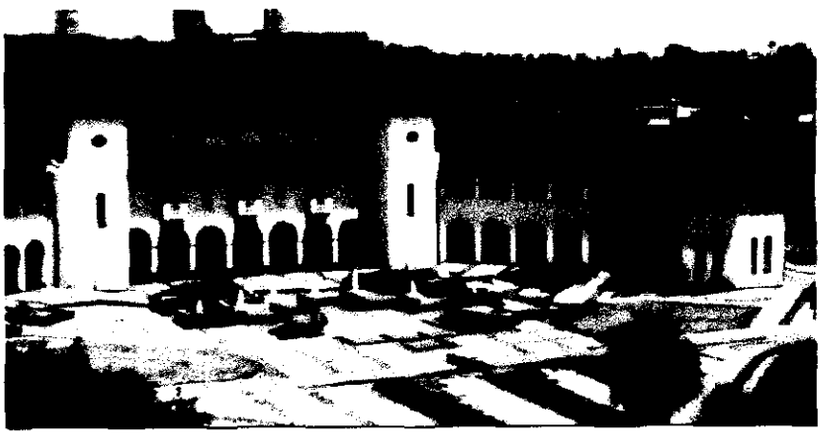
OPTIMISATION OF SUPERCRITICAL CARBON DIOXIDE DERIVED HIGH-VALUE BOTANICAL EXTRACTS OF *MELISSA OFFICINALIS*



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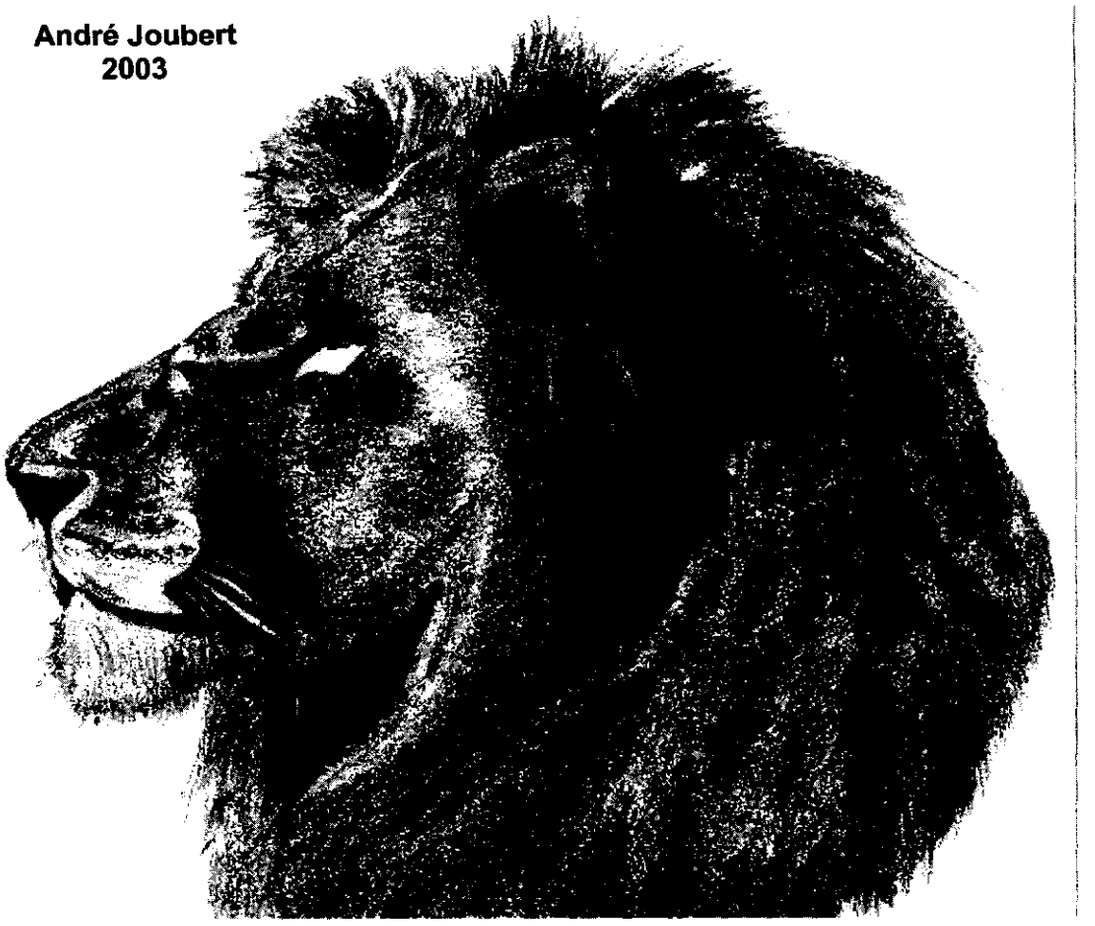

2 Corinthians 13:14. The grace (favor and spiritual blessing) of the Lord Jesus Christ and the love of God and the presence and fellowship (the communion and sharing together, and participation) in the Holy Spirit be with you all. Amen (so be it). (Amplified Bible)

Optimisation of sc-CO_2 derived high-value botanical extracts of *Melissa officinalis*

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Thesis submitted in partial fulfilment of the degree

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in

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ABSTRACT

This project dealt with the acquisition of supercritical carbon dioxide (sc-CO_2) derived extracts from *Melissa officinalis*. The actuality of such extracts lies in the diversity of its components, which are relevant to the pharmaceutical, cosmetic, fragrance/flavour and food industries.

Extractions were performed on selected dried plant material using a laboratory-scale supercritical fluid extractor. The runs were executed according to an orthogonal, rotatable statistical design in order to optimise conditions (pressure, temperature, duration) for a maximum yield of extract by computer-assisted surface response analysis.

The relationship between the yield of extract and the principal process parameters enabled conclusions to be drawn about the mechanism of extraction. The volatile components are physically desorbed from the plant matrix as a result of the diffusivity of low-density (gas-like) sc-CO_2, whereas the non-volatile components are chemically dissolved on account of the solvent strength of high-density (liquid-like) sc-CO_2.

An equation based on a dimensionless grouping of variables was derived to mathematically describe the extraction process in terms of all major contributing process parameters.

The extracts were analysed by GC/MS and GC-GC/TOF-MS. A total of 204 components were identified, many of which correspond with those extracted by traditional methods and which are reported in the literature.

The analytical results prove that component-rich botanical extracts can be obtained with sc-CO_2 as extractant. In addition, the fluid warrants solvent free extracts and clean technology for sustained environmental protection.

OPSOMMING

Die projek het gehandel oor die verkryging van ekstrakte van *Melissa officinalis* met superkritieke koolstofdioksied (sc-CO_2) as ekstraheermiddel. Die belangrikheid van sodanige ekstrakte is geleë in die diversiteit van komponente wat vir die farmaseutiese, kosmetiese, reuk- en geurmiddel- en voedselnywerhede van belang is.

Ekstraksies is op uitgesoekte, gedroogde plantmateriaal uitgevoer deur van 'n laboratoriumgrootte superkritieke-fluïed-ekstraktor gebruik te maak. Die lopies is volgens 'n ortogonale, roteerbare statistiese ontwerp uitgevoer met die doel om die kondisies (druk, temperatuur, tydsduur) vir 'n maksimum ekstrakopbrengs rekenaarmatig met behulp van oppervlakresponsanalise te bepaal.

Die verband tussen die ekstrakopbrengs en belangrikste prosesveranderlikes het gevolgtrekkinge oor die ekstraksiemeganisme moontlik gemaak. Die vlugtige komponente word fisies vanaf die plantmatrys gedesorbeer as gevolg van die diffusiwiteit van lae-digtheid (gassoortige) sc-CO_2, terwyl nie-vlugtige komponente chemies opgelos word as gevolg van die oplosmiddelsterkte van hoë-digtheid (vloeistofsoortige) sc-CO_2.

'n Vergelyking gebaseer op 'n dimensielose groepering van veranderlikes is afgelei om die ekstraksieproses in terme van alle bydraende prosesfaktore wiskundig te beskryf.

Die ekstrakte is met behulp van GC/MS en GC-GC/TOF-MS geanaliseer. 'n Totaal van 204 komponente is geïdentifiseer, waarvan baie ooreenstem met dié wat met behulp van tradisionele metodes verkry is en in die literatuur gerapporteer word.

Die analitiese resultate toon dat komponentryke botaniese ekstraksies met sc-CO_2 as ekstraheermiddel verkry kan word. Die fluïed verseker verder dat oplosmiddelvrye ekstrakte verkry word en dat skoon tegnologie volhoubare omgewingsbeskerming verleen.

ACKNOWLEDGEMENTS

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Chapter 0

ACTUALITY AND OBJECTIVES

It has been demonstrated that supercritical carbon dioxide (sc-CO_2) is effective in extracting essential oils from plant material.[1-4] The motivation for developing extractive separation techniques with sc-CO_2 is that mild conditions (temperature and pressure) are involved. The extracts are free from solvent residues as a result of simple and complete separation of the solvent when ambient conditions are restored. Hazardous traditional solvents (halogenated hydrocarbons, aromatic compounds) can be replaced as required by stricter regulations regarding the use of solvents in the food, flavour and fragrance industries.

sc-CO_2 is used as an extracting agent in several industrial processes, such as the decaffeination of coffee and tea and the production of hop(s) for beer brewing. In addition, many other industrial extractions on a smaller scale are based on sc-CO_2, viz. spices (pepper, paprika), aromatic substances (aniseed, citrus fruit, essential oils), fragrances (perfumes), pharmaceuticals (natural substances), seed oils (soybeans, sunflower, olives), contaminants (insecticides, nicotine, cholesterol).

In this study *Melissa officinalis* (Lemon balm) was selected for extraction with sc-CO_2 in view of the antioxidant, sedative, antispasmodic and antiflatulent characteristics of the botanical content.

The objectives of this investigation were

- to identify the components extracted from the plant material by sc-CO_2 for comparison to those obtained by other methods or investigators;
- to vary process parameters and acquire suitable extraction conditions for optimum yield;
- to reveal the principal features and mechanistic characteristics of the extraction for the purpose of process control and tunability;

- to add value to a developing process technology that warrants sustainable chemistry through environmentally friendly solvents.

The workplan comprised the following steps:

1. select suitable dried plant material and perform sc-CO_2 extractions using available laboratory scale supercritical fluid extractors;
2. compare results with available literature and data obtained by other investigators;
3. perform extraction runs as a function of time to establish the minimum duration of extraction for optimum yield at selected conditions;
4. design a statistical routine based on two independent variables (temperature, pressure) at constant flow rate and extraction time pursuant to the requirements of orthogonality and rotatibility;
5. construct a three-dimensional surface response graph by virtue of which optimum conditions for extraction can be determined;
6. analyse the composition of the extracts using suitable instrumental analytical techniques and make an assessment of the results of sc-CO_2 extraction in comparison to those of other methods reported in the literature;
7. utilise the acquired extraction data to calculate kinetic/termodynamic quantities revealing the mechanistic characteristics of the extraction and to validate a mathematical description of the process as a first step to process modelling.

The project was performed in the laboratories of Separation Science and Technology (SST) at the North-West University, Potchefstroom, and was part of a collaboration among the university, CSIR Bio/Chem Division and a private company, Clive Teubes (Pty) Ltd. It was funded by the NRF, Clive Teubes (Pty) Ltd, North-West University and THRIP.

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MELISSA OFFICINALIS (LEMON BALM)

Chapter 1

Essential oils are the key to aromatherapy and this term was assigned by a French cosmetic chemist Gattefosse in 1937.[1] When an explosion occurred while he was doing experiments, he burnt his hand and immediately immersed it into lavender oil. The rapid healing without scare tissue forming amazed him and he decided to look deeper into the antiseptic and antimicrobial properties of essential oils. The popularity of aromatherapy was boosted by Dr. Jean Valnet, with his publication on *The Practice of Aromatherapy* in 1964.[1] France mainly uses essential oils for medicinal aromatherapy internally in suppository and capsule form. England focuses on massage aromatherapy, while Germany focuses on the diffusing of essential oils. The British aromatherapist, Robert Tisserand, defined aromatherapy into five categories: psycho, aesthetic, holistic, nursing and medical aromatherapy.[1]

Essential oils have a history dating back much further than Gattefosse.[1] We read in Matthew 26: 6-8 "And when Jesus was in Bethany at the house of Simon the leper, a woman came to Him having an alabaster flask of very costly fragrant oil, and she poured it on His head as He sat at the table".[2] Matthew 2 : 11 "And when they had come into the house, they saw the young child with Mary His mother, and fell down and worshipped Him. And when they opened their treasures, they presented gifts to Him: gold, frankincense and myrrh".[2] The latter two were highly revered oils.[1] Later, alchemists searched for the elixir of life and distilled aromatic plants and called the oils "quintessence", the origin of the term "essential." They viewed essential oils as the spirit or soul of the plant. From a biochemical point of view, the oil can be considered part of the plant's immune system. Plants like frankincense and myrrh that grow in hot climates use the evaporated aura of the essential oil as a protective cloud to filter the sun's rays. *Dictamus fraxinella*, a plant of the same family that grows in Sinai, has such an abundance of resinous vapour around it that the vapour will burn with a brilliant glow when lit. Some people speculated that this was responsible for the burning bush of Moses.[1]

1.1 History and origin of the plant

There are only three species in the genus *Melissa*, and the botanical name for *Melissa officinalis* in the Greek language means "honey bee", referring to the strong attraction the plant holds for honeybees. "Balm" is the short for balsam, a term widely used for many fragrant plants.[3] "Lemon" comes from the fragrance as it smells like lemon. Lemon balm in Hebrew is "Bal-Smin", meaning "chief of oils".[4] Lemon balm has been cultivated in the Mediterranean region for more than twenty centuries and this fragrant herb has been used for its medicinal properties.[5] It was mostly used for its antibacterial, sedative and spasmodic characteristics.[6] Lemon balm was soaked in wine and used topically and orally in ancient Indian, Roman and Greek medicines. They used it to treat venomous bites and stings and as surgical dressing for wounds according to the writings of *Pliny the Elder and Dioscorides*.[7]

It is now recognised as a scientific fact that the balsamic oils of aromatic plants make excellent surgical dressings: they give off ozone and thus exercise anti-putrescent effects. Being chemically hydrocarbons, they contain so little oxygen that in wound dressing the germs of disease cannot survive. The resinous parts of these balsamic oils, as they dry upon the (sore) wound, seal it up and effectively exclude all noxious air.[8] Fourteenth century French King Charles V drank this tea to keep good health.[6]

Paracelsus (1493-1541), a famous alchemist and physician, made a statement regarding this plant : "This herb could completely revitalize the body" and he called it the "elixir of life".[1,4,6] Avicenna, the Muslim herbalist recommended lemon balm to make the heart merry. Sacred to the temple of Diana, lemon balm was called "heart's delight' in southern Europe.[6,7] The famous Carmelite Water was made between the fourteenth and seventeenth century by French Carmelite nuns to treat headache by combining lemon balm with lemon peel, nutmeg, angelica root and coriander. The moral excellence of this herb has been praised by herbal authors for centuries.[4,6]

Lemon balm also has a long history of use in Europe where it has been esteemed for curing all sorts of illnesses, wounds and sicknesses, both physical and psychological. According to *A Modern Herbal*, the London Dispensary, published in 1696,[3] "an essence of balm, given in canary wine, every morning will renew youth, strengthen the brain, relieve languishing nature and prevent baldness. " What more could you want"?.[3] John Evalyn (1620- 1706) described it as "sovereign for the brain, strengthening the

memory, and powerfully chasing away melancholy".[1,4] Patricia Davis, author of *Subtle Aromatherapy*, calls Melissa "the oil above all others which seems to be of supreme help to both the dying and those around". Gurudas wrote that Melissa was useful in past life regeneration.[1] The Arabs relied on it to treat depression and anxiety, while the English included it in furniture polish.[9] John Hussey, of Sydenham, who lived to the age of 116, breakfasted for fifty years on balm tea sweetened with honey. The same applies to Llewelyn Prince of Glamorgan who died at 108.[8]

1.2 Location of occurrence

Melissa officinalis is cultivated throughout the world.[7] Lemon balm occurs naturally in eastern, central and southern Europe,[10] especially southern France,[11] Italy,[12] south of England [8] and northern Africa, Tunisia to Morocco [13] and parts of Egypt.[4] This plant is also common in the Mediterranean areas, the near East[10] and western Asia.[7] It is also naturalised in most parts of the United States[11,14] and even in Bulgaria.[15] It grows wild in waste places, fields, along roadsides and disturbed lands from sea level into the mountains.[3] It is well known as a garden plant in the backyard.[11]

1.3 Classification

The classification of *Melissa officinalis* is as follows:[14]

Table 1.1 Classification of *Melissa officinalis*

Kingdom	***Plantae***	Plants
Subkingdom	***Tracheobionta***	Vascular plants
Super division	***Spermatophyta***	Seed plants
Division	***Magnoliophyta***	Flowering plants
Class	***Magnoliopsida***	Dicotyledons
Subclass	***Asteridae***	
Order	***Lamiales***	
Family	***Lameaceae (Labiatea)***	Mint family
Genus	***Melissa***	Balm
Species	***Officinalis***	Common balm

1.4 Description of the plant

Figure 1.1 Plant, seeds, flowers and leaves of *Melissa officinalis*

The *Labiatea* family consists of two hundred genera and about three thousand species, most of which are aromatic or perennial herbs.[16] Lemon balm is a herbaceous, aromatic perennial subshrub in the mint family.[3,7,9] It has an upright stem with tiny hairs all over. The stem and branches are square or quadrangular and, as a mature lemon balm plant, it can reach a height of about one meter with a spread of about a half to one meter as illustrated in Figure 1.1.[3,9,10,17] The square stems are characteristic of its clan.[18]

Typical of herbaceous mints, lemon balm leaves are arranged in opposing pairs on the stems.[3] They are light to deep green in colour. The lemon-scented leaves are broadly oval, rogues, obtuse, rounded or subcordate at the base, heart shaped and somewhat hairy with scalloped edges.[11,17,18] There are also characteristic glandular hairs which secrete oil. The development of these secretor trichomes was identified by electron microscopy.[16] Its foliage has a distinctive lemony fragrance when bruised. The youngest and tender rest lemon balm leaves have the most flavour and lemony fragrance.[3]

Little half-inch flowers are produced all summer in irregular coils at leaf nodes on upright stems.[3] These flowers are bisexual, zygomorphic, which are usually of the floral formula K5, C5, A4, G2, and are arranged in verticillasters. The corolla is bilabiate and the stamens are didynamous. Occasionally the number of stamens is reduced to two. The ovary becomes four-celled and has a single ovule in each loculus. The medicinally important species have a gynobasic style, and the four nut lets have only a small surface of contact with one another.[16] The flowers may range in colour from pale yellow to rose coloured, whitish or blue white.[9]

1.5 Cultivation

Lemon balm is not particular about soil or site, in fact, it can become as invasive as its mint relatives if left unattended. It prefers average, well drained soil but performs best in a fertile soil with a pH of 5 to 7.[6,18] It will also tolerate poor sandy soil[3]. It can be planted in full sun, but will do in partial shade. Good sun and moisture are necessary for the production of essential oil and good fragrance.[17] Plants can be started from seeds, cuttings or root division. Seeds are slow to germinate and are so fine that they hardly need covering at all.

Seeds are sown in early spring or autumn. An alternative method of propagation is to take stem cuttings from lush summer growth, root them in water and plant them into the garden in early autumn. It should be cut back to soil level in autumn to encourage strong growth. When the plant becomes overgrown, roots and divisions should be dug up and divided and replaced in either early spring or autumn.

They should be spaced 20-30 centimeters apart from one another.[17,18] The plant should be covered in countries with severe summer and winter temperatures[19]. The only care

required is to keep the plant clean from weeds and to cut off the decayed stalks in autumn. The soil among the roots should then be stirred.[8,20] The plant does not tolerate high humidity. lemon balm performs well in containers.[17,19]

Figure 1.2 *Melissa officinalis* (Lemon Balm)

1.6 Basis of vegetative propagation

Vegetative propagation is an important means of plant production for aromatic plants such as scented geranium, lavender, mint, marjoram, rosemary and lemon balm. The genetic information encoded in the deoxyribonucleic acid (DNA) of cells of an organism is considered to be uniform, allowing a cell or group of cells removed from parent organism to form, through regeneration processes, a completely new organism that would be identical to the original parent. This asexual production of new plants, identical to a "mother plant," is termed vegetative propagation with the descendants of the "mother plant" known as clones.

