of the compounds of interest as well as for eliminating or reducing those components that can interefere in an analysis (Teixeira *et al*., 2007). Three solvents, namely, hexane, dichloromethane and ethyl acetate, were investigated to determine which was the most suitable solvent for the extraction of the volatile organic compounds. The results obtained from the investigation to determine the most suitable solvent for the solvent and Soxhlet extraction of the five selected aroma components in *M. koenigii* is presented in this section.

3.2.2.1 Solvent extraction

The chromatograms of the solvent extraction of the aroma compounds of *M. koenigii* leaves obtained with hexane, dichloromethane, and ethyl acetate, after 68 hours, are shown in Figures 3.3 to 3.5 respectively. The peak areas of the selected analytes for a single extraction and analysis are shown in Table 3.2.

It needs to be mentioned that although the peak due to α-caryophyllene in Figure 3.4 (labelled F) is poorly-shaped the peak areas for the selected compounds of interest were manually integrated in order to account for baseline correction.

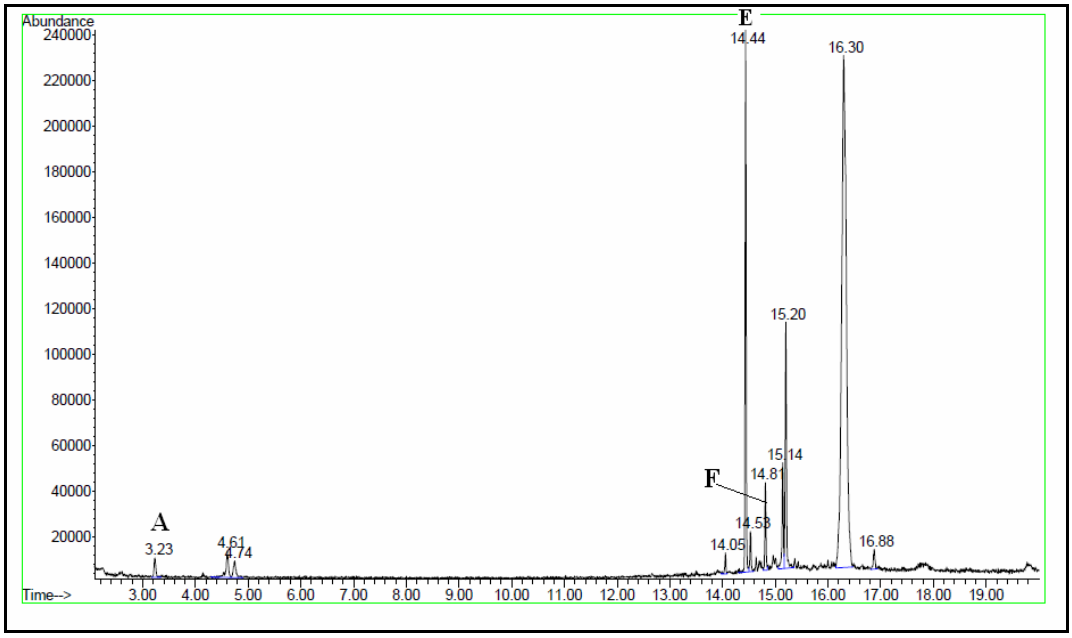


Figure 3.3 Total ion chromatogram for the essential oil obtained from the solvent extraction with hexane.

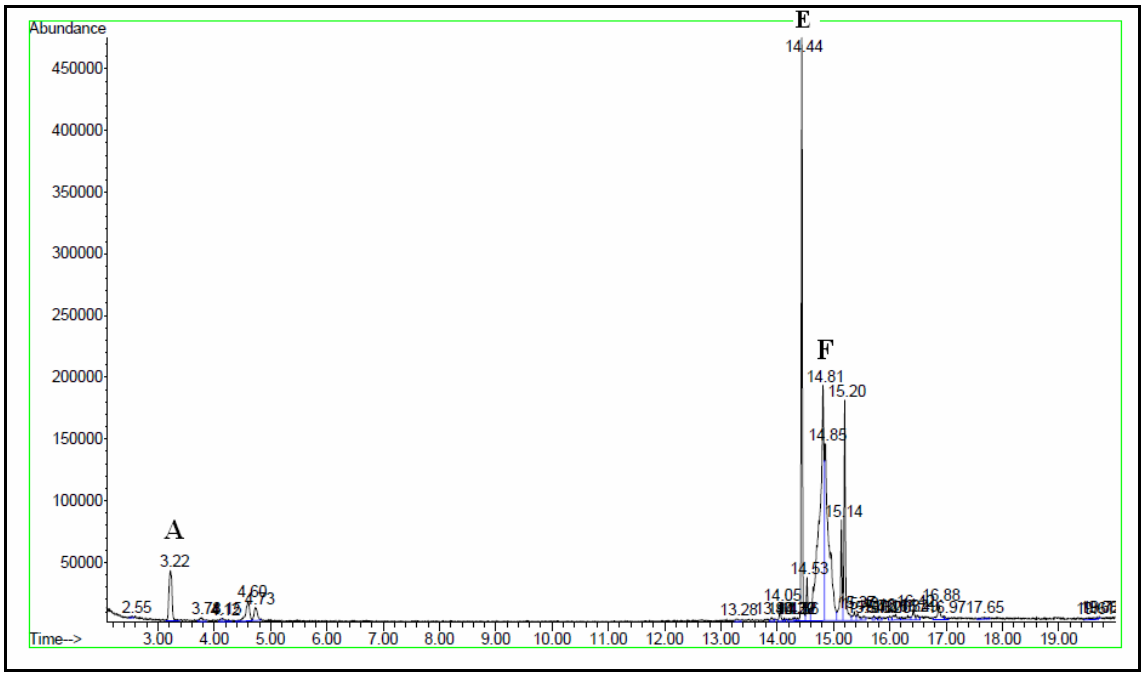


Figure 3.4 Total ion chromatogram for the essential oil obtained from the dichloromethane solvent extraction.

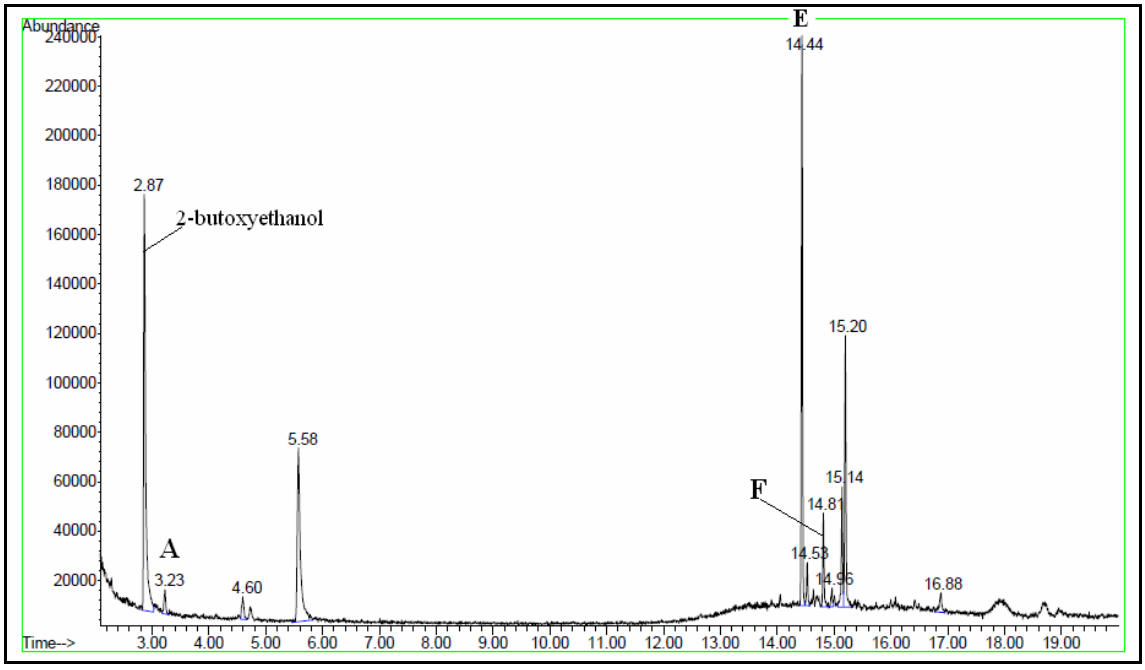


Figure 3.5 Total ion chromatogram for the essential oil obtained from the solvent extraction with ethyl acetate.

Table 3.2 Peak areas for the compounds in *M. koenigii* from solvent extraction.

Compound	**Hexane**	**Dichloromethane**	**Ethyl acetate**
α-pinene	1.99×10^5	1.54×10^6	2.24×10^5
β-pinene	ND*	ND*	ND*
α-phellandrene	ND*	ND*	ND*
β-caryophyllene	3.40×10^6	6.61×10^6	3.34×10^6
α-caryophyllene	6.05×10^5	8.49×10^6	5.94×10^5

*ND - not detected

A comparison of the peak areas for the compounds of interest, in Table 3.2, showed that the greatest extraction yield was obtained when dichloromethane was used as the extracting solvent. It needs to be pointed out that the compounds which were present in a low concentration, i.e. β-pinene and α-phellandrene, could not be seen in the

chromatogram, as the sample was introduced in its diluted form for this investigation, directly from the extraction flask, i.e. without preconcentration.

From the abundances obtained from the chromatograms shown in Figures 3.3 to 3.5, it can be seen that there is a preferential extraction of compounds depending on the polarity of the solvent, i.e. a larger number of compounds were extracted when dichloromethane was used, since due to its polarity, it was able to extract the hydrocarbons more efficiently. Hexane did not efficiently extract the compounds as a smaller amount of the target analytes were obtained when this solvent was used. Although ethyl acetate also extracted the same amount of the target analytes as hexane, it also extracted other compounds leading to a "noisy" or "complicated" chromatogram. The presence of too many compounds hinders the separation and a complex chromatogram will be obtained. Therefore balancing the number of compounds extracted against the target analytes extracted shows dichloromethane to be solvent of choice. Also, taking into account the peak areas of the compounds, it can be clearly seen that the dichloromethane showed a better extraction efficiency for the target compounds and was therefore, selected for all subsequent studies.

3.2.2.2 Soxhlet extraction

In this investigation, a trend similar to the results presented for solvent extraction was observed. Gas chromatographic analysis was performed immediately after the extraction. The peak areas for the selected compounds extracted with either hexane, dichloromethane or ethyl acetate are contained in Table 3.3. Once again, the compounds which were present in a low concentration, i.e. β-pinene and α-phellandrene, could not be observed in the chromatogram, as the sample was introduced into the GC-MS without concentration.

Table 3.3 Peak areas for the aroma components in *M. koenigii* obtained from the Soxhlet method for a single analysis.

Compound	Hexane	Dichloromethane	Ethyl acetate
α-pinene	1.19×10^6	1.34×10^6	3.89×10^5
β-pinene	ND*	ND*	ND*
α-phellandrene	ND*	ND*	ND*
β-caryophyllene	4.47×10^6	8.99×10^6	7.61×10^6
α-caryophyllene	9.98×10^5	9.65×10^5	1.36×10^6

*ND - not detected

From the data shown in Table 3.3, it can be observed that for the extraction with dichloromethane, the yields were relatively higher for two of the compounds, α-pinene (a monoterpene) and β-caryophyllene (a sesquiterpene). This investigation, also showed that dichloromethane was the most appropriate solvent for the extraction of the volatile compounds because of its physical properties, i.e. its volatility and ability to dissolve a wide range of organic compounds (http://en.wikipedia.org/wiki/Dichloromethane).

3.2.3 Headspace and HS-SPME extraction conditions

According to Monje *et al*. (2002), there are many variables which affect HS-SPME. Some of these factors are: extraction temperature, adsorption time, choice of fibre and desorption conditions (Sostaric *et al*., 2000). The sensitivity of the HS-SPME technique is dependent on these factors (Teixeira *et al*., 2007).

In initial studies, equilibration time, desorption time and fibre coating were investigated. The headspace mode was selected instead of the direct extraction mode for this study because, according to Demeestere *et al*. (2007), equilibration times for volatile compounds are shorter in the headspace mode than in direct extraction and the fibre is protected from any adverse effects caused by other substances present in the matrix.

3.2.3.1 Equilibration period

The plant material in this work consisted of high, medium and low volatility compounds. According to Torrens *et al.* (2004), a short extraction period would be required for highly volatile compounds and a longer extraction time for compounds with low volatility. Hamm *et al.* (2003) mentioned in their work that the extraction period of the compounds is dependent on the volatility of the compounds. Stashenko and Martinez (2007) reported in their work that, for samples with a large number of components, some compounds will reach equilibrium before others and will compete for sites on the fibre coating. Stashenko *et al*. (2004) used a 15 minute equilibration period in their study of the Mill, *Lippia Alba* plant.

Taking into consideration some of the findings reported in earlier work, two different extraction times were investigated with the PDMS fibre coating at room temperature: 15 and 70 minutes. The results obtained for the 15 and 70 minute extractions (single analysis) performed at room temperature are given in Table 3.4. The area percent reports can be found in Table 3.5 to 3.6.

Table 3.4 Peak areas for the five standards extracted by HS-SPME (PDMS coating) at room temperature for two different extraction periods.

Compound	**15 mins**	**70 mins**
α-pinene	2.39×10^7	1.56×10^7
β-pinene	2.79×10^6	1.39×10^6
α-phellandrene	2.35×10^6	1.19×10^6
β-caryophyllene	4.94×10^7	3.59×10^7
α-caryophyllene	5.94×10^6	4.27×10^6

From the results in Table 3.4, it was observed that there was a slight decrease in the amounts of all the selected compounds extracted with an increase in the equilibration

time. The results indicated that the 15-minute period was adequate for the analysis of the selected compounds.

Table 3.5 Area percent report for HS-SPME (PDMS fibre coating) at room temperature – 15 mins.

SPMERT1
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\171209\SPMERT1.D Vial: 27
Acq On : 5 Feb 2007 11:48 Operator: Patricia
Sample : spme fresh sample2 roomtemp. 15 min Inst : Instrumen
Misc : spme room temp. 15 minutes Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.344	7	45	56	BV 8	17323	1330041	2.69%	0.700%
2	2.620	56	92	134	PB 9	41562	3241092	6.56%	1.706%
3	3.348	167	216	237	BV 3	341519	23872261	48.33%	12.563%
4	3.895	267	309	330	BV 4	41746	2786391	5.64%	1.466%
5	4.288	330	376	395	VV 4	33767	2353129	4.76%	1.238%
6	4.764	395	457	467	PV 3	328243	26479804	53.61%	13.936%
7	4.894	467	479	506	VB 6	138569	7605993	15.40%	4.003%
8	10.346	1313	1407	1495	BV 6	55153	8957242	18.13%	4.714%
9	30.264	4690	4797	4829	BV 6	11167	1129280	2.29%	0.594%
10	30.464	4829	4831	4836	VV 6	2535	36101	0.07%	0.019%
11	35.687	5613	5720	5809	BV 6	276811	49396222	100.00%	25.996%
12	39.683	6337	6400	6403	BV 6	27056	2681759	5.43%	1.411%
13	39.706	6403	6404	6466	VB 8	25324	2018393	4.09%	1.062%
14	42.198	6759	6828	6875	PB 6	72304	5938346	12.02%	3.125%
15	43.749	7054	7092	7103	PV 6	14722	908754	1.84%	0.478%
16	43.913	7103	7120	7136	VV 6	20299	1300517	2.63%	0.684%
17	44.489	7198	7218	7261	VV 4	262760	12282250	24.86%	6.464%
18	44.859	7261	7281	7300	VV 3	551407	23006146	46.57%	12.108%
19	45.635	7394	7413	7424	BV 3	13137	606793	1.23%	0.319%
20	47.744	7760	7772	7807	VV 2	217303	7122831	14.42%	3.749%
21	49.442	8036	8061	8100	VV 3	68226	3072389	6.22%	1.617%
22	51.023	8286	8330	8341	PV 5	15242	230459	0.47%	0.121%
23	51.546	8404	8419	8434	PV 4	75863	2689315	5.44%	1.415%
24	52.874	8621	8645	8674	BV 5	23035	967964	1.96%	0.509%

Sum of corrected areas: 190013471

Table 3.6 Area percent report for HS-SPME (PDMS fibre coating) at room temperature – 70 mins.

SPMERT1A
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\171209\SPMERT1A.D Vial: 27
Acq On : 5 Feb 2007 13:01 Operator: Patricia
Sample : spme fresh sample2 roomtemp. 70 min Inst : Instrumen
Misc : spme room temp. 70 minutes Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.361	36	48	83	VB 3	20521	661211	1.89%	0.587%
2	2.655	84	98	134	BV 3	37251	1400398	4.00%	1.243%
3	3.366	191	219	251	BV	616503	15567064	44.52%	13.820%
4	3.930	302	315	345	VV 3	45566	1388998	3.97%	1.233%
5	4.318	360	381	405	VV 2	36928	1185273	3.39%	1.052%
6	4.635	414	435	439	PV 4	9417	279227	0.80%	0.248%
7	4.794	439	462	473	VV	361978	12492474	35.73%	11.091%
8	4.929	473	485	511	VV 3	140575	4575134	13.08%	4.062%
9	30.640	4824	4861	4910	PV 3	11325	1507746	4.31%	1.339%
10	35.746	5628	5730	5821	PV 3	199251	34966632	100.00%	31.043%
11	39.753	6353	6412	6477	PV 3	21534	3810776	10.90%	3.383%
12	42.227	6803	6833	6880	BV 7	53182	4271011	12.21%	3.792%
13	42.985	6918	6962	6992	BV 7	7975	570989	1.63%	0.507%
14	43.755	7078	7093	7109	VV 7	10078	484981	1.39%	0.431%
15	43.925	7109	7122	7141	VV 7	12034	543101	1.55%	0.482%
16	44.501	7200	7220	7250	PV 6	196476	8873863	25.38%	7.878%
17	44.871	7266	7283	7303	VV 5	440511	18972451	54.26%	16.843%
18	45.047	7303	7313	7333	PV 5	8635	415592	1.19%	0.369%
19	45.658	7397	7417	7427	PV 5	10746	477647	1.37%	0.424%
20	45.764	7427	7435	7444	VV 10	6545	195857	0.56%	0.174%

Sum of corrected areas: 112640425

3.2.3.2 Fibre desorption time

After the chromatogram was obtained from the first desorption, the fibre was desorbed a second time to verify that the first desorption was complete and that there were no traces of sample carryover. A representative chromatogram of the PA fibre desorption run can be found in Figure 3.6. As can be seen from the chromatogram, none of the compounds identified in this work can be seen in the chromatogram,thus confirming that there was no sample carryover from run to run and that the five minute desorption period was adequate for the desorption of the analytes.

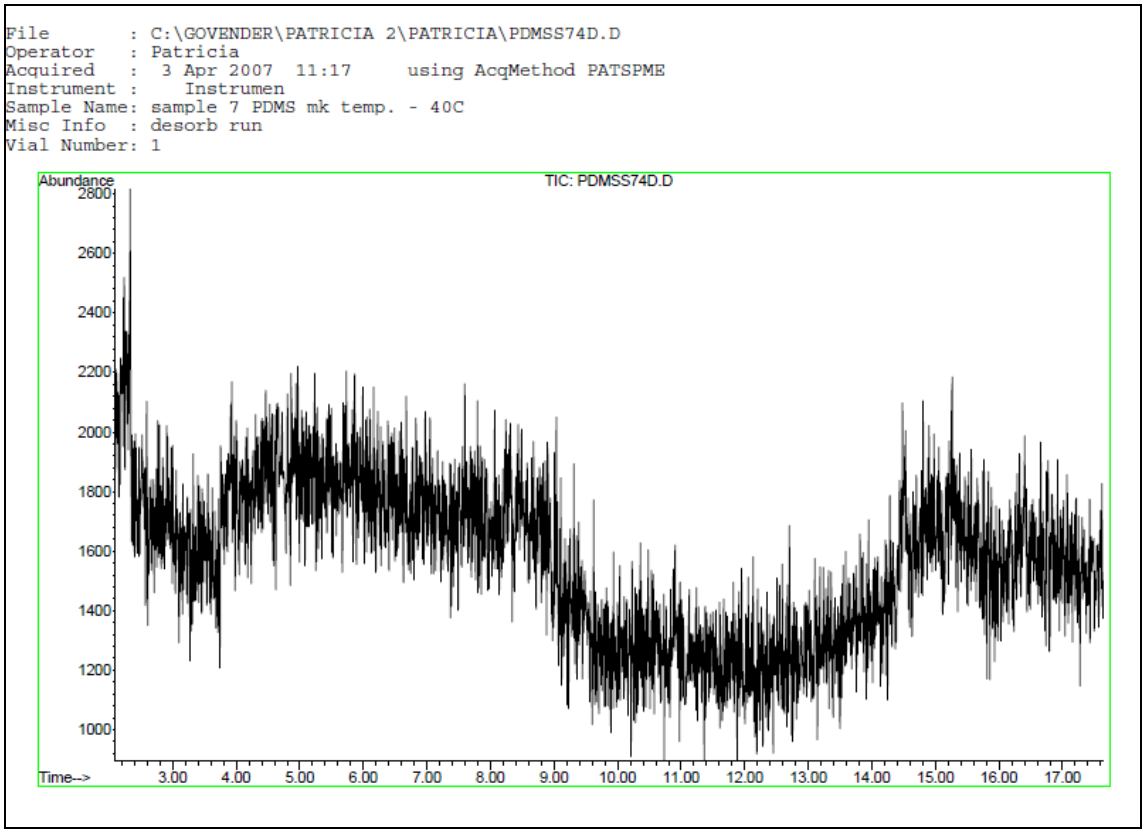


Figure 3.6 Total ion chromatogram for HS-SPME (PDMS coating) at 40 °C - desorption run.

3.2.3.3 PDMS and PA fibre coating

According to Contini and Esti (2006), it is difficult to determine which components cause the displacement of the compounds for adsorption sites on the fibre and also which compounds they displace. They also reported competition effects between analytes as well as linearity deviations (probably due to saturation of adsorption sites on the fibre) in relation to the sample matrix composition.

Therefore an investigation of the adsorption capacity of the compounds with the two different fibre coatings was conducted at room temperature. In this work, the individual standards for the selected compounds as well as a mixture comprising of the five chosen analytes as well as four other standard compounds, *p*-cymene, *d*-limonene, gurjunene

and camphene were investigated. The same sample size, namely, a volume of 20 µl was used. In addition, a sample in a headspace vessel, in which no fibre was present was also analysed and the results are discussed here.

The chromatograms of the combined standard mixture for the different headspace techniques are shown in Figures 3.7 to 3.9. The area percent reports for the combined standard mixture for this investigation can be found in Tables 3.7 to 3.9. A representative area percent report for the individual standard can be found in Appendix F, Table F3.

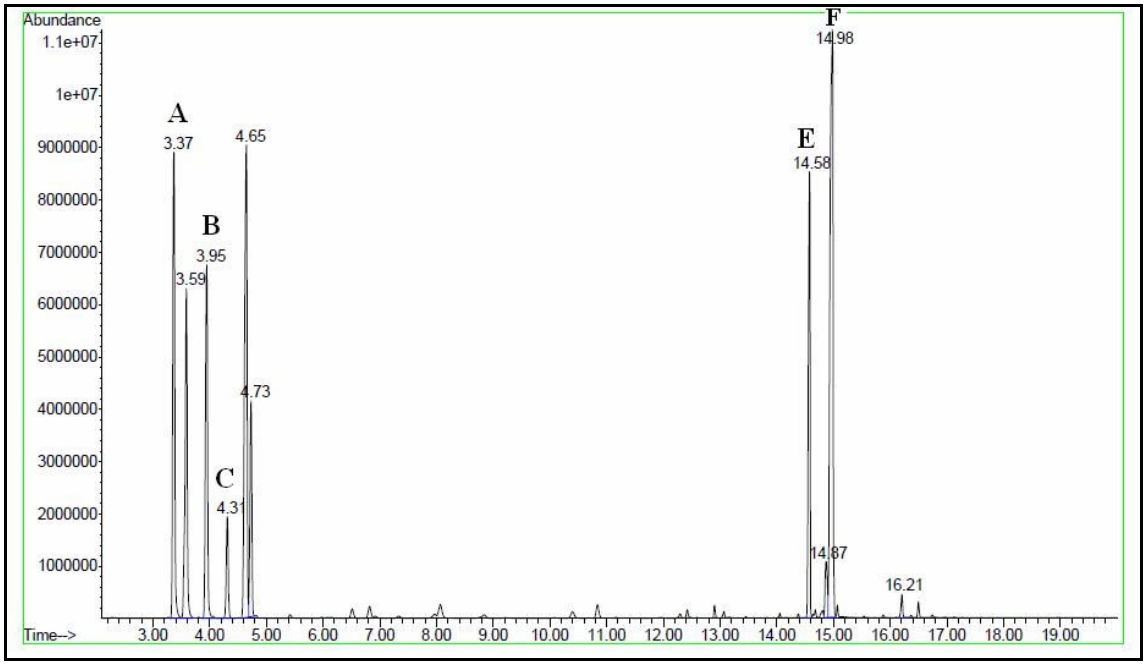


Figure 3.7 Total ion chromatogram for a standard mixture at room temperature for the HS-SPME-PDMS fibre coating.

Table 3.7 Area percent report for HS-SPME (PDMS fibre coating) for standard mixture.

PDSTDRT
Area Percent Report

Data File : C:\GOVENDER\PATRICIA\PDSTDRT.D Vial: 1
Acq On : 29 May 2007 11:01 Operator: Patricia
Sample : sample -standard -room temp. Inst : Instrumen
Misc : PDMS coating Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.371	191	220	242	BV	8932212	221833330	53.72%	13.679%
2	3.589	242	257	283	VB	6238395	169444314	41.03%	10.449%
3	3.947	305	318	344	BB	6685882	174615889	42.28%	10.767%
4	4.312	361	380	397	BB	1936742	46199454	11.19%	2.849%
5	4.646	420	437	444	BV	9026945	282660492	68.45%	17.430%
6	4.729	444	451	463	VB	4079461	96514430	23.37%	5.951%
7	14.576	2114	2127	2134	BV	8517317	179814658	43.54%	11.088%
8	14.876	2170	2178	2183	VV 2	1052639	28956728	7.01%	1.786%
9	14.982	2183	2196	2206	VV 2	11110391	412972854	100.00%	25.465%
10	16.210	2394	2405	2417	BB	440576	8686166	2.10%	0.536%

Sum of corrected areas: 1621698316

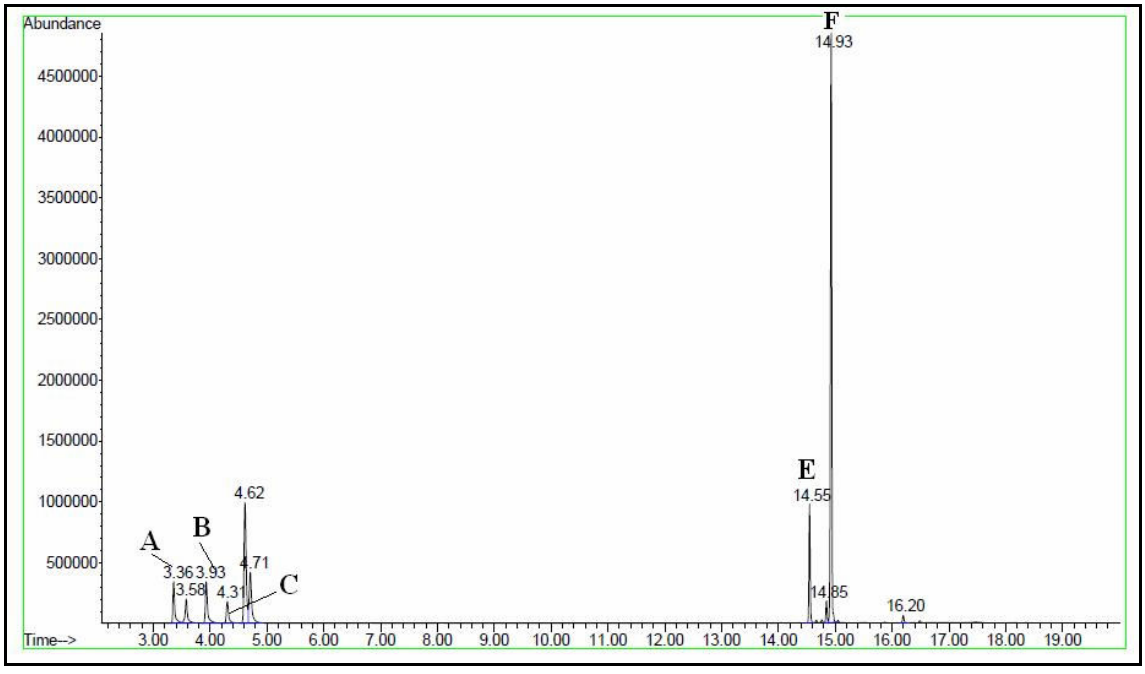


Figure 3.8 Total ion chromatogram for a standard mixture at room temperature for the HS-SPME-PA fibre coating.

Table 3.8 Area percent report for HS-SPME (PA fibre coating) for standard mixture.

PA1STDRT
Area Percent Report

Data File : C:\GOVENDER\PATRICIA\PA1STDRT.D Vial: 31
Acq On : 1 Jun 2007 10:22 Operator: Patricia
Sample : Pa coating standard mixture room temp. Inst : Instrumen
Misc : PA1 Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.360	210	218	243	BB	336675	7585656	9.54%	4.707%
2	3.583	244	256	276	BB	190804	4989664	6.28%	3.096%
3	3.936	306	316	340	BB	334981	8474830	10.66%	5.259%
4	4.306	369	379	399	BB	176706	4684457	5.89%	2.907%
5	4.617	420	432	441	BV	987103	26034072	32.76%	16.156%
6	4.711	441	448	475	VB	412990	12964394	16.31%	8.045%
7	14.547	2114	2122	2133	BB	945619	14180043	17.84%	8.800%
8	14.847	2167	2173	2179	BV	179594	2756169	3.47%	1.710%
9	14.929	2179	2187	2203	VV	4763965	79474874	100.00%	49.319%

Sum of corrected areas: 161144159

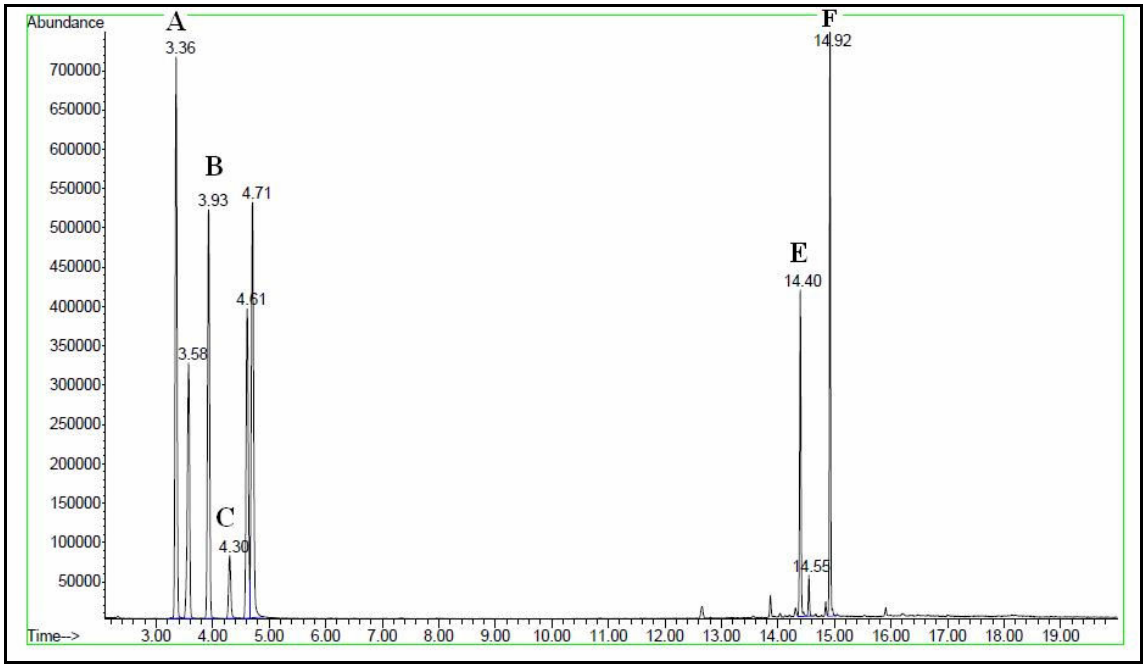


Figure 3.9 Total ion chromatogram for a standard mixture at room temperature for HSA.

Table 3.9 Area percent report for HSA for standard mixture.

HSSTDRT
Area Percent Report

Data File : C:\GOVENDER\PATRICIA\HSSTDRT.D Vial: 1
Acq On : 31 May 2007 11:17 Operator: Patricia
Sample : headspace standard room temp. Inst : Instrumen
Misc : 0.1 mL injection Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.354	206	217	232	BB	704522	16326060	100.00%	19.338%
2	3.577	239	255	270	BB	323622	8953281	54.84%	10.605%
3	3.930	303	315	330	BB	520708	13128958	80.42%	15.551%
4	4.306	367	379	395	BB	79164	2096654	12.84%	2.484%
5	4.611	415	431	439	BV	392593	10855544	66.49%	12.859%
6	4.705	439	447	471	VB 2	527091	15302297	93.73%	18.126%
7	14.400	2089	2097	2113	VB	405503	6617450	40.53%	7.838%
8	14.553	2117	2123	2133	BB 2	50065	720585	4.41%	0.854%
9	14.923	2178	2186	2192	PV	740685	10421892	63.84%	12.345%

Sum of corrected areas: 84422721

The results obtained for the peak areas of the individual standards (20 µl) as well as the combined standards consisting of 20 µl of each standard for the headspace methods are shown in Table 3.10 and these areas are represented graphically in Figures 3.10 to 3.12. The concentrations (in mg L^{-1}) of the individual standards and the combined standards are the same and are also shown in Table 3.10.

Table 3.10 Comparison of peak areas of individual standards and the combined standards for the headspace methods.

Compound	Concentration/ mg L^{-1}	HSA STD	HSA STD mixture	PDMS STD	PDMS STD mixture	PA STD	PA STD mixture
α-pinene	8.58×10^5	6.66×10^7	1.64×10^7	4.20×10^8	2.21×10^8	2.61×10^7	7.59×10^6
β-pinene	8.59×10^5	2.28×10^7	1.31×10^7	5.71×10^8	1.76×10^8	2.95×10^7	8.48×10^6
α-phellandrene	8.40×10^5	1.35×10^7	2.10×10^6	3.46×10^8	4.62×10^7	5.06×10^7	4.68×10^6
β-caryophyllene	9.02×10^5	1.58×10^6	6.62×10^6	7.44×10^7	1.80×10^8	6.92×10^6	1.42×10^7
α-caryophyllene	8.89×10^5	1.69×10^6	1.04×10^7	8.82×10^7	4.13×10^8	1.16×10^7	7.95×10^7

PDMS – polydimethylsiloxane fibre **HSA -** headspace analysis
PA – polyacrylate fibre **STD** – individual standard
STD mixture – combined standards

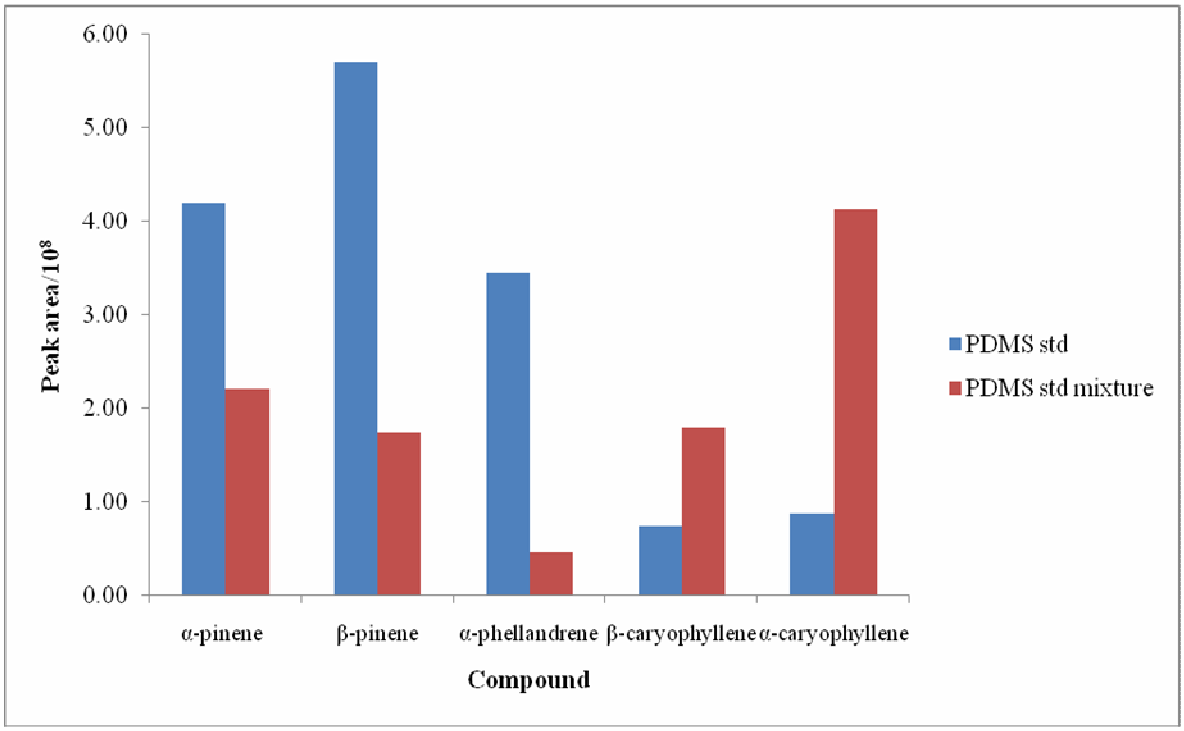


Figure 3.10 Comparison of the peak areas of the individual standards vs the adsorption capacity of the combined standards for HS-SPME (PDMS coating).

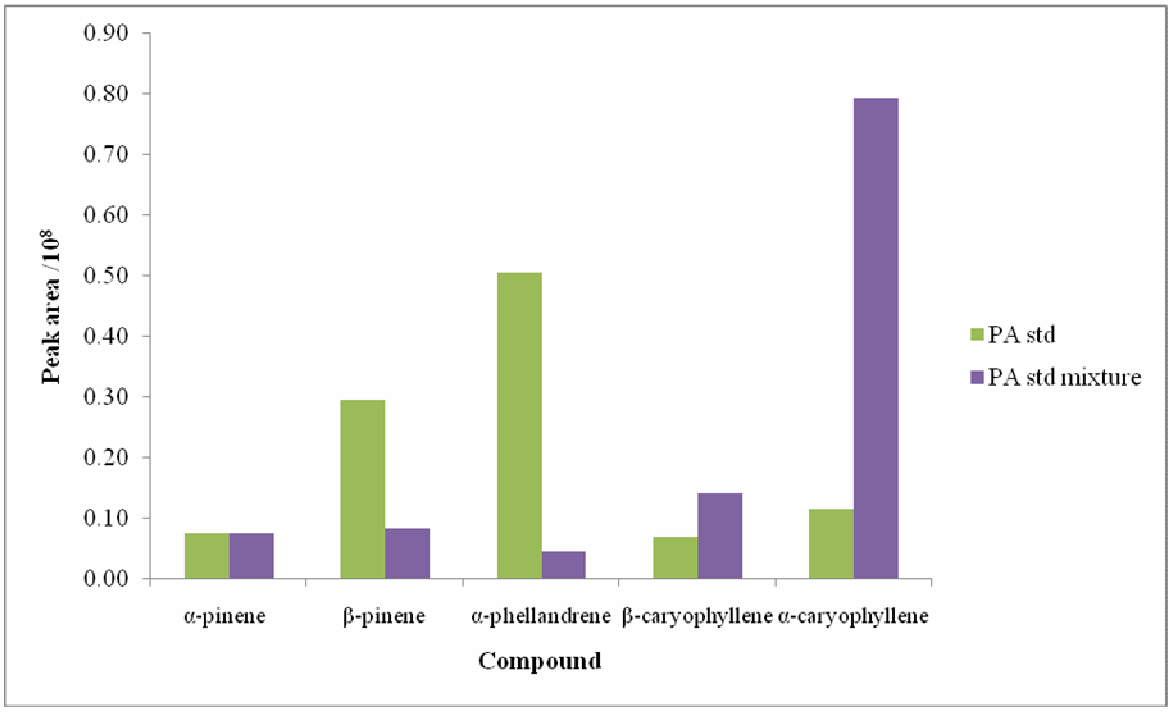


Figure 3.11 Comparison of the peak areas of the individual standards and the peak areas of the combined standards for HS-SPME (PA coating).

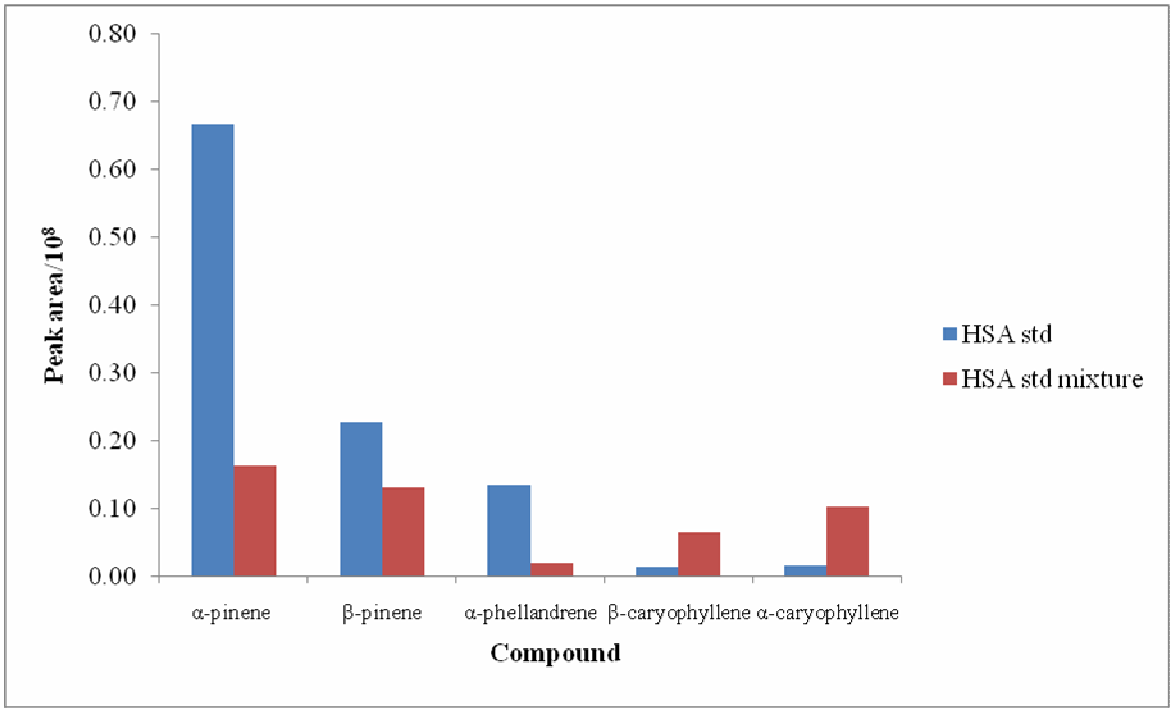


Figure 3.12 Comparison of the peak areas of the individual standards and the peak areas of the combined standards for HSA.

Examination of the results in Table 3.10, and the graphs in Figures 3.10 to 3.12, indicated that, for the monoterpenes, a larger amount of the compound was extracted for the individual standards and a smaller amount when present in the combined mixture. In contrast, for the sesquiterpenes β- and α-caryophyllene, larger amounts were extracted when the compounds were present in the combined mixture than when they were present individually.

For the HS-SPME analysis, these differences could be due to the adsorption capacity of the fibre as well as competition effects. However, this inconsistency is not unusual, since other researchers also found discrepancy in their results. Contini and Esti (2006) pointed out that competition between the compounds resulted in a loss in linearity for the compounds present in low concentration which led to distortion of the results for quantitative evaluation. Kalua *et al*. (2006) mentioned in their work that, due to the phenomenon of competition for the adsorption sites on the fibre, the results obtained will be greatly skewed. The results from this investigation indicated that competition exists between the compounds for the adsorption sites on the fibre, and the use of a single component standard to construct a calibration curve would not be recommended as it is not representative of the actual sample to be analysed which contains many components (Stashenko and Martinez, 2007). This needs to be taken into consideration for all future work. In this work a single component standard was used since the solvent, soxhlet and steam distillation methods were quantified only and not the headspace methods.

For the headspace analysis, the adsorption of high molar mass volatiles onto walls of the flask resulted in the loss of the sesquiterpenes. This problem can be circumvented by modifying the surface of the glass (e.g. polyethylene glycol to increase hydrophilicity) or by using headspace containers of other materials (Hachenberg and Schmidt, 1986).

3.3 Essential oil analysis by solvent and Soxhlet extraction

In this section the extraction of the essential oil with solvent and soxhlet extraction is discussed.

3.3.1 Extraction period

Three different extraction times were used in this investigation and these were: 24, 48 and 72 hours.

3.3.1.1 Extraction period for solvent extraction

The total ion chromatograms from the GC-MS analysis of the essential oil for the solvent extraction method are shown in Appendix D, Figures D3 to D8. A representative total ion chromatogram of the solvent extraction for the 48 hour extraction period and its area percent report is shown in Figure 3.13 and Table 3.11 respectively.

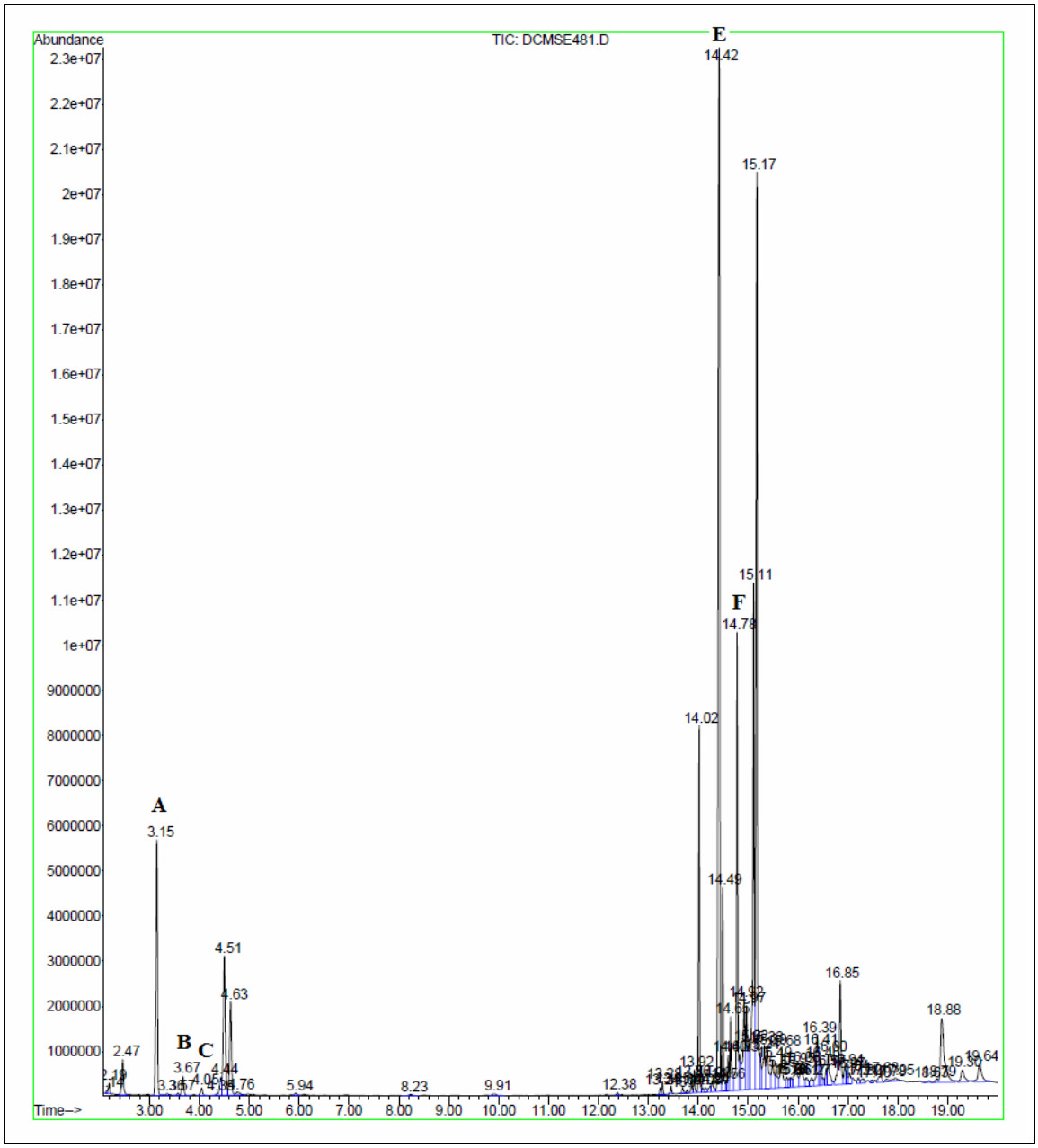


Figure 3.13 Total ion chromatogram for the essential oil obtained from the 48 hour solvent extraction.

Table 3.11 Area percent report for the essential oil obtained from the 48 hour solvent extraction.

DCMSE481
Area Percent Report

Data File : D:\PATRICIA\DCMSE481.D Vial: 50
Acq On : 1 Apr 2009 15:04 Operator: Patricia
Sample : DCM SOLVENT EXT. SAMPLE - 48 HOURS Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.144	5	11	13	BV	63760	774060	0.14%	0.030%
2	2.191	13	19	28	VV 2	225966	4580178	0.85%	0.175%
3	2.467	49	66	85	PV 2	778570	19345859	3.58%	0.741%
4	3.149	165	182	202	PV	5603421	122086931	22.60%	4.676%
5	3.354	202	217	225	VV 6	37786	1506245	0.28%	0.058%
6	3.572	237	254	260	VV 2	49637	1225198	0.23%	0.047%
7	3.672	260	271	295	VV	415912	10767699	1.99%	0.412%
8	4.048	320	335	358	VV	161111	4640575	0.86%	0.178%
9	4.347	368	386	389	VV 2	33336	815013	0.15%	0.031%
10	4.435	389	401	403	VV 2	393473	10599766	1.96%	0.406%
11	4.506	403	413	423	VV	3096496	89978574	16.66%	3.446%
12	4.629	423	434	448	VV	2063250	58264353	10.78%	2.232%
13	4.764	448	457	483	VV 3	64355	3499618	0.65%	0.134%
14	5.940	647	657	670	VV 4	45955	1467272	0.27%	0.056%
15	8.231	1019	1047	1073	BV 4	26866	1318410	0.24%	0.050%
16	9.911	1319	1333	1365	VV 6	41079	2416710	0.45%	0.093%
17	12.379	1747	1753	1761	VV	62777	1208157	0.22%	0.046%
18	13.237	1890	1899	1903	BV	151269	2541655	0.47%	0.097%
19	13.284	1903	1907	1917	VV	287817	4698006	0.87%	0.180%
20	13.454	1924	1936	1942	PV	172789	3048738	0.56%	0.117%
21	13.689	1968	1976	1985	VV	127147	2835054	0.52%	0.109%
22	13.772	1985	1990	1997	VV 3	77099	1471637	0.27%	0.056%
23	13.860	1997	2005	2011	VV	300843	5618742	1.04%	0.215%
24	13.924	2011	2016	2024	VV	501964	8658655	1.60%	0.332%
25	14.018	2024	2032	2040	VV	7993361	121883845	22.56%	4.668%
26	14.083	2040	2043	2048	VV 4	104645	2216984	0.41%	0.085%
27	14.142	2048	2053	2060	VV 7	102382	3230604	0.60%	0.124%
28	14.242	2060	2070	2072	VV 7	133543	4133768	0.77%	0.158%
29	14.277	2072	2076	2088	VV 2	246026	7154727	1.32%	0.274%
30	14.418	2088	2100	2107	VV	22992682	540249030	100.00%	20.692%
31	14.489	2107	2112	2122	VV 2	4384073	69215941	12.81%	2.651%
32	14.565	2122	2125	2127	VV 2	211274	3243334	0.60%	0.124%
33	14.600	2127	2131	2135	VV	780011	13171605	2.44%	0.504%
34	14.647	2135	2139	2150	VV	1634290	34362420	6.36%	1.316%
35	14.782	2150	2162	2167	VV	9869668	160459218	29.70%	6.146%
36	14.829	2167	2170	2174	VV	792886	17864679	3.31%	0.684%
37	14.923	2174	2186	2190	VV 3	1960672	58458479	10.82%	2.239%
38	14.964	2190	2193	2199	VV	1829725	39554900	7.32%	1.515%
39	15.017	2199	2202	2205	VV	1016331	20538660	3.80%	0.787%
40	15.111	2205	2218	2222	VV	11167634	216507177	40.08%	8.292%
41	15.176	2222	2229	2238	VV 2	20293825	415100516	76.84%	15.899%
42	15.241	2238	2240	2245	VV	796560	15295043	2.83%	0.586%
43	15.329	2245	2255	2258	VV	957886	30169062	5.58%	1.155%

DCMSE481

44	15.387	2258	2265	2276	VV	4	916746	32843091	6.08%	1.258%
45	15.487	2276	2282	2290	VV	3	604018	20251014	3.75%	0.776%
46	15.558	2290	2294	2303	VV		407219	13273188	2.46%	0.508%
47	15.675	2303	2314	2327	VV		855343	27812397	5.15%	1.065%
48	15.781	2327	2332	2336	VV	4	226665	6121343	1.13%	0.234%
49	15.834	2336	2341	2343	VV	6	213667	4630571	0.86%	0.177%
50	15.858	2343	2345	2350	VV	6	216556	5044244	0.93%	0.193%
51	15.952	2350	2361	2372	VV	7	458766	22868236	4.23%	0.876%
52	16.057	2372	2379	2393	VV	5	469789	21581190	3.99%	0.827%
53	16.163	2393	2397	2407	VV	9	186956	7606869	1.41%	0.291%
54	16.275	2407	2416	2423	VV	4	182614	7713352	1.43%	0.295%
55	16.386	2423	2435	2438	VV		1125494	26109833	4.83%	1.000%
56	16.416	2438	2440	2444	VV	2	858414	14809616	2.74%	0.567%
57	16.457	2444	2447	2454	VV	2	544584	11743648	2.17%	0.450%
58	16.563	2460	2465	2467	VV	2	367667	6549911	1.21%	0.251%
59	16.604	2467	2472	2486	VV		672543	20834285	3.86%	0.798%
60	16.851	2493	2514	2523	VV		2274880	65578005	12.14%	2.512%
61	16.933	2523	2528	2532	VV	5	368500	9520870	1.76%	0.365%
62	16.968	2532	2534	2540	VV	6	299925	5903328	1.09%	0.226%
63	17.039	2540	2546	2554	VV	5	235530	8032774	1.49%	0.308%
64	17.209	2569	2575	2581	VV	9	121119	3676010	0.68%	0.141%
65	17.303	2581	2591	2599	VV	6	99778	4598306	0.85%	0.176%
66	17.479	2614	2621	2626	VV	10	56793	1818953	0.34%	0.070%
67	17.632	2636	2647	2662	VV	10	126624	6934905	1.28%	0.266%
68	17.779	2662	2672	2683	PV	10	53809	2533697	0.47%	0.097%
69	17.949	2683	2701	2718	VV	10	59189	4045832	0.75%	0.155%
70	18.631	2793	2817	2827	BV	10	30152	851536	0.16%	0.033%
71	18.789	2827	2844	2848	PV	5	73530	2722536	0.50%	0.104%
72	18.878	2848	2859	2905	VV	4	1410097	74888207	13.86%	2.868%
73	19.295	2913	2930	2949	VV	3	257021	12706438	2.35%	0.487%
74	19.641	2966	2989	3029	VV	3	396812	21147753	3.91%	0.810%

Sum of corrected areas: 2610929033

Table 3.12 shows the average peaks areas for a single extraction of the essential oil during the solvent extraction for the different extraction periods as well as the relative standard deviations for the triplicate injections. The raw data can be found in Appendix E, Tables E11 to E15.

Table 3.12 Peak areas for the selected compounds for solvent extraction with dichloromethane for different extraction periods.

Extraction period/hours	**24**	**48**	**72**
α-pinene	9.8×10^7 (1.2)	1.2×10^8 (2.9)	9.5×10^7 (0.4)
β-pinene	9.2×10^6 (0.9)	1.0×10^7 (3.2)	8.7×10^6 (2.3)
α-phellandrene	5.3×10^6 (4.2)	4.4×10^6 (4.5)	3.0×10^6 (1.8)
β-caryophyllene	5.2×10^8 (1.7)	5.3×10^8 (2.7)	4.8×10^8 (3.4)
α-caryophyllene	1.5×10^8 (3.5)	1.6×10^8 (1.9)	1.4×10^8 (4.7)

Figures in parentheses are % RSD.

The results in Table 3.12 showed that the extraction after the 48 hour period produced the largest amount of the compounds of interest. This investigation was conducted on three separate samples. The results showed a decrease in the amounts of compounds extracted after 72 hours. It should be noted that this experiment was conducted on an orbital shaker on a bench top and was exposed to light. Therefore, this decrease could be due to the limited chemical stability of the terpenes due to photolysis, oxidation and other reactions as reported in literature. Work undertaken by Augusto *et al.* (2003) showed that the atmospheric chemical lifetime of monoterpenes during daylight conditions was found to be less than 5 minutes for α-terpinene to three hours for α-pinene, β-pinene and sabinene. Thus, to prevent loss in the amounts obtained, the flasks should be covered with foil in future investigations.

3.3.1.2 Extraction period for Soxhlet extraction

The total ion chromatograms obtained for the oil from the Soxhlet extraction method are shown in Appendix D, Figures D3 to D8. A representative total ion chromatogram of the Soxhlet extraction for the 48 hour extraction period together with its area percent report is shown in Figure 3.14 and Table 3.13 respectively.

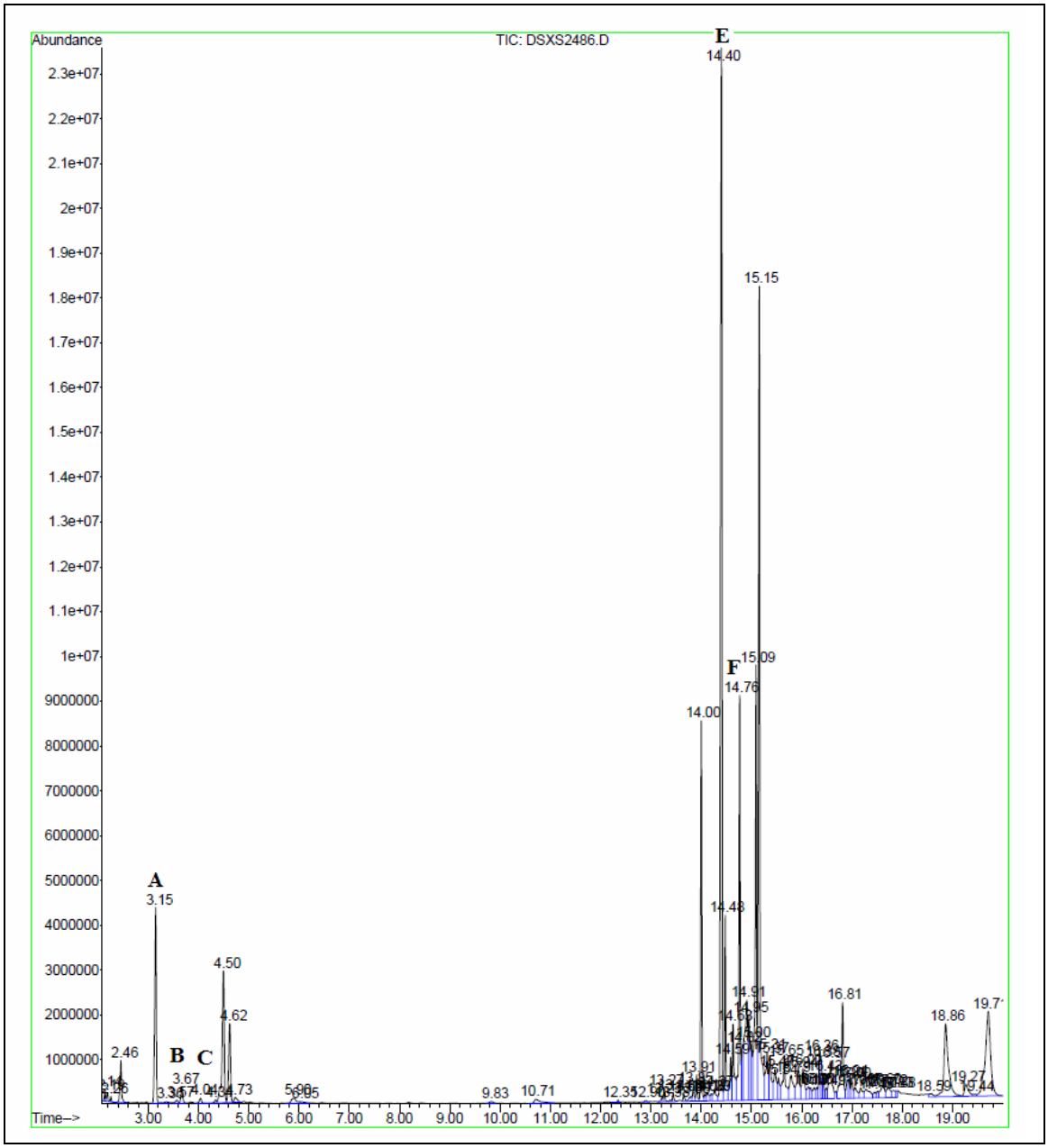


Figure 3.14 Total ion chromatogram for the essential oil obtained from the 48 hour Soxhlet extraction.

Table 3.13 Area percent report for the essential oil obtained from the 48 hour Soxhlet extraction.

DSXS2486
Area Percent Report

Data File : D:\PATRICIA\DSXS2486.D Vial: 34
Acq On : 26 Mar 2009 15:38 Operator: PATRICIA
Sample : DCM SOX. EXT. SAMPLE 2 48 HOURS Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.138	4	10	14	BV	245845	3259103	0.65%	0.115%
2	2.191	14	19	26	VV	192244	3713517	0.74%	0.131%
3	2.255	26	30	47	VV	117466	1579452	0.31%	0.056%
4	2.455	47	64	84	PV 2	917744	19752914	3.92%	0.699%
5	3.148	171	182	200	PV	4240338	90851725	18.03%	3.216%
6	3.360	207	218	231	VV	32584	1536408	0.30%	0.054%
7	3.571	244	254	261	VV	69892	2230917	0.44%	0.079%
8	3.671	261	271	282	VV	352459	8920841	1.77%	0.316%
9	4.041	318	334	355	VV	108213	3306111	0.66%	0.117%
10	4.341	355	385	390	PV	67979	1722374	0.34%	0.061%
11	4.500	390	412	422	VV	2952790	91121034	18.08%	3.226%
12	4.623	422	433	443	VV	1765037	47417132	9.41%	1.679%
13	4.735	443	452	473	VV 3	111471	4931677	0.98%	0.175%
14	5.898	621	650	671	PV 7	114364	8055949	1.60%	0.285%
15	6.051	671	676	703	VV 7	33803	1876858	0.37%	0.066%
16	9.829	1303	1319	1333	PV 5	38056	1759574	0.35%	0.062%
17	10.716	1446	1470	1530	BV 2	87531	7503339	1.49%	0.266%
18	12.350	1724	1748	1756	PV	49821	1114484	0.22%	0.039%
19	12.902	1832	1842	1853	VV 3	36398	1150778	0.23%	0.041%
20	13.225	1880	1897	1900	VV 3	121531	2737885	0.54%	0.097%
21	13.272	1900	1905	1919	VV	291398	5235931	1.04%	0.185%
22	13.384	1919	1924	1928	VV 5	38553	774093	0.15%	0.027%
23	13.442	1928	1934	1947	VV	200589	3906368	0.78%	0.138%
24	13.654	1963	1970	1981	VV	156928	3629774	0.72%	0.128%
25	13.795	1981	1994	1997	VV 2	110423	3849879	0.76%	0.136%
26	13.848	1997	2003	2008	VV	350496	7181032	1.43%	0.254%
27	13.907	2008	2013	2021	VV	571251	9968757	1.98%	0.353%
28	14.001	2021	2029	2037	VV	8273397	128948396	25.59%	4.565%
29	14.077	2037	2042	2045	VV 4	155427	3679681	0.73%	0.130%
30	14.124	2045	2050	2058	VV 3	178195	6095283	1.21%	0.216%
31	14.200	2058	2063	2066	VV 3	159697	4106702	0.81%	0.145%
32	14.265	2066	2074	2085	VV 3	266329	10970016	2.18%	0.388%
33	14.400	2085	2097	2103	VV	23463433	503919918	100.00%	17.839%
34	14.476	2103	2110	2119	VV 2	4038848	65095957	12.92%	2.304%
35	14.588	2119	2129	2132	VV	951921	25227291	5.01%	0.893%
36	14.635	2132	2137	2147	VV	1683352	41759501	8.29%	1.478%
37	14.764	2147	2159	2164	VV	9154847	151449347	30.05%	5.361%
38	14.823	2164	2169	2171	VV	1237030	24849438	4.93%	0.880%
39	14.905	2171	2183	2188	VV 4	2233024	98371717	19.52%	3.482%
40	14.952	2188	2191	2196	VV	1872064	42810481	8.50%	1.515%
41	14.999	2196	2199	2203	VV	1320760	28650997	5.69%	1.014%
42	15.093	2203	2215	2219	VV	9772229	189605136	37.63%	6.712%
43	15.152	2219	2225	2242	VV 2	17858382	358990584	71.24%	12.708%

DSXS2486

44	15.311	2242	2252	2258	VV	2	1062251	39815805	7.90%	1.409%
45	15.370	2258	2262	2272	VV	3	971083	29604328	5.87%	1.048%
46	15.469	2272	2279	2287	VV	4	677699	26242052	5.21%	0.929%
47	15.540	2287	2291	2297	VV	3	489298	14050453	2.79%	0.497%
48	15.652	2297	2310	2324	VV		910009	37949948	7.53%	1.343%
49	15.787	2324	2333	2345	VV	3	539660	28547085	5.67%	1.011%
50	15.940	2345	2359	2366	VV	5	643844	31155664	6.18%	1.103%
51	16.022	2366	2373	2384	VV	4	681783	25591119	5.08%	0.906%
52	16.133	2384	2392	2396	VV	10	274145	10649259	2.11%	0.377%
53	16.175	2396	2399	2404	VV	4	251010	6104191	1.21%	0.216%
54	16.245	2404	2411	2416	VV	4	256108	9334520	1.85%	0.330%
55	16.304	2416	2421	2424	VV	2	259213	6539210	1.30%	0.231%
56	16.357	2424	2430	2433	VV		1012717	21533213	4.27%	0.762%
57	16.386	2433	2435	2440	VV	3	887259	16092028	3.19%	0.570%
58	16.427	2440	2442	2448	VV		551351	11048058	2.19%	0.391%
59	16.492	2448	2453	2455	VV	5	239804	5873331	1.17%	0.208%
60	16.574	2455	2467	2481	VV		840221	35942739	7.13%	1.272%
61	16.815	2488	2508	2517	VV		2111833	66865763	13.27%	2.367%
62	16.903	2517	2523	2526	VV	4	417363	10315701	2.05%	0.365%
63	16.938	2526	2529	2534	VV	3	426392	9876363	1.96%	0.350%
64	16.997	2534	2539	2545	VV	3	395403	11349872	2.25%	0.402%
65	17.062	2545	2550	2562	VV	3	240922	10721053	2.13%	0.380%
66	17.173	2562	2569	2580	VV	6	226640	10382081	2.06%	0.368%
67	17.279	2580	2587	2609	VV	8	198052	14671729	2.91%	0.519%
68	17.444	2609	2615	2620	VV	9	129372	4219736	0.84%	0.149%
69	17.502	2620	2625	2629	VV	7	150855	4206334	0.83%	0.149%
70	17.602	2629	2642	2653	VV	7	245326	16028070	3.18%	0.567%
71	17.720	2653	2662	2673	VV	4	222981	12686397	2.52%	0.449%
72	17.831	2673	2681	2687	VV	4	154215	7257734	1.44%	0.257%
73	17.884	2687	2690	2693	VV	5	148516	3077645	0.61%	0.109%
74	18.584	2798	2809	2821	VV	5	70157	4120403	0.82%	0.146%
75	18.866	2821	2857	2909	VV	3	1620345	100798698	20.00%	3.568%
76	19.271	2909	2926	2947	VV	6	261134	14447176	2.87%	0.511%
77	19.441	2947	2955	2961	VV	6	38602	1920279	0.38%	0.068%
78	19.712	2961	3001	3045	VBA2		1882065	142223158	28.22%	5.035%

Sum of corrected areas: 2824859551

A discussion of the Soxhlet extraction performed for the different extraction periods is presented here. The peaks areas for the essential oil obtained from Soxhlet extraction for the various extraction periods are indicated in Table 3.14. Also included in this table are the relative standard deviations (RSD) for the triplicate injections. The raw data is contained in Appendix E, from Tables E16 to E20.

Table 3.14 Peak areas for the essential oil obtained from Soxhlet extraction with dichloromethane for differing extraction periods.

Extraction period/hours	**24**	**48**	**72**
α-pinene	6.6×10^7 (4.1)	9.3×10^7 (4.0)	9.0×10^7 (2.9)
β-pinene	5.4×10^6 (4.1)	8.7×10^6 (3.2)	8.2×10^6 (4.0)
α-phellandrene	1.8×10^6 (2.4)	3.3×10^6 (1.6)	2.6×10^6 (4.7)
β-caryophyllene	3.8×10^8 (4.8)	5.1×10^8 (1.6)	4.6×10^8 (2.0)
α-caryophyllene	9.1×10^7 (2.1)	1.6×10^8 (2.3)	1.3×10^8 (1.1)

Figures in parentheses are % RSD.

The average peak areas obtained for the essential oils in the fresh leaves of *M. koenigii* was the highest for the 48 hour extraction period for the selected compounds. The extraction was exposed to light since it was not covered with foil and therefore the decrease in the terpenes after 72 hours could be due to the same reasons already discussed in Section 3.3.1.1. In addition, the decrease could be due to thermal degradation. McGraw *et al*. (1999) showed in their work that the percent thermal degradation of β-pinene in a 72 hour extraction with heating at 120 °C, was 36%. Although McGraw *et al*. (1999) used a different technique, in our case the extraction took place at the boiling point of the solvent, 40 °C and a loss of ~6% for β-pinene indicated that thermal degradation possibly took place. Therefore to verify that the 48 hour period extracted the largest amount of analytes and to ensure that the compounds are not exposed to high temperatures for 72 hours, a sequential Soxhlet extraction was undertaken with dichloromethane and the results for this investigation are discussed in the next section.

3.3.1.3 Sequential Soxhlet extraction

In this study, Soxhlet extraction was performed for the verification of the optimium extraction period and the chromatograms for these extractions are displayed in Figures 3.15 to 3.17. After the first extraction with 300 mL of dichloromethane for a 24 hour period, the chromatogram in Figure 3.15 was obtained.

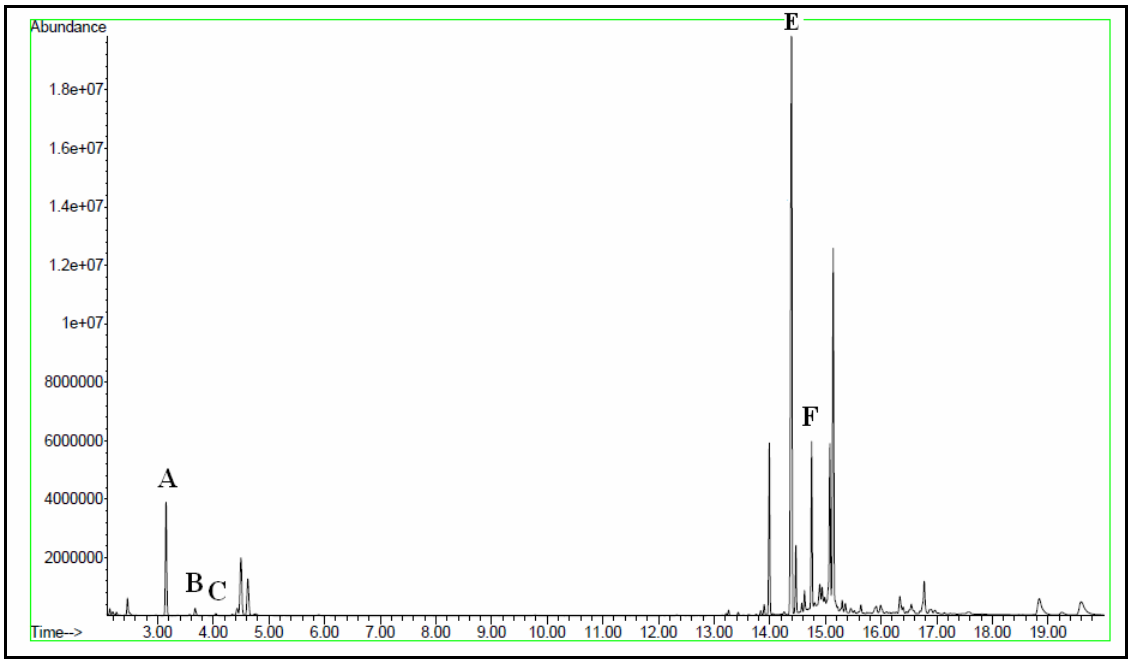


Figure 3.15 Total ion chromatogram for the first Soxhlet extraction, with 300 mL of dichloromethane, i.e. after 24 hours of extraction.

After the first 24 hour extraction period was complete, the dichloromethane extract was removed and a second 300 mL aliquot of dichloromethane was added to the same sample of leaves for a further 24 hour extraction (i.e 48 hours extraction on the same sample) and the chromatogram shown in Figure 3.16 was obtained.

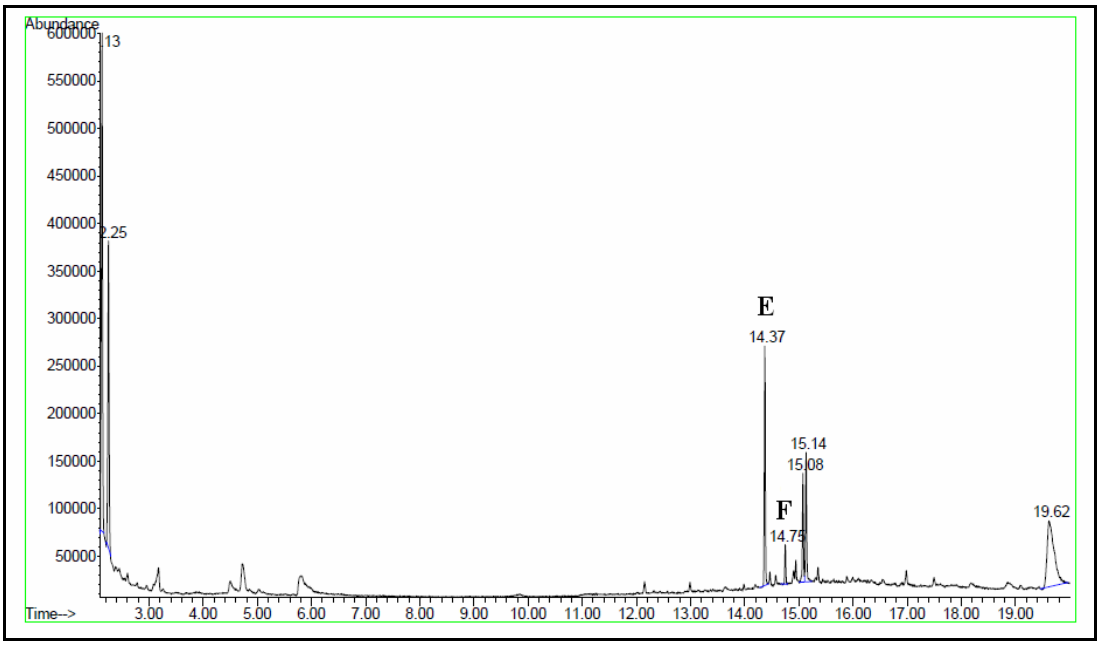


Figure 3.16 Total ion chromatogram for the second Soxhlet extraction, i.e the leaves were extracted for a total of 48 hours, with fresh dichloromethane on the same leaves as previously extracted.

The chromatogram in Figure 3.16 showed that β-caryophyllene (E) and α-caryophyllene (F) as well as the sesquiterpenes, β-selinene (15.08 min retention time) and valencene (15. 14 min) were still being extracted when the second aliquot of dichloromethane was added after the first 24 hour period, even though most hydrocarbon terpenes are extracted within 24 hours. This indicated that the 24 hour extraction was not complete. The peak at a retention time of 19.62 min is indicative of the compound, phytol.

After the second extraction process, a third 300 mL of dichloromethane was added to the same sample of leaves for a further 24 hour extraction (i.e. a total of 72 hours of extraction on the same sample) and the chromatogram in Figure 3.17 was produced.

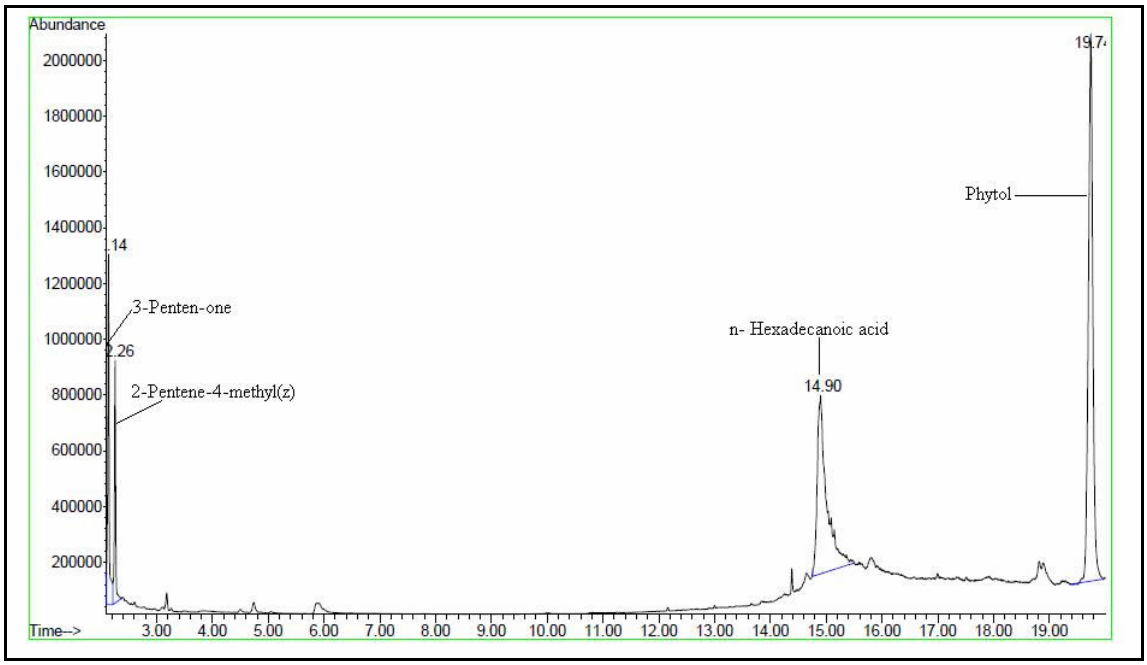


Figure 3.17 Total ion chromatogram for the third Soxhlet extraction, i.e the leaves were extracted for a total of 72 hours, with fresh dichloromethane on the same leaves as previously extracted.

The chromatogram in Figure 3.17 showed that although fresh solvent was used no compounds of interest were left after the 48 hour extraction, as no further hydrocarbons were being extracted. Other compounds identified are indicated in the above chromatogram. The presence of the large peak due to phytol, indicates that leaving the extraction period for longer periods can result in the hydrolysis of chlorophyll a to phytol (Krautler, 2002).

From these results as well as the results obtained for the earlier Soxhlet extraction in Table 3.14, the optimium extraction period for the five selected compounds was found to be 48 hours. All quantitation was performed for this extraction period for both solvent and Soxhlet extraction.

3.4 Steam distillation technique

The total ion chromatogram of the extraction of the leaves by steam distillation and its area percent report can be found in Figure 3.18 and Table 3.15 respectively.

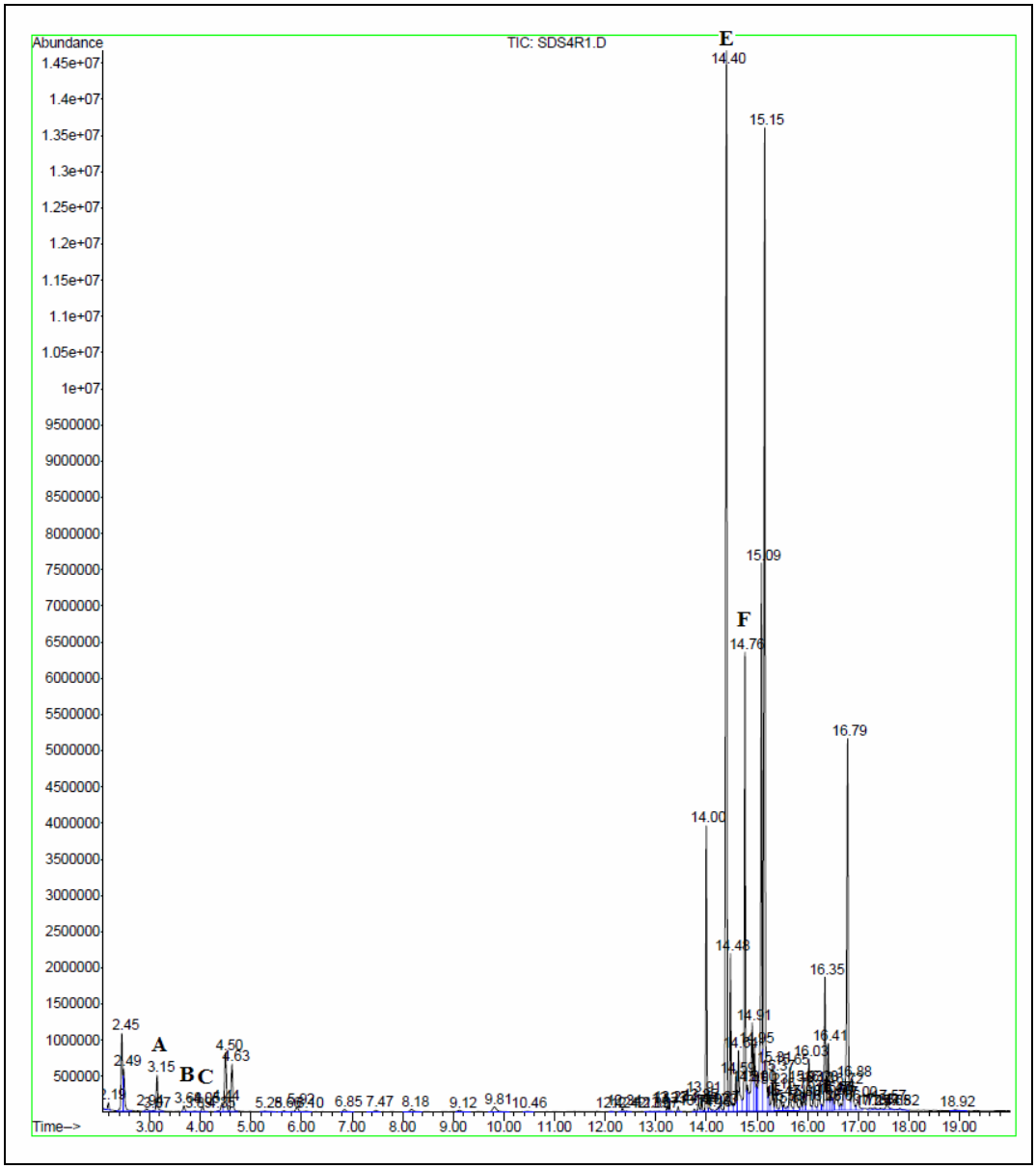


Figure 3.18 Total ion chromatogram for the essential oil obtained from the steam distillation.

Table 3.15 Area percent report for the essential oil obtained from the steam distillation.

SDS4R1
Area Percent Report

Data File : D:\PATRICIA\SDS4R1.D
Acq On : 9 Feb 2009 12:46
Sample : steam distillation run 1
Misc : 1 ul injection
Vial: 1
Operator: PATRICIA
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.191	4	19	27	BV 3	98454	1723699	0.59%	0.126%
2	2.455	46	64	68	PV	1072403	20499169	7.04%	1.495%
3	2.490	68	70	96	VV 3	579542	12356374	4.24%	0.901%
4	2.937	134	146	164	PV 5	32536	1345054	0.46%	0.098%
5	3.072	164	169	174	VV 5	10406	237590	0.08%	0.017%
6	3.154	174	183	204	VV	496121	10842140	3.72%	0.791%
7	3.683	265	273	290	VV 2	80051	1898630	0.65%	0.138%
8	3.894	290	309	323	VV 2	8188	283762	0.10%	0.021%
9	4.053	323	336	353	VV	73950	1891245	0.65%	0.138%
10	4.347	375	386	390	PV 3	11089	244046	0.08%	0.018%
11	4.435	390	401	405	VV 3	114370	3013617	1.03%	0.220%
12	4.505	405	413	425	VV	803034	20916518	7.18%	1.525%
13	4.629	425	434	453	VV	644921	16896283	5.80%	1.232%
14	5.281	530	545	581	BB	7953	355993	0.12%	0.026%
15	5.657	589	609	629	BV	13545	446788	0.15%	0.033%
16	5.916	629	653	669	VV 2	67806	2579430	0.89%	0.188%
17	6.098	669	684	701	VV 2	9775	556930	0.19%	0.041%
18	6.856	799	813	842	VV 4	32269	1284425	0.44%	0.094%
19	7.473	888	918	933	BV 5	21567	856571	0.29%	0.062%
20	8.178	1010	1038	1072	BV 2	33159	1714950	0.59%	0.125%
21	9.124	1187	1199	1234	VV 6	20769	1093804	0.38%	0.080%
22	9.817	1298	1317	1365	PV 7	67754	4025028	1.38%	0.294%
23	10.463	1415	1427	1447	VV 9	7865	384470	0.13%	0.028%
24	12.120	1702	1709	1717	PV 6	5784	140048	0.05%	0.010%
25	12.344	1734	1747	1755	BV	49648	986467	0.34%	0.072%
26	12.426	1755	1761	1776	VB 6	10112	227331	0.08%	0.017%
27	12.896	1822	1841	1847	VV 7	6868	182414	0.06%	0.013%
28	13.072	1847	1871	1883	VV 7	7729	325599	0.11%	0.024%
29	13.225	1883	1897	1901	VV	80386	1351230	0.46%	0.099%
30	13.272	1901	1905	1918	VV	98805	1606900	0.55%	0.117%
31	13.442	1928	1934	1946	VV	67539	1072046	0.37%	0.078%
32	13.765	1982	1989	1996	PV 3	34907	661212	0.23%	0.048%
33	13.848	1996	2003	2009	VV	120636	2260920	0.78%	0.165%
34	13.906	2009	2013	2023	VV	219666	3375228	1.16%	0.246%
35	14.000	2023	2029	2036	VV	3881945	55424516	19.03%	4.042%
36	14.071	2036	2041	2047	VV 6	54733	1518075	0.52%	0.111%
37	14.124	2047	2050	2059	VV 9	22093	662052	0.23%	0.048%
38	14.235	2059	2069	2071	VV 3	55995	1302132	0.45%	0.095%
39	14.271	2071	2075	2085	VV	95666	2503804	0.86%	0.183%
40	14.400	2085	2097	2103	VV	14465705	291303001	100.00%	21.243%
41	14.476	2103	2110	2119	VV 2	2093347	31915624	10.96%	2.327%
42	14.588	2119	2129	2133	VV	472383	9500149	3.26%	0.693%
43	14.641	2133	2138	2150	VV	808582	19307587	6.63%	1.408%

SDS4R1

44	14.764	2150	2159	2164	VV		6486061	94102364	32.30%	6.862%
45	14.811	2164	2167	2172	VV		359565	8022905	2.75%	0.585%
46	14.905	2172	2183	2188	VV	3	1205683	31205482	10.71%	2.276%
47	14.952	2188	2191	2197	VV		898950	18943279	6.50%	1.381%
48	14.999	2197	2199	2202	VV	2	370024	6161153	2.12%	0.449%
49	15.093	2202	2215	2219	VV		7386193	129379350	44.41%	9.435%
50	15.152	2219	2225	2236	VV	2	13362035	252908389	86.82%	18.443%
51	15.228	2236	2238	2243	VV		323460	5197548	1.78%	0.379%
52	15.311	2243	2252	2257	VV		629342	11299497	3.88%	0.824%
53	15.369	2257	2262	2272	VV	2	499651	8558732	2.94%	0.624%
54	15.469	2272	2279	2286	VV	4	158713	4474622	1.54%	0.326%
55	15.528	2286	2289	2296	VV	4	94370	2121746	0.73%	0.155%
56	15.651	2304	2310	2322	VV		601672	10697331	3.67%	0.780%
57	15.757	2322	2328	2343	VV	3	255859	7317059	2.51%	0.534%
58	15.875	2343	2348	2352	VV	4	153574	3096878	1.06%	0.226%
59	15.928	2352	2357	2363	VV	3	347775	6514282	2.24%	0.475%
60	16.027	2363	2374	2385	VV	2	704215	20252666	6.95%	1.477%
61	16.121	2385	2390	2401	VV		328979	7233840	2.48%	0.528%
62	16.233	2401	2409	2415	VV		347647	7869964	2.70%	0.574%
63	16.280	2415	2417	2421	VV	5	83773	1680749	0.58%	0.123%
64	16.345	2421	2428	2434	VV		1811149	32280734	11.08%	2.354%
65	16.409	2434	2439	2445	VV	2	929577	17712863	6.08%	1.292%
66	16.468	2445	2449	2452	VV	5	208603	4413101	1.51%	0.322%
67	16.562	2459	2465	2477	VV	4	212079	8879057	3.05%	0.647%
68	16.662	2477	2482	2485	VV	4	94942	2164971	0.74%	0.158%
69	16.715	2485	2491	2494	VV		321671	6174011	2.12%	0.450%
70	16.791	2494	2504	2514	VV		5071446	107236784	36.81%	7.820%
71	16.885	2514	2520	2531	VV		429075	11440728	3.93%	0.834%
72	16.997	2531	2539	2555	VV	3	133098	5226354	1.79%	0.381%
73	17.326	2588	2595	2605	VV	3	31602	1205866	0.41%	0.088%
74	17.438	2605	2614	2625	VV	3	22470	1065687	0.37%	0.078%
75	17.567	2625	2636	2648	VV	5	90606	3240092	1.11%	0.236%
76	17.655	2648	2651	2664	VV	5	17091	491814	0.17%	0.036%
77	17.825	2672	2680	2710	BV	5	19496	716942	0.25%	0.052%
78	18.924	2849	2867	2906	PV	5	15102	929199	0.32%	0.068%

Sum of corrected areas: 1371288887

The steam distillate obtained during this extraction displayed a crystalline form when recovered in its cold state. This crystalline form was obtained for all the extractions performed with a simple steam distillation set-up. The essential oil extracted from the steam distillation method was pale yellow in colour. The yields of the oil which were obtained in this work are recorded in Table 3.16.

Table 3.16 Yields of extracts of *M. koenigii* obtained from the steam distillation extraction.

Sample number	**1**	**2**	**3**
Mass of milled leaves/g	25.6	25.6	25.1
Yield of extract/g	0.50	0.43	0.59
Percentage of extract/%	1.94	1.67	2.37

The results obtained in Table 3.16 were lower for samples 1 and 2 compared to the amount obtained by Mitra (2.6%) (MacLeod and Pieris, 1982), but for sample 3 the amount obtained was close to the yield reported in literature. Studies done by other researchers, Rana *et al*. (2004), Paranagama *et al*. (2002) and Wong and Tie (1993) on *M. koenigii* leaves showed that the yield of oil obtained was in the range 0.23 % (m/m) to 0.42%(m/m). These differences in yield could be due to geographical location, environmental factors and seasonal variation. The variability in the results could also be due to the problems inherent with the steam distillation technique such as the vulnerability of the monoterpenes to steam distillation and loss of volatiles during the evaporation step.

3.4.1 Method precision

The precision was determined for the triplicate extractions (done in parallel) of the same batch of leaves and these results including the average peak areas and corresponding standard deviations are shown in Tables 3.17 to 3.19. The peak area, standard deviation as well as the RSD values for the individual samples can be found in Appendix E, Tables E21 to E25.

Table 3.17 Peak areas, standard deviation and relative standard deviation for the triplicate injections of the essential oil obtained from the steam distillation method for sample 1.

Sample	**1**	
Compound	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	$(4.8 \pm 0.1) \times 10^5$	2.6
β-pinene	$(2.20 \pm 0.06) \times 10^5$	2.8
α-phellandrene	$(2.5 \pm 0.1) \times 10^5$	4.4
β-caryophyllene	$(2.64 \pm 0.03) \times 10^8$	1.3
α-caryophyllene	$(9.1 \pm 0.1) \times 10^7$	1.8

Table 3.18 Peak areas, standard deviation and relative standard deviation for the triplicate injections of the essential oil obtained from the steam distillation method for sample 2.

Sample	**2**	
Compound	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	$(5.34 \pm 0.09) \times 10^6$	1.6
β-pinene	$(1.20 \pm 0.02) \times 10^6$	2.1
α-phellandrene	$(1.09 \pm 0.02) \times 10^6$	1.5
β-caryophyllene	$(2.65 \pm 0.01) \times 10^8$	0.5
α-caryophyllene	$(8.4 \pm 0.2) \times 10^7$	1.9

Table 3.19 Peak areas, standard deviation and relative standard deviation for the triplicate injections of the essential oil obtained from the steam distillation method for sample 3.

Sample	**3**	
Compound	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	$(1.05 \pm 0.03) \times 10^7$	3.0
β-pinene	$(1.90 \pm 0.002) \times 10^6$	0.1
α-phellandrene	$(1.84 \pm 0.05) \times 10^6$	3.0
β-caryophyllene	$(2.86 \pm 0.05) \times 10^8$	1.6
α-caryophyllene	$(9.1 \pm 0.2) \times 10^7$	2.4

From the tables, the percent relative standard deviation (%RSD) values obtained for the trplicate injections of the essential oils were found to be between 0.1 and 3.0%.

The reproducibility of the steam distillation method on the same batch of leaves, given by the percent relative standard deviation (%RSD) values obtained in Table 3.20 for the analysis of the sesquiterpenes (β-caryophyllene and α-caryophyllene), in the essential oils were found to be the same, i.e. 4.6%. The RSDs for the monoterpenes, however, were found to be high and this could be due to the loss of monoterpenes during the extraction step, as well as during the back extraction of the compounds into the organic phase using dichloromethane. As already mentioned in Chapter 1, Section 1.4.3, the monoterpenes are vulnerable to steam distillation and loss of the compounds can occur (Diaz-Maroto *et al*., 2002). Also, concentrating the oil by removing the solvent on the rotary evaporator could have resulted in the decrease of the monoterpenes during this evaporation step. Given that it was the same batch of homogenised leaves which was used it is unlikely to have any variation in the plants.

Table 3.20 Statistics for the reproducibility of the steam distillation method.

Compound	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	$(5.5 \pm 5.0) \times 10^6$	92.3
β-pinene	$(1.10 \pm 0.8) \times 10^6$	76.2
α-phellandrene	$(1.1 \pm 0.8) \times 10^6$	74.6
β-caryophyllene	$(2.7 \pm 0.1) \times 10^8$	4.6
α-caryophyllene	$(8.9 \pm 0.4) \times 10^7$	4.6

More discussion on the terpenoid profile of the oil follows in Section 3.7.

3.5 Headspace and HS-SPME extraction

3.5.1 Effect of temperature

According to researchers, Wang *et al*. (2004) and Pellati *et al*. (2005), temperature affects the equilibrium of analytes between the sample matrix and the coating of the fibre. Wang *et al*. (2004) maintained that the optimum temperature chosen for a complex matrix would depend on the analytes of interest or the compounds that need the most sensitivity, a finding which was also mentioned by Monje *et al*. (2002). The influence of temperature on the volatiles for HS-SPME and headspace analysis was investigated. For both the headspace analysis and HS-SPME, approximately 25 g milled curry leaves were extracted at four different temperatures: room temperature (~22 ºC), 40 °C, 60 °C and 80 °C. These temperatures were also chosen since it has been reported previously in literature that some terpenes, e.g. β-caryophyllene, can be oxidised at high temperatures (Hamm *et al*., 2003).

The average peak area counts and the percent relative standard deviation (%RSD) of the selected hydrocarbons for the HS-SPME and headspace analysis of *M. koenigii* at different temperatures are recorded in Table 3.21. Each result shows the mean for three separate sample analyses. The individual results for the mean, standard deviation and RSDs are shown in Appendix E, Tables E29 to E43.

Table 3.21 Average area count for HSA and HS-SPME extraction of the essential oil in *M. koenigii.*[†]

Compound and Extraction method	**Extraction Temperature RT (~22 °C)**	**40 °C**	**60 °C**	**80 °C**
α-pinene	**Average peak areas**	**Average peak areas**	**Average peak areas**	**Average peak areas**
PDMS	2.1×10^7 (9.2)	5.3×10^7 (9.5)	1.9×10^8 (7.7)	3.4×10^8 (16.9)
PA	3.5×10^5 (3.3)	2.0×10^6 (3.6)	1.2×10^7 (7.7)	7.1×10^7 (18.1)
HSA	2.6×10^6 (9.2)	7.0×10^6 (13.1)	4.0×10^7 (38.2)	9.7×10^7 (52.8)
β-pinene				
PDMS	1.6×10^6 (9.9)	3.7×10^6 (4.3)	2.4×10^7 (8.5)	7.9×10^7 (14.1)
PA	2.6×10^4 (4.3)	2.2×10^5 (7.3)	1.7×10^6 (3.3)	1.0×10^7 (16.3)
HSA	8.3×10^4 (13.2)	2.1×10^5 (8.8)	1.9×10^6 (13.1)	6.8×10^6 (79.6)
α-phellandrene				
PDMS	9.6×10^5 (4.3)	2.6×10^6 (5.1)	1.5×10^7 (2.8)	5.7×10^7 (19.7)
PA	4.6×10^4 (6.5)	2.0×10^5(6.2)	1.2×10^6 (2.8)	7.4×10^6 (14.6)
HSA	5.3×10^4 (17.8)	1.4×10^5 (15.3)	1.0×10^6 (6.5)	7.4×10^6 (71.5)
β-caryophyllene				
PDMS	1.1×10^8 (6.3)	1.3×10^8 (9.5)	2.9×10^8 (5.4)	8.9×10^8 (7.0)
PA	7.0×10^6 (17.4)	1.7×10^7 (19.0)	7.2×10^7 (9.8)	2.4×10^8 (17.7)
HSA	1.7×10^6 (14.2)	2.8×10^6 (62.6)	1.4×10^7 (21.7)	4.1×10^7 (90.7)
α-caryophyllene				
PDMS	2.0×10^7 (4.0)	2.1×10^7 (8.1)	5.4×10^7 (9.0)	2.3×10^8 (7.4)
PA	1.6×10^6 (18.4)	3.0×10^6 (17.1)	1.2×10^7 (9.7)	4.1×10^7 (22.2)
HSA	2.4×10^5 (11.8)	2.8×10^5 (57.5)	1.5×10^6 (27.9)	5.4×10^6 (95.6)

PDMS – polydimethylsiloxane fibre **HSA -** headspace analysis
PA – polyacrylate fibre **RT** - room temperature
[†] The numbers in parentheses refer to the % RSD.

In order to examine the extraction efficiency for the selected compounds present in *M. koenigii,* the peak areas in Table 3.21 for the HS-SPME and HSA extractions are represented graphically in Figures 3.19 to 3.21.

3.5.2 HS-SPME (PDMS fibre coating)

For the HS-SPME extraction with the PDMS coating, the results in Table 3.21 are illustrated in Figure 3.19.

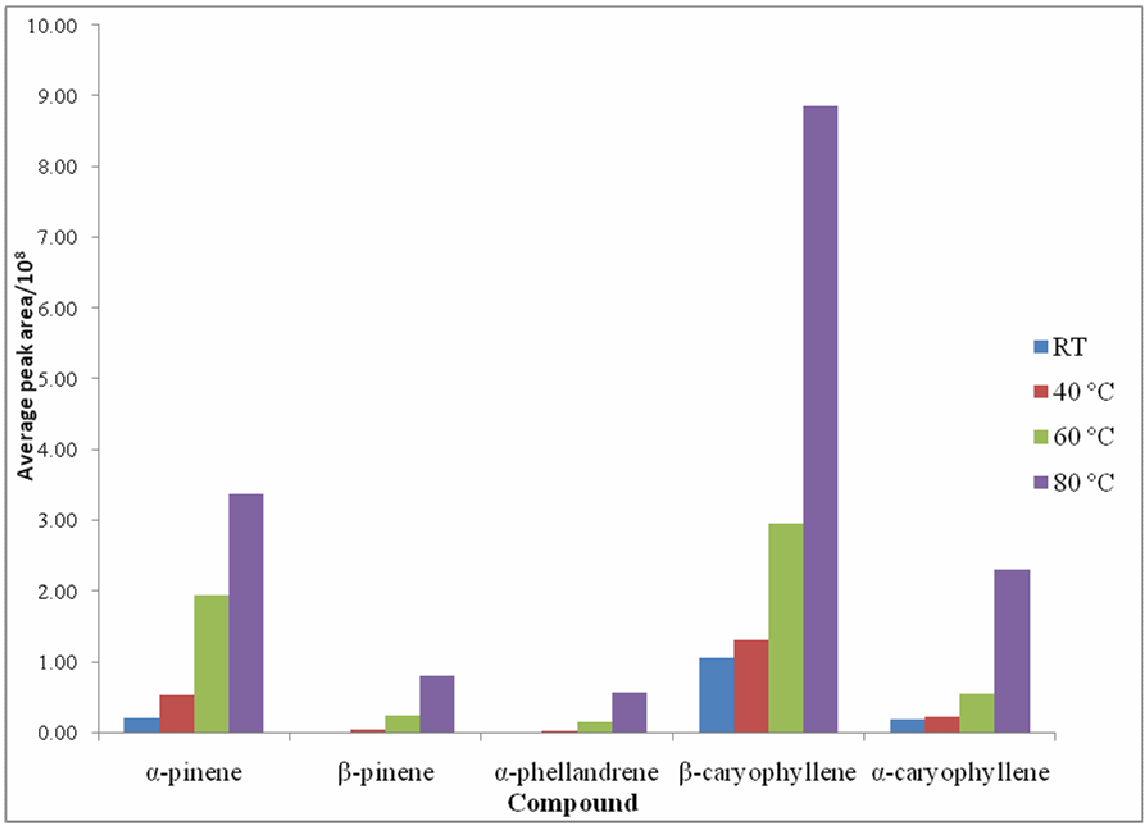


Figure 3.19 Extraction profile for the selected hydrocarbons for the HS-SPME (PDMS coating) method.

From Figure 3.19 as well as the peak areas in Table 3.21, it can be observed that there is a general increase in analyte enrichment into the fibre coating with an increase in

temperature, with the largest amount observed at 80 °C. Also, for the extraction of the sesquiterpene, β-caryophyllene, a relatively larger amount was extracted as compared to the other compounds and this could also be an indication that it may be one of the major components. A similar observation was noted from the results of the solvent extraction. Taking into account the RSD values in Table 3.21, a temperature of 60 °C will be most suitable for the extraction of these compounds, since it is a compromise between extraction efficiency and precision.

3.5.3 HS-SPME (PA fibre coating)

For the HS-SPME extraction with the PA coating, the results in Table 3.21 are represented graphically in Figures 3.20.

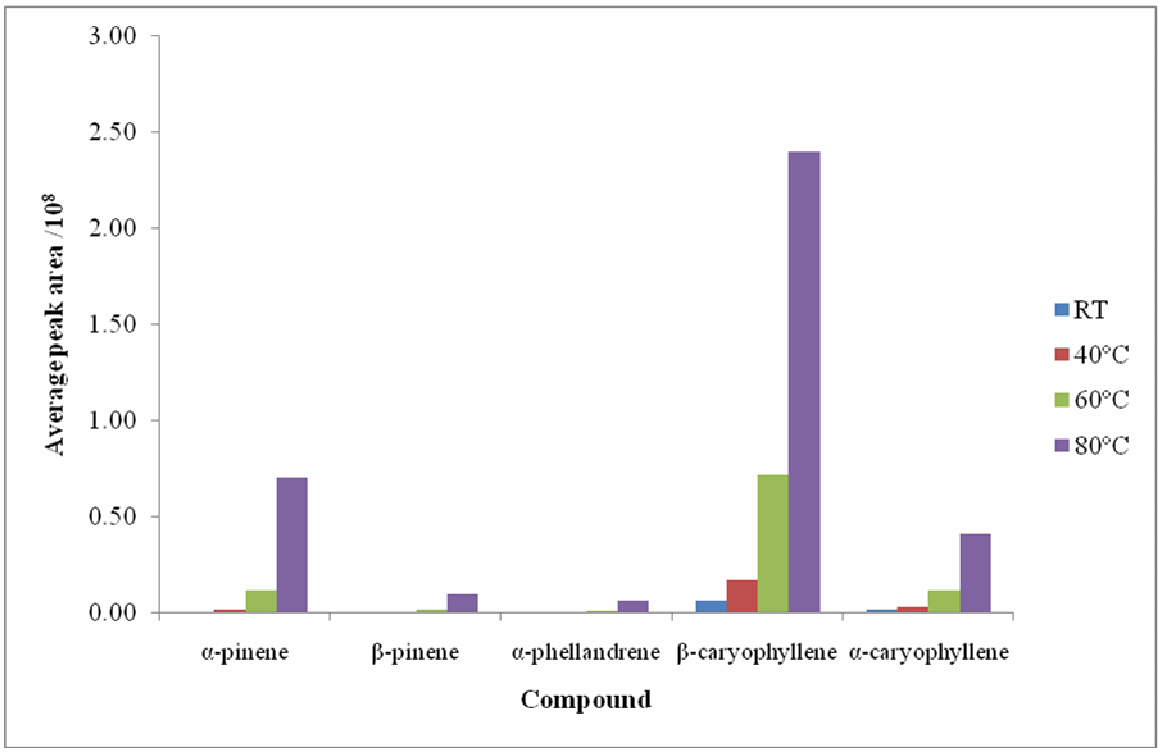


Figure 3.20 The extraction profile of the target analytes extracted by HS-SPME (PA coating) at different temperatures.

From Figure 3.20, as well as Table 3.21, a similar trend to the PDMS fibre coating was observed for the HS-SPME method with the PA coating, i.e. a general increase in the extraction efficiency with an increase in temperature. Comparing the extraction of β-caryophyllene, the amount extracted was relatively lower than the amount obtained when the PDMS coating was used. The most favourable temperature was found to be 60 °C.

3.5.4 Headspace technique

For the HSA extraction, the results in Table 3.21 are represented graphically in Figure 3.21.

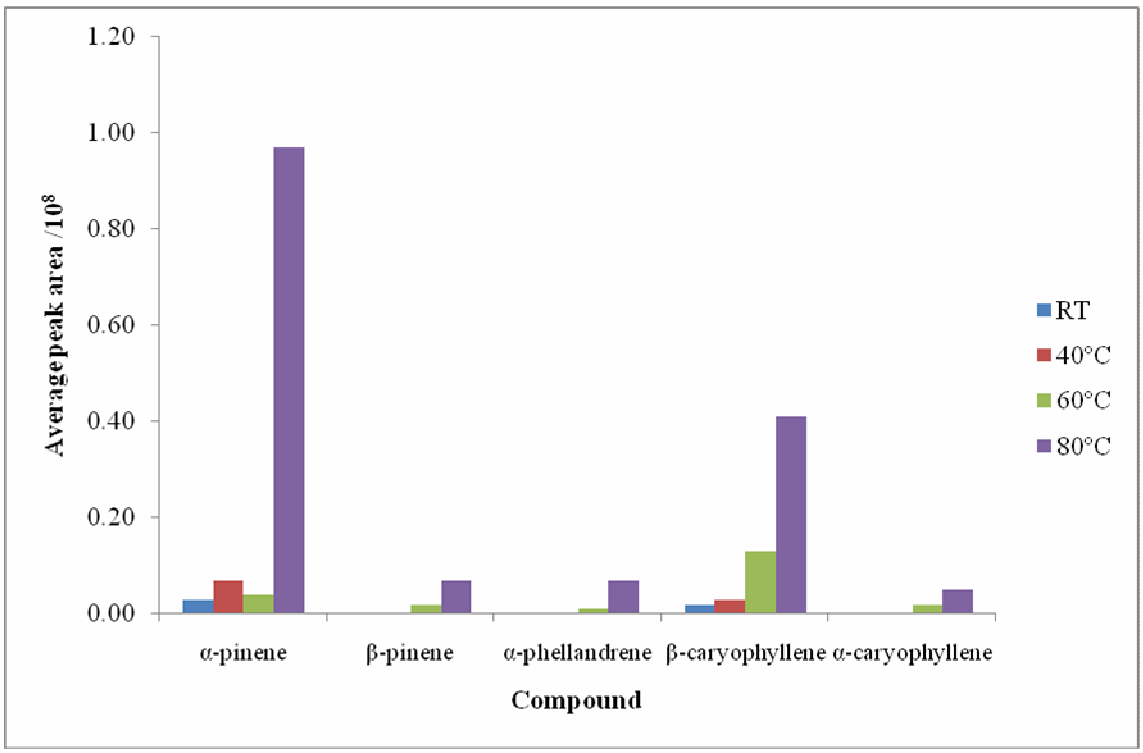


Figure 3.21 Extraction profile of the five analytes present in the headspace of *M. koenigii* at various temperatures.

From Figure 3.21, it can be seen that the largest amount extracted at a temperature of 80 °C was for the highly volatile monoterpene, α-pinene. These results differ from the results obtained for the HS-SPME with the PDMS and PA coatings, even though the same equilibration time and temperatures were used. The HS-SPME extractions showed a higher amount extracted for β-caryophyllene. The headspace method under these experimental conditions seems to favour the extraction of the more volatile compounds, e.g. α-pinene at a temperature of 80 °C. These results seem to be consistent with the findings of Manura and Overton (1999). According to them, headspace analysis is useful for the analysis of highly volatile organic compounds at the mg L^{-1} level, but less favourable for the analysis of higher boiling analytes. It should be noted that a comparison of the total amount of compounds extracted for the different headspace methods cannot be made because a 0.1 mL vapour phase sample was used for the headspace analysis and for HS-SPME, a fibre (1 cm in length) was desorbed into the GC-MS for analysis. Since no internal standard was added in these experiments, comparison can therefore only be based on the trends which were observed.

3.5.5 Extraction efficiency

In this section, the results for the investigation of the effect of temperature on extraction efficiency are discussed. Also included in this section is a discussion on the extraction profile of HS-SPME.

3.5.5.1 Influence of temperature

In order to examine the influence of temperature on the headspace composition and the extraction efficiency, the peak areas in Table 3.21 (Section 3.5.1.) for the selected compounds were plotted at the various temperatures and this can be seen in Figures 3.22 to 3.26.

The results shown in Figures 3.22 to 3.26 and in Table 3.21 indicated that the concentration of the selected hydrocarbons in the headspace and HS-SPME analyses increased with an increase in temperature and the extraction efficiency was the highest at 80 °C (as also observed in similar work investigated by Camara *et al.* (2006)).

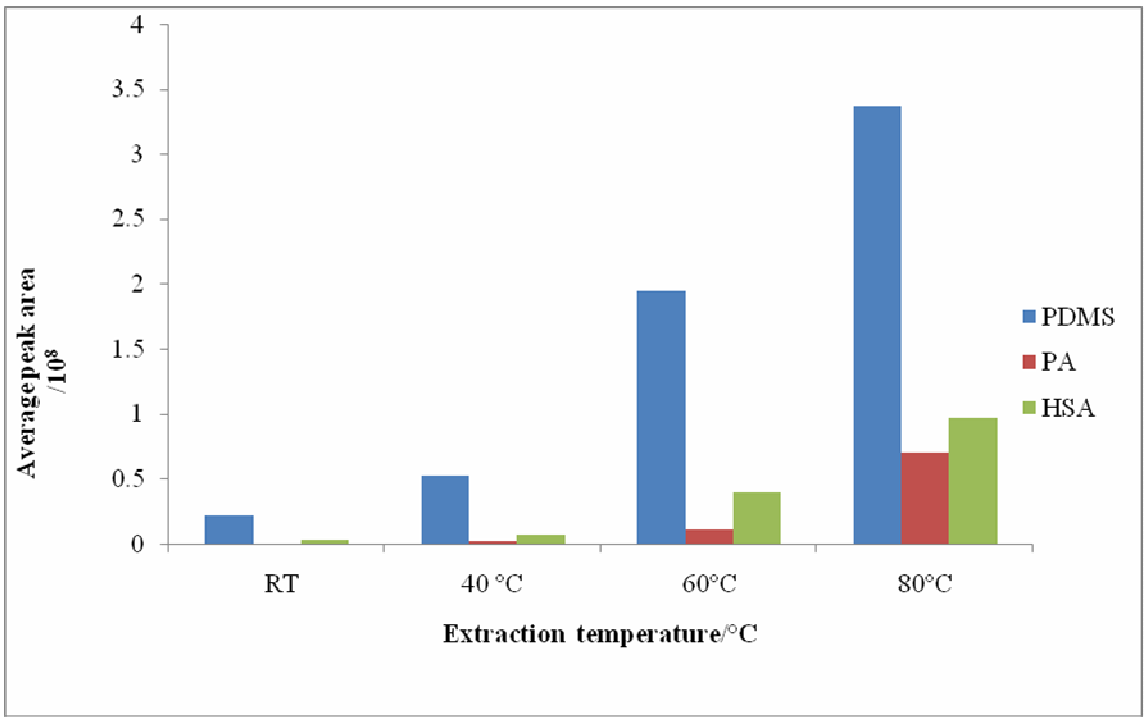


Figure 3.22 The effect of temperature on the headspace composition of α-pinene.

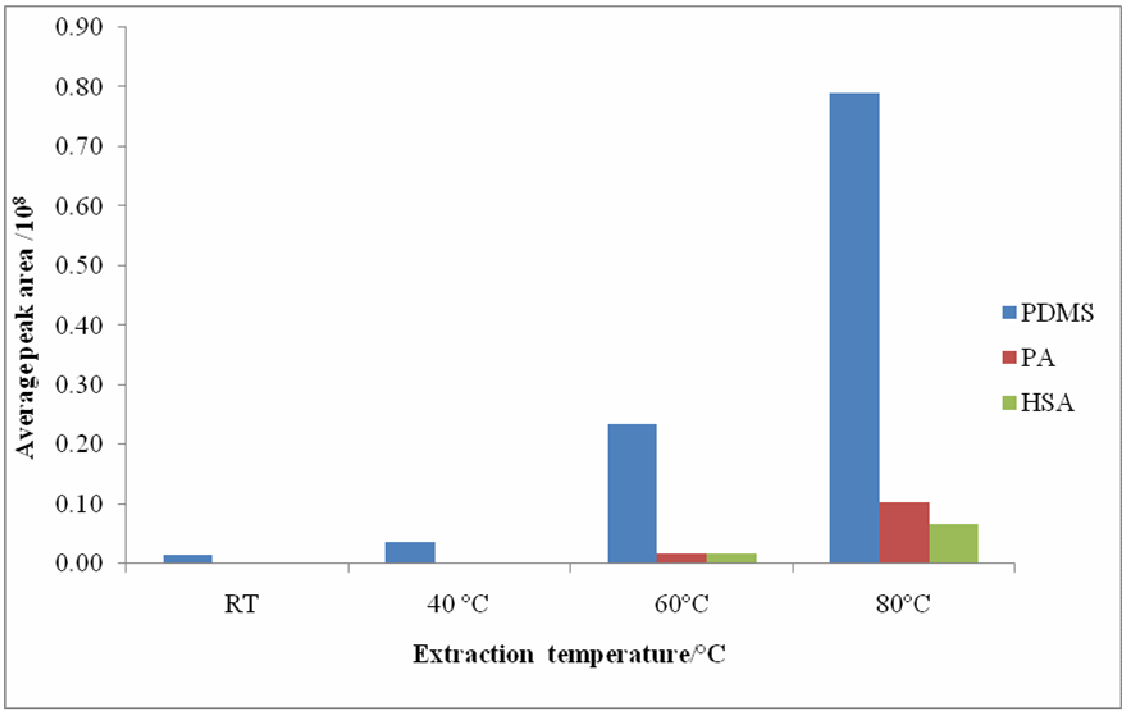


Figure 3.23 The influence of temperature on the headspace composition of β-pinene.

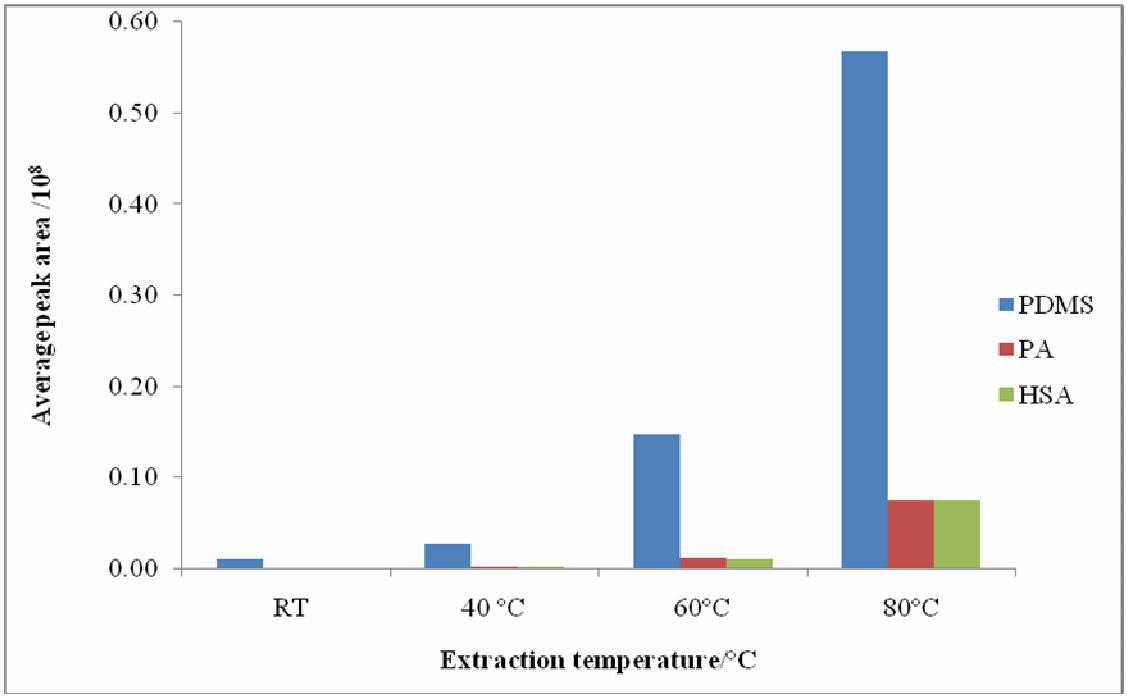


Figure 3.24 The influence of temperature on the headspace composition of α-phellandrene.

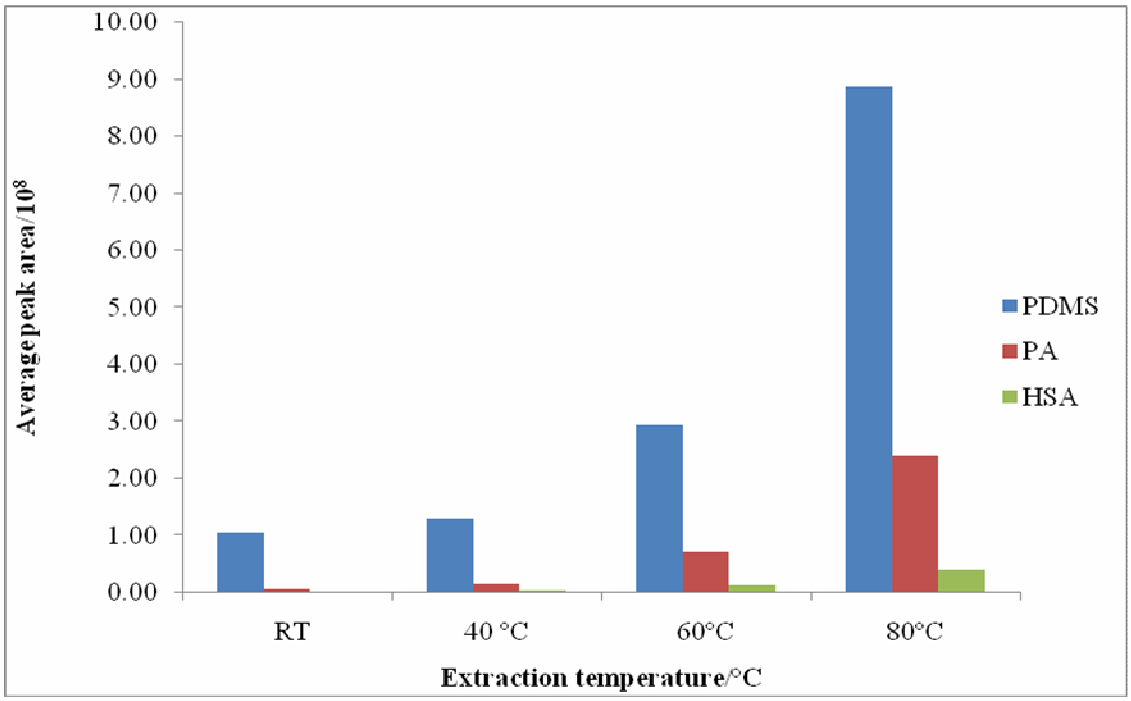


Figure 3.25 The influence of temperature on the headspace composition of β-caryophyllene.

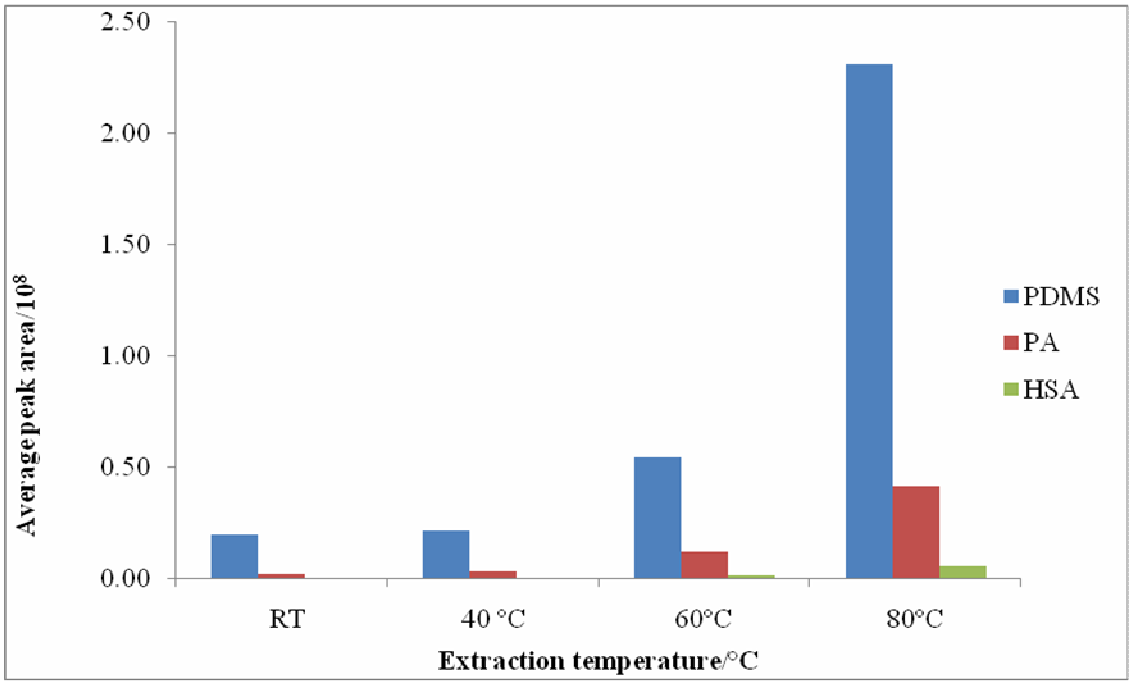


Figure 3.26 The influence of temperature on the headspace composition of α-caryophyllene.

In the present study, the increase in the hydrocarbons in the headspace at high temperatures was due to the increase of the concentration of compounds with low volatility (e.g. β-caryophyllene) in the gas phase. A similar reasoning was suggested by Castro *et al.* (2004). Also, the higher temperatures did not show any decomposition of the volatile compounds as no decomposition products could be seen in the chromatogram and this could be due to the short equilibration times used in this study. A similar trend was obtained by Pellati *et al*. (2005) in their work conducted on the aroma compounds of the *Evodia* fruit as well as by Castro *et al.* (2004). The results obtained for this investigation indicated that temperature is an important parameter for the extraction of the volatile organic compounds and that extraction increases with an increase in temperature.

3.5.5.2 Extraction profile

Headspace SPME involves the equilibration of the analytes between the fibre coating, headspace and sample matrix. The analyte enrichment of the fibre relies on the mass transfer from the matrix to the vapour phase and from this phase to the polymer coating (Bicchi *et al*., 2007). In this work, for optimum HS-SPME conditions, two different fibre coatings, PDMS and PA, were studied. Both fibres' performance was determined from the results of three individual samples. After the exposure of the fibres to the headspace above the milled leaves, at the temperatures mentioned earlier, namely, room temperature (~22 ºC), 40 °C, 60 °C and 80 °C, the analytes were extracted into the fibre coating.

From the results shown in Table 3.21 and in Figures 3.22-3.26, it can be seen that the peak areas for the analytes with the PDMS coating obtained were greater than those with the PA coating. The smaller amount extracted for the fibre with PA coating is expected as it is known to be more suitable for polar compounds and the compounds extracted here are nonpolar.

According to work done by Alpendurada (2000), the diffusion constants in the PA coating are much smaller than the PDMS fibre coating, and therefore a longer extraction time is necessary for the adsorption of the analytes. Also, the partition coefficients are different and this explains why (in this study), the yields for the PA fibre are much smaller than for the fibre with the PDMS coating. The results for the extraction with the PDMS coating showed better extraction efficiency for the selected compounds.

3.5.6 Method reproducibilty

For a method to be acceptable, it needs to provide scientific proof of consistency in the results obtained, within reasonable limits. One way in which this can be demonstrated is by examining the reproducibilty of the peak areas, expressed in terms of its precision (RSD value) for each of the headspace methods investigated. Replicate analysis with the same fiber can produce reproducible results, with a 20% variation in peak areas being reported. Also the differences in the results obtained for the HS-SPME with the different fibres is due to affinity of the compounds for the adsorption sites on the fibre.

In the analysis with the HS-SPME fibres, the fibres were exposed for a 15 minute duration, during which time the compounds was adsorbed onto the fibre coating and thereafter desorbed into the injection port of the GC-MS, whilst for the headspace analysis, a sample was taken out with the aid of a syringe after 15 minutes. Therefore, the differences in the results obtained could be due to this experimental difference. It has been reported that it is a lack of precision that has become problematic for quantitative determination when using SPME and headspace analysis (Stashenko and Martinez, 2007).

3.5.6.1 HS-SPME

The reproducibility values, expressed as percent relative standard deviations, of the HS-SPME methods with the different coatings are compared in Table 3.22. The results

were determined from the peak areas obtained from three separate experiments, which can be found in Appendix E, Tables E29 to E43.

Table 3.22 Comparative percent relative standard deviation (%RSD) of the peak areas for Headspace-SPME extraction of *M. koenigii* leaves at different temperatures.

Compound	**PDMS Fibre RSD/%**				**PA Fibre RSD/%**			
	RT	**40 °C**	**60 °C**	**80 °C**	**RT**	**40 °C**	**60 °C**	**80 °C**
α-pinene	9.2	9.5	7.7	16.9	3.3	3.6	7.7	18.1
β-pinene	9.9	4.3	8.5	14.1	4.3	7.3	3.3	16.3
α-phellandrene	4.3	5.1	2.8	19.7	6.5	6.2	2.8	14.6
β-caryophyllene	6.3	9.5	5.4	7.0	17.4	19.0	9.8	17.7
α-caryophyllene	4.0	8.1	9.0	7.4	18.4	17.1	9.7	22.2

RT – room temperature

From Table 3.22, it can be seen that the precision obtained for the HS-SPME extraction with the PDMS fibre coating did not exceed 10% up to a temperature of 60 °C (ranging from 2.8 to 9.9%). According to Stashenko and Martinez (2007), the RSDs for HS-SPME are usually below 10%, however, RSDs below 20% have been obtained for aroma compound determinations. The RSDs in this work were higher at 80 °C for the monoterpenes and lower for the sesquiterpenes.

For the extraction with the PA fibre coating, the RSDs for the monoterpenes at room temperature and at 40 °C were lower than the RSDs for the sequiterpenes. The RSDs' at 60 °C were all below 10%; and the precision at 80 °C, was between 14.6 and 22.2%. The reproducibility obtained in this study compares well with work done by other researchers with the same fibre coating. In a study of flavour volatiles conducted by Steffen and Pawliszyn (1996) the percent relative standard deviation values for the fibre with the PA coating ranged between 1 and 18%.

The RSDs for α-pinene, β-pinene, and β-caryophyllene obtained in this work are comparable to the RSDs obtained for the same compounds from the analysis of sage leaves. Extraction of these compounds by Bicchi *et al*. (2007) using HS-SPME with the PDMS fibre coating at 50 °C showed that the RSDs obtained for these compounds were in the following ranges: α-pinene (3.3-9.3%), β-pinene (3.2-9.8%) and β-caryophyllene (3.2-8.4%). Examination of the RSDs for the same compounds in Table 3.22 at 40 °C and 60 °C shows that the RSDs were all within this range, even though the sage leave extraction time was 30 minutes with sample agitation every 10 minutes. The precision of the results are in agreement with what has been reported for similar systems.

In this work, for the extraction of the compounds with the PDMS coating, it can be seen that room temperature, 40 °C and 60 °C could be used, but since temperature affects the extraction efficiency (discussed earlier in Section 3.5.5.1.), the more suitable temperature would be 60 °C and this could also probably be the optimum temperature for the extraction of the volatile compounds with the PA coating.

Further discussion on the terpenoid profile of the essential oil in *M. koenigii* can be found in Section 3.7.2.

3.5.6.2 HSA

The chromatograms obtained by the headspace method used for the extraction of the essential oils from the leaves of *M. koenigii* can be found in Appendix D, Figures D18 to D21. A representative chromatogram obtained for the headspace analysis at 60 °C is shown in Figure 3.27.

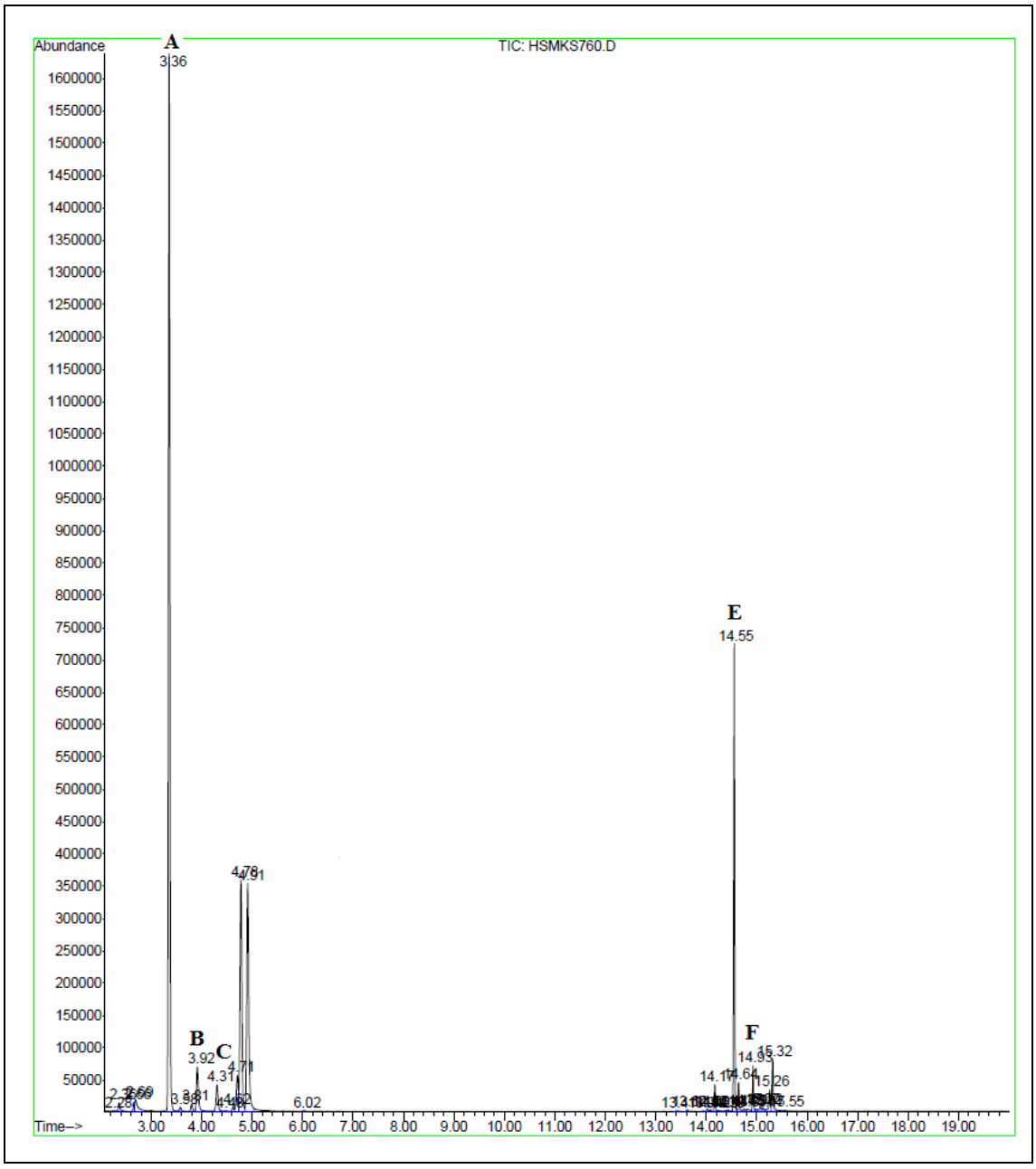


Figure 3.27 Total ion chromatogram for the headspace analysis at 60 °C.

In this study, the reproducibility of the headspace analysis was poor between replicates as can be seen from the high relative standard deviations for the method, shown in Table 3.23. From the examination of the RSDs, headspace analysis appears to be more precise for the extraction of the high volatiles e.g. α-pinene. The precision was better at a lower temperature. The headspace GC shows a better response for the more highly volatile

analytes than those of lesser volatility and this can be seen by examining the terpenoid profile of the essential oils in Section 3.8.2.

Table 3.23 Relative standard deviation (RSD) for the headspace extracts of the leaves of *M. koenigii* at the various temperatures.

Compound	**HSA RSD/%**			
	RT	**40 °C**	**60 °C**	**80 °C**
α-pinene	9.2	13.1	38.2	52.8
β-pinene	13.2	8.8	13.1	79.6
α-phellandrene	17.8	15.3	6.5	71.5
β-caryophyllene	14.2	62.6	21.7	90.7
α-caryophyllene	11.8	57.5	27.9	95.6

The high RSD values in Tables 3.22 and 3.23 for HS-SPME and headspace analysis can be accounted for. In the case of headspace analysis condensation inside the barrel of the syringe was observed for the extraction at 80 °C and this could play a contributory role to the poor RSD values obtained. This problem can be overcome in future work, by making use of a heated syringe for sample injection into the GC-MS. In HS-SPME, the high RSDs are also due to condensation on the SPME fibres. However the condensation in HS-SPME will be lower (due to competition of compounds) than headspace analysis and the syringe can also be heated before a sample is obtained for GC-MS analysis. Since the PDMS coating is non-polar, better RSD values were obtained than for the polar PA coating due to less condensation on the PDMS fibre coating.

Also, due to the low concentration of some of the compounds present, the loss of volatile organic compounds could be due to sample collection and handling and

measuring errors become unavoidable, as reported by Barbier *et al*. (2004) in their work.

Since the flask was sealed with a rubber septum, the reproducibility and accuracy of the analysis could be affected, since a large amount of trace components can be absorbed by rubber septa (Hachenberg and Schmidt, 1986), as well as adsorptive losses onto walls of the flask.

3.6 Fragmentation and identification of components of extracts

Before the different extraction methods could be compared in terms of the terpenoid profile extracted, the various components needed to be identified. This was done by comparing the retention time and mass spectrum of each component in the sample with those of standard compounds as explained earlier in Section 3.2.1. In this section a discussion of the fragmentation patterns observed in this work is given and how they led to the identification of the compounds extracted.

From the fragmentation patterns in Table 3.24, it would appear that compounds belonging to two different types of terpenes, the monoterpenes and sesquiterpenes were present. It should be added that the mode of fragmentation was very similar except that the base peak was formed via different routes for the terpenes mentioned.

Table 3.24 Mass spectra of some hydrocarbons identified in *M. koenigii*.

Compound	**Molar mass/g mol^{-1}**	**B.P./°C**	**Base peak *m/z***	**Main fragment ions *m/z***
α-pinene	136	154-156	93	93, 77, 41, 121
camphene	136	159-160	93	93, 121, 79, 41, 107
β-pinene	136	164-169	93	93, 41, 69, 121,107,55
β-myrcene	136	164-169	93	41, 93, 69, 27, 53
α-phellandrene	136	171-174	93	93, 77, 121, 55, 107,39
d-limonene	136	176	68	68, 93, 41, 79, 136
copaene	204	246-251	119	119, 105, 161, 93, 41
β-elemene	204	251-253	93	81, 93, 68, 41, 107
β-caryophyllene	204	262-264	93	93, 41, 69, 133, 107
α-farnesene	204	260-262	93	41, 93, 69, 55, 107
α-caryophyllene	204	266-268	93	93, 79, 41, 121, 79,107
γ-elemene	204	257-259	121	121, 93, 41, 107, 67

B.P.– boiling point (http://www.thegoodscentscompany.com/data/rw1014751.html., http://en.wikipedia.org/wiki/Limonene).

3.6.1 Fragmentation pattern of bicyclic terpenes

The fragmentation pattern of α- and β-pinene closely resembled that of the bicyclic terpenes (refer to Scheme 1 on page 125). A representative mass spectrum of α-pinene, in comparison to the mass spectrum in the NIST library, is shown in Figure 3.28.

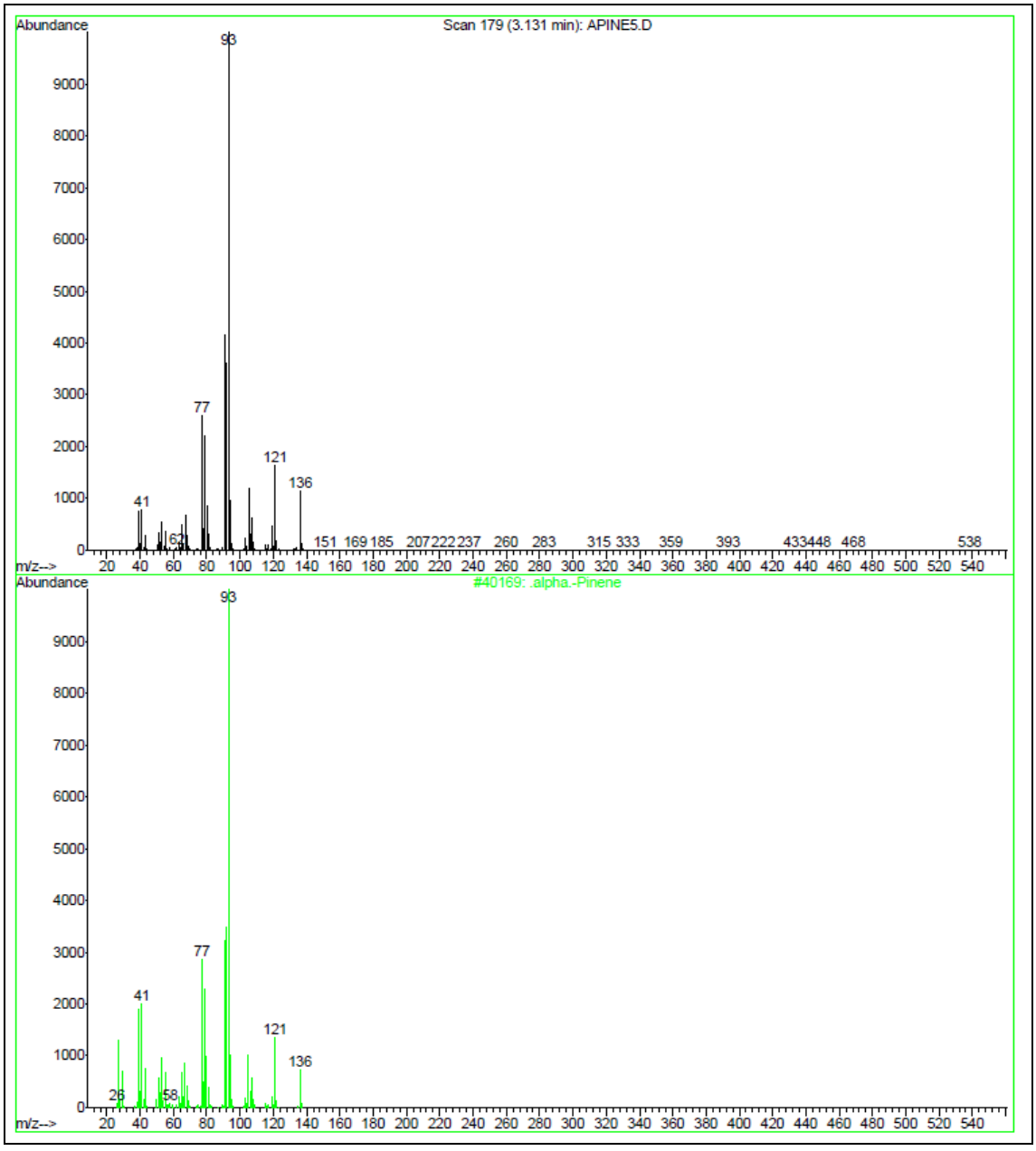


Figure 3.28 Comparison of mass spectrum of standard α-pinene with the mass spectrum contained in the NIST library.

For α-pinene, a bicyclic terpene containing a gem-dimethyl group, it was likely that the breakdown reaction $136^+ \rightarrow 93^+ + 43$ arises through the expulsion of the propylene group ($C_3H_5 + 2H^+$, i.e. 43 mass units) and the second reaction $93^+ \rightarrow 91^+ + 2$ is not clearly interpreted (Ryhage and von Snydow, 1963; Budzikiewicz *et al*., 1964). Thereafter, the fragmentation pattern of these bicyclic compounds is very similar to that

of the monoterpenes (see Schemes 1 and 2 on pages 125 and 126). It should be mentioned that the Schemes are merely suggested pathways, with various parts of the routes extracted from Hill (1969), Reed (1966), Ryhage and von Snydow (1963) and Budzikiewicz *et al*., (1964). The structural features of bicyclic terpenes were such that once forty three mass units were lost the resulting monocyclic moiety which rearranged again produced strong peaks at 79 and 77 pointing to the formation of conjugated systems. Some common fragment ions are listed in Table 3.25.

Table 3.25 Mass composition table of some common fragment ions.

m/z	**Fragment**
136	M^+
121	M-15
93	C_7H_9
91	Tropylium ion, C_7H_7
79	C_6H_7
77	Phenyl, C_6H_5
65	$C_5H_5^+$ retro-Diels-Alder of 91 (C_2H_2)
51	$C_4H_3^+$ retro-Diels-Alder of 77 (C_2H_2)
43	$C_3H_7^+$
41	$C_3H_5^+$
39	$C_3H_3^+$
29	$C_2H_5^+$
27	$C_2H_3^+$
15	CH_3^+

3.6.2 Fragmentation pattern of monocyclic terpenes

The second fragmentation pattern resembles those belonging to the monocyclic terpenes similar to that of the phellandrenes (refer to Scheme 2 on page 126). In addition, it

suggested that the detected monoterpenes contained a conjugated system (e.g. phellandrene) or a conjugated system which was easily formed by rearrangement within the mass spectrum as indicated by strong peaks at 79, 77. The mass spectrum of α-phellandrene is shown in Figure 3.29 and includes the mass spectrum in the NIST library.

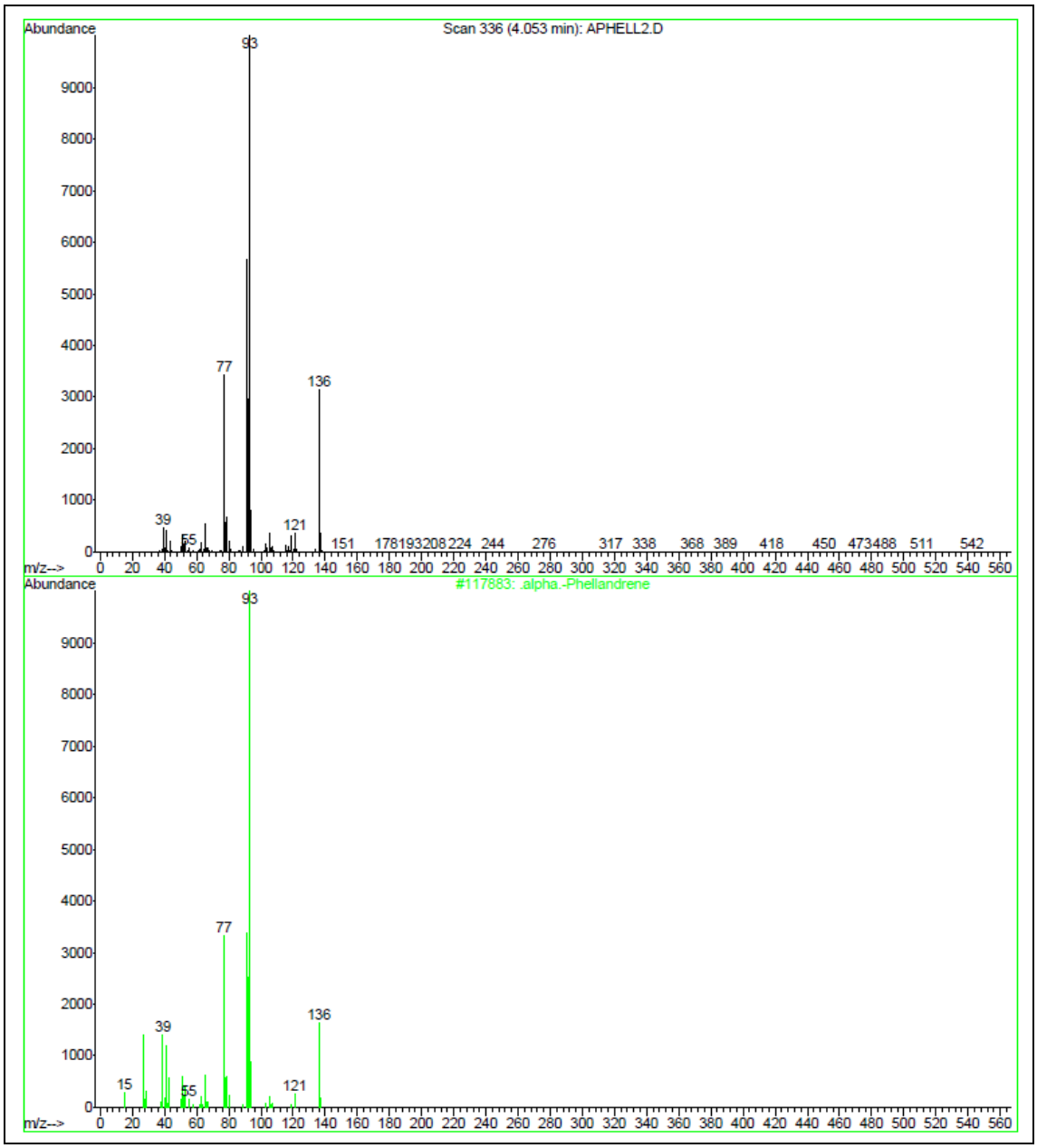


Figure 3.29 Comparison of the mass spectrum of α-phellandrene with the spectrum in the NIST library.

3.6.3 Comparison of mass spectra of detected compounds

Comparison of the mass spectra of the similar terpene compounds detected, showed that there was a striking similarity amongst all the spectra (shown below for the monoterpenes in Figures 3.30 and 3.31), which made positive identification difficult. However, some interesting patterns of fragmentation are mentioned in Sections 3.6.4 to 3.6.6.

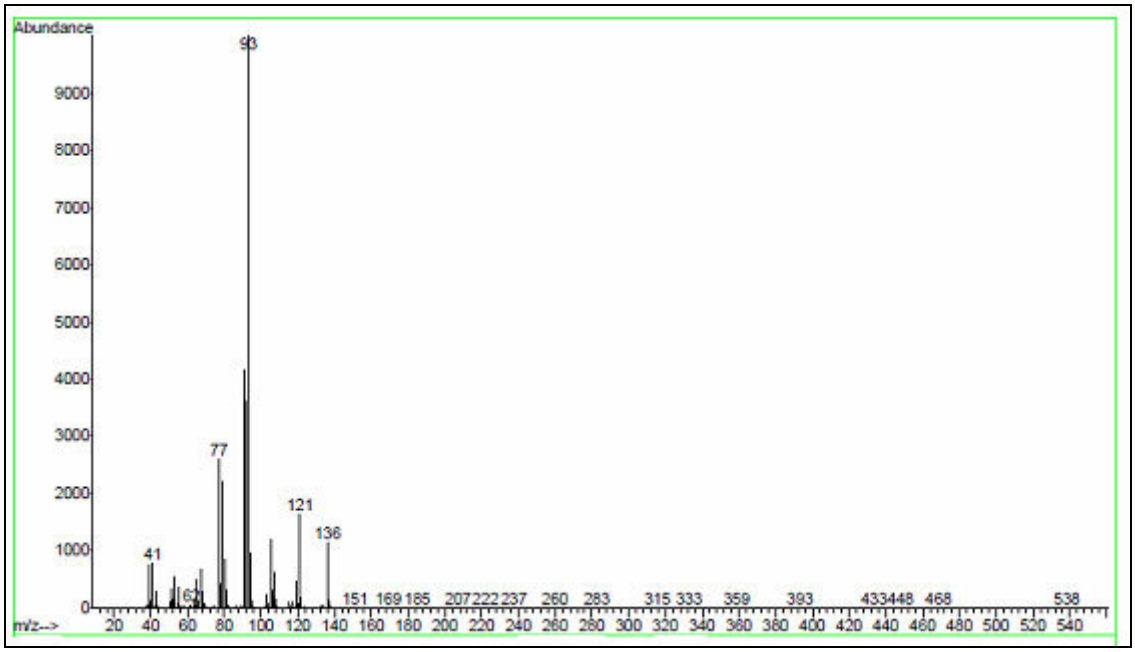


Figure 3.30 Mass spectrum of standard α-pinene.