Rooting of cuttings, planting of vegetative parts, and *in vitro* culturing of sterile selections are the important vegetative propagation techniques applying to aromatic plants. Vegetative cuttings of many Mediterranean plants such as *Melissa officinalis, Salvia officinalis, Rosmarinus officinalis and Salvia fruticosa* that propagate directly in the field during winter will usually root faster and at a higher percentage under indoor propagating conditions where there is less water loss. The removal of the upper,

herbaceous part of the stem helps minimise water loss and increases rooting in semi-hardwood cuttings of sage and laurel under mist.[21]

1.7 What is an essential oil?

An essential oil is a completely natural product which is extracted from the fragrant part, or "essence", of the plant such as special secreted glands, ducts or cells and from the sap and tissues of certain trees. It is present in the roots, stems, barks, leaves and flowers and are more abundant in certain plants than others. Because an essential oil evaporates when exposed to the air at ordinary temperatures, it is called a volatile oil, an ethereal oil, or an essential oil. The last term is applied, since a volatile oil represents the "essence" or the active constituents of a plant. [22]

Volatile oils are colourless as a rule, particularly when they are fresh, but on long standing they may oxidise and resinify, thus darkening in colour. To prevent this, essential oils should be stored in a cool, dry place in tightly stoppered, preferably in full (not half emptied) amber glass containers. Depending on the plant family, volatile oils may occur in specialised secretory structures such as glandular hairs (*Labiatae*), modified parenchyma cells (*Piperaceae*), oil-tubes called vittae (*Umbelliferae*), or in lysignous or schizogenous passages (*Pinaceae, Rutaceae*).[22]

Volatile oils may act as repellents to insects, thus preventing the destruction of the flowers and leaves; or they may serve as insect attractants, thus aiding in cross-fertilization of the flowers. Chemical constituents of volatile oils may be divided on the basis of their biosynthetic origin into two broad classes: (1) terpene derivatives formed via the acetatemevalonic acid pathway, and (2) aromatic compounds formed via the shikimic acid phenylpropanoid route.

The biosynthesis of relatively few of these compounds has been investigated in any detail. Although volatile oils differ largely in their chemical constitution, they have a number of physical properties in common. They have characteristic odours, they are characterised by high refractive indices, most of them are optically active, and the specific rotation is often a valuable diagnostic property (natural menthol is levorotatory, synthetic forms are usually racemic; natural camphor is dextrorotatory, synthetic camphor is racemic). As a rule, volatile oils are immiscible with water, but they are sufficiently soluble to impart their odour to water. The aromatic waters are dependent

on this slight solubility. Volatile oils, however, are soluble in alcohol and most organic solvents.

Several differences exist between volatile and fixed oils. The former can be distilled from their natural sources; they do not consist of glyceryl esters of fatty acids, hence they do not leave a permanent grease spot on paper and cannot be saponified with alkali(e)s. Volatile oils do not become rancid as the fixed oils, but instead, on exposure to light and air, they oxidise and resinify.[22]

Practically all volatile oils consist of mixtures of chemicals which are often quite complex; they vary widely in chemical composition. Almost any type of organic compound may be found in volatile oils (hydrocarbons, alcohols, ketones, aldehydes, ethers, and others), and only a few possess a single component in a high percentage. Volatile oils are usually obtained by distillation of the plant parts containing the oil, the method depending on the condition of the plant material. There are three types of distillation used in industry, viz: (1) water, (2) water and steam, (3) direct steam.[22]

1.8 Chemistry of volatile oils

In only a very few cases do volatile oils consist of a single chemical compound with high purity. In most cases they are mixtures containing compounds of diverse types. These compounds may be separated in various ways: low temperatures which crystallise out the stearoptenes; fractional distillation; fractional crystallization from poor solvents; removal by chemical action. In the last method, compounds with free acidic groups may be removed from the oil by sodium carbonate, basic compounds may be removed with hydrochloric acid, phenols with sodium hydroxide, aldehydes with sodium bisulfate, and so forth.[22]

Most volatile oils largely consist of terpenes, which are isomeric hydrocarbons having molecular formula $C_{10}H_{16}$. Closely related are the sesquiterpenes $C_{15}H_{24}$ and diterpenes $C_{20}H_{32}$. The most common terpenes are limonene and pinene, limonene being probably the most widely distributed of the monocyclic terpenes. Oxygenation of the terpene hydrocarbons would naturally account for the presence of alcohols, aldehydes, ketones, phenols, phenolic ethers, esters and oxides. Since these oxygenated compounds are responsible for the characteristic odours, tastes and therapeutic properties of the volatile oils, a chemical classification of the oils should be based on the principal chemical constituents.[22]

As the constituents are so diverse and so numerous, it is often difficult to assign a volatile oil or oil-bearing drug to a definite classification. Although unoxygenated terpenes sometimes account for a large percentage of the oil, the stearoptene present in smaller quantity represents the principal constituent. The following are the categories in which volatile oils and oil-containing drugs are placed: (1) hydrocarbons, (2) alcohols, (3) phenolic ethers, (4) ketones, (5) phenols, (6) aldehydes, (7) oxides and (8) esters.[22]

1.9 Health benefits of oils

Many plants have health qualities, among which are antiseptic, antibiotic, anti-fungal, anti-inflammatory, anti-neuralgic, anti-rheumatic, anti-venomous, anti-depressant, anti-toxic, nervine, digestive, and expectorant. These oils work through olfactory nerve (inhalation) and skin absorption. Essential oils are carried through the circulatory system to all the organs and elimination system. Each organ takes only the components it needs from the essential oils and the balance is discarded through the eliminatory systems.[23,24]

1.10 Foreign names of *Melissa officinalis*

Other names include:

common balm; lemon balm; Melissa; sweet balm; bee balm; heart's delight; cure-all; dropsy plant; blue balm; Zitronenmelisse; Melisse; Herztrost (German); citronelle; baume; mélisse; Herbe citron melissa (Italian); Sidrunmeliss (Estonian); Sitronmelisse (Norwegian); Melisa lekarska (Polish); Melissa limonnaya, Limonnik (Russian); Toranjil (Spanish); Citronmeliss, Hjärtansfröjd (Swedish); Melisa, Ogul out (Turkish); Touroudjan; Tzndjan; Louiza (Arabic); Mèlisse; Citronelle; Mèlisse officinale; Herb du citron; Thè de France (France).[6,9,13]

1.11 Medicinal uses of lemon balm

Its very name suggests the primary application of balm – a soothing, calming agent for stressed nerves. lemon balm, like the various members of the mint family, is invigorating and yet relaxing to the nervous system because it is highly aromatic. These

results are felt throughout the body, and have earned balm the reputation of being a mild panacea. Balm is a common constituent of relaxants, nerving and sleeping aids throughout the world. Seldomly used alone, it seems to interact with and enhance the activity of other beneficial tonics for the nervous system. It works as a calmative as well.

As mentioned before, lemon balm relaxes and strengthens the mind and nervous system and produces sleep. It can be used for sedation and contains at least five sedative compounds. It is also used for epilepsy, other nerve disorders, insomnia, fainting, hysteria, phobias, migraine headaches, hypochondria and vertigo. Lemon balm has a tonic effect on the heart and circulatory system, causing mild vasodilatation of the peripheral vessels and thus lowers blood pressure and helps for heat palpitations. It is good for depression and tension. Its emmenagogue activity promotes menstrual flow. It helps support the female reproductive system and relieves tension, especially for women troubled by PMS or by the emotional upset that may accompany menstrual or menopausal problems.

Balm is also used to ease menstrual cramps and pain. It helps for emotional upset. It can clear mucous from the longs. Balm is also used as a stoma chic (to reduce turmoil in the gastro-intestinal tract), and it is antispasmodic (to reduce tension and cramping in both smooth and striated muscle throughout the body), carminative (to neutralise the effects of gas on the stomach and intestines) and anti-flatulent. Being a diaphoretic, it can be used to induce sweating. It is also well known for its anodyne, antihistamine, febrifuge, antiviral, antibacterial, antimicrobial spasm, sternutatory and stimulant sudorific activities.[25,26]

Lemon balm has the ability to soothe and heal minor wounds.[10] Old European reference books, such as those on medicinal herbs, document a variety of other plants such as *Melissa officinalis* with memory improving properties. Cholinergic activities have recently been identified in extracts from the plant.[27]

In aromatherapy, Lemon balm is primarily used and applied for stress headaches, soothing emotions, to regulate blood pressure and for its sedative properties.[23] It is also used for certain fungal infections.[28] It can block the attachment to the thyroid cells by the antibodies that cause Grave's disease. The brain signal to the thyroid (thyroid-stimulating hormone or TSH) is also blocked from further stimulating the excessively

active thyroid gland in this disease.[29] Lemon balm can be used for insect bites and stings by taking crushed leaves and put it on the site. It is well used in the perfume and cosmetic industries. A strong infusion makes a good rinse for oily hair.

It is used as a facial steam for dry skin and is said to reduce the ageing process. It also makes a refreshing skin toner. Dried leaves add a lemon scent to potpouris.[3,20,22] Because of the lemon scented fragrance, it has the advantage of repelling mosquitoes.[10] In vapour therapy Melissa oil assists fever, headaches hysteria, stress and anxiety.[4] It is also used in the food industry. Fresh leaves are used in salads, salad dressings or as a garnish. Leaves are added to chicken and fish or sprinkled over vegetables for flavour. It is widely used to prepare a hot or cold tea.[6,11]

Lemon balm is formulated in tablets, ointments, creams and suppositories, compressed pillows for rinsing in an infusion, tinctures and massage oil.[26,29] It is suggested not to use lemon balm when pregnant because of its effect on the menstrual cycle. It may irritate skin if used in large quantities.[11] Beyond these, there are no other side-effects dated, and the herb is safe for human consumption.[30]

1.12 Chemical compounds found in lemon balm

It is found that constituents of the essential oil include citronellal, citronellol, citral-A, citral-B, linalol, linolol, rosmarinic, ferulic and caffeic acid, beta-caryphyllene oxide, beta-caryophyllene eugenol, geraniol and geranial (about 96 % of oil ingredients).[31,32] There are also tannins, terpenic alcohols, aldehydes, phenolic acids, organic acids, flavanoids and triterpenes, such as 3-octanol, 3-octanone, alpha-cubebene, alpha-humulene, beta-bourbonene, catecthins, chlorogenic acid, cis-3-hexenol, cis-ocimene, copaene, delta-and gamma-cadinene, germacrene-D, isogeranial, luteolin-7-o-glucoside, neral, nerol, octyl-benzoate, methyl-heptenone, oleanolic acid, pomolic acid, protocatechuic acid, phamnazin, rosmarinic acid, rosmaric acid, stachyose succinic acid, thymol, trans-ocimene, ursolic acid, quercitrin, rhamnocitrin, kaemferol and quercetin.[7,25] The structures of the main compounds are given in Figures 1.3 to 1.7.

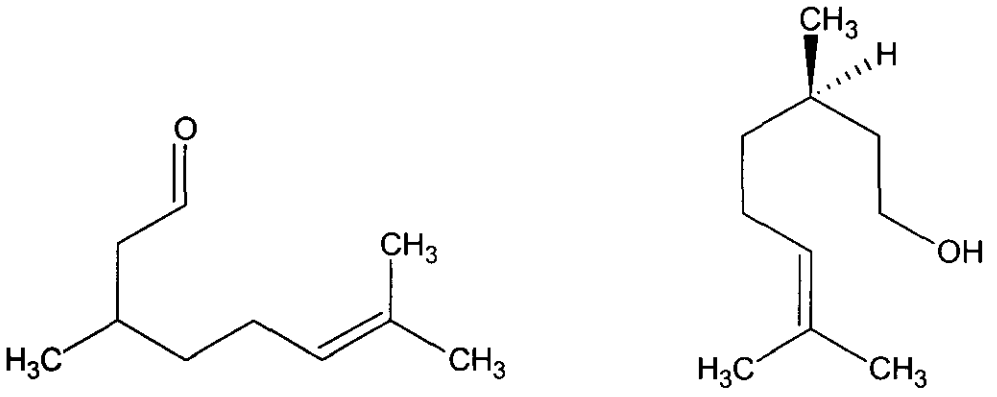

Figure 1.3 Citronellal (30 – 40 % of the essential oil) and citronellol

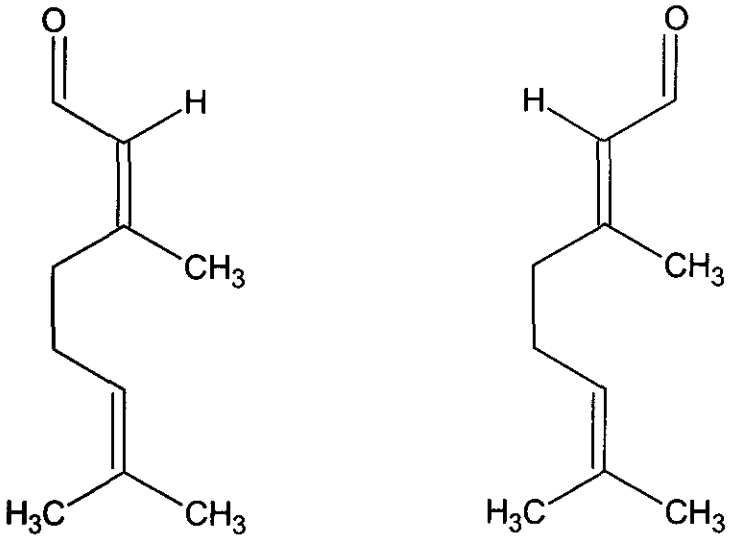

Figure 1.4 Neral and geranial (10 – 30 %)

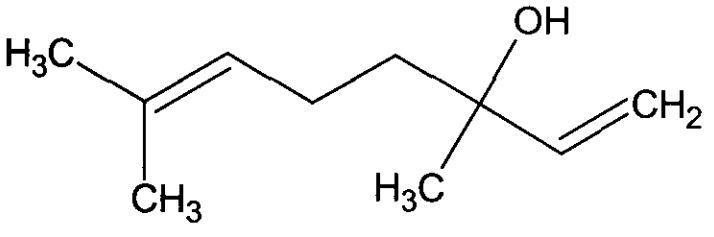

Figure 1.5 Linalol

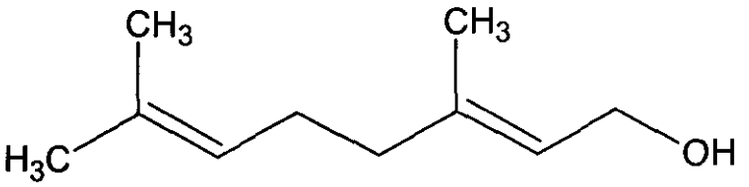

Figure 1.6 Geraniol

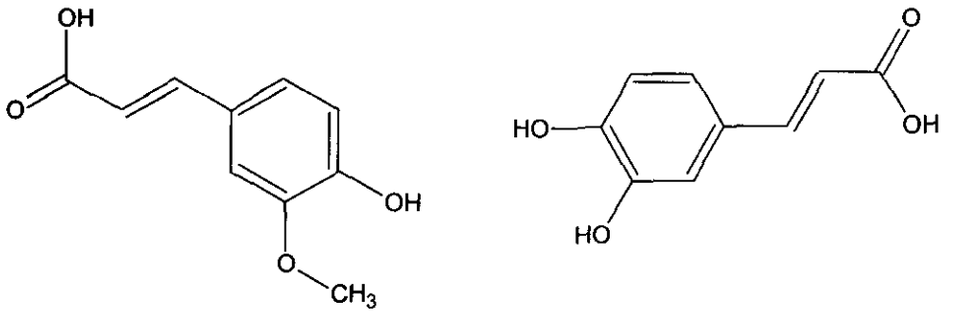

Figure 1.7 Ferulic acid and caffeic acid

The extract obtained by any method may in addition to an essential oil also contains other types of botanical ingredients such as waxes, chlorophyll and others. The essential oil is the prime interest as it is a low-volume high-value product, but an extraction method is seldom so selective that only the pure essential oil is obtained. One of the objectives of this investigation indeed was to properly analyse the composition of the sc-CO_2 derived extract.

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Chapter 2

INTRODUCTION TO SUPERCRITICAL FLUIDS

In 1822, Cagniard de la Tourin described his observation of the liquid phase disappearing when various liquids were heated in close vessels. Upon cooling, the liquids were restored. That study is considered to be the first describing the phenomenon of the supercritical state. The definition of the critical point was introduced by Andrews in 1869. At that point in time it was possible to determine very precisely the critical temperature of 30.9°C (304 K) and the critical pressure of 73.0 atm (7.3 MPa) for carbon dioxide. In the following decades, numerous studies were published, especially about the solubility of inorganic and organic substances in condensed and supercritical gases.[1,2,3] However, it took approximately a hundred years from the discovery of the unusual solvent strength of supercritical fluids, especially for substances of low volatility, to their industrial use as extractants.

2.1 Supercritical fluid technology

In recent years there has been a tremendous amount of attention paid to supercritical fluid extraction (SFE) and supercritical fluid chromatography (SFC), and the interest continues to grow. This interest has been evoked by changes in environmental regulations; conventionally used solvents are being replaced by "cleaner" processes such as those which utilise a supercritical fluid (SF).[4,5,6] It is only since the 1980's and 1990's that the number of research papers published in the field of SFC and SFE has largely increased.[4,5,6,7] SFE and SFC are two complementary techniques of practical interest to analysts in toxicology and therapeutic drug monitoring (TDM). As compared to the more established and widely used gas chromatography (GC) and high-performance liquid chromatography (HPLC), SFC may be regarded as a more recent entry. Although SFC is relatively "older" than SFE, the more recent applications in SFE may have actually enhanced the further application of SFC. Currently, SFC may be characterised as a complementary methodology for analysis not readily performed by GC and HPLC, whereas SFE offers unique advantages compared to traditional extraction methods.[8,9]

The first industrial use of compressed gases as solvents for separation was the deasphalting of heavy mineral oil fractions by means of dense propane in the petrochemical industry in the late 1930's. Since the 1950's, studies and development efforts have been focused on new ways of separating substances by making use of extraordinary properties of supercritical fluids. In this context, mention must be made of the excellent solvent strengths resulting from the liquid-like densities of supercritical fluids. In addition, their favourable transport properties (gas-like viscosity and much higher diffusion coefficients than liquids) are noteworthy with respect to successful applications in extraction and separation processes. An extractive separation process using compressed gases differs from usual solid-liquid and liquid-liquid extractions mainly by the need to make provision for high pressures. This results in capital costs being higher than in traditional extraction methods.[1]

While supercritical ethylene has been used for decades as a medium for polymerisation to low density polyethylene, excitement about supercritical fluids in the 1970's and 1980's focused on their use for extractions and separations. In fact, millions of kilograms per year of coffee and tea is now being decaffeinated using supercritical carbon dioxide (sc-CO_2).[10] Supercritical fluid chromatography, in addition, is widely used in difficult separations in industrial analytical laboratories. A large number of new applications has been developed over the past few years. These include natural product processing applications, environmental applications, biotechnology uses, material processing applications and new possibilities for reaction chemistry, pharmaceutical industries, food and essential oil industries.[10]

Researchers at the National Centre for Agricultural Utilization Research (NCAUR) in Peoria, Illinois, have expanded supercritical fluid technology far beyond decaffeinating coffee and extracting hop for beer flavouring. They have used supercritical fluid extraction (SFE) to enrich nutritionally beneficial compounds, called nutraceuticals, from rice bran, corn fibre or bran and soybeans. The goals were to find alternative sources of nutraceuticals and to find value-added uses for byproducts of oilseed and milling industries. Plant-derived oils contain nutraceuticals that have recently been shown to enhance human health. For example, rice bran, soybean and cofibre oils all contain significant levels of compounds called phytosterols which has cholesterol-lowering properties.[11]

Finally, SFE serves as an alternative sample preparation method with reduced usage of organic solvents and increased sample throughput as a result of decreased extraction time and fewer sample handling steps.[4]

2.2 Nature of supercritical fluids

The nature of a supercritical fluid (SF) may be understood in terms of a phase diagram presenting the solid, liquid and gas phase boundaries of the substance as shown in Figure 2.1. The distinction between the liquid and gas phases vanishes at and above the critical point and the substance only has one new, supercritical phase. A supercritical fluid therefore exhibits both gas-like and liquid-like properties. It is gas-like since it is highly compressible, and it is liquid-like since it has solvent strengths capable of dissolving substances from matrices. A SF can, in view of the foregoing, be considered either a highly compressed gas or a highly mobile liquid for which the liquid and gaseous states are indistinguishable.[12,13,14]

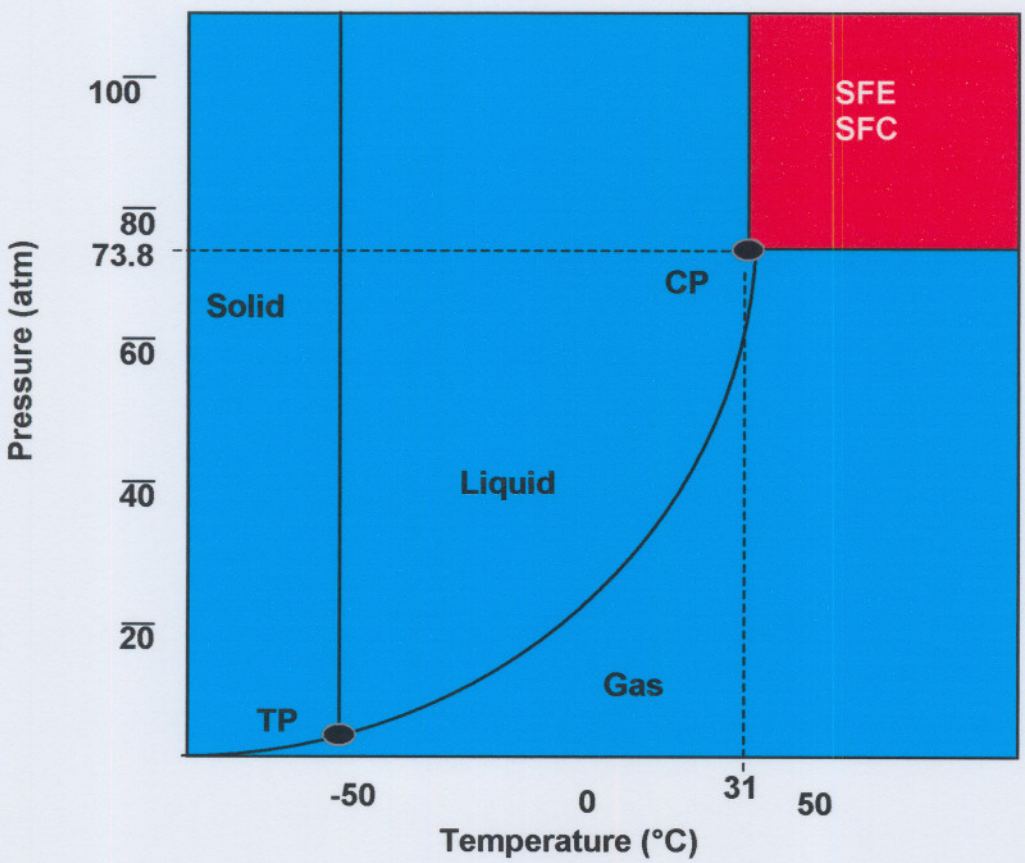

Figure 2.1 Phase diagram of CO_2. *TP* = triple point. *CP* = critical point. P_c = critical pressure (73.8 atm). T_c = critical temperature (31°C).

Figure 2.2 illustrates two different ways of accessing the supercritical state. One involves starting at point A in the liquid region, increasing the pressure above P_c and then raising the temperature above T_c until the state denoted by C is reached. The other way entails starting in the vapour region (point B), heating the substance above its T_c and then raising the pressure above its critical value. The critical point is characteristic for each substance. In Table 2.1 the critical pressure and temperature for various solvents classified according to their chemical nature are listed, together with the fluid density at the critical point, which is called the critical density. Figure 2.3 compares several of these solvents with respect to their critical parameters.[12]

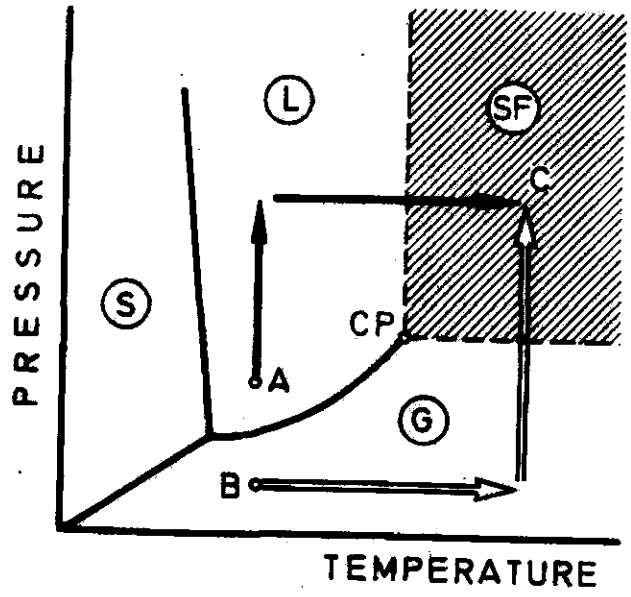

Figure 2.2 Technical ways of reaching a supercritical state (point C) from a liquid (point A) and a gas (point B). CP = critical point.

Table 2.1 Features of various solvents at the critical point

Solvents	Critical temperature (°C)	Critical pressure (bar)	Critical density (g/mL)
Inorganic			
CO_2	31.1	72	0.47
N_2O	36.5	70.6	0.45
NO_2	158	98.7	0.27
Ammonia	132.5	109.8	0.23
Water	374.2	214.8	0.32

Sulphur hexafluride	45.5	38	
Helium	-268	2.2	0.07
Hydrogen	-240	12.6	0.03
Xenon	17	56.9	1.11
Hydrogen chloride	51	83.3	0.45
Sulphur dioxide	157	76.8	0.52
Hydrocarbons			
Methane	-82	46	0.169
Ethane	32.3	47.6	0.2
Propane	96.7	42.4	0.22
n-Butane	152	70.6	0.288
n-Pentane	196	32.9	0.23
n-Hexane	234.2	28.9	0.23
2,3-Dimethylbutane	226.8	42.4	0.241
Ethylene	11	50.5	0.2
Propylene	92	45.4	0.22
Benzene	288.9	98.7	0.302
Toluene	319	41.1	0.292
Alcohols			
Methanol	239	78.9	0.27
Ethanol	243.4	72	0.276
Isopropyl alcohol	235.3	47.6	0.273
Ethers			
Diethyl ether	193.6	63.8	0.267
Ethyl methyl ether	164.7	47.6	0.272
Tetrahydrofuran	267	50.5	0.32
Halides			
Trifluoromethane	26	46.9	0.52
Dichlorodifluoromethane	111.7	109.8	0.558
Dichlorofluoromethane	178.5	32.9	0.522
Chlorotrifluoromethane	28.8	214.8	0.58
Trichlorofluoromethane	196.6	28.9	0.554
1,2-Dichlorotetrafluoroethane	146.1	78.9	0.582
Miscellaneous			
Acetone	235	47	0.279
Acetonitrile	275	47	0.25
Pyridine	347	56.3	0.312

Interesting conclusions can be drawn from the three-dimensional diagram in Figure 2.3[12]:

- Many solvents lie in a specific region, notable exceptions being hydrogen (no.8), helium (no.7), xenon (no.9), $CCIF_3$ (no.32) and water (no.5).
- The critical values for inorganic solvents increase from hydrogen and helium to xenon and water.
- The critical pressure and density of hydrocarbons are very similar. The increase in the number of carbon atoms will increase their critical temperature considerably.
- Critical densities of fluorocarbon solvents are quite similar despite large differences in critical temperature and critical pressure in a few cases.
- Oxygen-containing organic solvents lie in the same region of the diagram.
- All aromatic solvents have a similar critical density and high critical temperature.

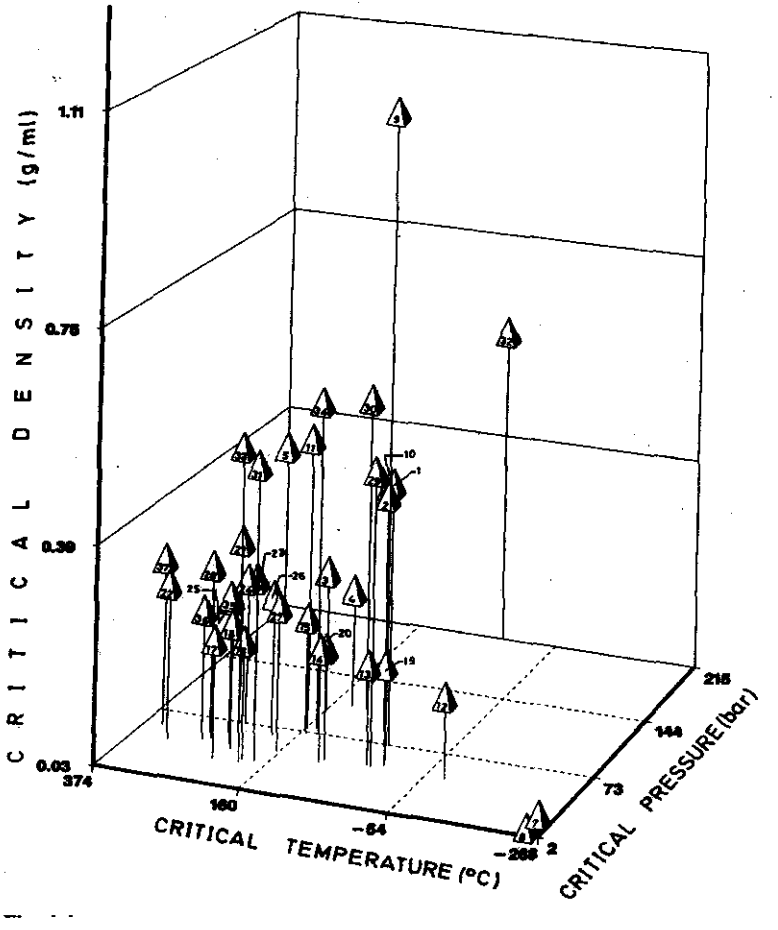

Figure 2.3 Three-dimensional graph of the critical parameters (density, temperature and pressure) for various substances. The numbers refer to those in Table 2.1

2.3 Physical properties of supercritical fluids

Most properties of substances vary widely in the vicinity of the critical point in the phase diagram. Such variability should be taken into account in studying the behaviour of supercritical fluids and can be exploited for some applications. Sharp variations are often observed in the close vicinity of the critical point. The following sections discuss the influence of temperature and pressure on several essential properties of supercritical fluids, namely: dielectric constant, diffusivity, density and viscosity.[12]

2.3.1 Dielectric constant

The dielectric constant is one of the most relevant physico-chemical properties for defining the solubility in fluids. The dielectric constant of water is relative high (78.5 at 25 ^{0} C and 1 atm), thereby facilitating dissolution of ionic compounds. It decreases with increasing temperature and decreasing pressure. Because of its low dielectric constant at high temperatures, water shields the electrostatic potential between ions only weakly, so that dissolved ions can freely form ion-pairs. Supercritical water behaves as a non-polar rather than a polar solvent under these conditions. These properties partly account for its ability to dissolve non-polar organic compounds.[12]

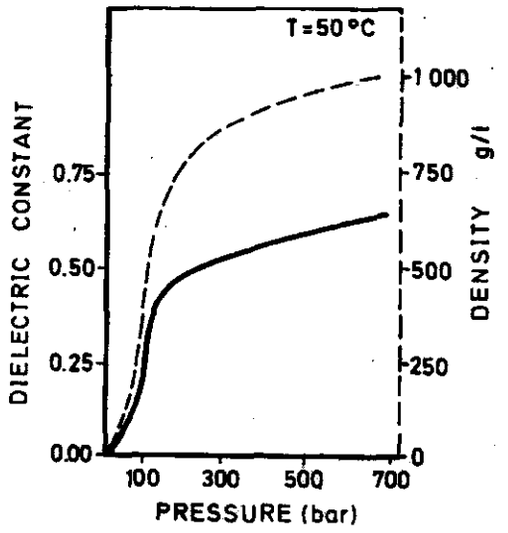

Figure 2.4 Pressure influence on the dielectric constant and density of supercritical CO_2 at a constant temperature.

Figure 2.4 shows the influence of pressure on the dielectric constant and density of sc-CO_2 at constant temperature. The dielectric constant in a very dense state (200 bar and 40°C) is ca.1.5, which means that CO_2 is a highly non-polar solvent capable of dissolving non-polar substances. The key physico-chemical property of a SF is without doubt its density, which is determined by pressure ($P > P_c$) and temperature. The density relates directly to the dielectric constant and the viscosity, and has a decisive effect on the solvent strength and diffusivity.[12]

2.3.2 Diffusivity

Figure 2.5 depicts the diffusivity of solutes in CO_2 as a function of temperature at different pressure values. The typical diffusivity range for ordinary liquids is also shown. There are three conclusions to be drawn from the figure:[12]

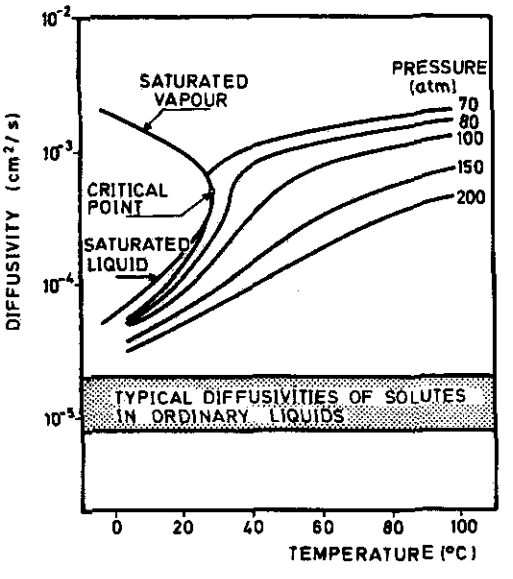

Figure 2.5 Variation in diffusivity of CO_2 as function of temperature at different pressures

- The diffusivity of a solute in a supercritical fluid always exceeds that in an ordinary liquid solvent.
- The diffusivity increases with an increase in temperature, especially in the vicinity of the critical point.
- An increase in pressure is responsible for a decrease in the diffusivity of a SF.

2.3.3 Viscosity

The viscosity of a substance (gas, liquid or supercritical fluid) is temperature dependent. While pressure has little effect on the viscosity of liquids, it markedly influences that of supercritical fluids. Figure 2.6 illustrates that at a given constant temperature, viscosity increases with pressure. Increased SF viscosity results in diminished solute diffusivity and transport, but in increased solubility through decreased density.[12]

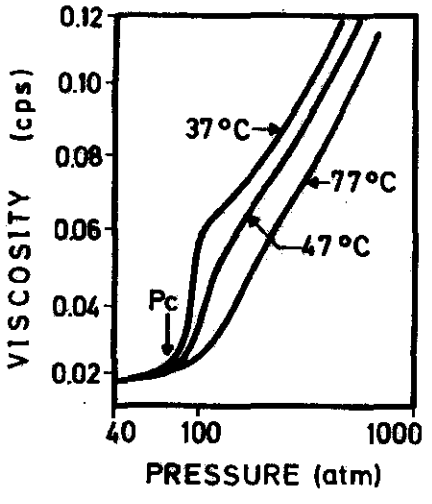

Figure 2.6 Variation of viscosity of CO_2 with pressure at three different temperatures. P_c = critical point

2.3.4 Density

The density of a supercritical fluid is markedly dependent on its pressure and temperature. The variation of the density of a SF with pressure at a constant temperature is typically non-linear. The variables in Figure 2.7 are all reduced variables given as ratios between their actual values and those at the critical point. The critical point occurs at the intersection where the three reduced variables have unity value. Density in the supercritical region increases sharply with increasing pressure at a constant temperature. It also decreases with increasing temperature at a constant pressure. The steep slopes of the curves in the vicinity of the critical point imply that a small pressure rise will result in a sharp increase in solvent density. The slopes of the curves decrease sharply with increasing distance from the critical point.

The zone immediately above the critical point provides the largest density changes and thus the most effective region to effect changes by minimal temperature and/or pressure variations. The darker area at the bottom left of the CP is also useful for extraction purposes. The slopes of the curves in this region are not that steep in the supercritical region, yet the density varies sufficiently with pressure for useful purposes.

The solvent strength of a given fluid depends on its density. Even though the density of a fluid strongly influences the solute-solvent interactions in the bulk of a supercritical solvent, little is known about the nature of the interactions themselves or how they change with density. The solvent strength of an ordinary organic liquid solvent is essentially independent of pressure, but the density of a SF can be varied over a wide range by changing the pressure, the temperature, or both.[12]

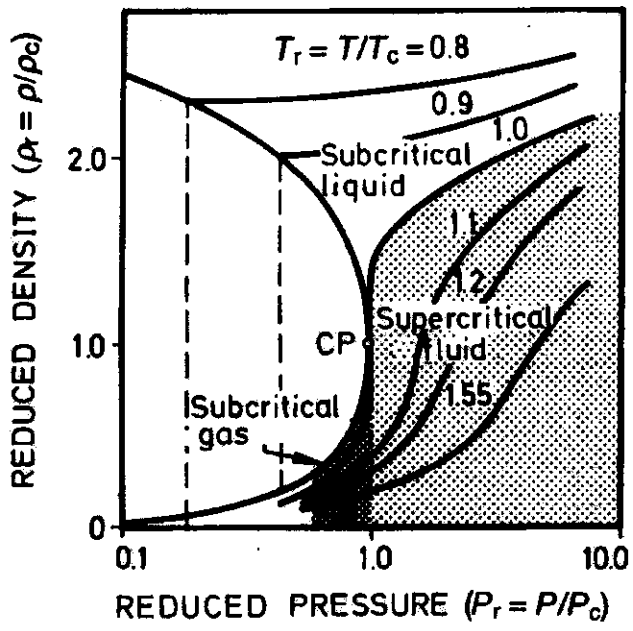

Figure 2.7 Two-dimensional reduced density-reduced pressure isotherms. CP = critical point

The physical properties of supercritical fluids in relation to extraction are summarised in Figure 2.8.[12]

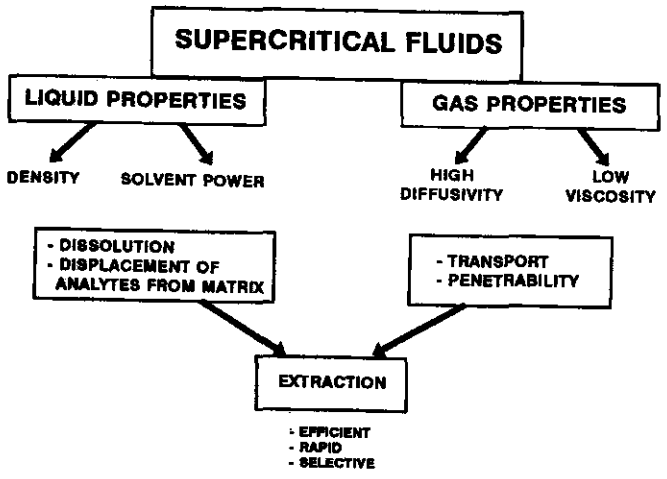

Figure 2.8 Physical properties of SFs in relation to extraction

2.4 Choosing an appropriate supercritical extractant

The choice of substance to be used as a supercritical solvent is determined by the polarity of the target analyte. Practical considerations, such as the pressure and temperature required to take a substance to its critical region are also important in the choice of a SF. A number of substances have critical parameters that allow SFE to be performed. These substances span a wide range of solvent strengths but, for practical reasons, the range generally used is more restricted. A few fluids, such as ammonia, are usually ruled out in view of corrosion or toxicity hazards. Ethane and methane are flammable and their use requires special safety considerations. Methanol, although having a high solvent strength, is not generally employed as a SF due to its rather high critical temperature; it is, however, commonly used with other fluids as a cosolvent. On the basis of solubility alone, nitrous oxide (N_2O) is quite similar to CO_2 in terms of the range of readily extractable analytes. However, notable differences in extraction efficiencies of these two fluids have been reported.[13] Although N_2O possesses a small permanent dipole whereas CO_2 has no permanent dipole, differences in the interaction with the matrix are probably responsible for the observations made. Promising results

have been obtained using chlorodifluoro-methane, which has a larger dipole moment than CO_2 and can thus be expected to solubilise more polar analytes.

The main problem with the most widely used SF, CO_2, is the relatively low solvent strength at typical extraction pressures (80-600 atm). This limits extraction to non-polar analytes such as hydrocarbons, halogenated hydrocarbons, steroids, fats, organochlorine and pesticides. For more polar analytes, addition of modifiers or cosolvents is usually required. The contribution to the overall effect of the addition of a polar modifier, such as methanol, lies in its ability to displace analyte molecules from adsorption sites on the matrix, particularly when small quantities are used. The presence of small quantities of water also assist this displacement process where highly polar matrices are involved. This was recently demonstrated with the water entrained sc-CO_2 extraction of polar flavonoids from rooibos tea.[15] The type and quantity of modifier used with CO_2 is generally arrived at by trial and error, since limited solubility data for analytes in modified CO_2 is available. Common modifiers include methanol, propanol and dichloromethane.[13]

2.5 Advantages of carbon dioxide as extractant

The advantage of CO_2 over organic solvents lies primarily in the health safety extractions that can be performed with it. It is found everywhere in nature, e.g. in water and in the atmosphere. It is breathed by plants and produced when organic substances ferment. It is formed in many manufacturing processes in the food industry, e.g. in the production of beer and wine, in the baking of bread, etc., and remains in these food products. It is also used artificially in carbonated mineral waters. Being an "inert" gas, even in the supercritical state, CO_2 does not react in any way with food constituents. This is also the reason for its universal use for quick-freezing foods in the liquid and solid state. Recent legislation[1,6,9,16] does not regard CO_2 as a foreign substance, and together with air, nitrogen, and distilled or demineralised water, CO_2 is excluded from a list of prohibited additives.