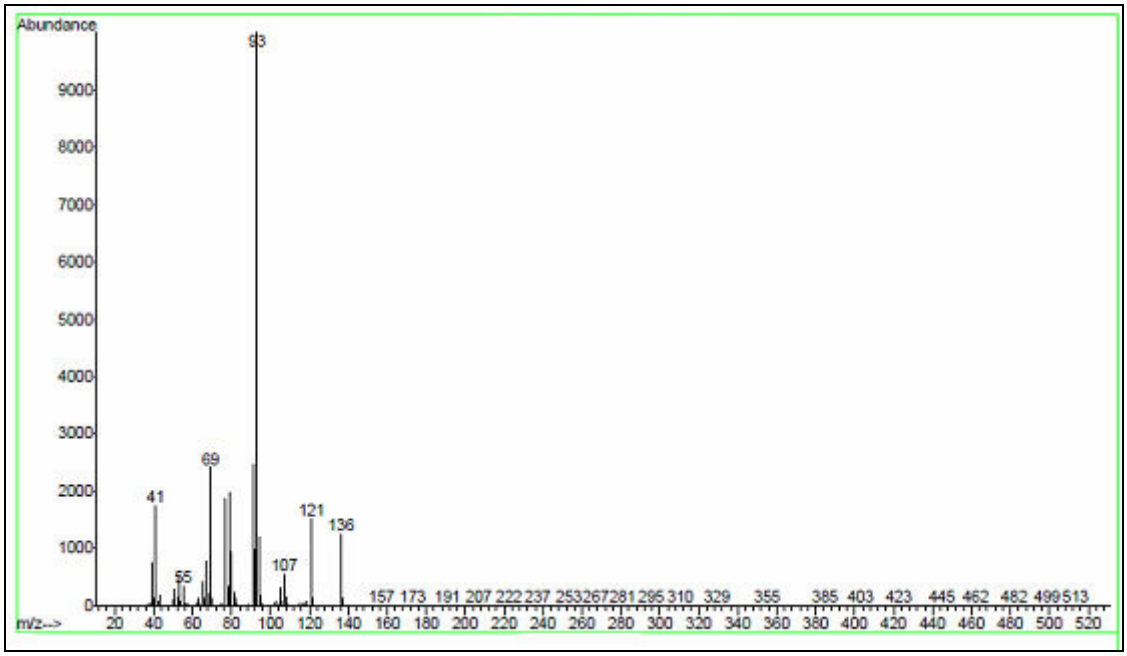
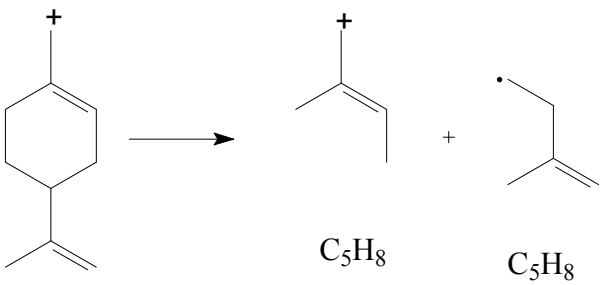


Figure 3.31 Mass spectrum of standard β-pinene.

3.6.4 Limonene

A further interesting point concerning fragmentation arises in the spectrum of α-1.8(9)-*p*-menthadiene (limonene, one of the compounds identified in this work), namely the formation of the ion *m/z* = 68 (as shown below), which was also the base peak of the spectrum (Reed, 1966). None of the spectra of the compounds detected produced this type of fragmentation pattern that suggested that the isopropyl substituent rather than the isopropylene moiety was present in the detected monocylic terpenes.



Limonene *m/z* = 68

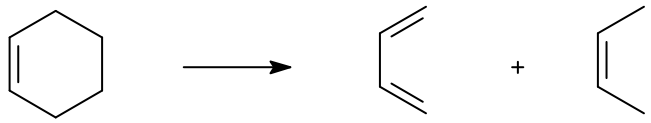
Certain fragmentation patterns, like the retro-Diels-Alder reaction, in Section 3.6.6, occurred repeatedly in the mass spectra of the compounds detected. This can be seen from the main fragment ions of, for example, α-pinene, β- pinene, *d*- limonene and α-phellandrene in Section 3.6, Table 3.24, as well as in Schemes 1 and 2.

3.6.5 Simple β-fission initiated by an aromatic system

When a substituent is present on an aromatic system, or when an aromatic nucleus is part of a large cyclic system, fission of the bond β to the aromatic system is favoured. The driving force for "*β-fission"* in this case - appears to be the high stability of the resulting aromatic ion (Hill, 1969). This reaction has been discussed in Chapter 1, Section 1.8.2.2.

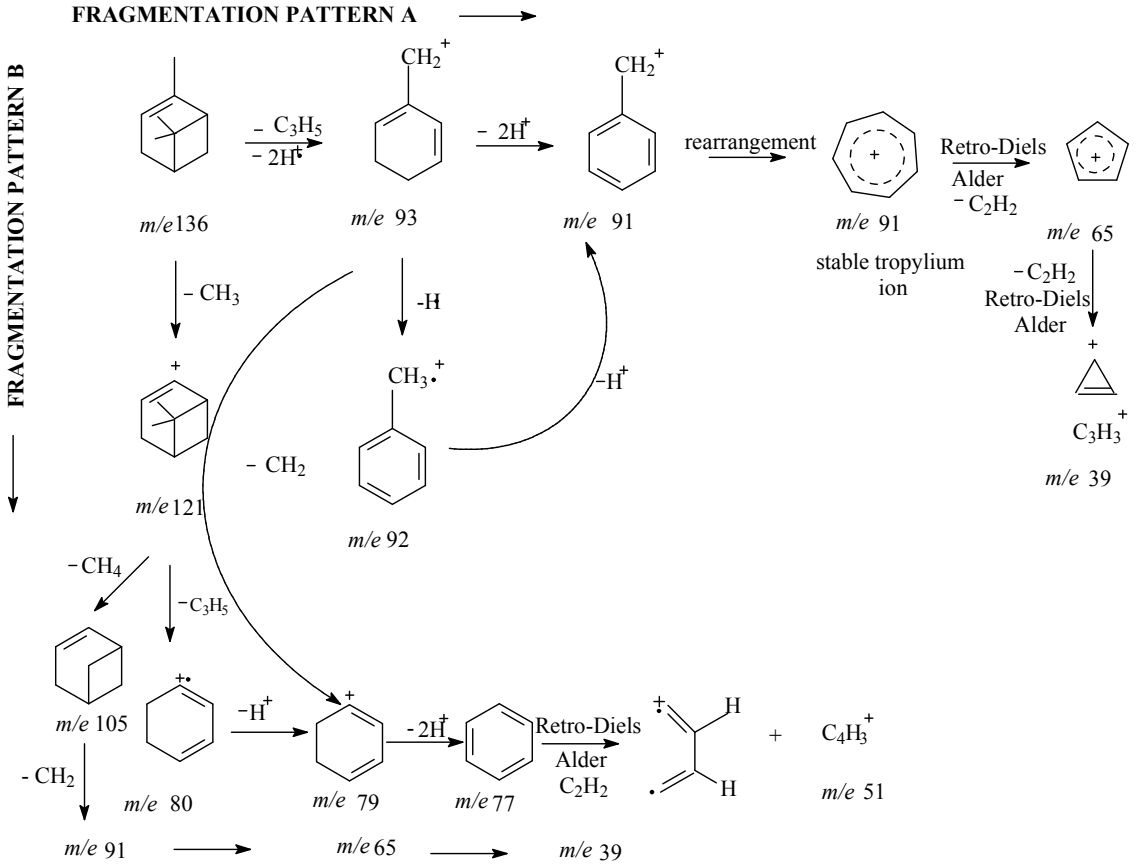
3.6.6 Retro-Diels-Alder

A double bond in a cyclic system could migrate, if it was suitably positioned to produce energetically favourable fragments by the retro-Diels-Alder process, (Hill, 1969). This is represented as follows:

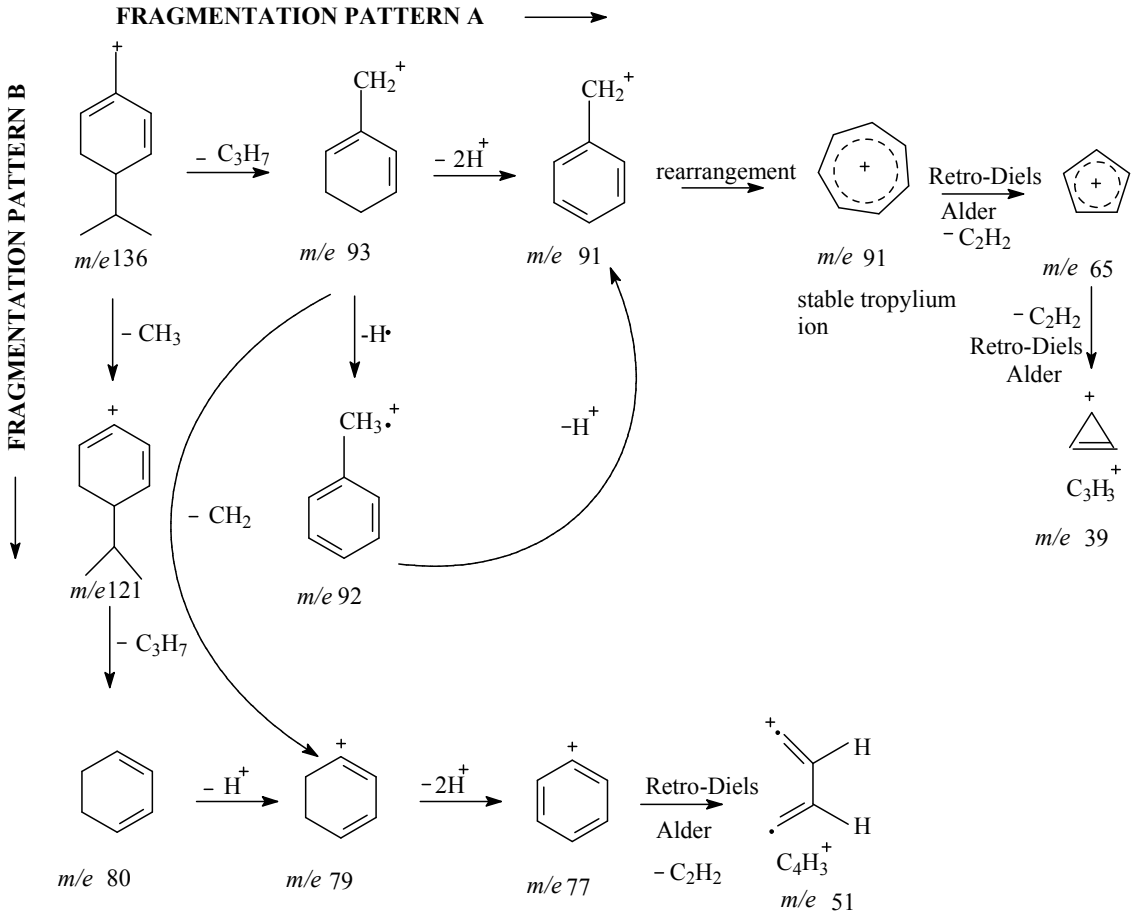


This process has been useful in rationalizing the spectra of terpenes of all classes.

Examination of the mass spectrum of *d*-limonene, shows that the formation of the ion at *m/z* = 68 is the diene fragment formed as a result of this reaction (Donald *et al*., 2009).



Scheme 1 Proposed fragmentation pattern for α-pinene.



Scheme 2 Proposed fragmentation pattern for α-phellandrene.

3.7 Terpenoid profile of the essential oil in *M. koenigii*

The volatile profile of the essential oil depended on the different methods of extraction used. For HS-SPME, the terpenoid profile depended on the fibre coating which was used. Different proportions were observed for the different compounds and this is discussed in Sections 3.7.1 and 3.7.2.

3.7.1 Comparison of the oil extracted by means of solvent, Soxhlet and steam distillation methods

The relative percentage distribution of the terpene compounds in the essential oil (listed in order of elution) obtained for the 48 hour solvent and Soxhlet extractions as well as the steam distillation, is shown in Table 3.26. The results are displayed graphically in Figure 3.32. The results were evaluated by using the ratio of the area of each peak to the total peak area calculated as a percentage. Individual results for the different extraction methods can be found in Appendix E, Tables E41 to E43.

An overlay chromatogram for the comparison of the essential oil obtained by steam distillation and the 48 hour solvent and Soxhlet extractions methods is shown in Figure 3.33.

Table 3.26 Relative percentage distribution (peak area) of the terpene compounds in the essential oil in the fresh leaves of *M. koenigii*.

Method	**Solvent extraction (SE)/%**	**Soxhlet extraction (SOX)/%**	**Steam distillation (SD)/%**
Compound			
α-pinene	4.16	3.41	0.42
β-pinene	0.37	0.32	0.09
α-phellandrene	0.16	0.12	0.09
d-limonene	0.36	0.26	0.20
β-phellandrene	3.02	3.21	1.32
Z-(β)-ocimene	1.95	1.79	1.00
Total monoterpenes	**10.0**	**9.11**	**3.12**
copaene	3.93	4.81	2.19
β-caryophyllene	18.4	18.6	20.9
(E)-α-bergamotene	2.41	2.35	2.59
β-farnesene	0.51	1.32	2.12
α-caryophyllene	1.24	5.70	1.26
γ-selinene	5.54	1.18	6.62
α-guaiene	2.39	2.61	2.20
(+)-epi-bicyclosesquiphellandrene	1.83	1.47	0.98
sesquiterpene hydrocarbon	1.11	1.00	13.5
β-selinene	7.83	7.05	17.4
valencene	14.3	13.3	1.08
cadinene	1.31	1.23	2.25
sesquiterpene hydrocarbon	1.40	0.96	2.26
sesquiterpene hydrocarbon	–	1.05	1.18
sesquiterpene hydrocarbon	1.23	0.85	6.61
caryophyllene oxide	1.11	1.01	–
α-farnesene	0.81	1.11	–
sesquiterpene hydrocarbon	1.06	1.27	–
sesquiterpene hydrocarbon	0.62	2.41	–
sesquiterpene hydrocarbon	2.60	–	–
Total sesquiterpenes	**69.6**	**69.3**	**83.1**
Other	**19.4**	**21.5**	**13.8**

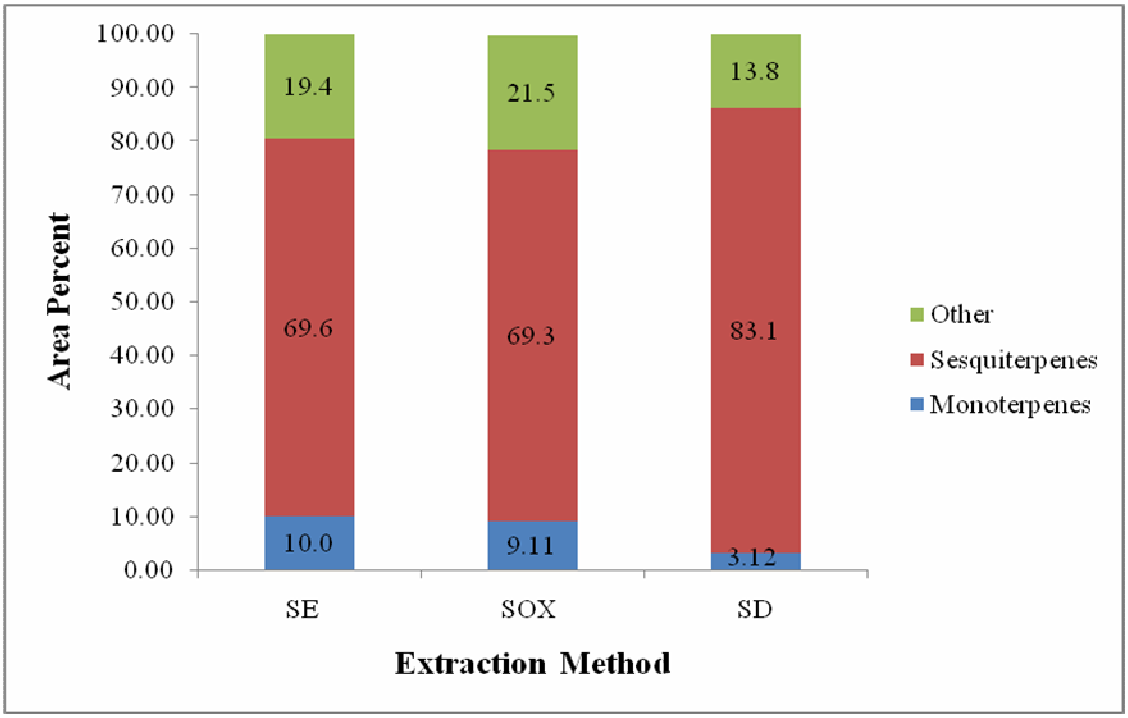


Figure 3.32 The terpenoid profile of the oil composition from solvent and Soxhlet extraction as well as steam distillation.

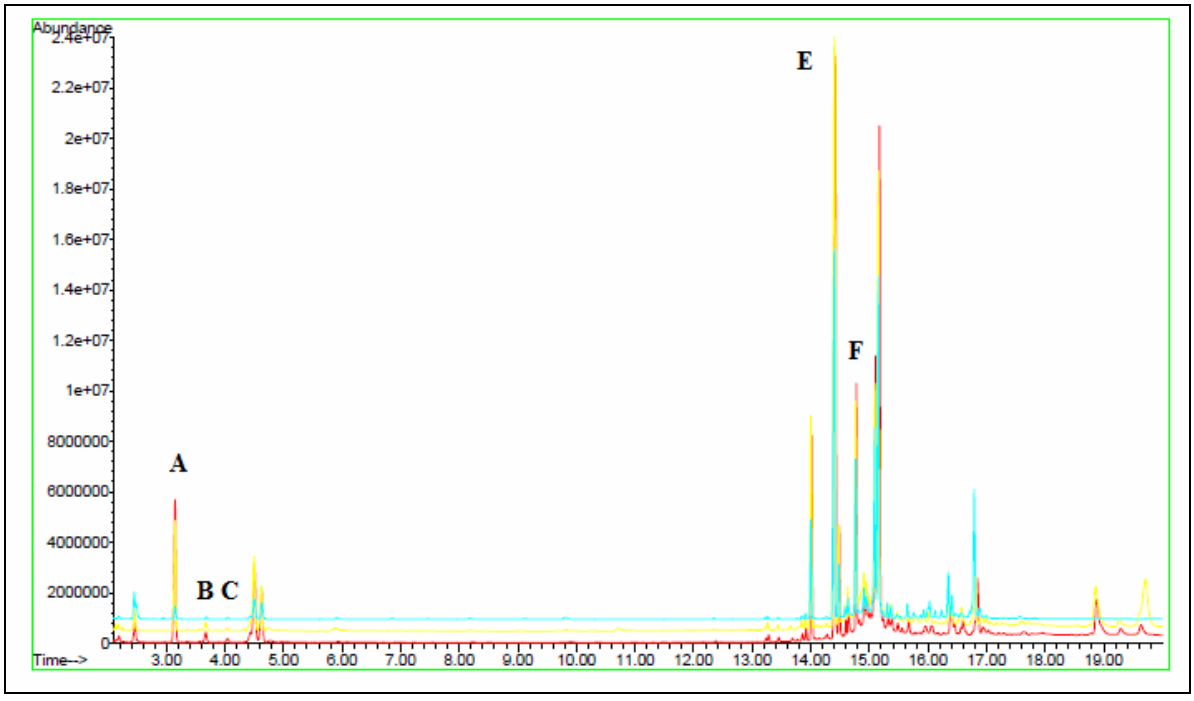


Figure 3.33 An overlay chromatogram of the essential oil obtained during steam distillation (—) solvent (—) and Soxhlet extraction (—).

Discussion on the comparison of the terpenoid profile of the essential oil obtained from the steam distillation, solvent and Soxhlet extractions can be found in Section 3.7.3.

3.7.2 The terpenoid profile of the oil with headspace analysis and HS-SPME

An overlay chromatogram showing the oil obtained by headspace analysis and HS-SPME with each of the two fibres is shown in Figure 3.34.

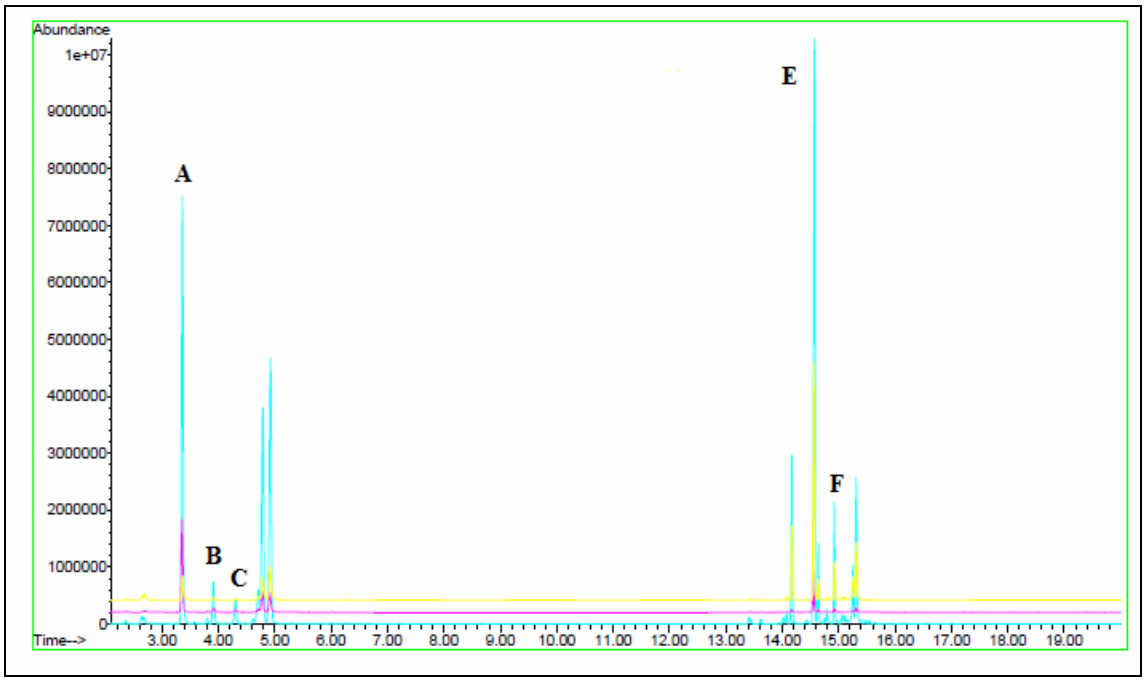


Figure 3.34 An overlay chromatogram of the essential oil obtained with headspace analysis (——) and HS-SPME with both the PDMS (——) and PA fibre coatings (——).

The average percentage distribution of the terpene compounds in the essential oil extracted at the various temperatures for the headspace and HS-SPME methods is shown in Tables 3.27 to 3.28 and in Figure 3.35. The results for the individual samples

can be found in Appendix E, Table E44 to E55. The results were evaluated by using the ratio of the area of each peak to the total peak area.

Table 3.27 Relative percentage distribution (peak area) of the terpene compounds in the different headspace methods at room temperature and at 40 °C.

Temperature	**RT**			**40 °C**		
Method	**HSA/%**	**PDMS/%**	**PA/%**	**HSA/%**	**PDMS/%**	**PA/%**
Compound						
α-pinene	35.9	7.31	1.47	47.2	12.5	3.93
β-pinene	1.17	0.55	0.11	1.40	0.90	0.44
α-phellandrene	0.84	0.33	0.19	1.10	0.63	0.41
d-limonene	1.05	0.51	0.17	1.38	1.01	0.53
β-phellandrene	7.67	3.44	1.92	9.98	6.32	4.26
Z-(β)-ocimene	5.74	3.28	2.27	8.31	6.03	5.84
Total monoterpenes	**52.3**	**15.4**	**6.12**	**69.3**	**27.4**	**15.4**
δ-elemene	9.79	–	–	–	–	–
copaene	1.39	–	–	–	–	–
β-elemene	2.00	12.6	18.8	1.28	9.89	13.3
β-caryophyllene	22.8	36.1	28.0	17.5	31.4	32.8
(E)-α-bergamotene	2.25	–	–	–	–	–
α-gurjunene	2.46	3.35	3.18	1.38	3.54	3.22
β-farnesene	1.35	1.00	–	–	0.91	–
α-caryophyllene	3.28	6.71	6.53	1.79	5.06	5.80
isocaryophyllene	1.53	–	1.19	–	–	–
β-selinene	2.45	4.73	6.76	1.52	4.08	5.15
cadinene	–	–	–	–	–	–
γ-elemene	6.14	13.0	19.8	3.98	10.7	14.4
Total sesquiterpenes	**44.6**	**77.4**	**85.1**	**27.5**	**65.6**	**74.6**
Other	**3.06**	**7.10**	**8.76**	**2.90**	**6.64**	**8.58**

RT – room temperature

Table 3.28 Relative percentage distribution (peak area) of the terpene compounds in the different headspace methods at 60 °C and 80 °C.

Temperature	**60 °C**			**80 °C**		
Method	**HSA/%**	**PDMS/%**	**PA/%**	**HSA/%**	**PDMS/%**	**PA/%**
Compound						
α-pinene	44.3	16.9	5.51	39.7	9.25	8.54
β-pinene	2.20	2.05	0.82	2.48	2.18	1.25
α-phellandrene	1.29	1.29	0.57	1.54	1.56	0.90
d-limonene	1.78	1.94	0.96	2.09	1.40	1.40
β-phellandrene	11.5	10.9	6.69	12.9	11.4	9.03
Z-(β)-ocimene	10.9	11.0	9.30	12.7	10.7	11.8
Total monoterpenes	**71.9**	**44.1**	**23.8**	**71.4**	**36.5**	**32.9**
δ-elemene	–	–	–	–	–	–
copaene	–	–	–	–	–	–
β-elemene	1.04	5.83	8.82	**1.56**	7.09	6.78
β-caryophyllene	15.6	25.7	34.1	14.0	24.5	28.9
(E)-α-bergamotene	–	–	–	–	–	–
α-gurjunene	1.20	3.20	3.28	1.11	2.06	2.86
β-farnesene	–	0.77	–	–	–	–
α-caryophyllene	1.74	4.70	5.49	1.80	6.39	4.83
isocaryophyllene	–	–	–	–	–	–
β-selinene	1.73	2.83	3.94	1.74	1.15	3.70
cadinene	–	–	–	–	4.98	–
γ-elemene	3.34	6.75	10.0	4.00	8.96	8.64
Total sesquiterpenes	**23.8**	**49.8**	**65.6**	**23.7**	**55.1**	**55.7**
Other	**4.21**	**6.2**	**10.4**	**4.5**	**8.03**	**11.3**

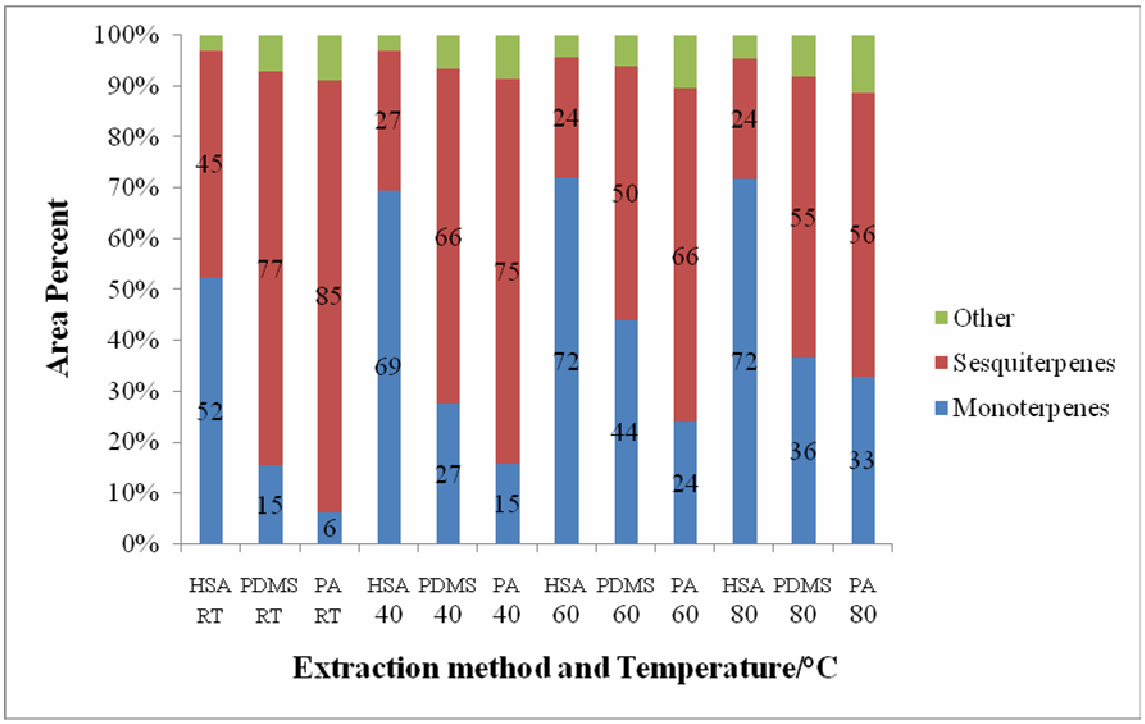


Figure 3.35 The relative distribution of the hydrocarbons in the essential oils from the headspace and HS-SPME analyses.

Although the qualitative profile of the essential oil showed a similar range of compounds for the headspace and HS-SPME methods, the relative abundances showed differences. A similar observation was reported by Pourmortazavi *et al*. (2005), for the essential oil analysis of black cumin. According to Pourmortazavi *et al*. (2005), as well as other researchers mentioned in their work, extracts obtained from natural products utilising different methods showed differences in their composition.

From Figure 3.35, it can be seen that the HS-SPME extraction with the PA fibre at room temperature favoured the extraction of high molar mass compounds, the sesquiterpenes (85%), but these were the lowest for headspace extraction at a temperature of 80 °C. The amount of monoterpenes extracted were greatest for the headspace analysis at all the different temperatures studied, ranging from 52% to 72%. More discussion on the comparison of the terpenoid profile of the essential oil obtained from the HS-SPME and headspace analysis is in Section 3.7.3.

3.7.3 Comparison of the composition of the essential oil obtained by different methods

The differences between the different extraction methods can be seen from an examination of the terpenoid content, shown in Figure 3.36. In this figure, only the headspace extractions at 60 °C are shown as this temperature was found to be most reproducible for the extraction of the highly volatile compounds.

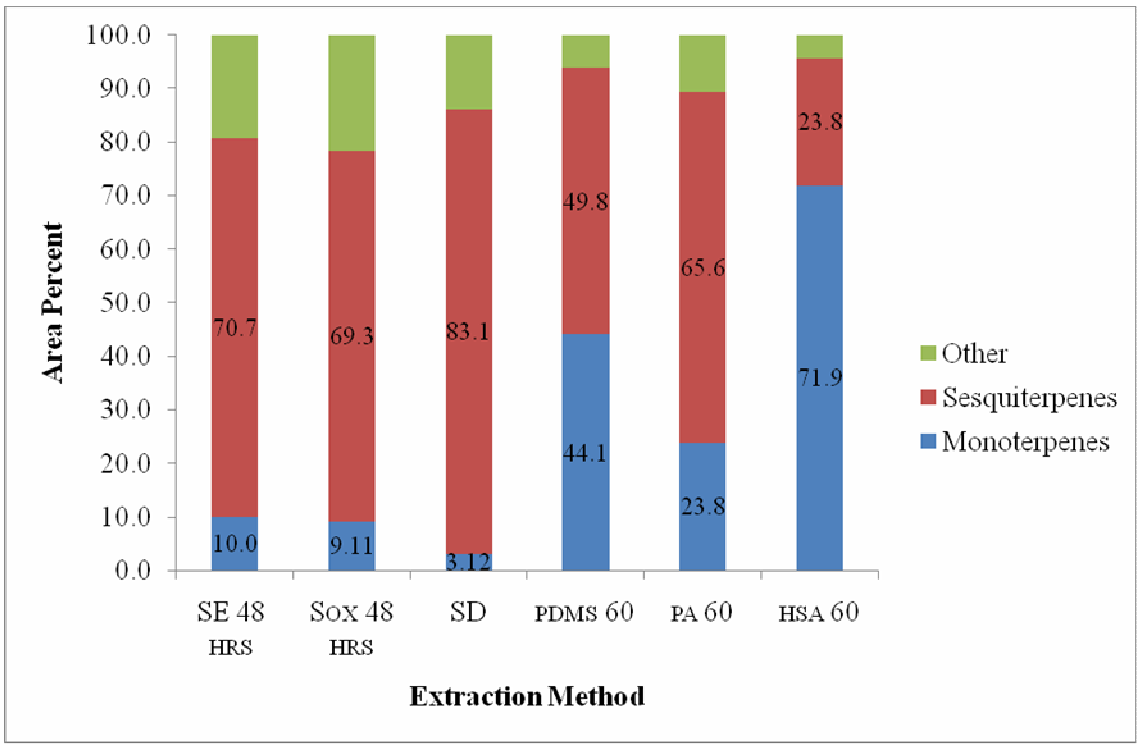


Figure 3.36 Comparison of the relative percentages of the hydrocarbons for all the extraction methods studied.

The terpenoid profile of the oil in *M. koenigii* leaves differed from that found in earlier studies. In the oil obtained from the leaves in China and North India, the main component was α-pinene whereas β-phellandrene was the main component in the leaves from Malaysia (Paranagama *et al*., 2002). In the oil from the leaves found in Sri Lanka, β-caryophyllene was the main component. In these earlier studies, different methods and solvents were used. Paranagama *et al.* (2002) used a modified Likens and Nickerson apparatus and isopentane to trap the volatiles. In the extraction of the essential oils from the leaves in China, Wong and Tie (1993) used the method of steam

distillation, followed by back extraction with dichloromethane. These differences, as reported by Paranagama *et al.* (2002), could be due to genetic variation as well as changes in the environment.

In this study the main aroma component was β-caryophyllene, since it was present in the highest amount. Its presence also gives an indication of the freshness of the leaves, (post harvest), as reported in the work of Paranagama *et al*. (2002). Other major constituents identified in this work include α-caryophyllene, α-pinene, and β- and γ-elemene.

A comprehensive list of compounds identified in the fresh leaves of *M. koenigii* from all the extraction methods is shown in Table 3.29. The retention times are recorded in a range as the column was cut twice during the course of this work and hence the retention times differed slightly. The compounds are listed according to the names contained in the NIST Library (contained in the software of the instrument), as well as their matching natural product name to be found in the reference http://www.thegoodscentscompany.com/data/rw1014751.html, date accessed: 30/11/2009.

Table 3.29 Volatile constituents tentatively identified in fresh leaves of *M. koenigii*.

Elution Order	Retention Time	Compound (Natural product name)	Compound (NIST Library)
1	2.349-2.37	hexanal	Hexanal[a]
2	2.455	3-hexen-1-ol	3-hexen-1-ol
3	2.49	2-hexen-1-ol	2-hexen-1-ol
4	2.626-2.67	2-hexenal,(E)	2-hexenal [a]
5	2.679	1-hexanol	1-hexanol
6	3.243	α-thujene	α-thujene
7	3.148-3.360	α-pinene	α-pinene[a]
8	3.58	camphene	camphene[a]
9	3.64	myrcene	myrcene
10	3.677-3.901	β-pinene	β-pinene[a]
11	3.81	β-thujene (sabinene)	β-thujene (sabinene) [a]
12	4.041-4.300	α-phellandrene	α-phellandrene[a]
13	4.623-4.635	*p*-cymene	1,4- dimethyl benzene[a]
14	4.714-4.723	*d*-limonene	*d*-limonene[a]
15	4.782-4.784	β-phellandrene	β-phellandrene[a]
16	4.917-4.92	Z-(β)-ocimene	1,3,6 octatriene,3,7-dimethyl-(Z) [a]-
17	5.305	γ-terpinene	gamma terpinene[a]
18	5.904	linalool	1,6- octadien-3-ol-3,7-dimethyl-
19	6.004	α-terpinolene	cyclohexene,1-methyl-4-(1-methylethylidene)- [a]
20	6.844	3-terpinenol	4-isopropyl-1-methyl-3-cyclohexen-1-ol
21	8.178	cis-sabinene hydrate	4-(hexen-1-ol,5-methyl-2-(1-methylethyl)-(R)
22	9.124	terpinen-4-ol	3-cyclohexen-1-ol,4-methyl-1-(1-methylethyl)-
23	10.387	(+)-α-terpineol	p-menth-1-en-8-ol
24	12.338	monoterpene	monoterpene[a]
25	13.23	α-terpinene	1,3-cyclohexadiene,1-methyl-4-(1-methylethyl)
26	13.272	δ-elemene	cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)- [a]
27	13.454	isoterpinolene	isoterpinolene
28	13.442-13.625	α-cubebene	α-cubebene[a]
29	13.765	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon[a]
30	13.848	6-epi-β-cubebene	1H-cyclopenta(1,3)cyclopropa(1,2)benzene,octahyd[a]
31	13.91-13.995	β-elemene[a]	cyclohexane, 1-ethenyl-1-methyl-2, 4-bis-(1-methylethenyl) [a]
32	13.94	ylangene	ylangene[a]
33	14.024	copaene	copaene[a]
34	14.071	isocaryophyllene	bicyclo [5.3.0.] decane, 2-methylene-5-(1-methylvinyl)
35	14.13	α-selinene	sesquiterpene hydrocarbon[a]
36	14.23	α-gurjunene	1H-cycloprop(e)azulene,1a,2,3,4,4a,5,6,7b-octahyd[a]

Table 3.29 Contd. Volatile constituents tentatively identified in fresh leaves of *M. koenigii*.

Elution Order	Retention Time	Compound (Natural product name)	Compound (NIST Library)
37	14.388-	β-caryophyllene	caryophyllene[a]
38	14.482-14.84	(E)-α-bergamotene	bicyclo [3.1.1.] hept-2-ene, 2, 6-dimethyl-6-(4-methylpent-3-en-1-yl) [a]
39	14.57	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon[a]
40	14.582	β-farnesene	1,6,10 dodecatriene,7,11-dimethyl-3-methylene-(Z)- [a]
41	14.635	γ-bisabolene	cyclohexene-3-(1,5-dimethyl-4-hexenyl)-6-methylen
42	14.758-14.99	α-caryophyllene	α-caryophyllene[a]
43	14.817	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
44	14.905	γ-selinene	naphtalene,decahydro-4a-methyl-1-methylene-7-(1-)
45	14.952	α-guaiene	azulene,1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-
46	14.98	(+)-epi-bicyclosesquiphellandrene	(+)-epi-bicyclosesquiphellandrene[a]
47	15.076	β-selinene	eudesma-4(14),11-diene
48	15.164	valencene	naphthalene-1, 2, 3, 4, 4a, 5, 6,8a-octahydro-4a-b-dimethyl[a]
49	15.228	longifolene	longifolene[a]
50	15.311	cadinene	naphthalene-1,2,3,4,4a,5,6,8a-hexahydro-4,7- dimethyl
51	15.320	γ-elemene	gamma elemene[a]
52	15.425	muurolene	naphthalene-1, 2, 3, 4, 4a,-7-hexahydro-1, 6-dimethyl-4
53	15.369	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
54	15.469	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
55	15.528	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
56	15.646	(+)-nerolidol	1,6,10 dodecatrien-3-ol,3,7,11-dimethyl-(E)-
57	15.763	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
58	15.875	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon[a]
59	15.998	caryophyllene oxide	caryophyllene oxide
60	16.027	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
61	16.122	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
62	16.227	α-farnesene	α-farnesene[a]
63	16.286	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
64	16.339	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
65	16.41	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon
66	16.55	bicyclo (4, 4, and 0) dec-1-ene, 2-isopropyl-5-methyl-9-methylene	bicyclo (4, 4, and 0) dec-1-ene, 2-isopropyl-5-methyl-9-methylene
67	16.56	sesquiterpene hydrocarbon	sesquiterpene hydrocarbon(204) [a]
68	16.656	unknown	unknown
69	16.715	unknown	unknown

Table 3.29 Contd. Volatile constituents tentatively identified in fresh leaves of *M. koenigii*.

Elution Order	Retention Time	Compound (Natural product name)	Compound (NIST Library)
70	16.785	unknown	unknown
71	16.885	unknown	unknown
72	16.797	unknown	unknown
73	17.138	unknown	unknown
74	17.22	unknown	unknown
75	17.33	unknown	unknown
76	17.4	unknown	unknown
77	17.57	unknown	unknown
78	17.67	unknown	unknown
79	17.83	unknown	unknown
80	19.33	unknown	unknown

[a] - Refers to compounds identified in HS-SPME.

In the present study, the oil composition from the solvent and Soxhlet extractions was similar. For the solvent extraction the amounts extracted at room temperature for both the monoterpenes and sesquiterpenes were 10.0% and 70.7% respectively. This method was also both time- and cost-saving. In comparison, Soxhlet extraction showed a 9.11 % monoterpene and 69.3 % sesquiterpene distribution. In the oil from the dichloromethane extractions, a total of 51 compounds were tentatively identified.

In the oil obtained from steam distillation, the sesquiterpenes (83.1%) were the major fraction present, containing 20.9% β-caryophyllene. MacLeod and Pieris (1982), obtained 80.2% sesquiterpenes from their steam distillation-extraction of the leaves. The yield of β-caryophyllene in this work was slightly lower when compared to earlier work done by other researchers. Previous work carried out by Paranagama *et al.* (2002) and MacLeod and Pieris (1982) showed β-caryophyllene to be one of the major components, with 23.3% and 28% respectively. Steam distillation carried out by Walde *et al*. (2006) also yielded 26.3% β-caryophyllene. Comparing the oil from the steam distillation with the oil obtained from the solvent and Soxhlet extractions, it was observed that the amount of β-selinene from the steam distillation (17.4%) was proportionately higher than the amount obtained with the other two methods (7.05-7.83%).

In the steam distillation, the amount of monoterpenes extracted relative to the sequiterpenes was proportionately low (3.12%), indicating the vulnerability of the monoterpenes to this technique. A similar finding was observed in the work of Diaz-Maroto *et al*. (2002). Also this low yield of the monoterpenes could be due to the fact that loss of the low molar mass compounds could take place when removing the solvent during the evaporation step. This is consistent with studies done by other researchers using this method of extraction (Barra *et al*., 2007). A study undertaken by Orav *et al*. (2001) showed variation in the composition of the essential oil of *Matricaria recutita* L. In their work, the monoterpenes and other volatile compounds showed a decrease in content with time during a 3-hour distillation.

In the HS-SPME extraction with the PDMS fibre, the compounds which were found to be absent at room temperature and 40 °C, namely, γ-terpinene, α-terpinolene, ylangene, (+)-epi-bicyclosesquiphellandrene, were found to be present in the extraction at 60 °C. This demonstrated the effect of temperature on the extraction of the compounds. Also the loss of the monoterpenes, *p*-cymene and *d*-limonene at 80 °C demonstrates the vulnerability of some of the monoterpenes to high temperatures. Thirty-five compounds were separated and tentatively identified in the headspace analysis at 60 °C and fifty-five compounds were detected in the HS-SPME extract (refer to Table 3.29).

Examining the headspace analysis, it can be seen from Figure 3.36, that the headspace analysis showed a much greater response for the more volatile analytes, the monoterpenes (71.9%) at a temperature of 60 °C than for the sesquiterpenes. In this work, the total monoterpenoid fraction obtained at the various temperatures, in Tables 3.19 and 3.20, as well as the corresponding graphs, for the headspace analysis was greater than that from the PDMS and PA extractions.

The PDMS extraction of monoterpenes was relatively greater when compared to the extraction with the PA coating. In contrast, the total amount of extracted sesquiterpenes was relatively greater for the extraction with the PA fibre coating (despite it being a polar coating), than the PDMS extraction. This result was unexpected as the PDMS coating is more sensitive to non-polar compounds and should be more efficient for the

total extraction of the hydrocarbons but it appears that the polar PA coating was much more efficient for the total extraction of the terpenes. In addition, the PDMS coating is 100 μm in thickness and is more suited for the retention of the highly volatile compounds when compared to the PA coating which is 85 μm in thickness. This can only be explained with respect to the equilibration times, i.e. thinner coatings require a shorter equilibration period (in this work 15 mins was used) and thicker coatings, a longer period (Stashenko and Martinez, 2007). However, since the coating thickness was not investigated in this work, it could form the basis for future work.

Comparing HS-SPME to the steam distillation, it can be seen that HS-SPME was more favourable for the extraction of the monoterpenes than the steam distillation extraction. This could be due to the loss of the more highly volatile compounds during the evaporation step in the steam distillation (Garcia-Estebana *et al*., 2004).

One must also keep in mind that these slight differences in the composition of the oil could be due to the fact that although the fresh leaves were collected from the same garden, they are variable in nature (Barbieri *et al*., 2004). It has also been reported by Stashenko *et al.* (2004) that the freshness of the plant plays a role in the volatile profile and more especially in the case of the headspace profile.

3.8 Calibration

Quantification by GC-MS can be problematic because of the significant differences in detector response as a function of the chemistry of the analytes. Quantification using mass spectrometry is usually done only when there is a specific standard for the compound of interest (Rose and Johnstone, 1982).

In this work, calibration curves were obtained for the selected analytes at two concentration ranges, a lower calibration range (3.4 to 173 mg L^{-1}) in order to quantify the compounds present in low amounts and a higher range (330 to 1.80×10^4 mg L^{-1}) for the major components.

The internal standard method was used for the quantitative analysis of the chosen volatile compounds. Dodecane, a non terpenoid volatile, was chosen as the internal standard to avoid interference with the terpenes, as reported in the study by Lluisa and Penuelas (2000). The response factor was determined from the ratio of the peak areas of the standards and the internal standard. The peak areas of the five selected analytes and the internal standard were integrated manually, whilst the other compounds were integrated automatically by the software contained in the instrument.

The calibration data for the five compounds quantified in this work is presented in Tables 3.31 to 3.35 which contain the data for the standards in the concentration range from 3.4 to 173 mg L^{-1} and Tables 3.36 to 3.40 which contains the data in the range from 330 to 1.80×10^4 mg L^{-1}. The working solutions of the standards were prepared in dichloromethane. The calibration curves were obtained by plotting the area ratios versus the concentration (mg L^{-1}) of the target analytes and regression analysis was used to analyse the results. The calibration curves are shown in Figures 3.38 to 3.47 together with the residual plots for the compounds present in the low concentration range and in Figures 3.48 to 3.52 for the compounds in the high concentration range.

The chromatograms and mass spectra of the individual standards as well as the standard mixtures can be found in Appendix C. The area percent reports can be found in Appendix F, Tables F1 to F10. A representative chromatogram of a standard mixture is shown in Figure 3.37 and the corresponding concentrations for the five selected compounds are shown in Table 3.30. The concentration of the other standard mixtures can be found in Appendix C, Tables C1 to C10.

Table 3.30 Concentration of compounds in of a standard mixture consisting of the five selected compounds and the internal standard.

Compound	**Concentration/mg L^{-1}**
α-pinene	13.7
β-pinene	13.7
α-phellandrene	13.4
dodecane	29.9
β-caryophyllene	14.4
α-caryophyllene	14.2

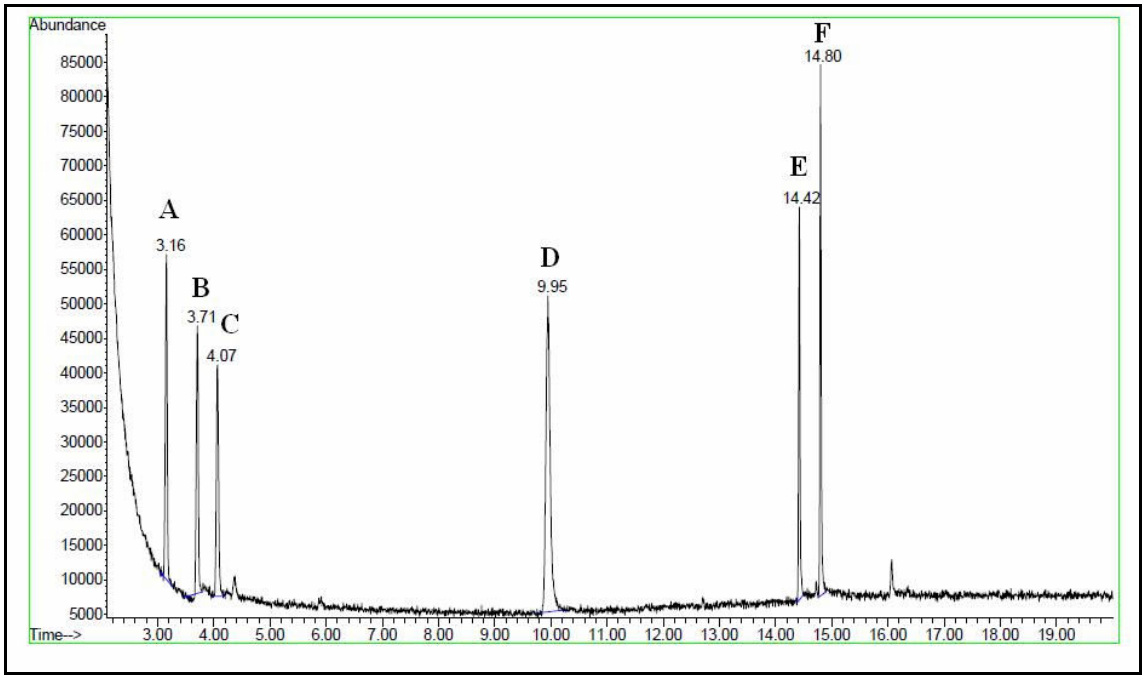


Figure 3.37 Typical total ion chromatogram of a standard mixture consisting of the five selected compounds and the internal standard.

The calibration curves for the standards in the lower concentration range showed linearity, whilst the graphs for standards in the higher concentration range were non-linear. The correlation coefficients for the analytes in the lower range were between 0.996 and 0.999, except for α-phellandrene which had a correlation coefficient of 0.970 when all five sets of data points were plotted. The residual plot for the data points also confirmed that there was bias in the data and the data points for the 80.6 mg L^{-1} standard were outliers which led to the distortion of the results. However, a plot of the concentration versus peak area only without the internal standard showed a correlation coefficient of 0.984 for α-phellandrene. This meant that the α-phellandrene standard was not added carefully in the 80.6 mg L^{-1} standard. Therefore a calibration curve and a residual plot of the area ratios of the 3.4 mg L^{-1}, 13.4 mg L^{-1}, 40.3 mg L^{-1} and 161 mg L^{-1} standards were constructed and is shown in Figures 3.42 and 3.43 respectively. The correlation coefficient obtained with these four standards for α-phellandrene was 0.999. Also, from the examination of the data in Table 3.25, it can be observed that a fairly good reproducibility was obtained.

The values obtained for the response factor covered a wide range. The variation in the response factor for the standards present in the low concentration range was smaller when compared to the variation of the response factor for the compounds present in the high concentration range. This variation could be due to peak tailing which was evident in the total ion chromatogram in Figure 3.37. The peak tailing, seen by the sloping baseline, was also observed in the chromatograms in Appendix C, Figures C13 to C17, although it is not as pronounced in Figure C17 when compared to Figure 3.37.

The non-linearity for the higher concentration range was due to the fact that the detector used in this study was an ion trap mass spectrometer which has a limited dynamic range (Pawliszyn, 1997e). This is as a result of the decomposition of the primary ions which are produced from the analyte due to secondary reactions which occur in the ion trap (Pawliszyn, 1997e). The RSDs for the compounds in the lower concentration range were all below 5%. The relative standard deviations for the compounds in the concentration range up to 9.02×10^3 mg L^{-1} were below 5%, but above this concentration, the RSDs were higher than 5% but did not exceed 10%.

Table 3.31 Calibration data for the determination of α-pinene.

Concentration/mg L^{-1}		3.4	13.7	41.2	82.4	165
Area Ratio	**1**	0.14	0.43	1.24	2.71	5.29
	2	0.15	0.47	1.26	2.57	5.27
	3	0.14	0.44	1.35	2.57	5.41
Mean		0.14	0.45	1.29	2.60	5.32
Std Dev		0.0061	0.0170	0.0602	0.0961	0.0752
RSD/%		4.3	3.8	4.7	3.7	1.4
Response Factor		0.82	1.03	1.07	1.06	1.03
Regression line		y = 0.032x – 0.006				
R^2		0.999				

Table 3.32 Calibration data for the determination of β-pinene.

Concentration/mg L^{-1}		3.4	13.7	41.2	82.5	165
Area Ratio	**1**	0.13	0.41	1.17	2.71	5.32
	2	0.12	0.41	1.23	2.52	5.32
	3	0.12	0.38	1.27	2.60	5.41
Mean		0.12	0.40	1.22	2.61	5.35
Std Dev		0.0044	0.0168	0.0489	0.0935	0.0499
RSD/%		3.6	4.2	4.00	3.6	0.9
Response Factor		0.93	1.15	1.13	1.06	1.03
Regression line		y = 0.032x – 0.051				
R^2		0.998				

Table 3.33 Calibration data for the determination of α-phellandrene.

Concentration/mg L^{-1}		3.4	13.4	40.3	80.6[†]	161
Area Ratio	**1**	0.12	0.38	1.13	1.61	4.32
	2	0.12	0.41	1.14	1.51	4.29
	3	0.12	0.38	1.21	1.51	4.40
Mean		0.12	0.39	1.16	1.54	4.34
Std Dev		0.0018	0.0140	0.0463	0.0583	0.0547
RSD/%		1.5	3.6	4.00	3.8	1.3
Response Factor		0.95	1.15	1.16	1.75	1.24
Regression line		y = 0.025x – 0.035				
R^2		0.970				

[†] - The values in this column were not used in the construction of the calibration curve.

Table 3.34 Calibration data for the determination of β-caryophyllene.

Concentration/mg L^{-1}		3.6	14.4	43.3	86.6	173
Area Ratio	**1**	0.12	0.39	1.19	2.70	5.75
	2	0.12	0.40	1.24	2.54	5.47
	3	0.12	0.39	1.29	2.56	5.81
Mean		0.12	0.39	1.24	2.60	5.68
Std Dev		0.0043	0.0097	0.0519	0.0870	0.182
RSD/%		3.5	2.5	4.2	3.4	3.2
Response Factor		0.99	1.23	1.17	1.11	1.02
Regression line		$y = 0.032x - 0.109$				
R^2		0.996				

Table 3.35 Calibration data for the determination of α-caryophyllene.

Concentration/mg L^{-1}		3.6	14.2	42.7	85.3	171
Area Ratio	**1**	0.15	0.52	1.50	3.50	7.32
	2	0.15	0.53	1.58	3.32	6.90
	3	0.14	0.50	1.65	3.33	7.39
Mean		0.15	0.51	1.58	3.38	7.21
Std Dev		0.0012	0.0162	0.0714	0.0998	0.268
RSD/%		0.8	3.1	4.5	3.0	3.7
Response Factor		0.82	0.92	0.91	0.84	0.79
Regression line		$y = 0.042x - 0.122$				
R^2		0.996				

The calibration curves and the residual plots used in this investigation are shown in Figures 3.38 to 3.47

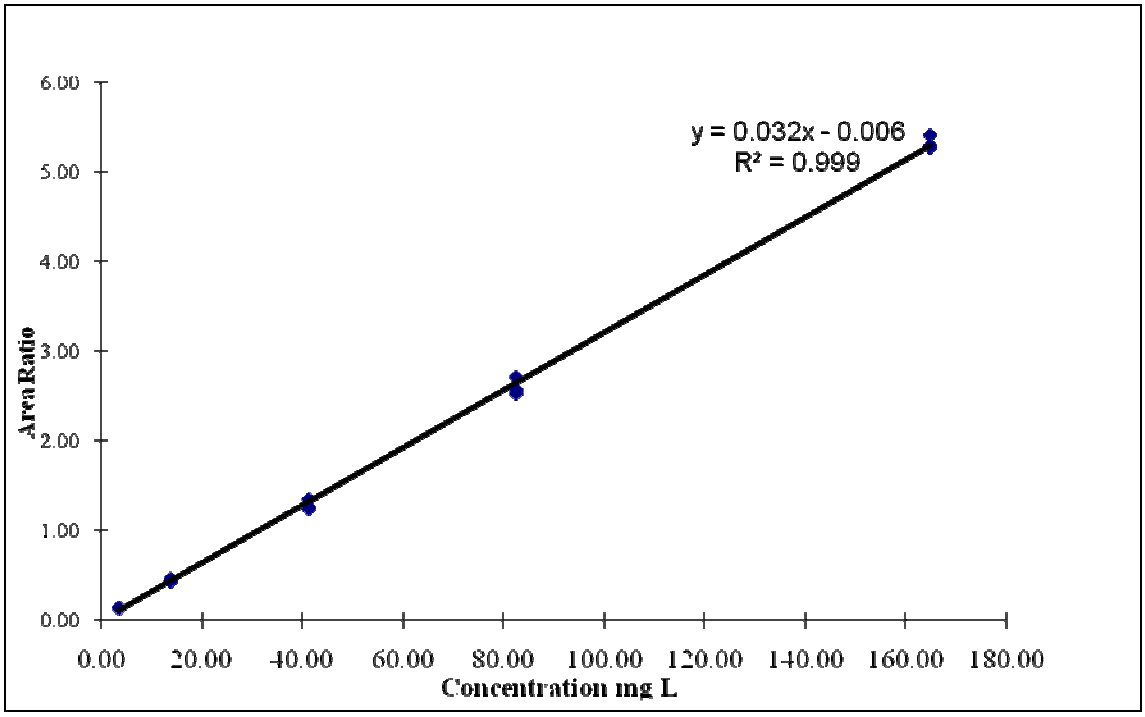


Figure 3.38 Calibration curve for α-pinene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1μL.

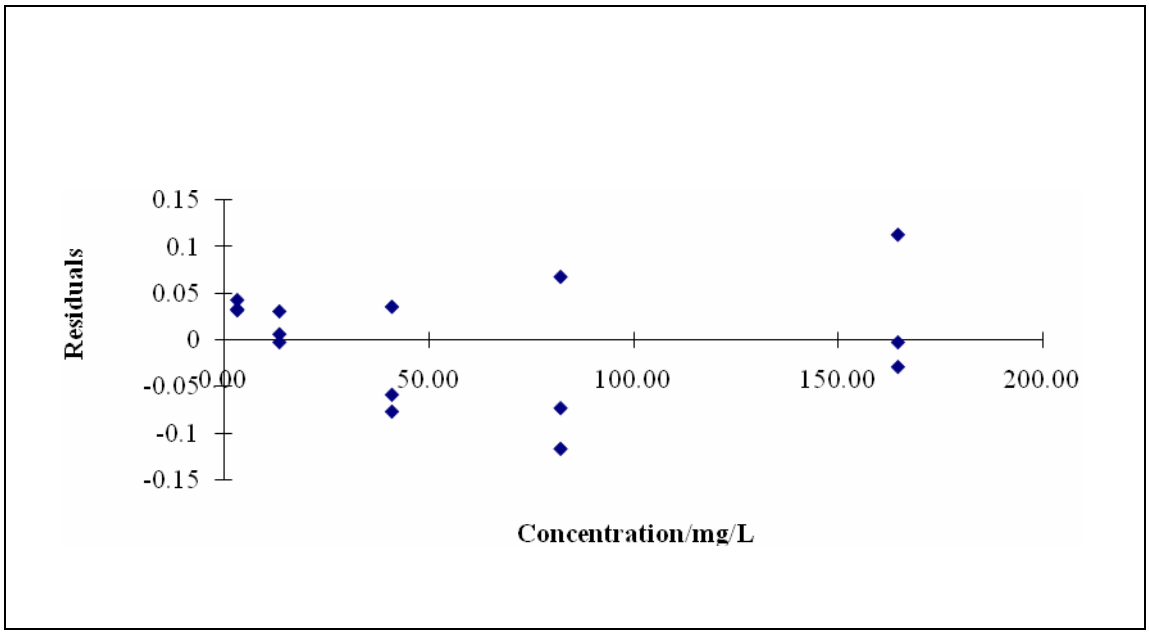


Figure 3.39 Residual plot for the calibration curve of α-pinene.

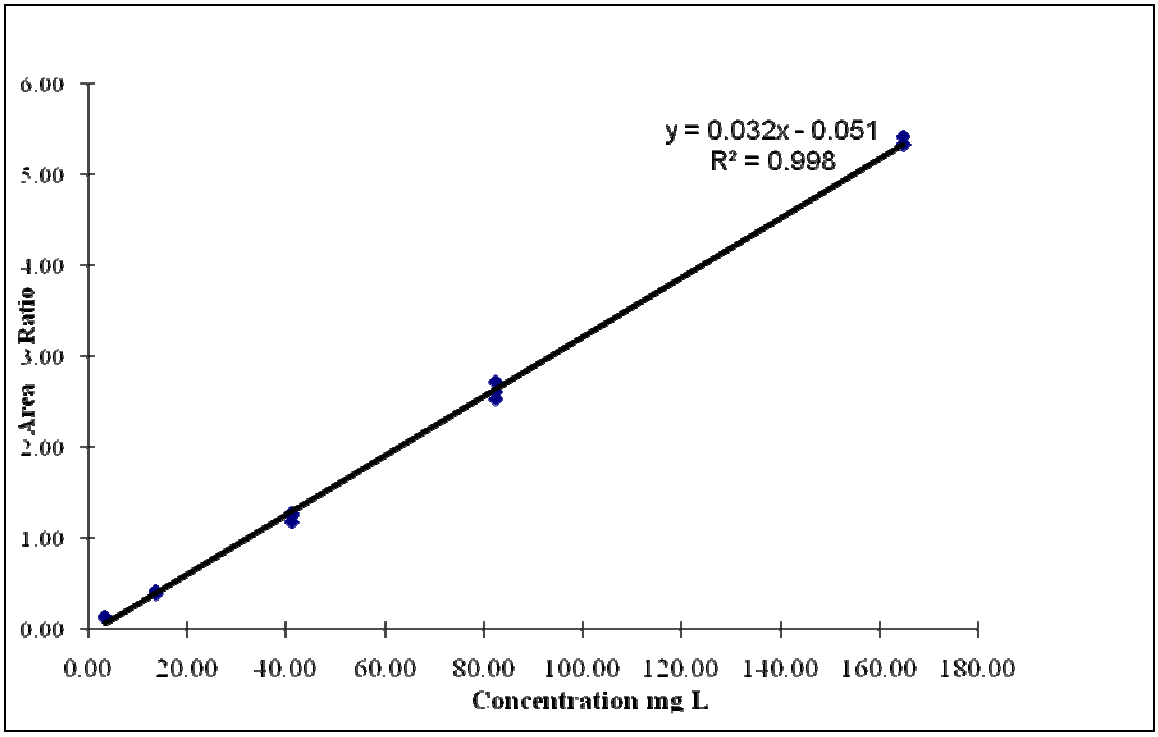


Figure 3.40 Calibration curve for β-pinene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1µL.

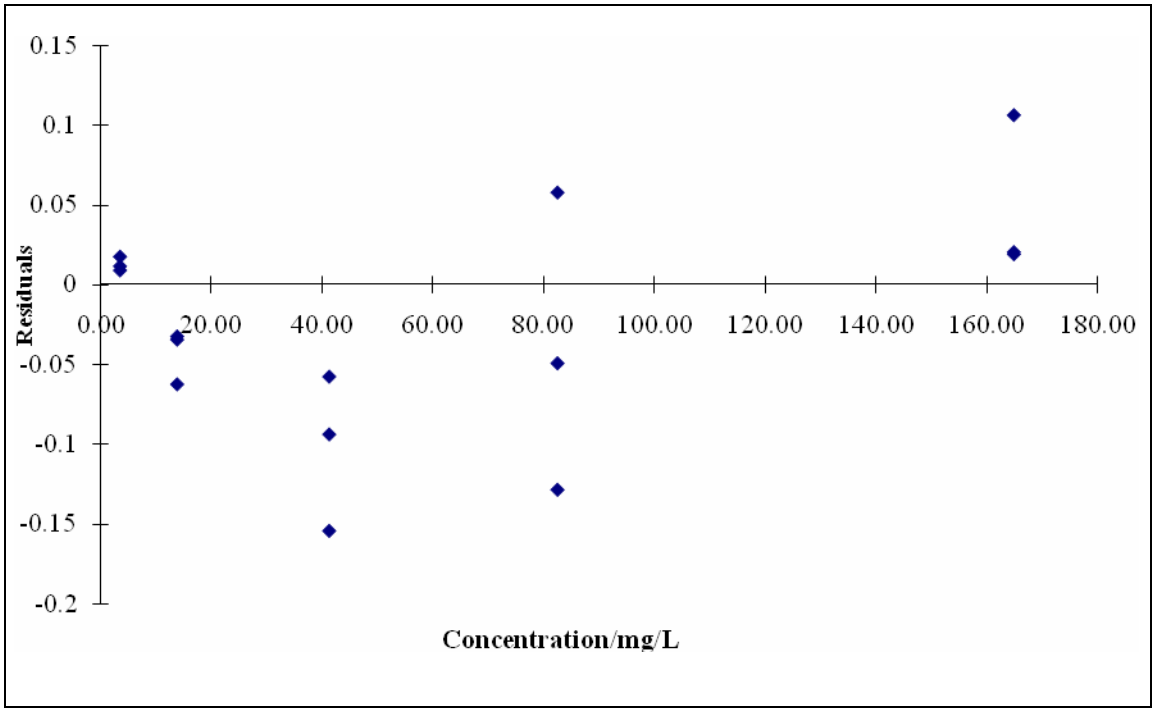


Figure 3.41 Residual plot for the calibration curve of β-pinene.

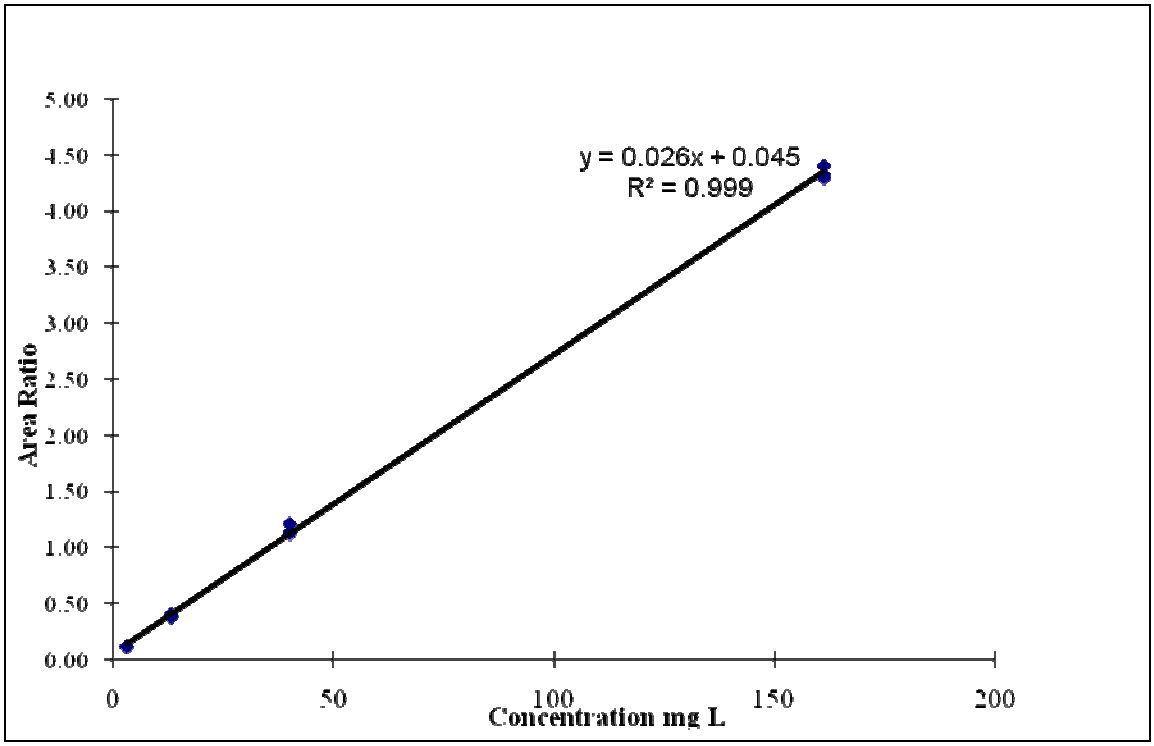


Figure 3.42 Calibration curve for α-phellandrene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1μL.

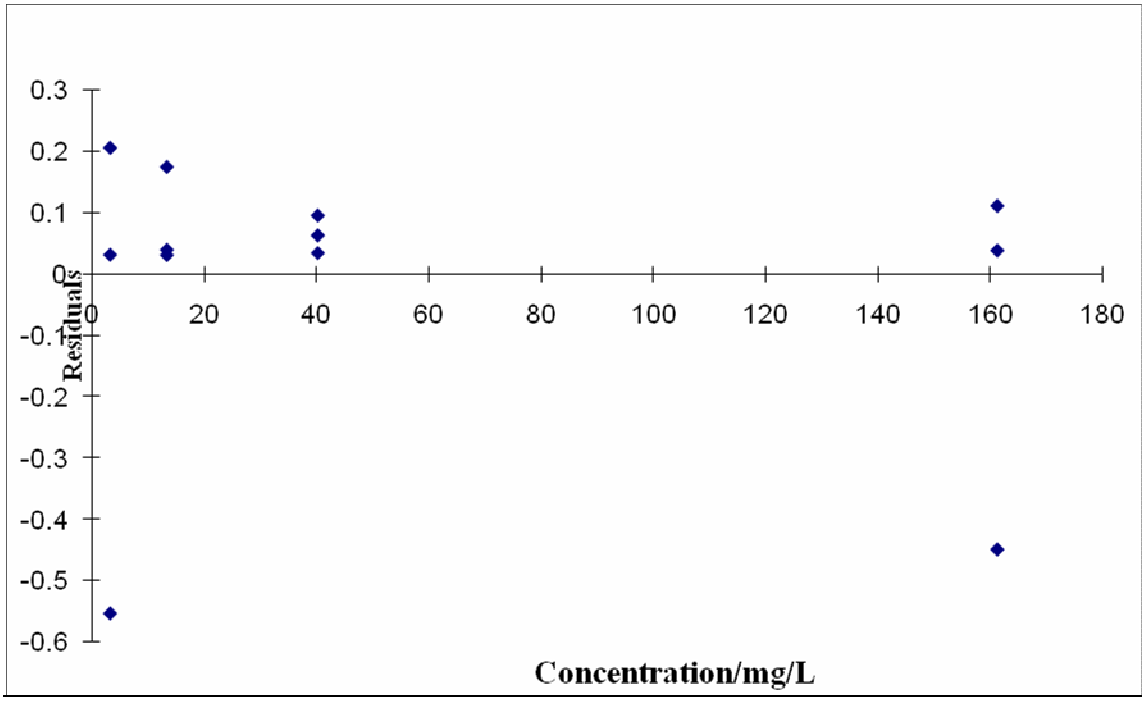


Figure 3.43 Residual plot for the calibration curve of α-phellandrene.

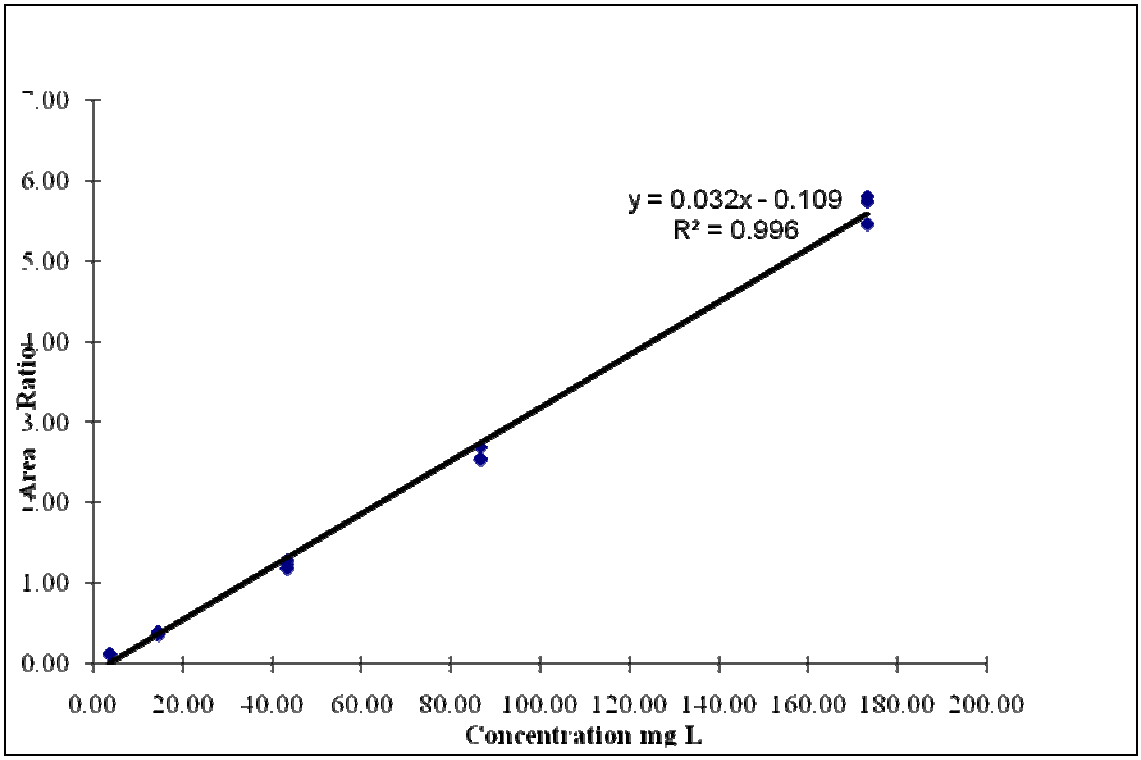


Figure 3.44 Calibration curve for β-caryophyllene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1μL.

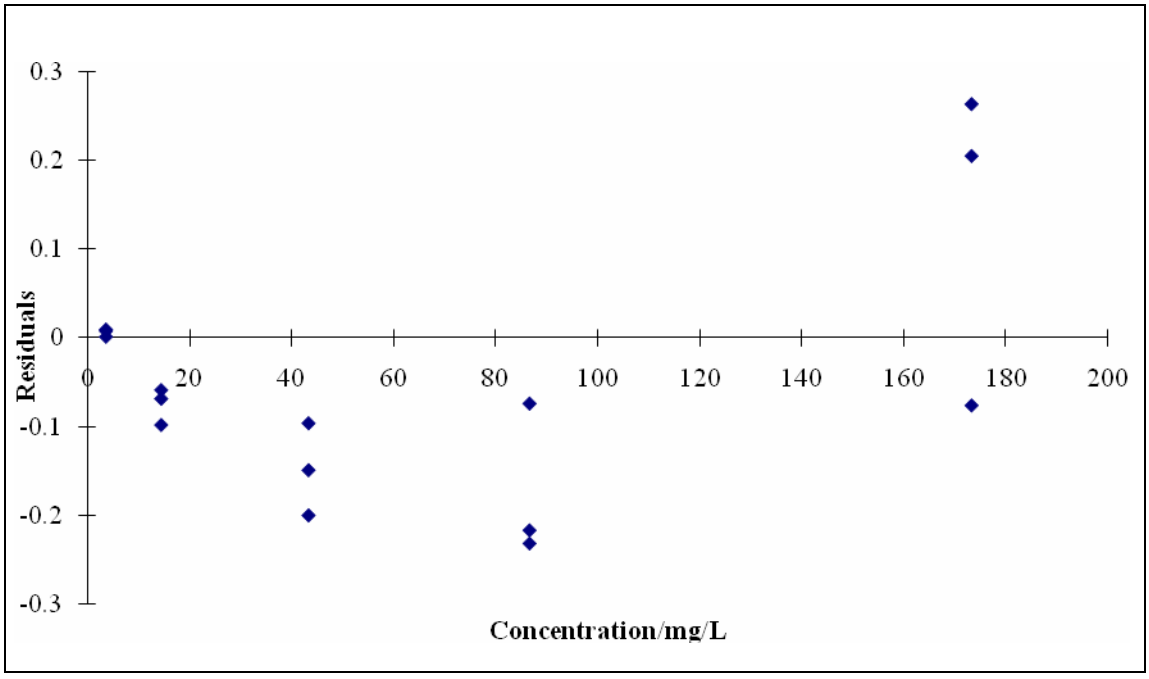


Figure 3.45 Residual plot for the calibration curve of β-caryophyllene.