In addition to the advantages mentioned above, CO_2 is neither flammable nor toxic, not maintaining combustion, and also not exerting a corrosive effect in combination with moisture on materials (stainless steel or plastics) used in processing natural products. It is inexpensive and readily available in large quantities and high purity. For all these

reasons, natural products have to date been processed predominantly with CO_2. It can be eliminated at rather low temperatures without leaving any toxic residue. All these advantages have made CO_2 the substance of choice for a variety of extraction processes. sc-CO_2 is used for extracting non-polar and slightly polar species but is not effective for polar compounds. However, the addition of modifiers to increase its polarity has resulted in the solvation of slightly polar species. The addition of other compounds soluble in sc-CO_2 has led to some interesting reaction chemistry and related applications.[1,6,9,17]

2.6 Where are supercritical fluids best applied?

It is generally recommended that supercritical fluids should be used where

- environmental compliance pressures require a change in a process;
- regulatory pressures require a change in product purity;
- increased product performance is required;
- an improved product can create a new market position;
- none of the previous can be achieved by industry's more traditional processes.[18]

Care should be taken not to force fit supercritical fluids into an application that is adequately handled by traditional industrial operations such as solvent extraction, distillation, wiped film evaporation, and the like.

2.7 Major advantages of SFE

The advantages of SFE are briefly summarised in this final paragraph :[5,18,19,20,21,22,23]

- The lower viscosity and higher diffusivity of supercritical fluids result in more effective penetration of porous solid materials and thus faster extractions than with traditional solvents.
- In dynamic SFE, fresh fluid continuously flows through the sample and can therefore result in quantitative or complete extraction.

- The solvent strength of a supercritical fluid can be manipulated by changing pressure and/or temperature; therefore SFE may achieve high selectivity.

- Solutes dissolved in sc-CO_2 can be easily separated by depressurisation. SFE therefore eliminates sample concentration, which is usually time-consuming and often results in loss of volatile components.

- SFE is usually (especially with CO_2) performed at low temperatures, so it may be an ideal technique to study thermally labile compounds. This may in turn lead to the discovery of new natural compounds.

- Compared to 20-100 g samples typically required in liquid-solid (LS) extractions, samples as small as 0.5-1.5 g are needed for SFE.

- SFE uses no or significantly less environmentally hazardous organic solvents.

- SFE may allow direct coupling with a chromatographic column, which can be a useful means to extract and directly quantify highly volatile compounds.

- In large-scale SFE the fluid, usually CO_2, can be recycled or reused, thus minimising waste generation.

- SFE can be applied to systems of different scales, for instance, from analytical scale (less than a gram to a few grams of samples), to preparative scale (several hundred grams of samples), to pilot plant scale (kilograms of samples) and up to large industrial scale (tons of raw material).

- When CO_2 is the solvent, SFE has the added advantage of being non-toxic.

- SFE eliminates hot spots in catalytic reactions which cause coking or other unwanted side-reaction products.

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Chapter 3

EXPERIMENTAL : PRINCIPLES, METHODOLOGY AND PROCEDURE

This chapter covers all experimental aspects of the investigation. It includes, in quasi-chronological order of execution, the topics of plant material selection and sample preparation, performance of extraction runs, analysis of extracts, and principles of experimental design, data processing and process modelling.

3.1 Instrumentation used in SFE

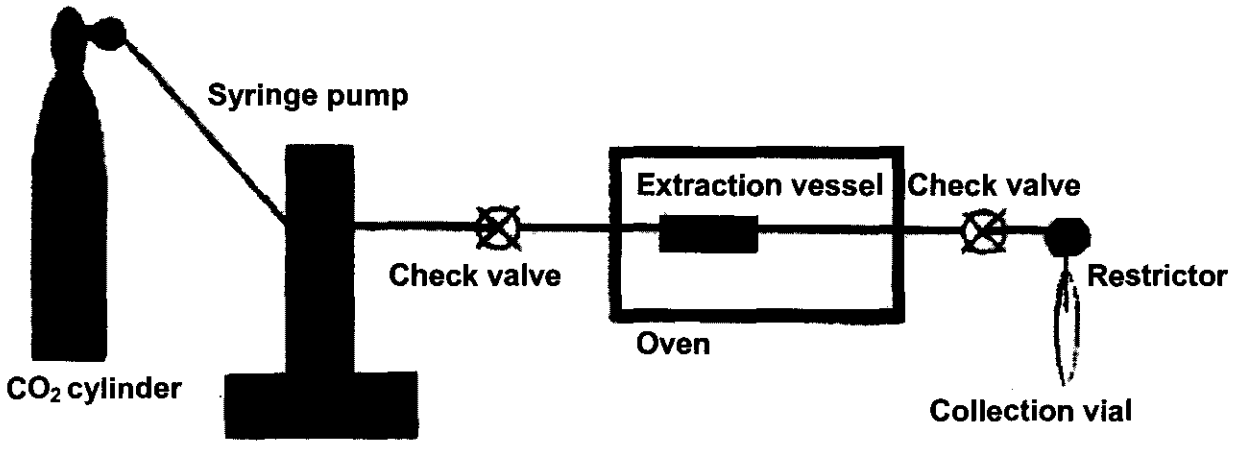

Figure 3.1 Instrumentation for SFE

SFE instruments are simple in design. Modern commercial instruments range from manually operated systems to highly sophisticated automated devices. In principle, these instruments all comprise five basic components as shown in Figure 3.1: a pump system, a constant temperature chamber, an extraction vessel containing the sample matrix, a restrictor to decompress the supercritical fluid and a trapping device to collect analytes.

The gas is pre-cooled and entered into the instrument in the liquid state. It is compressed by a double syringe pump to the appropriate pressure and thermostatted to the required temperature to warrant supercritical conditions before passing through the extraction vessel containing the sample matrix. The compressed fluid diffuses through the sample matrix and exits the vessel loaded with extracted analyte after either a preselected residence time (static mode) or at a preadjusted flow rate (dynamic mode). It finally passes on to the restrictor, where it is decompressed and bubbled through a solvent in a cold trap collector. The extracted material is deposited in the collection solvent while the gas is vented into the atmosphere.[1,2,3]

3.2 Plant selection and sample preparation

The selection of suitable plant material was done in collaboration with Clive Teubes (Pty) Ltd in Randburg, Johannesburg, a company known for the formulation and production of fragrances and flavours. The herbal plant *Melissa officinalis* was chosen for this investigation. Its specifications are listed in Table 3.1.

Table 3.1 Plant specifications

Date received	Plant species	Farmer	District	Moisture content	Mass Received (kg)	Oil content (dry mass)
2002-06-11	Lemon Balm	Andrew van Lingen	Schoombee, Eastern Cape	11%	5	0.02%

The plant material was harvested during March–April and then dried at room temperature until June. It was then kept in a store room. The stems and leaves were used for extraction. Figure 3.2 shows how the stripping was done. A mask was worn to prevent inhalation of plant "dust".

Figure 3.2 Stripping leaves from the stems

The leaves and stems were ground separately. This was done initially by using a ball and mill system shown in Figure 3.3. This process was tedious, as an entire day was needed to obtain uniform particles from a limited amount of plant material. A household Waring Blendor food processor shown in Figure 3.4 was a better option, providing particles of uniform size distribution at a much faster rate.

Figure 3.3 Ball crusher

Figure 3.4 Food processor used for crushing

3.3 Extraction

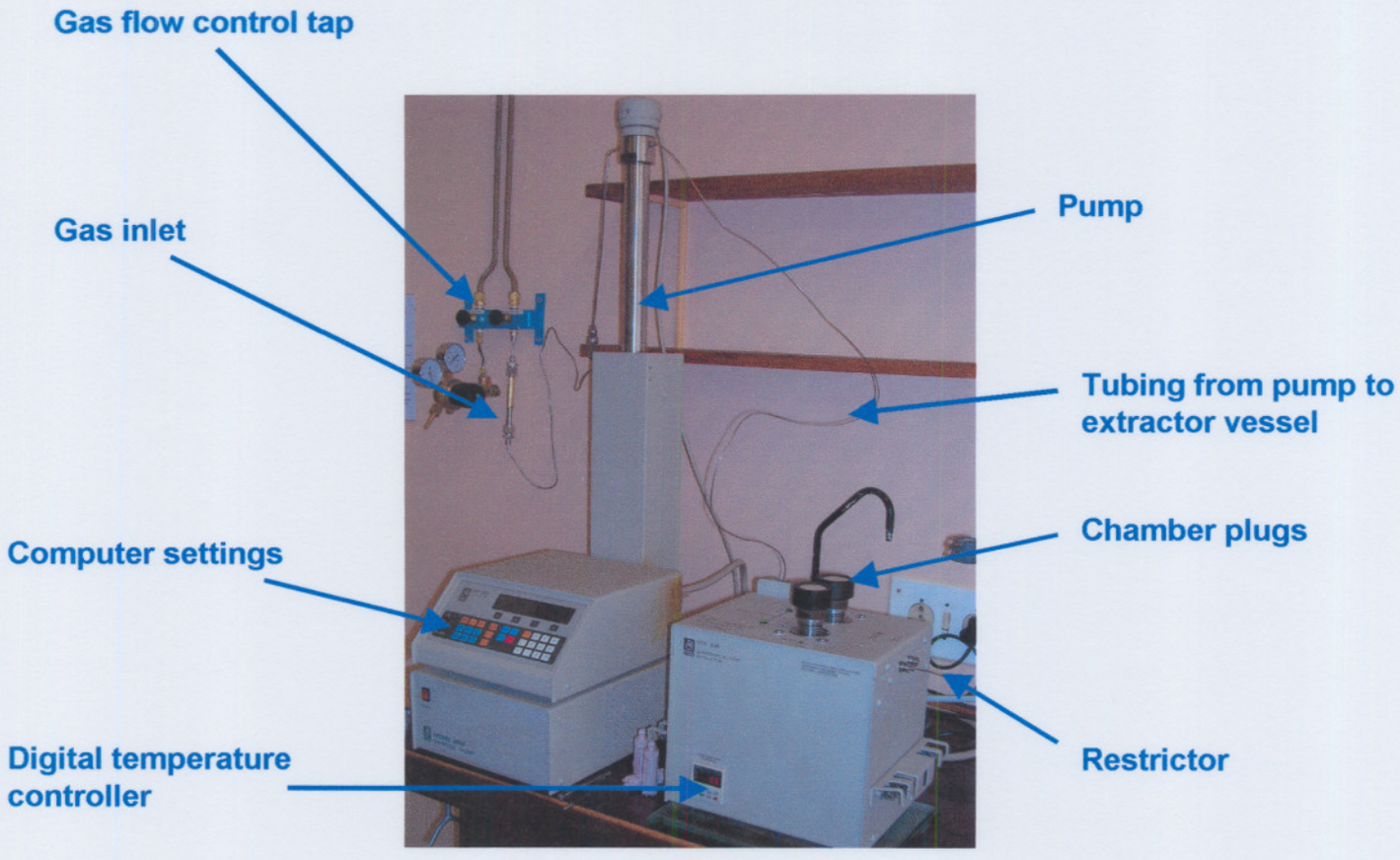

Figure 3.5 Bench-top scale extractor (ISCO SFX™ 220)

The extractor shown in Figure 3.5 (ISCO SFX™ 220) was used to perform the extraction runs. The setup does not include a modifier pump as no cosolvent was used during extraction. The helium compressed CO_2 was supplied by Afrox. On switching on the instrument, a main menu is displayed. At this module functions like flow rate, pressure, time of extraction, mode (static and dynamic) and auto refill timing can be selected. The temperature within the sample compartment is also programmed on the on-board computer. The prepared plant material was entered into preweighed disposable polymer cartridges shown in Figure 3.6 and weighed by means of an electronic mass meter.

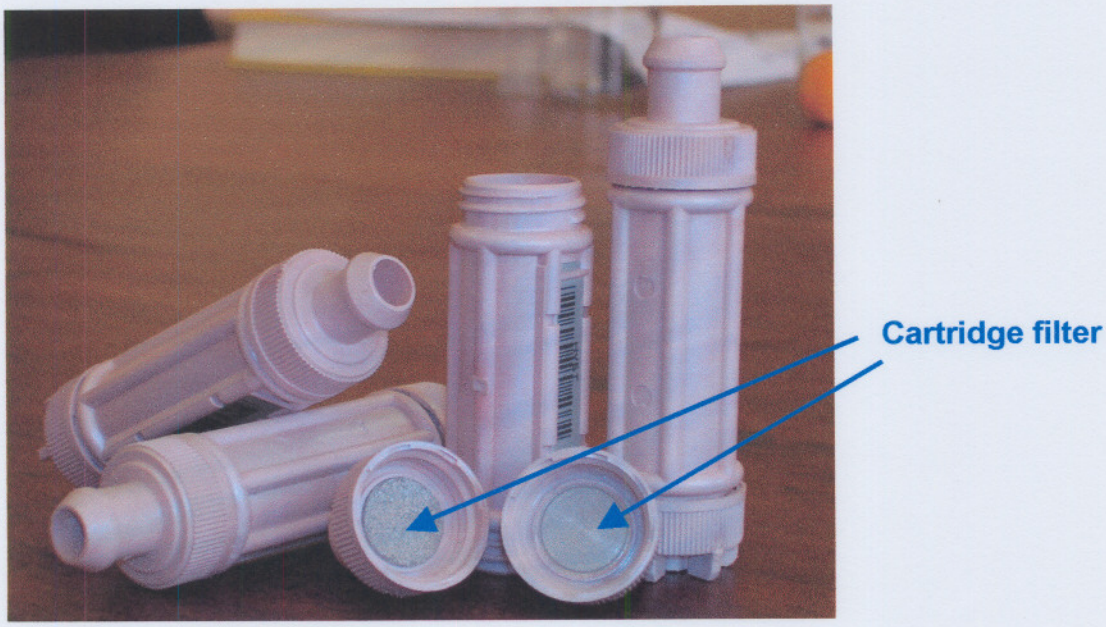

Figure 3.6 Disposable polymer cartridges

The filled cartridge was then hooked onto the chamber plugs and screwed into the oven compartment. When all the set conditions were attained, the extraction run was initiated. The pump is initially filled with carbon dioxide precooled to its liquid state. The liquid is channelled to the extraction chamber where supercritical conditions are established. The resulting fluid is led through the disposable polymer cartridge equipped with filters (Figure 3.6) to prevent sample particles from entering the flow line by physical displacement.[2,3] At the restrictor ambient conditions are restored. The dissolved/desorbed plant components are collected while the gas is released into the atmosphere.

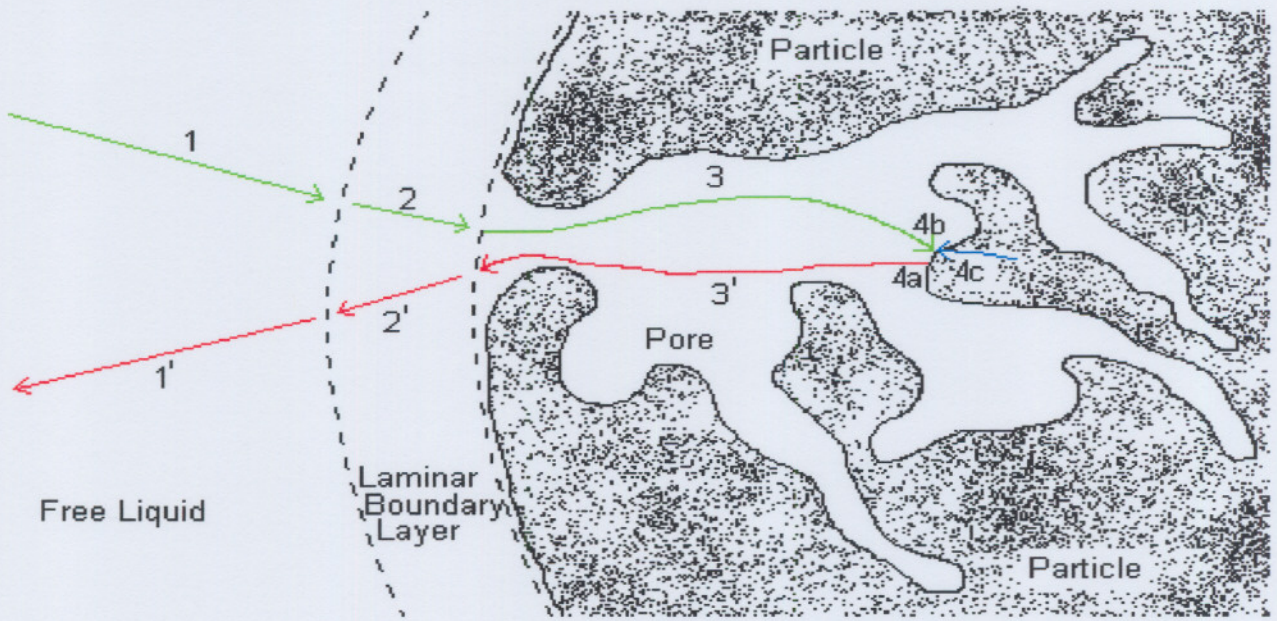

Figure 3.7 Mechanism of extraction

Extraction of substances from the bulk of the plant material can be visualised in terms of the pore structure of the plant matrix as shown in Figure 3.7. sc-CO_2 is transported to the matrix by convention (1) and crosses the laminar boundary layer by film diffusion (2). On reaching a pore, transport changes to pore diffusion (3). Within a pore, dissolution (4a) or desorption (4b) takes place, or prior to these, diffusive transport of components through the solid material to the pore surface (4c) occurs. The loaded solvent reaches the bulk of the fluid, initially by pore diffusion (3'), subsequently by film diffusion (2') and finally by convention (1').

Figure 3.8 Extracted oil

The extracted oil (Figure 3.8) was trapped in a specially designed vial (Figure 3.9) to optimise sample collection. An outlet in the cap of the vial relieved some of the pressure build-up in the vial. The inlet and outlet plastic tubes were cut to perfection to fit the restrictor tightly. A GC vial with properly sealed cap was put on top of the assembly to quantitatively trap the extracted material without any pressure build-up problem.

Initially, extractions were performed without a water bath. The extracted oil froze completely in the restrictor as a result of its small diameter and the Joule-Thompson cooling of the expanding gas. By connecting a water bath held at the same temperature as that used for extraction (Figure 3.10), blockage of extracted material was minimised.[2.3]

When the flow lines were rinsed with either n-hexane or acetone (extracted material dissolved well in both of them), it was obvious that extract was left behind in the instrument. Rinsing was repeated two to three times to ensure that all the extract was recovered. The dissolved extract was weighed once the solvent had been evaporated and the mass was added to that of the main extract.

Equipment was finally rinsed with acetone (technical grade) and then with distilled water prepared with a Milli-Q Plus system (supplied by Millipore). Distilled water and acetone were also used in an ultrasonic bath (UMC-5 Integral systems) to clean obstructed flow line tubing or restrictor parts.

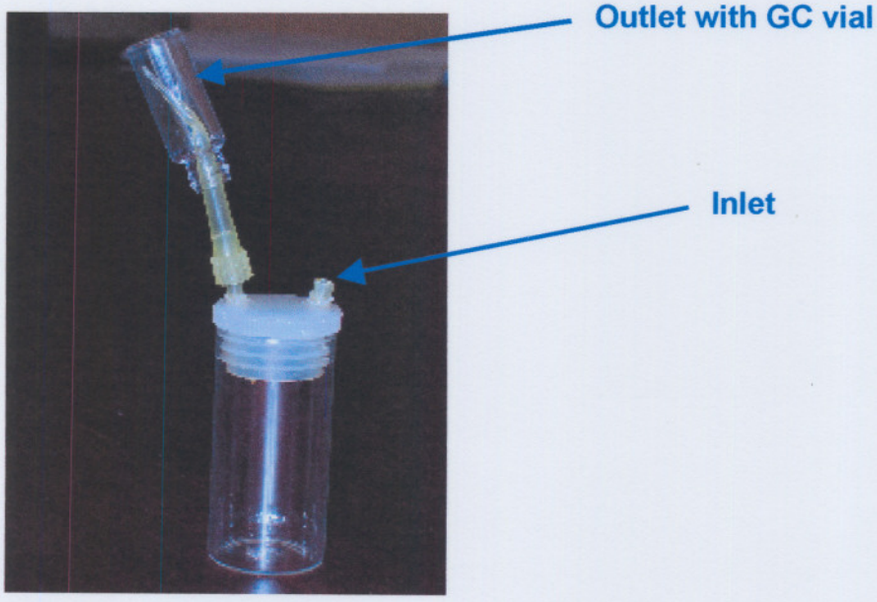

Figure 3.9 Trapping vial

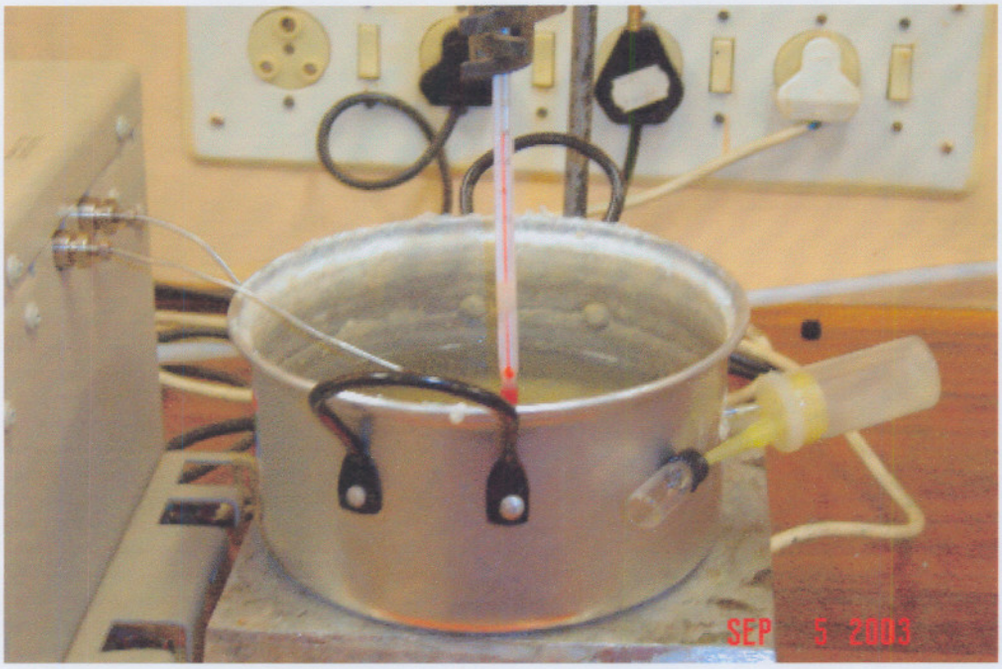

Figure 3.10 Restrictor with collecting vial

3.4 Analysis

Three variants of chromatography were used as analytical tool for qualitative and quantitative analysis of extracts in this investigation. These are ordinary gas chromatography (GC), combined gas chromatography and mass spectrometry (GC-MS) and two-dimensional gas chromatography in either its GC-GC/FID (flame ionisation detector) or GC-GC/TOF-MS (time-of-flight mass spectrometry) version.

Generally, chromatography is based on the separation of a multicomponent mixture into its distinct compounds as a result of their different retention by two phases in a suitable column. One is the stationary phase and the other one the mobile phase. The interaction of each compound with these two phases at specific conditions gives rise to different retention times (the time needed for the components to move through the column). A peak is registered once the detector perceives a component which exits the column. The different retention times cause the components to be detected in different regions of the resulting chromatogram. The chromatographic peaks can be used for qualitative and quantitative analysis.

The samples are injected and vaporised at the head of the chromatographic column. Elution is effected by the flow of an inert gas as mobile phase. In contrast to most other types of chromatography, the mobile phase does not interact with the molecules of the analyte. Its only function is to transport the analyte through the column. There are two types of gas chromatography, viz. gas-liquid chromatography (GLC) and gas-solid chromatography (GSC). GLC is mostly used and is generally referred to as gas chromatography (GC). In this investigation analysis was performed by GC. The technique is based upon the partition of the analyte between a gaseous mobile phase (argon, carbon dioxide, helium, hydrogen and nitrogen) and a liquid phase immobilised on the surface of an inert solid. There are generally two types of column, viz. capillary and packed/open tubular columns. These can vary in length from less than 2 meters to more than 50 meters and are made of teflon, fused silica or stainless steel.

GC is often coupled with selected techniques of spectroscopy and electrochemistry. The so-called hyphenated methods (for instance, GC-MS and GC-IR) are powerful tools to identify the components of complex mixtures. Once a molecule from the GC is introduced into the MS, it is ionised and fragmented. The ions are selected, counted and their abundance plotted as a function of the mass-to-charge ratio to yield a mass spectrum. Figures 3.11 and 3.12 jointly show a typical GC-MS spectrum.[4,5,6]

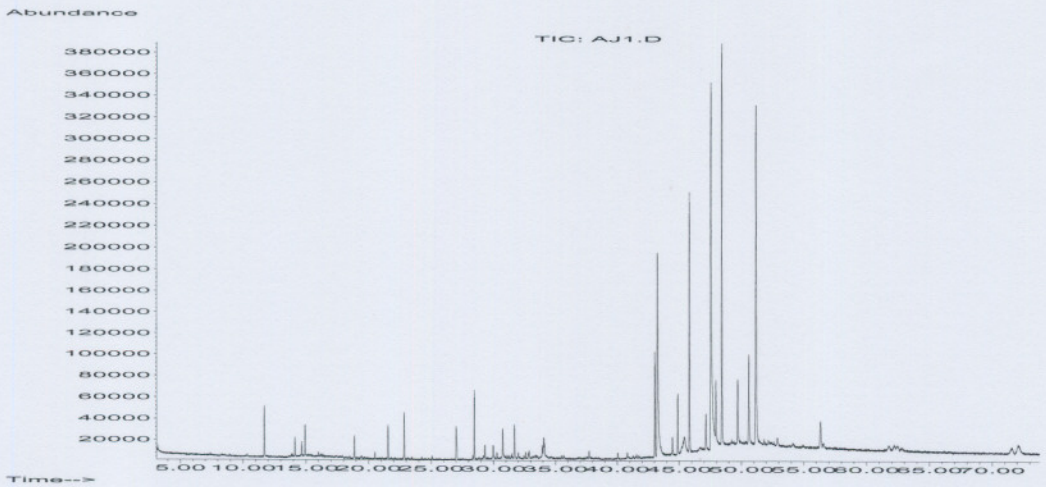

Figure 3.11 GC spectrum

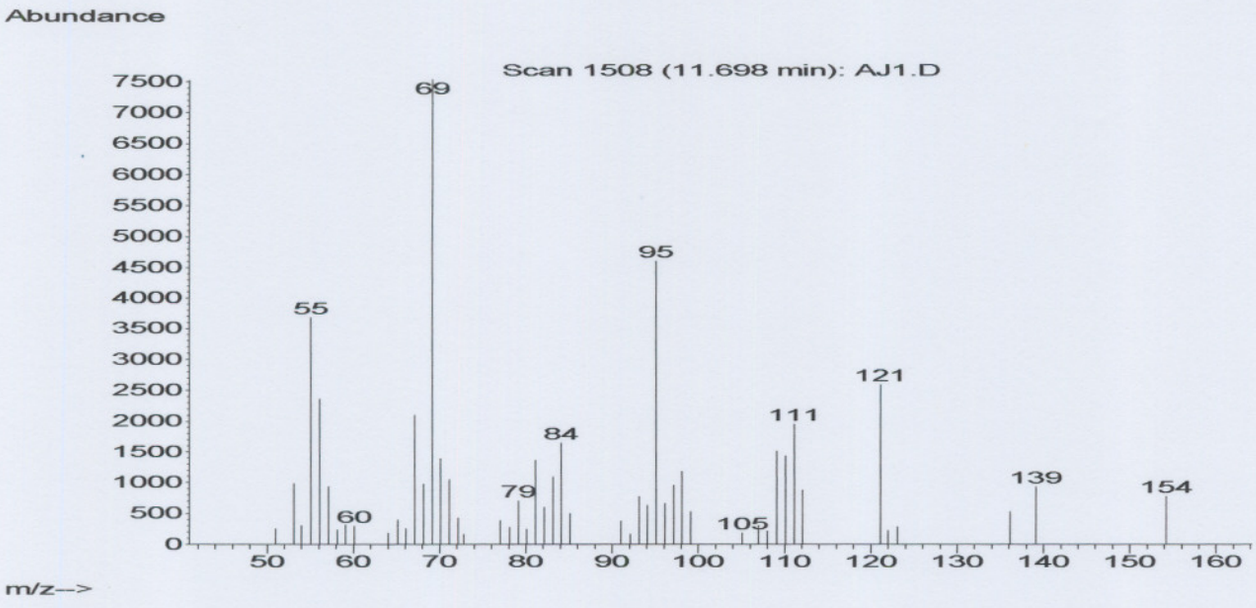

Figure 3.12 MS spectrum

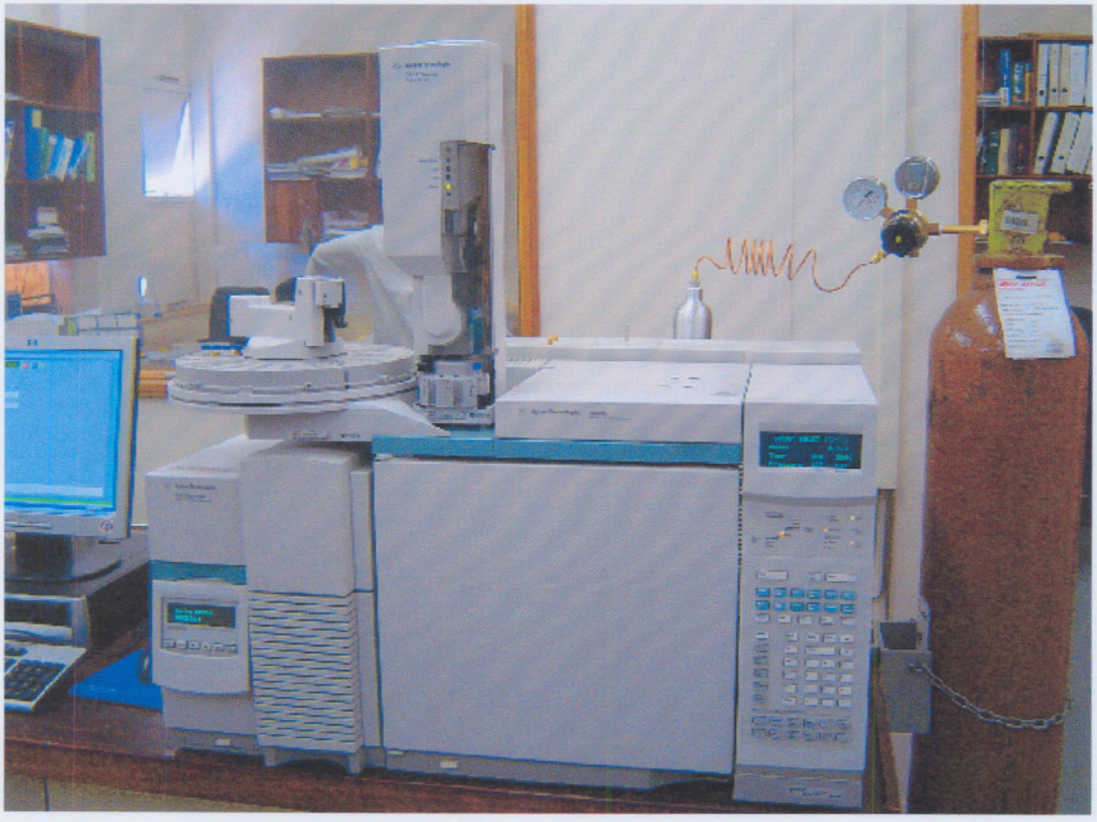

Figure 3.13 HP6890 GC-MS

The instrumental setup shown in Figure 3.13 comprises a GC, MS, autosampler, injector and computer hardware/software from Agilent Technologies. The 6890 GC is connected to an MS having a 5973 network mass selective detector. An autosampler (6890 Series ALS tray) and injector (7673 Series injector) are included in the setup. A computer is connected to the system with a software program that runs the entire analysis. The analysis protocol is summarised in Table 3.2.

Table 3.2 Protocol for GC-MS

OVEN :					
	Initial temp:	**50°C (on)**			
	Initial time:	**1.00 min**			
	Ramps:				
	#	Rate	Final temp		Final time
	1	**5**	**290°C**		**30.00 min**
	Run time:	**79.00 min**			
FRONT INLET (Split/Splitless):					
	Mode:	1.1 Split			
	Initial temp:	**220° C (on)**			
	Pressure:	**75.4 kPa (on)**			
	Split flow:	**49.0 ml/min**			
	Total flow:	**53.2 ml/min**			
	Gas type:	**Helium**			
COLUMN:					
	Capillary column				
	Model num:	**Agilent 19091S-433**			
	specifications:	**HP-5MS**	**0.25mm**	**30m**	**0.25µm**
	Max. temperature:	**350° C**			
	Nominal length:	**30.0 m**			
	Nomnal diameter:	**250.00 µm**			
		MS Quad:	**150°C maximum 200°C**		
		MS Source:	**230°C maximum 250°C**		

When an extraction run was completed, the collection vial was removed from the restrictor and sealed. n-Hexane (GC/HPLC standard, 99.99% pure) was used to dissolve the extract and to dilute it to a fixed volume (1 mL). This was then placed onto the autosampler tray, which moved it automatically to the injector.

GC-GC/TOF-MS is used to analyse highly complex mixtures and to provide results not possible with conventional one-dimensional GC-MS systems. When a GC-GC system is used, two separation mechanisms are employed. Typically the first column in the system is a non-polar column that produces a boiling point separation. The second column is a much shorter one employing a polarity based separation mechanism.

The increased chromatographic resolution is extremely powerful when complemented by automated peak find and spectral deconvolution algorithms to identify the components of highly complex mixtures. The deconvolution can only be properly implemented on a time-of-flight mass spectrometer. The compounds present in a sample are identified by matching the recorded spectrum to those contained in standard libraries used routinely in GC-MS analysis. Table 3.3 summarises the protocol used for GC-GC/TOF-MS analysis.

Table 3.3 Protocol for GC-GC/TOF-MS

Detector:	LECO Pegasus 4D Time-of -Flight Mass Spectrometer
Acquisition Rate:	125 spectra/sec
Stored Mass Range:	35 to 350 u
Transfer Line Temperature:	225°C
Source Temperature:	200°C
Detector Voltage:	-1750 Volts
GC:	Modified Hewlett Packard 6890N*
Column 1:	SPB-1, 30 m x 0.25 mm ID, 0.25 µm film thickness
Column 2:	Rtx 1701, 2 m x 0.1 mm ID, 0.1 µm film thickness
Column 1 Oven:	50°C for 1 min, to 275°C at 10°C/min., hold for 2 min.
Column 2 Oven:	55°C for 1 min, to 280°C at 10°C/min., hold for 2 min.
Second Dimension Separation Time:	4 sec.
Inlet:	Split at 200°C; split ratio 100:1
Injection:	1 µL
Carrier Gas:	Helium, 1.0 mL/min. constant flow

3.5 Experimental statistical design

The primary goal of statistical design in scientific research is to establish the statistical significance of the effect a particular factor exerts on a dependent variable of interest. It finds increasing use in manufacture to optimise a production process. Supercritical fluid ectraction is mainly influenced by pressure, temperature, flow rate and extraction time. For extraction of plant material, the % yield of extract may strongly depend on temperature and pressure (or density) of the supercritical fluid employed.

At least three different values are necessary to determine a second-order, quadratic or non-linear correlation between dependent and independent variables. The design should be as orthogonal as possible to retrieve maximum information from it. In principle, a number of pressure and temperature parameter pairs need to be selected for extraction runs to be performed at. The % yield of extract for these runs are then fitted by appropriate software (Statistica for Windows) as a function of the independent variables (pressure and temperature) according to a mathematical model.[7,8]

To derive a reliable mathematical model from the parameter pairs, the design should comply with certain requirements. The basic one is that the individual and interactive effects of the two parameters of interest should be independent of each other, implying that the design matrix should be orthogonal as shown in Table 3.4.

Table 3.4 Orthogonal design

	Independent Variable 1	**Independent Variable 2**
Run 1	1	1
Run 2	1	-1
Run 3	-1	1
Run 4	-1	-1

The second requirement is that the design should allow

1. a maximum amount of unbiased information to be retrieved from the experimental region of interest;

2. the prediction of values with the least uncertainty, where uncertainty depends on the variability of the design points and their covariance over the runs.

For the 4-run orthogonal design shown above, the information is the same on a circle centred at the origin. Thus any kind of rotation of the original design points will generate the same information. The 2-by-2 orthogonal design is therefore said to be rotatable. However, such a design is not feasible to detect a curvature in the relationship between the independent variables (so-called input factors) and the dependent variables. In order to estimate a higher order (second-order, quadratic, or non-linear component) relationship between the input factors and dependent variables, one needs at least three levels of interdependence for the respective factors. The information function then becomes more complex and non-rotatable, implying that different rotations of the design points will render different information from the experimental design. However, by adding so-called star points to the simple 2-by-2 orthogonal design points, one can achieve rotatable and often orthogonal or near orthogonal designs. For example, by adding appropriate points to the simple 2-by-2 orthogonal design shown in Table 3.4 a rotatable design listed in Table 3.5 is obtained.

Table 3.5 Rotatable design

	Independent Variable 1	**Independent Variable 2**
Run 1	1	1
Run 2	1	-1
Run 3	-1	1
Run 4	-1	-1
Run 5	-1.414	0
Run 6	1.414	0
Run 7	0	-1.414
Run 8	0	1.414
Run 9	0	0
Run 10	0	0

The first 4 runs represent the previous 2-by-2 orthogonal design. The added star points are represented by runs 5 to 8, and runs 9 and 10 are the centre points. The information function of this second-order design is rotatable, i.e. constant on a circle around the origin.

Rotatability refers to the capability of the design to render the same information in all directions of the fitted surface. The rotatable, orthogonal design can be displayed by a surface response graph, an example of which is shown in Figure 3.14. This graph presents the values of the variables in a three-dimensional fashion with the two horizontal axes (x and y) showing the independent variables 1 and 2 (temperature and pressure) and the vertical axis (z) the dependent variable (% yield of extract).

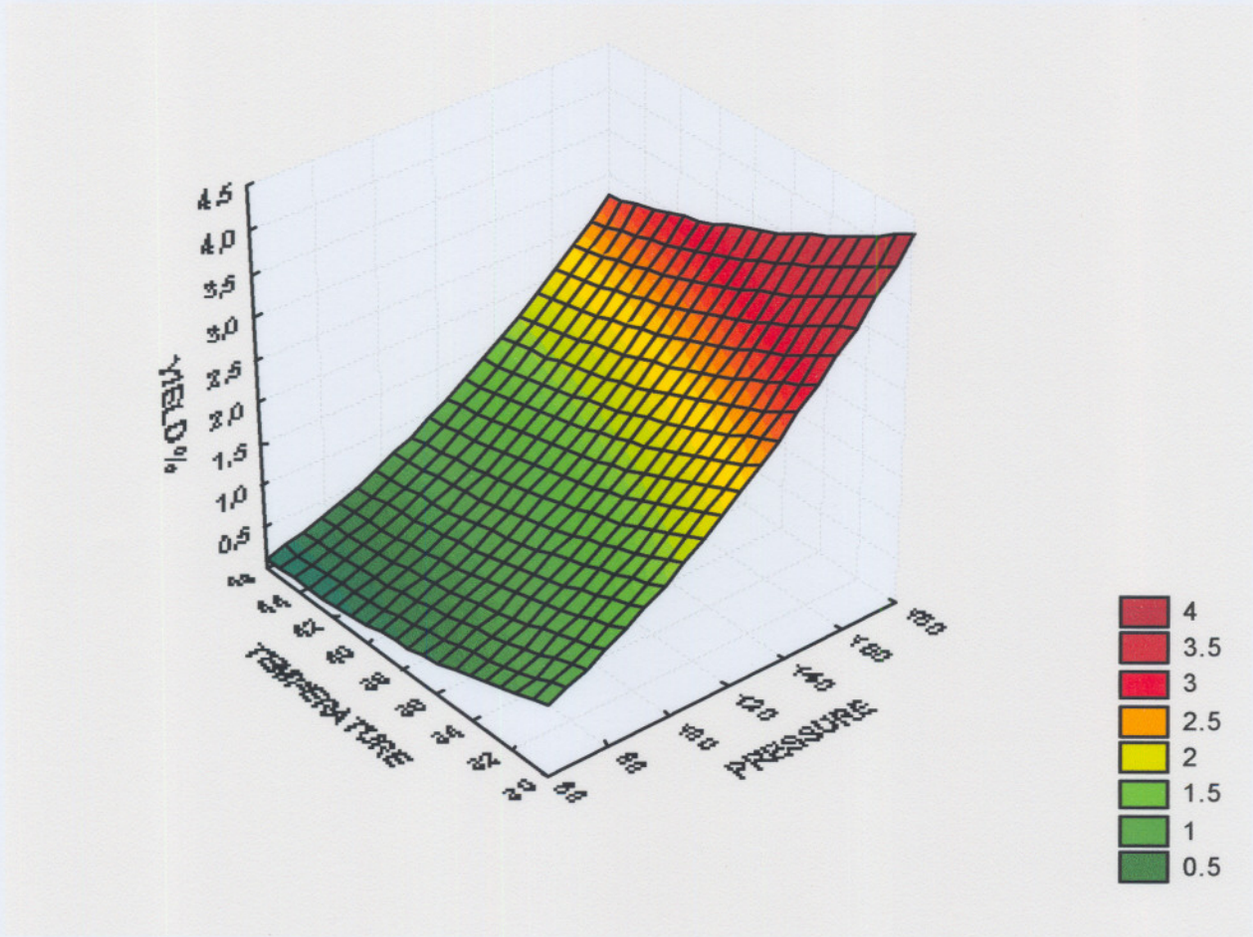

Figure 3.14 Surface response graph

The accuracy of a statistical design such as the one presented in Table 3.5, refers to the extent to which calculated values correspond to measured values. It can be gathered from a graph showing predicted (model) vs. observed (experimental) values. The ability to fit data to a straight line will determine the reliability of the model. If all data points fall on a straight line, the design can be considered perfect.[7,8]

3.6 Process description and modelling

3.6.1 Optimum extraction time

It was necessary to first find the required extraction time and to use the acquired optimum value as a constant in all subsequent extraction runs. This was done by plotting the % yield of extract as a function of time while keeping all other parameters constant at preliminary, preselected values. There is a region were the curve flattens off and forms a plateau as shown schematically in Figure 3.16. The time corresponding to the attainment of a plateau is the optimum extraction time.