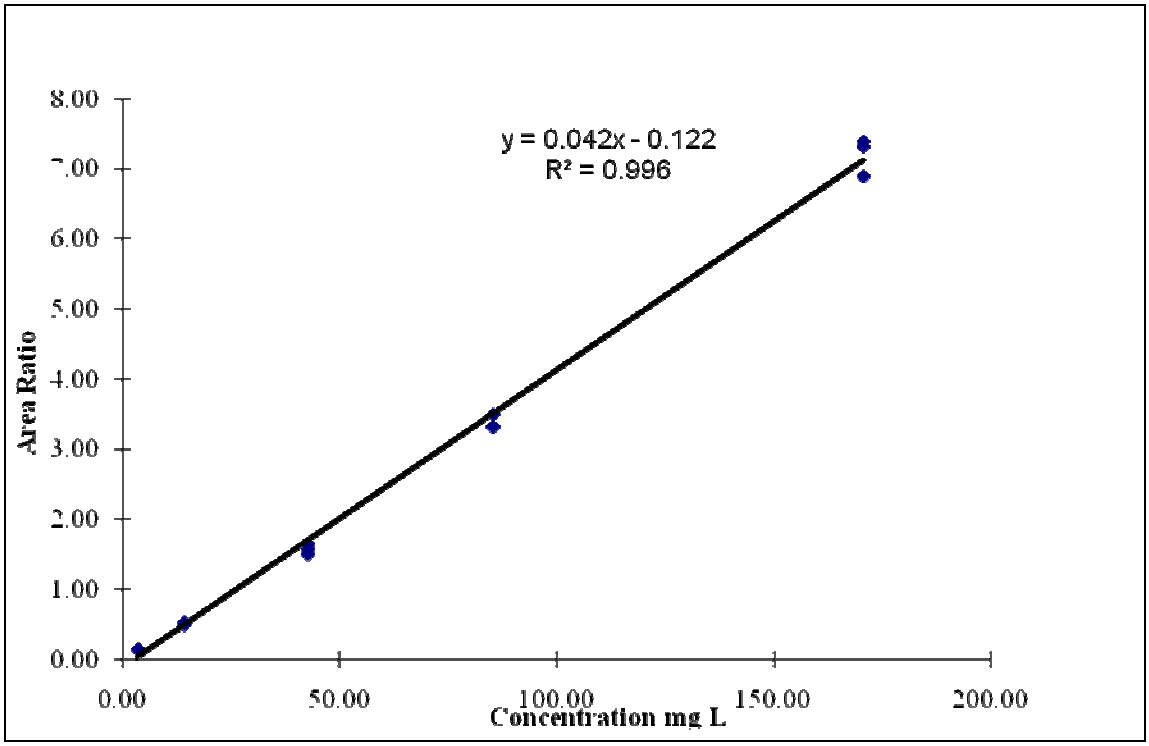


Figure 3.46 Calibration curve for α-caryophyllene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1μL.

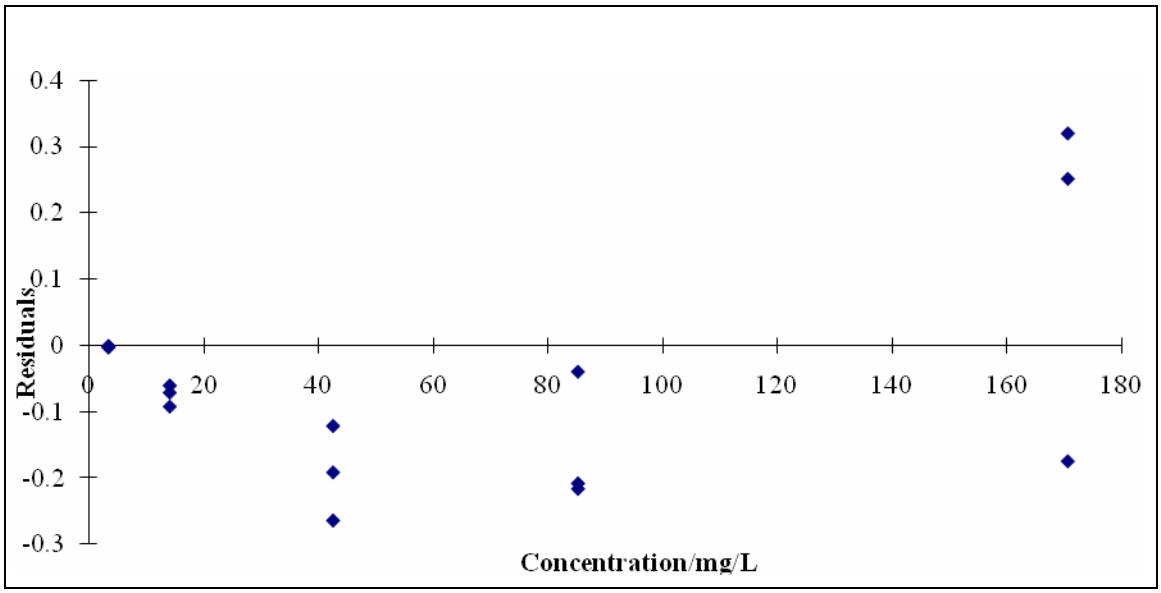


Figure 3.47 Residual plot for the calibration curve of α-caryophyllene.

Table 3.36 Calibration data for the determination of α-pinene.

Concentration/mg L^{-1}		330	858	2.57×10^3	8.58×10^3	1.72×10^4
Area Ratio	**1**	14.3	39.1	73.2	98.1	98.5
	2	13.8	39.9	73.3	96.5	99.1
	3	13.8	39.6	73.8	99.1	107
Mean		13.9	39.5	73.4	97.9	102
Std Dev		0.26	0.39	0.35	1.30	5.00
RSD/%		1.9	1.0	0.5	1.3	4.9
Response Factor		0.79	0.73	1.17	2.93	5.64

Table 3.37 Calibration data for the determination of β-pinene.

Concentration/mg L^{-1}		330	859	2.58×10^3	8.59×10^3	1.72×10^4
Area Ratio	**1**	14.5	45.2	82.3	105	107
	2	14.1	46.0	82.6	111	111
	3	14.0	45.5	83.4	114	117
Mean		14.2	45.6	82.8	110	112
Std Dev		0.27	0.42	0.57	4.25	5.15
RSD/%		1.9	0.9	0.7	3.9	4.6
Response Factor		0.78	0.63	1.04	2.60	5.13

Table 3.38 Calibration data for the determination of α-phellandrene.

Concentration/mg L^{-1}		323	840	2.52×10^3	8.40×10^3	1.68×10^4
Area Ratio	**1**	11.8	45.0	64.0	92.9	97.8
	2	11.2	45.7	64.0	92.1	98.5
	3	11.2	45.2	64.3	94.9	106
Mean		11.4	45.3	64.1	93.3	101
Std Dev		0.32	0.38	0.13	1.42	4.64
RSD/%		2.8	0.8	0.2	1.5	4.6
Response Factor		0.95	0.62	1.31	3.01	5.57

Table 3.39 Calibration data for the determination of β-caryophyllene.

Concentration/mg L^{-1}		346	902	2.71×10^3	9.02×10^3	1.80×10^4
Area Ratio	**1**	15.3	42.4	70.4	79.2	89.5
	2	14.8	42.5	71.9	76.1	92.9
	3	14.9	43.1	70.3	79.9	96.4
Mean		15.0	42.7	70.9	78.4	92.9
Std Dev		0.28	0.38	0.92	2.02	3.45
RSD/%		1.9	0.9	1.3	2.6	3.7
Response Factor		0.77	0.71	1.28	3.85	6.49

Table 3.40 Calibration data for the determination of α-caryophyllene.

Concentration/mg L^{-1}		341	889	2.67×10^3	8.89×10^3	1.78×10^4
Area Ratio	**1**	18.7	57.7	88.3	124	128
	2	18.1	58.0	90.4	119	133
	3	18.2	58.4	88.9	124	137
Mean		18.3	58.0	89.2	123	133
Std Dev		0.34	0.31	1.07	2.69	4.60
RSD/%		1.8	0.5	1.2	2.2	3.5
Response Factor		0.62	0.51	1.00	2.42	4.48

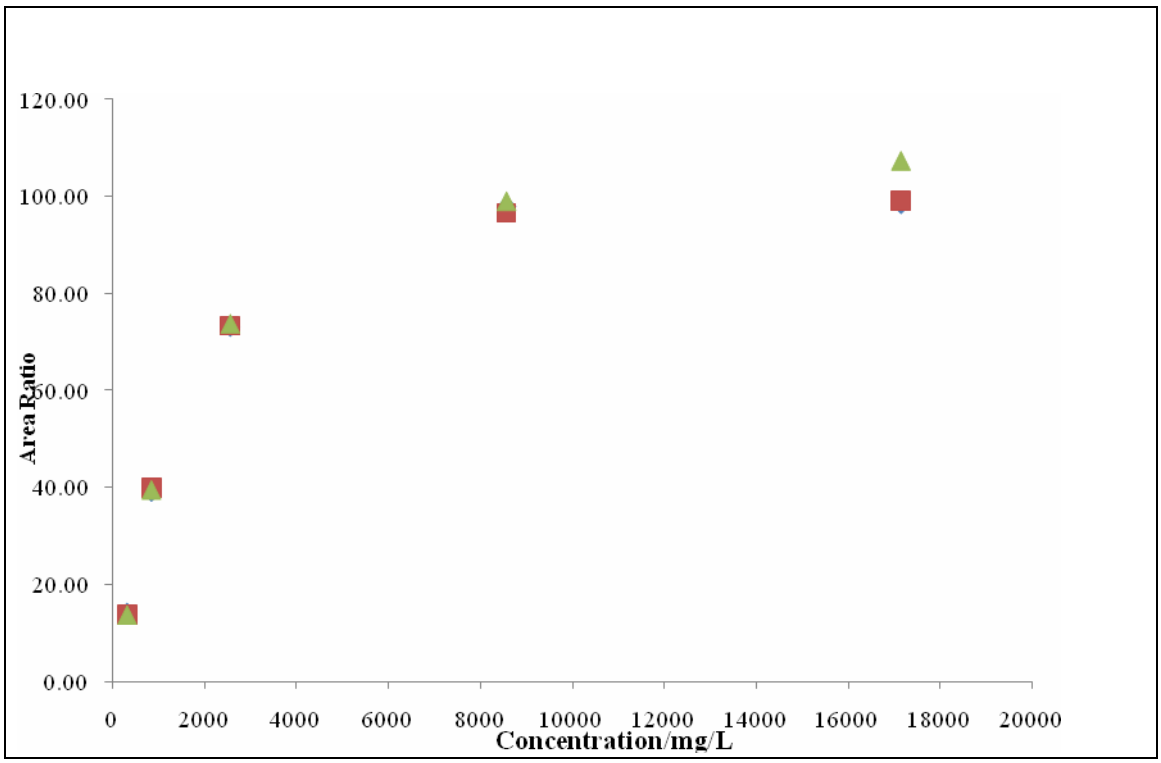


Figure 3.48 Calibration curve for α-pinene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1μL.

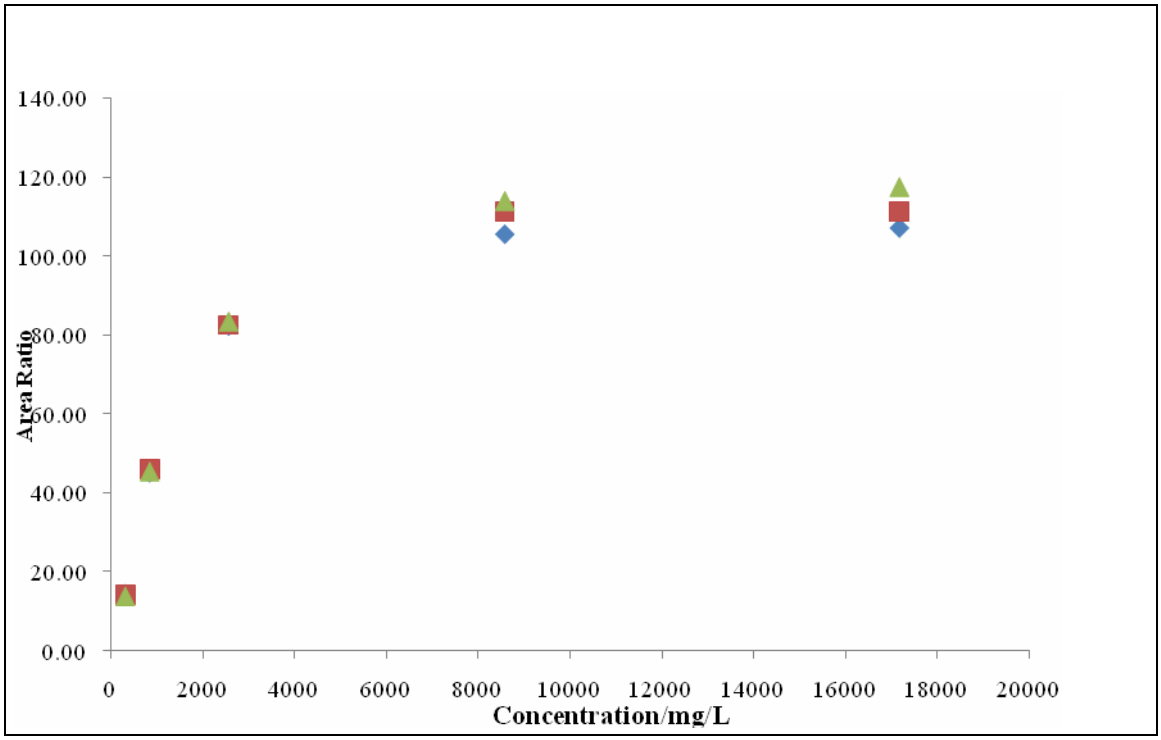


Figure 3.49 Calibration curve for β-pinene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1µL.

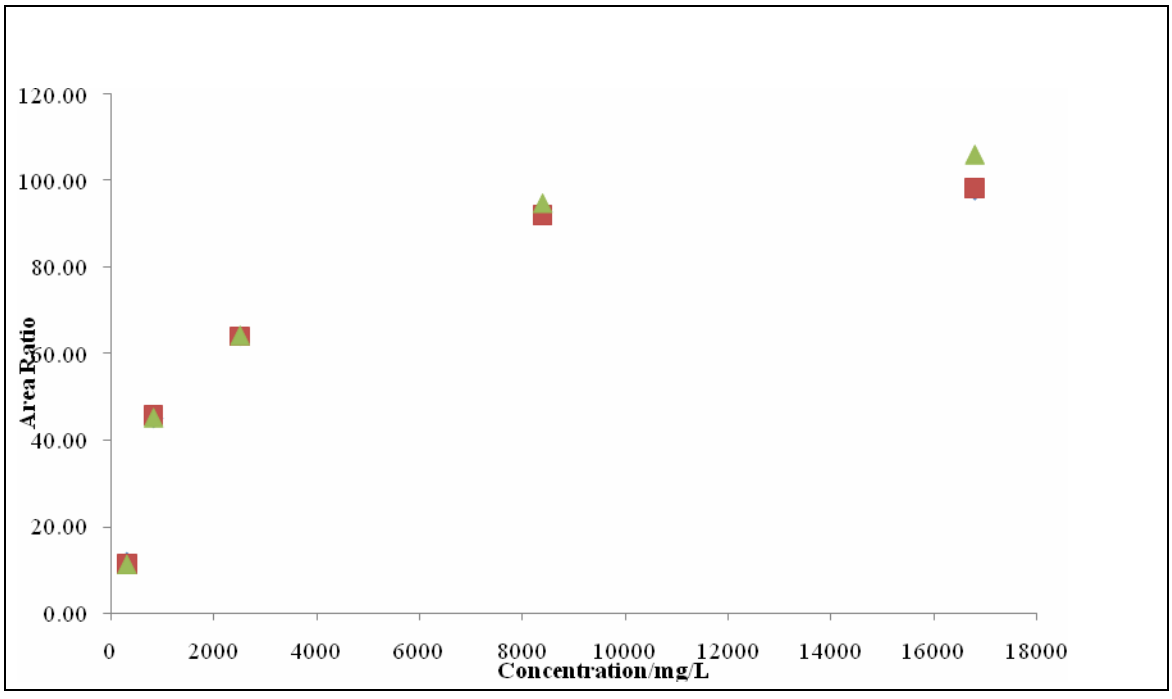


Figure 3.50 Calibration curve for α-phellandrene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1µL.

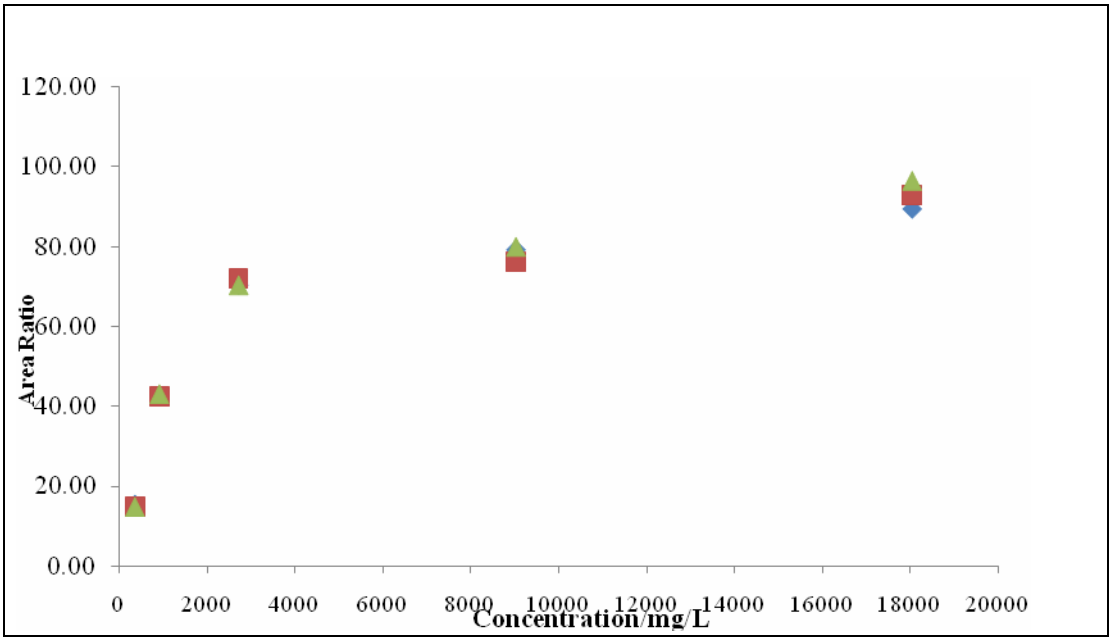


Figure 3.51 Calibration curve for β-caryophyllene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1µL.

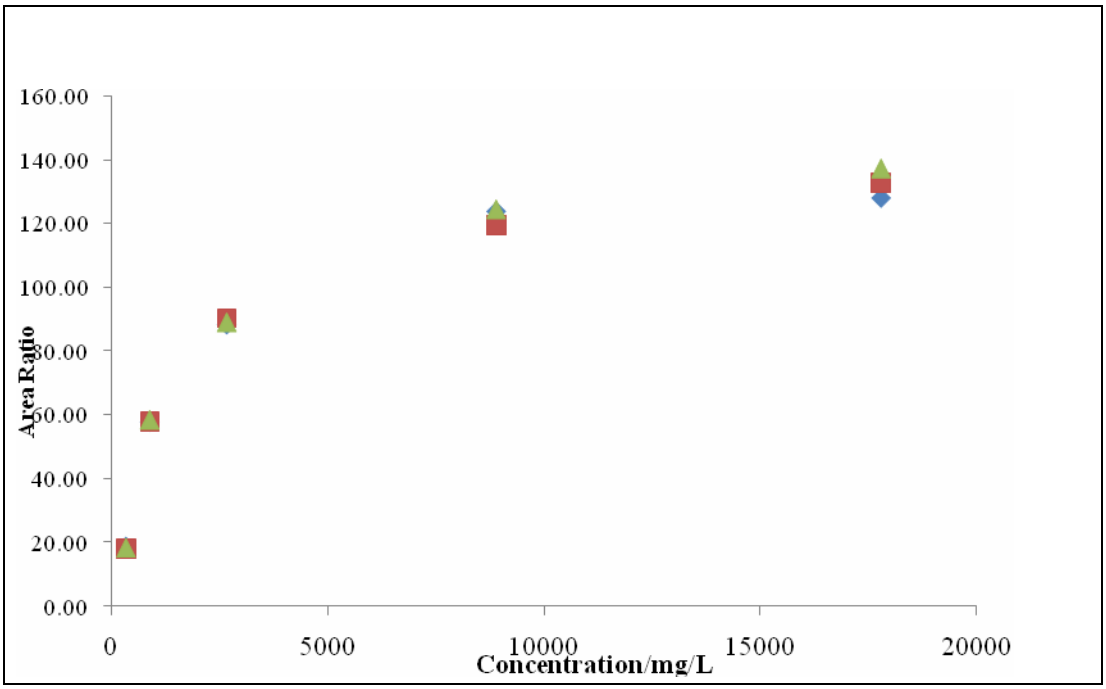


Figure 3.52 Calibration curve for α-caryophyllene. The chromatographic conditions used were: a non-polar DB-5 (methyl phenyl siloxane) capillary column, injection volume – 1µL.

3.9 Quantitation of the volatile compounds

Quantification was performed for the liquid phase extracts obtained by steam distillation, Soxhlet extraction and solvent extraction only and these results are discussed in Section 3.10. The concentrations of the five selected aroma compounds, α-pinene, β-pinene, α-phellandrene, β-caryophyllene, and α-caryophyllene, were determined in this study.

Even though HS-SPME has many advantages when compared to other extraction methods, quantitative studies using this method of extraction have been difficult. In the study discussed earlier in Section 3.5.5.2 it was observed that competition for the adsorption sites on the PDMS and PA fibres exist. According to Pino *et al*. (2002), the competition for the sites on the fibre could be due to the low volatiles displacing the high volatiles. They also reported that there may be headspace depletion of some low volatiles without reaching equilibrium and concluded that the use of SPME for the quantification of complex systems may be limited. However, they did mention that that quantitation may be possible for some matrices if biases due to competition are controlled. Although Contini and Esti (2006) performed quantitation in their work by ensuring that the slopes which were obtained were consistent, they maintained that selecting analytical conditions for the highest amount of the compounds extracted, without proper controls may lead to errors in quantitation.

Tholl *et al*. (2006) also reported that in order to obtain reproducible quantitative results, the analytes must reach equilibrium. Since the volatile fraction present in the curry leaves is complex and the compounds are present in a wide range, true equilibrium was difficult to establish. Since it was the pre-equilibrium period that was used in this work, quantification during this extraction period would therefore be inaccurate. According to Bichi *et al.* (2007), pre-equilibrium conditions must be used when working with aromatic plants with a complicated matrix, since equilibrium for all the components will be difficult to attain for compounds of varying polarity and volatility. Moreover, they maintained that due to the unavailability of a standard matrix, results, in the case of solid samples, cannot be accurately quantified.

Also, according to Vas and Vekey (2004), SPME was applied primarily for compound identification and screening purposes. According to Howard, Mike and Riesen (2005) SPME analysis has not been devoid of problems. They make mention of the competition of the volatiles for the sites on the fibre with the low molar mass compounds being displaced by the high molar mass compounds and that sometimes there may be a depletion of some high molar mass components, even before equilibrium has been reached. They also found that when different standardisation methods were used, the results obtained were not consistent and concluded that SPME is limited in its use for the quantification of complex systems. Furthermore, due to the lack of available certified reference materials to be used as standards for direct analysis, the quantification of solid samples is problematic.

Therefore, the headspace methods were not quantified since a broad range of volatiles were studied and according to Tholl *et al*. (2006), quantification by SPME can be both difficult and impractical when dealing with compounds present in a large range with varying distribution constants. A similar finding was reported by Ferreira *et al*. (1996) in their analysis of wine volatiles.

3.9.1 Quantification of the volatiles in the essential oil obtained from the solvent, Soxhlet and steam distillation techniques

The chromatograms in Figures 3.53 to 3.59 are representative of the solvent and Soxhlet extractions and steam distillation, with the addition of the internal standard, dodecane, at the start of the extraction procedure and at the end of extraction. The addition of the internal standard at the start of the extraction procedure was done to look at the performance of the extraction technique relative to the analytical procedure. The area percent reports can be found in Appendix F, Tables F37 to F42.

For the compounds which were present in a low concentration range, a 29.9 mg L^{-1} concentration of the internal standard was used so as not to suppress the ionization of the analytes present in the lower region. It has been reported that molecules with higher

mass cause the supression of the signal of compounds present in a smaller amount (Annesley, 2003). The peak in the chromatograms, due to the internal standard, dodecane, is small relative to the compounds in the high concentration range as shown in Figure 3.53. To overcome this problem, for future work, a second internal standard should be employed for the analytes present in the high concentration range. In Figure 3.54, the abundance scale has been adjusted to show the peak due to the internal standard, labelled D, in expanded form.

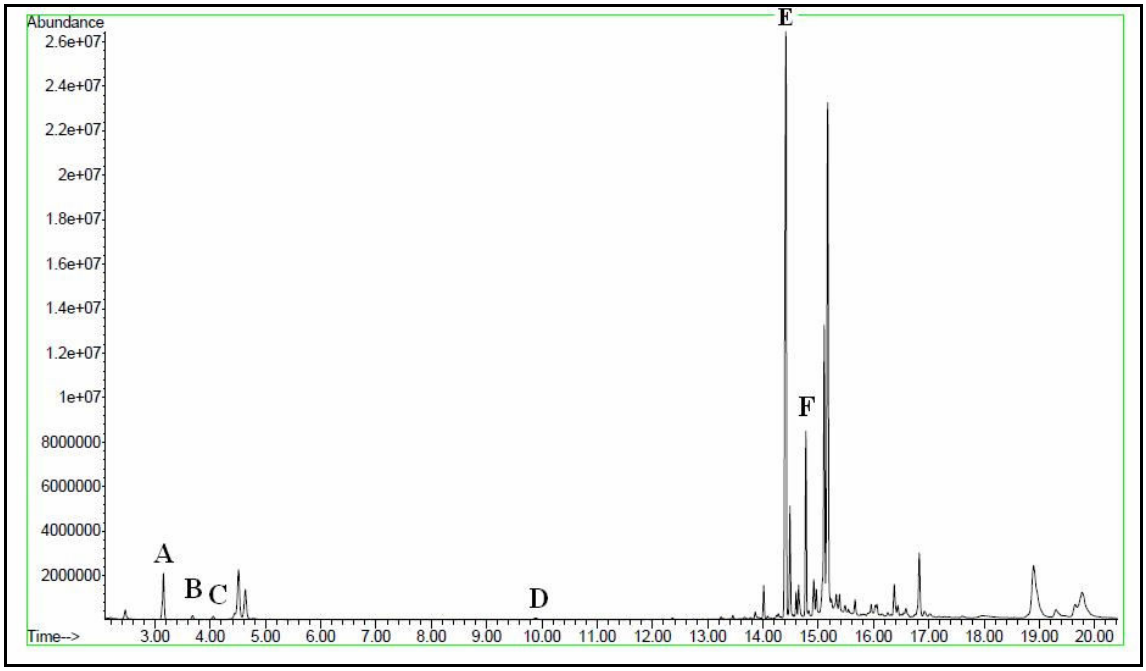


Figure 3.53 Total ion chromatogram for the essential oil obtained from the solvent extraction with the internal standard added at the start of the extraction.

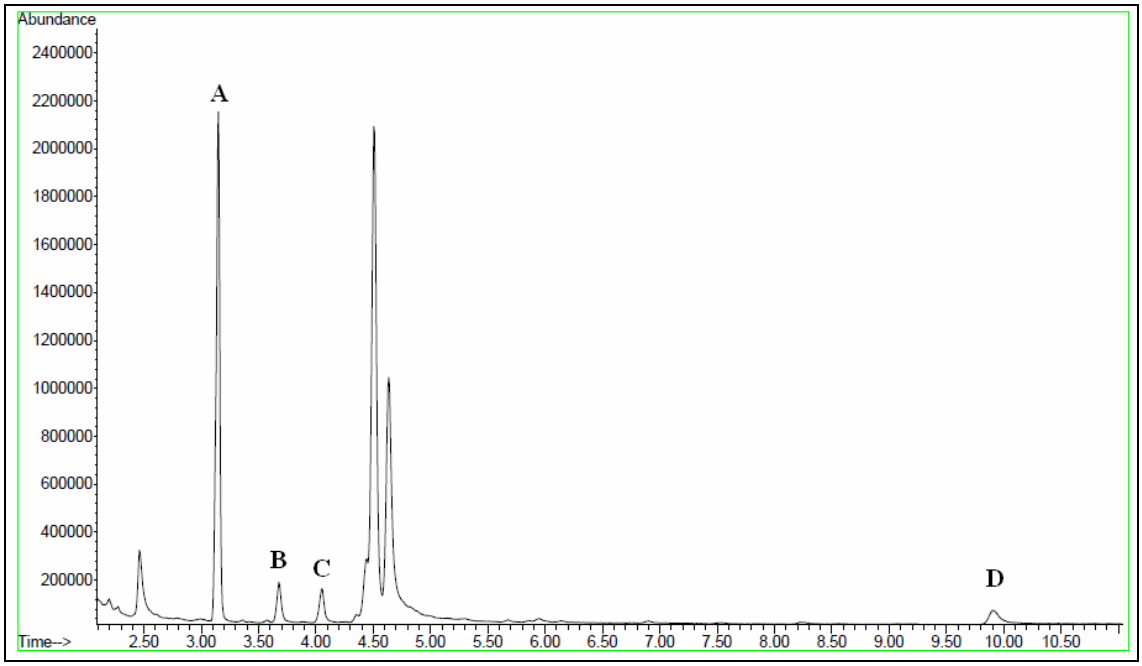


Figure 3.54 Expanded chromatogram of Figure 3.53 showing the internal standard, D.

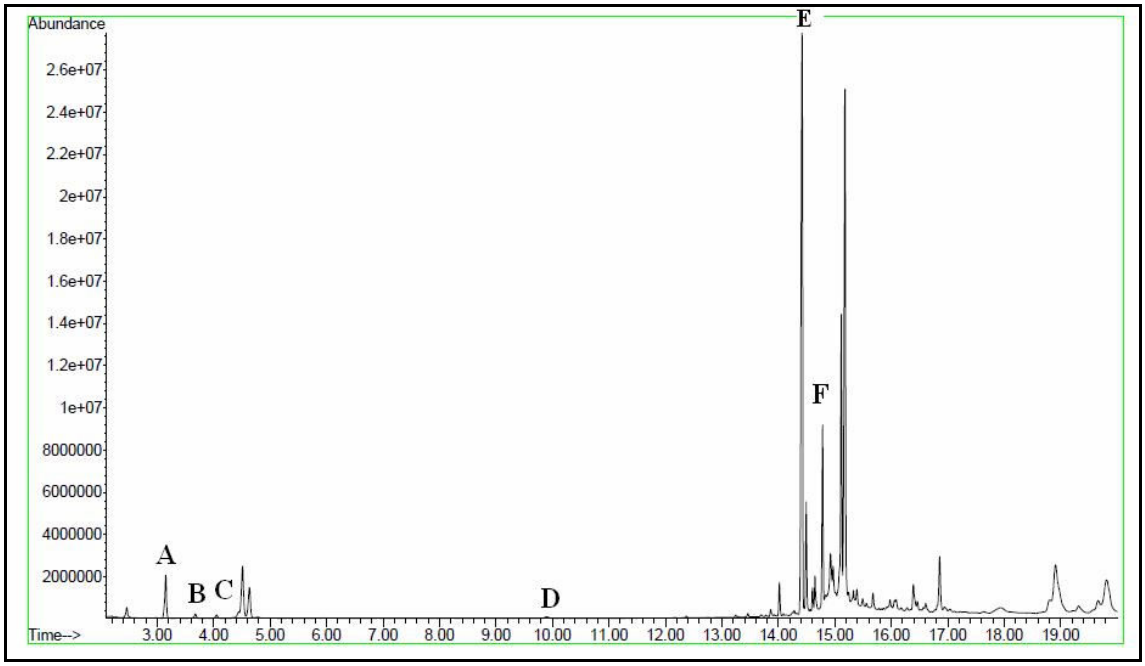


Figure 3.55 Total ion chromatogram for the essential oil obtained from the solvent extraction with the internal standard added at the end of the extraction.

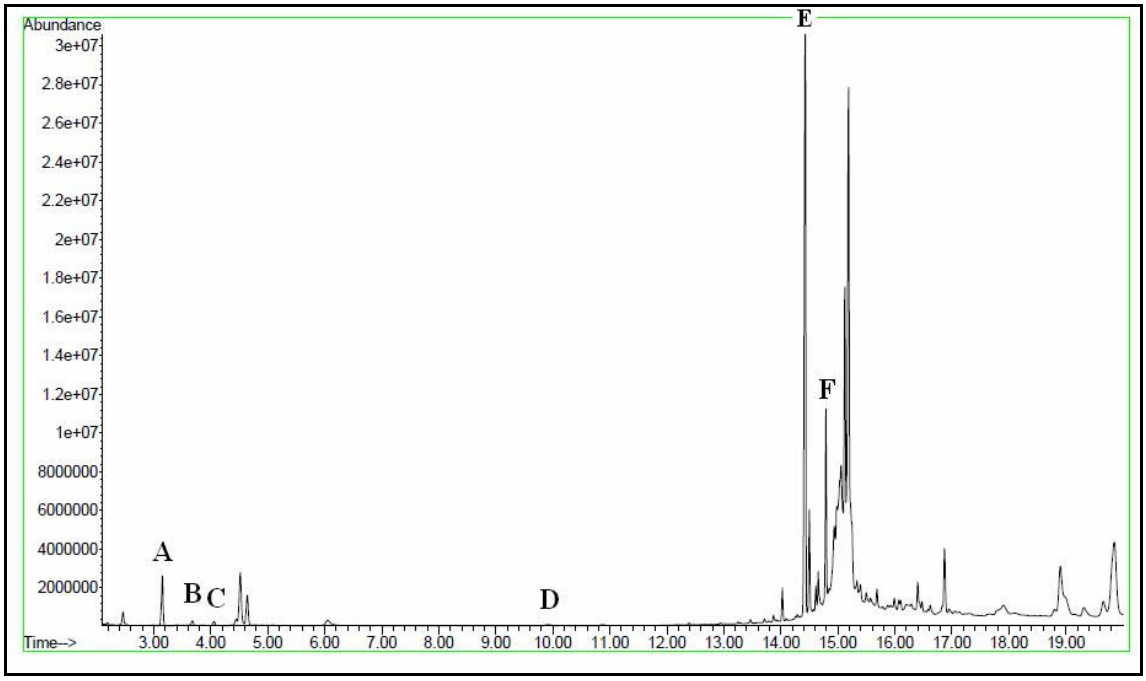


Figure 3.56 Total ion chromatogram for the essential oil obtained from the Soxhlet extraction with the internal standard added at the start of the extraction.

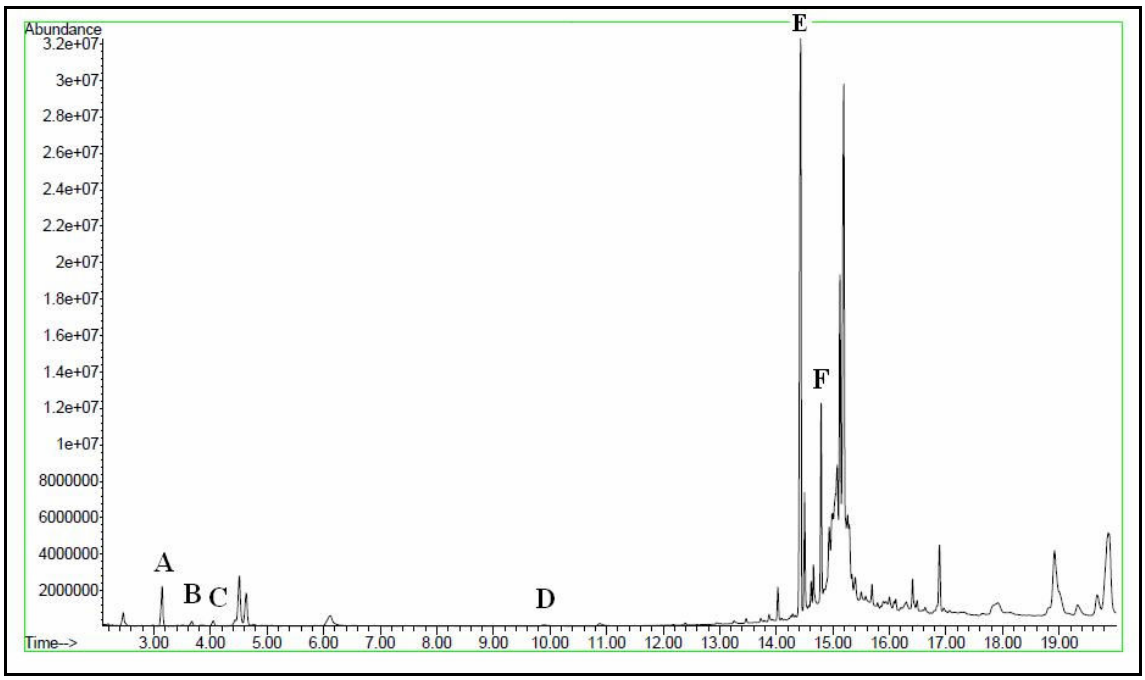


Figure 3.57 Total ion chromatogram for the essential oil obtained from the Soxhlet extraction with the internal standard added at the end of the extraction.

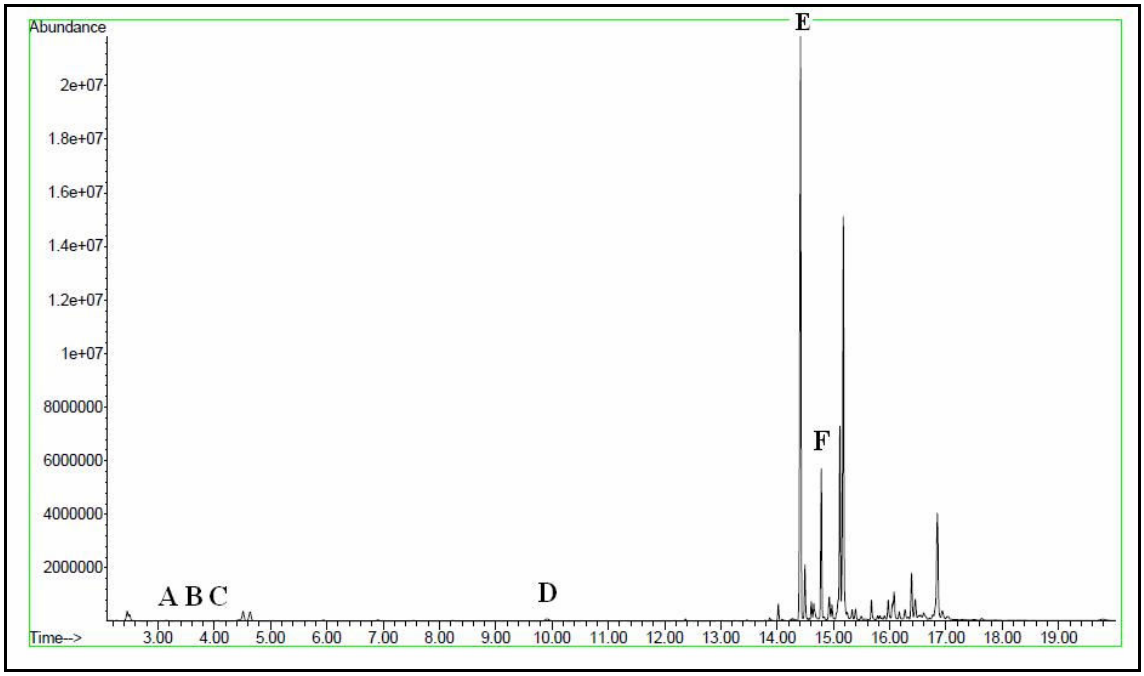


Figure 3.58 Total ion chromatogram for the essential oil obtained from the steam distillation extraction with the internal standard added at the start of the extraction.

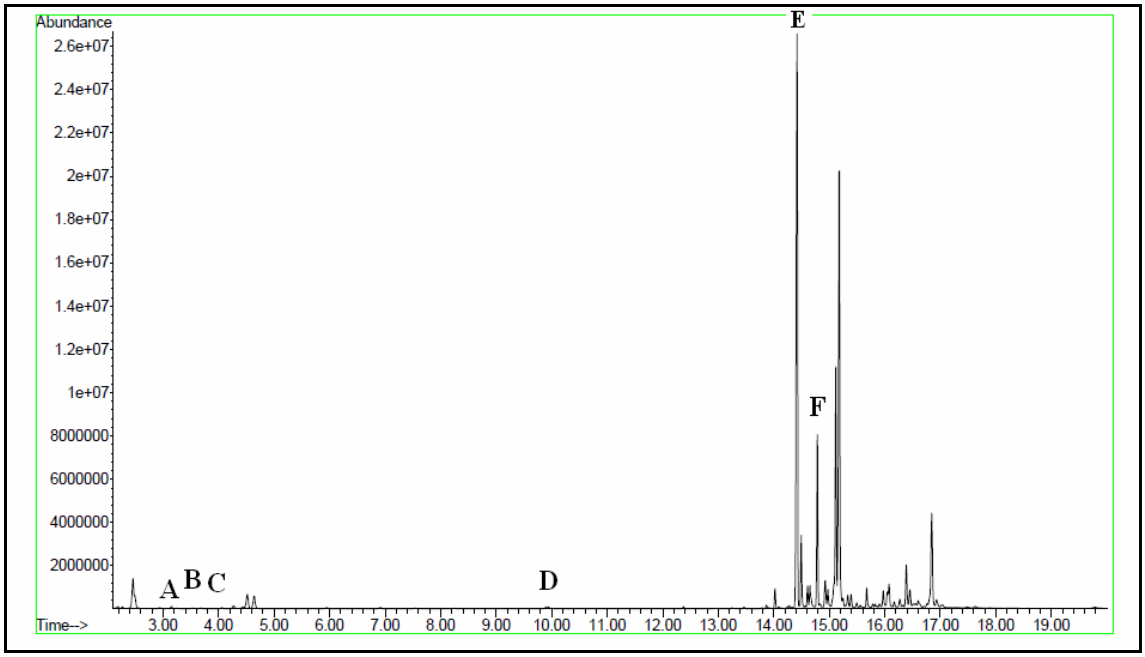


Figure 3.59 Total ion chromatogram for the essential oil obtained from the steam distillation extraction with the internal standard added at the end of the extraction.

Tables 3.41 to 3.43 show the average with the standard (STD) deviation and the percent relative standard deviations of the peak area ratios of the five selected aroma contributing compounds in curry leaves for the three extraction methods with the internal standard added at the start of the extraction and at the end of the extraction. The data shown are the mean values of three injections. The concentrations of the five compounds are shown in Table 3.44.

Table 3.41 Average peak area ratios with the standard deviation and RSD for solvent extraction.

Compound	**Solvent Extraction-internal standard added at start of extraction**		**Solvent Extraction-internal standard added at end of extraction**	
	Average ± STD Deviation (n = 3)	**RSD/%**	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	10.1 ± 0.4	4	9.3 ± 0.2	2
β-pinene	1.20 ± 0.01	1	1.18 ± 0.04	3
α-phellandrene	1.05 ± 0.03	3	1.01 ± 0.03	3
β-caryophyllene	122 ± 4	3	120 ± 1	1
α-caryophyllene	30.1 ± 0.7	2	30.6 ± 0.9	3

Table 3.42 Average peak area ratios with the standard deviation and RSD for Soxhlet extraction.

Compound	**Soxhlet Extraction-internal standard added at start of extraction**		**Soxhlet Extraction-internal standard added at end of extraction**	
	Average ± STD Deviation (n = 3)	**RSD/%**	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	11.9 ± 0.4	3	10.0 ± 0.4	4
β-pinene	1.55 ± 0.01	1	1.70 ± 0.06	3
α-phellandrene	1.32 ± 0.02	1	1.88 ± 0.02	1
β-caryophyllene	138 ± 1	1	135 ± 3	3
α-caryophyllene	46.5 ± 2.2	5	46.7 ± 1.9	4

Table 3.43 Average peak area ratios with the standard deviation and RSD for steam distillation.

Compound	**Steam Distillation-internal standard added at start of extraction**		**Steam Distillation-internal standard added at end of extraction**	
	Average ± STD Deviation (n = 3)	**RSD/%**	**Average ± STD Deviation (n = 3)**	**RSD/%**
α-pinene	0.13 ± 0.003	2	0.39 ± 0.003	1
β-pinene	0.10 ± 0.002	2	0.19 ± 0.01	4
α-phellandrene	0.15 ± 0.002	1	0.25 ± 0.002	1
β-caryophyllene	63.4 ± 1.3	2	80.7 ± 0.8	1
α-caryophyllene	15.3 ± 0.6	4	19.1 ± 0.1	0.3

Table 3.44 Concentration of the five volatile compounds determined in the essential oil of *M. Koenigii* by steam distillation, Soxhlet extraction and solvent extraction with the internal standard added at the start and at the end of the extraction.

Compound	**Solvent Extraction**		**Soxhlet Extraction**		**Steam Distillation**	
	Concentration/ mg kg^{-1} (n = 3)		**Concentration/ mg kg^{-1} (n = 3)**		**Concentration/ mg kg^{-1} (n = 3)**	
	A	**B**	**A**	**B**	**A**	**B**
α-pinene	46.7 ± 2.0	42.9 ± 0.9	54.6 ± 1.8	46.3 ± 1.7	1.02 ± 0.02	2.32 ± 0.02
β-pinene	7.39 ± 0.09	7.27 ± 0.23	9.51 ± 0.09	10.5 ± 0.4	0.73 ± 0.02	1.30 ± 0.05
α-phellandrene	10.7 ± 0.3	10.3 ± 0.3	8.86 ± 0.11	13.6 ± 0.2	1.33 ± 0.03	1.65 ± 0.01
β-caryophyllene	4609 ± 152	4551 ± 45	5204 ± 38	5115 ± 129	1838 ± 41	1803 ± 18
α-caryophyllene	90.0 ± 2.2	91.3 ± 2.8	271 ± 13	272 ± 11	71.9 ± 1.6	68.9 ± 0.2

A - refers to samples in which the internal standard was added at the start of extraction.
B - refers to samples in which the internal standard was added at the end of extraction

The qualitative composition of the oil obtained from the different methods was almost similar, but the relative concentrations for some compounds shown in Table 3.36 differed.

The oil obtained from the Soxhlet extraction (Table 3.44) showed the highest amount of β-caryophyllene (5204 mg L^{-1}) followed by solvent extraction (4609 mg L^{-1}) and with steam distillation giving the lowest yield (1803 mg L^{-1}). Once again, Soxhlet showed a higher amount of compound extracted when compared to solvent extraction. This could be due to the compounds being extracted at a higher temperature in the Soxhlet extraction when compared to the solvent extraction which was conducted at room temperature. The Soxhlet extraction showed a higher yield of oil extracted when compared to steam distillation, since Soxhlet extraction is an exhaustive process whilst steam distillation is not.

Although the extraction of the highly volatile compounds with the steam distillation was low, the results are consistent with work done by other researchers. Results obtained by MacLeod and Pieris for the steam distillation-extraction technique on the fresh leaves of *M. koenigii* showed the presence of the following amounts for the high volatile compounds: β-pinene, 66.1 ppb; α-phellandrene, 52.3 ppb, and for the low volatile, β-caryophyllene, 2563.2 ppb.

A statistical analysis to determine whether the results obtained from the different extraction methods are significantly different for the five compounds in which the internal standard was added at the start of the extraction and at the end of the extraction is given in the next section.

3.9.2 Statisical evaluation

The results in Table 3.44 were subjected to an analysis of variance (ANOVA) test, by using Microsoft Excel. Comparison between the methods was performed with the

ONE-WAY ANOVA procedure (Hibbert and Gooding, 2006) and the results are shown in Table 3.45.

Table 3.45 Statistical evaluation using the ONE-WAY ANOVA for comparison between the methods.

Compound	Sum of Squares	Degrees of Freedom	Mean Square	F_{exp}	P-value	F_{crit}
α-pinene	8669.57	5	1733.91	981.58	3.08×10^{-15}	3.11
β-pinene	257.51	5	51.50	1583.87	1.76×10^{-16}	3.11
α-phellandrene	387.14	5	77.42	1993.20	4.43×10^{-17}	3.11
β-caryophyllene	38219923	5	7643985	1012.12	2.56×10^{-15}	3.11
α-caryophyllene	146857.9	5	29371.57	575.65	7.47×10^{-14}	3.11

The F-experimental value (F_{exp}) was compared to the F-critical (F_{crit}) value at the 95% confidence level to establish if the null hypothesis can be accepted or rejected, i.e. if there is a significant difference between the extraction methods and also the compounds being extracted.

The results in Table E86 showed that F_{exp} was greater than F_{crit}. The F_{exp} value together with its associated probability values from Table E86 in Appendix E, indicated that the null hypothesis may be rejected at the 95 % level and thus, it can be deduced that there was a significant difference ($p<0.05$) between the different extraction methods. Although the ONE-WAY ANOVA showed that there was a significant difference between the methods, it did not indicate which means (average values) for which compounds and methods were different and therefore further statistical evaluation was done.

Further statistical treatment of the results was carried out with Duncan's Multiple Range test using the SAS Program (Statistical Analysis System, Version 9.1, SAS Institute

Inc., Cary, NC, USA). This test was used to determine whether the results obtained from the different extraction methods are similar for the five compounds in the absence and presence of the internal standard. The results obtained with the Duncan's Test are shown in Table 3.46.

Table 3.46 Statistical analysis for the mean concentrations (in mg kg^{-1}) of the essential oils with the various extraction methods.

Compound	**α-pinene**	**β-pinene**	**α-phellandrene**	**β-caryophyllene**	**α-caryophyllene**
Method					
Solvent Extraction A	46.7 b	7.39 c	10.7 b	4609 b	90.0 b
Solvent Extraction B	42.9 c	7.27 c	10.3 c	4551 b	91.3 b
Soxhlet Extraction A	54.6 a	9.51 b	8.86 d	5204 a	271 a
Soxhlet Extraction B	46.3 b	10.5 a	13.6 a	5115 a	272 a
Steam Distillation A	1.02 d	0.73 e	1.33 e	1838 c	71.9 c
Steam Distillation B	2.32 d	1.30 d	1.65 e	1803 c	68.9 c

A - refers to samples in which the internal standard was added at the start of extraction.
B - refers to samples in which the internal standard was added at the end of extraction
Least squares mean values marked with the same letter in the same column are not significantly different ($p<0.05$) according to the Duncan's Multiple Comparison Test (Barrera-Necha, *et al*., 2008).

The results in Table 3.46 indicate that no significant difference in the mean concentrations existed in the case of α-pinene for the steam distillation method in which the internal standard was added at the start of the extraction and at the end of the extraction as well as for the solvent extraction in which the internal standard was added at the start of the extraction and at the end of the extraction. For β-pinene, there was no significant difference in the results obtained for the solvent extraction with the internal standard added at the start of the extraction and at the end of the extraction. For the extraction of β-pinene with steam distillation, there was a significant difference in the results, within experimental error, when the internal standard was added at the start and

end of the extraction. Also, the steam distillation technique was not an efficient technique as it did not extract as much as Soxhlet and solvent extraction, since the lowest amounts for all the compounds were obtained with this procedure. For α-phellandrene, the steam distillation with the internal standard added at the start of the extraction as well as after the extraction did not show any significant difference. All the methods, namely, steam distillation, solvent and Soxhlet extractions with the internal standard added at the start and at the end of the extraction showed no significant difference in the amounts extracted for the sesquiterpenes, α- and β-caryophyllene. This indicated that the addition of the internal standard at the start of the extraction and at the end of the extraction made no difference and that satisfactory results were obtained for both these compounds. The data in Table 3.46 also showed that the Soxhlet method was suitable for the extraction of all the compounds investigated, since a relatively larger amount was extracted with this technique. Taking these results into consideration, quantitation can be performed for any of the above three methods.

3.10 Comparison of the methods of extraction

In this section the different analytical methods adopted for the determination of the volatile organic compounds are compared. The results obtained from the different methods have been presented and discussed in Sections 3.2 to 3.9, pages 64 to 166.

3.10.1 Solvent and Soxhlet extraction

The Soxhlet extraction technique, according to Naude *et al*. (1998), is not environmentally friendly, as it produces toxic fumes which adds to pollution. It requires a large amount of a hazardous substance which is not ideal for 'green chemistry' (Demeestere *et al*., 2007). To make use of more "greener" processes, instead of hazardous substances, supercritical CO_2 extraction can be used instead, as reported by Wenqiang *et al*. (2007).

Also the exposure of fellow co-workers to hazardous dichloromethane and the high cost of solvent removal have to be taken into account. It is also a time-consuming process with more sample handling and the extraction times are long. The disadvantage of this technique is the loss of solvent during the extraction process which can lead to errors in the analysis. However, based on analyte volatility it does not discriminate, as the results obtained have shown that compounds of high volatility (the monoterpenes). medium and low volatility (the sesquiterpenes) can be extracted.

An advantage of solvent extraction (with a typical chromatogram shown in Figure 3.60) is that the extraction can be carried out at a low temperature, i.e. at room temperature, with no high energy consumption required, unlike steam distillation and Soxhlet extraction. However, there can be co-extraction of non-volatile compounds and attempts to clean the sample can result in the loss of volatile analytes (Teixeira *et al*., 2007).

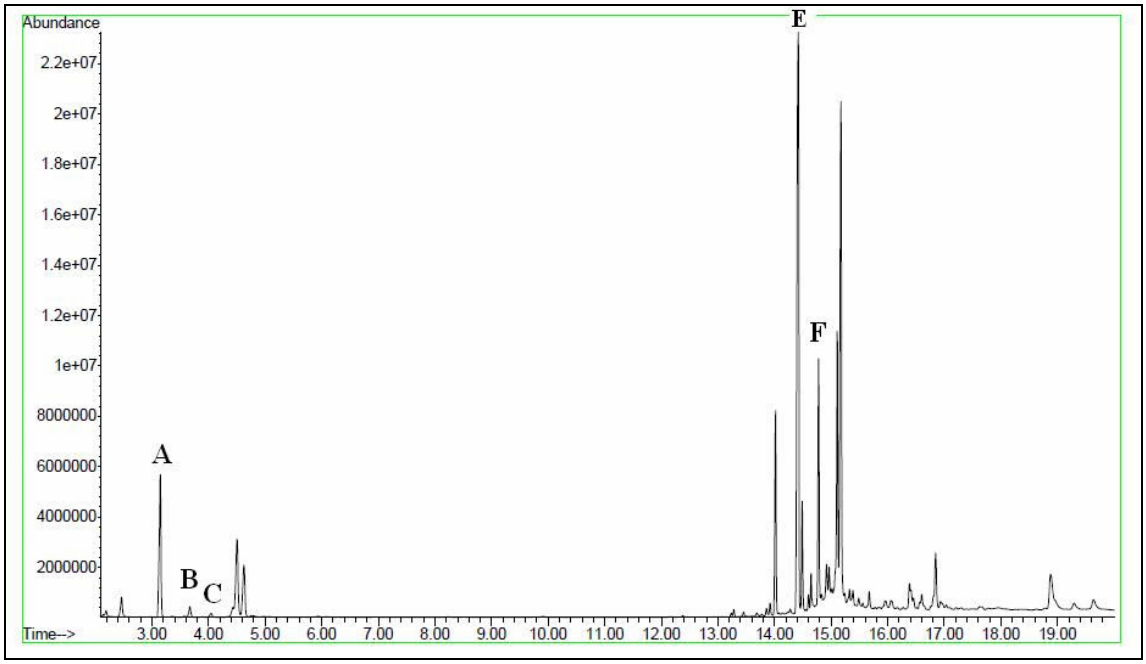


Figure 3.60 A typical total ion chromatogram for the solvent extraction after the 48 hour extraction.

3.10.2 Steam distillation method

In this work, the monoterpenes were found to be vulnerable to steam distillation demonstrated by the loss of the light volatiles. Due to the steam distillate being subjected to further liquid-liquid extraction, followed by evaporation and concentration of the organic phase, further loss of analytes occurred during this multi-step process and therefore, the sample preparation step contributes to the major source of error. This trend was similar to the observation by Alpendurada (2000). Also, extraction at a high temperature (100 °C) could cause thermal decomposition of some compounds, resulting in a change in some components of the essential oil and ultimately a change in aroma (Romanik *et al*., 2007). In addition, this technique lacks efficiency.

However, the pale yellow oil obtained is much purer than the extracts from the solvent and Soxhlet extractions which contained chlorophyll, making it more selective than solvent and Soxhlet extraction. The presence of chlorophyll could be seen in the solvent and Soxhlet extraction chromatograms, i.e the peak at retention time 19.60 corresponding to phytol, making these methods non-discriminatory.

3.10.3 Headspace analysis and HS-SPME

This method of analysis as used in this work was a solvent free determination, thereby eliminating solvent contamination of the samples. Sample preparation was performed with ease which therefore makes this a cost- and time-saving method. Also, since the headspace was used, there was a reduction in sample interference and GC contamination was eliminated.

The advantage of using SPME is that no preconcentrion step is required as the analytes are enriched directly into the fibre coating (Steffen and Pawliszyn, 1996). Due to the simple experimental set-up used, both headspace analysis and HS-SPME is useful for the qualitative determination of aroma compounds as shown by a typical ion chromatogram in Figure 3.61.

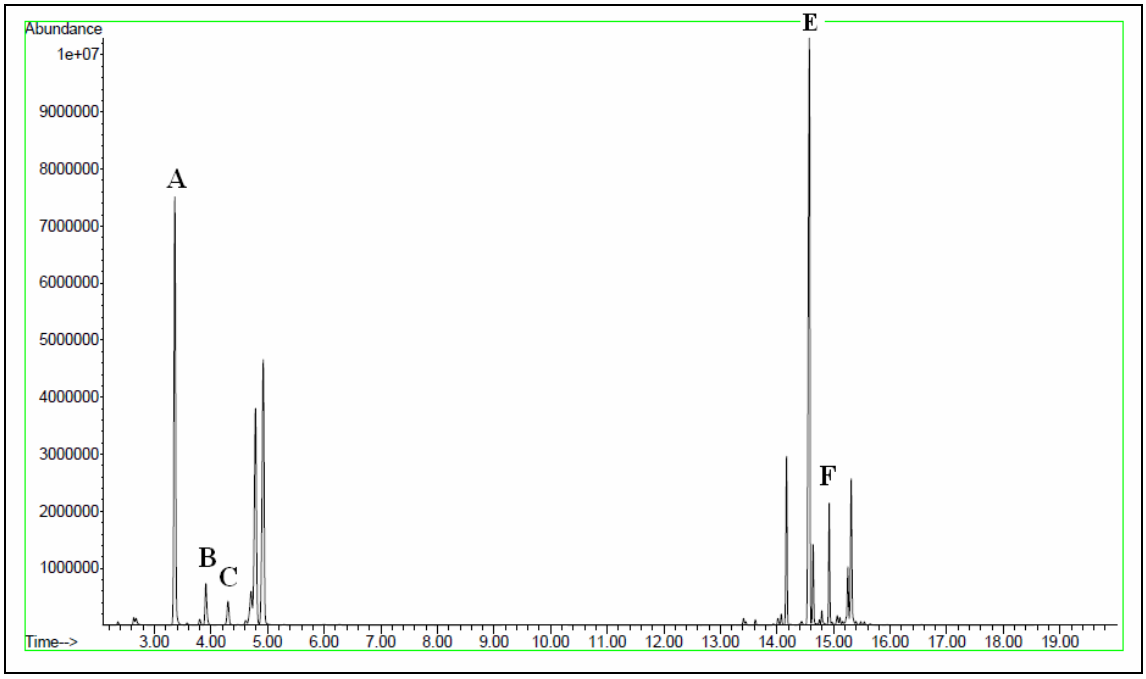


Figure 3.61 A typical total ion chromatogram for the HS-SPME (PDMS fibre coating) at 60 °C.

From the above discussion, for the selection of the appropriate technique consideration should be given to the quantitation levels obtained from each of these techniques for the compounds with different volatilities as outlined in Table 3.47.

Table 3.47 Method comparison for the quantitation of compounds with different volatilities.

Method	**Extraction period**	**Extraction of compounds**		**Quantitation**	
		Low volatility	**High volatility**	**Low levels**	**High levels**
Solvent extraction	48 hours	good	good	satisfactory	poor
Soxhlet extraction	48 hours	good	good	satisfactory	poor
Steam distillation	3 hours	satisfactory	poor	poor	poor
HS-SPME (PDMS coating)	15 mins	good	good	difficult	difficult
HS-SPME (PA coating)	15 mins	satisfactory	satisfactory	difficult	difficult
Headspace analysis	15 mins	satisfactory	good	difficult	difficult

The conclusions drawn from this study are discussed in Chapter 4.

CHAPTER 4

CONCLUSIONS

The aim of the project was to compare the different extraction methods for the analysis of volatile compounds of *M. koenigii* by using the GC-MS analytical technique.

Results from this work have shown that the volatile profile of the essential oil in *M. koenigii* was dependent on the extraction technique employed. According to Cao *et al*. (2007), different extraction techniques used for natural products exhibit different efficiencies. In this work, the solvent and Soxhlet extractions showed no difference between the quantities obtained for α-pinene and either technique can be used for the extraction of this compound. The Soxhlet extraction was generally favourable for the extraction of the compounds studied, i.e. α-pinene, β-pinene, α-phellandrene as well as α- and β-caryophyllene. The results have also shown that the extraction yield is determined by the solvent used for the extraction, the extraction temperature as well as the duration of the heating period. A similar finding has been reported Zhu *et al*. (2006).

Of the different techniques studied for the extraction of volatile compounds in *M. koenigii* leaves, Soxhlet extraction was the most efficient technique. This extraction technique can be used for a wide range of volatile and semi-volatile organics. A disadvantage of the Soxhlet extraction is that it can be a costly and time-consuming technique. It can also be difficult to obtain a product with the required characteristics with this method (Castro *et al*., 2004). This means that a further step in sample preparation would be needed. These important considerations weigh heavily against the selection of this extraction technique and perhaps, solvent extraction could be used instead, since a large amount of effort can be saved. According to Malundo *et al*. (1997), steam distillation, Soxhlet and solvent extraction might produce other compounds and artefacts that do not contribute to the aroma. According to Wenqiang *et*

al. (2007), thermal degradation, hydrolysis and water solubility of some constituents of essential oils are found to occur during these extraction processes.

The steam distillation method showed much lower extraction efficiency for the monoterpenes as compared to the Soxhlet and solvent extraction methods. A disadvantage of this method was that further solvent extraction was required to recover the essential oils.

As reported by Miller and Stuart (1999), the headspace technique lacks the sensitivity for adequate performance. However, it proved effective in the detection of the lowest boiling analytes and is a good technique for the detection of the very volatile analytes. It was also cost-effective as simple apparatus can be employed for the extraction process.

The use of HS-SPME combined with GC-MS can be used for the analysis of volatile organic compounds. The PDMS fibre coating was found to be superior for the compounds which were present in larger amounts, the monoterpenes, and this result could be due to the equilibration period used.

No sample preparation step was necessary for HS-SPME as the analytes adsorbed into the fibre were sufficient for direct analysis to be done. A temperature of 60 °C was found to be suitable for compounds present at a low concentration. Headspace-SPME combined with GC-MS is a simple, quick method used for the extraction and identification of monoterpenes and sesquiterpenes of *M. koenigii*.

However, the use of headspace gas chromatography for the quantitative determination of volatile compounds is limited (Zhu and Chai, 2005). In the quantitative analysis for the HS-SPME of complex matrixes, such as food products, it is essential that the method utilises the correct conditions, taking into consideration the fibre coating, the competition that exists between the components for adsorption sites on the fibre, as well as the number of components which are present. Quantitation errors can result if

conditions without using proper and appropriate controls are selected (Contini and Esti, 2006).

The results from this study have shown that there is no single optimal method that exists for the extraction of volatile organic compounds present in a wide concentration range and, therefore, it may be necessary to use a combination of methods for the extraction of all the volatile constituents. A similar finding was observed by Mamede and Pastore (2006). Various factors have to be considered in the selection of an optimal technique. Some of these factors are the physical properties of the sample, the sample matrix, the number of analytes of interest present in the sample, interfering compounds, the thermal stability of the sample, the cost and range of the equipment available, and the cost and time of the analysis (Manura and Manura, 1998).

FUTURE WORK

In future studies, multiple headspace solid-phase microextraction, for the quantification of the analytes present in the headspace of a complex mixture, can be investigated. This solvent-free method, based on the exhaustive extraction of the analytes, can be used to quantify volatile organic compounds in solid samples. In this method of analysis the total peak area is determined by adding the areas of each individual extraction for the respective compounds. The method can be employed only if the following three criteria are met:

i) The relationship between the peak area and the amount extracted must be linear over the range of volatiles studied.
ii) The distribution constants between the sample, coating and headspace must be constant.
iii) The equilibrium must be established for the analytes (Ezquerro *et al*., 2003). (More information about this method can be found in Ezquerro *et al*. (2003).

Also, future studies can be conducted to investigate the use of two internal standards to improve the analysis of the quantitative determination of analytes present in a wide

concentration range. A better choice of internal standard would be one for the compounds with high volatility, the monoterpenes and one for the compounds with a high molar mass, the sesquiterpenes.

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APPENDIX A

MATERIALS

The chemicals used in this work, are shown below, together with the manufacturer's details and grade of the chemical.

A1 Chemicals used for the quantitation of the volatile compounds.

Dichloromethane	(99%)	-	BDH HiperSolv^{TM} Chemicals, Ltd.
α-pinene	(98.5%)	-	Sigma-Aldrich
β-pinene	(99%)	-	Sigma-Aldrich
α-phellandrene	(95%)	-	Sigma-Aldrich
β-caryophyllene	(98.5%)	-	Sigma-Aldrich
α-caryophyllene	(98.0%)	-	Sigma-Aldrich

A2 Chemicals used for Gas Chromatography-Mass Spectrometry

Helium	(99.999%)	-	Afrox

APPENDIX B

EQUIPMENT

All equipment used in this study is indicated below.

B1 Equipment

AB 204 Mettler Toledo balance

Labcon Orbital shaker (3100U)

Julabo MD with Labotec bath

Heidolph Rotary Evaporator and a Memmert water- bath

Hamliton syringe, extended barrel, 1800 series, 1801N

SGE gas tight syringe

Supelco TM solid phase microextraction assembly

Agilent 6890 Series GC System together with a Hewlett Packard Kayak XM600 Microsoft Windows NT system

Agilent 5973 Network Mass Selective Detector

APPENDIX C

CHROMATOGRAMS AND MASS SPECTRA OF STANDARDS

In this section the representative chromatograms of the individual standards, including the internal standard, and their corresponding mass spectra together with the mass spectra contained in the library are shown in Figures C1 to C12. Also included in this section are the chromatograms for the standard mixtures shown in Figures C13 to C22. The concentrations of the compounds in the various standard mixtures are shown in Tables C1 to C10. The concentration of the internal standard, dodecane, was the same in all the standard mixtures, 29.9 mg L^{-1}.

Table C1 Concentration of compounds in standard mixture 1.

Compound	**Concentration/mg L^{-1}**
α-pinene	3.43
β-pinene	3.44
α-phellandrene	3.36
β-caryophyllene	3.61
α-caryophyllene	3.56

Table C2 Concentration of compounds in standard mixture 2.

Compound	**Concentration/mg L^{-1}**
α-pinene	13.7
β-pinene	13.7
α-phellandrene	13.4
β-caryophyllene	14.4
α-caryophyllene	14.2

Table C3 Concentration of compounds in standard mixture 3.

Compound	**Concentration/mg L^{-1}**
α-pinene	41.2
β-pinene	41.2
α-phellandrene	40.3
β-caryophyllene	43.3
α-caryophyllene	42.7

Table C4 Concentration of compounds in standard mixture 4.

Compound	**Concentration/mg L^{-1}**
α-pinene	82.4
β-pinene	82.5
α-phellandrene	80.6
β-caryophyllene	86.6
α-caryophyllene	85.3

Table C5 Concentration of compounds in standard mixture 5.

Compound	**Concentration/mg L^{-1}**
α-pinene	165
β-pinene	165
α-phellandrene	161
β-caryophyllene	173
α-caryophyllene	171

Table C6 Concentration of compounds in standard mixture 6.

Compound	**Concentration/mg L^{-1}**
α-pinene	329
β-pinene	330
α-phellandrene	323
β-caryophyllene	346
α-caryophyllene	341

Table C7 Concentration of compounds in standard mixture 7.

Compound	**Concentration/mg L^{-1}**
α-pinene	858
β-pinene	859
α-phellandrene	840
β-caryophyllene	902
α-caryophyllene	889

Table C8 Concentration of compounds in standard mixture 8.

Compound	**Concentration/mg L^{-1}** ($\times 10^3$)
α-pinene	2.57
β-pinene	2.58
α-phellandrene	2.52
β-caryophyllene	2.71
α-caryophyllene	2.67

Table C9 Concentration of compounds in standard mixture 9.

Compound	**Concentration/mg L^{-1}** ($\times 10^3$)
α-pinene	8.58
β-pinene	8.59
α-phellandrene	8.40
β-caryophyllene	9.02
α-caryophyllene	8.89

Table C10 Concentration of compounds in standard mixture 10.

Compound	**Concentration/mg L^{-1}** ($\times 10^4$)
α-pinene	1.72
β-pinene	1.72
α-phellandrene	1.68
β-caryophyllene	1.80
α-caryophyllene	1.78

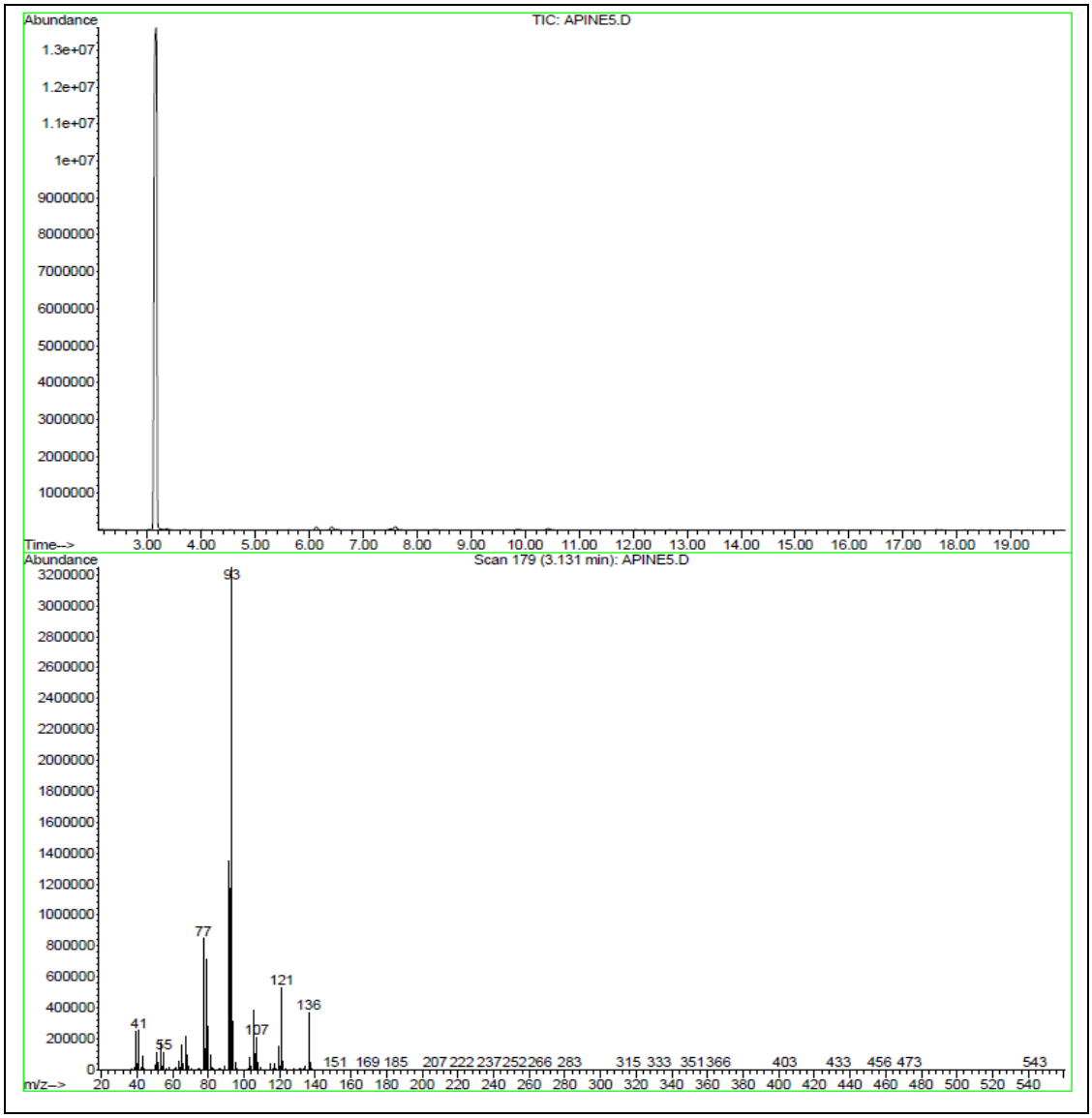

Figure C1 Total ion chromatogram and corresponding mass spectrum of α-pinene.

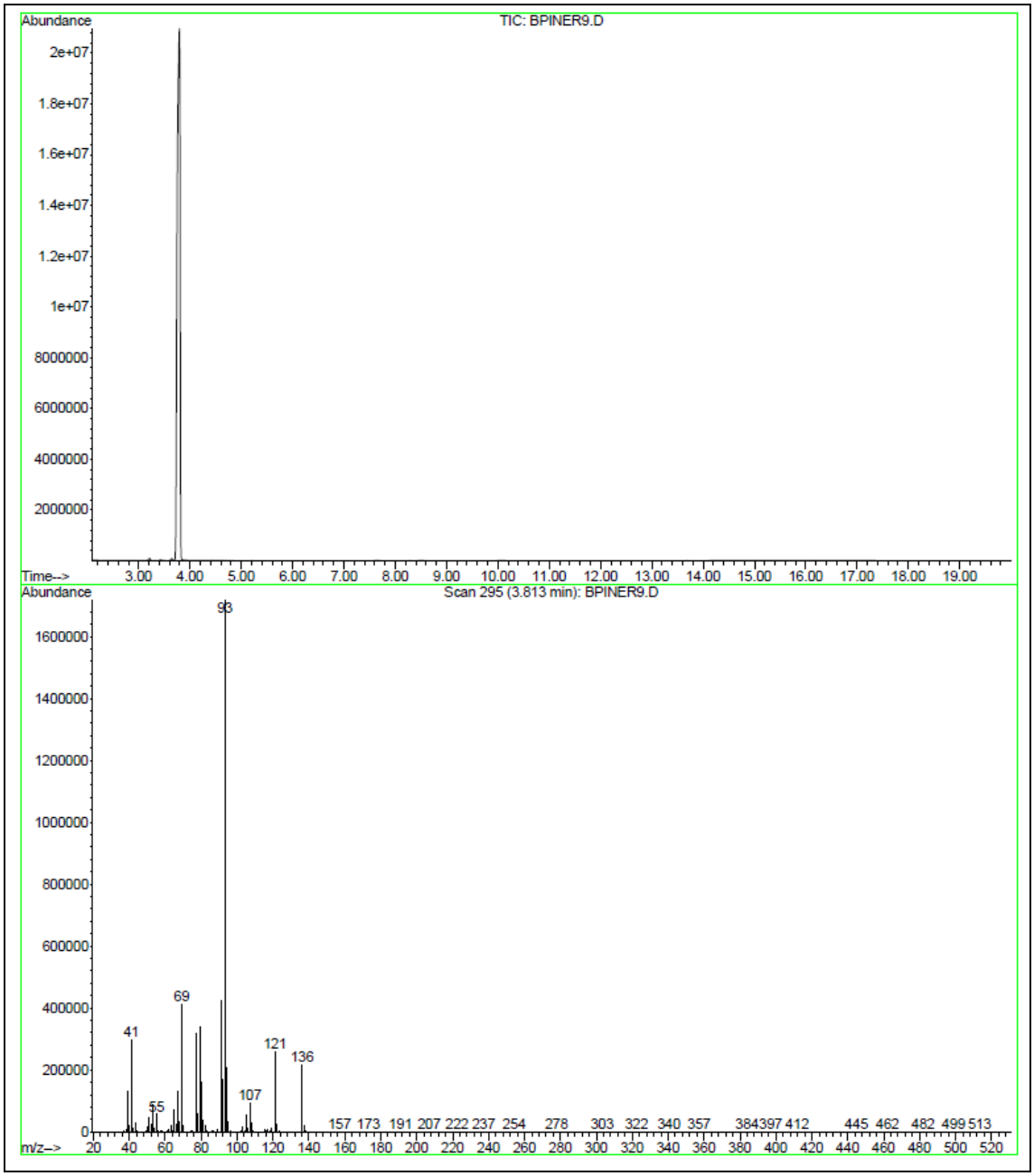

Figure C2 Total ion chromatogram and corresponding mass spectrum of β-pinene.

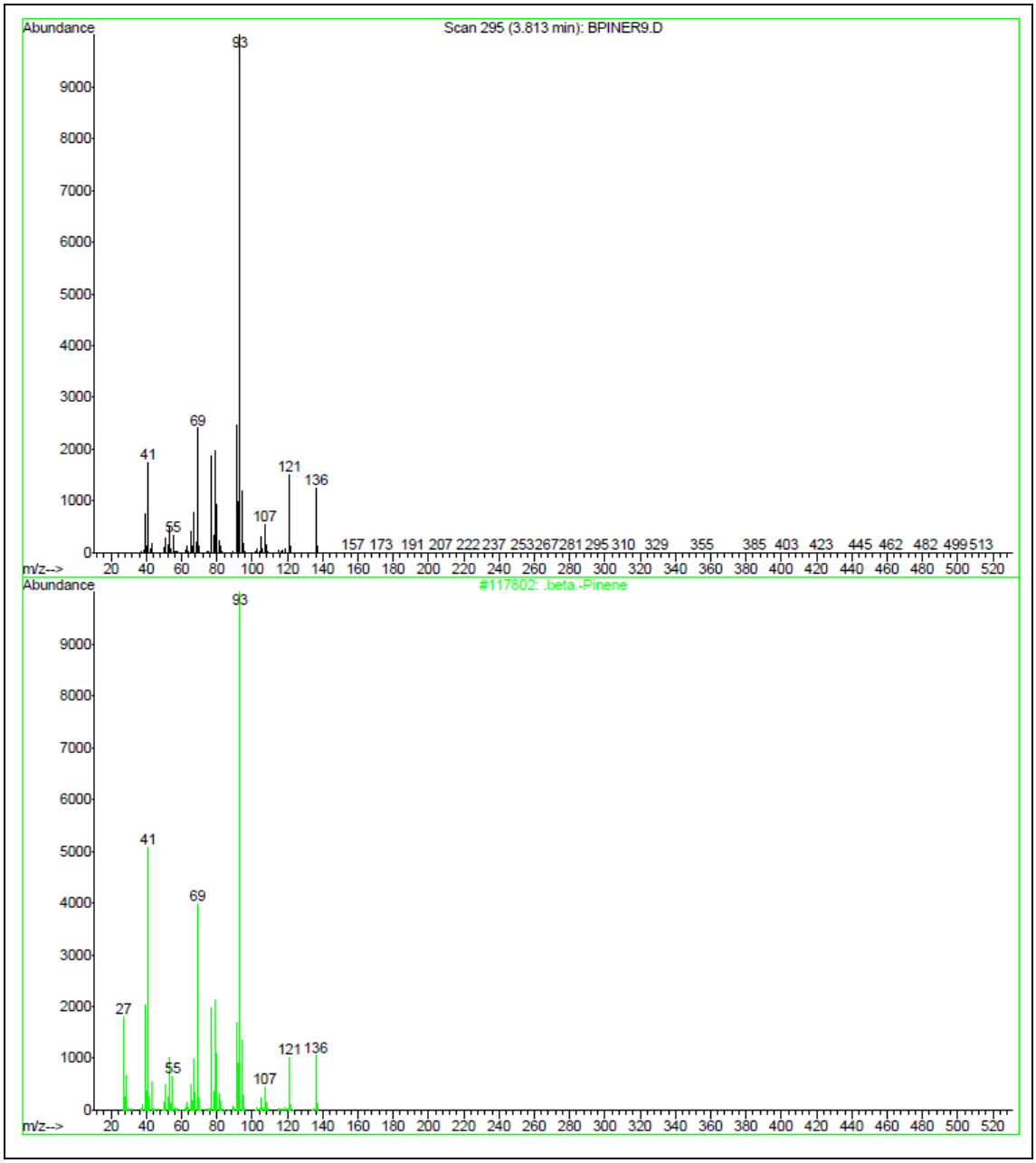

Figure C3 Comparison of mass spectrum of standard β-pinene with the spectrum contained in the NIST library.

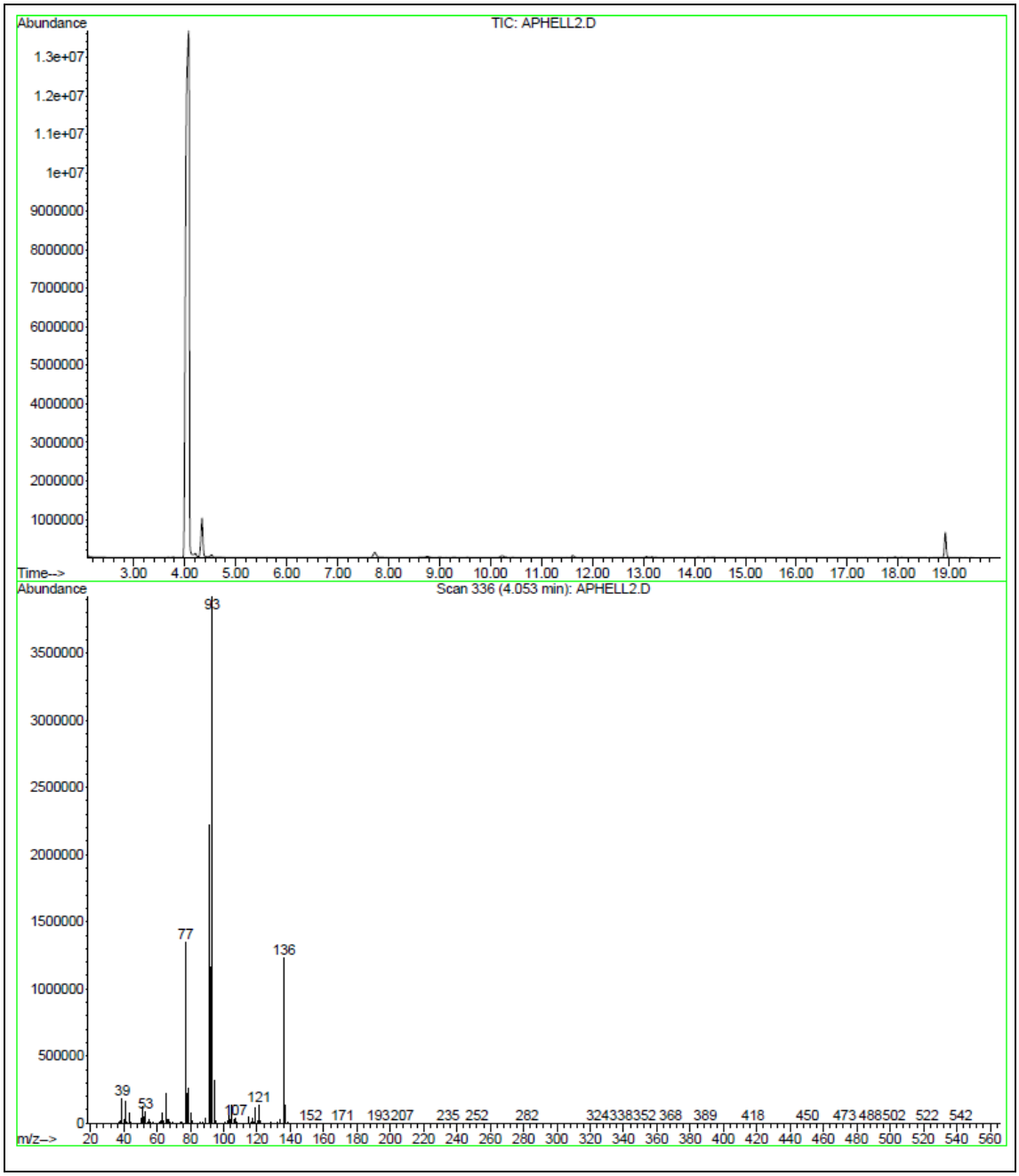

Figure C4 Total ion chromatogram and corresponding mass spectrum of α-phellandrene.

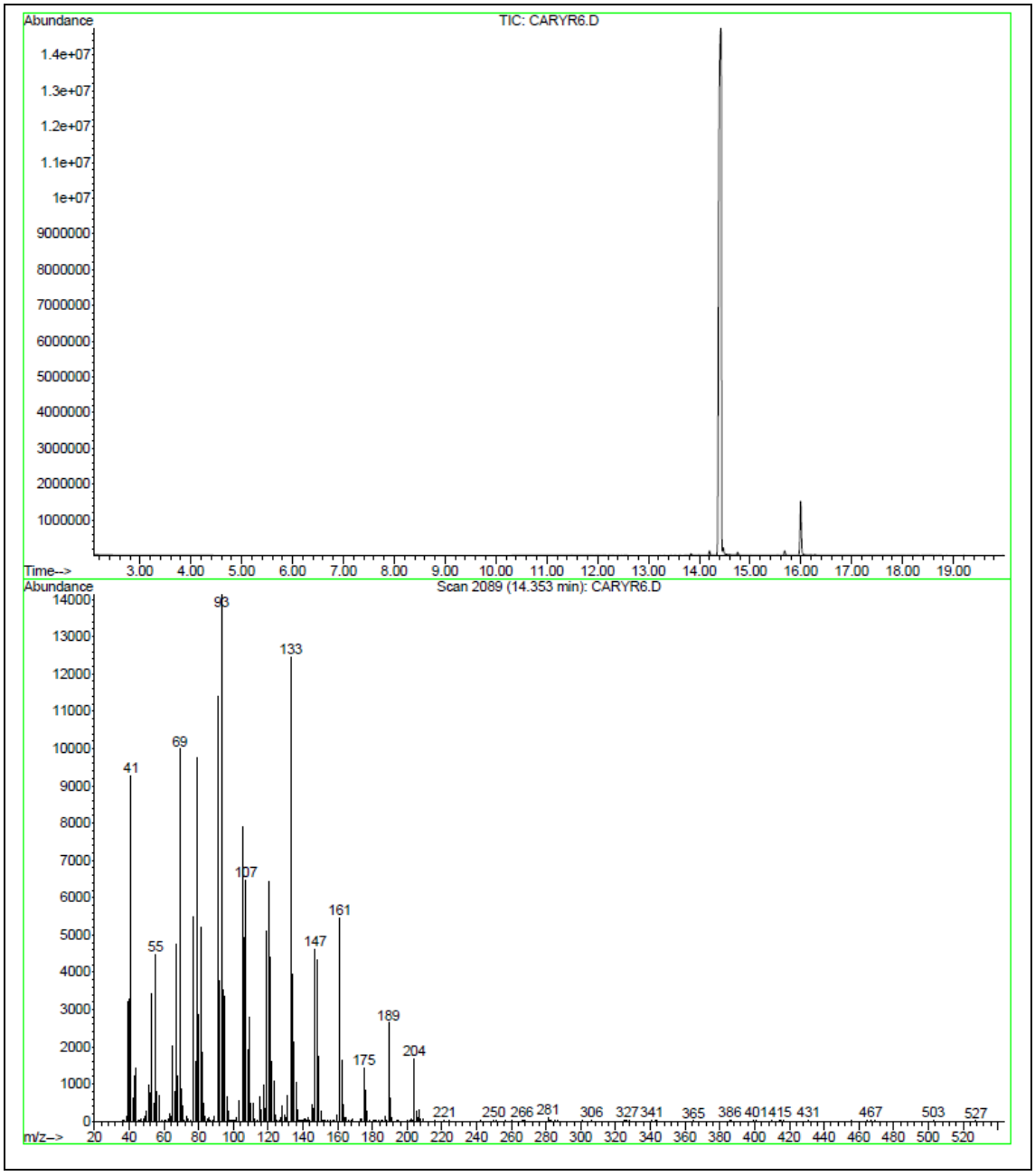

Figure C5 Total ion chromatogram and corresponding mass spectrum of β-caryophyllene.

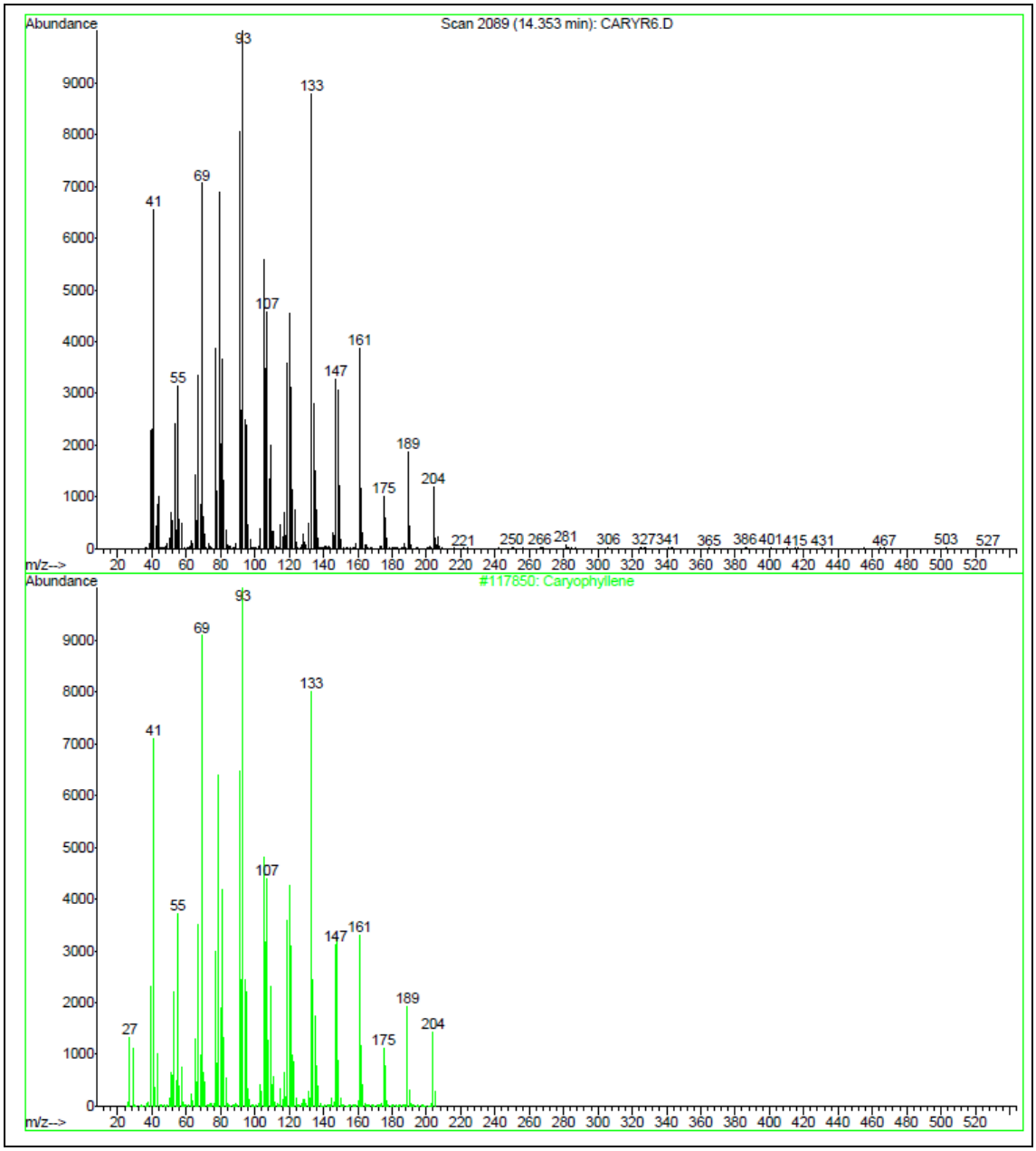

Figure C6 Comparison of mass spectrum of β-caryophyllene with the mass spectrum in the software library.

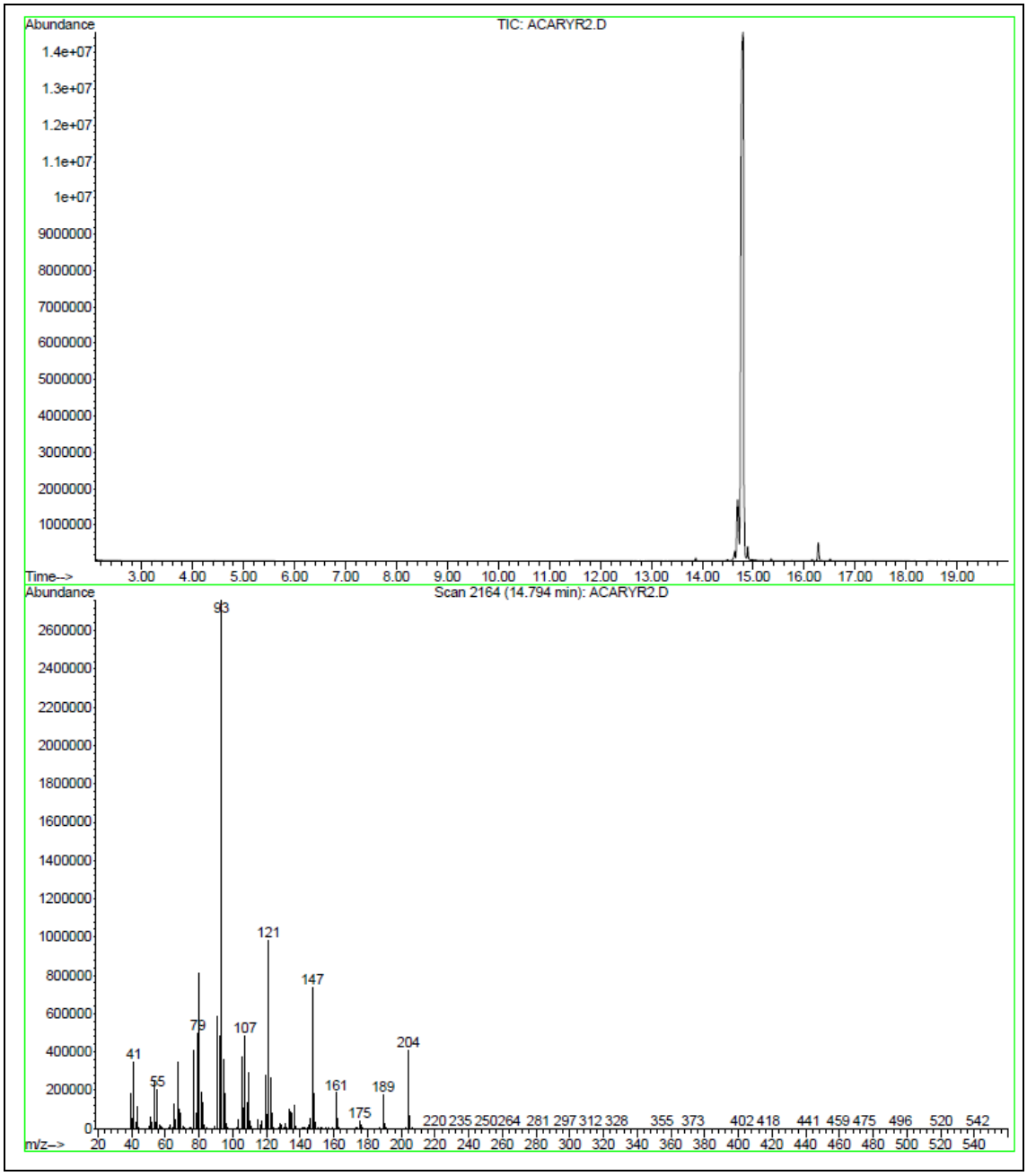

Figure C7 Total ion chromatogram and corresponding mass spectrum of α-caryophyllene.

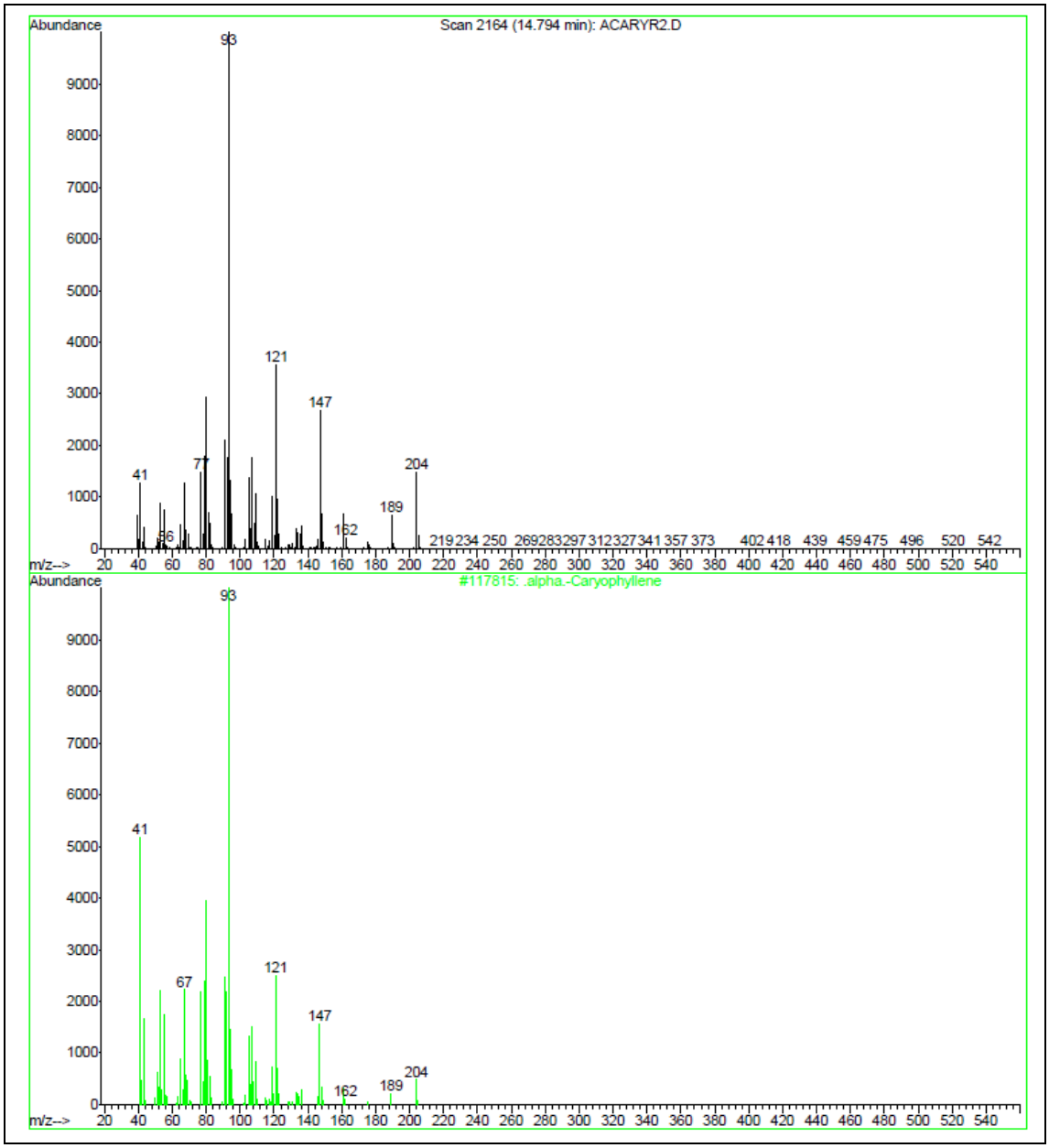

Figure C8 Comparison of mass spectrum of α-caryophyllene with the mass spectrum contained in the NIST library.

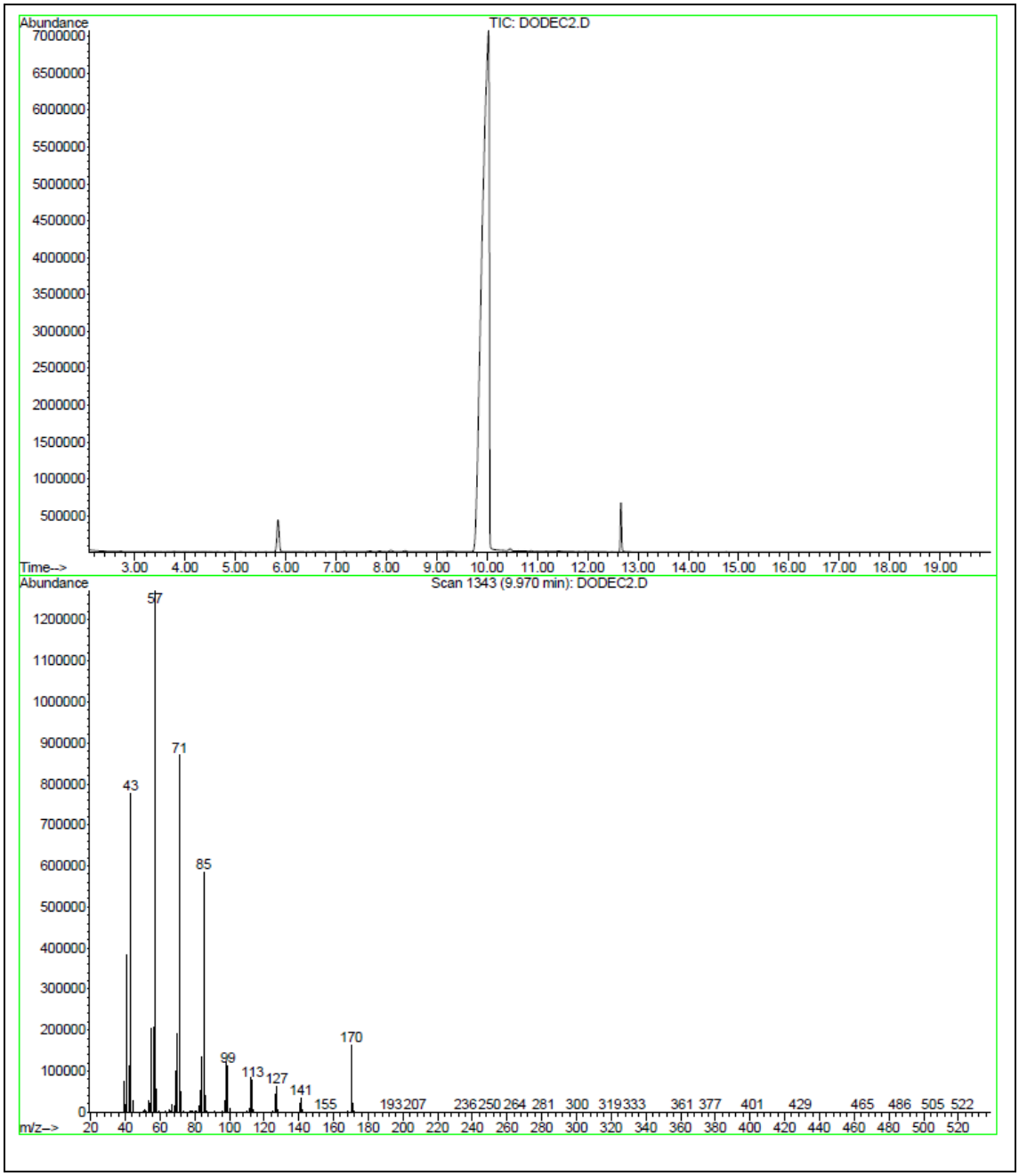

Figure C9 Total ion chromatogram and corresponding mass spectrum of the internal standard, dodecane.

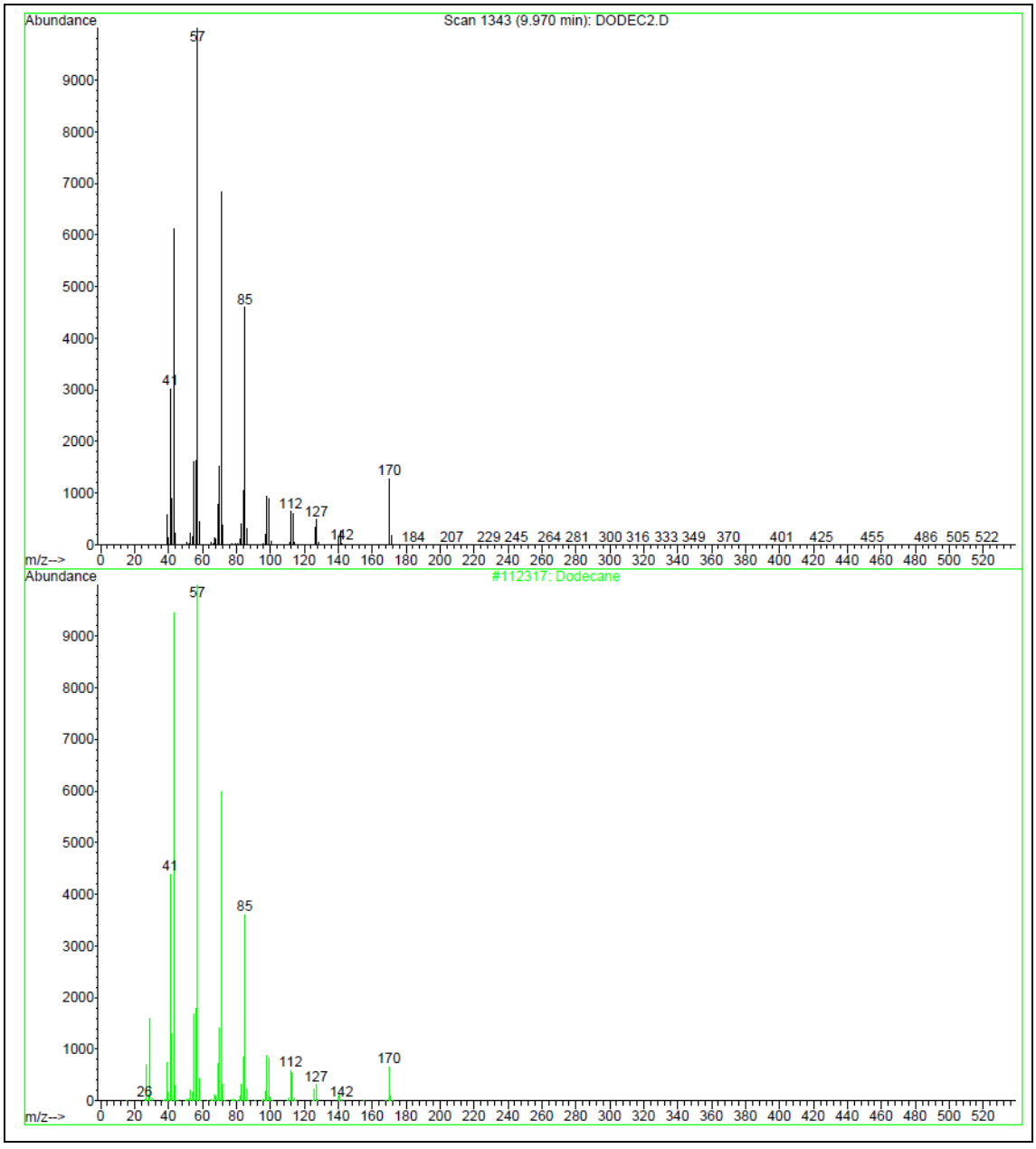

Figure C10 Comparison of mass spectrum of internal standard, dodecane, with the mass spectrum in the NIST library.

The representative chromatograms of the standard mixtures together with the internal standard are shown in Figures C13 to C22. For convenience, the selected peaks of interest on some of the chromatograms are labelled with an alphabet (A to F) as points of reference for the compounds as indicated in Table C11.

Table C11 Peak labels for compounds of interest

Compound	**Alphabet**
α-pinene	A
β-pinene	B
α-phellandrene	C
dodecane	D
β-caryophyllene	E
α-caryophyllene	F

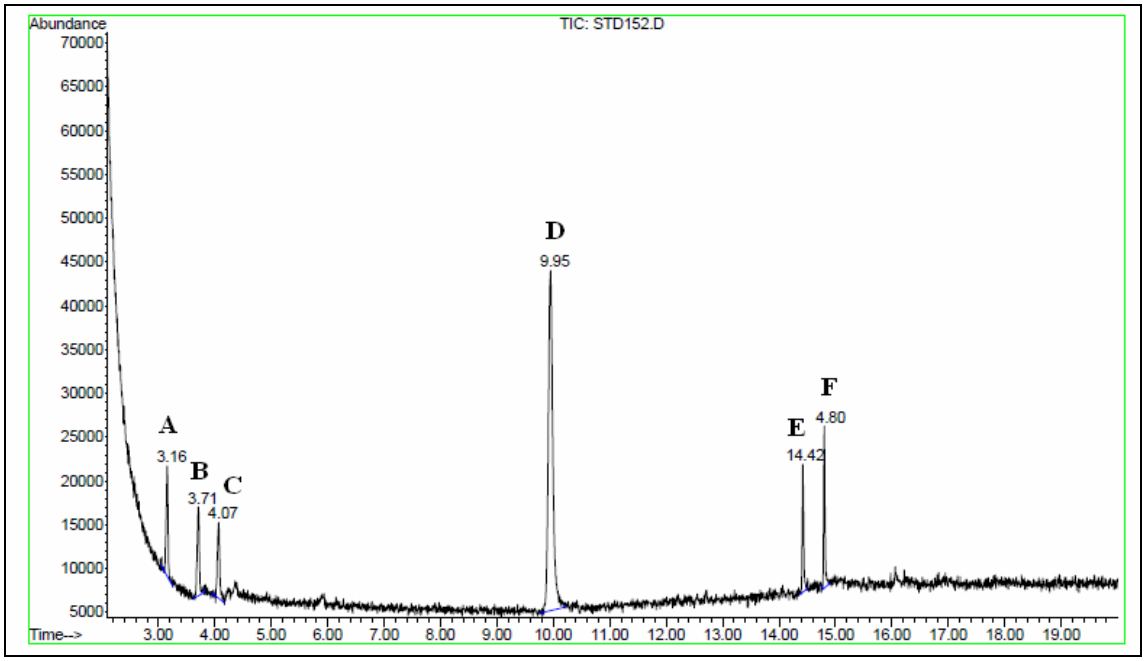

Figure C11 Typical total ion chromatogram of a standard mixture 1.

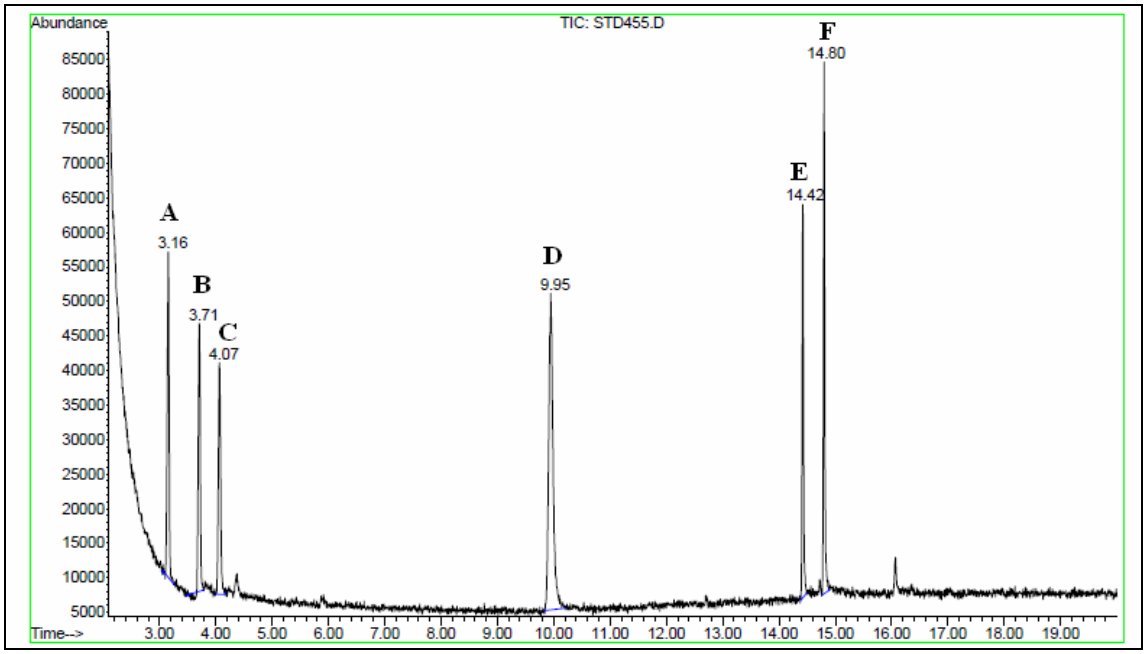

Figure C12 Typical total ion chromatogram of a standard mixture 2.

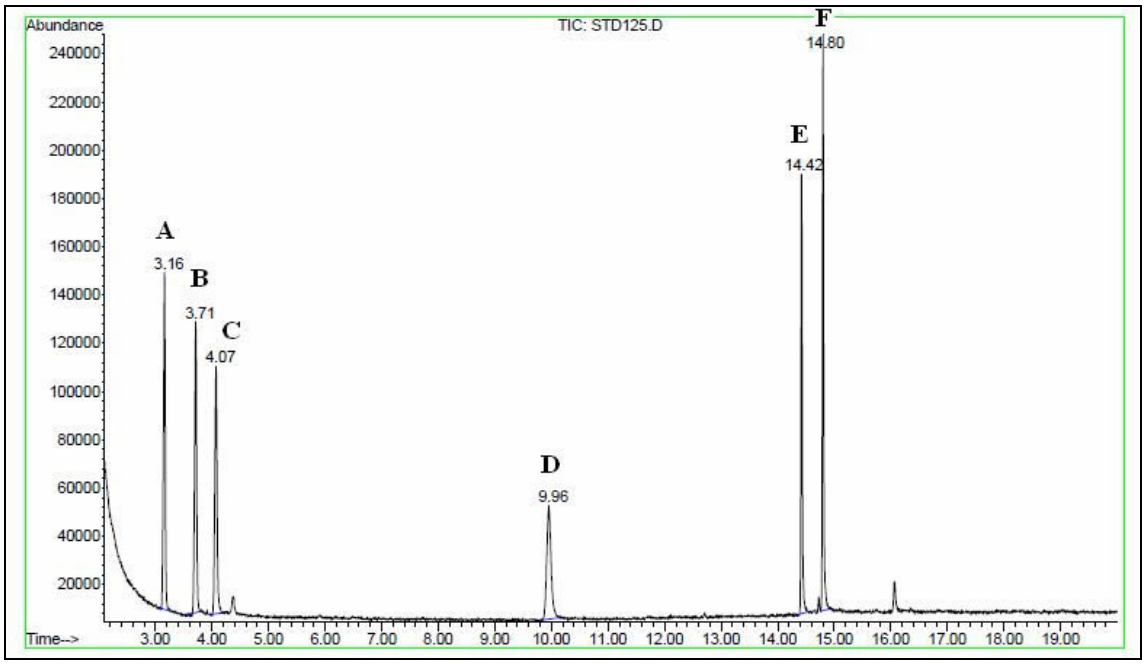

Figure C13 Total ion chromatogram for a standard mixture 3.

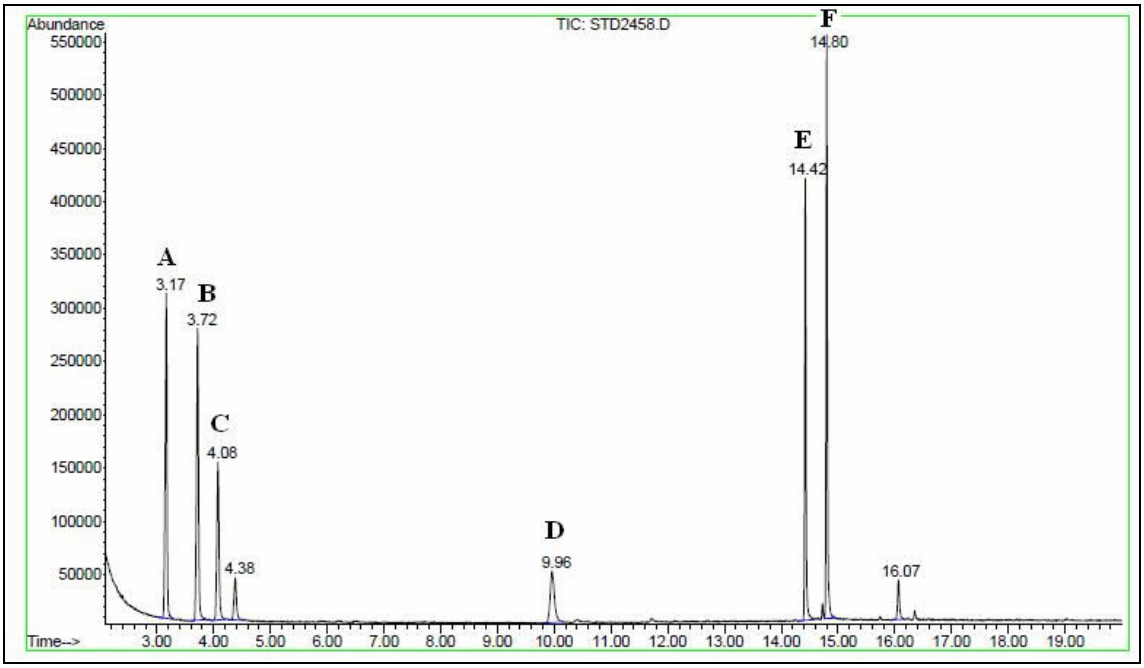

Figure C14 Total ion chromatogram for a standard mixture 4.

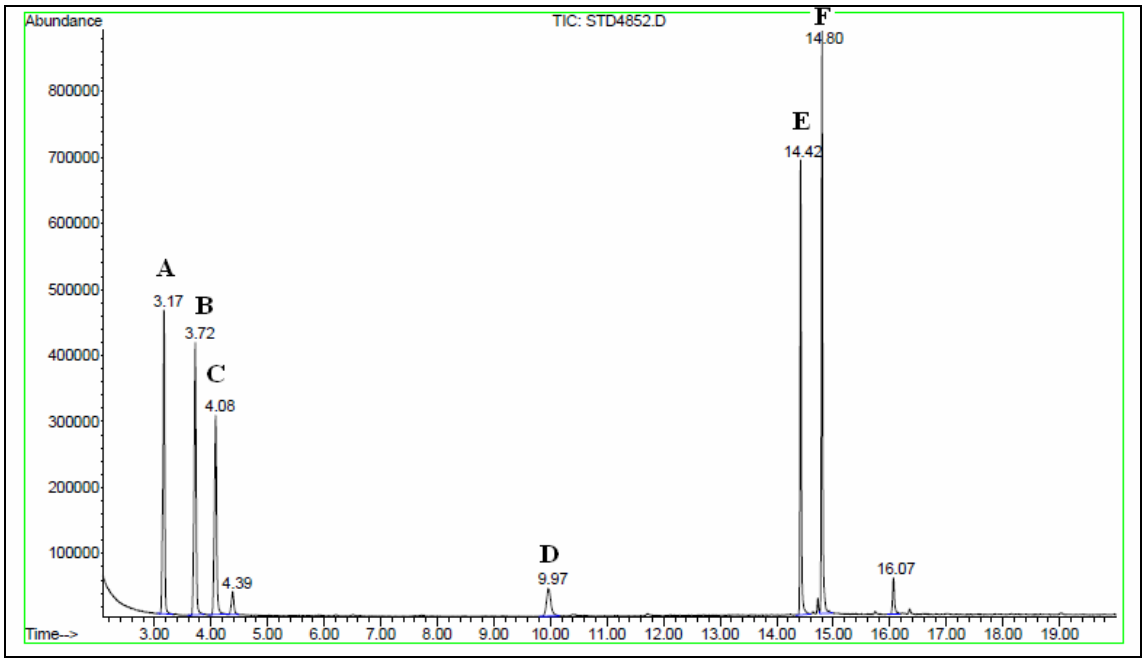

Figure C15 Total ion chromatogram for a standard mixture 5.

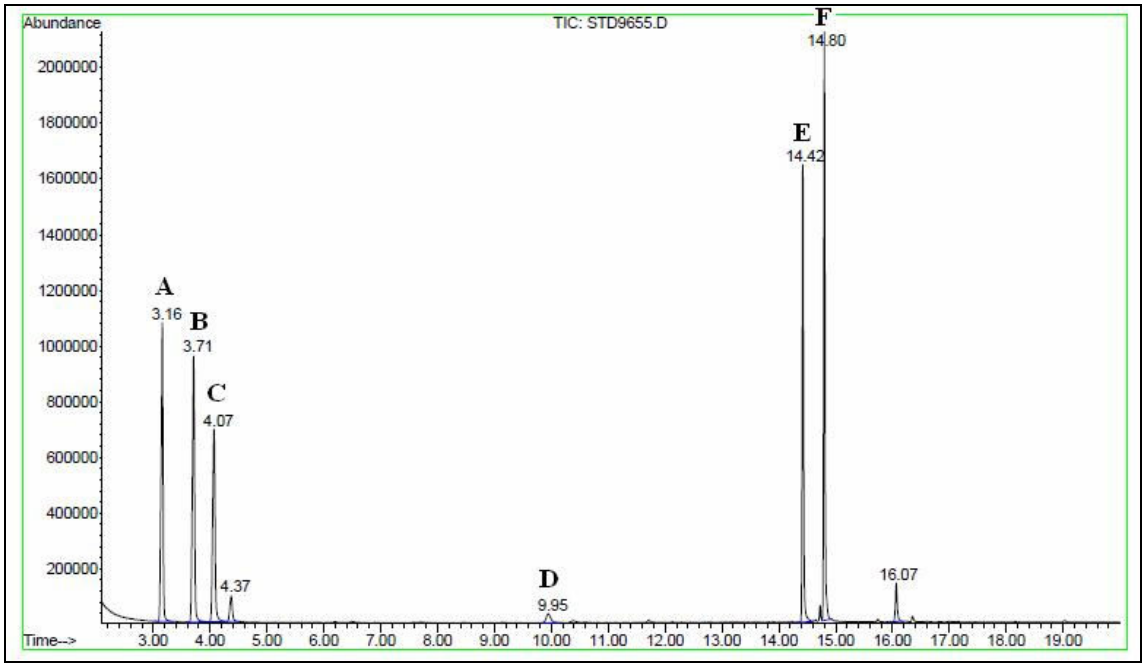

Figure C16 Total ion chromatogram for a standard mixture 6.

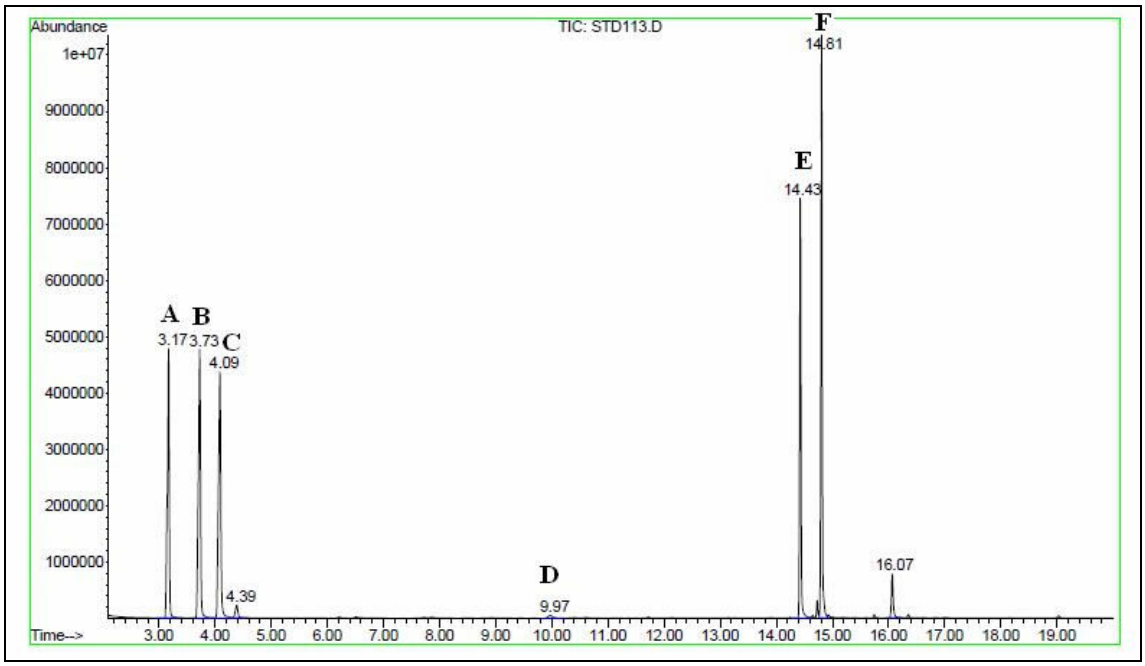

Figure C17 Total ion chromatogram for a standard mixture 7.

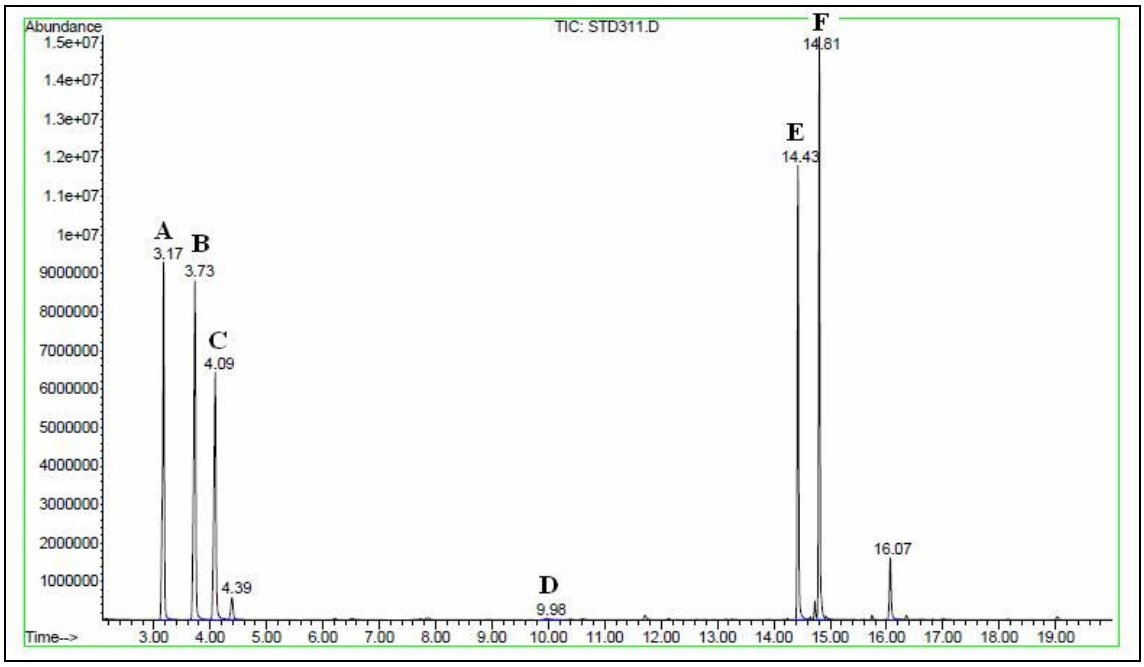

Figure C18 Total ion chromatogram for a standard mixture 8.

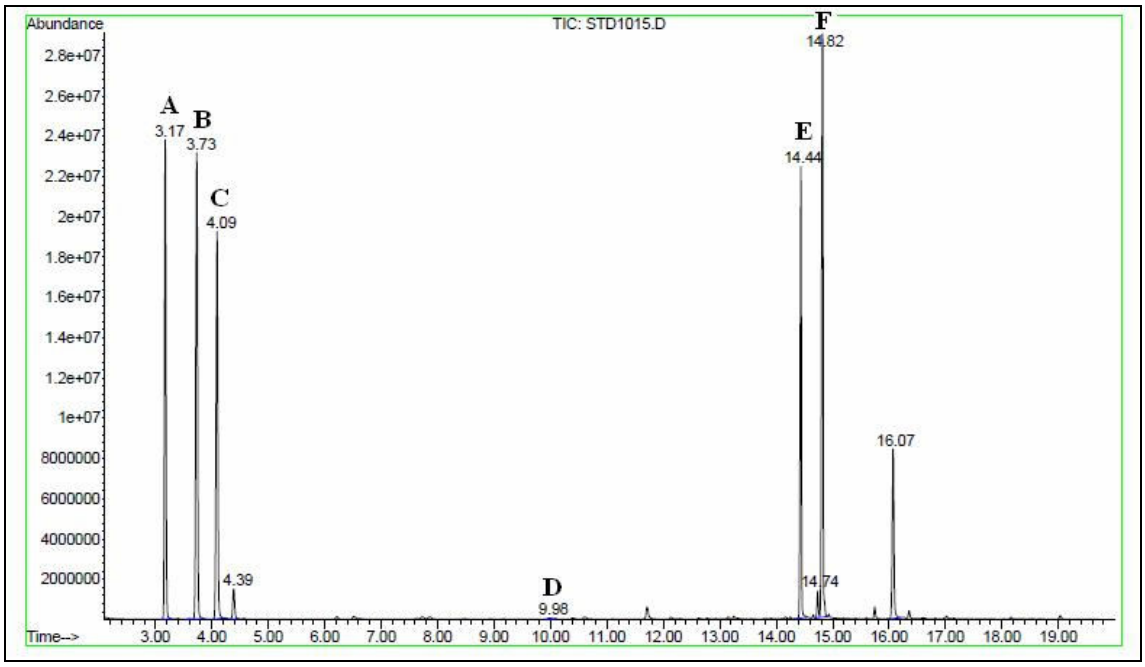

Figure C19 Total ion chromatogram for a standard mixture 9.

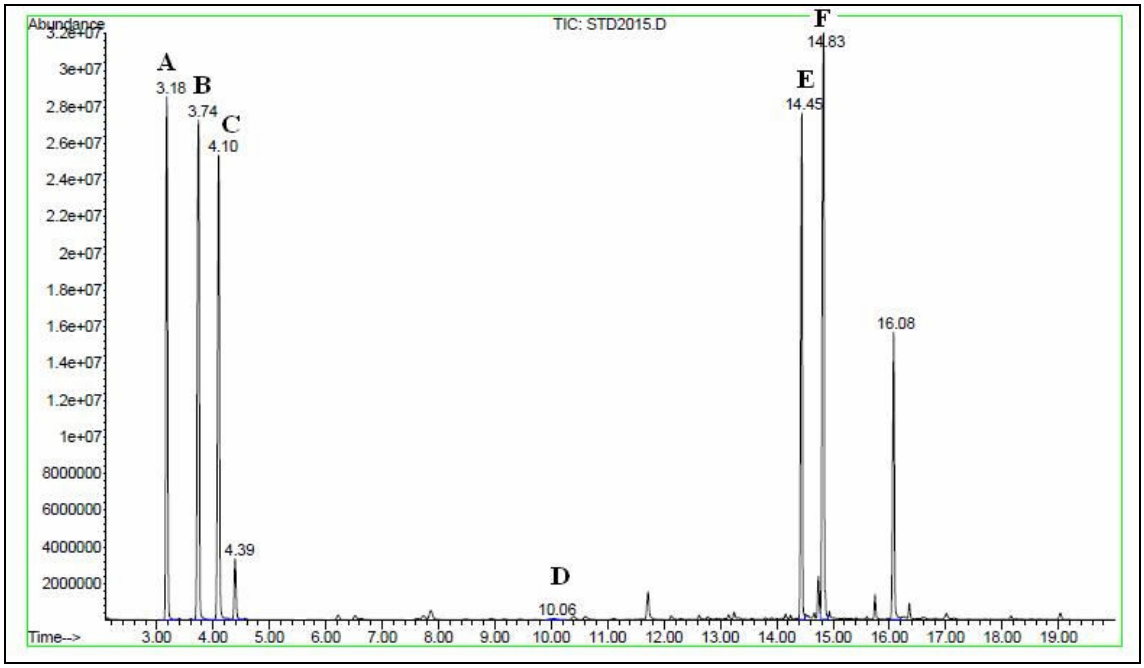

Figure C20 Total ion chromatogram for a standard mixture 10.

APPENDIX D

CHROMATOGRAMS

Representative chromatograms for the essential oil obtained from the different extraction methods shown in Figures D1 to D21 are presented in this section.

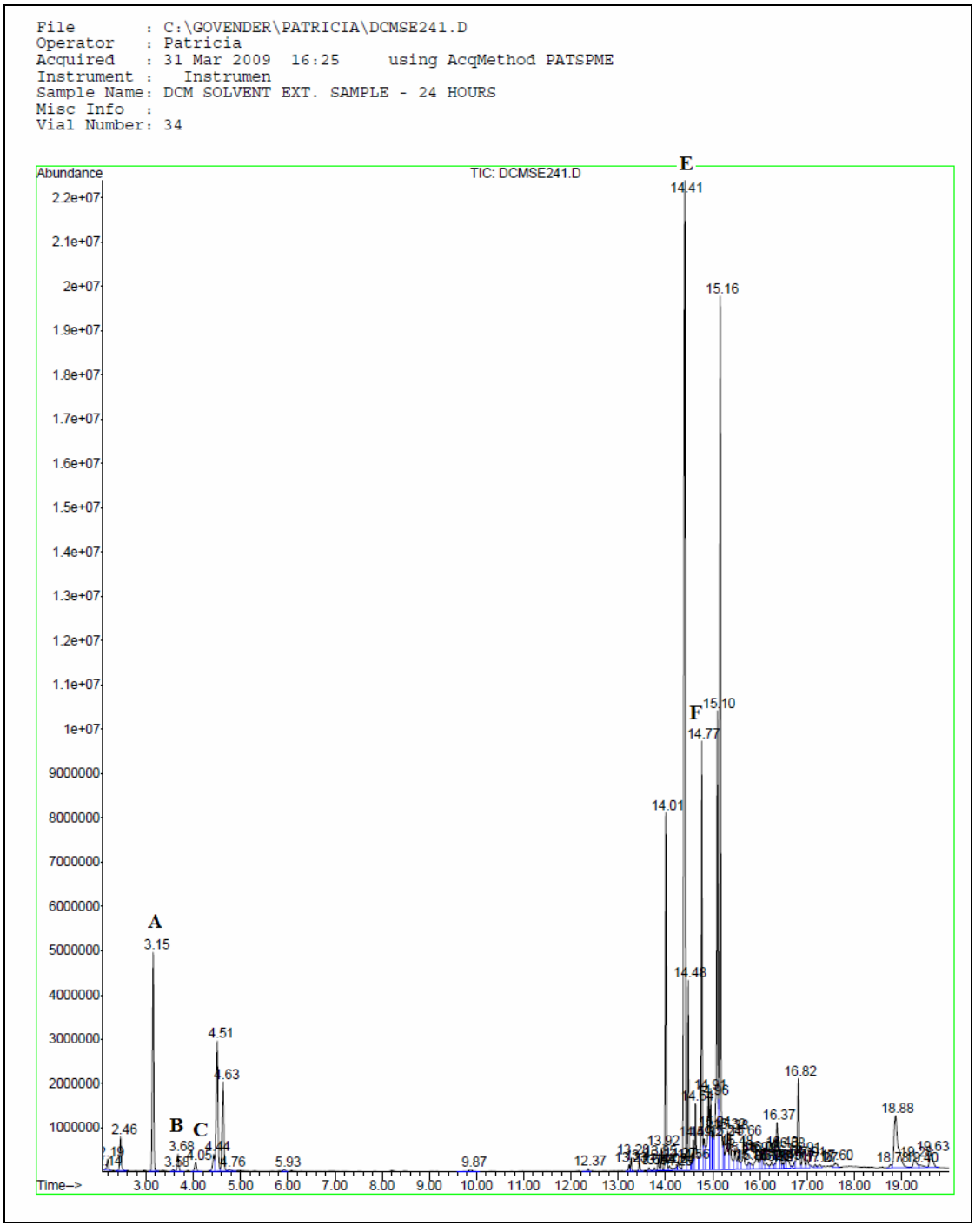

Figure D1 Total ion chromatogram for the essential oil obtained from the 24 hour solvent extraction.

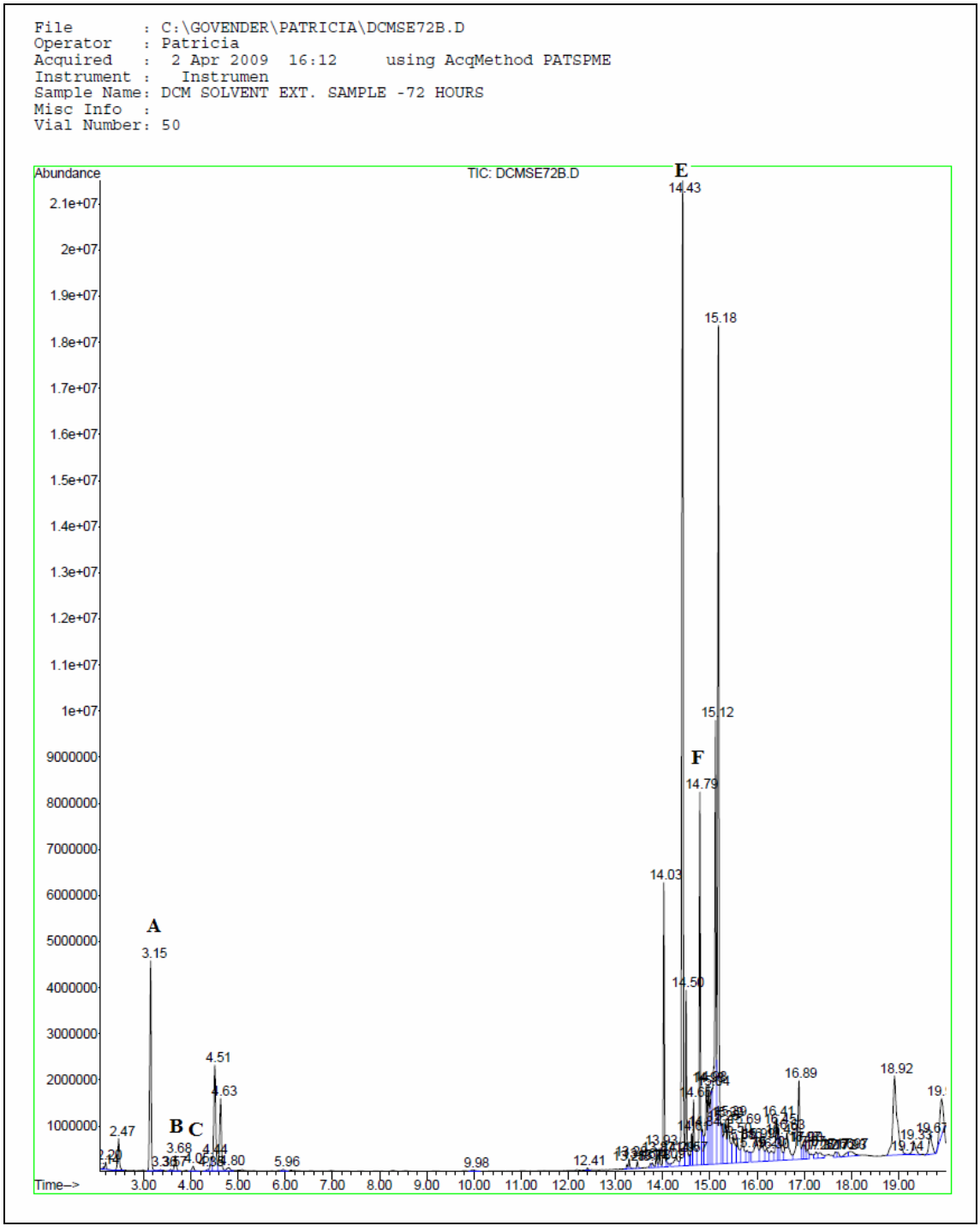

Figure D2 Total ion chromatogram for the essential oil obtained from the 72 hour solvent extraction.

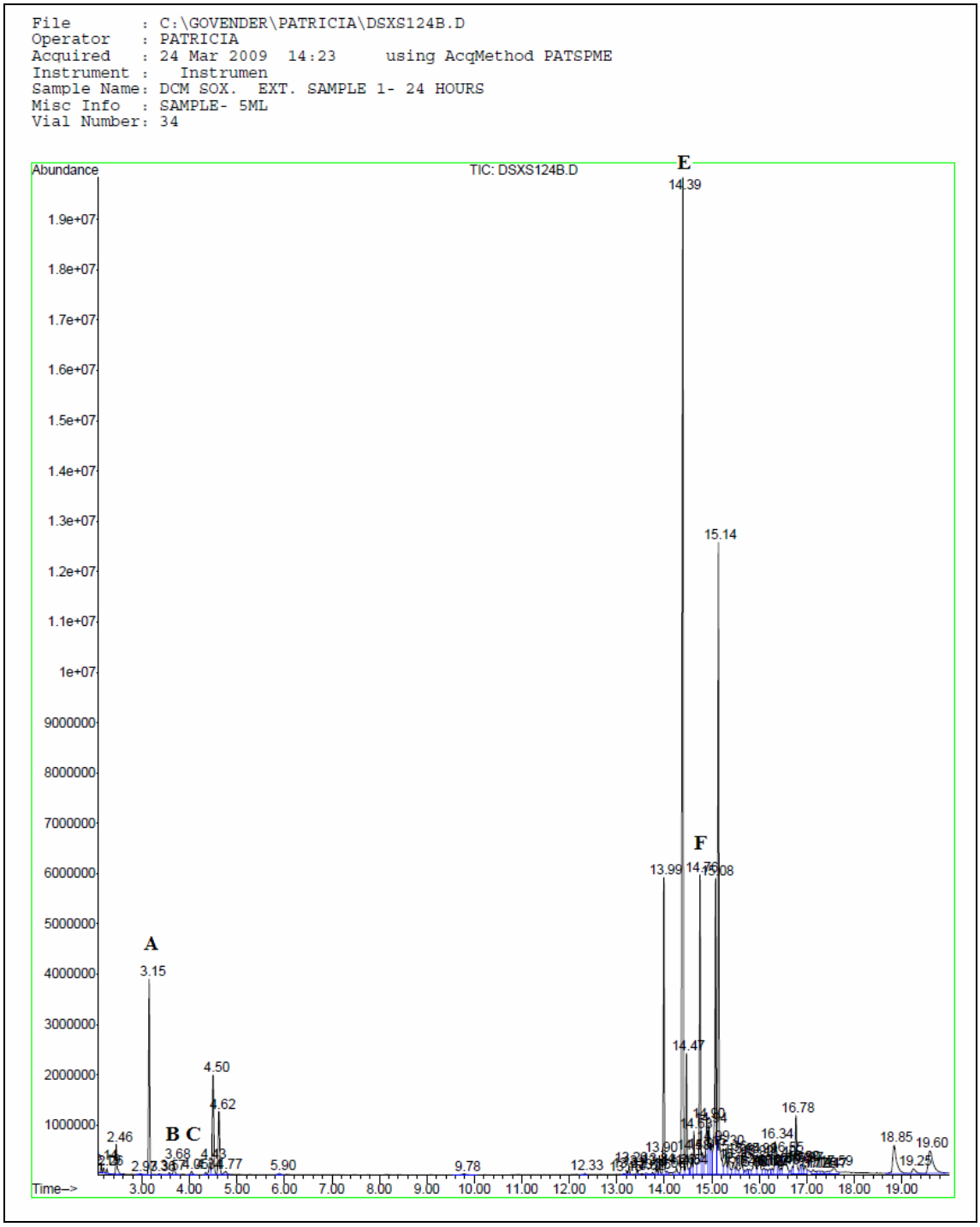

Figure D3 Total ion chromatogram for the essential oil obtained from the 24 hour Soxhlet extraction.

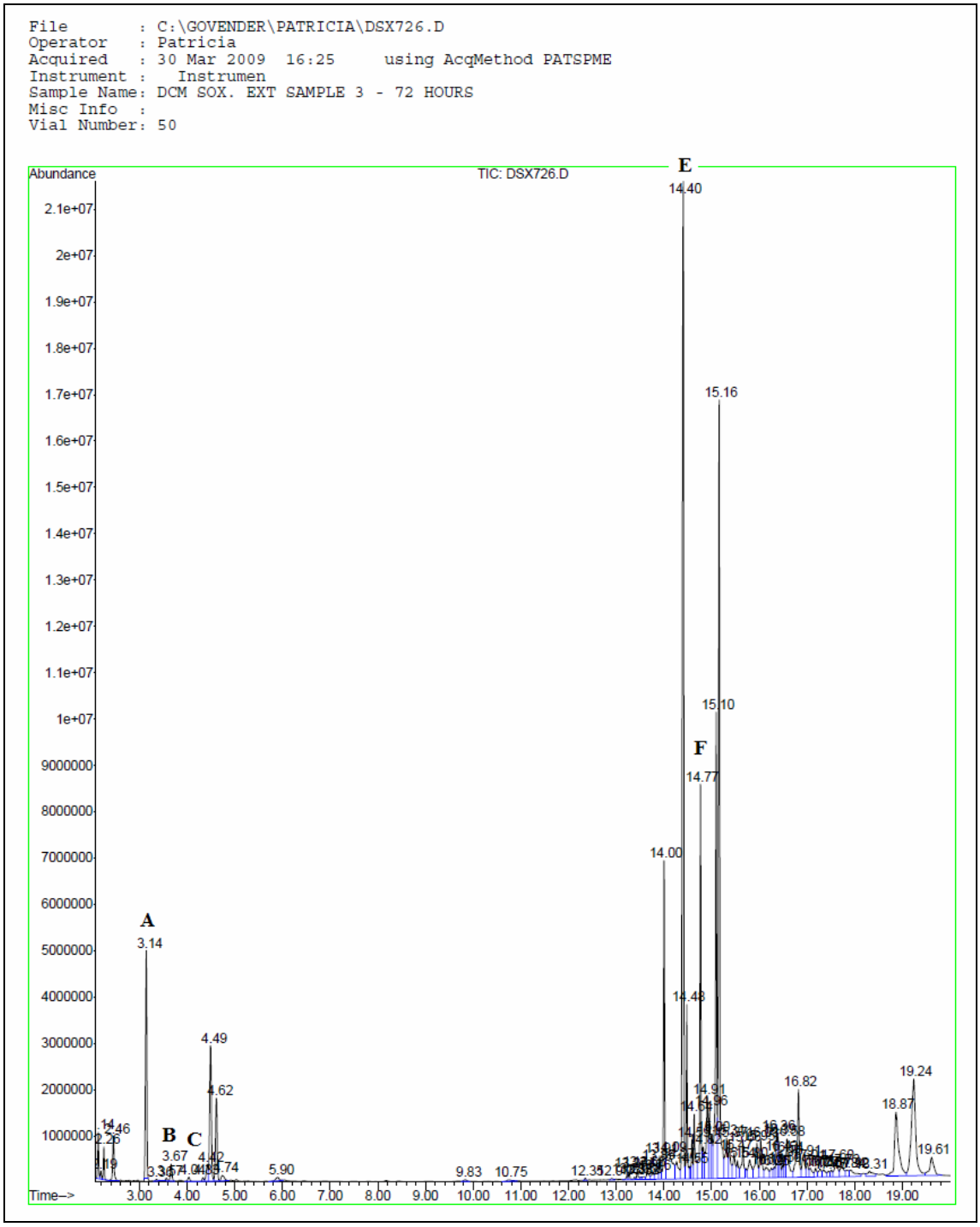

Figure D4 Total ion chromatogram for the essential oil obtained from the 72 hour Soxhlet extraction.

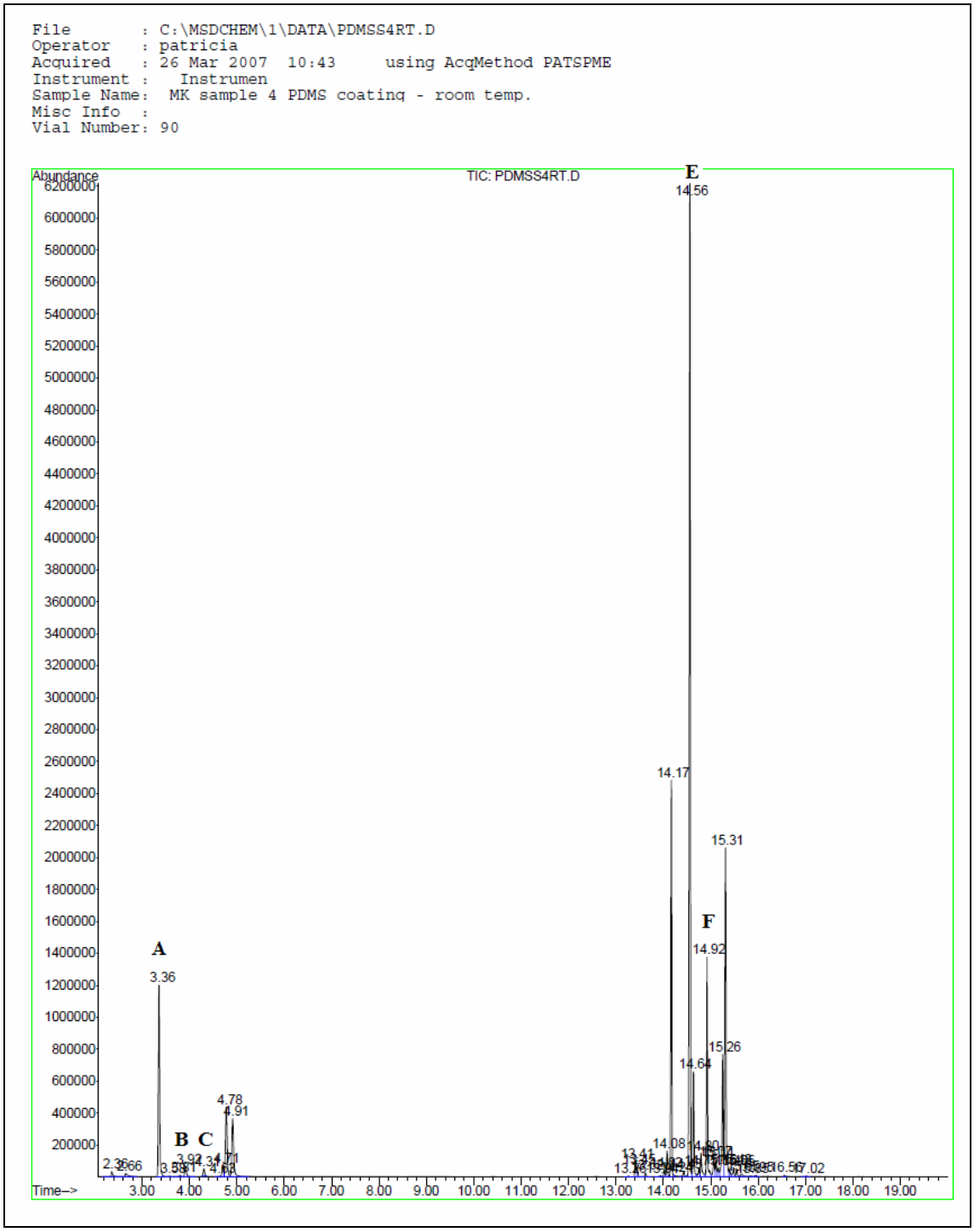

Figure D5 Total ion chromatogram for HS-SPME (PDMS fibre coating) at room temperature.