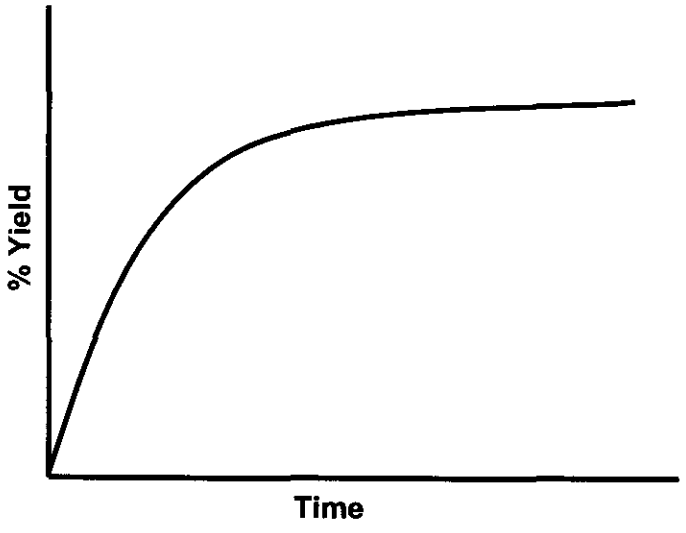

Figure 3.16 Optimisation of extraction time

3.6.2 Yield of extract

A first attempt to comprehend the nature of the extraction process was to correlate the amount of extract with different factors (density, pressure and temperature). A strong dependence on pressure (or density) of the fluid could be indicative of a chemical dissolution process as the solvent strength of the fluid is related to these two factors. An independency of density to the contrary, could point to a physical rather than a chemical extraction mechanism. The mutual contribution of temperature and pressure to the extraction yield should provide information on the extraction process since these two factors have opposing effects on the density of the extractant.

3.6.3 Activation parameters[9,10]

The activation parameters of the extraction can be derived from the temperature and pressure dependence of % yield of extract. Svante Arrhenius described the temperature dependency of the rate constant of a reaction as

$$k = Ae^{-Ea/RT}$$

where E_a is the activation energy, R = 8.314 J/mol K the gas constant, T the temperature in kelvin and A the frequency factor.

The logarithmic form

$$\ln k = \ln A - E_a/RT$$

makes it possible to determine E_a from the gradient = $-E_a/R$ of the straight line obtained when ln k is plotted against 1/T. In this particular case the rate constant k can be substituted by % yield without changing the magnitude of the slope, and thus the correct value of E_a can be obtained.

The empirical equation

$$\ln k = -\frac{\Delta V^{\neq}}{RT} p + \text{constant}$$

can likewise be plotted to determine the volume of activation $\Delta V^{\neq}$ from the slope of a graph of ln k against p, where p is the pressure of the extracting fluid and k the rate

constant of the extraction. The latter can, once again, be substituted by % yield of extract without changing the magnitude of the slope of the resulting straight line.

3.6.4 Dimensionless multivariant analysis[11,12]

In mathematical modelling it is attempted to write a mathematical equation which summarises all the factors that play a role in the process under consideration. In SFE there are quite a few factors that may play a role. It is crucial for a proper process description to include all process variables in a model. In addition to pressure and temperature (or density) of the fluid, other factors such as flow rate, extraction time, type of modifier, natural moisture content of the plant material, waxes and oil present in the plant material, and others, may be variables in the extraction process.

Suppose a simple function y = f(x) needs 4 points to properly represent y. If a second variable is added, the function y = f(x,z) needs 4 more values of z to be presented properly. This requires a total of 16 experiments to be performed in order to determine a good value for y. The number of experiments needed for n variables will thus be 4^n. A total of 64 experiments, for example, are required for 3 variables.

The number of experiments can be reduced by making it dependent of the number of arguments in the function rather than on the number of selected variables. To illustrate this, consider the function y = f(x,z) where x = uv/w, with u, v and w further variables that can be put together or grouped into a "bundle" of variables.

SFE can be presented by a function that consists of the following variables:

σ = amount of waxes and oil in plant material

t = extraction time

T = extraction temperature

w = moisture content of plant material

p = extraction pressure

ρ = density of fluid

r = amount of extract

f = flow rate of fluid

Each of these factors may play a role in the extraction. The extraction yield y may then be presented by a function

$$y = F\left\{\frac{f^2\rho}{T^3 t^4}, \frac{p}{T}, r, w, \sigma\right\}$$

which can be expanded into

$$y = m_0 + m_1 \frac{f^2\rho}{T^3 t^4} + m_2 \frac{p}{T} + m_3 r + m_4 w + m_5 \sigma$$

were m_0, ..., m_5 are constants.

If the physical units of the different variables are considered, it is possible to express these variables as shown in the table below.

Variables	f	p	T	ρ	t
Dimension	$\frac{M}{T}$	$\frac{M}{LT^2}$	$\frac{ML^2}{LT^2}$	$\frac{M}{L^3}$	T

Here M is the mass in kg, L the length in m, t the time in s and T the temperature in K. A product of the variables can be written in the form

$$\left(\frac{f^a \rho^e}{T^c t^d}\right)\left(\frac{p^b}{T^c}\right)$$

which will be dimensionless (i.e. without units) if

a + b + c + e = 0

- b - c - 3e = 0

- a - 2b - 2c + d = 0

A solution for these equations are

a = 1; b = 0; c = -3/2; d = -2; e = ½. Thus

$$\prod_1 = \sqrt{\frac{f^2 \rho}{T^3 t^4}}$$

and

$$\prod_2 = \frac{p}{T}$$

An equation is dimensional homogeneous according to Buckingham if and only if it can be written in the form

$$f(\prod_1, \prod_2, ..., \prod_n) = 0$$

where f is a certain function with a number of arguments presenting a complete set of dimensionless products. $\prod_2$ stays fairly constant for the extraction in this investigation as p and T conversely affect the density; therefore only $\prod_1$ needs to be considered.

The relationship between y and $\prod_1$ is given by the function $f(y, \prod_1) = 0$, which can be solved to give $y = g(\prod_1)$. Thus ln $y = m_2 \prod_1 + m_1$ with m_1 and m_2 being dimensionless constants. This equation can be presented by a graph which, if a straight line is obtained, proves that all important process factors have been taken into account by the particular model.

The straight line is described by the equation

$$\ln[\%yield] = m_1 + m_2 \sqrt{\frac{f^2 \rho}{T^3 t^4}}$$

or

$$y = e^{m_1 + m_2 \sqrt{\frac{f^2 \rho}{T^3 t^4}}}$$

or

$$y = Ae^{m_2\sqrt{\frac{f^2\rho}{T^3t^4}}}$$

with A = e^{m_2} a constant.

The graph of ln (%yield) as a function of $\sqrt{\frac{f^2\rho}{T^3t^4}}$ can be used to validate the model concerned if the experimental extraction data can be fitted to a straight line.

3.7 References

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[12] GIORDANO, F. R. & WEIR, M. D. 1985. Example 2, How long should you roast a turkey. (*In* Giordano, F. R., *ed.* A first course in mathematical modelling. Monterey : Brooks/Cole. 382 p.)

Chapter 4
RESULTS & INTERPRETATION

The results obtained in this investigation are presented, interpreted and discussed in this chapter. A principal feature of this chapter is to derive from the acquired results the characteristic features and mechanistic details of the extraction process as these are important for maximum process control and tunability.

4.1 Optimum extraction time

A very first issue to deal with was the required duration of an extraction run to obtain an optimum yield of extract at preselected conditions.

These conditions were as follows :

- The temperature was kept at 45°C for all the time runs;
- The flow rate was set at 2 mL/min and the pressure was maintained at 100 bar.

The only variable that was changed from run to run was the extraction time. The results of the time dependence study is shown in Figure 4.1. The loss in mass of the plant material in the sample cartridge instead of the mass of extracted product is plotted versus time since part of the extract was lost either by escaped volatiles or by viscous/waxy material staying behind in the flow-line as discussed previously (Par. 3.3).

The observed tendency was the same regardless of whether the mass loss from the sample cartridge or the gain in mass in the collection vial was measured; only the numerical values along the ordinate axis were different.

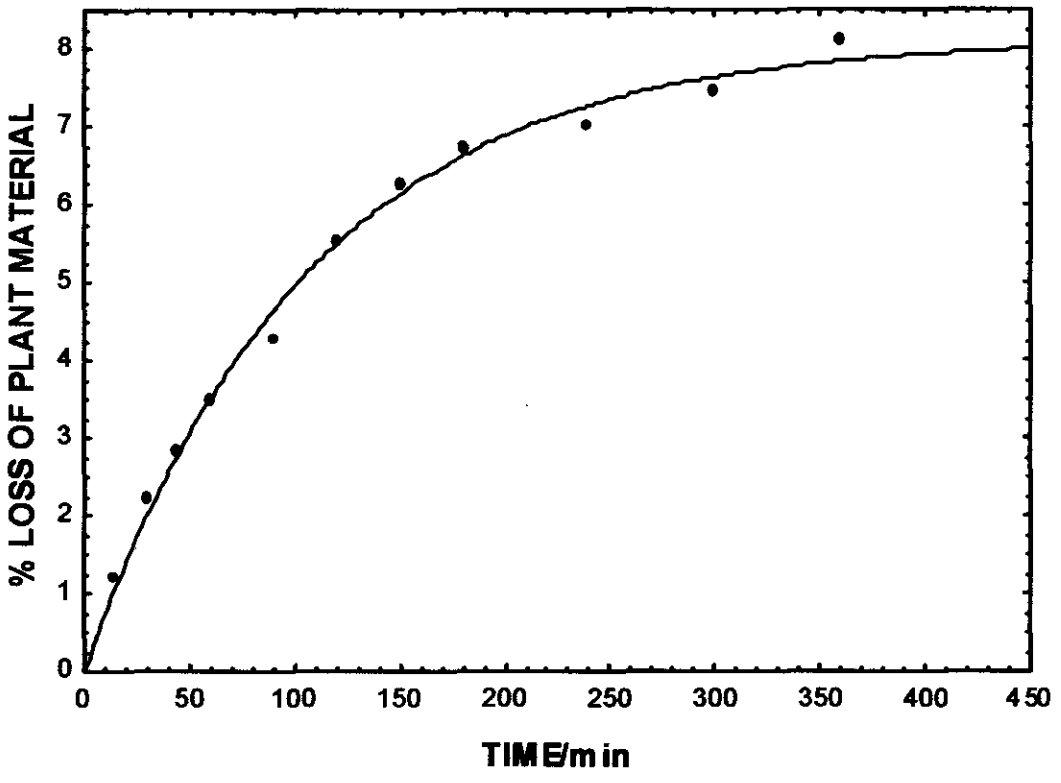


Figure 4.1 % Loss of plant material versus extraction time

The graph reaches a plateau just above 8% loss of plant material from the sample cartridge. The mass of extracted material was less than this figure, but the maximum was also obtained after about 360 minutes of extraction. At this point the plant material is exhausted in terms of extractable substances and continued extraction would not lead to any further loss in mass.

The preselected conditions for the time dependence study were quite moderate, and one could expect to accelerate the extraction by selecting more stringent conditions, especially temperature and/or pressure. However, by establishing a minimum extraction time of 3 hours at fairly moderate conditions means that an extraction of this duration at any other selected conditions would be sufficient to warrant complete removal of all extractable components.

Finally, the rate of extraction can be determined from the initial slope of the mass loss vs extraction time curve in Figure 4.1. The acceleration of the extraction by various parameters can therefore be studied in terms of the variation of the initial slope with a change in these parameters. This, however, was not considered within the scope of this investigation.

4.2 Statistical surface response analysis

Once the optimum extraction time of 3 hours was established at a moderate and easy to maintain flow rate of 2 mL/min, the two most important remaining parameters were optimised by surface response analysis. These parameters are temperature and pressure, which jointly determine the density of the fluid and, for that matter, the solvent strength of the sc-CO_2.

The extraction runs needed to be performed according to the statistical design in Table 3.5 to optimise pressure and temperature in terms of % yield of extract, led to the results in Table 4.1. A total of 16 extraction runs were performed at different combinations of temperature/pressure as required by the statistical design. The densities and solubilities at the various combinations of temperature and pressure were calculated from computer software (SF solver®) supplied by ISCO. In this table yields of extract rather than material loss are listed in order to reflect the extent of removal of extractables from the sample of plant material.

Table 4.1 Yield at different Temperatures/Pressures or Densities

	Pressure (bar)	Temperature (^{0}C)	Density (g/mL)	Yield (%)	Solubility (g/L)
1	73	32	0.359	0.76	3.059
2	75	32	0.402	0.97	3.427
3	78	32	0.454	1.09	3.867
4	100	45	0.482	0.73	4.111
5	80	32	0.483	1.25	4.116
6	100	40	0.607	0.54	5.176
7	100	35	0.713	0.70	6.079
8	100	32	0.767	1.38	6.541
9	105	32	0.782	1.26	6.667
10	150	40	0.788	2.33	6.721
11	150	45	0.75	1.63	6.392
12	175	45	0.793	2.48	6.764
13	130	32	0.823	2.54	7.012
14	125	40	0.739	1.76	6.297
15	150	32	0.847	2.67	7.222
16	125	35	0.785	1.17	6.694

The data in Table 4.1 can be plotted in a three-dimensional way to produce a so-called surface response graph as shown in Figure 4.2. The graph shows that temperature and pressure have opposite effects on the acquired yield. There is generally a slight decrease in yield with an increase in temperature at a fixed pressure, whereas the yield increases fairly significantly with pressure at any constant temperature. The opposing effect of these two variables strongly suggests that they work together in establishing the density and that the latter is the real factor that determines/limits the process. This can be proven by virtue of the increasing yield with increasing density along the diagonal of the surface response graph in Figure 4.2 or, even better, by the graph of % yield versus density in Figure 4.3.

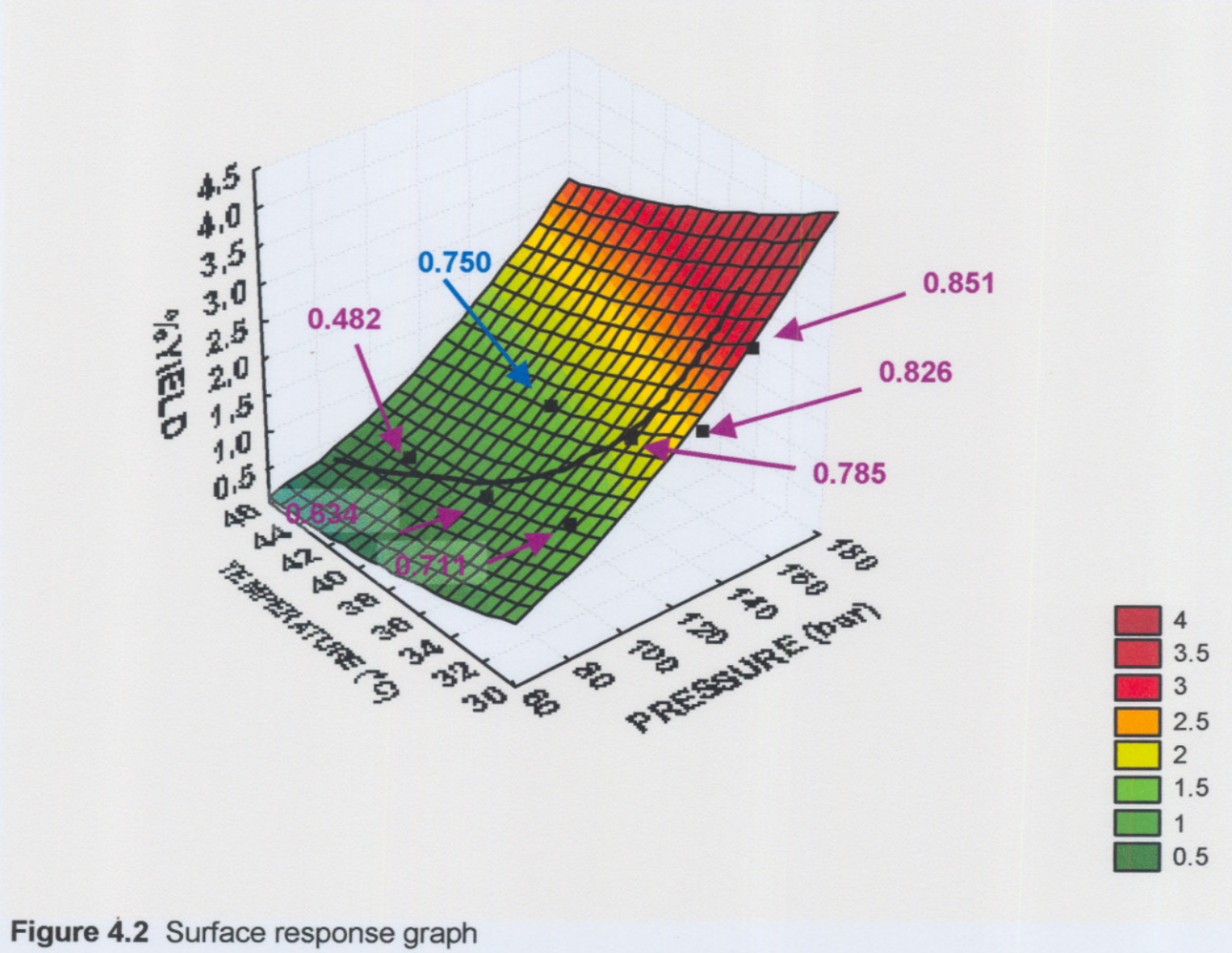


Figure 4.2 Surface response graph

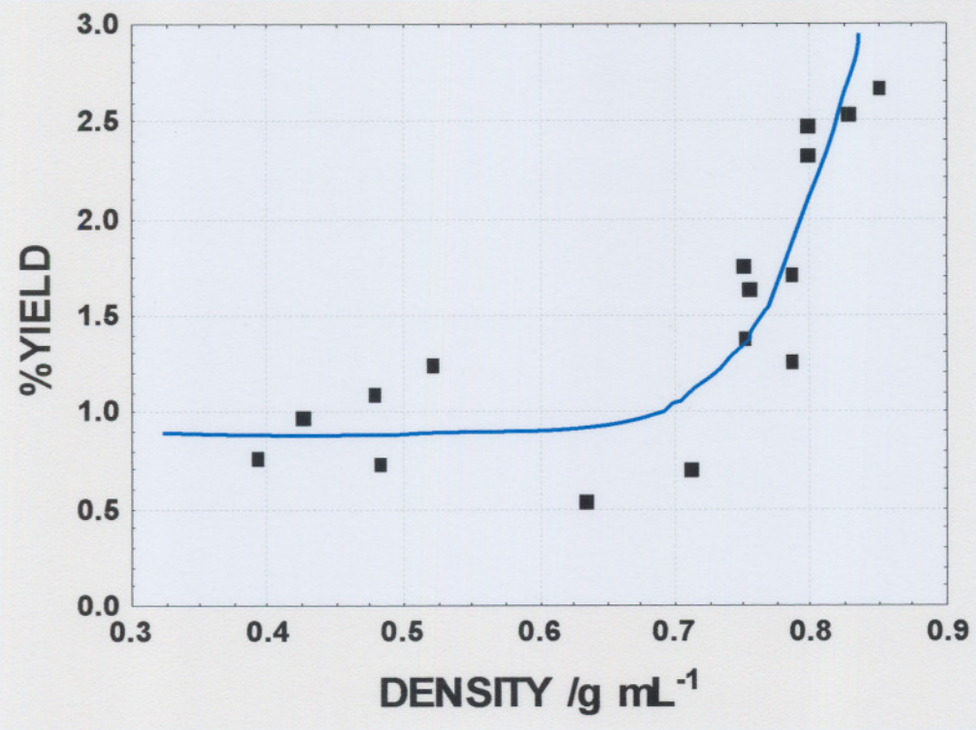


Figure 4.3 %Yield vs density

The highest yield obtained was 2.67% at a density value of 0.851 g/mL. This density value was derived by combining a fairly high pressure (bar) with a low temperature (^{0}C) just falling within the supercritical domain. An increase in temperature at low pressure is responsible for a decrease in density and yield, but the effect is smaller at high pressures. This is because the positive contribution of pressure towards density outweighs the negative effect of temperature on density. The opposing effects of temperature and pressure are probably larger than what is observed since an increase in temperature on the one hand decreases the density but at the same time provides activation energy for the dissolution process by lowering of the energy barrier. These two opposing temperature effects lead to the observed much smaller effect of temperature in comparison to that of pressure on the acquired extraction yield.