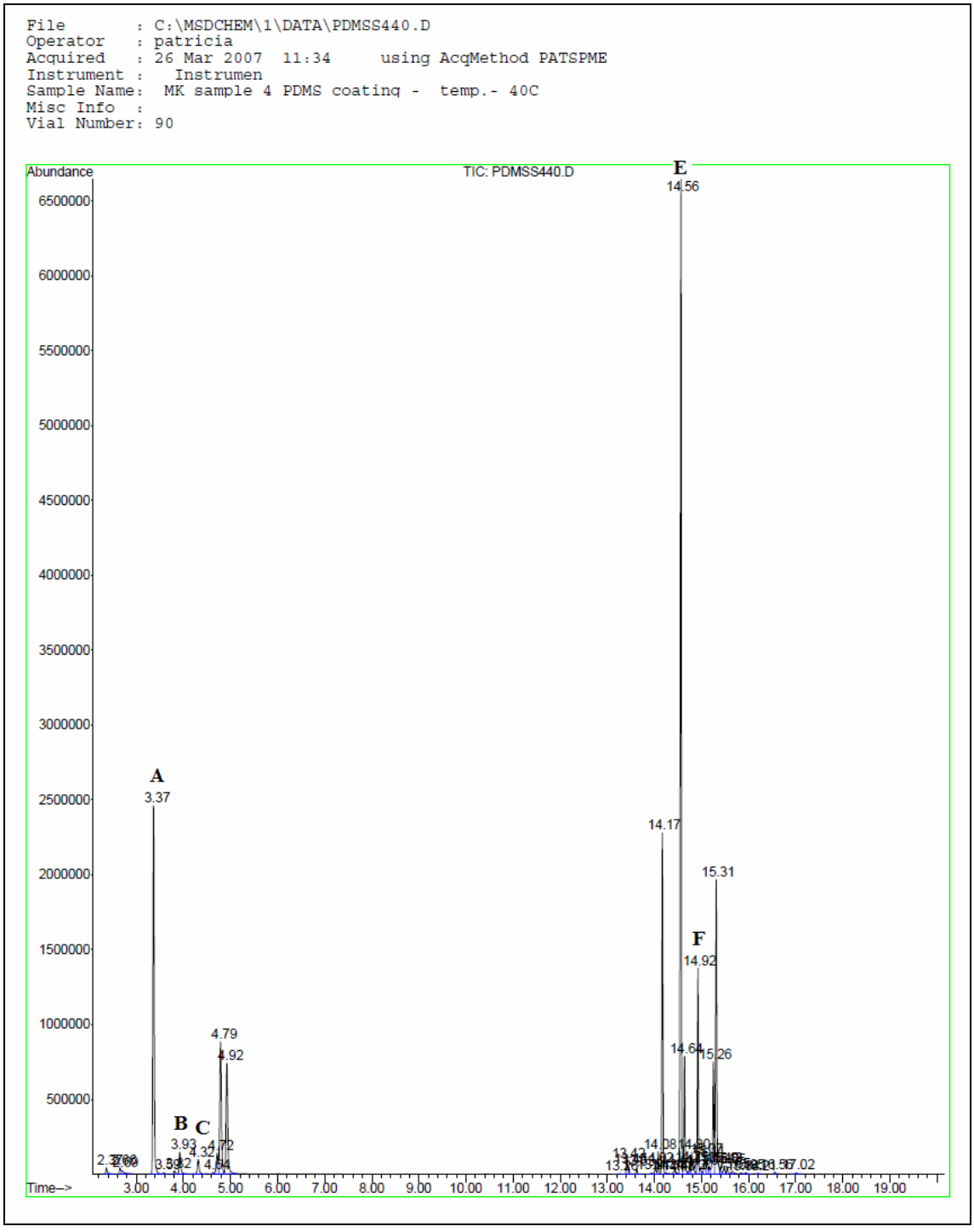

Figure D6 Total ion chromatogram for HS-SPME (PDMS fibre coating) at 40 °C.

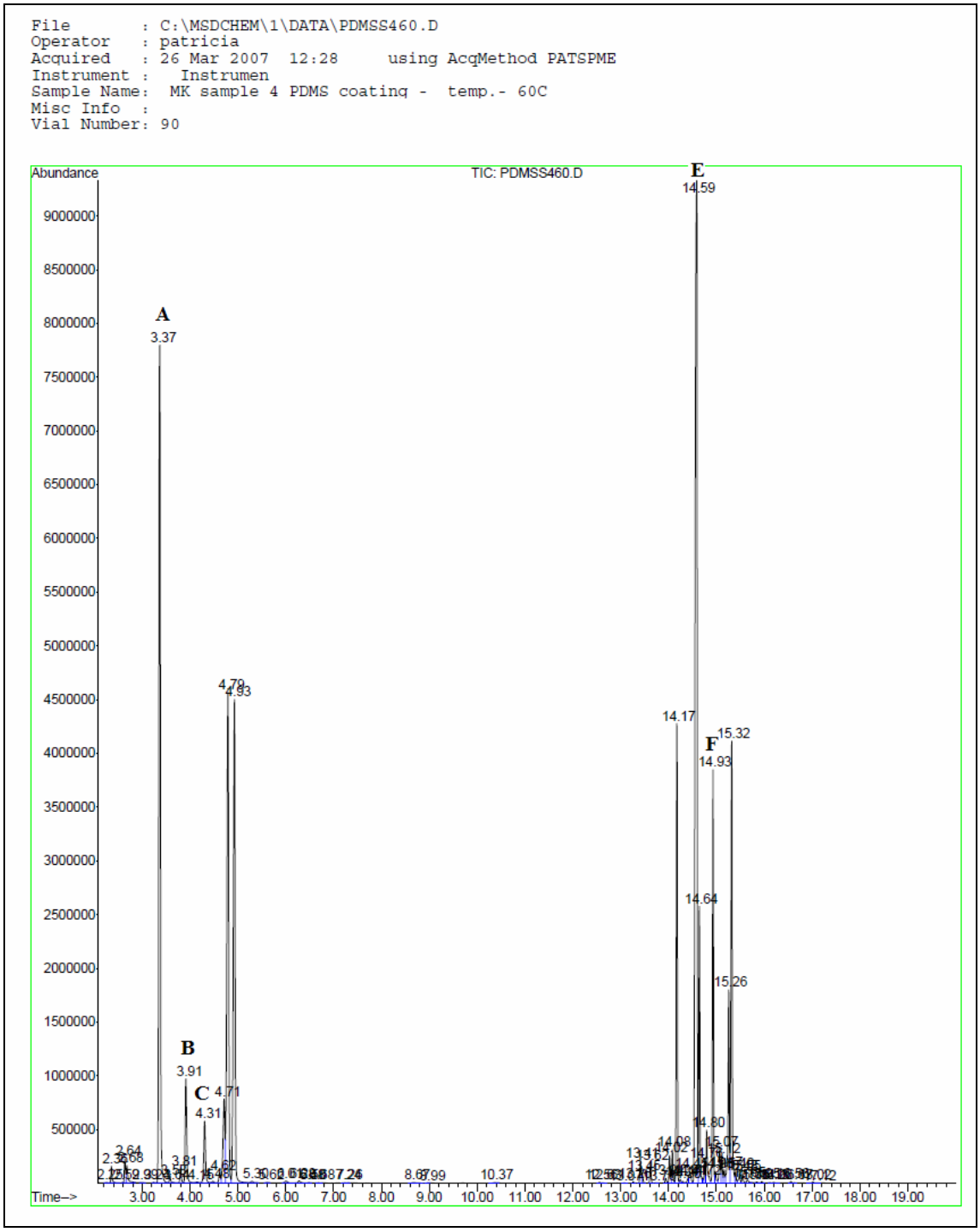

Figure D7 Total ion chromatogram for HS-SPME (PDMS fibre coating) at 60 °C.

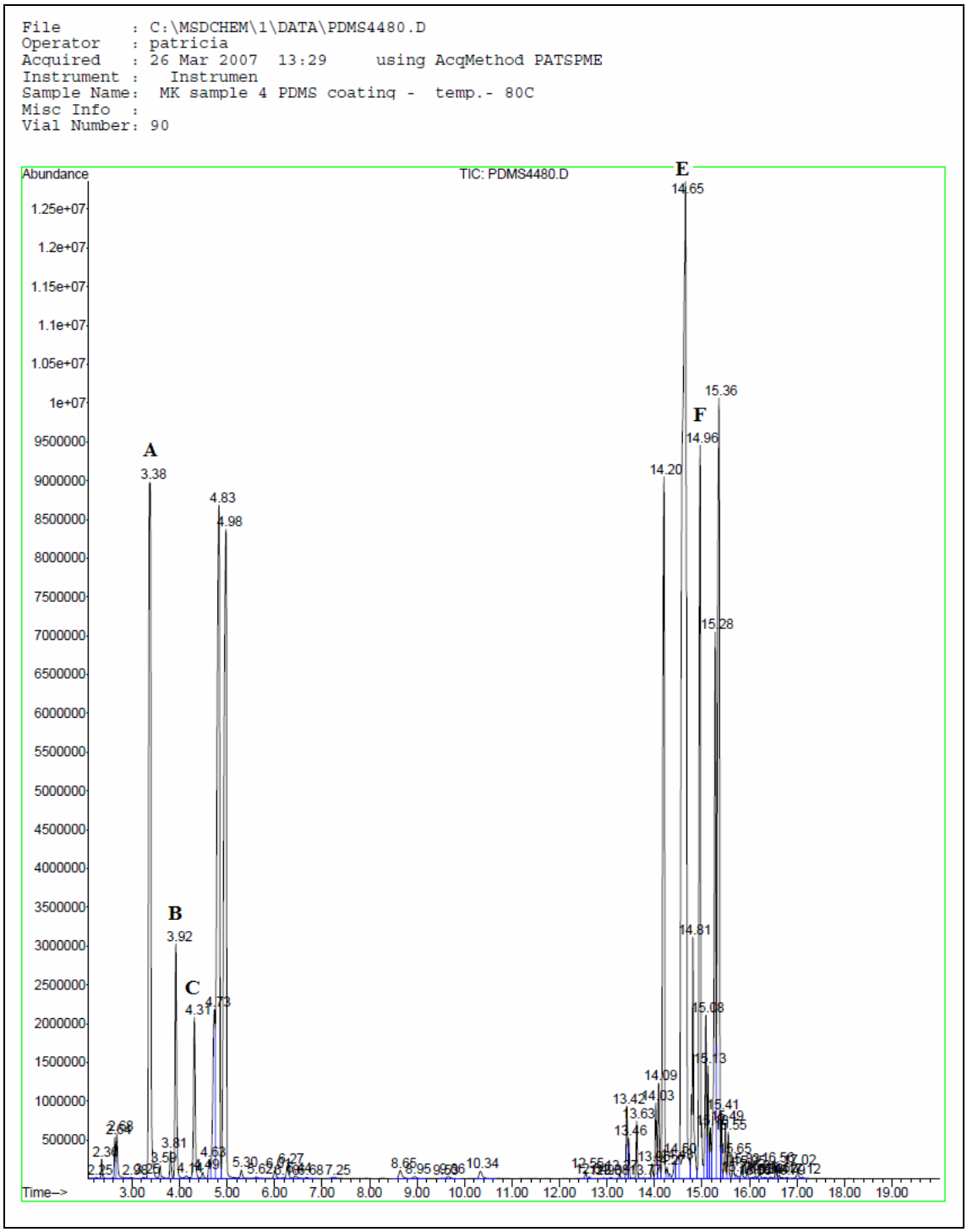

Figure D8 Total ion chromatogram for HS-SPME (PDMS fibre coating) at 80 °C.

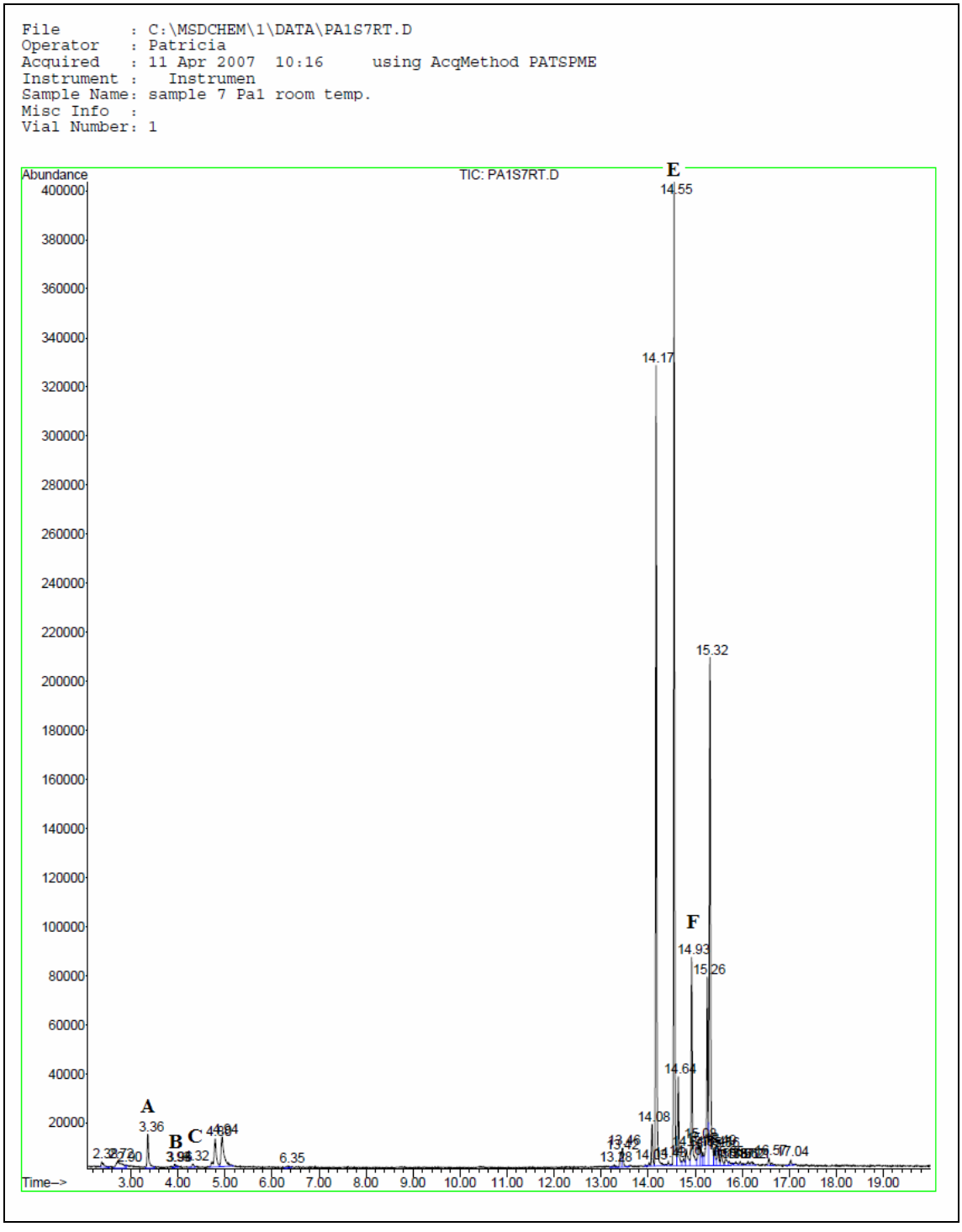

Figure D9 Total ion chromatogram for HS-SPME (PA fibre coating) at room temperature.

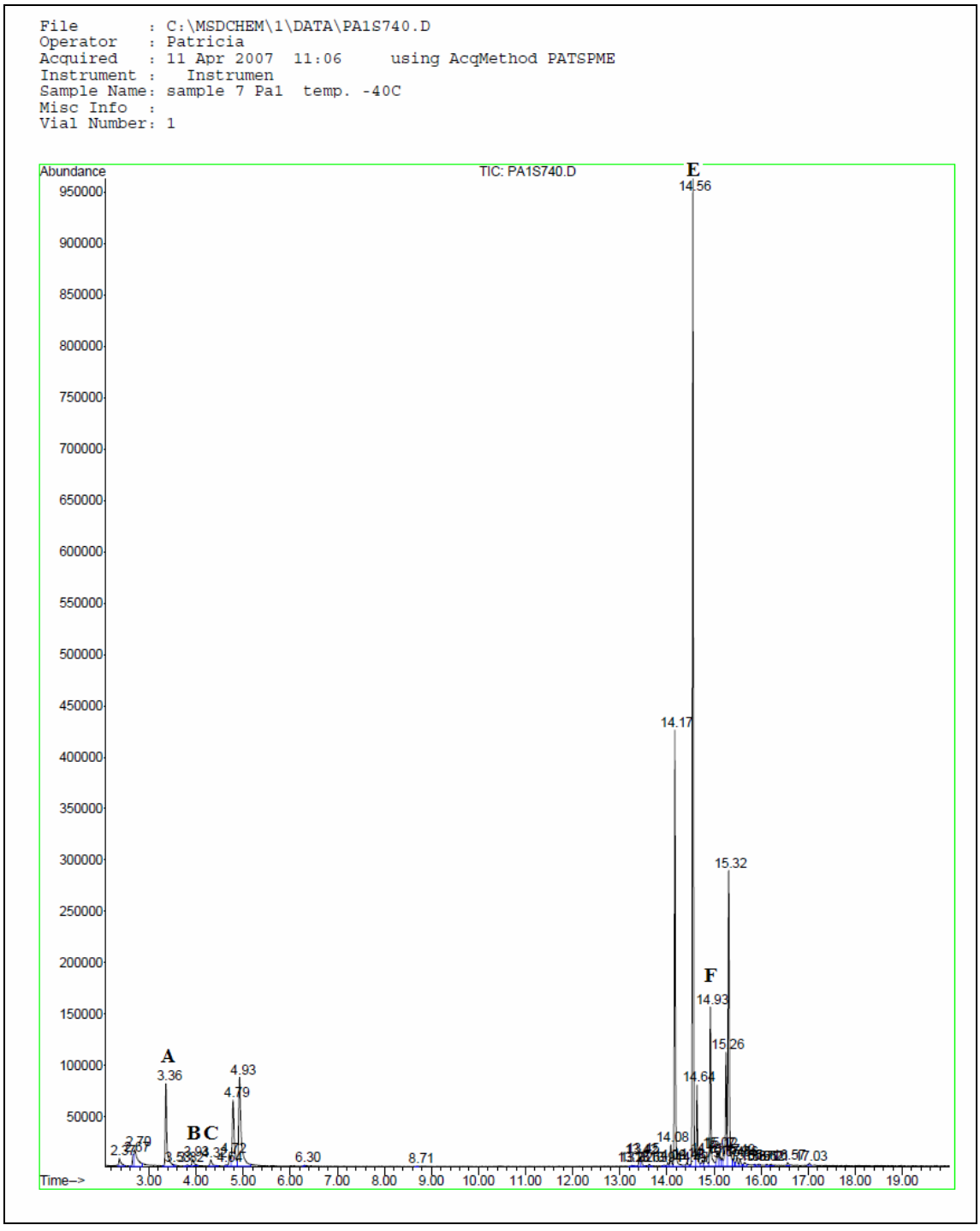

Figure D10 Total ion chromatogram for HS-SPME (PA fibre coating) at 40 °C.

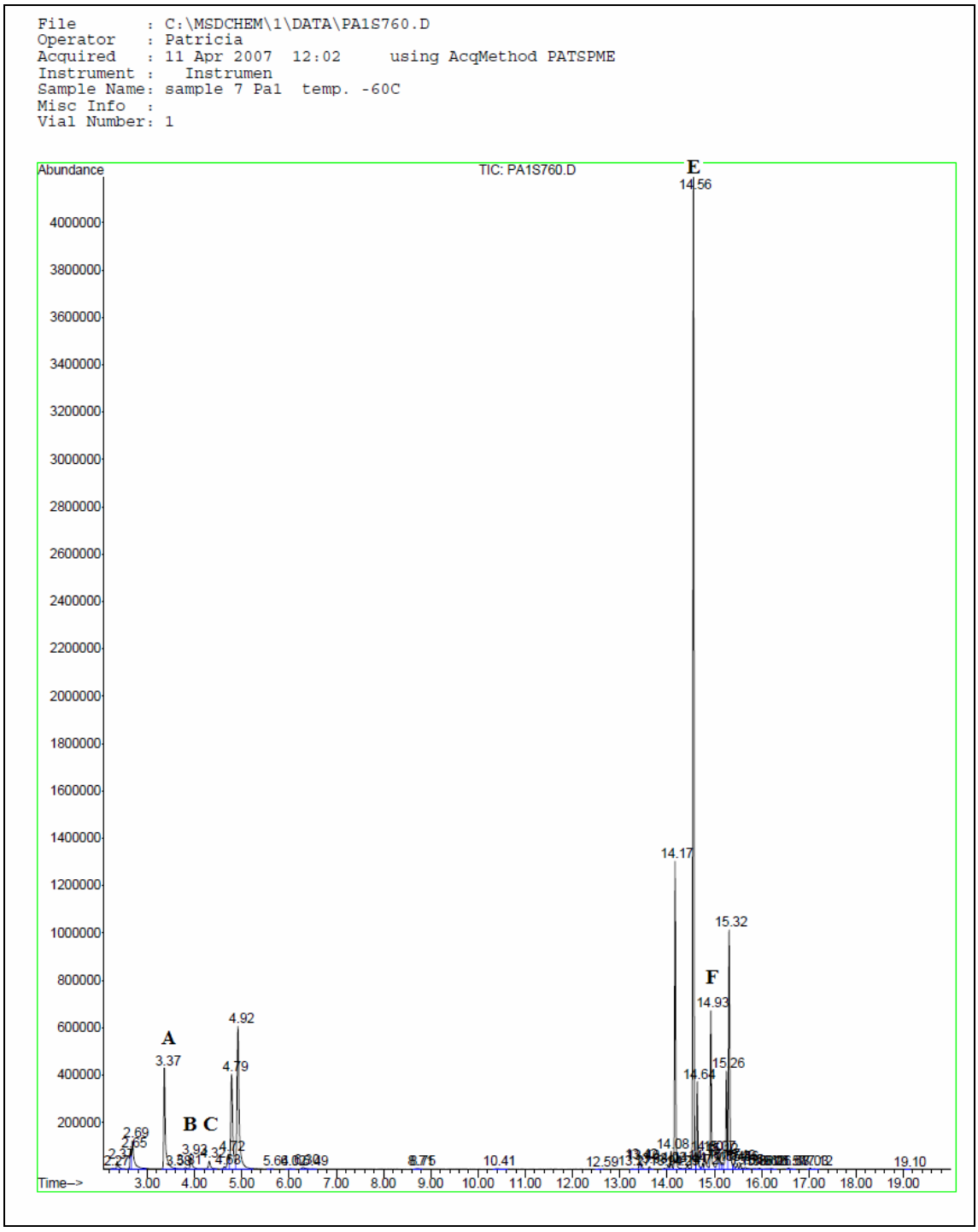

Figure D11 Total ion chromatogram for HS-SPME (PA fibre coating) at 60 °C.

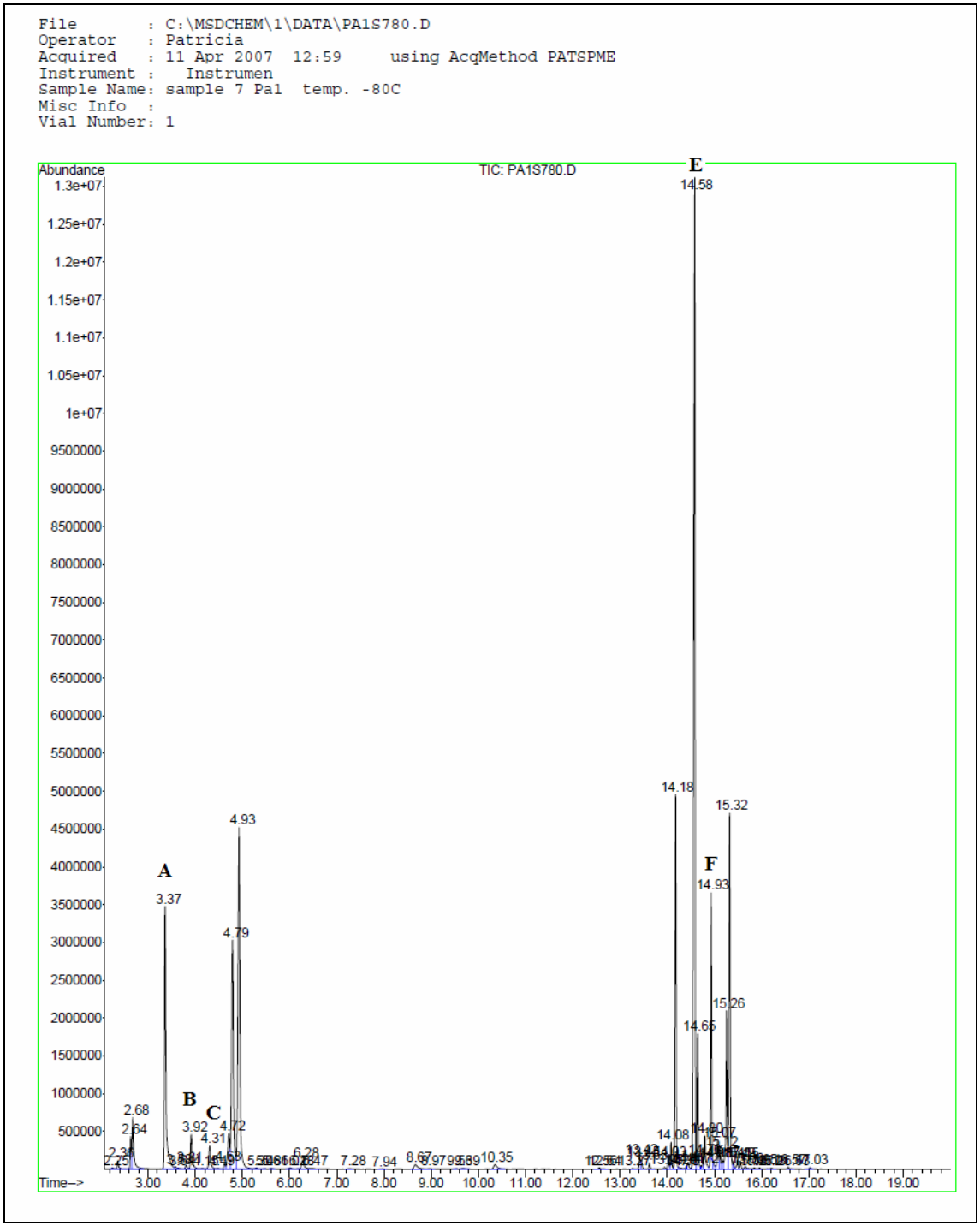

Figure D12 Total ion chromatogram for HS-SPME (PA fibre coating) at 80 °C.

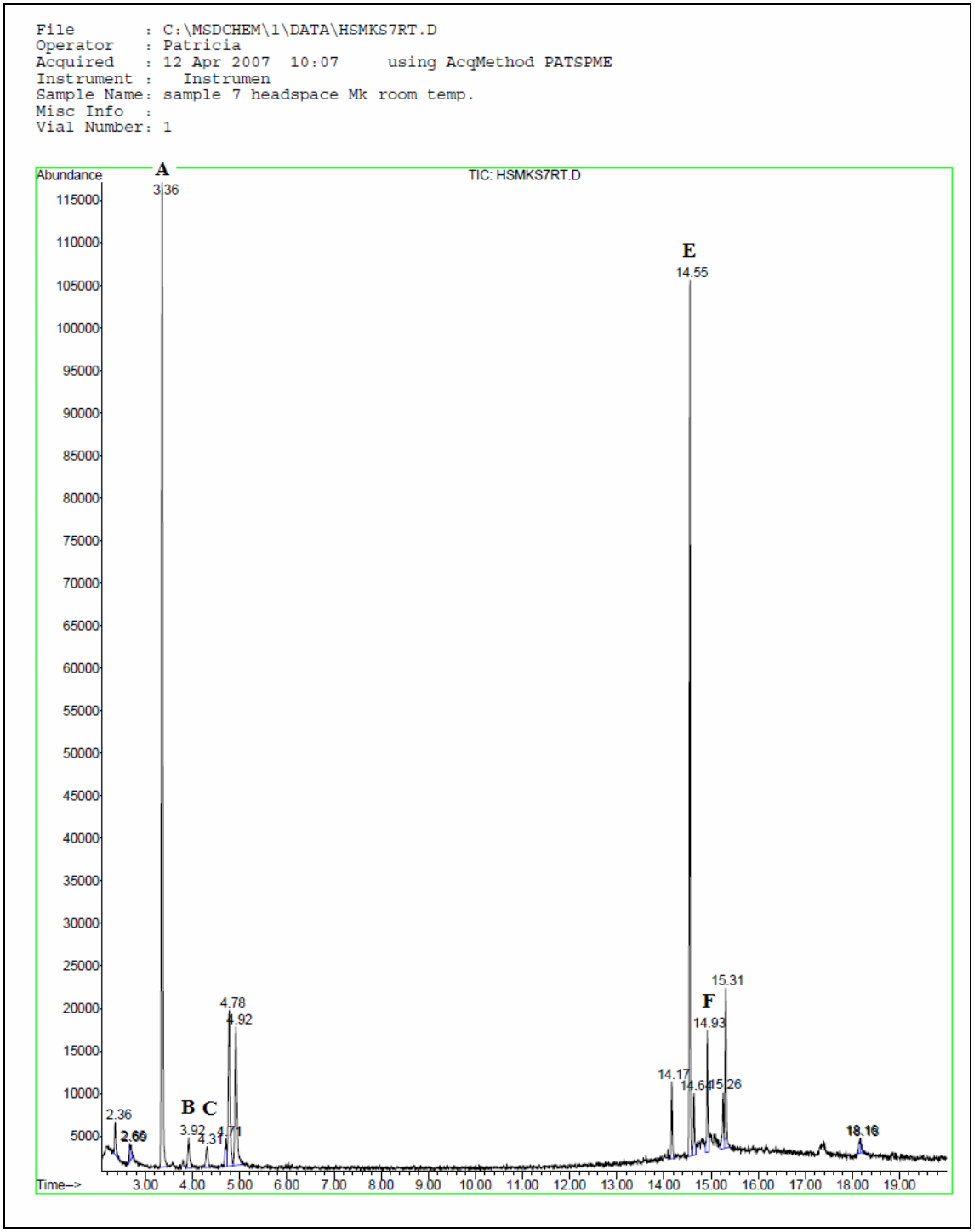

Figure D13 Total ion chromatogram for the headspace analysis at room temperature.

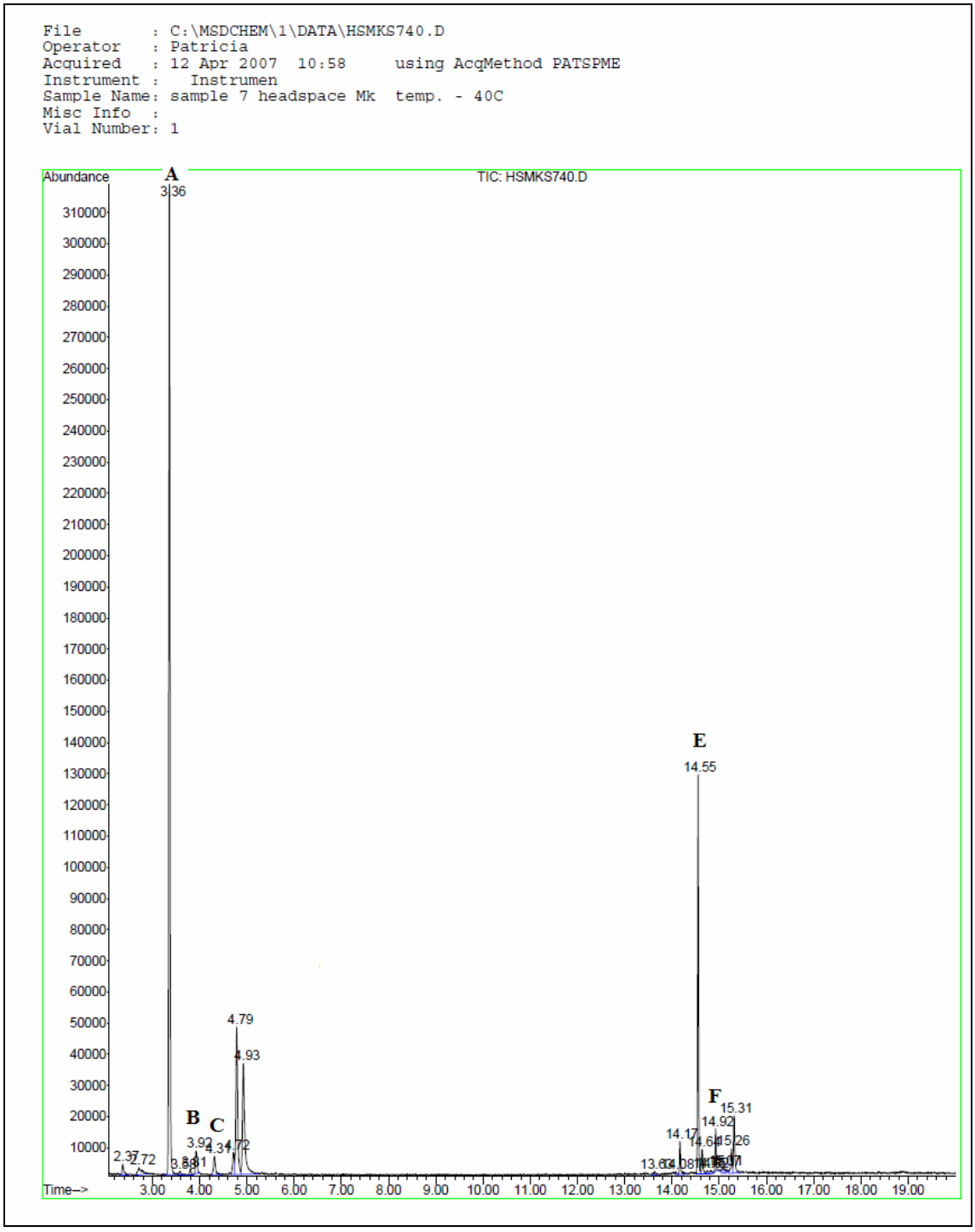

Figure D14 Total ion chromatogram for the headspace analysis at 40 °C.

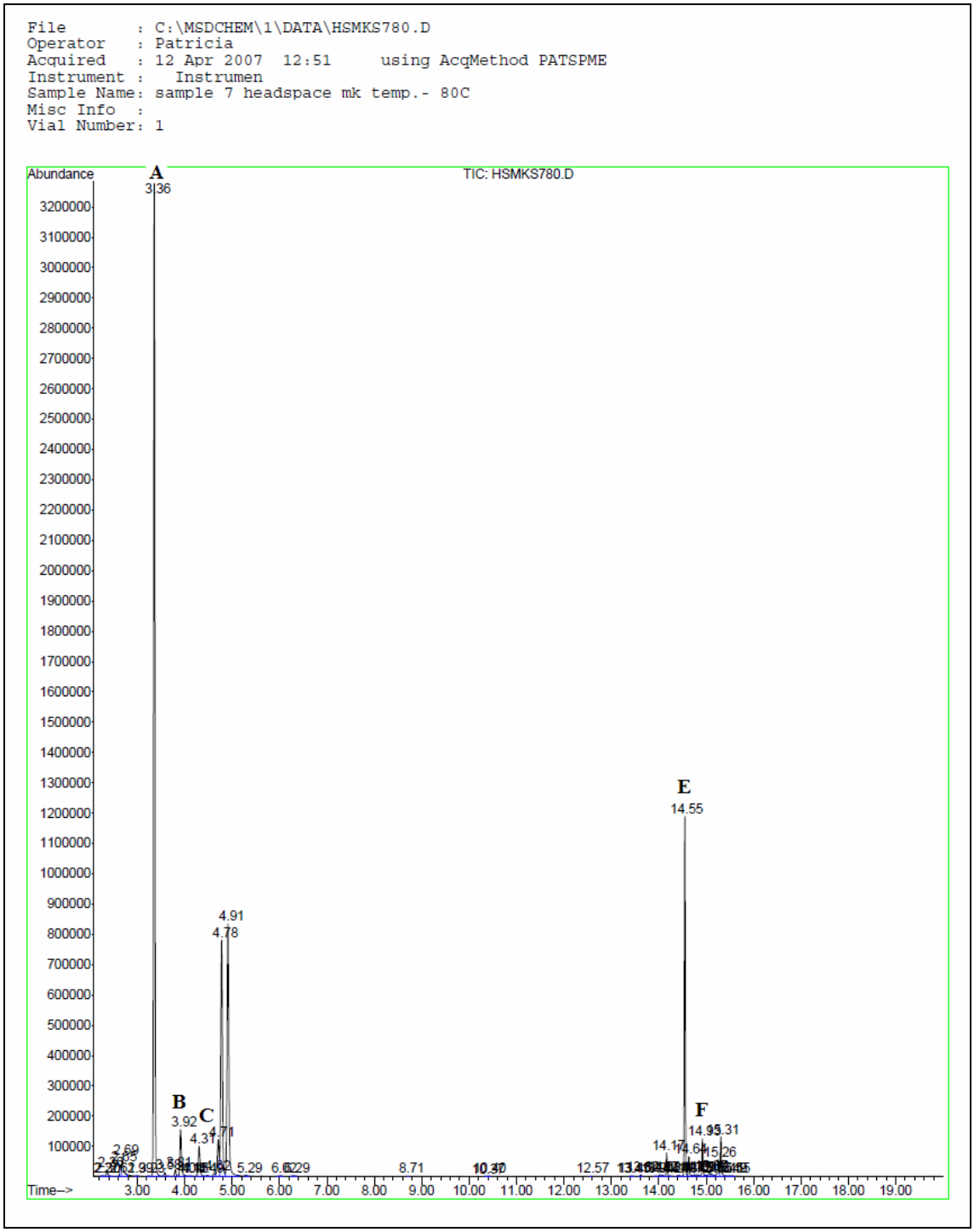

Figure D15 Total ion chromatogram for the headspace analysis at 80 °C.

APPENDIX E

RAW DATA FOR THE ESSENTIAL OILS

Tables E1 to E10 show the peak areas obtained for the five standard compounds.

Data for standards (range 3.36 – 173 mg L^{-1})

Table E1 α-pinene

Concentration /mg L^{-1}		3.43	13.7	41.2	82.4	165
Peak Area	**1**	2.98×10^5	1.06×10^6	3.10×10^6	6.71×10^6	1.01×10^7
	2	3.31×10^5	1.07×10^6	3.10×10^6	6.16×10^6	1.14×10^7
	3	3.02×10^5	1.07×10^6	3.10×10^6	6.54×10^6	1.15×10^7
Mean		3.10×10^5	1.07×10^6	3.10×10^6	6.47×10^6	1.10×10^7
Std Dev		1.78×10^4	5.88×10^3	3.87×10^3	2.78×10^5	7.90×10^5
RSD/%		5.8	0.6	0.1	4.3	7.2

Table E2 β-pinene.

Concentration/mg L^{-1}		3.44	13.7	41.2	82.5	165
Peak Area	**1**	2.78×10^5	1.00×10^6	2.92×10^6	6.70×10^6	1.01×10^7
	2	2.75×10^5	9.38×10^5	3.03×10^6	6.15×10^6	1.15×10^7
	3	2.66×10^5	9.21×10^5	2.90×10^6	6.62×10^6	1.15×10^7
Mean		2.73×10^5	9.54×10^5	2.95×10^6	6.49×10^6	1.10×10^7
Std Dev		6.47×10^3	4.35×10^4	7.16×10^4	2.96×10^5	7.92×10^5
RSD/%		2.4	4.6	2.4	4.6	7.2

Table E3 α-phellandrene.

Concentration/mg L^{-1}		3.36	13.4	40.3	80.6	161
Peak Area	**1**	2.55×10^5	9.39×10^5	2.80×10^6	3.98×10^6	8.22×10^6
	2	2.63×10^5	9.36×10^5	2.81×10^6	3.67×10^6	9.27×10^6
	3	2.68×10^5	9.27×10^5	2.77×10^6	3.85×10^6	9.34×10^6
Mean		2.62×10^5	9.34×10^5	2.79×10^6	3.84×10^6	8.95×10^6
Std Dev		6.21×10^3	5.83×10^3	1.95×10^4	1.55×10^5	6.27×10^5
RSD/%		2.4	0.6	0.7	4.1	7.1

Table E4 β-caryophyllene.

Concentration/mg L^{-1}		3.61	14.4	43.3	86.6	173
Peak Area	**1**	2.54×10^5	9.65×10^5	2.96×10^6	6.68×10^6	1.09×10^7
	2	2.77×10^5	9.28×10^5	3.05×10^6	6.20×10^6	1.18×10^7
	3	2.77×10^5	8.84×10^5	2.95×10^6	6.50×10^6	1.23×10^7
Mean		2.69×10^5	9.26×10^5	2.99×10^6	6.46×10^6	1.17×10^7
Std Dev		1.36×10^4	4.03×10^4	5.35×10^4	2.42×10^5	7.09×10^5
RSD/%		5.1	1.0	1.8	3.8	6.1

Table E5 α-caryophyllene.

Concentration/mg L^{-1}		3.56	14.2	42.7	85.3	171
Peak Area	**1**	3.17×10^5	1.27×10^6	3.75×10^6	8.65×10^6	1.39×10^7
	2	3.29×10^5	1.22×10^6	3.88×10^6	8.09×10^6	1.49×10^7
	3	3.20×10^5	1.21×10^6	3.76×10^6	8.46×10^6	1.57×10^7
Mean		3.22×10^5	1.23×10^6	3.80×10^6	8.40×10^6	1.48×10^7
Std Dev		6.22×10^3	3.37×10^4	7.26×10^4	2.82×10^5	8.88×10^5
RSD/%		1.9	2.7	1.9	3.4	6.0

Data for standards (range 323 – 1.80×10^4 mg L^{-1})

Table E6 α-pinene.

Concentration/mg L^{-1}		329	858	2.57×10^3	8.58×10^3	1.72×10^4
Peak Area	**1**	2.61×10^7	1.11×10^8	2.21×10^8	4.52×10^8	6.26×10^8
	2	2.53×10^7	1.16×10^8	1.95×10^8	4.29×10^8	6.12×10^8
	3	2.79×10^7	1.19×10^8	1.71×10^8	4.42×10^8	5.73×10^8
Mean		2.64×10^7	1.15×10^8	1.95×10^8	4.41×10^8	6.04×10^8
Std Dev		1.37×10^6	3.97×10^6	2.51×10^7	1.14×10^7	2.74×10^7
RSD/%		5.2	3.5	12.9	2.6	4.5

Table E7 β-pinene.

Concentration/mg L^{-1}		330	859	2.58×10^3	8.59×10^3	1.72×10^4
Peak Area	**1**	2.66×10^7	1.28×10^8	2.49×10^8	4.87×10^8	6.81×10^8
	2	2.58×10^7	1.33×10^8	2.19×10^8	4.95×10^8	6.88×10^8
	3	2.84×10^7	1.37×10^8	1.93×10^8	5.07×10^8	6.26×10^8
Mean		2.69×10^7	1.33×10^8	2.20×10^8	4.96×10^8	6.65×10^8
Std Dev		1.36×10^6	4.38×10^6	2.78×10^7	1.04×10^7	3.39×10^7
RSD/%		5.1	3.3	12.6	2.1	5.1

Table E8 α-phellandrene.

Concentration/mg L^{-1}		323	840	2.52×10^3	8.40×10^3	1.68×10^4
Peak Area	**1**	2.16×10^7	1.28×10^8	1.93×10^8	4.28×10^8	6.22×10^8
	2	2.04×10^7	1.33×10^8	1.70×10^8	4.10×10^8	6.08×10^8
	3	2.28×10^7	1.36×10^8	1.49×10^8	4.23×10^8	5.66×10^8
Mean		2.16×10^7	1.32×10^8	1.71×10^8	4.20×10^8	5.99×10^8
Std Dev		1.18×10^6	4.12×10^6	2.24×10^7	9.60×10^6	2.89×10^7
RSD/%		5.5	3.1	13.1	2.3	4.8

Table E9 β-caryophyllene.

Concentration/mg L^{-1}		346	902	2.71×10^3	9.02×10^3	1.80×10^4
Peak Area	**1**	2.80×10^7	1.20×10^8	2.13×10^8	3.65×10^8	5.69×10^8
	2	2.70×10^7	1.23×10^8	1.91×10^8	3.39×10^8	5.73×10^8
	3	3.02×10^7	1.29×10^8	1.63×10^8	3.56×10^8	5.14×10^8
Mean		2.84×10^7	1.24×10^8	1.89×10^8	3.53×10^8	5.52×10^8
Std Dev		1.62×10^6	4.70×10^6	2.51×10^7	1.36×10^7	3.30×10^7
RSD/%		5.7	3.8	13.3	3.8	6.0

Table E10 α-caryophyllene.

Concentration/mg L^{-1}		341	889	2.67×10^3	8.89×10^3	1.78×10^4
Peak Area	**1**	3.44×10^7	1.64×10^8	2.67×10^8	5.71×10^8	8.14×10^8
	2	3.31×10^7	1.68×10^8	2.40×10^8	5.31×10^8	8.20×10^8
	3	3.69×10^7	1.75×10^8	2.06×10^8	5.54×10^8	7.32×10^8
Mean		3.48×10^7	1.69×10^8	2.37×10^8	5.52×10^8	7.88×10^8
Std Dev		1.93×10^6	5.78×10^6	3.05×10^7	1.97×10^7	4.91×10^7
RSD/%		5.5	3.4	12.9	3.6	6.2

Tables E11 to E25 contain the data for the different extraction methods.

Solvent extraction

Table E11 α-pinene.

Sample/hours	**24**	**48**	**72**
Peak Area	9.94×10^7	1.22×10^8	9.53×10^7
	9.84×10^7	1.16×10^8	9.45×10^7
	9.70×10^7	1.17×10^8	9.52×10^7
Mean	9.83×10^7	1.18×10^8	9.50×10^7
Std Dev	1.19×10^6	3.41×10^6	4.21×10^5
RSD/%	1.2	2.9	0.4

Table E12 β-pinene

.

Sample/hours	**24**	**48**	**72**
Peak Area	9.11×10^6	1.08×10^7	8.48×10^6
	9.29×10^6	1.01×10^7	8.87×10^6
	9.22×10^6	1.03×10^7	8.65×10^6
Mean	9.21×10^6	1.04×10^7	8.67×10^6
Std Dev	8.65×10^4	3.31×10^5	1.97×10^5
RSD/%	0.9	3.2	2.3

Table E13 α-phellandrene.

Sample/hours	**24**	**48**	**72**
Peak Area	4.56×10^6	4.64×10^6	3.01×10^6
	4.23×10^6	4.28×10^6	3.08×10^6
	4.27×10^6	4.31×10^6	2.98×10^6
Mean	4.36×10^6	4.41×10^6	3.02×10^6
Std Dev	1.83×10^5	2.00×10^5	5.35×10^4
RSD/%	4.2	4.6	1.8

Table E14 β-caryophyllene.

Sample/hours	**24**	**48**	**72**
Peak Area	5.05×10^8	5.40×10^8	4.78×10^8
	5.22×10^8	5.20×10^8	4.92×10^8
	5.17×10^8	5.14×10^8	4.60×10^8
Mean	5.15×10^8	5.25×10^8	4.77×10^8
Std Dev	8.83×10^6	1.39×10^7	1.64×10^7
RSD/%	1.7	2.7	3.4

Table E15 α-caryophyllene.

Sample/hours	24	48	72
Peak Area	1.45×10^8	1.60×10^8	1.35×10^8
	1.54×10^8	1.58×10^8	1.48×10^8
	1.53×10^8	1.54×10^8	1.43×10^8
Mean	1.51×10^8	1.58×10^8	1.42×10^8
Std Dev	5.24×10^6	3.03×10^6	6.66×10^6
RSD/%	3.5	1.9	4.7

Soxhlet extraction

Table E16 α-pinene.

Sample/hours	24	48	72
Peak Area	6.32×10^7	9.71×10^7	8.77×10^7
	6.74×10^7	9.06×10^7	9.30×10^7
	6.84×10^7	9.09×10^7	9.04×10^7
Mean	6.63×10^7	9.28×10^7	9.04×10^7
Std Dev	2.71×10^6	3.71×10^6	2.66×10^6
RSD/%	4.1	4.00	2.9

Table E17 β-pinene.

Sample/hours	24	48	72
Peak Area	5.25×10^6	8.89×10^6	7.86×10^6
	5.34×10^6	8.42×10^6	8.31×10^6
	5.67×10^6	8.92×10^6	8.49×10^6
Mean	5.42×10^6	8.74×10^6	8.22×10^6
Std Dev	2.21×10^5	2.79×10^5	3.26×10^5
RSD/%	4.1	3.2	4.0

Table E18 α-phellandrene.

Sample/hours	24	48	72
Peak Area	1.79×10^6	3.31×10^6	2.46×10^6
	1.81×10^6	3.22×10^6	2.69×10^6
	1.87×10^6	3.31×10^6	2.64×10^6
Mean	1.82×10^6	3.28×10^6	2.59×10^6
Std Dev	4.43×10^4	5.24×10^4	1.22×10^5
RSD/%	2.4	1.6	4.7

Table E19 β-caryophyllene.

Sample/hours	24	48	72
Peak Area	3.60×10^8	5.16×10^8	4.56×10^8
	3.70×10^8	5.01×10^8	4.60×10^8
	3.96×10^8	5.04×10^8	4.73×10^8
Mean	3.75×10^8	5.07×10^8	4.63×10^8
Std Dev	1.82×10^7	8.13×10^6	9.22×10^6
RSD/%	4.8	1.6	2.0

Table E20 α-caryophyllene.

Sample/hours	24	48	72
Peak Area	8.91×10^7	1.58×10^8	1.32×10^8
	9.04×10^7	1.56×10^8	1.31×10^8
	9.30×10^7	1.51×10^8	1.34×10^8
Mean	9.08×10^7	1.55×10^8	1.32×10^8
Std Dev	1.95×10^6	3.52×10^6	1.42×10^6
RSD/%	2.1	2.3	1.1

Steam distillation

Table E21 α-pinene.

Sample	1	2	3
Peak Area	4.97×10^5	5.28×10^6	1.08×10^7
	4.81×10^5	5.44×10^6	1.06×10^7
	4.73×10^5	5.29×10^6	1.02×10^7
Mean	4.84×10^5	5.34×10^6	1.05×10^7
Std Dev	1.24×10^4	8.70×10^4	3.17×10^5
RSD/%	2.6	1.6	3.0

Table E22 β-pinene.

Sample	1	2	3
Peak Area	2.14×10^5	1.17×10^6	1.90×10^6
	2.21×10^5	1.22×10^6	1.90×10^6
	2.26×10^5	1.21×10^6	1.89×10^6
Mean	2.20×10^5	1.20×10^6	1.90×10^6
Std Dev	6.21×10^3	2.53×10^4	2.16×10^3
RSD/%	2.8	2.1	0.1

Table E23 α-phellandrene.

Sample	1	2	3
Peak Area	2.67×10^5	1.07×10^6	1.89×10^6
	2.51×10^5	1.10×10^6	1.83×10^6
	2.45×10^5	1.10×10^6	1.78×10^6
Mean	2.55×10^5	1.09×10^6	1.84×10^6
Std Dev	1.13×10^4	1.68×10^4	5.42×10^4
RSD/%	4.4	1.5	3.0

Table E24 β-caryophyllene.

Sample	1	2	3
Peak Area	2.65×10^8	2.66×10^8	2.91×10^8
	2.60×10^8	2.66×10^8	2.85×10^8
	2.66×10^8	2.64×10^8	2.82×10^8
Mean	2.64×10^8	2.65×10^8	2.86×10^8
Std Dev	3.48×10^6	1.28×10^6	4.67×10^6
RSD/%	1.3	0.5	1.6

Table E25 α-caryophyllene.

Sample	1	2	3
Peak Area	9.17×10^7	8.34×10^7	9.41×10^7
	8.91×10^7	8.60×10^7	9.08×10^7
	9.21×10^7	8.31×10^7	8.98×10^7
Mean	9.10×10^7	8.42×10^7	9.16×10^7
Std Dev	1.64×10^6	1.61×10^6	2.24×10^6
RSD/%	1.8	1.9	2.4

The following tables show the peak areas of the five selected compounds for the headspace extraction methods.

HS-SPME (PDMS fibre coating)

Tables E26 to E30 show the peak areas obtained for the five selected compounds during the HS-SPME (PDMS fibre coating) analysis.

Table E26 α-pinene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	1.91×10^7	5.43×10^7	2.09×10^8	3.08×10^8
	2.29×10^7	4.69×10^7	1.79×10^8	3.00×10^8
	2.20×10^7	5.63×10^7	1.95×10^8	4.02×10^8
Mean	2.13×10^7	5.25×10^7	1.94×10^8	3.37×10^8
Std Dev	1.97×10^6	4.96×10^6	1.49×10^7	5.69×10^7
RSD/%	9.2	9.5	7.7	16.9

Table E27 β-pinene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	1.42×10^6	3.72×10^6	2.58×10^7	7.41×10^7
	1.66×10^6	3.55×10^6	2.23×10^7	7.13×10^7
	1.71×10^6	3.87×10^6	2.24×10^7	9.19×10^7
Mean	1.60×10^6	3.72×10^6	2.35×10^7	7.91×10^7
Std Dev	1.58×10^5	1.59×10^5	2.00×10^6	1.12×10^7
RSD/%	9.9	4.3	8.5	14.1

Table E28 α-phellandrene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	9.26×10^5	2.75×10^6	1.46×10^7	4.81×10^7
	9.39×10^5	2.50×10^6	1.44×10^7	5.29×10^7
	1.00×10^6	2.56×10^6	1.52×10^7	6.94×10^7
Mean	9.56×10^5	2.61×10^6	1.47×10^7	5.68×10^7
Std Dev	4.11×10^4	1.32×10^5	4.09×10^5	1.12×10^7
RSD/%	4.3	5.1	2.8	19.7

Table E29 β-caryophyllene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	9.94×10^7	1.38×10^8	2.93×10^8	8.95×10^8
	1.04×10^8	1.17×10^8	2.80×10^8	8.21×10^8
	1.13×10^8	1.39×10^8	3.11×10^8	9.44×10^8
Mean	1.05×10^8	1.31×10^8	2.94×10^8	8.87×10^8
Std Dev	6.68×10^6	1.25×10^7	1.58×10^7	6.20×10^7
RSD/%	6.3	9.5	5.4	7.00

Table E30 α-caryophyllene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	1.87×10^7	2.29×10^7	5.83×10^7	2.50×10^8
	1.99×10^7	1.95×10^7	5.45×10^7	2.20×10^8
	2.01×10^7	2.09×10^7	4.87×10^7	2.21×10^8
Mean	1.96×10^7	2.11×10^7	5.38×10^7	2.30×10^8
Std Dev	7.72×10^5	1.71×10^6	4.84×10^6	1.71×10^7
RSD/%	4.00	8.1	9.00	7.4

HS-SPME (PA fibre coating)

Tables E31 to E35 show the peak areas obtained for the five selected compounds during the HS-SPME (PA fibre coating) analysis.

Table E31 α-pinene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	3.56×10^5	1.94×10^6	1.26×10^7	7.43×10^7
	3.62×10^5	2.06×10^6	1.15×10^7	5.67×10^7
	3.39×10^5	1.93×10^6	1.08×10^7	8.17×10^7
Mean	3.52×10^5	1.98×10^6	1.16×10^7	7.09×10^7
Std Dev	1.17×10^4	7.07×10^4	8.91×10^5	1.28×10^7
RSD/%	3.3	3.6	7.7	18.1

Table E32 β-pinene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	2.50×10^4	2.37×10^5	1.72×10^6	1.02×10^7
	2.67×10^4	2.07×10^5	1.77×10^6	8.74×10^6
	2.47×10^4	2.14×10^5	1.66×10^6	1.21×10^7
Mean	2.55×10^4	2.19×10^5	1.72×10^6	1.03×10^7
Std Dev	1.09×10^3	1.59×10^4	5.63×10^4	1.68×10^6
RSD/%	4.3	7.3	3.3	16.3

Table 33 α-phellandrene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	4.41×10^4	2.16×10^5	1.22×10^6	6.61×10^6
	4.91×10^4	1.92×10^5	1.19×10^6	7.00×10^6
	4.39×10^4	1.98×10^5	1.16×10^6	8.64×10^6
Mean	4.57×10^4	2.02×10^5	1.19×10^6	7.42×10^6
Std Dev	2.96×10^3	1.24×10^4	3.30×10^4	1.08×10^6
RSD/%	6.5	6.2	2.8	14.6

Table E34 β-caryophyllene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	6.62×10^6	1.56×10^7	7.99×10^7	2.34×10^8
	8.31×10^6	2.05×10^7	6.98×10^7	2.01×10^8
	5.96×10^6	1.45×10^7	6.63×10^7	2.85×10^8
Mean	6.96×10^6	1.68×10^7	7.20×10^7	2.40×10^8
Std Dev	1.21×10^6	3.21×10^6	7.06×10^6	4.25×10^7
RSD/%	17.4	19.0	9.8	17.7

Table E35 α-caryophyllene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	1.39×10^6	2.72×10^6	1.29×10^7	4.25×10^7
	1.95×10^6	3.55×10^6	1.09×10^7	3.16×10^7
	1.50×10^6	2.64×10^6	1.10×10^7	4.98×10^7
Mean	1.61×10^6	2.97×10^6	1.16×10^7	4.13×10^7
Std Dev	2.96×10^5	5.07×10^5	1.13×10^6	9.17×10^6
RSD/%	18.4	17.1	9.7	22.2

HSA

Tables E36 to E40 show the peak areas obtained for the five selected compounds during the headspace analysis.

Table E36 α-pinene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	2.30×10^6	5.98×10^6	3.25×10^7	6.06×10^7
	2.75×10^6	7.16×10^6	5.75×10^7	7.52×10^7
	2.65×10^6	7.76×10^6	2.98×10^7	1.56×10^8
Mean	2.57×10^6	6.97×10^6	3.99×10^7	9.73×10^7
Std Dev	2.36×10^5	9.09×10^5	1.53×10^7	5.14×10^7
RSD/%	9.2	13.1	38.2	52.8

Table E37 β-pinene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	9.15×10^4	1.86×10^5	1.86×10^6	3.73×10^6
	8.67×10^4	2.19×10^5	2.18×10^6	3.63×10^6
	7.06×10^4	2.16×10^5	1.68×10^6	1.31×10^7
Mean	8.29×10^4	2.07×10^5	1.91×10^6	6.82×10^6
Std Dev	1.09×10^4	1.82×10^4	2.50×10^5	5.43×10^6
RSD/%	13.2	8.8	13.1	79.6

Table E38 α-phellandrene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	7.21×10^4	1.86×10^5	1.15×10^6	2.50×10^6
	5.52×10^4	1.52×10^5	1.13×10^6	2.28×10^6
	5.25×10^4	1.39×10^5	1.02×10^6	7.42×10^6
Mean	5.99×10^4	1.59×10^5	1.10×10^6	4.06×10^6
Std Dev	1.06×10^4	2.44×10^4	7.10×10^4	2.91×10^6
RSD/%	17.8	15.3	6.5	71.5

Table E39 β-caryophyllene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	1.51×10^6	1.85×10^6	1.01×10^7	1.71×10^7
	1.51×10^6	4.83×10^6	1.46×10^7	2.18×10^7
	1.92×10^6	1.73×10^6	1.57×10^7	8.32×10^7
Mean	1.65×10^6	2.81×10^6	1.35×10^7	4.07×10^7
Std Dev	2.34×10^5	1.76×10^6	2.92×10^6	3.69×10^7
RSD/%	14.2	62.6	21.7	90.7

Table E40 α-caryophyllene.

Sample temperature	RT	40°C	60°C	80°C
Peak Area	2.55×10^5	1.91×10^5	1.03×10^6	1.74×10^6
	2.03×10^5	4.73×10^5	1.78×10^6	3.15×10^6
	2.47×10^5	1.89×10^5	1.74×10^6	1.13×10^7
Mean	2.35×10^5	2.84×10^5	1.52×10^6	5.39×10^6
Std Dev	2.77×10^4	1.63×10^5	4.22×10^5	5.15×10^6
RSD/%	11.8	57.5	27.9	95.6

Terpenoid profile of the essential oil

Tables E41 to E43 show the terpenoid profile of the essential oil in *M. koenigii* leaves for the steam distillation, solvent and Soxhlet extraction methods.

Table E41 Terpenoid profile of the essential oil by means of the solvent extraction method for the 48 hour extraction.

	1	2	3	average
Compound/%				
α-pinene	4.68	3.83	3.97	4.16
β-pinene	0.41	0.34	0.35	0.37
α-phellandrene	0.18	0.14	0.15	0.16
d-limonene	0.41	0.34	0.34	0.36
β-phellandrene	3.45	2.76	2.84	3.02
Z-(β)-ocimene	2.23	1.78	1.84	1.95
Total monoterpenes/%	**11.4**	**9.18**	**9.49**	**10.0**
copaene	4.67	3.69	3.44	3.93
β-caryophyllene	20.7	17.2	17.4	18.4
(E)-α-bergamotene	2.65	2.28	2.29	2.41
β-farnesene	1.32	1.08	1.32	1.24
α-caryophyllene	6.15	5.24	5.24	5.54
γ-selinene	0.68	0.74	0.78	0.73
α-guaiene	2.24	2.28	2.65	2.39
(+)-epi-bicyclosesquiphellandrene	1.52	1.86	2.10	1.83
sesquiterpene hydrocarbon	0.79	1.30	1.25	1.11
β-selinene	8.29	7.66	7.54	7.83
valencene	15.9	13.4	13.4	14.3
cadinene	1.16	1.37	1.40	1.31
sesquiterpene hydrocarbon	1.26	1.41	1.52	1.40
sesquiterpene hydrocarbon	–	1.12	–	–
sesquiterpene hydrocarbon	1.07	1.31	1.33	1.23
sesquiterpene hydrocarbon	0.88	1.24	1.21	1.11
α-farnesene	0.83	0.53	1.06	0.81
sesquiterpene hydrocarbon	1.00	1.09	1.08	1.06
sesquiterpene hydrocarbon	0.80	1.08	0.00	0.62
sesquiterpene hydrocarbon	–	–	1.58	–
sesquiterpene hydrocarbon	2.51	2.70	2.60	2.60
Total sesquiterpenes/%	**74.4**	**68.6**	**69.2**	**70.7**
Other/%	**14.4**	**22.4**	**21.4**	**19.4**

Table E42 Terpenoid profile of the essential oil using Soxhlet extraction after the 48 hour extraction period.

	1	2	3	average
Compound/%				
α-pinene	3.38	3.22	3.62	3.41
β-pinene	0.31	0.32	0.33	0.32
α-phellandrene	0.12	0.12	0.12	0.12
d-limonene	0.36	0.06	0.37	0.26
β-phellandrene	3.08	3.23	3.32	3.21
Z-(β)-ocimene	1.78	1.68	1.91	1.79
Total monoterpenes/%	**9.03**	**8.62**	**9.68**	**9.11**
copaene	4.85	4.57	5.03	4.81
β-caryophyllene	18.7	17.8	19.3	18.6
(E)-α-bergamotene	2.33	2.30	2.43	2.35
β-farnesene	1.27	1.48	1.21	1.32
α-caryophyllene	5.83	5.36	5.91	5.70
γ-selinene	1.67	0.88	0.98	1.18
α-guaiene	2.16	3.48	2.19	2.61
(+)-epi-bicyclosesquiphellandrene	1.48	1.52	1.43	1.47
sesquiterpene hydrocarbon	1.02	1.01	0.96	1.00
β-selinene	7.10	6.71	7.34	7.05
valencene	13.4	12.7	13.8	13.3
cadinene	1.21	1.41	1.08	1.23
sesquiterpene hydrocarbon	0.97	1.05	0.87	0.96
sesquiterpene hydrocarbon	0.97	1.34	0.84	1.05
sesquiterpene hydrocarbon	0.86	1.01	0.68	0.85
caryophyllene oxide	1.02	1.10	0.91	1.01
α-farnesene	1.35	0.76	1.21	1.11
sesquiterpene hydrocarbon	1.32	1.27	1.22	1.27
sesquiterpene hydrocarbon	2.46	2.37	2.42	2.41
Total sesquiterpenes/%	**70.0**	**68.2**	**69.8**	**69.3**
Other/%	**21.0**	**23.2**	**20.2**	**21.5**

Table E43. Terpenoid profile of the essential oil by means of the steam distillation method.

	1	2	3	average
Compound/%				
α-pinene	0.42	0.43	0.41	0.42
β-pinene	0.09	0.10	0.09	0.09
α-phellandrene	0.08	0.09	0.09	0.09
d-limonene	0.20	0.21	0.20	0.21
β-phellandrene	1.30	1.35	1.30	1.32
Z-(β)-ocimene	0.99	1.03	0.98	1.00
Total monoterpenes/%	**3.08**	**3.21**	**3.07**	**3.12**
copaene	2.20	2.18	2.19	2.19
β-caryophyllene	20.9	21.2	20.6	20.9
(E)-α-bergamotene	2.60	2.58	2.59	2.59
β-farnesene	1.88	2.30	2.17	2.12
α-caryophyllene	6.56	6.84	6.47	6.62
γ-selinene	1.21	1.27	1.31	1.26
α-guaiene	2.21	2.19	2.21	2.20
(+)-epi-bicyclosesquiphellandrene	0.98	0.98	0.99	0.98
sesquiterpene hydrocarbon	1.07	1.08	1.10	1.08
β-selinene	17.5	17.3	17.3	17.4
valencene	13.5	13.6	13.4	13.5
cadinene	2.28	2.22	2.26	2.25
sesquiterpene hydrocarbon	2.30	2.22	2.26	2.26
sesquiterpene hydrocarbon	1.20	1.16	1.19	1.18
sesquiterpene hydrocarbon	6.76	6.46	6.61	6.61
caryophyllene oxide	–	–	–	–
α-farnesene	–	–	–	–
sesquiterpene hydrocarbon	–	–	–	–
Total sesquiterpenes/%	**83.1**	**83.5**	**82.6**	**83.1**
Other/%	**13.8**	**13.3**	**14.4**	**13.8**

Tables E44 to E47 show the terpenoid profile of the essential oil in *M. koenigii* leaves for the headspace extraction and HS-SPME at the different temperatures.

Table E44 Terpenoid profile of the essential oil with the HS-SPME (PDMS fibre coating) method at room temperature.

Room Temperature				**average**
Compound/%				
α-pinene	7.17	7.82	6.94	7.31
β-pinene	0.53	0.57	0.54	0.55
α-phellandrene	0.35	0.32	0.32	0.33
d-limonene	0.44	0.57	0.52	0.51
β-phellandrene	2.94	3.83	3.54	3.44
Z-(β)-ocimene	2.65	3.47	3.72	3.28
Total monoterpenes/%	**14.1**	**16.6**	**15.6**	**15.4**
β-elemene	12.1	12.5	13.1	12.6
β-caryophyllene	37.3	35.6	35.5	36.1
α-gurjunene	3.26	3.39	3.38	3.35
β-farnesene	1.04	1.01	0.94	1.00
α-caryophyllene	7.00	6.79	6.34	6.71
β-selinene	4.87	4.57	4.76	4.73
γ-elemene	13.3	12.7	13.1	13.0
Total sesquiterpenes/%	**78.7**	**76.5**	**77.1**	**77.4**
Other/%	**7.15**	**6.92**	**7.23**	**7.10**

Table E45 Terpenoid profile of the oil with the HS-SPME (PDMS fibre coating) at 40 °C.

Temperature/40 °C				**average**
Compound/%				
α-pinene	12.1	13.2	12.3	12.5
β-pinene	0.83	1.00	0.87	0.90
α-phellandrene	0.62	0.71	0.58	0.63
d-limonene	0.89	0.97	1.18	1.01
β-phellandrene	5.30	6.28	7.39	6.32
Z-(β)-ocimene	4.63	5.45	8.00	6.03
Total monoterpenes/%	**24.4**	**27.6**	**30.3**	**27.4**
β-elemene	10.4	9.53	9.72	9.89
β-caryophyllene	30.8	32.9	30.3	31.4
α-gurjunene	3.74	3.35	3.54	3.54
β-farnesene	1.06	0.84	0.84	0.91
α-caryophyllene	5.12	5.50	4.57	5.06
β-selinene	4.63	3.75	3.86	4.08
γ-elemene	12.1	9.99	10.1	10.7
Total sesquiterpenes/%	**67.9**	**65.9**	**62.9**	**65.6**
Other/%	**6.89**	**6.24**	**6.80**	**6.64**

Table E46 Terpenoid profile of the essential oil employing the HS-SPME (PDMS fibre coating) method at 60 °C.

Temperature/60 °C				**average**
Compound/%				
α-pinene	18.0	16.1	16.8	16.9
β-pinene	2.22	2.01	1.93	2.05
α-phellandrene	1.26	1.30	1.31	1.29
d-limonene	1.92	1.98	1.91	1.94
β-phellandrene	10.4	11.2	11.0	10.9
Z-(β)-ocimene	9.69	10.6	12.6	11.0
Total monoterpenes/%	**43.4**	**43.2**	**45.5**	**44.1**
β-elemene	5.87	6.04	5.59	5.83
β-caryophyllene	25.2	25.2	26.7	25.7
α-gurjunene	3.17	3.43	3.00	3.20
β-farnesene	0.81	0.82	0.66	0.77
α-caryophyllene	5.02	4.91	4.19	4.70
β-selinene	3.06	2.89	2.52	2.83
γ-elemene	7.15	6.94	6.16	6.75
Total sesquiterpenes/%	**50.3**	**50.2**	**48.9**	**49.8**
Other/%	**6.29**	**6.57**	**5.65**	**6.17**

Table E47 Terpenoid profile of the oil with the HS-SPME (PDMS fibre coating) at 80 °C.

Temperature/80 °C				**average**
Compound/%				
α-pinene	8.67	8.97	10.1	9.25
β-pinene	2.09	2.13	2.31	2.18
α-phellandrene	1.36	1.58	1.75	1.56
d-limonene	1.94	2.11	0.15	1.40
β-phellandrene	9.13	10.6	14.5	11.4
Z-(β)-ocimene	8.52	9.86	13.6	10.7
Total monoterpenes/%	**31.7**	**35.2**	**42.4**	**36.5**
β-elemene	7.51	7.10	6.65	7.09
β-caryophyllene	25.2	24.5	23.7	24.5
α-gurjunene	2.39	2.16	1.63	2.06
α-caryophyllene	7.04	6.58	5.55	6.39
β-selinene	1.38	1.22	0.85	1.15
cadinene	5.84	5.14	3.97	4.98
γ-elemene	9.84	9.17	7.86	8.96
Total sesquiterpenes/%	**59.2**	**55.9**	**50.3**	**55.1**
Other/%	**8.19**	**8.86**	**7.03**	**8.03**

Tables E48 to E51 show the terpenoid profile of the essential oil in *M. koenigii* leaves for the HS-SPME (PA fibre coating) analysis at the different temperatures.

Table E48 Terpenoid profile of the essential oil using the HS-SPME (PA fibre coating) method at room temperature.

Room Temperature				**average**
Compound/%				
α-pinene	1.83	1.10	1.47	1.47
β-pinene	0.13	0.08	0.11	0.11
α-phellandrene	0.23	0.15	0.19	0.19
d-limonene	0.33	0.19	0.00	0.17
β-phellandrene	2.48	1.47	1.79	1.92
Z-(β)-ocimene	3.10	1.35	2.35	2.27
Total monoterpenes/%	**8.10**	**4.34**	**5.92**	**6.12**
isocaryophyllene	**-**	**1.16**	**1.22**	**1.19**
β-elemene	15.3	18.8	22.3	18.8
β-caryophyllene	33.0	25.2	25.9	28.0
α-gurjunene	-	3.48	2.87	3.18
γ-selinene	1.18	1.27	0.98	1.14
α-caryophyllene	7.17	5.92	6.49	6.53
valencene	1.19	1.14	1.05	1.12
β-selinene	6.76	7.69	5.84	6.76
γ-elemene	18.7	22.5	18.2	19.8
Total sesquiterpenes/%	**83.3**	**87.2**	**84.9**	**85.1**
Other/%	**8.62**	**8.47**	**8.91**	**8.67**

Table E49 Terpenoid profile of the essential oil employing the HS-SPME (PA fibre coating) method at 40 °C.

Temperature/40 °C				**average**
Compound/%				
α-pinene	4.43	3.10	4.27	3.93
β-pinene	0.54	0.31	0.48	0.44
α-phellandrene	0.49	0.29	0.44	0.41
d-limonene	0.53	0.49	0.55	0.53
β-phellandrene	4.38	4.05	4.34	4.26
Z-(β)-ocimene	4.94	5.38	7.21	5.84
Total monoterpenes/%	**15.3**	**13.6**	**17.3**	**15.4**
β-elemene	12.0	13.2	14.7	13.3
β-caryophyllene	35.5	30.9	32.1	32.8
α-gurjunene	3.40	3.40	2.87	3.22
α-caryophyllene	6.19	5.36	5.85	5.80
β-selinene	5.07	5.99	4.38	5.15
γ-elemene	14.0	16.6	12.6	14.4
Total sesquiterpenes/%	**76.1**	**75.5**	**72.4**	**74.6**
Other/%	**8.63**	**10.9**	**9.52**	**9.69**

Table E50 Terpenoid profile of the essential oil with the HS-SPME (PA fibre coating) method at 60 °C.

Temperature/60 °C				**average**
Compound/%				
α-pinene	5.45	5.62	5.48	5.51
β-pinene	0.75	0.87	0.84	0.82
α-phellandrene	0.53	0.58	0.59	0.57
d-limonene	1.10	0.92	0.85	0.96
β-phellandrene	7.55	6.54	5.97	6.69
Z-(β)-ocimene	9.10	8.93	9.88	9.30
Total monoterpenes/%	**24.5**	**23.5**	**23.6**	**23.8**
β-elemene	7.78	8.09	10.6	8.82
β-caryophyllene	34.5	34.2	33.5	34.1
α-gurjunene	3.57	3.31	2.97	3.28
α-caryophyllene	5.58	5.35	5.54	5.49
β-selinene	3.79	4.24	3.78	3.94
γ-elemene	9.50	10.7	9.78	10.0
Total sesquiterpenes/%	**64.8**	**65.9**	**66.2**	**65.6**
Other/%	**10.5**	**10.6**	**10.3**	**10.4**

Table E51 Terpenoid profile of the essential oil by means of the HS-SPME (PA fibre coating) method at 80 °C.

Temperature/80 °C				**average**
Compound/%				
α-pinene	9.20	8.36	8.08	8.54
β-pinene	1.26	1.29	1.20	1.25
α-phellandrene	0.82	1.03	0.86	0.90
d-limonene	1.31	1.50	1.39	1.40
β-phellandrene	8.55	9.89	8.65	9.03
Z-(β)-ocimene	9.39	13.0	13.0	11.8
Total monoterpenes/%	**30.5**	**35.1**	**33.2**	**32.9**
β-elemene	6.93	5.36	8.04	6.78
β-caryophyllene	29.0	29.6	28.2	28.9
α-gurjunene	3.02	2.78	2.77	2.86
α-caryophyllene	5.27	4.65	4.57	4.83
β-selinene	4.06	3.25	3.78	3.70
γ-elemene	9.57	7.61	8.75	8.64
Total sesquiterpenes/%	**57.8**	**53.3**	**56.1**	**55.7**
Other/%	**11.7**	**11.7**	**10.5**	**11.3**

Tables E52 to E55 show the terpenoid profile of the essential oil in *M. koenigii* leaves for the headspace analysis at the different temperatures.

Table E52 Terpenoid profile of the essential oil with headspace method at room temperature.

Room Temperature				**average**
Compound/%				
α-pinene	33.7	41.1	32.8	35.9
β-pinene	1.34	1.30	0.87	1.17
α-phellandrene	1.06	0.82	0.65	0.84
d-limonene	1.16	1.40	0.58	1.05
β-phellandrene	7.64	9.50	5.86	7.67
Z-(β)-ocimene	8.02	6.87	2.33	5.74
Total monoterpenes/%	**52.9**	**61.0**	**43.10**	**52.3**
δ-elemene	-	-	9.79	9.79
copaene	-	-	1.39	1.39
β-elemene	2.23	1.74	2.03	2.00
β-caryophyllene	22.1	22.6	23.7	22.8
(E)-α-bergamotene	-	-	2.25	2.25
α-gurjunene	2.74	1.75	2.88	2.46
β-farnesene	1.35	-	-	1.35
α-caryophyllene	3.73	3.04	3.06	3.28
isocaryophyllene	1.53	-	-	1.53
β-selinene	2.62	2.16	2.55	2.45
γ-elemene	5.95	5.63	6.83	6.14
Total sesquiterpenes/%	**42.3**	**36.9**	**54.5**	**44.6**
Other/%	**4.80**	**2.09**	**2.29**	**3.06**

Table E53 Terpenoid profile of the essential oil using the headspace method at 40 °C.

Temperature/40 °C				**average**
Compound/%				
α-pinene	48.2	37.1	56.17	47.2
β-pinene	1.50	1.14	1.56	1.40
α-phellandrene	1.50	0.79	1.01	1.10
d-limonene	1.42	1.28	1.44	1.38
β-phellandrene	10.1	9.01	10.8	9.98
Z-(β)-ocimene	9.86	8.48	6.60	8.31
Total monoterpenes/%	**72.6**	**57.8**	**77.6**	**69.3**
β-elemene	1.50	1.53	0.83	1.28
β-caryophyllene	14.9	25.1	12.5	17.5
α-gurjunene	1.24	1.81	1.08	1.38
α-caryophyllene	1.54	2.45	1.37	1.79
β-selinene	1.21	2.08	1.27	1.52
γ-elemene	3.10	5.46	3.39	3.98
Total sesquiterpenes/%	**23.5**	**38.4**	**20.5**	**27.5**
Other/%	**3.30**	**3.76**	**1.65**	**2.90**

Table E54 Terpenoid profile of the essential oil with headspace methods at 60 °C.

Temperature/60 °C				**average**
Compound/%				
α-pinene	43.8	49.8	39.2	44.3
β-pinene	2.51	1.88	2.21	2.20
α-phellandrene	1.55	0.98	1.34	1.29
d-limonene	1.97	1.54	1.82	1.78
β-phellandrene	13.0	10.2	11.2	11.5
Z-(β)-ocimene	13.42	9.42	9.96	10.9
Total monoterpenes/%	**76.3**	**73.8**	**65.7**	**71.9**
β-elemene	-	1.07	1.01	1.04
β-caryophyllene	13.7	12.6	20.6	15.6
α-gurjunene	0.99	1.13	1.49	1.20
α-caryophyllene	1.39	1.54	2.29	1.74
β-selinene	-	1.69	1.78	1.73
γ-elemene	2.00	3.81	4.19	3.34
Total sesquiterpenes/%	**18.1**	**21.9**	**31.4**	**23.8**
Other/%	**5.58**	**4.12**	**2.94**	**4.21**

Table E55 Terpenoid profile of the essential oil using the headspace method at 80 °C.

Temperature/80 °C				**average**
Compound/%				
α-pinene	42.6	44.5	32.0	39.7
β-pinene	2.63	2.15	2.68	2.48
α-phellandrene	1.76	1.35	1.52	1.54
d-limonene	2.11	1.59	2.55	2.09
β-phellandrene	13.8	10.9	14.0	12.9
Z-(β)-ocimene	15.3	9.52	13.4	12.7
Total monoterpenes/%	**78.3**	**70.0**	**66.1**	**71.4**
β-elemene		**1.72**	**1.40**	**1.56**
β-caryophyllene	12.0	12.9	17.1	14.0
α-gurjunene	0.73	1.22	1.39	1.11
α-caryophyllene	1.23	1.86	2.31	1.80
β-selinene	0.72	2.19	2.33	1.74
γ-elemene	1.72	5.16	5.12	4.00
Total sesquiterpenes/%	**16.4**	**25.0**	**29.6**	**23.7**
Other/%	**4.45**	**4.89**	**4.09**	**4.48**

The following results are for quantitation.

Solvent extraction with internal standard

Table E56 α-pinene.

Sample	**at start of extraction**	**at end of extraction**
Peak Area	4.31×10^7	4.26×10^7
	4.95×10^7	4.34×10^7
	4.40×10^7	4.47×10^7
Peak Area Ratio	10.6	9.41
	10.2	9.42
	9.70	9.08
Mean	10.1	9.30
Std Dev	0.42	0.19
RSD/%	4.2	2.1

Table E57 β-pinene.

Sample	at start of extraction	at end of extraction
Peak Area	4.88×10^6	5.26×10^6
	5.93×10^6	5.63×10^6
	5.41×10^6	5.71×10^6
Peak Area Ratio	1.19	1.16
	1.22	1.22
	1.19	1.16
Mean	1.20	1.18
Std Dev	0.01	0.04
RSD/%	1.2	3.1

Table E58 α-phellandrene.

Sample	at start of extraction	at end of extraction
Peak Area	4.39×10^6	4.47×10^6
	5.17×10^6	4.79×10^6
	4.58×10^6	4.93×10^6
Peak Area Ratio	1.07	0.99
	1.06	1.04
	1.01	1.00
Mean	1.05	1.01
Std Dev	0.03	0.03
RSD/%	3.2	2.8

Table E59 β-caryophyllene.

Sample	at start of extraction	at end of extraction
Peak Area	5.16×10^8	5.49×10^8
	5.75×10^8	5.55×10^8
	5.49×10^8	5.86×10^8
Peak Area Ratio	126	121
	118	121
	121	119
Mean	122	120
Std Dev	4.01	1.19
RSD/%	3.3	1.00

Table E60 α-caryophyllene.

Sample	at start of extraction	at end of extraction
Peak Area	1.20×10^8	1.43×10^8
	1.48×10^8	1.40×10^8
	1.39×10^8	1.47×10^8
Peak Area Ratio	29.3	31.6
	30.4	30.4
	30.7	29.8
Mean	30.1	30.6
Std Dev	0.72	0.92
RSD/%	2.4	3.0

Soxhlet extraction with internal standard

Table E61 α-pinene.

Sample	at start of extraction	at end of extraction
Peak Area	6.05×10^7	4.30×10^7
	5.03×10^7	4.86×10^7
	5.13×10^7	4.32×10^7
Peak Area Ratio	12.3	10.4
	11.8	9.66
	11.5	10.1
Mean	11.9	10.0
Std Dev	0.39	0.36
RSD/%	3.3	3.6

Table E62 β-pinene.

Sample	at start of extraction	at end of extraction
Peak Area	7.53×10^6	7.29×10^6
	6.67×10^6	8.27×10^6
	6.87×10^6	7.33×10^6
Peak Area Ratio	1.53	1.76
	1.56	1.64
	1.55	1.71
Mean	1.55	1.70
Std Dev	0.01	0.06
RSD/%	0.9	3.4

Table E63 α-phellandrene.

Sample	at start of extraction	at end of extraction
Peak Area	6.48×10^6	7.86×10^6
	5.73×10^6	9.49×10^6
	5.81×10^6	7.94×10^6
Peak Area Ratio	1.32	1.89
	1.34	1.89
	1.31	1.86
Mean	1.32	1.88
Std Dev	0.02	0.02
RSD/%	1.2	1.1

Table E64 β-caryophyllene.

Sample	at start of extraction	at end of extraction
Peak Area	6.75×10^8	5.74×10^8
	5.85×10^8	6.62×10^8
	6.17×10^8	5.80×10^8
Peak Area Ratio	137	138
	137	132
	139	136
Mean	138	135
Std Dev	1.01	3.42
RSD/%	0.7	2.5

Table E65 α-caryophyllene.

Sample	at start of extraction	at end of extraction
Peak Area	2.26×10^8	2.02×10^8
	2.09×10^8	2.26×10^8
	1.98×10^8	1.99×10^8
Peak Area Ratio	45.9	48.7
	48.9	44.9
	44.5	46.4
Mean	46.5	46.7
Std Dev	2.23	1.90
RSD/%	4.8	4.1

Steam distillation with the addition of the internal standard

Table E66 α-pinene.

Sample	at start of extraction	at end of extraction
Peak Area	6.78×10^5	2.21×10^6
	7.38×10^5	2.17×10^6
	7.18×10^5	2.03×10^6
Peak Area Ratio	0.13	0.39
	0.13	0.39
	0.13	0.39
Mean	0.13	0.39
Std Dev	0.003	0.003
RSD/%	2.1	0.9

Table E67 β-pinene.

Sample	at start of extraction	at end of extraction
Peak Area	5.33×10^5	1.09×10^6
	5.81×10^5	1.13×10^6
	5.51×10^5	9.83×10^5
Peak Area Ratio	0.10	0.19
	0.10	0.20
	0.10	0.19
Mean	0.10	0.19
Std Dev	0.002	0.01
RSD/%	2.2	3.6

Table E68 α-phellandrene.

Sample	at start of extraction	at end of extraction
Peak Area	8.01×10^5	1.42×10^6
	8.56×10^5	1.37×10^6
	8.28×10^5	1.27×10^6
Peak Area Ratio	0.15	0.25
	0.15	0.25
	0.15	0.25
Mean	0.15	0.25
Std Dev	0.002	0.002
RSD/%	1.2	0.7

Table E69 β-caryophyllene.

Sample	at start of extraction	at end of extraction
Peak Area	3.27×10^8	4.61×10^8
	3.55×10^8	4.45×10^8
	3.47×10^8	4.21×10^8
Peak Area Ratio	61.9	80.6
	64.2	80.0
	64.0	81.6
Mean	63.4	80.7
Std Dev	1.30	0.79
RSD/%	2.1	1.0

Table E70 α-caryophyllene.

Sample	at start of extraction	at end of extraction
Peak Area	7.69×10^7	1.09×10^8
	8.59×10^7	1.06×10^8
	8.51×10^7	9.80×10^7
Peak Area Ratio	14.5	19.1
	15.5	19.1
	15.7	19.0
Mean	15.3	19.1
Std Dev	0.62	0.06
RSD/%	4.1	0.3

The concentrations of the five volatile compounds determined in the essential oil of *M. koenigii* by steam distillation, Soxhlet extraction and solvent extraction with the internal standard added at the start and end of the extraction are shown in Tables E71 to E85.

Solvent Extraction

Table E71 α-pinene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	48.6	43.3
	46.9	43.4
	44.7	41.8
Mean	46.7	42.9
Std Dev	1.95	0.90
RSD/%	4.2	2.1

Table E72 β-pinene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	7.34	7.14
	7.50	7.53
	7.34	7.14
Mean	7.39	7.27
Std Dev	0.09	0.23
RSD/%	1.2	3.1

Table E73 α-phellandrene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	10.9	10.1
	10.8	10.6
	10.3	10.2
Mean	10.7	10.3
Std Dev	0.34	0.29
RSD/%	3.2	2.8

Table E74 β-caryophyllene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	4771	4588
	4469	4564
	4586	4501
Mean	4607	4551
Std Dev	152	45.1
RSD/%	3.3	1.0

Table E75 α-caryophyllene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	87.5	94.3
	90.8	90.9
	91.6	88.9
Mean	90.0	91.3
Std Dev	2.15	2.75
RSD/%	2.4	3.0

Soxhlet Extraction

Table E76 α-pinene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	56.6	47.8
	54.1	44.5
	53.1	46.5
Mean	54.6	46.3
Std Dev	1.81	1.7
RSD/%	3.3	3.6

Table E77 β-pinene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	9.42	10.8
	9.59	10.1
	9.51	10.5
Mean	9.51	10.5
Std Dev	0.09	0.36
RSD/%	0.9	3.4

Table E78 α-phellandrene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	8.83	13.7
	8.98	13.7
	8.77	13.4
Mean	8.86	13.6
Std Dev	0.11	0.15
RSD/%	1.2	1.1

Table E79 β-caryophyllene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	5192	5238
	5173	4980
	5247	5128
Mean	5204	5115
Std Dev	38.2	130
RSD/%	0.7	2.5

Table E80 α-caryophyllene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	268	284
	285	262
	259	271
Mean	271	272
Std Dev	13.0	11.1
RSD/%	4.8	4.1

Steam Distillation

Table E81 α-pinene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	1.04	2.30
	1.00	2.32
	1.02	2.34
Mean	1.02	2.32
Std Dev	0.02	0.02
RSD/%	2.0	0.9

Table E82 β-pinene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	0.74	1.28
	0.71	1.36
	0.73	1.27
Mean	0.73	1.30
Std Dev	0.02	0.05
RSD/%	2.1	3.8

Table E83 α-phellandrene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	1.36	1.66
	1.30	1.64
	1.32	1.65
Mean	1.33	1.65
Std Dev	0.03	0.01
RSD/%	2.3	0.6

Table E84 β-caryophyllene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	1.88×10^3	1.80×10^3
	1.80×10^3	1.79×10^3
	1.83×10^3	1.82×10^3
Mean	1.84×10^3	1.80×10^3
Std Dev	41.0	17.7
RSD/%	2.2	1.0

Table E85 α-caryophyllene.

Sample	at start of extraction	at end of extraction
Concentration/mg kg^{-1}	73.6	69.1
	70.4	68.9
	71.8	68.6
Mean	71.9	68.9
Std Dev	1.60	0.22
RSD/%	2.2	0.3

APPENDIX F

AREA PERCENT REPORTS

In this section, the area percent reports for each of the extraction methods are presented.

Table F1 Area percent report for SPME extraction with frozen leaves.

Area Percent Report

Data File : C:\MSDCHEM\1\DATA\SPMERT2.D Vial: 27
Acq On : 5 Feb 2007 14:10 Operator: Patricia
Sample : spme frozen sample2 40 C -15 min Inst : Instrumen
Misc : spme 40 C 15 minutes Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.361	35	48	78	VV 3	56268	1351758	1.73%	0.538%
2	2.643	78	96	144	PV 2	146113	4934372	6.31%	1.963%
3	3.366	176	219	251	BV	1457314	34676871	44.34%	13.792%
4	3.589	251	257	283	VV	8814	356357	0.46%	0.142%
5	3.812	283	295	301	VV 5	8274	215552	0.28%	0.086%
6	3.924	301	314	341	VV	148981	3999720	5.11%	1.591%
7	4.312	364	380	401	VV	106743	3212854	4.11%	1.278%
8	4.629	419	434	439	PV 2	30976	891924	1.14%	0.355%
9	4.794	439	462	473	VV	1083073	35788616	45.76%	14.234%
10	4.923	473	484	539	VV	489300	15005310	19.19%	5.968%
11	6.286	693	716	740	BV 9	9668	435352	0.56%	0.173%
12	30.652	4809	4863	4941	BB 9	19817	2981206	3.81%	1.186%
13	35.834	5629	5745	5864	BB 9	443083	78211045	100.00%	31.107%
14	39.759	6326	6413	6528	BB 9	49810	8936740	11.43%	3.554%
15	42.239	6794	6835	6876	BV 5	107947	8485807	10.85%	3.375%
16	42.967	6876	6959	6995	PV 5	15627	1126077	1.44%	0.448%
17	43.449	6995	7041	7060	PV 5	10662	561096	0.72%	0.223%
18	43.755	7060	7093	7107	VV 9	18104	922170	1.18%	0.367%
19	43.925	7107	7122	7141	VV 9	21687	975798	1.25%	0.388%
20	44.507	7188	7221	7262	BV 6	330747	15143045	19.36%	6.023%
21	44.871	7262	7283	7303	VV 3	764957	31094331	39.76%	12.367%
22	45.053	7303	7314	7332	PV 3	12749	564663	0.72%	0.225%
23	45.652	7387	7416	7428	BV 3	14651	685961	0.88%	0.273%
24	45.770	7428	7436	7446	VV 3	9893	366518	0.47%	0.146%
25	45.882	7446	7455	7488	VB 7	11560	504676	0.65%	0.201%

Sum of corrected areas: 251427818

Table F2 Area percent report for SPME extraction with fresh leaves.

Area Percent Report

Data File : C:\MSDCHEM\1\DATA\SPMERTB1.D Vial: 27
Acq On : 6 Feb 2007 13:07 Operator: Patricia
Sample : spme fresh sample 40C- 15 mins. Inst : Instrumen
Misc : spme 40C -15 mins Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.367	42	49	73	BB 4	7191	202040	0.13%	0.051%
2	2.690	77	104	169	BB 4	128129	6540792	4.16%	1.664%
3	3.372	183	220	252	BV	1052512	21842373	13.91%	5.558%
4	3.818	276	296	306	BV 2	14974	333746	0.21%	0.085%
5	3.930	306	315	343	VV 2	114185	2851005	1.82%	0.725%
6	4.318	362	381	405	VV 2	87966	2328980	1.48%	0.593%
7	4.635	425	435	439	BV 3	15659	382800	0.24%	0.097%
8	4.794	439	462	476	VV	899671	26927340	17.14%	6.852%
9	4.929	476	485	541	VV	1045548	27993783	17.82%	7.123%
10	6.298	689	718	738	BB	7820	334841	0.21%	0.085%
11	21.968	3336	3385	3434	BV	11872	1432174	0.91%	0.364%
12	29.365	4579	4644	4714	BB	15605	2292920	1.46%	0.583%
13	30.711	4767	4873	4976	BB	260786	38598915	24.58%	9.822%
14	35.940	5584	5763	5880	BB	836365	157062959	100.00%	39.966%
15	39.783	6317	6417	6528	BB	51990	9589369	6.11%	2.440%
16	42.245	6798	6836	6880	BV 7	259949	21138252	13.46%	5.379%
17	42.967	6922	6959	7010	BB 10	30385	2056199	1.31%	0.523%
18	43.455	7012	7042	7060	BV 9	10419	636997	0.41%	0.162%
19	43.772	7078	7096	7111	VV 9	23130	1412354	0.90%	0.359%
20	43.925	7111	7122	7138	VV 9	26086	1411699	0.90%	0.359%
21	44.125	7138	7156	7170	VV 9	10471	637812	0.41%	0.162%
22	44.507	7197	7221	7251	BV 5	343014	16404874	10.44%	4.174%
23	44.877	7264	7284	7303	VV 3	1023573	43524235	27.71%	11.075%
24	45.053	7303	7314	7332	VV 3	27100	1648513	1.05%	0.419%
25	45.194	7332	7338	7347	VV 3	13748	539353	0.34%	0.137%
26	45.329	7347	7361	7387	VB 3	22598	1011866	0.64%	0.257%
27	45.652	7392	7416	7427	BV 10	29719	1388989	0.88%	0.353%
28	45.770	7427	7436	7446	VV 10	13873	527495	0.34%	0.134%
29	45.882	7446	7455	7482	VB 5	27657	977466	0.62%	0.249%
30	46.234	7491	7515	7525	BV 6	10195	223486	0.14%	0.057%
31	47.027	7637	7650	7668	BV 6	6404	211367	0.13%	0.054%
32	48.520	7843	7904	7913	BV 6	11203	241714	0.15%	0.062%
33	49.336	8006	8043	8055	BV 6	10228	284825	0.18%	0.072%

Sum of corrected areas: 392991532

Table F3 Area percent report for HS-SPME (PDMS fibre coating) for α-pinene.

ALPPINPD
Area Percent Report

Data File : C:\GOVENDER\PATRCIA1\ALPPINPD.D Vial: 1
Acq On : 6 Jun 2007 13:11 Operator: Patricia
Sample : alpha pinene PDMS room temp. Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.401	210	225	248	VV	11761961	419768441	100.00%	94.065%
2	3.607	248	260	288	VB 4	307977	10382020	2.47%	2.326%
3	3.948	300	318	330	BB	127186	2685381	0.64%	0.602%
4	4.629	422	434	443	BV 2	106334	2475481	0.59%	0.555%
5	7.350	879	897	922	BB 2	163873	6650451	1.58%	1.490%
6	10.887	1486	1499	1520	BB 2	110560	4291902	1.02%	0.962%

Sum of corrected areas: 446253675

Table F4 Area percent report for standard mixture 1.

STD152
Area Percent Report

Data File : D:\PATRICIA1\STD152.D Vial: 50
Acq On : 26 May 2009 12:31 Operator: D:\PATRICIA1\
Sample : STD MIX. 1UL/5ML - 1UL DODECANE -STOCK Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.160	166	184	200	BB 4	12450	297761	13.71%	8.330%
2	3.713	262	278	295	BB 2	10089	278389	12.81%	7.788%
3	4.071	320	339	356	BB 4	8591	255261	11.75%	7.141%
4	9.953	1312	1340	1383	BB 5	38661	2172620	100.00%	60.783%
5	14.424	2093	2101	2113	BV 6	14003	253516	11.67%	7.093%
6	14.800	2160	2165	2181	BB 2	17975	316823	14.58%	8.864%

Sum of corrected areas: 3574370

Table F5 Area percent report for standard mixture 2.

STD455
Area Percent Report

Data File : D:\PATRICIA1\STD455.D Vial: 50
Acq On : 26 May 2009 15:44 Operator: D:\PATRICIA1\
Sample : STD MIX. 4UL/5ML - 1UL DODECANE -STOCK Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.160	166	184	201	BB	46680	1072604	44.17%	14.416%
2	3.707	238	277	291	BV 2	38363	921477	37.95%	12.385%
3	4.065	326	338	355	BB	33495	927366	38.19%	12.464%
4	9.953	1318	1340	1384	BB 4	45194	2428297	100.00%	32.636%
5	14.424	2050	2101	2115	BB	55156	884187	36.41%	11.884%
6	14.800	2158	2165	2181	PB	73627	1206516	49.69%	16.216%

Sum of corrected areas: 7440446

Table F6 Area percent report for standard mixture 3.

STD125
Area Percent Report

Data File : D:\PATRICIA1\STD125.D Vial: 50
Acq On : 26 May 2009 11:38 Operator: D:\PATRICIA1\
Sample : STD MIX. 12UL/5ML - 1UL DODECANE -STOCK Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.160	166	184	201	BB	138053	3095093	82.64%	17.187%
2	3.712	244	278	292	BB 2	119439	2917236	77.89%	16.200%
3	4.071	320	339	359	BB 2	101760	2802994	74.84%	15.565%
4	9.958	1313	1341	1372	BB 3	46658	2490642	66.50%	13.831%
5	14.424	2086	2101	2121	BB	182199	2956801	78.95%	16.419%
6	14.800	2158	2165	2183	PB	230994	3745115	100.00%	20.797%

Sum of corrected areas: 18007882

Table F7 Area percent report for standard mixture 4.

STD2458
Area Percent Report

Data File : D:\PATRICIA1\STD2458.D
Acq On : 28 May 2009 12:38
Sample : STD. MIX. 24UL/5ML DCM
Misc :
Vial: 50
Operator: D:\PATRICIA1\
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.166	166	185	209	BB	300906	6707016	77.57%	18.037%
2	3.719	259	279	304	BB	270294	6698554	77.47%	18.014%
3	4.077	316	340	360	M	148313	4038718	46.71%	10.861%
4	4.383	372	392	411	BB	38993	1191195	13.78%	3.203%
5	9.958	1326	1341	1364	M3	47792	2368666	27.40%	6.370%
6	14.424	2083	2101	2126	BB	406037	6677413	77.23%	17.957%
7	14.800	2158	2165	2190	VB	533248	8646226	100.00%	23.252%
8	16.063	2364	2380	2398	BB 3	36515	857810	9.92%	2.307%

Sum of corrected areas: 37185598

Table F8 Area percent report for standard mixture 5.

STD4852
Area Percent Report

Data File : D:\PATRICIA1\STD4852.D
Acq On : 27 May 2009 10:14
Sample : STD. MIX. 48UL/5ML DCM
Misc :
Vial: 50
Operator: D:\PATRICIA1\
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.172	166	186	211	BB	464740	10066159	72.27%	17.510%
2	3.724	258	280	305	BB	412030	10119094	72.65%	17.602%
3	4.083	320	341	369	BB	298311	8223947	59.05%	14.305%
4	4.388	374	393	410	BB	34062	1045363	7.51%	1.818%
5	9.958	1326	1341	1361	M4	39857	1901602	13.65%	3.308%
6	14.424	2088	2101	2131	BB	687678	10941675	78.56%	19.033%
7	14.800	2158	2165	2197	VB	860328	13927968	100.00%	24.228%
8	16.063	2363	2380	2399	BB 2	53947	1262256	9.06%	2.196%

Sum of corrected areas: 57488064

Table F9 Area percent report for standard mixture 6.

STD9655
Area Percent Report

Data File : D:\PATRICIA1\STD9655.D
Acq On : 27 May 2009 15:20
Sample : STD. MIX. 96UL/5ML DCM
Misc :
Vial: 50
Operator: D:\PATRICIA1\
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.160	162	184	214	BB	1068830	25250904	76.24%	17.999%
2	3.713	256	278	315	BB	956311	25761932	77.78%	18.364%
3	4.071	315	339	376	BB	692915	20415763	61.64%	14.553%
4	4.371	376	390	412	BB	91880	2920631	8.82%	2.082%
5	9.953	1316	1340	1368	BB 4	32169	1829514	5.52%	1.304%
6	14.424	2084	2101	2132	BV	1648404	26998040	81.51%	19.245%
7	14.729	2145	2153	2158	PV 2	55649	871536	2.63%	0.621%
8	14.800	2158	2165	2183	VV	2043761	33120941	100.00%	23.609%
9	16.069	2364	2381	2399	BB 2	137083	3118399	9.42%	2.223%

Sum of corrected areas: 140287661

Table F10 Area percent report for standard mixture 7.

STD113
Area Percent Report

Data File : D:\PATRICIA1\STD113.D
Acq On : 29 May 2009 12:07
Sample : STD. MIX 1UL/1ML DCM
Misc :
Vial: 50
Operator: D:\PATRICIA1\
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.172	151	186	214	BV	4708909	110929694	67.75%	16.365%
2	3.724	265	280	326	PV	4716394	128025394	78.19%	18.887%
3	4.089	326	342	380	VV	4366671	127608741	77.94%	18.826%
4	4.388	380	393	414	VV	223858	7137556	4.36%	1.053%
5	9.970	1317	1343	1382	BB 3	46529	2835831	1.73%	0.418%
6	14.430	2078	2102	2133	PV	7393964	120150216	73.38%	17.725%
7	14.806	2158	2166	2184	VV	10016792	163732011	100.00%	24.155%
8	16.069	2370	2381	2404	PV	780863	17419492	10.64%	2.570%

Sum of corrected areas: 677838934

Table F11 Area percent report for standard mixture 8.

STD311
Area Percent Report

Data File : D:\PATRICIA1\STD311.D
Acq On : 29 May 2009 16:32
Sample : STD. MIX 3UL/1ML DCM
Misc :
Vial: 50
Operator: D:\PATRICIA1\
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.172	174	186	215	BV	9046758	194695985	81.16%	18.197%
2	3.730	266	281	326	PV	8795211	219359805	91.44%	20.502%
3	4.089	326	342	380	VV	6354578	169991460	70.86%	15.888%
4	4.388	380	393	415	VV	573514	16560411	6.90%	1.548%
5	9.976	1319	1344	1389	BB 6	39003	2655138	1.11%	0.248%
6	14.430	2092	2102	2134	BV	11611014	190951488	79.60%	17.847%
7	14.812	2159	2167	2184	VV	14813020	239896977	100.00%	22.422%
8	16.069	2370	2381	2403	PV	1611097	35818696	14.93%	3.348%

Sum of corrected areas: 1069929960

Table F12 Area percent report for standard mixture 9.

STD1015
Area Percent Report

Data File : D:\PATRICIA1\STD1015.D
Acq On : 1 Jun 2009 15:11
Sample : STD. MIX 10UL/1ML DCM WITH DODECANE
Misc :
Vial: 50
Operator: Patricia
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.172	174	186	205	BB	23455594	441613397	79.67%	17.702%
2	3.736	250	282	302	BB	22975323	507255618	91.52%	20.334%
3	4.094	326	343	363	BB	19145062	422795253	76.28%	16.948%
4	4.388	383	393	408	BB	1476326	34158887	6.16%	1.369%
5	9.976	1333	1344	1374	M8	64731	4456246	0.80%	0.179%
6	14.435	2089	2103	2121	BB	22097254	355979020	64.22%	14.270%
7	14.823	2160	2169	2184	VV	29020939	554280937	100.00%	22.219%
8	16.075	2371	2382	2403	BB	8405294	174113011	31.41%	6.979%

Sum of corrected areas: 2494652368

Table F13 Area percent report for standard mixture 10.

STD2015
Area Percent Report

Data File : D:\PATRICIA1\STD2015.D
Acq On : 2 Jun 2009 13:06
Sample : STD. MIX. 20UL/1ML DCM
Misc :
Vial: 50
Operator: PATRICIA
Inst : Instrumen
Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	3.178	175	187	214	PV	28476119	626168702	76.95%	16.770%
2	3.742	269	283	325	VV	27067554	680975665	83.68%	18.238%
3	4.100	325	344	366	VV	25264907	621758320	76.41%	16.652%
4	4.394	383	394	417	VV	3302895	79430230	9.76%	2.127%
5	10.064	1330	1359	1391	BV 5	66170	6357823	0.78%	0.170%
6	14.447	2094	2105	2114	VV	27933014	568947716	69.92%	15.238%
7	14.829	2160	2170	2184	VV	31915647	813753925	100.00%	21.794%
8	16.075	2371	2382	2403	VV	15485765	336434044	41.34%	9.010%

Sum of corrected areas: 3733826424

Table F14 Area percent report for the essential oil obtained from the 24 hour solvent extraction.

DCMSE241
Area Percent Report

Data File : D:\PATRICIA\DCMSE241.D Vial: 34
Acq On : 31 Mar 2009 16:25 Operator: Patricia
Sample : DCM SOLVENT EXT. SAMPLE - 24 HOURS Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.144	5	11	13	BV 2	29781	258492	0.05%	0.011%
2	2.191	13	19	30	VV 2	238725	4697890	0.93%	0.207%
3	2.461	48	65	85	BV 2	751025	16582608	3.28%	0.730%
4	3.154	168	183	201	PV	4927662	99423693	19.68%	4.378%
5	3.577	245	255	262	BV 2	46992	1038517	0.21%	0.046%
6	3.677	262	272	293	VB	384981	9114404	1.80%	0.401%
7	4.047	327	335	343	M	192567	4564756	0.90%	0.201%
8	4.441	389	402	405	VV 2	376501	9988405	1.98%	0.440%
9	4.511	405	414	424	VV	2903810	76762177	15.19%	3.380%
10	4.629	424	434	449	VV	1992083	52195077	10.33%	2.298%
11	4.764	449	457	481	VV 5	41785	2013688	0.40%	0.089%
12	5.927	646	655	667	VV 3	42891	1325767	0.26%	0.058%
13	9.876	1279	1327	1358	BB 6	38300	2372565	0.47%	0.104%
14	12.367	1731	1751	1760	PV	60670	1152364	0.23%	0.051%
15	13.231	1888	1898	1902	PV 2	144131	2603439	0.52%	0.115%
16	13.278	1902	1906	1915	VV	301069	4900284	0.97%	0.216%
17	13.448	1929	1935	1948	VV	165953	2889680	0.57%	0.127%
18	13.654	1964	1970	1983	VV	72308	1647960	0.33%	0.073%
19	13.771	1983	1990	1997	VV 2	66646	1295618	0.26%	0.057%
20	13.854	1997	2004	2010	VV	280689	5023637	0.99%	0.221%
21	13.918	2010	2015	2023	VV	502261	8048307	1.59%	0.354%
22	14.012	2023	2031	2039	VV	7951476	115802705	22.92%	5.099%
23	14.083	2039	2043	2057	VV 6	100506	3743907	0.74%	0.165%
24	14.200	2057	2063	2065	VV 4	50610	1177407	0.23%	0.052%
25	14.235	2065	2069	2071	VV 4	92167	1680582	0.33%	0.074%
26	14.271	2071	2075	2087	VV	200791	5355127	1.06%	0.236%
27	14.412	2087	2099	2105	VV	22260482	505183372	100.00%	22.245%
28	14.482	2105	2111	2121	VV 2	4090010	61902032	12.25%	2.726%
29	14.559	2121	2124	2126	VV 2	175837	2691001	0.53%	0.118%
30	14.594	2126	2130	2134	VV	681286	11486806	2.27%	0.506%
31	14.641	2134	2138	2149	VV	1517760	31139126	6.16%	1.371%
32	14.770	2149	2160	2166	VV	9354928	144545653	28.61%	6.365%
33	14.823	2166	2169	2174	VV	698196	18193499	3.60%	0.801%
34	14.911	2174	2184	2189	VV 3	1728572	49784068	9.85%	2.192%
35	14.958	2189	2192	2197	VV	1582510	33198579	6.57%	1.462%
36	15.005	2197	2200	2204	VV	892131	18400128	3.64%	0.810%
37	15.099	2204	2216	2220	VV	9896201	188199542	37.25%	8.287%
38	15.164	2220	2227	2236	VV 2	19342984	377115769	74.65%	16.606%
39	15.234	2236	2239	2243	VV	695224	12480757	2.47%	0.550%
40	15.317	2243	2253	2259	VV 2	852336	28233796	5.59%	1.243%
41	15.375	2259	2263	2275	VV 3	804228	21371981	4.23%	0.941%
42	15.475	2275	2280	2288	VV 2	477916	13593051	2.69%	0.599%
43	15.540	2288	2291	2301	VV 3	307292	9261534	1.83%	0.408%

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44	15.657	2301	2311	2324	VV		687504	19410158	3.84%	0.855%
45	15.769	2324	2330	2334	VV	4	164591	4210335	0.83%	0.185%
46	15.804	2334	2336	2346	VV	10	128303	4514779	0.89%	0.199%
47	15.945	2346	2360	2368	VV	6	319230	14855328	2.94%	0.654%
48	16.028	2368	2374	2377	VV	2	336309	7756635	1.54%	0.342%
49	16.051	2377	2378	2388	VV	4	314013	7044767	1.39%	0.310%
50	16.139	2388	2393	2404	VV	4	124716	5127539	1.01%	0.226%
51	16.251	2404	2412	2418	VV	4	136043	4689472	0.93%	0.206%
52	16.315	2418	2423	2426	VV	4	106368	2510740	0.50%	0.111%
53	16.368	2426	2432	2440	VV	5	1034969	28953608	5.73%	1.275%
54	16.433	2440	2443	2449	VV		438519	8948308	1.77%	0.394%
55	16.492	2449	2453	2456	VV	6	114465	2550181	0.50%	0.112%
56	16.539	2456	2461	2463	VV	3	218654	4230932	0.84%	0.186%
57	16.580	2463	2468	2482	VV	2	390645	12154585	2.41%	0.535%
58	16.738	2489	2495	2497	VV	3	153415	2970911	0.59%	0.131%
59	16.821	2497	2509	2518	VV		1990037	48538189	9.61%	2.137%
60	16.909	2518	2524	2535	VV	6	293451	10167335	2.01%	0.448%
61	17.015	2535	2542	2551	VV	5	187333	6301511	1.25%	0.277%
62	17.179	2563	2570	2576	VV	6	76511	2069498	0.41%	0.091%
63	17.273	2576	2586	2595	VV	4	69612	3102380	0.61%	0.137%
64	17.596	2631	2641	2657	VV	8	92787	4776691	0.95%	0.210%
65	18.777	2828	2842	2846	PV	6	69911	2478947	0.49%	0.109%
66	18.877	2846	2859	2908	VV	4	1176246	72039505	14.26%	3.172%
67	19.277	2914	2927	2943	VV	5	195770	9875036	1.95%	0.435%
68	19.394	2943	2947	2974	VV	5	57875	4088495	0.81%	0.180%
69	19.629	2974	2987	3013	VV	3	312166	15216464	3.01%	0.670%

Sum of corrected areas: 2271022077

Table F15 Area percent report for the essential oil obtained from the 72 hour solvent extraction.

DCMSE72B
Area Percent Report

Data File : D:\PATRICIA\DCMSE72B.D Vial: 50
Acq On : 2 Apr 2009 16:12 Operator: Patricia
Sample : DCM SOLVENT EXT. SAMPLE -72 HOURS Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.144	4	11	14	BV 2	48572	487989	0.10%	0.020%
2	2.197	14	20	50	VV 2	151805	2953139	0.62%	0.119%
3	2.473	50	67	85	PV 3	683296	17290752	3.62%	0.698%
4	3.149	169	182	203	PV	4438117	95338760	19.96%	3.851%
5	3.360	203	218	224	PV 9	17566	425821	0.09%	0.017%
6	3.572	243	254	260	BV 2	35686	760561	0.16%	0.031%
7	3.677	260	272	290	VV	324821	8475510	1.77%	0.342%
8	4.053	321	336	358	VV	102244	3012654	0.63%	0.122%
9	4.353	372	387	390	VV 2	30145	735756	0.15%	0.030%
10	4.441	390	402	405	VV 2	299234	8146146	1.71%	0.329%
11	4.512	405	414	425	VV	2279673	67871533	14.21%	2.741%
12	4.635	425	435	450	VV	1551277	44201804	9.25%	1.785%
13	4.800	450	463	481	VV 4	61996	3273355	0.69%	0.132%
14	5.957	651	660	677	VV 3	35062	1304457	0.27%	0.053%
15	9.976	1330	1344	1370	VB 8	28462	1736953	0.36%	0.070%
16	12.409	1751	1758	1769	PV	49660	969607	0.20%	0.039%
17	13.249	1890	1901	1904	PV 2	101775	1774885	0.37%	0.072%
18	13.290	1904	1908	1924	VV	215750	4070977	0.85%	0.164%
19	13.460	1931	1937	1949	VV	152396	2790273	0.58%	0.113%
20	13.742	1978	1985	1989	VV 2	96221	2230800	0.47%	0.090%
21	13.783	1989	1992	2000	VV 4	100549	2595766	0.54%	0.105%
22	13.866	2000	2006	2012	VV	264881	5500845	1.15%	0.222%
23	13.930	2012	2017	2026	VV	404207	7502006	1.57%	0.303%
24	14.024	2026	2033	2042	VV	6016455	95712448	20.04%	3.866%
25	14.089	2042	2044	2050	VV 5	116329	2571763	0.54%	0.104%
26	14.283	2057	2077	2089	VV 2	244701	13382840	2.80%	0.541%
27	14.424	2089	2101	2108	VV	21436198	477625902	100.00%	19.292%
28	14.494	2108	2113	2123	VV 2	3687178	62166443	13.02%	2.511%
29	14.571	2123	2126	2128	VV 2	234894	3721892	0.78%	0.150%
30	14.612	2128	2133	2136	VV	686652	12158935	2.55%	0.491%
31	14.653	2136	2140	2152	VV	1363229	32122991	6.73%	1.298%
32	14.788	2152	2163	2169	VV	8014537	134561517	28.17%	5.435%
33	14.841	2169	2172	2177	VV	774460	18828063	3.94%	0.761%
34	14.929	2177	2187	2191	VV 2	1705317	52862387	11.07%	2.135%
35	14.976	2191	2195	2199	VV	1746213	38688068	8.10%	1.563%
36	15.035	2199	2205	2207	VV 4	1618995	38546682	8.07%	1.557%
37	15.117	2207	2219	2224	VV	9376331	215950707	45.21%	8.723%
38	15.182	2224	2230	2240	VV 2	18185503	364562901	76.33%	14.726%
39	15.252	2240	2242	2246	VV	874548	16999138	3.56%	0.687%
40	15.335	2246	2256	2261	VV	953082	35986973	7.53%	1.454%
41	15.393	2261	2266	2278	VV 3	961872	38086038	7.97%	1.538%
42	15.499	2278	2284	2295	VV 4	600887	25454124	5.33%	1.028%
43	15.581	2295	2298	2309	VV 3	438892	17349340	3.63%	0.701%

DCMSE72B

44	15.693	2309	2317	2328	VV		781540	26474275	5.54%	1.069%
45	15.787	2328	2333	2340	VV	10	258086	9767749	2.05%	0.395%
46	15.987	2348	2367	2377	VV	8	451764	31510168	6.60%	1.273%
47	16.110	2377	2388	2397	VV	8	426152	22862189	4.79%	0.923%
48	16.204	2397	2404	2412	VV	8	270331	11801924	2.47%	0.477%
49	16.304	2412	2421	2430	VV	5	215181	11876578	2.49%	0.480%
50	16.410	2430	2439	2443	VV		911566	22103055	4.63%	0.893%
51	16.451	2443	2446	2450	VV	4	735665	16586928	3.47%	0.670%
52	16.486	2450	2452	2465	VV	3	511084	15481585	3.24%	0.625%
53	16.633	2465	2477	2498	VV		587482	31520263	6.60%	1.273%
54	16.886	2498	2520	2529	VV		1687842	58313748	12.21%	2.355%
55	16.968	2529	2534	2538	VV	7	315520	8289538	1.74%	0.335%
56	17.003	2538	2540	2546	VV	5	307968	7793906	1.63%	0.315%
57	17.068	2546	2551	2558	VV	5	214380	6767444	1.42%	0.273%
58	17.250	2572	2582	2589	VV	5	134412	6030075	1.26%	0.244%
59	17.326	2589	2595	2612	VV	7	118138	7334445	1.54%	0.296%
60	17.661	2644	2652	2657	VV	6	116683	3840750	0.80%	0.155%
61	17.714	2657	2661	2673	VV	6	102678	3152346	0.66%	0.127%
62	17.926	2673	2697	2699	PV	6	92298	4246111	0.89%	0.172%
63	17.973	2699	2705	2737	VV	6	101511	7806105	1.63%	0.315%
64	18.913	2837	2865	2903	PV	3	1699985	108939328	22.81%	4.400%
65	19.142	2903	2904	2914	VB	10	19508	316213	0.07%	0.013%
66	19.330	2914	2936	2959	BV	5	259570	13582971	2.84%	0.549%
67	19.665	2975	2993	3013	PV	2	414440	20046642	4.20%	0.810%
68	19.912	3013	3035	3045	PBA	3	773934	38482965	8.06%	1.554%

Sum of corrected areas: 2475716797

Table F16 Area percent report for the essential oil from the 24 hour Soxhlet extraction.

DSXS124B
Area Percent Report

Data File : D:\PATRICIA\DSXS124B.D Vial: 34
Acq On : 24 Mar 2009 14:23 Operator: PATRICIA
Sample : DCM SOX. EXT. SAMPLE 1- 24 HOURS Inst : Instrumen
Misc : SAMPLE- 5ML Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.143	3	11	15	BV	195442	2417705	0.67%	0.170%
2	2.196	15	20	27	VV 2	105019	1819339	0.51%	0.128%
3	2.261	27	31	48	VV	95351	1364498	0.38%	0.096%
4	2.461	59	65	86	VV	582743	11064507	3.08%	0.776%
5	2.972	127	152	171	PV 8	18125	667274	0.19%	0.047%
6	3.154	171	183	199	PV	3852368	63247470	17.58%	4.435%
7	3.360	211	218	224	VV 3	18360	360016	0.10%	0.025%
8	3.577	247	255	265	PV 2	36704	850922	0.24%	0.060%
9	3.677	265	272	283	VV	248646	4713680	1.41%	0.356%
10	4.047	319	335	353	PV 2	67555	1510855	0.49%	0.124%
11	4.341	376	385	390	PV	47683	1047769	0.29%	0.073%
12	4.429	390	400	405	VV	250637	5904612	1.64%	0.414%
13	4.500	405	412	424	VV	1986895	45077461	12.53%	3.161%
14	4.623	424	433	445	VV	1255216	28341238	7.88%	1.988%
15	4.770	445	458	487	VV 2	59039	2804424	0.78%	0.197%
16	5.898	636	650	663	VV 5	28717	1004478	0.28%	0.070%
17	9.782	1286	1311	1340	BV 7	23596	1288804	0.36%	0.090%
18	12.332	1737	1745	1751	PV 2	25117	445976	0.12%	0.031%
19	13.148	1877	1884	1889	PV 5	15976	329854	0.09%	0.023%
20	13.219	1889	1896	1899	VV 2	70289	1208340	0.34%	0.085%
21	13.260	1899	1903	1913	VV	179416	2768307	0.77%	0.194%
22	13.436	1925	1933	1942	VV	109683	1806933	0.50%	0.127%
23	13.624	1959	1965	1976	VV 4	25436	705737	0.20%	0.049%
24	13.754	1980	1987	1994	VV 2	38005	696472	0.19%	0.049%
25	13.842	1994	2002	2007	VV 2	153504	2668711	0.74%	0.187%
26	13.901	2007	2012	2021	VV	373442	5672765	1.58%	0.398%
27	13.995	2021	2028	2036	VV	5887756	82434771	22.91%	5.781%
28	14.065	2036	2040	2045	VV 4	55498	1262156	0.35%	0.089%
29	14.194	2059	2062	2065	VV 4	36149	645541	0.18%	0.045%
30	14.224	2065	2067	2069	VV 3	43449	597328	0.17%	0.042%
31	14.259	2069	2073	2084	VV 2	121311	2894886	0.80%	0.203%
32	14.394	2084	2096	2102	VV	19990872	359806778	100.00%	25.233%
33	14.470	2102	2109	2115	VV 2	2394449	34531028	9.60%	2.422%
34	14.512	2115	2116	2118	VV 2	101224	1185220	0.33%	0.083%
35	14.541	2118	2121	2123	VV 3	120691	1717313	0.48%	0.120%
36	14.576	2123	2127	2131	VV 2	402677	7234166	2.01%	0.507%
37	14.629	2131	2136	2146	VV	826209	17334112	4.82%	1.216%
38	14.705	2146	2149	2151	VV 2	231316	3907054	1.09%	0.274%
39	14.752	2151	2157	2163	VV	5735396	84361611	23.45%	5.916%
40	14.805	2163	2166	2171	VV	425188	10326415	2.87%	0.724%
41	14.899	2171	2182	2186	VV 2	1045322	32486694	9.03%	2.278%
42	14.946	2186	2190	2194	VV	945925	19651405	5.46%	1.378%
43	14.993	2194	2198	2201	VV	602890	12959899	3.60%	0.909%

DSXS124B

44	15.082	2201	2213	2217	VV		5828665	105269991	29.26%	7.382%
45	15.140	2217	2223	2235	VV	2	12257304	211746842	58.85%	14.849%
46	15.223	2235	2237	2241	VV		281001	4502567	1.25%	0.316%
47	15.305	2241	2251	2255	VV		496528	12480846	3.47%	0.875%
48	15.364	2255	2261	2272	VV	2	395572	9979777	2.77%	0.700%
49	15.458	2272	2277	2284	VV	3	225344	6645409	1.85%	0.466%
50	15.522	2284	2288	2296	VV		154657	3808773	1.06%	0.267%
51	15.640	2296	2308	2314	VV		343095	9038781	2.51%	0.634%
52	15.751	2322	2327	2330	VV	5	86964	1995012	0.55%	0.140%
53	15.798	2330	2335	2341	VV	10	77043	2621620	0.73%	0.184%
54	15.916	2341	2355	2361	VV	5	292364	11466464	3.19%	0.804%
55	15.992	2361	2368	2379	VV	5	331145	10914147	3.03%	0.765%
56	16.104	2379	2387	2392	VV	7	101958	3526374	0.98%	0.247%
57	16.157	2392	2396	2400	VV	7	77388	2001354	0.56%	0.140%
58	16.221	2400	2407	2412	VV	4	88107	2676524	0.74%	0.188%
59	16.274	2412	2416	2421	VV	4	93692	2451758	0.68%	0.172%
60	16.339	2421	2427	2434	VV	6	623219	15934900	4.43%	1.117%
61	16.398	2434	2437	2443	VV	2	261548	5262151	1.46%	0.369%
62	16.462	2443	2448	2451	VV	6	103435	2298575	0.64%	0.161%
63	16.509	2451	2456	2458	VV	4	168554	2913623	0.81%	0.204%
64	16.545	2458	2462	2477	VV	2	358165	11559572	3.21%	0.811%
65	16.703	2483	2489	2491	VV	3	132724	2494654	0.69%	0.175%
66	16.780	2491	2502	2512	VV		1135261	28964241	8.05%	2.031%
67	16.874	2512	2518	2521	VV	4	167392	3794500	1.05%	0.266%
68	16.903	2521	2523	2529	VV	3	173465	3758173	1.04%	0.264%
69	16.968	2529	2534	2542	VV	4	148110	4463284	1.24%	0.313%
70	17.138	2556	2563	2570	VV	8	58197	1769303	0.49%	0.124%
71	17.250	2576	2582	2588	VV	8	37597	1121112	0.31%	0.079%
72	17.308	2588	2592	2601	VV	8	33189	1043028	0.29%	0.073%
73	17.467	2601	2619	2623	VV	8	27408	1243035	0.35%	0.087%
74	17.567	2623	2636	2657	VV	8	79871	4700200	1.31%	0.330%
75	18.754	2823	2838	2840	PV	6	17679	504688	0.14%	0.035%
76	18.848	2840	2854	2893	VV	2	561145	31373170	8.72%	2.200%
77	19.247	2905	2922	2945	VV	5	94740	4892155	1.36%	0.343%
78	19.600	2962	2982	3045	PBA	5	446746	33006132	9.17%	2.315%

Sum of corrected areas: 1425965367

Table F17 Area percent report for the essential oil obtained from the 72 hour Soxhlet extraction.

DSX726
Area Percent Report

Data File : C:\GOVENDER\PATRICIA\DSX726.D Vial: 50
Acq On : 30 Mar 2009 16:25 Operator: Patricia
Sample : DCM SOX. EXT SAMPLE 3 - 72 HOURS Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.138	3	10	15	BV	1023271	15025750	3.27%	0.585%
2	2.191	15	19	24	VV	170337	2993285	0.65%	0.117%
3	2.255	24	30	48	VV	697839	10406926	2.26%	0.405%
4	2.455	48	64	84	PV 2	954311	19902227	4.33%	0.775%
5	3.143	174	181	187	M	4899938	93028680	20.24%	3.625%
6	3.354	208	217	232	PB 8	30436	902275	0.20%	0.035%
7	3.566	235	253	262	BV	55210	1359975	0.30%	0.053%
8	3.666	262	270	282	VV	360467	8309128	1.81%	0.324%
9	4.036	320	333	350	PV	92432	2686787	0.58%	0.105%
10	4.335	350	384	389	PV	77524	1872148	0.41%	0.073%
11	4.424	389	399	402	VV	356698	9102135	1.98%	0.355%
12	4.494	402	411	422	VV	2920450	75832297	16.50%	2.955%
13	4.617	422	432	443	VV	1770920	44473665	9.67%	1.733%
14	4.741	443	453	476	VV 2	112551	5376773	1.17%	0.209%
15	5.898	619	650	670	PV 7	73863	3720348	0.81%	0.145%
16	9.829	1306	1319	1334	VB 6	33950	1592254	0.35%	0.062%
17	10.752	1451	1476	1505	BV 2	29943	2021927	0.44%	0.079%
18	12.350	1740	1748	1755	VV 2	46682	847457	0.18%	0.033%
19	12.908	1833	1843	1853	VV 2	31920	1000059	0.22%	0.039%
20	13.225	1880	1897	1900	VV 2	85238	1895093	0.41%	0.074%
21	13.272	1900	1905	1913	VV	194828	3302575	0.72%	0.129%
22	13.384	1913	1924	1928	VV 4	50013	1337296	0.29%	0.052%
23	13.443	1928	1934	1944	VV	208429	4699127	1.02%	0.183%
24	13.519	1944	1947	1949	VV 4	51732	945378	0.21%	0.037%
25	13.584	1949	1958	1963	VV 4	59216	2540755	0.55%	0.099%
26	13.654	1963	1970	1980	VV 2	216018	6851100	1.49%	0.267%
27	13.766	1980	1989	1994	VV 4	139450	5109967	1.11%	0.199%
28	13.848	1994	2003	2009	VV 3	369591	10287923	2.24%	0.401%
29	13.913	2009	2014	2019	VV	493734	9719179	2.11%	0.379%
30	14.007	2019	2030	2036	VV	6839043	108198784	23.54%	4.216%
31	14.089	2036	2044	2068	VV	532318	48042084	10.45%	1.872%
32	14.265	2068	2074	2086	VV 3	378073	18524716	4.03%	0.722%
33	14.406	2086	2098	2104	VV	21441591	459726921	100.00%	17.912%
34	14.477	2104	2110	2120	VV 2	3609526	59648725	12.97%	2.324%
35	14.553	2120	2123	2124	VV 2	273658	4077849	0.89%	0.159%
36	14.588	2124	2129	2133	VV	842166	15580751	3.39%	0.607%
37	14.635	2133	2137	2148	VV	1363553	31514909	6.86%	1.228%
38	14.765	2148	2159	2165	VV	8237327	130933637	28.48%	5.101%
39	14.817	2165	2168	2173	VV	681395	16210044	3.53%	0.632%
40	14.911	2173	2184	2188	VV 2	1759102	52639824	11.45%	2.051%
41	14.953	2188	2191	2196	VV	1479864	31666857	6.89%	1.234%
42	15.005	2196	2200	2203	VV 2	951836	20659000	4.49%	0.805%
43	15.094	2203	2215	2220	VV	9731191	178438235	38.81%	6.952%

DSX726

44	15.158	2220	2226	2243	VV	2	16800170	320474520	69.71%	12.486%
45	15.317	2243	2253	2258	VV	2	856092	31667638	6.89%	1.234%
46	15.376	2258	2263	2272	VV	2	812856	22703797	4.94%	0.885%
47	15.476	2272	2280	2287	VV	4	541142	19506080	4.24%	0.760%
48	15.540	2287	2291	2298	VV	3	372277	10608584	2.31%	0.413%
49	15.658	2298	2311	2319	VV		736175	23851639	5.19%	0.929%
50	15.799	2325	2335	2346	VV	3	389995	19517934	4.25%	0.760%
51	15.951	2346	2361	2367	VV	4	756573	27684519	6.02%	1.079%
52	16.028	2367	2374	2385	VV	3	810039	26338765	5.73%	1.026%
53	16.139	2385	2393	2405	VV	9	227691	13030641	2.83%	0.508%
54	16.251	2405	2412	2418	VV	5	207261	7600027	1.65%	0.296%
55	16.316	2418	2423	2425	VV	2	231951	5533931	1.20%	0.216%
56	16.363	2425	2431	2434	VV		953098	18612101	4.05%	0.725%
57	16.392	2434	2436	2441	VV	2	884988	16667130	3.63%	0.649%
58	16.433	2441	2443	2449	VV	2	506551	10022160	2.18%	0.390%
59	16.498	2449	2454	2457	VV	5	281100	6616597	1.44%	0.258%
60	16.539	2457	2461	2463	VV	3	482194	9030825	1.96%	0.352%
61	16.580	2463	2468	2489	VV		826878	29052670	6.32%	1.132%
62	16.821	2489	2509	2518	VV	2	1873637	60730758	13.21%	2.366%
63	16.909	2518	2524	2535	VV	7	402134	17962646	3.91%	0.700%
64	17.009	2535	2541	2547	VV	5	307835	9757995	2.12%	0.380%
65	17.068	2547	2551	2563	VV	3	193116	8443064	1.84%	0.329%
66	17.179	2563	2570	2576	VV	5	188370	6259292	1.36%	0.244%
67	17.273	2576	2586	2593	VV	4	173642	7788030	1.69%	0.303%
68	17.344	2593	2598	2610	VV	3	153771	7579719	1.65%	0.295%
69	17.450	2610	2616	2621	VV	9	120218	3989489	0.87%	0.155%
70	17.514	2621	2627	2631	VV	7	147082	4259622	0.93%	0.166%
71	17.602	2631	2642	2654	VV	8	297982	15948215	3.47%	0.621%
72	17.732	2654	2664	2676	VV	4	206912	12418064	2.70%	0.484%
73	17.843	2676	2683	2689	VV	4	135276	6431915	1.40%	0.251%
74	17.920	2689	2696	2732	VV	4	138871	12591455	2.74%	0.491%
75	18.313	2749	2763	2785	VV	4	108571	9036015	1.97%	0.352%
76	18.866	2821	2857	2893	VV	3	1364719	84818750	18.45%	3.305%
77	19.242	2893	2921	2960	VV	2	2067969	132350074	28.79%	5.157%
78	19.612	2960	2984	3021	VV	3	386713	24716256	5.38%	0.963%

Sum of corrected areas: 2566605746

Table F18 Area percent report for HS-SPME (PDMS fibre coating) at room temperature.

PDMSS4RT
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PDMSS4RT.D Vial: 90
Acq On : 26 Mar 2007 10:43 Operator: patricia
Sample : MK sample 4 PDMS coating - room temp. Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.367	16	49	81	BV 2	31528	755657	0.73%	0.258%
2	2.661	81	99	139	PV 4	19651	799559	0.77%	0.273%
3	3.360	209	218	247	BV	1181431	22896118	21.99%	7.821%
4	3.577	247	255	265	VV 3	4658	105841	0.10%	0.036%
5	3.806	265	294	304	VV 4	9920	211182	0.20%	0.072%
6	3.918	304	313	350	PV 2	64071	1661312	1.60%	0.567%
7	4.306	372	379	386	M2	43719	938546	0.90%	0.321%
8	4.629	419	434	438	PV 3	3278	77995	0.07%	0.027%
9	4.711	438	448	452	VV 2	68747	1667060	1.60%	0.569%
10	4.782	452	460	473	VV	437832	11213038	10.77%	3.830%
11	4.917	473	483	540	VV	361708	10142742	9.74%	3.465%
12	13.260	1889	1903	1916	BV 6	5377	107994	0.10%	0.037%
13	13.413	1916	1929	1933	PV	92329	1562379	1.50%	0.534%
14	13.454	1933	1936	1959	VV	63854	1100942	1.06%	0.376%
15	13.624	1959	1965	1985	VB	25560	450143	0.43%	0.154%
16	13.942	1996	2019	2026	BV 7	6260	129324	0.12%	0.044%
17	14.018	2026	2032	2037	VV 2	38384	664380	0.64%	0.227%
18	14.083	2037	2043	2050	VV	158694	2491960	2.39%	0.851%
19	14.171	2050	2058	2067	VV	2492019	36561364	35.11%	12.489%
20	14.241	2067	2070	2075	VV 7	9128	191394	0.18%	0.065%
21	14.435	2086	2103	2113	VV 2	32841	804927	0.77%	0.275%
22	14.564	2113	2125	2131	VV	6130171	104141448	100.00%	35.574%
23	14.641	2131	2138	2148	VV	659691	9931883	9.54%	3.393%
24	14.752	2148	2157	2160	VV 3	51132	1038037	1.00%	0.355%
25	14.799	2160	2165	2178	VV	143097	2969575	2.85%	1.014%
26	14.923	2178	2186	2202	VV	1306141	19880015	19.09%	6.791%
27	15.070	2202	2211	2215	VV 3	112233	2475180	2.38%	0.845%
28	15.117	2215	2219	2223	VV 2	99095	1768612	1.70%	0.604%
29	15.164	2223	2227	2231	VV	58023	1104203	1.06%	0.377%
30	15.258	2231	2243	2247	VV	746992	13389942	12.86%	4.574%
31	15.311	2247	2252	2264	VV 2	2012832	37101038	35.63%	12.673%
32	15.405	2264	2268	2273	VV 2	54677	935484	0.90%	0.320%
33	15.481	2273	2281	2287	VV 2	56131	1195591	1.15%	0.408%
34	15.552	2287	2293	2303	VV	49664	960088	0.92%	0.328%
35	15.651	2303	2310	2328	VV 5	16936	640805	0.62%	0.219%
36	15.845	2335	2343	2354	VV 5	5081	169575	0.16%	0.058%
37	15.951	2354	2361	2374	VV 5	9821	203714	0.20%	0.070%
38	16.556	2458	2464	2472	PV 7	9058	170337	0.16%	0.058%
39	17.020	2526	2543	2555	BV 7	5104	139221	0.13%	0.048%

Sum of corrected areas: 292748479

Table F19 Area percent report for HS-SPME (PDMS fibre coating) at 40 °C.

PDMSS440A
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PDMSS440.D Vial: 90
Acq On : 26 Mar 2007 11:34 Operator: patricia
Sample : MK sample 4 PDMS coating - temp.- 40C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.367	39	49	74	BV	42147	933508	0.80%	0.263%
2	2.655	93	98	103	PV 3	41204	789360	0.68%	0.222%
3	2.696	103	105	135	VV 3	22951	798154	0.68%	0.225%
4	3.366	208	219	249	PV	2417344	46878933	40.14%	13.213%
5	3.589	249	257	268	VV 2	9683	220870	0.19%	0.062%
6	3.818	287	296	305	PV 2	21094	462113	0.40%	0.130%
7	3.924	305	314	346	VV	144449	3554169	3.04%	1.002%
8	4.318	357	381	404	BV	97026	2500757	2.14%	0.705%
9	4.635	427	435	440	PV 2	10378	242409	0.21%	0.068%
10	4.723	440	450	454	VV 2	137384	3445044	2.95%	0.971%
11	4.793	454	462	475	VV	874727	22267984	19.07%	6.276%
12	4.923	475	484	519	VV	729999	19326667	16.55%	5.447%
13	13.266	1897	1904	1912	PV 5	5231	100835	0.09%	0.028%
14	13.413	1920	1929	1933	VV	86391	1481001	1.27%	0.417%
15	13.454	1933	1936	1949	VV	50856	887620	0.76%	0.250%
16	13.624	1959	1965	1979	VV 2	37033	659665	0.56%	0.186%
17	13.942	2003	2019	2026	VV 5	9540	208619	0.18%	0.059%
18	14.024	2026	2033	2038	VV	55939	955948	0.82%	0.269%
19	14.083	2038	2043	2051	VV	147707	2327379	1.99%	0.656%
20	14.171	2051	2058	2067	VV	2255600	33809126	28.95%	9.529%
21	14.241	2067	2070	2076	VV 6	10940	235423	0.20%	0.066%
22	14.435	2085	2103	2108	VV	39282	945592	0.81%	0.267%
23	14.476	2108	2110	2114	VV 4	6437	96068	0.08%	0.027%
24	14.565	2114	2125	2131	VV	6593497	116778576	100.00%	32.915%
25	14.641	2131	2138	2148	VV	789738	11872182	10.17%	3.346%
26	14.717	2148	2151	2153	VV 4	15391	209405	0.18%	0.059%
27	14.753	2153	2157	2161	VV 3	61086	992227	0.85%	0.280%
28	14.800	2161	2165	2178	VV	142001	2983221	2.55%	0.841%
29	14.923	2178	2186	2192	VV	1319982	19499507	16.70%	5.496%
30	14.976	2192	2195	2204	VV 2	39092	929902	0.80%	0.262%
31	15.070	2204	2211	2215	VV 3	119441	2447911	2.10%	0.690%
32	15.117	2215	2219	2223	VV 2	98502	1747416	1.50%	0.493%
33	15.164	2223	2227	2231	VV	54078	989862	0.85%	0.279%
34	15.258	2231	2243	2247	VV	739021	13302360	11.39%	3.749%
35	15.317	2247	2253	2265	VV 2	1937584	35429273	30.34%	9.986%
36	15.405	2265	2268	2273	VV	52336	927547	0.79%	0.261%
37	15.481	2273	2281	2287	VV 2	53029	1172839	1.00%	0.331%
38	15.552	2287	2293	2303	VV 2	47929	920126	0.79%	0.259%
39	15.652	2303	2310	2318	VV 5	16757	504901	0.43%	0.142%
40	15.845	2338	2343	2355	VV 6	6271	192406	0.16%	0.054%
41	15.951	2355	2361	2376	VV 7	9903	204507	0.18%	0.058%
42	16.204	2397	2404	2414	VV 7	4005	116997	0.10%	0.033%
43	16.556	2458	2464	2471	BV 3	12260	222760	0.19%	0.063%

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                                        PDMSS440A
44  17.021  2526 2543 2556 PV 7    8166    214389   0.18%   0.060%

                      Sum of corrected areas:   354785559
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Table F20 Area percent report for HS-SPME (PDMS fibre coating) at 60 °C.

PDMSS460
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PDMSS460.D Vial: 90
Acq On : 26 Mar 2007 12:28 Operator: patricia
Sample : MK sample 4 PDMS coating - temp.- 60C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.361	39	48	84	VV	150455	2735667	0.98%	0.246%
2	2.637	84	95	99	PV	222249	3744295	1.34%	0.337%
3	2.678	99	102	149	VV 2	162351	4762388	1.70%	0.429%
4	3.242	191	198	207	VV 4	5249	114366	0.04%	0.010%
5	3.366	207	219	248	PV	7778861	178935384	64.01%	16.110%
6	3.583	248	256	263	VV	54166	1151670	0.41%	0.104%
7	3.642	263	266	282	VV 10	5760	184413	0.07%	0.017%
8	3.806	282	294	304	VV	135836	2817554	1.01%	0.254%
9	3.912	304	312	342	VV	957013	22304294	7.98%	2.008%
10	4.153	342	353	366	VV	8304	429012	0.15%	0.039%
11	4.306	366	379	402	VV	573075	14383360	5.15%	1.295%
12	4.482	402	409	422	VV 4	15104	428407	0.15%	0.039%
13	4.617	422	432	437	VV	93084	2199127	0.79%	0.198%
14	4.711	437	448	453	VV	774846	21952675	7.85%	1.976%
15	4.794	453	462	473	VV	4599699	124476230	44.53%	11.207%
16	4.929	473	485	536	VV	4490785	118190810	42.28%	10.641%
17	5.299	536	548	586	VV 4	15466	651567	0.23%	0.059%
18	6.004	649	668	697	BV 3	17656	655497	0.23%	0.059%
19	6.280	704	715	738	VV 4	17988	683291	0.24%	0.062%
20	7.238	866	878	905	PV 4	3327	180224	0.06%	0.016%
21	8.671	1098	1122	1162	BV 4	6500	404291	0.14%	0.036%
22	10.369	1386	1411	1434	PV 9	4607	253179	0.09%	0.023%
23	12.561	1770	1784	1791	PV	8633	194279	0.07%	0.017%
24	12.632	1791	1796	1808	VV 8	3667	93126	0.03%	0.008%
25	13.260	1887	1903	1919	PV 2	13720	267613	0.10%	0.024%
26	13.413	1919	1929	1933	VV	206319	3646425	1.30%	0.328%
27	13.454	1933	1936	1953	VV	97884	1647572	0.59%	0.148%
28	13.624	1953	1965	1985	VV	187685	3137405	1.12%	0.282%
29	13.942	2011	2019	2026	VV 3	45237	904896	0.32%	0.081%
30	14.024	2026	2033	2038	VV 2	251957	4452909	1.59%	0.401%
31	14.083	2038	2043	2051	VV	305916	5155244	1.84%	0.464%
32	14.177	2051	2059	2066	VV	4220392	67087554	24.00%	6.040%
33	14.247	2066	2071	2076	VV 2	34773	712417	0.25%	0.064%
34	14.294	2076	2079	2086	VV 3	17226	397914	0.14%	0.036%
35	14.447	2086	2105	2115	VV 3	109022	3876392	1.39%	0.349%
36	14.588	2115	2129	2134	VV 2	9342179	279534406	100.00%	25.167%
37	14.647	2134	2139	2149	VV	2538525	38038003	13.61%	3.425%
38	14.717	2149	2151	2153	VV	47405	623409	0.22%	0.056%
39	14.753	2153	2157	2161	VV 2	201486	3000007	1.07%	0.270%
40	14.794	2161	2164	2179	VV 2	477284	9132753	3.27%	0.822%
41	14.929	2179	2187	2193	VV	3661520	54481467	19.49%	4.905%
42	14.976	2193	2195	2203	VV 2	106905	2076198	0.74%	0.187%
43	15.070	2203	2211	2215	VV 2	315967	6367978	2.28%	0.573%

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44	15.117	2215	2219	2224	VV		253825	4406219	1.58%	0.397%
45	15.164	2224	2227	2231	VV	2	124572	2273012	0.81%	0.205%
46	15.258	2231	2243	2247	VV		1804288	32122335	11.49%	2.892%
47	15.317	2247	2253	2264	VV	3	4072750	77080956	27.57%	6.940%
48	15.399	2264	2267	2273	VV		111465	1919566	0.69%	0.173%
49	15.481	2273	2281	2287	VV		94285	2057396	0.74%	0.185%
50	15.552	2287	2293	2303	VV	2	94264	1742861	0.62%	0.157%
51	15.652	2303	2310	2319	VV	4	27553	851178	0.30%	0.077%
52	15.716	2319	2321	2336	VV	4	7768	283777	0.10%	0.026%
53	15.840	2336	2342	2355	VV	7	9425	320000	0.11%	0.029%
54	15.951	2355	2361	2377	VV	4	15021	352307	0.13%	0.032%
55	16.204	2396	2404	2421	VV	4	6177	210514	0.08%	0.019%
56	16.556	2456	2464	2471	VV	4	13930	288365	0.10%	0.026%
57	16.627	2471	2476	2490	VB	4	3684	99522	0.04%	0.009%
58	17.021	2526	2543	2554	PV	9	10340	259718	0.09%	0.023%

Sum of corrected areas: 1110733395

Table F21 Area percent report for HS-SPME (PDMS fibre coating) at 80 °C.

PDMS4480
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PDMS4480.D Vial: 90
Acq On : 26 Mar 2007 13:29 Operator: patricia
Sample : MK sample 4 PDMS coating - temp.- 80C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.249	10	29	40	BV 3	10999	158693	0.02%	0.005%
2	2.361	40	48	80	VV	248053	4291305	0.52%	0.128%
3	2.637	80	95	98	PV	530516	8814290	1.07%	0.263%
4	2.684	98	103	141	VV 2	570081	14023315	1.71%	0.419%
5	2.984	149	154	168	VV 4	12735	463526	0.06%	0.014%
6	3.248	184	199	207	VV 2	16672	467432	0.06%	0.014%
7	3.378	207	221	249	VV 2	8973993	300342822	36.59%	8.973%
8	3.589	249	257	280	VV	168022	4203790	0.51%	0.126%
9	3.812	280	295	304	VV	355691	7615859	0.93%	0.228%
10	3.924	304	314	338	VV	2992101	71289838	8.68%	2.130%
11	4.141	338	351	360	VV 6	34191	1341604	0.16%	0.040%
12	4.312	366	380	401	VV	2053202	52907624	6.45%	1.581%
13	4.488	401	410	424	VV	78428	2116178	0.26%	0.063%
14	4.629	424	434	438	VV	242821	6280820	0.77%	0.188%
15	4.735	438	452	455	VV 3	2173543	70755599	8.62%	2.114%
16	4.829	455	468	476	VV	8655479	354264279	43.16%	10.584%
17	4.976	476	493	537	VV	8345737	329890771	40.19%	9.856%
18	5.299	537	548	572	VV 2	104695	3194617	0.39%	0.095%
19	5.616	591	602	633	VB 8	17095	759002	0.09%	0.023%
20	6.004	656	668	690	PV 2	99132	3343197	0.41%	0.100%
21	6.180	690	698	703	VV 7	8742	296382	0.04%	0.009%
22	6.268	703	713	732	VV 2	146344	5083166	0.62%	0.152%
23	6.439	732	742	773	VV 4	30961	1685332	0.21%	0.050%
24	6.685	773	784	818	VB 3	12615	602323	0.07%	0.018%
25	7.255	864	881	896	PV 8	13927	829425	0.10%	0.025%
26	8.648	1093	1118	1154	BV 3	103090	5123928	0.62%	0.153%
27	8.948	1154	1169	1209	VV 6	25119	1581944	0.19%	0.047%
28	9.523	1256	1267	1276	VV 8	8022	396456	0.05%	0.012%
29	9.664	1276	1291	1330	VV 5	26248	1614495	0.20%	0.048%
30	10.334	1390	1405	1459	VV 3	94908	4665200	0.57%	0.139%
31	12.549	1763	1782	1791	BV	98602	2022542	0.25%	0.060%
32	12.637	1791	1797	1822	VB 2	23110	544158	0.07%	0.016%
33	12.931	1833	1847	1861	BB 4	7888	143203	0.02%	0.004%
34	13.078	1862	1872	1883	BV 6	13007	373578	0.05%	0.011%
35	13.266	1894	1904	1917	VV 2	61931	1302052	0.16%	0.039%
36	13.419	1917	1930	1934	VV	934143	18546335	2.26%	0.554%
37	13.454	1934	1936	1952	VV	512266	8657238	1.05%	0.259%
38	13.625	1952	1965	1977	VV	714353	12865742	1.57%	0.384%
39	13.771	1984	1990	2003	VV 9	8867	278523	0.03%	0.008%
40	13.948	2012	2020	2027	VV 2	190943	4211181	0.51%	0.126%
41	14.030	2027	2034	2039	VV	960152	20027731	2.44%	0.598%
42	14.089	2039	2044	2052	VV	1225032	26176235	3.19%	0.782%
43	14.200	2052	2063	2070	VV	9002997	237628796	28.95%	7.100%

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44	14.265	2070	2074	2079	VV	2	144422	3241566	0.39%	0.097%
45	14.429	2089	2102	2105	VV	4	218991	6648979	0.81%	0.199%
46	14.500	2105	2114	2117	VV	3	286564	11066967	1.35%	0.331%
47	14.653	2117	2140	2157	VV	5	12836148	820876279	100.00%	24.526%
48	14.811	2157	2167	2181	VV	2	3128888	72144681	8.79%	2.155%
49	14.958	2181	2192	2206	VV		9480877	220191578	26.82%	6.579%
50	15.082	2206	2213	2217	VV	2	2102973	40903449	4.98%	1.222%
51	15.129	2217	2221	2226	VV		1421920	25383090	3.09%	0.758%
52	15.176	2226	2229	2233	VV		646737	12804839	1.56%	0.383%
53	15.281	2233	2247	2251	VV		7009213	172146461	20.97%	5.143%
54	15.358	2251	2260	2266	VV	4	10059739	307068574	37.41%	9.174%
55	15.411	2266	2269	2274	VV		857293	12794515	1.56%	0.382%
56	15.493	2274	2283	2288	VV		719244	13579680	1.65%	0.406%
57	15.552	2288	2293	2303	VV	2	585695	11699256	1.43%	0.350%
58	15.657	2303	2311	2318	VV	2	274085	5724258	0.70%	0.171%
59	15.716	2318	2321	2327	VV	3	53247	1239847	0.15%	0.037%
60	15.834	2335	2341	2354	VV		157064	3393601	0.41%	0.101%
61	15.951	2354	2361	2376	VV		120610	2579346	0.31%	0.077%
62	16.081	2376	2383	2387	VV	8	15400	404673	0.05%	0.012%
63	16.128	2387	2391	2397	VV	3	30071	671028	0.08%	0.020%
64	16.204	2397	2404	2421	VV	5	61662	1984847	0.24%	0.059%
65	16.333	2421	2426	2435	VV	6	15801	406726	0.05%	0.012%
66	16.445	2435	2445	2450	VV	5	21838	522372	0.06%	0.016%
67	16.556	2457	2464	2471	VV	2	165750	3212109	0.39%	0.096%
68	16.627	2471	2476	2482	VV	3	42063	944803	0.12%	0.028%
69	16.786	2497	2503	2516	VV	8	10432	389470	0.05%	0.012%
70	17.015	2532	2542	2554	VV	2	139025	3362751	0.41%	0.100%
71	17.121	2554	2560	2572	VV	8	17268	451427	0.05%	0.013%

Sum of corrected areas: 3347017703

Table F22 Area percent report for HS-SPME (PA fibre coating) at room temperature.