The exponential increase in yield obtained at densities approaching those of liquids ($0.8 < \rho < 1.0$ g/mL) strongly suggests that the essential mechanism of extraction is chemical dissolution. The more liquid-like the sc-CO_2 becomes, the better its solvent strength or dissolving capability becomes and the higher yield of extract through dissolution is obtained. However, at densities where sc-CO_2 is essentially gas-like ($0.3 < \rho < 0.7$ g/mL), the extraction yield is well above zero (> 0.5%), which proves that part

of the extracted material is removed physically (by desorption, displacement or effective transport) rather than chemically (by dissolving in sc-CO_2 as an effective solvent). It can thus be concluded that more than one mechanism is operative to remove various constituents of the overall extract but that a major contribution can be assigned to the capability of the fluid to chemically dissolve substances present in the plant matrix.

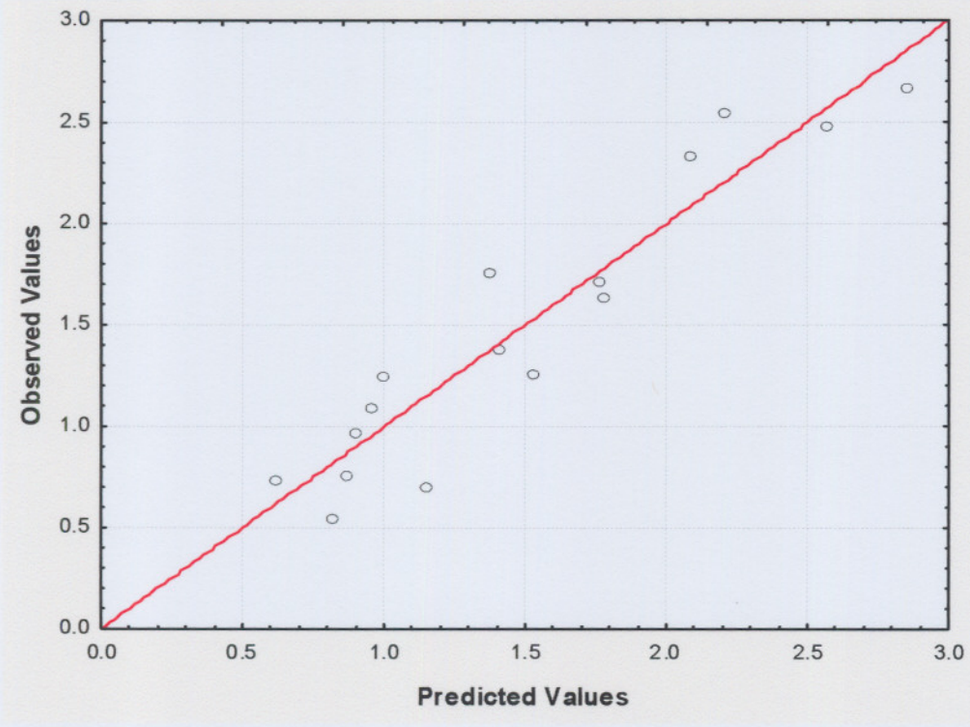


Figure 4.4 Graph of observed vs predicted values

Finally, the reliability of the statistical design on which Figures 4.2 and 4.3 are based, was tested by plotting the observed yields of extract versus the values predicted by the mathematical model. The result is shown in Figure 4.4. It is concluded from the reasonable fit of the data that a reliable optimisation of the extraction conditions was obtained by the statistical design.

4.3 Activation parameters

An Arrhenius plot can be compiled by selected data from Table 4.1 in order to determine the energy of activation for the extraction process. The resulting straight lines obtained at two different pressures are shown in Figure 4.5.

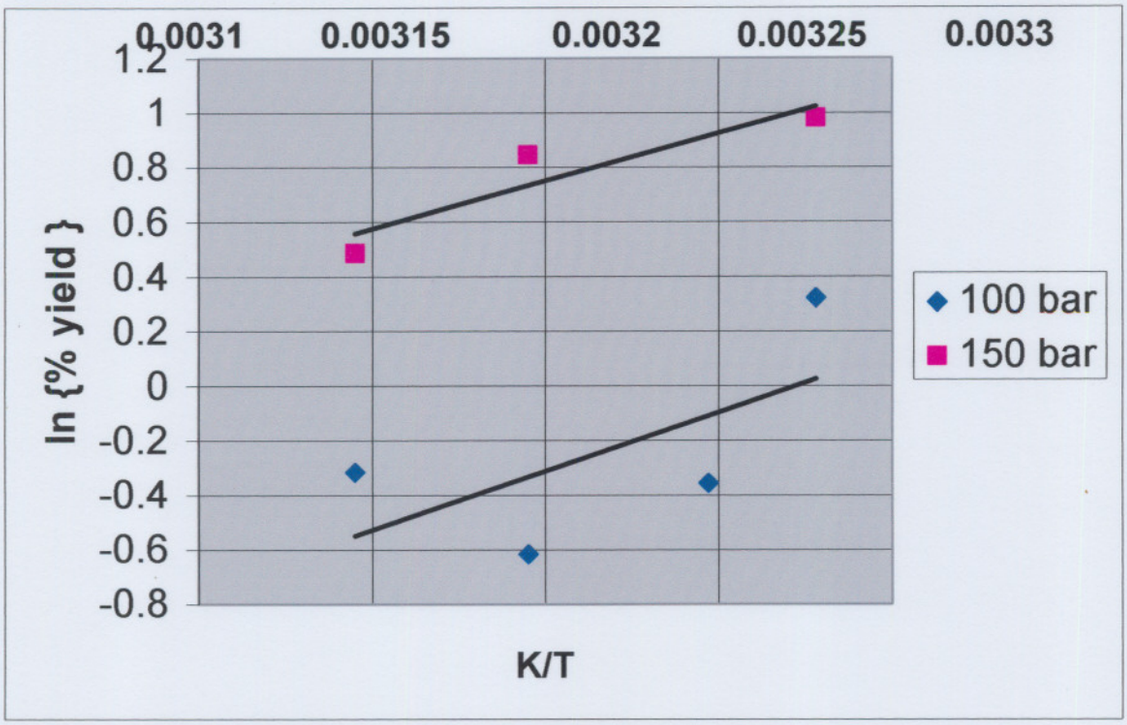


Figure 4. 5 Graphical determination of energy of activation E_a

The slopes of the straight lines at two different pressures (100 and 150 bar) yield two values for the energy of activation, viz. E_a = -29,1 kJ/mol at 150 bar and E_a = -35,9 kJ/mol at 100 bar. The energy of activation is negative since an increase in temperature results in a decrease in density (and solvent strength) and thus a decrease in the yield of extract.

Generally, the solubility of a substance increases with an increase in temperature since the activation energy of dissolution is lowered by a higher temperature, but with sc-CO_2 as solvent, temperature has an opposite effect since an increase in temperature results in a decrease in density and thus a decrease in the solvent strength of the fluid. The decrease in activation energy normally encountered with an increase in temperature is thus counteracted, depleted or exceeded by a decrease in fluid density. Since the calculated activation energy values are negative, the loss in density exceeds the gain in reduced energy requirement.

Depending on the conditions at which an extraction run is performed, the observed activation energy will be determined by the magnitude of the two opposing effects, implying that the calculated values above are not absolute values but will change at

different conditions. This explains why the value of E_a is smaller at 100 bar than at 150 bar. At the lower pressure, the density and thus the solvent strength of the fluid is lower and thus the energy of activation is larger negative. The activation energy for a chemical dissolution process should be in the order of 50-100 kJ/mol. The negative values calculated above thus reflect to what extent the negative effect of an increase in temperature, viz. density decrease, exceeds the positive effect of an increase in temperature, viz. activation energy increase, implying that the numerical values reported have no real significance.

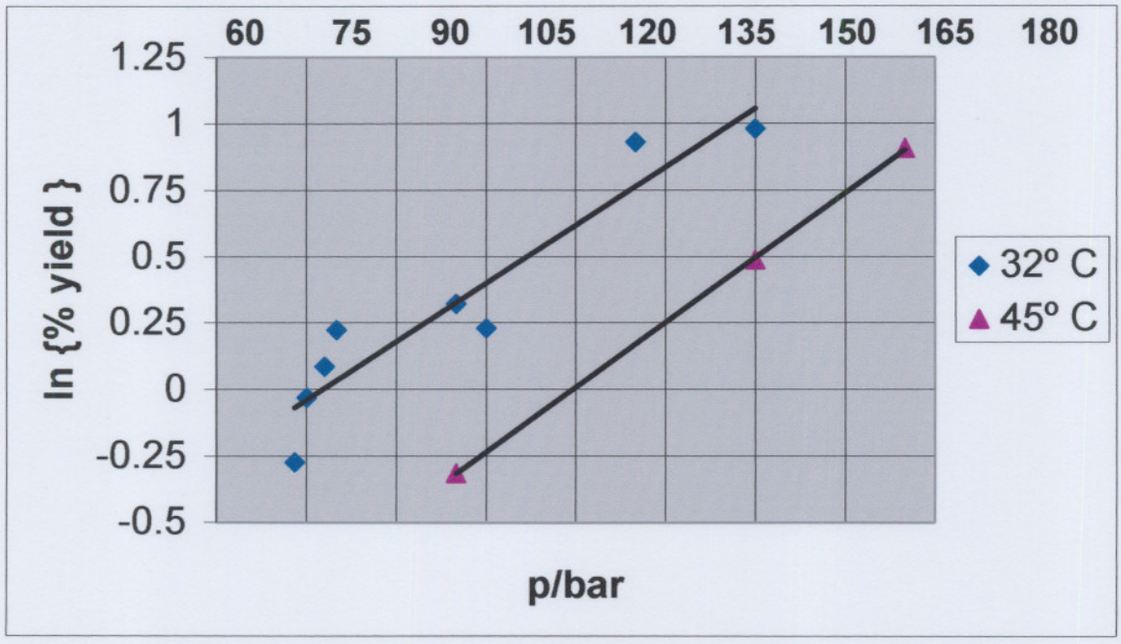


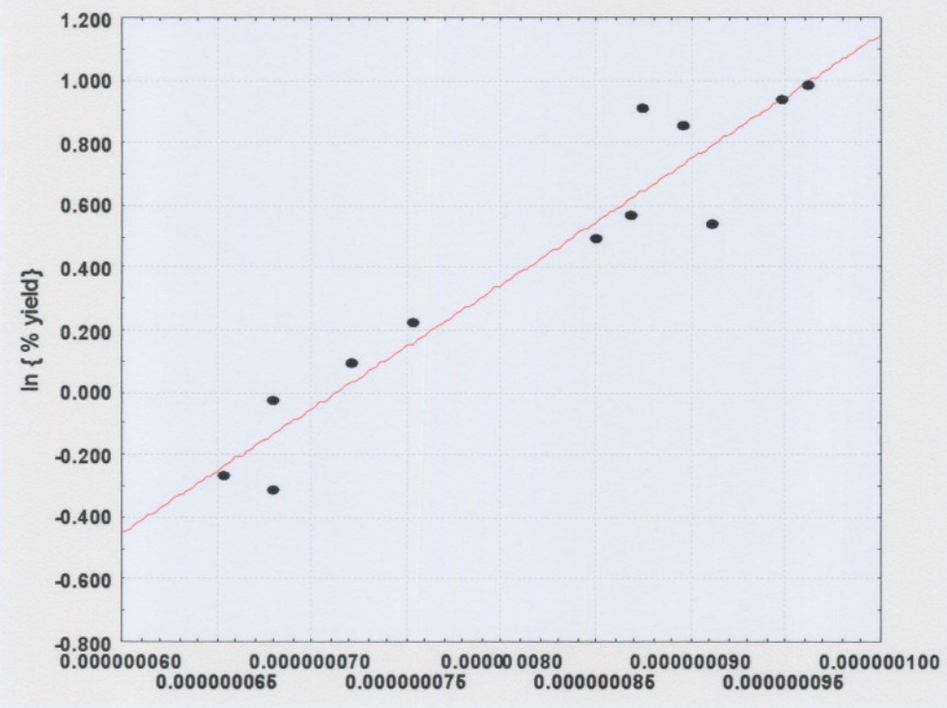
Figure 4.6 Graphical determination of volume of activation $\Delta V^{\neq}$

The straight lines in Figure 4.6 allow the volume of activation to be calculated from the respective slopes at the two temperatures specified. The values turned out to be $\Delta V^{\neq}$ = - 365 mL/mol at 32°C and $\Delta V^{\neq}$ = -425 mL/mol at 45°C. The large negative values should to be understood in terms of the intrinsic and solvational changes that occur when plant components dissolve in sc-CO_2. A first step in any dissolution process is melting, which leads to a negative $\Delta V^{\neq}_{intrinsic}$ as a result of the collapse of molecules when bonds and bond angles are ruptured. This is followed by a solvational step for which significantly negative $\Delta V^{\neq}_{solvational}$ occurs as a result of the solvation of molecules by the highly compressed supercritical fluid. Since no modifier was used, no charge

neutralisation brought some relief and thus two large negative contributions finally resulted in large negative values of $\Delta V^{\neq}$.

4.4 Multivariable analysis

The extraction data in Table 4.1 was used to prove whether the specific dimensionless grouping of variables as outlined in Paragraph 3.6.4 indeed provides a satisfactory mathematical description of the extraction process. The result is shown in Figure 4.7 where ln (% yield) is plotted against the dimensionless grouping of variables in the derived mathematical equation.



$$\sqrt{\frac{f^2\rho}{t^4T^3}}$$

Figure 4.7 ln {% yield } vs dimensionless grouping of variables

The straight line through the data points illustrates the validity of the proposed model and confirms that the variables under consideration are indeed the most important ones among the many factors that may play a role in botanical extraction. The flow rate and extraction time was kept constant for the data fit.

The presentation in Figure 4.7 can be used to obtain valuable information of the extraction process. It can, for instance, be used to predict the % yield expected at specific conditions, provided that these conditions fall within the plotted range. The extrapolation of the straight line to values beyond the plotted data is uncertain.

4.5 Extract analysis

As mentioned earlier (Par. 3.4), the appropriate method for extract analysis in this investigation was GC-MS. The required analytical protocol was outlined previously. In addition to the basic GC-MS method, analysis was also done with GC-GC/TOF-MS to obtain a better separation of the multiple components in the sc-CO_2 derived extracts. The results obtained with these two methods are presented in the subparagraphs below.

4.5.1 Results of GC-MS analysis

The primary goal with the analysis was to compare the results obtained by sc-CO_2 extraction with those obtained by traditional extraction methods.

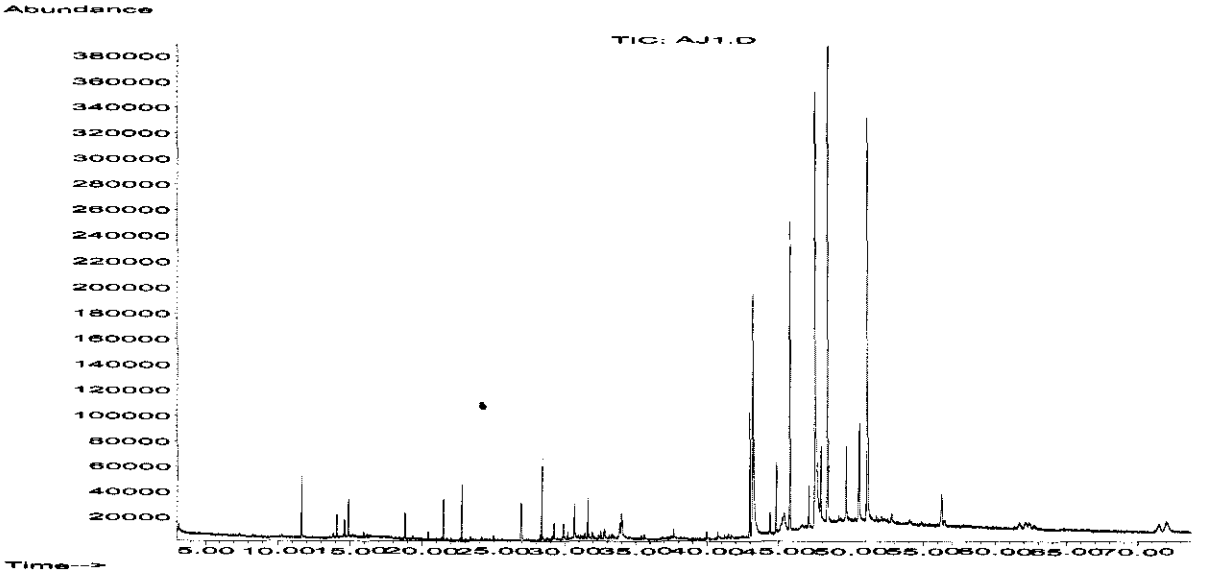


Figure 4.8 GC analysis of components

A typical one-dimensional chromatogram is shown in Figure 4.8. Every peak represents a component found in the extract. The x-axis shows the retention time at which the component appeared and the y-axis shows the abundance of the component found in the extract.

The retention time is specific for a given component and is therefore a helpful tool to identify components and to compare them to retention times and components published in the literature. The retention time for a given component may slightly differ from one instrument to another, depending on the type of column used and the relative purity of the extracted component. If the retention time differs by up to 3 min. the analysis is still viable.

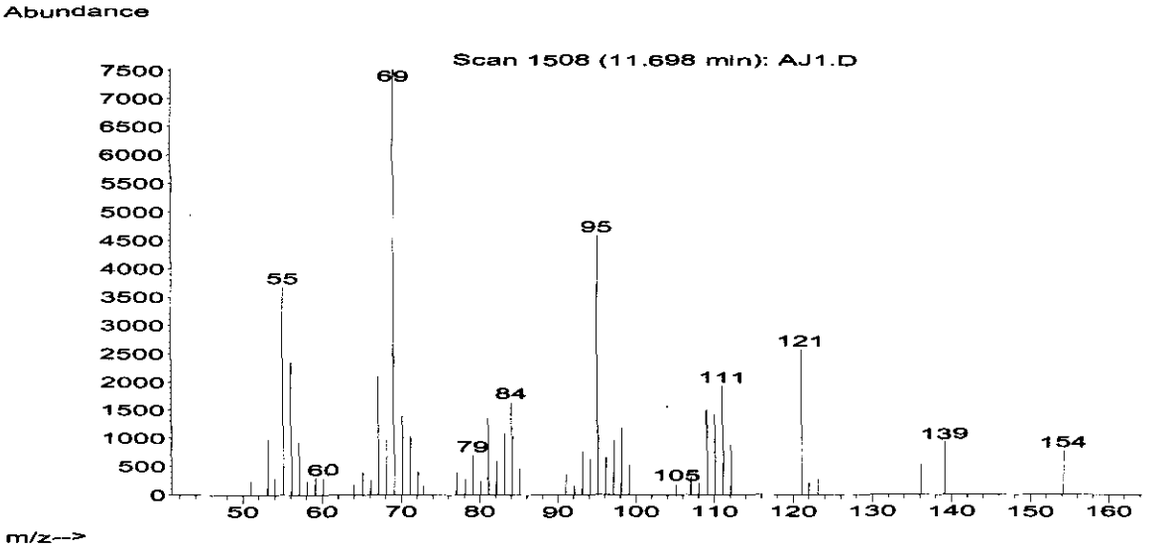


Figure 4.9 MS spectrum

The MS spectrum in Figure 4.9 shows the mass fragments of a component with mole mass of 154 g/mol occurring at a retention time of 11.70 min. as shown on an enlarged section of Figure 4.8 in Figure 4.10. More than one component may have a mole mass of this value, but the library of the analysis program is very helpful to yield a correct analysis based on the fragmentation pattern. Once a component is detected by the MSD, it does a library search and gives an accuracy figure (%) for each identified component. These figures are included in Table 4.2.

Table 4.2 Library results

Best match	Component Name	Molar Mass g/mol	Molar Formula	Accuracy %	CAS-Number
1	6-Octenal, 3,7-dimethyl-, (R)-	154	$C_{10}H_{18}O$	94	002385-77-5
2	6-Octenal, 3,7-dimethyl-	154	$C_{10}H_{18}O$	94	000106-23-0
3	6-Octenal, 3,7-dimethyl-	154	$C_{10}H_{18}O$	87	000106-23-0
4	7-Octenal, 3,7-dimethyl-	154	$C_{10}H_{18}O$	80	000141-26-4
5	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	154	$C_{10}H_{18}O$	64	007786-67-6

The library gives the IUPAC names of the components. The program can be set to give the number of best matches found, and for this investigation the best five matches were considered enough. In Table 4.2 best match 1, for example, has an accuracy of 94 %. The CAS-numbers are the international registered numbers of the listed components.