PA1S7RT
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PAT-HS-2007\PA1S7RT.D Vial: 1
Acq On : 11 Apr 2007 10:16 Operator: Patricia
Sample : sample 7 Pa1 room temp. Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.384	47	52	79	VV	2098	90262	1.51%	0.392%
2	2.725	95	110	135	PV	2704	218087	3.66%	0.947%
3	2.896	135	139	142	VV 5	1312	23494	0.39%	0.102%
4	3.366	209	219	241	PV 3	13606	339126	5.69%	1.472%
5	3.930	306	315	318	VV 7	1160	24709	0.41%	0.107%
6	3.959	318	320	327	VV 7	1155	22451	0.38%	0.097%
7	4.317	366	381	389	VV 10	1377	43876	0.74%	0.190%
8	4.793	440	462	478	PV 2	11354	412913	6.92%	1.793%
9	4.940	478	487	528	VV 2	12110	541901	9.09%	2.353%
10	6.350	713	727	738	PV 2	774	33818	0.57%	0.147%
11	13.278	1890	1906	1919	VV 2	1174	41004	0.69%	0.178%
12	13.419	1919	1930	1933	PV 6	6207	135126	2.27%	0.587%
13	13.454	1933	1936	1961	VV 4	7697	171056	2.87%	0.743%
14	14.030	2016	2034	2037	BV 4	1468	22531	0.38%	0.098%
15	14.083	2037	2043	2052	VV 3	16682	279978	4.70%	1.216%
16	14.171	2052	2058	2088	VV	321043	5138424	86.18%	22.309%
17	14.429	2094	2102	2112	VV	1990	45611	0.76%	0.198%
18	14.553	2112	2123	2132	VV	390070	5962657	100.00%	25.888%
19	14.647	2132	2139	2151	VV 2	35753	661649	11.10%	2.873%
20	14.758	2151	2158	2163	VV 10	2663	88707	1.49%	0.385%
21	14.811	2163	2167	2180	VV 7	6803	224511	3.77%	0.975%
22	14.929	2180	2187	2205	VV	84115	1495109	25.07%	6.491%
23	15.076	2205	2212	2216	VV 5	9923	240781	4.04%	1.045%
24	15.117	2216	2219	2224	VV 5	8065	179728	3.01%	0.780%
25	15.170	2224	2228	2231	VV 6	5459	112487	1.89%	0.488%
26	15.258	2231	2243	2247	VV 2	74709	1344023	22.54%	5.835%
27	15.317	2247	2253	2266	VV 3	208341	4200701	70.45%	18.238%
28	15.411	2266	2269	2276	VV 7	6631	147746	2.48%	0.641%
29	15.487	2276	2282	2289	VV 5	7100	173416	2.91%	0.753%
30	15.557	2289	2294	2304	VV 5	5913	140015	2.35%	0.608%
31	15.651	2304	2310	2319	VV 5	2460	95511	1.60%	0.415%
32	15.728	2319	2323	2333	VV 5	1731	47934	0.80%	0.208%
33	15.863	2339	2346	2355	VV 5	1513	51016	0.86%	0.221%
34	15.957	2355	2362	2371	VV 5	1611	40995	0.69%	0.178%
35	16.121	2379	2390	2397	VV 5	1631	45772	0.77%	0.199%
36	16.215	2397	2406	2419	VV 5	1410	53887	0.90%	0.234%
37	16.568	2460	2466	2485	VV 5	2960	92198	1.55%	0.400%
38	17.038	2528	2546	2558	PV 5	2319	49671	0.83%	0.216%

Sum of corrected areas: 23032880

Table F23 Area percent report for HS-SPME (PA fibre coating) at 40 °C.

PA1S740
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PA1S740.D Vial: 1
Acq On : 11 Apr 2007 11:06 Operator: Patricia
Sample : sample 7 Pa1 temp. -40C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.373	45	50	69	VV 4	7342	226730	1.57%	0.503%
2	2.672	86	101	102	PV 4	11645	211473	1.46%	0.469%
3	2.702	102	106	133	VV 5	16280	828321	5.73%	1.836%
4	3.366	206	219	246	VV 2	79190	1925779	13.32%	4.269%
5	3.536	246	248	252	VV 4	1484	26030	0.18%	0.058%
6	3.818	283	296	300	PV 9	1199	33383	0.23%	0.074%
7	3.936	306	316	334	VV 4	6398	214456	1.48%	0.475%
8	4.317	372	381	407	PV 3	5954	197777	1.37%	0.438%
9	4.641	429	436	440	PV 6	1174	25555	0.18%	0.057%
10	4.723	440	450	454	VV 4	10083	248964	1.72%	0.552%
11	4.788	454	461	476	VV 2	63616	1959096	13.55%	4.343%
12	4.929	476	485	525	VV 3	86333	3252376	22.49%	7.210%
13	6.303	712	719	721	PV 7	454	5263	0.04%	0.012%
14	8.712	1118	1129	1130	PV 8	601	8766	0.06%	0.019%
15	13.260	1891	1903	1909	PV 10	1084	28782	0.20%	0.064%
16	13.313	1909	1912	1918	VV 6	967	15193	0.11%	0.034%
17	13.419	1924	1930	1933	VV 4	8771	166175	1.15%	0.368%
18	13.454	1933	1936	1950	VV 4	9680	208862	1.44%	0.463%
19	13.624	1962	1965	1977	VV 4	2036	50467	0.35%	0.112%
20	13.942	2009	2019	2027	VV 4	1100	38864	0.27%	0.086%
21	14.024	2027	2033	2037	VV 8	3786	74386	0.51%	0.165%
22	14.083	2037	2043	2051	VV 3	20612	374273	2.59%	0.830%
23	14.171	2051	2058	2086	VV	415921	6627870	45.84%	14.693%
24	14.435	2095	2103	2111	VV 8	4091	108577	0.75%	0.241%
25	14.494	2111	2113	2115	VV 3	1482	17161	0.12%	0.038%
26	14.553	2115	2123	2133	VV	933786	14458365	100.00%	32.053%
27	14.641	2133	2138	2150	VV 2	77051	1293756	8.95%	2.868%
28	14.752	2150	2157	2162	VV 9	5658	145443	1.01%	0.322%
29	14.811	2162	2167	2180	VV 4	10230	338828	2.34%	0.751%
30	14.929	2180	2187	2206	VV	156187	2638861	18.25%	5.850%
31	15.076	2206	2212	2216	VV 6	14832	348766	2.41%	0.773%
32	15.117	2216	2219	2225	VV 3	14535	297667	2.06%	0.660%
33	15.170	2225	2228	2232	VV 5	8607	166441	1.15%	0.369%
34	15.258	2232	2243	2247	VV 2	107989	1974445	13.66%	4.377%
35	15.317	2247	2253	2266	VV 2	288510	5671579	39.23%	12.573%
36	15.411	2266	2269	2274	VV 6	7066	144694	1.00%	0.321%
37	15.493	2277	2283	2289	VV 5	8717	206589	1.43%	0.458%
38	15.557	2289	2294	2301	VV 6	6584	150778	1.04%	0.334%
39	15.646	2305	2309	2319	VV 10	3132	107480	0.74%	0.238%
40	15.869	2344	2347	2351	VV 6	1587	28967	0.20%	0.064%
41	15.963	2357	2363	2366	VV 8	1729	39102	0.27%	0.087%
42	16.116	2386	2389	2399	VV 8	1488	42836	0.30%	0.095%
43	16.216	2399	2406	2415	VV 8	1607	50287	0.35%	0.111%

PA1S740

44	16.568	2460	2466	2474	VV	8	3016	73804	0.51%	0.164%
45	17.032	2538	2545	2553	VV	8	2203	54284	0.38%	0.120%

Sum of corrected areas: 45107548

Table F24 Area percent report for HS-SPME (PA fibre coating) at 60 °C.

PA1S760
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PA1S760.D Vial: 1
Acq On : 11 Apr 2007 12:02 Operator: Patricia
Sample : sample 7 Pa1 temp. -60C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.273	26	33	39	PV 8	2319	68726	0.10%	0.035%
2	2.367	39	49	75	VV	31485	817637	1.23%	0.413%
3	2.649	86	97	100	PV 3	79133	1330301	2.01%	0.672%
4	2.690	100	104	151	VV	118876	4274471	6.45%	2.160%
5	3.366	210	219	254	VV	430207	10834562	16.34%	5.475%
6	3.583	254	256	285	VV	4666	194671	0.29%	0.098%
7	3.812	285	295	306	VV 4	7502	214835	0.32%	0.109%
8	3.924	306	314	356	VV 2	48041	1656875	2.50%	0.837%
9	4.318	365	381	406	VV	33977	1158465	1.75%	0.585%
10	4.635	421	435	439	VV 3	9942	256228	0.39%	0.129%
11	4.717	439	449	453	VV 3	61751	1675720	2.53%	0.847%
12	4.788	453	461	475	VV	399463	11818416	17.83%	5.973%
13	4.923	475	484	536	VV	599048	19547780	29.49%	9.879%
14	5.645	589	607	609	VV	1380	63352	0.10%	0.032%
15	6.016	656	670	688	PV	2042	98593	0.15%	0.050%
16	6.298	707	718	748	PV 9	7181	390030	0.59%	0.197%
17	6.486	748	750	766	VV 9	866	31413	0.05%	0.016%
18	8.707	1115	1128	1131	BV 9	1628	47606	0.07%	0.024%
19	8.754	1131	1136	1146	VV 9	1268	35291	0.05%	0.018%
20	10.416	1404	1419	1453	PV 9	1867	153686	0.23%	0.078%
21	12.584	1772	1788	1796	PV 9	1190	53518	0.08%	0.027%
22	13.266	1896	1904	1922	VV 9	2426	90973	0.14%	0.046%
23	13.419	1922	1930	1934	VV 3	29574	590024	0.89%	0.298%
24	13.454	1934	1936	1958	VV 2	25811	579353	0.87%	0.293%
25	13.630	1958	1966	1978	VV 4	11180	260804	0.39%	0.132%
26	13.942	2013	2019	2027	VV 9	4308	111688	0.17%	0.056%
27	14.024	2027	2033	2038	VV	19836	371232	0.56%	0.188%
28	14.083	2038	2043	2051	VV 2	74205	1321017	1.99%	0.668%
29	14.171	2051	2058	2075	VV	1272289	20934563	31.58%	10.580%
30	14.294	2075	2079	2089	VV 10	4371	145912	0.22%	0.074%
31	14.435	2089	2103	2113	VV	20362	556382	0.84%	0.281%
32	14.559	2113	2124	2133	VV	4105443	66289211	100.00%	33.500%
33	14.641	2133	2138	2149	VV	360821	5866754	8.85%	2.965%
34	14.717	2149	2151	2153	VV 3	8546	128360	0.19%	0.065%
35	14.758	2153	2158	2161	VV 4	25421	432912	0.65%	0.219%
36	14.805	2161	2166	2180	VV 2	65531	1586405	2.39%	0.802%
37	14.929	2180	2187	2203	VV	673938	10959875	16.53%	5.539%
38	15.076	2203	2212	2216	VV 4	64184	1456211	2.20%	0.736%
39	15.117	2216	2219	2224	VV 2	54430	1060803	1.60%	0.536%
40	15.170	2224	2228	2232	VV 2	29584	583277	0.88%	0.295%
41	15.258	2232	2243	2247	VV	406437	7483370	11.29%	3.782%
42	15.317	2247	2253	2265	VV 2	1017164	19358621	29.20%	9.783%
43	15.411	2265	2269	2274	VV 3	24206	493716	0.74%	0.250%

PA1S760

44	15.487	2274	2282	2288	VV	2	24382	586533	0.88%	0.296%
45	15.558	2288	2294	2305	VV	3	21790	494773	0.75%	0.250%
46	15.652	2305	2310	2327	VV	9	7628	349972	0.53%	0.177%
47	15.863	2339	2346	2354	VV	9	3173	130826	0.20%	0.066%
48	15.957	2354	2362	2380	VV	9	4432	195755	0.30%	0.099%
49	16.116	2380	2389	2398	VV	9	2517	115439	0.17%	0.058%
50	16.216	2398	2406	2422	VV	9	3730	157825	0.24%	0.080%
51	16.574	2460	2467	2474	VV	9	5140	141439	0.21%	0.071%
52	16.633	2474	2477	2490	VV	9	2016	82110	0.12%	0.041%
53	17.032	2535	2545	2557	VV	9	4230	146132	0.22%	0.074%
54	17.126	2557	2561	2576	VV	10	1684	70328	0.11%	0.036%
55	19.101	2886	2897	2903	PV	9	888	23284	0.04%	0.012%

Sum of corrected areas: 197878057

Table F25 Area percent report for HS-SPME (PA fibre coating) at 80 °C.

PA1S780
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\PA1S780.D Vial: 1
Acq On : 11 Apr 2007 12:59 Operator: Patricia
Sample : sample 7 Pa1 temp. -80C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.249	19	29	42	PV 3	15595	465397	0.16%	0.046%
2	2.361	42	48	79	VV	108295	2243301	0.79%	0.221%
3	2.637	84	95	98	PV 2	432402	6922308	2.43%	0.682%
4	2.684	98	103	140	VV 2	668955	17582635	6.16%	1.733%
5	3.366	208	219	252	VV	3454584	81687927	28.64%	8.052%
6	3.583	252	256	264	VV 4	30281	839868	0.29%	0.083%
7	3.642	264	266	286	VV 4	11729	479239	0.17%	0.047%
8	3.812	286	295	305	VV	52421	1273208	0.45%	0.126%
9	3.918	305	313	345	VV	454547	12080685	4.24%	1.191%
10	4.153	345	353	367	VV	10130	414384	0.15%	0.041%
11	4.312	367	380	403	VV	307128	8644034	3.03%	0.852%
12	4.488	403	410	424	VV 4	9182	269393	0.09%	0.027%
13	4.629	424	434	438	PV	69067	1586650	0.56%	0.156%
14	4.717	438	449	453	VV	470180	14033010	4.92%	1.383%
15	4.793	453	462	475	VV	3040705	87442783	30.66%	8.619%
16	4.929	475	485	539	VV	4485099	131530646	46.11%	12.965%
17	5.299	539	548	570	VV 4	13470	487787	0.17%	0.048%
18	5.475	570	578	580	PV 8	3791	81371	0.03%	0.008%
19	5.610	594	601	615	VV 8	7427	280560	0.10%	0.028%
20	6.016	655	670	685	BV 6	15734	635612	0.22%	0.063%
21	6.180	691	698	703	VV 6	7020	212907	0.07%	0.021%
22	6.274	703	714	737	VV 3	113972	4722269	1.66%	0.465%
23	6.468	737	747	776	VV 3	11599	866774	0.30%	0.085%
24	7.279	870	885	896	PV 3	7861	372203	0.13%	0.037%
25	7.937	987	997	1016	PV 3	3709	174770	0.06%	0.017%
26	8.665	1106	1121	1161	PV 3	56772	3539382	1.24%	0.349%
27	8.971	1161	1173	1189	VV 3	6624	429772	0.15%	0.042%
28	9.529	1257	1268	1270	VV 10	4529	98771	0.03%	0.010%
29	9.688	1279	1295	1314	VV 10	8862	530066	0.19%	0.052%
30	10.352	1394	1408	1443	PV 5	56922	2985402	1.05%	0.294%
31	12.561	1765	1784	1793	PV 4	17856	471583	0.17%	0.046%
32	12.643	1793	1798	1814	VV 6	5450	165995	0.06%	0.016%
33	13.266	1897	1904	1920	VV 4	9920	302799	0.11%	0.030%
34	13.419	1920	1930	1934	VV	156905	3090294	1.08%	0.305%
35	13.454	1934	1936	1949	VV 3	123508	2388478	0.84%	0.235%
36	13.630	1958	1966	1983	VV	82721	1603532	0.56%	0.158%
37	13.948	2013	2020	2027	VV 3	26923	606424	0.21%	0.060%
38	14.024	2027	2033	2038	VV 2	135552	2508712	0.88%	0.247%
39	14.083	2038	2043	2051	VV	348348	6084267	2.13%	0.600%
40	14.177	2051	2059	2069	VV	4831417	81281826	28.50%	8.012%
41	14.247	2069	2071	2076	VV 3	26261	481495	0.17%	0.047%
42	14.300	2076	2080	2089	VV 6	13342	394334	0.14%	0.039%
43	14.412	2089	2099	2100	VV 3	38326	670027	0.23%	0.066%

PA1S780

44	14.441	2100	2104	2115	VV	3	79599	2120473	0.74%	0.209%
45	14.582	2115	2128	2134	VV		12985302	285233004	100.00%	28.116%
46	14.647	2134	2139	2149	VV		1814307	28041030	9.83%	2.764%
47	14.717	2149	2151	2154	VV	2	41170	590096	0.21%	0.058%
48	14.758	2154	2158	2161	VV		158622	2427031	0.85%	0.239%
49	14.800	2161	2165	2179	VV		434738	8736068	3.06%	0.861%
50	14.929	2182	2187	2193		M	3599628	49774136	17.45%	4.906%
51	14.982	2194	2196	2203	VV		110155	2310664	0.81%	0.228%
52	15.076	2203	2212	2216	VV	2	381187	7914673	2.77%	0.780%
53	15.117	2216	2219	2224	VV	2	261617	4904017	1.72%	0.483%
54	15.170	2224	2228	2231	VV	2	128026	2432220	0.85%	0.240%
55	15.264	2231	2244	2248	VV		2081475	38242731	13.41%	3.770%
56	15.322	2248	2254	2265	VV	2	4644262	88395848	30.99%	8.713%
57	15.405	2265	2268	2274	VV		111112	1893852	0.66%	0.187%
58	15.487	2274	2282	2288	VV		112977	2430955	0.85%	0.240%
59	15.552	2288	2293	2304	VV	3	105636	2005129	0.70%	0.198%
60	15.657	2304	2311	2319	VV	4	33750	953648	0.33%	0.094%
61	15.716	2319	2321	2334	VV	4	10683	357638	0.13%	0.035%
62	15.845	2334	2343	2356	VV	3	17746	596501	0.21%	0.059%
63	15.951	2356	2361	2370	VV	5	18770	413354	0.14%	0.041%
64	16.133	2388	2392	2398	VV	9	5804	141668	0.05%	0.014%
65	16.210	2398	2405	2421	VV	10	13183	419125	0.15%	0.041%
66	16.568	2459	2466	2473	VV	3	26959	570666	0.20%	0.056%
67	16.633	2473	2477	2485	VV	10	6552	170419	0.06%	0.017%
68	17.026	2527	2544	2554	PV	5	19248	455755	0.16%	0.045%

Sum of corrected areas: 1014493578

Table F26 Area percent report for the headspace analysis at room temperature.

HSMKS7RT
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\HSMKS7RT.D Vial: 1
Acq On : 12 Apr 2007 10:07 Operator: Patricia
Sample : sample 7 headspace Mk room temp. Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.361	43	48	80	VV 5	4335	120592	5.24%	1.767%
2	2.666	95	100	124	VV 5	2388	115321	5.01%	1.689%
3	3.354	209	217	247	PV	113511	2300421	100.00%	33.698%
4	3.918	302	313	324	PV 5	3411	91451	3.98%	1.340%
5	4.306	335	379	391	BV 4	2376	72106	3.13%	1.056%
6	4.711	440	448	452	VV 8	3191	79365	3.45%	1.163%
7	4.782	452	460	474	VV 3	18005	521618	22.67%	7.641%
8	4.917	474	483	545	VB 3	16078	547785	23.81%	8.024%
9	14.171	2041	2058	2068	BV 5	9019	151944	6.61%	2.226%
10	14.553	2112	2123	2133	PV 2	101763	1511485	65.70%	22.141%
11	14.641	2133	2138	2153	VV 5	7545	187196	8.14%	2.742%
12	14.805	2153	2166	2169	VV 5	2007	91836	3.99%	1.345%
13	14.923	2178	2186	2192	VV 2	14365	254798	11.08%	3.732%
14	14.982	2192	2196	2207	VV 2	2625	104168	4.53%	1.526%
15	15.258	2226	2243	2247	VV 2	6968	178907	7.78%	2.621%
16	15.317	2247	2253	2291	VV 3	18918	405914	17.65%	5.946%
17	18.160	2713	2737	2751	PV 2	2001	91578	3.98%	1.342%

Sum of corrected areas: 6826486

Table F27 Area percent report for the headspace analysis at 40 °C.

HSMKS740
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\HSMKS740.D Vial: 1
Acq On : 12 Apr 2007 10:58 Operator: Patricia
Sample : sample 7 headspace Mk temp. - 40C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.373	36	50	70	PV 6	3393	93739	1.57%	0.755%
2	2.714	97	108	139	PV 6	2194	132691	2.22%	1.069%
3	3.360	198	218	238	BV	318896	5977910	100.00%	48.151%
4	3.577	247	255	273	VV	1143	37764	0.63%	0.304%
5	3.806	283	294	303	PV 6	1792	46942	0.79%	0.378%
6	3.918	307	313	323	M3	7390	186075	3.11%	1.499%
7	4.312	369	380	404	VV 3	5909	186469	3.12%	1.502%
8	4.717	440	449	453	VV 4	6879	176368	2.95%	1.421%
9	4.788	453	461	475	VV	46605	1257931	21.04%	10.132%
10	4.923	475	484	532	VV 3	35244	1223473	20.47%	9.855%
11	13.636	1954	1967	1971	PV 9	864	23766	0.40%	0.191%
12	14.083	2039	2043	2050	VV 8	874	15827	0.26%	0.127%
13	14.171	2050	2058	2075	PV 3	10614	185697	3.11%	1.496%
14	14.553	2116	2123	2132	VV	128672	1852951	31.00%	14.925%
15	14.641	2132	2138	2152	VV 4	7766	153823	2.57%	1.239%
16	14.747	2152	2156	2162	VV 9	988	23363	0.39%	0.188%
17	14.817	2162	2168	2178	VV 9	1054	34959	0.58%	0.282%
18	14.923	2181	2186	2191	M3	13606	191487	3.20%	1.542%
19	15.076	2206	2212	2215	VV 8	1695	43856	0.73%	0.353%
20	15.117	2215	2219	2224	VV 8	1619	35039	0.59%	0.282%
21	15.258	2231	2243	2247	VV 7	7418	150168	2.51%	1.210%
22	15.317	2247	2253	2274	VV 3	18216	384538	6.43%	3.097%

Sum of corrected areas: 12414837

Table F28 Area percent report for the headspace analysis at 60 °C.

HSMKS760
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\HSMKS760.D Vial: 1
Acq On : 12 Apr 2007 11:53 Operator: Patricia
Sample : sample 7 headspace mk temp.- 60C Inst : Instrumen
Misc : 0.1 mL injection Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.279	28	34	41	VV 8	843	20935	0.06%	0.028%
2	2.367	41	49	65	VV 2	13032	298154	0.92%	0.402%
3	2.661	89	99	101	PV 4	14060	237789	0.73%	0.321%
4	2.690	101	104	136	VV 5	18301	804743	2.48%	1.085%
5	3.360	207	218	238	VV	1617277	32513138	100.00%	43.831%
6	3.577	242	255	265	VV 6	6536	167456	0.52%	0.226%
7	3.806	286	294	304	VV	11547	261461	0.80%	0.352%
8	3.918	304	313	341	VV 2	68548	1857897	5.71%	2.505%
9	4.306	363	379	400	VV	40987	1146702	3.53%	1.546%
10	4.482	400	409	417	VV 9	1185	35018	0.11%	0.047%
11	4.623	423	433	437	VV 3	5723	136796	0.42%	0.184%
12	4.711	437	448	452	VV 2	55455	1463739	4.50%	1.973%
13	4.782	452	460	472	VV	355893	9630402	29.62%	12.983%
14	4.911	472	482	503	VV	349632	9956642	30.62%	13.423%
15	6.016	667	670	678	VV 8	578	13374	0.04%	0.018%
16	13.413	1921	1929	1942	PV 8	1203	43819	0.13%	0.059%
17	13.624	1956	1965	1970	PV 4	3073	57476	0.18%	0.077%
18	13.936	2014	2018	2026	VV 9	1045	23182	0.07%	0.031%
19	14.024	2026	2033	2039	VV 7	3758	73808	0.23%	0.100%
20	14.083	2039	2043	2051	VV 9	2727	47211	0.15%	0.064%
21	14.171	2051	2058	2069	PV 2	41850	635052	1.95%	0.856%
22	14.247	2069	2071	2076	VV 6	1224	14946	0.05%	0.020%
23	14.377	2089	2093	2095	BV 5	1226	15440	0.05%	0.021%
24	14.429	2095	2102	2107	VV 5	2592	53505	0.16%	0.072%
25	14.553	2114	2123	2132	VV	713372	10149304	31.22%	13.682%
26	14.641	2132	2138	2153	VV	44860	730632	2.25%	0.985%
27	14.753	2153	2157	2163	VV 7	3696	83827	0.26%	0.113%
28	14.811	2163	2167	2181	VV 7	3444	136104	0.42%	0.183%
29	14.929	2181	2187	2193	VV	67965	1028695	3.16%	1.387%
30	14.976	2193	2195	2203	VV 9	3460	87000	0.27%	0.117%
31	15.070	2203	2211	2216	VV 8	6695	173864	0.53%	0.234%
32	15.117	2216	2219	2225	VV 8	6066	125298	0.39%	0.169%
33	15.170	2225	2228	2232	VV 6	2790	60285	0.19%	0.081%
34	15.258	2232	2243	2247	VV 3	33292	595759	1.83%	0.803%
35	15.317	2247	2253	2266	VV 2	79762	1486673	4.57%	2.004%
36	15.552	2288	2293	2296	PV 7	1142	12026	0.04%	0.016%

Sum of corrected areas: 74178153

Table F29 Area percent report for the headspace analysis at 80 °C.

HSMKS780
Area Percent Report

Data File : C:\MSDCHEM\1\DATA\HSMKS780.D Vial: 1
Acq On : 12 Apr 2007 12:51 Operator: Patricia
Sample : sample 7 headspace mk temp.- 80C Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.267	24	32	36	VV 9	1595	43966	0.07%	0.031%
2	2.302	36	38	43	VV 4	1221	18004	0.03%	0.013%
3	2.361	43	48	72	VV 2	19740	436416	0.72%	0.307%
4	2.608	82	90	91	VV 5	1600	16036	0.03%	0.011%
5	2.649	91	97	99	VV 2	38174	640365	1.06%	0.451%
6	2.684	99	103	121	VV	60975	1782733	2.94%	1.254%
7	2.984	147	154	161	VV	2380	85149	0.14%	0.060%
8	3.236	191	197	208	VV	1423	44951	0.07%	0.032%
9	3.360	208	218	240	PV	3242582	60588924	100.00%	42.626%
10	3.583	248	256	274	BV 3	12335	259519	0.43%	0.183%
11	3.806	282	294	304	PV	21475	436427	0.72%	0.307%
12	3.918	304	313	333	VV	152967	3734569	6.16%	2.627%
13	4.041	333	334	343	VV 9	1615	38430	0.06%	0.027%
14	4.124	343	348	351	VV 7	1304	24753	0.04%	0.017%
15	4.153	351	353	356	VV 4	1478	18232	0.03%	0.013%
16	4.306	366	379	398	VV	97866	2496320	4.12%	1.756%
17	4.488	401	410	421	VV 8	3105	97781	0.16%	0.069%
18	4.623	425	433	437	VV 3	9924	234983	0.39%	0.165%
19	4.711	437	448	452	VV 2	120657	3212675	5.30%	2.260%
20	4.782	452	460	472	VV	775103	19635603	32.41%	13.814%
21	4.917	472	483	520	VV	825184	21807761	35.99%	15.342%
22	5.293	540	547	558	VV	3874	144673	0.24%	0.102%
23	6.021	658	671	680	VV	2328	88519	0.15%	0.062%
24	6.292	707	717	731	PV	2822	124414	0.21%	0.088%
25	8.712	1127	1129	1132	PV 4	406	2895	0.00%	0.002%
26	10.363	1405	1410	1412	PV 5	620	8000	0.01%	0.006%
27	10.399	1412	1416	1425	VV 9	1107	21112	0.03%	0.015%
28	12.573	1784	1786	1789	VV 4	625	6771	0.01%	0.005%
29	13.413	1924	1929	1934	PV 7	1711	35616	0.06%	0.025%
30	13.454	1934	1936	1941	VV 6	1432	19364	0.03%	0.014%
31	13.624	1957	1965	1971	VV 5	5022	87745	0.14%	0.062%
32	13.936	2013	2018	2025	VV 8	1845	34207	0.06%	0.024%
33	14.024	2025	2033	2039	VV 6	5882	115987	0.19%	0.082%
34	14.083	2039	2043	2048	VV 6	4331	75537	0.12%	0.053%
35	14.171	2051	2058	2066	VV 2	76411	1136230	1.88%	0.799%
36	14.241	2066	2070	2076	VV 6	1908	40138	0.07%	0.028%
37	14.429	2089	2102	2110	VV 10	3803	113788	0.19%	0.080%
38	14.553	2114	2123	2132	VV	1176363	17096903	28.22%	12.028%
39	14.641	2132	2138	2149	VV	65272	1035657	1.71%	0.729%
40	14.717	2149	2151	2153	VV 3	2206	30379	0.05%	0.021%
41	14.752	2153	2157	2162	VV 6	6384	118207	0.20%	0.083%
42	14.811	2162	2167	2180	VV 10	5985	203560	0.34%	0.143%
43	14.923	2180	2186	2193	VV	117841	1742334	2.88%	1.226%

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44	14.976	2193	2195	2201	VV	5	4550	100724	0.17%	0.071%
45	15.070	2205	2211	2216	VV	7	10644	254618	0.42%	0.179%
46	15.117	2216	2219	2224	VV	5	7694	164691	0.27%	0.116%
47	15.164	2224	2227	2231	VV	6	3575	75119	0.12%	0.053%
48	15.258	2231	2243	2247	VV	2	54916	1017250	1.68%	0.716%
49	15.317	2247	2253	2267	VV	2	129004	2440532	4.03%	1.717%
50	15.411	2267	2269	2274	VV	6	2348	39140	0.06%	0.028%
51	15.487	2274	2282	2289	VV	6	2271	70584	0.12%	0.050%
52	15.552	2289	2293	2303	VV	6	1825	41686	0.07%	0.029%

Sum of corrected areas: 142139977

Table F30 Area percent report for the essential oil obtained from the solvent extraction with the internal standard added at the start of the extraction.

DCMSEA1
Area Percent Report

Data File : D:\PATRICIA1\DCMSEA1.D Vial: 42
Acq On : 18 Jun 2009 11:01 Operator: Patricia
Sample : dcm solvent ext. a- int.std. added at st Inst : Instrumen
Misc : solvent ext.a int.std. added at start of Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.197	4	20	28	BV 5	45398	717274	0.14%	0.028%
2	2.461	50	65	87	PV 2	409740	10644107	2.06%	0.423%
3	3.154	171	183	207	PV	2063288	43131175	8.37%	1.712%
4	3.683	261	273	296	VV	185118	4876948	0.95%	0.194%
5	4.059	321	337	355	PV	154946	4390974	0.85%	0.174%
6	4.359	373	388	391	PV 2	37016	907931	0.18%	0.036%
7	4.512	391	414	425	VV	2223500	72211886	14.01%	2.867%
8	4.635	425	435	452	VV	1341324	40370526	7.83%	1.603%
9	4.788	452	461	490	VV 7	50245	2739733	0.53%	0.109%
10	5.928	648	655	665	VV 5	18318	522694	0.10%	0.021%
11	8.207	1025	1043	1076	BB 6	15401	819163	0.16%	0.033%
12	9.888	1300	1329	1366	BV 4	73318	4089172	0.79%	0.162%
13	12.173	1687	1718	1728	PV 7	20444	718092	0.14%	0.029%
14	12.361	1728	1750	1759	PV	71848	1469893	0.29%	0.058%
15	12.749	1810	1816	1826	VV 8	11795	280673	0.05%	0.011%
16	12.914	1826	1844	1850	VV 8	13431	324587	0.06%	0.013%
17	13.008	1850	1860	1864	VV 9	17036	318757	0.06%	0.013%
18	13.243	1889	1900	1905	VV 2	109207	2057363	0.40%	0.082%
19	13.284	1905	1907	1915	VV 2	36090	605364	0.12%	0.024%
20	13.401	1915	1927	1930	VV 2	14469	354061	0.07%	0.014%
21	13.454	1930	1936	1949	VV	171915	3159135	0.61%	0.125%
22	13.666	1966	1972	1985	VV 2	79364	2493578	0.48%	0.099%
23	13.777	1985	1991	1998	VV 3	73401	1761437	0.34%	0.070%
24	13.860	1998	2005	2013	VV	319245	6835958	1.33%	0.271%
25	13.924	2013	2016	2025	VV 2	87092	2133587	0.41%	0.085%
26	14.012	2025	2031	2038	VV	1473537	22309856	4.33%	0.886%
27	14.083	2038	2043	2048	VV 4	129859	2613360	0.51%	0.104%
28	14.130	2048	2051	2059	VV 5	56156	1555646	0.30%	0.062%
29	14.277	2059	2076	2081	VV 2	236407	8248760	1.60%	0.328%
30	14.318	2081	2083	2088	VV 5	112287	2087774	0.40%	0.083%
31	14.412	2088	2099	2106	VV	26179448	515590408	100.00%	20.471%
32	14.488	2106	2112	2122	VV 2	4940550	76027553	14.75%	3.019%
33	14.559	2122	2124	2126	VV 2	188925	2684261	0.52%	0.107%
34	14.600	2126	2131	2135	VV	1182722	17880048	3.47%	0.710%
35	14.647	2135	2139	2151	VV 2	1517488	31280341	6.07%	1.242%
36	14.776	2151	2161	2167	VV	8130570	119897867	23.25%	4.760%
37	14.829	2167	2170	2177	VV	377886	9042939	1.75%	0.359%
38	14.917	2177	2185	2190	VV 2	1735867	35447995	6.88%	1.407%
39	14.964	2190	2193	2203	VV	1287628	28109385	5.45%	1.116%
40	15.111	2203	2218	2222	VV	13203869	236273206	45.83%	9.381%
41	15.170	2222	2228	2237	VV 2	23014258	447510451	86.80%	17.768%
42	15.240	2237	2240	2245	VV	888527	18271390	3.54%	0.725%
43	15.329	2245	2255	2260	VV 2	1085636	36187406	7.02%	1.437%

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44	15.387	2260	2265	2276	VV	3	1091334	31483250	6.11%	1.250%
45	15.487	2276	2282	2289	VV	3	604487	19030725	3.69%	0.756%
46	15.552	2289	2293	2304	VV	5	432404	16524617	3.20%	0.656%
47	15.669	2304	2313	2326	VV		847503	25095709	4.87%	0.996%
48	15.775	2326	2331	2334	VV	5	205184	5181599	1.00%	0.206%
49	15.816	2334	2338	2347	VV	5	211785	7792508	1.51%	0.309%
50	15.957	2347	2362	2370	VV	4	620769	24625629	4.78%	0.978%
51	16.063	2370	2380	2390	VV	2	635308	27398700	5.31%	1.088%
52	16.151	2390	2395	2405	VV	7	212995	8654222	1.68%	0.344%
53	16.257	2405	2413	2420	VV	3	265720	8952503	1.74%	0.355%
54	16.322	2420	2424	2427	VV	5	177672	4033457	0.78%	0.160%
55	16.374	2427	2433	2440	VV		1502861	34375486	6.67%	1.365%
56	16.439	2440	2444	2450	VV	3	580337	12579509	2.44%	0.499%
57	16.504	2450	2455	2458	VV	5	189950	4553169	0.88%	0.181%
58	16.551	2458	2463	2465	VV	4	264866	5458492	1.06%	0.217%
59	16.586	2465	2469	2483	VV	2	450206	14270759	2.77%	0.567%
60	16.692	2483	2487	2491	VV	6	95200	2347378	0.46%	0.093%
61	16.750	2491	2497	2499	VV	3	181409	3933966	0.76%	0.156%
62	16.833	2499	2511	2520	VV		2901092	65954132	12.79%	2.619%
63	16.921	2520	2526	2536	VV	6	319490	11501541	2.23%	0.457%
64	17.021	2536	2543	2562	VV	7	202756	10463858	2.03%	0.415%
65	17.185	2562	2571	2578	VV	7	93765	3588042	0.70%	0.142%
66	17.291	2578	2589	2597	VV	7	72528	4023016	0.78%	0.160%
67	17.373	2597	2603	2612	VV	7	62358	2542974	0.49%	0.101%
68	17.479	2612	2621	2625	VV	10	41501	1476259	0.29%	0.059%
69	17.614	2633	2644	2658	VV	8	96383	4742904	0.92%	0.188%
70	17.961	2670	2703	2764	VV	8	111410	18794438	3.65%	0.746%
71	18.372	2764	2773	2794	VV	8	28472	1808871	0.35%	0.072%
72	18.560	2794	2805	2812	VV	8	18962	542799	0.11%	0.022%
73	18.895	2824	2862	2915	PV	2	2349798	166890512	32.37%	6.626%
74	19.300	2915	2931	2951	VV	4	363334	21537734	4.18%	0.855%
75	19.441	2951	2955	2975	VV	4	87569	5891545	1.14%	0.234%
76	19.647	2975	2990	2996	VV	3	595987	24908619	4.83%	0.989%
77	19.776	2996	3012	3078	VV	2	1128730	98711063	19.15%	3.919%

Sum of corrected areas: 2518622696

Table F31 Area percent report for the essential oil obtained from the solvent extraction with the internal standard added at the end of the extraction.

DCMSEB1
Area Percent Report

Data File : D:\PATRICIA1\DCMSEB1.D Vial: 42
Acq On : 18 Jun 2009 14:02 Operator: Patricia
Sample : dcm sol. ext. b- int.std. added at start Inst : Instrumen
Misc : sol. ext.a int.std. added at start of in Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.196	14	20	27	VV 5	47004	806298	0.15%	0.027%
2	2.461	50	65	86	BV 2	478049	11773926	2.14%	0.396%
3	3.154	170	183	204	PV	2008662	42630473	7.76%	1.434%
4	3.677	260	272	290	VV	201294	5258771	0.96%	0.177%
5	4.053	317	336	357	BV	160778	4470680	0.81%	0.150%
6	4.359	377	388	391	BV	40662	998687	0.18%	0.034%
7	4.447	391	403	405	VV	296195	7900752	1.44%	0.266%
8	4.511	405	414	425	VV	2441169	69984609	12.74%	2.355%
9	4.635	425	435	451	VV	1454606	41235161	7.51%	1.388%
10	4.788	451	461	472	VV 5	41103	1814063	0.33%	0.061%
11	9.899	1281	1331	1381	BV 5	77676	4532024	0.82%	0.152%
12	12.179	1704	1719	1726	PV 8	22119	617699	0.11%	0.021%
13	12.379	1726	1753	1761	PV	84761	1720360	0.31%	0.058%
14	13.248	1893	1901	1905	PV	108990	1916926	0.35%	0.065%
15	13.290	1905	1908	1915	VV 2	39542	666747	0.12%	0.022%
16	13.460	1925	1937	1952	VV	171667	3597371	0.65%	0.121%
17	13.695	1970	1977	1986	VV 2	107628	2772095	0.50%	0.093%
18	13.783	1986	1992	2000	VV 4	86089	2092304	0.38%	0.070%
19	13.865	2000	2006	2014	VV	340972	7394060	1.35%	0.249%
20	13.930	2014	2017	2026	VV 5	101892	2680977	0.49%	0.090%
21	14.024	2026	2033	2040	VV	1580247	24215944	4.41%	0.815%
22	14.089	2040	2044	2048	VV 2	143102	2957196	0.54%	0.100%
23	14.136	2048	2052	2061	VV 2	79615	2969339	0.54%	0.100%
24	14.253	2061	2072	2074	VV 4	206739	5265852	0.96%	0.177%
25	14.283	2074	2077	2082	VV	292004	6105310	1.11%	0.205%
26	14.329	2082	2085	2089	VV 3	157147	3277643	0.60%	0.110%
27	14.424	2089	2101	2107	VV	27820767	549369164	100.00%	18.486%
28	14.494	2107	2113	2123	VV 2	5258845	85088954	15.49%	2.863%
29	14.565	2123	2125	2128	VV 2	325778	5374238	0.98%	0.181%
30	14.606	2128	2132	2136	VV	1325978	22131026	4.03%	0.745%
31	14.653	2136	2140	2151	VV	1884535	41816162	7.61%	1.407%
32	14.788	2151	2163	2168	VV	8785895	143093332	26.05%	4.815%
33	14.841	2168	2172	2175	VV 2	1001393	23542879	4.29%	0.792%
34	14.929	2175	2187	2191	VV 3	2943331	100405074	18.28%	3.379%
35	14.970	2191	2194	2205	VV 2	2338687	73538712	13.39%	2.474%
36	15.117	2205	2219	2223	VV	14160772	269587589	49.07%	9.071%
37	15.181	2223	2230	2238	VV 2	24920546	476287520	86.70%	16.026%
38	15.246	2238	2241	2246	VV 2	1120908	25337570	4.61%	0.853%
39	15.334	2246	2256	2262	VV 2	1189555	45608248	8.30%	1.535%
40	15.393	2262	2266	2278	VV 3	1245227	42460304	7.73%	1.429%
41	15.493	2278	2283	2291	VV 3	801962	26359794	4.80%	0.887%
42	15.563	2291	2295	2306	VV 4	580335	23812887	4.33%	0.801%
43	15.681	2306	2315	2328	VV	1065140	38514614	7.01%	1.296%

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44	15.787	2328	2333	2337	VV	5	338383	9398054	1.71%	0.316%
45	15.834	2337	2341	2343	VV	4	324479	6882469	1.25%	0.232%
46	15.869	2343	2347	2351	VV	2	340255	8060283	1.47%	0.271%
47	15.922	2351	2356	2358	VV	5	389928	9732838	1.77%	0.327%
48	15.981	2358	2366	2374	VV	3	740048	26247486	4.78%	0.883%
49	16.063	2374	2380	2382	VV	2	717427	15976947	2.91%	0.538%
50	16.086	2382	2384	2395	VV	5	742908	21459007	3.91%	0.722%
51	16.180	2395	2400	2408	VV	7	338198	13182692	2.40%	0.444%
52	16.280	2408	2417	2425	VV	3	358761	15669132	2.85%	0.527%
53	16.345	2425	2428	2430	VV	4	268817	4692522	0.85%	0.158%
54	16.392	2430	2436	2445	VV	2	1435897	40195358	7.32%	1.353%
55	16.468	2445	2449	2454	VV	3	637535	14809729	2.70%	0.498%
56	16.574	2462	2467	2469	VV	6	354781	7656826	1.39%	0.258%
57	16.615	2469	2474	2487	VV	2	536252	18668633	3.40%	0.628%
58	16.774	2494	2501	2503	VV	5	240470	6259687	1.14%	0.211%
59	16.862	2503	2516	2525	VV	2	2714244	69521325	12.65%	2.339%
60	16.944	2525	2530	2540	VV	8	363505	15374805	2.80%	0.517%
61	17.044	2540	2547	2556	VV	6	253897	10687227	1.95%	0.360%
62	17.220	2570	2577	2583	VV	6	138966	5076588	0.92%	0.171%
63	17.649	2638	2650	2666	VV	6	114986	7561078	1.38%	0.254%
64	17.943	2666	2700	2741	VV	6	295599	41121990	7.49%	1.384%
65	18.190	2741	2742	2750	VV	8	63503	1755095	0.32%	0.059%
66	18.689	2805	2827	2829	VV	8	68465	2893239	0.53%	0.097%
67	18.801	2829	2846	2848	VV	2	621627	18757455	3.41%	0.631%
68	18.824	2848	2850	2853	VV	3	624211	10427272	1.90%	0.351%
69	18.912	2853	2865	2908	VV	3	2284549	160790126	29.27%	5.410%
70	19.224	2908	2918	2924	VV	3	108227	4936274	0.90%	0.166%
71	19.318	2924	2934	2964	VV	3	329315	18377663	3.35%	0.618%
72	19.665	2964	2993	3003	PV	2	539771	30651254	5.58%	1.031%
73	19.811	3003	3018	3045	VBA2		1480094	103071020	18.76%	3.468%

Sum of corrected areas: 2971877407

Table F32 Area percent report for the essential oil obtained from the Soxhlet extraction with the internal standard added at the start of the extraction.

DSOXAA7
Area Percent Report

Data File : D:\PATRICIA1\DSOXAA7.D Vial: 1
Acq On : 26 Jun 2009 12:46 Operator: PATRICIA
Sample : DCM SOXA.I.S. ADDED AT START Inst : Instrumen
Misc : INT. STD. ADDED AT START OF EXTRACTION Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.191	4	19	27	BV 4	109938	1711429	0.21%	0.035%
2	2.461	49	65	86	PV 2	683431	16708592	2.05%	0.343%
3	2.984	146	154	166	VV 2	48468	1286219	0.16%	0.026%
4	3.154	166	183	203	PV	2563370	51295625	6.29%	1.052%
5	3.407	214	226	235	PV 5	49014	1706657	0.21%	0.035%
6	3.507	235	243	251	VV 3	25733	1169895	0.14%	0.024%
7	3.683	258	273	285	VV	245450	6873617	0.84%	0.141%
8	4.059	322	337	359	BV	215871	5814372	0.71%	0.119%
9	4.359	377	388	392	BV 2	40221	972404	0.12%	0.020%
10	4.447	392	403	407	VV	332282	8899523	1.09%	0.182%
11	4.517	407	415	426	VV	2728356	75582347	9.26%	1.550%
12	4.641	426	436	451	VV	1557266	42066113	5.15%	0.863%
13	4.899	472	480	489	VV 4	37061	1417173	0.17%	0.029%
14	5.087	505	512	531	VV 3	40040	1497592	0.18%	0.031%
15	5.857	630	643	652	PV 5	23880	857253	0.11%	0.018%
16	6.051	652	676	739	VV 3	280185	19986264	2.45%	0.410%
17	9.917	1287	1334	1358	BV 7	70512	4445088	0.54%	0.091%
18	10.869	1467	1496	1520	BV 3	56679	2641389	0.32%	0.054%
19	12.185	1691	1720	1730	PV 3	25816	833554	0.10%	0.017%
20	12.391	1730	1755	1763	VV	100239	2091597	0.26%	0.043%
21	12.479	1763	1770	1777	VV	8070	207625	0.03%	0.004%
22	12.696	1796	1807	1814	PV	13932	455658	0.06%	0.009%
23	12.778	1814	1821	1826	VV 8	11267	259283	0.03%	0.005%
24	12.943	1826	1849	1856	PV 2	70629	1631141	0.20%	0.033%
25	13.013	1856	1861	1867	VV 8	26010	654253	0.08%	0.013%
26	13.119	1867	1879	1883	VV 8	14084	611910	0.07%	0.013%
27	13.254	1893	1902	1906	VV	104558	1974182	0.24%	0.040%
28	13.296	1906	1909	1915	VV 4	56131	1186091	0.15%	0.024%
29	13.466	1915	1938	1956	VV 2	211130	7233445	0.89%	0.148%
30	13.630	1956	1966	1972	VV 2	48153	2454026	0.30%	0.050%
31	13.713	1972	1980	1988	VV	226228	5752270	0.70%	0.118%
32	13.789	1988	1993	1999	VV 4	122414	3638077	0.45%	0.075%
33	13.871	1999	2007	2015	VV	411645	10068900	1.23%	0.206%
34	13.936	2015	2018	2026	VV 5	161921	5091406	0.62%	0.104%
35	14.024	2026	2033	2040	VV	1767223	28361865	3.48%	0.582%
36	14.095	2040	2045	2049	VV 3	220128	5465195	0.67%	0.112%
37	14.147	2049	2054	2058	VV 6	154018	4518480	0.55%	0.093%
38	14.288	2058	2078	2083	VV 3	426344	21670466	2.66%	0.444%
39	14.335	2083	2086	2089	VV 4	335759	7310176	0.90%	0.150%
40	14.430	2089	2102	2108	VV	30637217	616710586	75.57%	12.646%
41	14.500	2108	2114	2124	VV 2	5818027	105813954	12.97%	2.170%
42	14.612	2124	2133	2137	VV	1848339	45934653	5.63%	0.942%
43	14.659	2137	2141	2153	VV	2504748	76622252	9.39%	1.571%

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44	14.788	2153	2163	2169	VV		10751057	198001690	24.26%	4.060%
45	14.847	2169	2173	2175	VV		1722452	32108773	3.93%	0.658%
46	14.935	2175	2188	2192	VV	3	4924614	183853919	22.53%	3.770%
47	14.982	2192	2196	2198	VV	3	5969927	128547111	15.75%	2.636%
48	15.058	2198	2209	2215	VV	2	8101118	401948224	49.26%	8.242%
49	15.123	2215	2220	2224	VV		17276028	338169276	41.44%	6.934%
50	15.187	2224	2231	2250	VV	2	27414680	816037403	100.00%	16.734%
51	15.334	2250	2256	2261	VV	2	2099537	68231798	8.36%	1.399%
52	15.399	2261	2267	2278	VV	3	1889799	83606875	10.25%	1.714%
53	15.499	2278	2284	2292	VV	2	1471417	58870022	7.21%	1.207%
54	15.575	2292	2297	2310	VV	4	1203664	62217660	7.62%	1.276%
55	15.687	2310	2316	2330	VV		1659125	64895180	7.95%	1.331%
56	15.787	2330	2333	2339	VV	4	737409	21531442	2.64%	0.442%
57	15.875	2339	2348	2353	VV	4	803289	35241138	4.32%	0.723%
58	15.934	2353	2358	2363	VV	8	784081	25604245	3.14%	0.525%
59	15.992	2363	2368	2376	VV	4	1158783	37832473	4.64%	0.776%
60	16.069	2376	2381	2384	VV		1058160	24914302	3.05%	0.511%
61	16.104	2384	2387	2392	VV		1036840	22463124	2.75%	0.461%
62	16.204	2392	2404	2414	VV		826674	56614055	6.94%	1.161%
63	16.286	2414	2418	2432	VV	5	848879	42934731	5.26%	0.880%
64	16.404	2432	2438	2447	VV		1933967	56701515	6.95%	1.163%
65	16.474	2447	2450	2456	VV	2	941175	22964239	2.81%	0.471%
66	16.539	2456	2461	2464	VV	6	464081	12058540	1.48%	0.247%
67	16.586	2464	2469	2471	VV	5	544925	12260134	1.50%	0.251%
68	16.621	2471	2475	2488	VV	2	759674	29258475	3.59%	0.600%
69	16.874	2488	2518	2526	VV		3647676	114913060	14.08%	2.356%
70	16.956	2526	2532	2542	VV	8	550995	23771323	2.91%	0.487%
71	17.056	2542	2549	2556	VV	5	442789	18701477	2.29%	0.383%
72	17.132	2556	2562	2574	VV	9	388685	21591504	2.65%	0.443%
73	17.232	2574	2579	2583	VV	7	297248	9049772	1.11%	0.186%
74	17.303	2583	2591	2614	VV	7	294762	26406689	3.24%	0.541%
75	17.655	2635	2651	2665	VV	7	253178	22271562	2.73%	0.457%
76	17.796	2665	2675	2678	VV	5	440295	16353508	2.00%	0.335%
77	17.908	2678	2694	2716	VV	6	677818	62270168	7.63%	1.277%
78	18.102	2716	2727	2757	VV	6	263862	30380549	3.72%	0.623%
79	18.801	2827	2846	2851	VV	3	413777	17882643	2.19%	0.367%
80	18.901	2851	2863	2901	VV	3	2632139	176465165	21.62%	3.619%
81	19.148	2901	2905	2922	VV	3	141155	7295675	0.89%	0.150%
82	19.312	2922	2933	2970	VV	6	477169	28186742	3.45%	0.578%
83	19.659	2970	2992	3003	PV	4	734270	34176846	4.19%	0.701%
84	19.853	3003	3025	3045	VBA2		3712458	228589428	28.01%	4.687%

Sum of corrected areas: 4876655050

Table F33 Area percent report for the essential oil obtained from the Soxhlet extraction with the internal standard added at the end of the extraction.

DSOXBB1
Area Percent Report

Data File : D:\PATRICIA1\DSOXBB1.D Vial: 1
Acq On : 26 Jun 2009 13:36 Operator: PATRICIA
Sample : DCM SOXB .I.S. ADDED BEFORE INJ Inst : Instrumen
Misc : INT. STD. ADDED BEFORE INJECTION Multiplr: 1.00
Sample Amount: 0.00
MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.191	4	19	26	BV 3	73050	1319524	0.18%	0.024%
2	2.461	50	65	86	PV 3	713904	19349976	2.58%	0.348%
3	2.984	145	154	167	VV 2	55516	1565994	0.21%	0.028%
4	3.148	167	182	204	PV	2159641	48584000	6.48%	0.874%
5	3.413	212	227	235	PV 3	49467	1696272	0.23%	0.031%
6	3.507	235	243	259	VV 4	46070	2887844	0.39%	0.052%
7	3.671	259	271	286	VV	258478	8265498	1.10%	0.149%
8	3.830	286	298	317	VV 3	53705	3511632	0.47%	0.063%
9	4.053	317	336	357	VV	288997	9491204	1.27%	0.171%
10	4.441	375	402	404	PV 2	335610	10014266	1.34%	0.180%
11	4.512	404	414	425	VV	2717576	83990738	11.21%	1.511%
12	4.635	425	435	449	VV	1798208	53861729	7.19%	0.969%
13	4.770	449	458	473	VV 2	79210	3291332	0.44%	0.059%
14	5.857	627	643	651	VV 6	28520	1048973	0.14%	0.019%
15	5.957	651	660	665	VV 6	32007	1059372	0.14%	0.019%
16	6.116	665	687	747	VV 3	561923	43732199	5.84%	0.787%
17	6.539	747	759	789	VB 5	29774	1805605	0.24%	0.032%
18	9.899	1294	1331	1363	BV 6	71662	5029668	0.67%	0.091%
19	10.881	1467	1498	1544	BV 2	135434	8441332	1.13%	0.152%
20	12.179	1693	1719	1737	PV 5	40996	1277268	0.17%	0.023%
21	12.391	1737	1755	1764	PV 2	112986	2546415	0.34%	0.046%
22	12.479	1764	1770	1782	VV 2	11047	384622	0.05%	0.007%
23	12.608	1782	1792	1798	VV 2	8968	256930	0.03%	0.005%
24	12.696	1798	1807	1810	PV 9	12686	302373	0.04%	0.005%
25	12.784	1810	1822	1827	VV 9	13804	396512	0.05%	0.007%
26	12.955	1827	1851	1858	VV 2	88861	2615278	0.35%	0.047%
27	13.014	1858	1861	1869	VV 4	45396	1173402	0.16%	0.021%
28	13.108	1869	1877	1881	VV 4	24126	797674	0.11%	0.014%
29	13.254	1881	1902	1919	VV	168306	6740687	0.90%	0.121%
30	13.401	1919	1927	1929	VV 9	29187	1001046	0.13%	0.018%
31	13.466	1929	1938	1944	VV	279097	5823458	0.78%	0.105%
32	13.519	1944	1947	1951	VV 5	46591	1052650	0.14%	0.019%
33	13.636	1951	1967	1972	VV 5	56398	3441972	0.46%	0.062%
34	13.730	1972	1983	1989	VV	266993	7556880	1.01%	0.136%
35	13.789	1989	1993	2001	VV 5	152583	4703613	0.63%	0.085%
36	13.877	2001	2008	2015	VV	479321	12207831	1.63%	0.220%
37	13.936	2015	2018	2027	VV 7	197720	6849159	0.91%	0.123%
38	14.030	2027	2034	2041	VV	1927706	34162348	4.56%	0.615%
39	14.095	2041	2045	2050	VV 3	261957	6406882	0.85%	0.115%
40	14.153	2050	2055	2060	VV 7	184147	5860818	0.78%	0.105%
41	14.289	2060	2078	2083	VV 3	474151	23015052	3.07%	0.414%
42	14.336	2083	2086	2090	VV 3	380977	8842076	1.18%	0.159%
43	14.430	2090	2102	2108	VV	32120040	661831401	88.31%	11.909%

DSOXBB1

44	14.500	2108	2114	2123	VV	6906700	125749409	16.78%	2.263%
45	14.576	2123	2127	2129	VV 2	921941	17144102	2.29%	0.308%
46	14.618	2129	2134	2137	VV	2250671	42672281	5.69%	0.768%
47	14.659	2137	2141	2153	VV	3139769	87840544	11.72%	1.581%
48	14.794	2153	2164	2170	VV	12179906	225732497	30.12%	4.062%
49	14.853	2170	2174	2176	VV 2	1794229	38637835	5.16%	0.695%
50	14.935	2176	2188	2192	VV 3	5159745	178284209	23.79%	3.208%
51	14.988	2192	2197	2199	VV 2	5880617	130405074	17.40%	2.347%
52	15.076	2199	2212	2216	VV 3	8460441	404247823	53.94%	7.274%
53	15.129	2216	2221	2225	VV	19075162	373873460	49.89%	6.728%
54	15.193	2225	2232	2240	VV 2	29441917	749442570	100.00%	13.486%
55	15.264	2240	2244	2261	VV 3	5809511	300884420	40.15%	5.414%
56	15.399	2261	2267	2277	VV 4	2391095	97021865	12.95%	1.746%
57	15.505	2277	2285	2294	VV 3	1590991	73387475	9.79%	1.321%
58	15.581	2294	2298	2311	VV 3	1355709	68787253	9.18%	1.238%
59	15.693	2311	2317	2329	VV	1984238	74714901	9.97%	1.344%
60	15.793	2329	2334	2340	VV 2	976388	29834516	3.98%	0.537%
61	15.893	2340	2351	2355	VV 2	1038309	46635170	6.22%	0.839%
62	15.940	2355	2359	2364	VV 8	1018174	32178986	4.29%	0.579%
63	16.004	2364	2370	2378	VV 3	1269432	45734135	6.10%	0.823%
64	16.110	2378	2388	2398	VV 2	1138395	56211159	7.50%	1.011%
65	16.204	2398	2404	2408	VV 8	676888	22006652	2.94%	0.396%
66	16.298	2408	2420	2429	VV 5	985509	59475491	7.94%	1.070%
67	16.410	2429	2439	2448	VV	2200553	68483720	9.14%	1.232%
68	16.486	2448	2452	2460	VV 4	1032667	29504078	3.94%	0.531%
69	16.592	2466	2470	2473	VV 5	512406	11436149	1.53%	0.206%
70	16.633	2473	2477	2490	VV 3	658969	29127879	3.89%	0.524%
71	16.733	2490	2494	2497	VV 6	361032	8405522	1.12%	0.151%
72	16.886	2497	2520	2528	VV	4074232	120649083	16.10%	2.171%
73	16.962	2528	2533	2545	VV 7	601053	26954043	3.60%	0.485%
74	17.068	2545	2551	2558	VV 6	453015	18284802	2.44%	0.329%
75	17.156	2558	2566	2575	VV 9	350798	20652408	2.76%	0.372%
76	17.250	2575	2582	2586	VV 8	352424	11697716	1.56%	0.210%
77	17.297	2586	2590	2618	VV 10	348371	30484714	4.07%	0.549%
78	17.532	2626	2630	2637	VV 9	182754	5951567	0.79%	0.107%
79	17.661	2637	2652	2659	VV 10	244305	15533872	2.07%	0.280%
80	17.720	2659	2662	2667	VV 7	202819	5858926	0.78%	0.105%
81	17.914	2667	2695	2716	VV 6	816390	93300047	12.45%	1.679%
82	18.049	2716	2718	2726	VV 9	267991	9202712	1.23%	0.166%
83	18.131	2726	2732	2772	VV 9	276265	27683380	3.69%	0.498%
84	18.919	2828	2866	2922	VV 2	3598603	268074838	35.77%	4.824%
85	19.330	2922	2936	2972	VV 3	593888	35083630	4.68%	0.631%
86	19.671	2972	2994	3007	PV 2	1046031	45925399	6.13%	0.826%
87	19.870	3007	3028	3045	VBA2	4338725	285916618	38.15%	5.145%

Sum of corrected areas: 5557306440

Table F34 Area percent report for the essential oil obtained from the steam distillation extraction with the internal standard added at the start of the extraction.

SDSAA2
Area Percent Report

Data File : D:\PATRICIA1\SDSAA2.D Vial: 1
Acq On : 30 Jun 2009 13:40 Operator: PATRICIA
Sample : STEAM DISTILL. INT. STD ADDED AT START Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.273	25	33	45	PV 2	20029	428053	0.13%	0.034%
2	2.455	45	64	68	PV	370843	8088258	2.47%	0.648%
3	2.496	68	71	86	VV 2	243735	5413656	1.65%	0.433%
4	2.954	133	149	162	PV 10	13626	691172	0.21%	0.055%
5	3.154	174	183	188	PV 2	34610	677721	0.21%	0.054%
6	3.677	256	272	289	VV 2	18042	533163	0.15%	0.039%
7	4.059	322	337	347	PV 2	29881	800610	0.24%	0.064%
8	4.447	370	403	406	VV 8	54608	2203725	0.67%	0.176%
9	4.517	406	415	425	VV	358280	9811110	3.00%	0.786%
10	4.641	425	436	450	VV	342298	10017879	3.06%	0.802%
11	4.764	450	457	472	VV 5	20582	808771	0.25%	0.065%
12	5.687	589	614	622	PV 9	14417	490275	0.15%	0.039%
13	5.857	622	643	649	VV 6	18142	795490	0.24%	0.064%
14	5.945	649	658	701	VV 3	44213	1808299	0.55%	0.145%
15	6.909	797	822	852	BB 3	53337	2086209	0.64%	0.167%
16	7.537	891	929	961	BV 7	32757	1387330	0.42%	0.111%
17	8.231	1027	1047	1083	BV 5	27545	1431112	0.44%	0.115%
18	9.083	1177	1192	1200	VV 5	6973	309737	0.09%	0.025%
19	9.206	1200	1213	1249	VV 9	17666	949160	0.29%	0.076%
20	9.917	1311	1334	1398	VV 8	83979	5290629	1.62%	0.424%
21	10.546	1425	1441	1466	BV 6	14573	654335	0.20%	0.052%
22	11.022	1512	1522	1552	PB 6	6964	440506	0.13%	0.035%
23	11.268	1553	1564	1606	BV 2	11100	863241	0.26%	0.069%
24	12.179	1707	1719	1730	PV 2	26054	691780	0.21%	0.055%
25	12.373	1741	1752	1762	VV	79171	1655508	0.51%	0.133%
26	12.461	1762	1767	1776	VV 10	9482	215878	0.07%	0.017%
27	12.761	1811	1818	1832	VV 4	11411	276375	0.08%	0.022%
28	12.955	1832	1851	1856	VV 7	14281	596783	0.18%	0.048%
29	13.013	1856	1861	1870	VV 3	23995	536100	0.16%	0.043%
30	13.248	1895	1901	1905	VV 2	26863	431914	0.13%	0.035%
31	13.290	1905	1908	1921	VV 9	12662	240927	0.07%	0.019%
32	13.466	1931	1938	1948	VV 2	46139	755777	0.23%	0.061%
33	13.789	1985	1993	2000	PV 3	28461	551361	0.17%	0.044%
34	13.871	2000	2007	2015	VV 2	108756	2278503	0.70%	0.182%
35	13.930	2015	2017	2020	VV 4	19155	285782	0.09%	0.023%
36	13.965	2020	2023	2026	VV 5	12687	207915	0.06%	0.017%
37	14.024	2026	2033	2039	VV	627693	8539701	2.61%	0.684%
38	14.095	2039	2045	2060	VV 7	62861	1779901	0.54%	0.143%
39	14.253	2060	2072	2074	VV 3	76674	1537765	0.47%	0.123%
40	14.283	2074	2077	2081	VV	94982	1587286	0.48%	0.127%
41	14.330	2081	2085	2089	VV 4	49933	939802	0.29%	0.075%
42	14.412	2089	2099	2107	VV	21290972	327397197	100.00%	26.216%
43	14.494	2107	2113	2123	VV	2061023	31516842	9.63%	2.524%

SDSAA2

44	14.612	2123	2133	2136	VV		707488	11943788	3.65%	0.956%
45	14.653	2136	2140	2153	VV	2	639863	15286826	4.67%	1.224%
46	14.782	2153	2162	2168	VV		5492774	76939689	23.50%	6.161%
47	14.835	2168	2171	2177	VV	3	143238	3005240	0.92%	0.241%
48	14.929	2177	2187	2191	VV	2	896515	16973600	5.18%	1.359%
49	14.976	2191	2195	2204	VV	2	588865	10925549	3.34%	0.875%
50	15.117	2204	2219	2223	VV		7309217	122128737	37.30%	9.779%
51	15.176	2223	2229	2248	VV		14893012	242414728	74.04%	19.411%
52	15.334	2248	2256	2261	VV		415839	8616518	2.63%	0.690%
53	15.393	2261	2266	2275	VV	2	432183	8936125	2.73%	0.716%
54	15.493	2275	2283	2290	VV	3	169520	4413142	1.35%	0.353%
55	15.558	2290	2294	2298	VV	4	73827	1595558	0.49%	0.128%
56	15.599	2298	2301	2306	VV	7	54761	1337388	0.41%	0.107%
57	15.675	2306	2314	2326	VV		749413	13943214	4.26%	1.117%
58	15.787	2326	2333	2336	VV	2	179314	3635820	1.11%	0.291%
59	15.828	2336	2340	2349	VV	2	186034	4928643	1.51%	0.395%
60	15.910	2349	2354	2359	VV	3	179885	4146588	1.27%	0.332%
61	15.975	2359	2365	2372	VV	3	773363	14856787	4.54%	1.190%
62	16.075	2372	2382	2393	VV	2	1052879	29718215	9.08%	2.380%
63	16.169	2393	2398	2409	VV		328486	8057479	2.46%	0.645%
64	16.274	2409	2416	2422	VV		409713	9189627	2.81%	0.736%
65	16.333	2422	2426	2429	VV	2	136732	2925010	0.89%	0.234%
66	16.386	2429	2435	2441	VV		1727071	30326345	9.26%	2.428%
67	16.457	2441	2447	2453	VV	4	797309	17004480	5.19%	1.362%
68	16.515	2453	2457	2460	VV	4	194843	4152233	1.27%	0.332%
69	16.551	2460	2463	2467	VV	3	206838	4643061	1.42%	0.372%
70	16.603	2467	2472	2485	VV	5	293559	10148305	3.10%	0.813%
71	16.709	2485	2490	2494	VV	5	98770	2310308	0.71%	0.185%
72	16.768	2494	2500	2502	VV	2	223973	4680999	1.43%	0.375%
73	16.850	2502	2514	2523	VV		3956192	88997157	27.18%	7.126%
74	16.932	2523	2528	2538	VV	4	368207	10670593	3.26%	0.854%
75	17.032	2538	2545	2564	VV	6	149014	7479793	2.28%	0.599%
76	17.203	2570	2574	2580	VV	8	38653	1098442	0.34%	0.088%
77	17.309	2580	2592	2600	VV	8	41979	2271564	0.69%	0.182%
78	17.385	2600	2605	2614	VV	8	29501	1225175	0.37%	0.098%
79	17.497	2614	2624	2638	VV	10	38756	2170470	0.66%	0.174%
80	17.638	2638	2648	2666	VV	6	80439	3110605	0.95%	0.249%
81	17.878	2680	2689	2699	VV	6	20078	835063	0.26%	0.067%
82	18.307	2748	2762	2769	PV	8	7219	233090	0.07%	0.019%
83	19.788	2984	3014	3045	PBA	6	51708	3599453	1.10%	0.288%

Sum of corrected areas: 1248828946

Table F35 Area percent report for the essential oil obtained from the steam distillation extraction with the internal standard added at the end of the extraction.

SDSBBB5
Area Percent Report

Data File : D:\PATRICIA1\SDSBBB5.D Vial: 1
Acq On : 1 Jul 2009 16:06 Operator: Patricia
Sample : STEAM DISTILL. INT. STD BEFORE INJECT. Inst : Instrumen
Misc : Multiplr: 1.00
Sample Amount: 0.00

MS Integration Params: autoint1.e

Method : C:\MSDCHEM\1\METHODS\DEFAULT.M (Chemstation Integrator)
Title :

Signal : TIC

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	2.191	4	19	25	BV 4	79338	1544156	0.34%	0.088%
2	2.273	25	33	43	PV 2	65460	1261779	0.27%	0.072%
3	2.461	43	65	94	PV 5	1382583	42505662	9.23%	2.423%
4	2.661	94	99	107	VV 6	15540	383197	0.08%	0.022%
5	2.931	131	145	162	PV 8	46232	1752959	0.38%	0.100%
6	3.078	162	170	175	VV 4	20142	469350	0.10%	0.027%
7	3.154	175	183	189	VV	104441	2205388	0.48%	0.126%
8	3.242	189	198	214	VV 5	24793	1086018	0.24%	0.062%
9	3.530	235	247	265	VV 4	32855	1677036	0.36%	0.096%
10	3.683	265	273	288	VV 3	38954	1090830	0.24%	0.062%
11	4.059	324	337	349	PV 2	54006	1421756	0.31%	0.081%
12	4.271	349	373	383	VV	134934	3869751	0.84%	0.221%
13	4.359	383	388	392	VV 2	18913	491639	0.11%	0.028%
14	4.447	392	403	407	VV 2	91306	2875033	0.62%	0.164%
15	4.517	407	415	426	VV	663024	18013379	3.91%	1.027%
16	4.641	426	436	460	VV	588208	16914169	3.67%	0.964%
17	5.311	531	550	583	VB	13841	689892	0.15%	0.039%
18	5.687	590	614	626	BV 8	11952	486067	0.11%	0.028%
19	5.845	626	641	648	VV 8	11125	536586	0.12%	0.031%
20	5.945	648	658	677	VV 4	54404	1980298	0.43%	0.113%
21	6.157	677	694	711	VV 4	9119	499636	0.11%	0.028%
22	6.915	807	823	869	VB 4	62375	2607338	0.57%	0.149%
23	7.543	908	930	953	BV 5	33379	1350267	0.29%	0.077%
24	8.237	1030	1048	1083	PV 4	29082	1433692	0.31%	0.082%
25	9.089	1175	1193	1202	VV 4	6873	344268	0.07%	0.020%
26	9.212	1202	1214	1234	VV 7	13836	755813	0.16%	0.043%
27	9.917	1315	1334	1393	VV 7	95504	5713113	1.24%	0.326%
28	10.552	1427	1442	1479	BB 3	13427	626062	0.14%	0.036%
29	11.033	1484	1524	1540	BV 3	5806	230053	0.05%	0.013%
30	11.280	1550	1566	1590	BV 2	6564	370366	0.08%	0.021%
31	12.150	1706	1714	1731	BB 2	8480	218670	0.05%	0.012%
32	12.373	1731	1752	1763	BV	97093	1931674	0.42%	0.110%
33	12.461	1763	1767	1779	VV 10	7673	216759	0.05%	0.012%
34	12.761	1809	1818	1833	PV 4	13554	352577	0.08%	0.020%
35	12.937	1833	1848	1869	PV 8	13456	632987	0.14%	0.036%
36	13.249	1887	1901	1905	BV 2	45379	798088	0.17%	0.045%
37	13.290	1905	1908	1919	VV 3	16153	293228	0.06%	0.017%
38	13.466	1927	1938	1951	VV	74035	1268027	0.28%	0.072%
39	13.789	1987	1993	2000	VV 3	38920	753528	0.16%	0.043%
40	13.871	2000	2007	2015	VV	166150	3475856	0.75%	0.198%
41	13.930	2015	2017	2026	VV 5	35758	826589	0.18%	0.047%
42	14.024	2026	2033	2040	VV	933745	13123931	2.85%	0.748%
43	14.095	2040	2045	2061	VV 4	86108	2374804	0.52%	0.135%

SDSBBB5

44	14.253	2061	2072	2074	VV		108214	2121831	0.46%	0.121%
45	14.283	2074	2077	2082	VV		143995	2483447	0.54%	0.142%
46	14.330	2082	2085	2090	VV	3	71544	1358378	0.29%	0.077%
47	14.418	2090	2100	2107	VV		26047438	460717303	100.00%	26.262%
48	14.494	2107	2113	2123	VV		3290079	49335649	10.71%	2.812%
49	14.571	2123	2126	2128	VV		135973	1986427	0.43%	0.113%
50	14.612	2128	2133	2137	VV		1049026	15080854	3.27%	0.860%
51	14.653	2137	2140	2153	VV	2	1046540	22745496	4.94%	1.297%
52	14.788	2153	2163	2168	VV		7956038	109217402	23.71%	6.226%
53	14.835	2168	2171	2179	VV	2	212512	5175970	1.12%	0.295%
54	14.929	2179	2187	2191	VV	2	1301123	24931809	5.41%	1.421%
55	14.976	2191	2195	2204	VV		881457	16410760	3.56%	0.935%
56	15.117	2204	2219	2223	VV		10933793	182523501	39.62%	10.404%
57	15.182	2223	2230	2239	VV	2	20787625	354939424	77.04%	20.232%
58	15.246	2239	2241	2248	VV		468144	8835557	1.92%	0.504%
59	15.334	2248	2256	2261	VV		617890	13028466	2.83%	0.743%
60	15.393	2261	2266	2276	VV	3	637041	12708736	2.76%	0.724%
61	15.493	2276	2283	2290	VV	2	262472	5972533	1.30%	0.340%
62	15.558	2290	2294	2306	VV	3	151213	4609215	1.00%	0.263%
63	15.675	2306	2314	2327	VV		922300	17131619	3.72%	0.977%
64	15.787	2327	2333	2336	VV		213782	4331879	0.94%	0.247%
65	15.828	2336	2340	2349	VV	2	199936	5379690	1.17%	0.307%
66	15.910	2349	2354	2359	VV	5	209484	5006854	1.09%	0.285%
67	15.975	2359	2365	2372	VV	3	824359	16443822	3.57%	0.937%
68	16.075	2372	2382	2393	VV	2	1095693	33523022	7.28%	1.911%
69	16.169	2393	2398	2408	VV	2	306424	7832226	1.70%	0.446%
70	16.274	2408	2416	2422	VV		422774	10014684	2.17%	0.571%
71	16.333	2422	2426	2429	VV	2	159191	3383548	0.73%	0.193%
72	16.386	2429	2435	2441	VV		1961330	34898475	7.57%	1.989%
73	16.457	2441	2447	2453	VV	3	850855	18197166	3.95%	1.037%
74	16.515	2453	2457	2460	VV	4	216047	4740267	1.03%	0.270%
75	16.551	2460	2463	2467	VV	4	225260	5022253	1.09%	0.286%
76	16.604	2467	2472	2485	VV	5	352355	11810567	2.56%	0.673%
77	16.703	2485	2489	2493	VV	4	105076	2501916	0.54%	0.143%
78	16.768	2493	2500	2502	VV	3	233448	4837318	1.05%	0.276%
79	16.844	2502	2513	2523	VV		4314397	95260364	20.68%	5.430%
80	16.933	2523	2528	2538	VV	3	391618	11676211	2.53%	0.666%
81	17.038	2538	2546	2563	VV	5	164732	7752551	1.68%	0.442%
82	17.203	2569	2574	2579	VV	8	45419	1323733	0.29%	0.075%
83	17.309	2579	2592	2600	VV	8	45624	2665475	0.58%	0.152%
84	17.379	2600	2604	2612	VV	8	30646	1124257	0.24%	0.064%
85	17.485	2612	2622	2636	VV	8	61940	3378387	0.73%	0.193%
86	17.632	2636	2647	2682	VV	9	89295	4809626	1.04%	0.274%
87	17.867	2682	2687	2718	VV	9	21061	1571590	0.34%	0.090%
88	18.302	2748	2761	2788	VV	9	9854	802326	0.17%	0.046%
89	19.794	2987	3015	3045	PBA9		49230	3248806	0.71%	0.185%

Sum of corrected areas: 1754303663

APPENDIX G

PRESENTATION

A portion of this work was presented at the Faculty of Science and Agriculture Research Day, University of KwaZulu-Natal (Westville Campus), 2004.