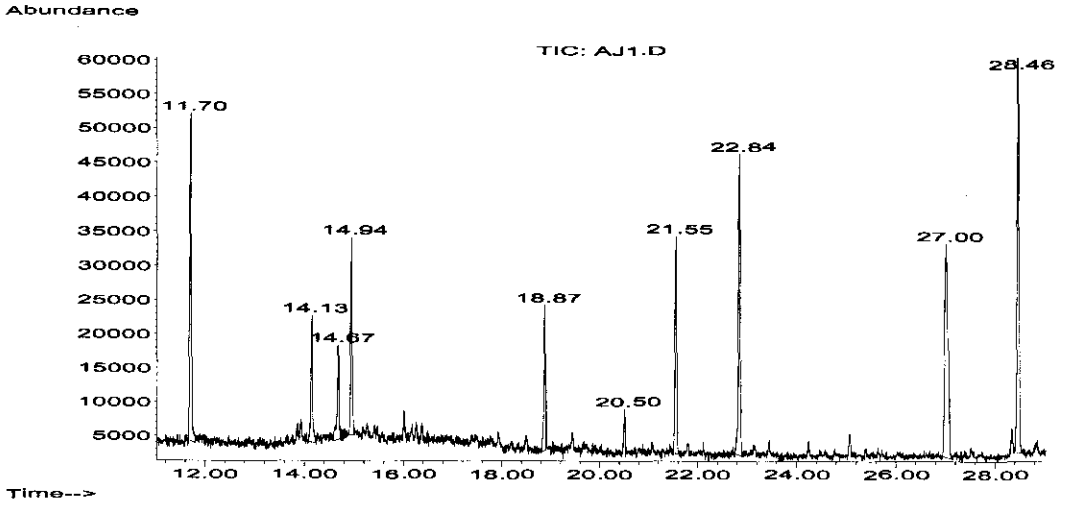


Figure 4.10 Enlargement of a section of Figure 4.8 showing component with mole mass of 154 g/mol at a retention time of 11.70 min.

RT: 13.58 KI: 1153 **Citronellal**
CAS#: 106-23-0 MF: C10 H18 O FW: 154 MSD LIB#: 0953 ITD LIB#: 0365
CN: dimethyl-6-octenal<3,7->
Synonyms: 2,3-dihydrocitral; rhodinal;3,7-dimethyl-6-octenal

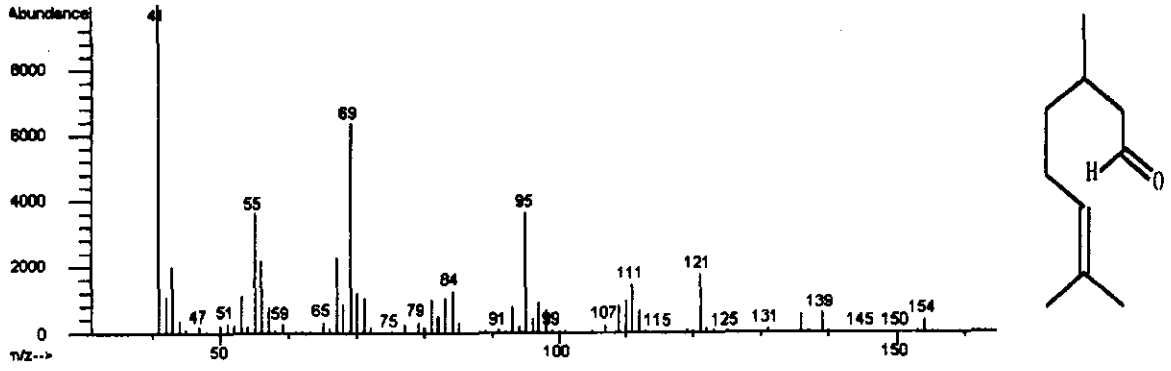


Figure 4.11 MS spectrum from literature

On comparing Figure 4.9 (obtained in this investigation) and Figure 4.11 (taken from the literature) one sees that the spectra are almost exact fingerprints of one another. The retention time is 13.58 min. according to the literature.[3] The correspondence between the MS spectra in the two figures mentioned confirms the successful identification. The remaining results can be found in appendix A.

The names of essential oil components can be written in IUPAC, synonyms and common names. The common names are not the same worldwide. This makes a search more complex as the IUPAC and common names need to be matched. The search was done on internet, the literature and other databases.[1,2] In appendix B a list of common names with their synonyms and IUPAC analogues is presented.

4.5.2 GC-GC/TOF-MS analysis[3]

One sample was analysed. This was run on the Pegasus 4D instrument at the NML, CSIR. Once data acquisition was complete, the sample file was processed with automated *Peak Finding* and *Spectral Deconvolution* software, followed by library searching of the NIST (National Institutes of Standards and Technology) library.

For *Peak Finding* a second dimension column peak width of 0.1 seconds and a signal-to-noise cut off of 500:1 were used. Only those peaks meeting these criteria were added to the peak table. The threshold values were set at these values in order to simplify the results by reducing the number of located peaks to a level which could be easily assimilated and to locate the major components in the sample. These values resulted in 204 located peaks in the sample. The data file can readily be reprocessed with different signal-to-noise and peak width cutoffs, if necessary, without sacrificing the results from previous data processing. This allows the analyst to adjust the number of located analytes in a given sample to a number that is most appropriate for the degree of information required from the analysis without losing any previous information.

Figures 4.12 and 4.13 show the total ion chromatogram (TIC) by virtue of a contour plot and a surface plot respectively.

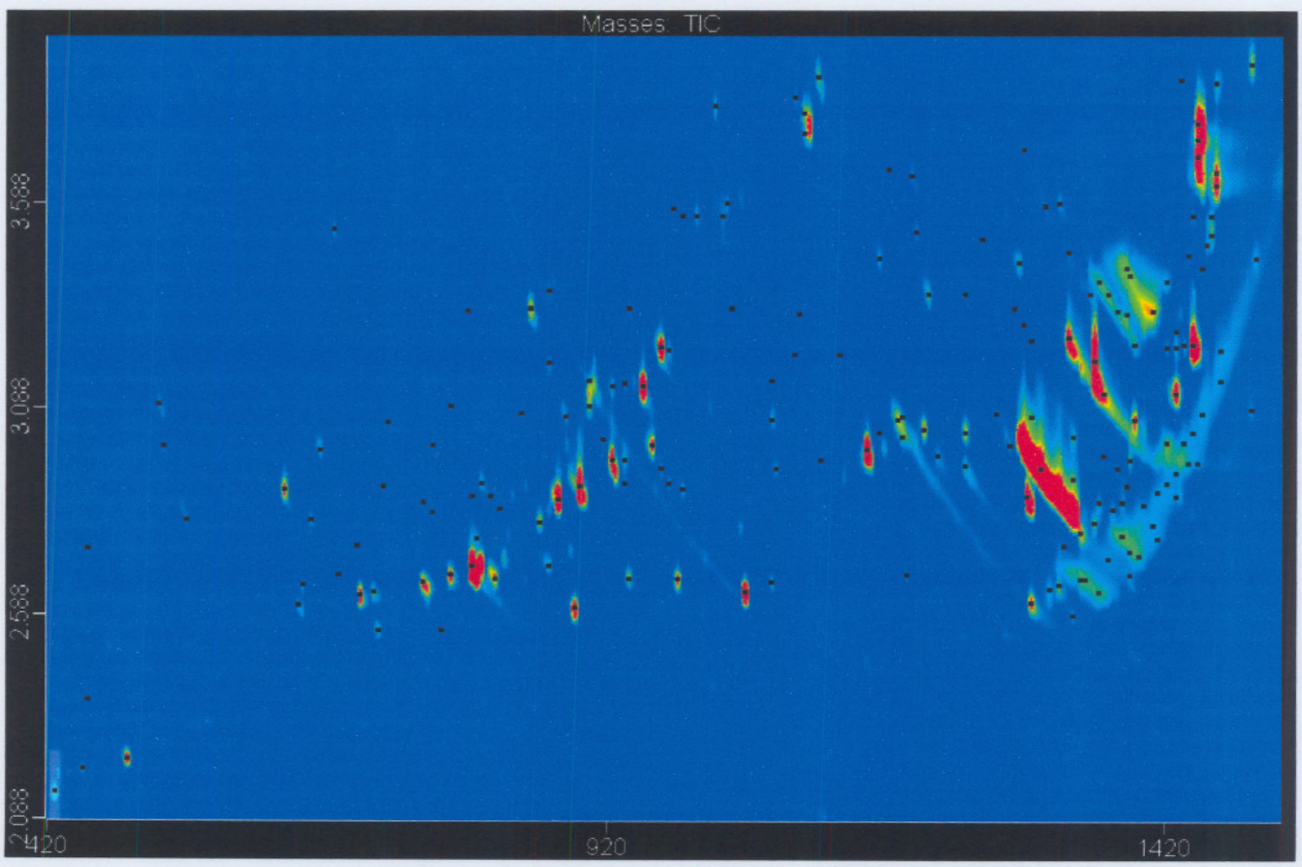


Figure 4.12 Contour plot

In Figure 4.12 very low level peaks at the selected settings are only faintly visible, but their position is indicated by a black dot. The x-axis shows the separation on a SPB-1 column (boiling point based). The y-axis shows the separation on a Rtx 1701 column (based on polarity).

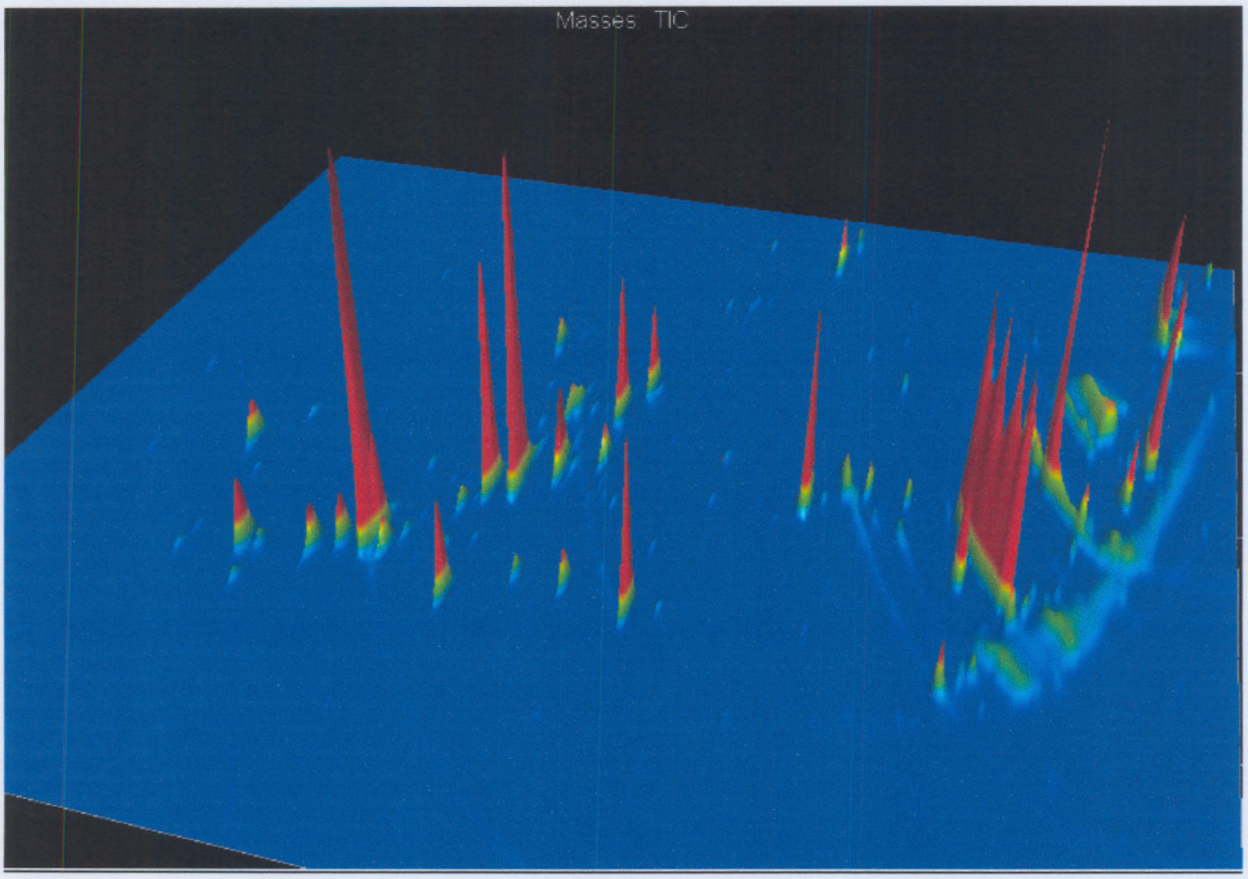


Figure 4.13 Surface plot

Figure 4.13 shows the TIC as a surface plot. The angle from which the plot is observed (this is fully rotatable) shows the low level peaks between the major components. However, some peaks are hidden behind the large peaks. Rotation of the plot brings these into view.

Table 4.3 shows a remarkable increase in the number of identified components when compared to ordinary GC-MS analysis. The multiple components illustrate how complex and component-rich sc-CO_2 derived botanical extracts are.

The library results in Table 4.3. gives the IUPAC names of the components found in the analysis. The similarity number reflects the accuracy of the spectral match with the database spectrum. This number is based on a scale of 1 to 1 000, where 1 000 represents a perfect match. If the analyte name in the table is incorrect, the true analyte identification may be further down the library hit list, or the compound may not be present in the mass spectral databases used to search the spectrum. It may occur that the same analyte is a match for more than one peak in the table. This can occur when two or more analytes in the sample are structurally similar and consequently result in similar mass spectra. Editing of the table should be done by someone familiar with the chromatography of the sample being analysed to produce a table in which multiple assignments of the same compound and nonsensical assignments have been eliminated. No editing has been attempted on these results.

Table 4.3 Components from GC-GC/TOF-MS analysis

Peak	Name	R.T. (s)	Similarity	Quant Masses	Area	CAS
1	Hexane, 2,2-dimethyl-	428 , 2.160	933	57	182435	590-73-8
2	Pentane, 2,3,4-trimethyl-	452 , 2.216	940	71	38082	565-75-3
3	Toluene	456 , 2.384	920	91	32975	108-88-3
4	Butanoic acid	456 , 2.752	936	60	61761	107-92-6
5	Heptane, 2,4-dimethyl-	492 , 2.240	905	43	560198	2213-23-2
6	Acetone	520 , 3.104	798	43	375129	67-64-1
7	5,9-Dodecadien-2-one, 6,10-dimethyl-, (E)-	524 , 3.000	933	72	25614	0-00-0
8	Pentanoic acid	544 , 2.824	951	60	219708	109-52-4
9	Hexanoic acid	632 , 2.896	938	60	932645	142-62-1
10	1-Octen-3-ol	644 , 2.616	955	57	273981	3391-86-4
11	5-Hepten-2-one, 6-methyl-	648 , 2.664	875	108	13570	110-93-0
12	2,4-Heptadienal, (E,E)-	656 , 2.824	894	81	94467	3/5/4313
13	2-Hexenoic acid, (E)-	664 , 2.992	963	73	150975	13419-69-7
14	2,5-Furandione, 3,4-dimethyl-	676 , 3.528	932	54	77877	766-39-2
15	2-Propanol, 1-(2-methoxypropoxy)-	680 , 2.688	890	59	58121	13429-07-7
16	Pentanamide	696 , 2.760	775	102	6358	626-97-1
17	O-Menthan-8-ol	700 , 2.640	802	59	1891857	0-00-0
18	5-Heptenal, 2,6-dimethyl-	712 , 2.648	874	82	147963	106-72-9
19	Cyclohexane, 1,1,3,5-tetramethyl-, trans-	716 , 2.552	772	125	83368	50876-31-8
20	Heptanoic acid	720 , 2.904	928	60	88841	111-14-8
21	2,3-Nonadiene	724 , 3.056	723	68	88729	22433-34-7
22	Nonanal	756 , 2.672	944	57	365727	124-19-6
23	6-Methyl-3,5-heptadiene-2-one	756 , 2.864	930	109	20725	1604-28-0
24	Hexanoic acid, 2-ethyl-	764 , 2.840	948	88	28602	149-57-5
25	Phenylethyl Alcohol	764 , 3.000	923	91	68068	60-12-8
26	2H-Pyran, tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-	772 , 2.552	902	139	15802	16409-43-1
27	5-Hepten-1-ol, 2,6-dimethyl-	780 , 2.688	904	67	310890	4234-93-9
28	2,4-Octadienoic acid, 7-hydroxy-6-methyl-, [r-[r*,s*-(E,E)]]-	780 , 3.096	768	97	27815	86845-56-9
29	Benzoic Acid	796 , 3.328	920	122	27277	65-85-0
30	6-Octenal, 3,7-dimethyl-, (R)-	800 , 2.712	940	68	696493	2385-77-5
31	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	800 , 2.880	758	59	47151	5989-33-3

32	1-Cyclohexene-1-acetaldehyde, à,2-dimethyl-	804 , 2.776	823	123	24740	53155-80-9
33	Octanoic Acid	808 , 2.912	862	60	135170	124-07-2
34	Benzoyl bromide	816 , 2.880	875	105	35228	618-32-6
35	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-, [1R-(1à,2á,5à)]-	820 , 2.680	931	67	251354	89-79-2
36	3-Isoxazolecarboperoxoic acid, 4,5-dihydro-5-phenyl-, 1,1-dimethylethyl ester	824 , 2.848	623	59	26421	35145-84-7
37	Propanoic acid, 2-hydroxy-2-methyl-, ethyl ester	832 , 0.680	715	59	79749	80-55-7
38	2-(1-Hydroxy-1-methylethyl)cyclohexanol	844 , 3.080	793	59	29535	0-00-0
39	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	852 , 3.336	802	139	257729	20189-42-8
40	Ethanone, 1-(1,2,2,3-tetramethylcyclopentyl)-, (1R-cis)-	860 , 2.816	734	59	135717	59642-07-8
41	Tetrahydroionol	868 , 2.712	944	67	56288	4361-23-3
42	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, [1R-(1à,2á,5à)]-	868 , 3.200	792	95	24576	2216-51-5
43	1,4-Methanoazulene, decahydro-4,8,8-trimethyl-9-methylene-, [1S-(1à,3aá,4à,8aá)]-	868 , 3.376	776	91	40991	475-20-7
44	2,6-Octadienal, 3,7-dimethyl-, (Z)-	876 , 2.872	905	41	3118684	106-26-3
45	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	884 , 3.072	780	111	30895	5989-33-3
46	6-Octenoic acid, 3,7-dimethyl-, methyl ester	892 , 2.608	899	110	185789	2270-60-2
47	2,6-Octadienal, 3,7-dimethyl-, (E)-	896 , 2.899	937	69	4108917	141-27-5
48	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	904 , 3.096	747	152	3323	5989-33-3
49	Epoxy-linalooloxide	904 , 3.160	761	147	1657	0-00-0
50	Chrysandemic acid	916 , 3.016	771	123	17484	0-00-0
51	6-Octenoic acid, 3,7-dimethyl-, methyl ester	924 , 2.968	791	170	91141	2270-60-2
52	trans,trans-2,6-Dimethyl-2,6-octadiene-1,8-diol	924 , 3.144	736	126	3705	26488-97-1
53	2,4-Decadienal	936 , 2.912	790	152	2474	2363-88-4
54	Neric acid	936 , 2.968	865	100	29806	4613-38-1
55	2,4-Dimethyl-1-penten-3-ol	936 , 3.152	771	71	54127	19781-54-5
56	2,6-Octadienoic acid, 3,7-dimethyl-, methyl ester	940 , 2.680	906	114	64120	2349-14-6
57	Pulegone	940 , 3.336	754	81	49770	89-82-7
58	Cyclohexanol, 2-(2-hydroxy-2-propyl)-5-methyl-	952 , 3.144	889	59	1301423	138663-70-4
59	Geranic acid	960 , 3.000	916	123	74626	459-80-3
60	n-Decanoic acid	968 , 2.944	825	60	49165	334-48-5
61	Cyclohexanol, 2-(2-hydroxy-2-propyl)-5-methyl-	968 , 3.240	862	59	1240144	138663-70-4
62	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	976 , 2.912	890	71	69120	74367-33-2
63	8-Hydroxycarvotanacetone	976 , 3.232	757	110	44698	7712-46-1
64	4,4'-Biscyclohexanone, 2,2',6,6'-tetramethyl-	980 , 3.576	697	126	11243	0-00-0

65	Propylene Glycol	984 , 1.792	722	45	953252	57-55-6
66	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate	984 , 2.680	938	69	330298	16409-44-2
67	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	988 , 2.896	926	71	100851	74367-34-3
68	Vanillin	988 , 3.560	907	151	14645	121-33-5
69	2(1H)-Naphthalenone, octahydro-8a-methyl-, cis-	1000 , 3.560	699	166	16095	2530-17-8
70	1,2-Propanediol, 3-methoxy-	1008 , 1.816	648	75	111428	623-39-2
71	5-Octen-2-one, 3,6-dimethyl-	1016 , 3.824	725	126	29508	0-00-0
72	5,9-Dimethyl-3-decanol	1024 , 3.560	740	59	146910	19550-53-9
73	2(5H)-Furanone, 3-methyl-	1028 , 3.592	818	98	79804	22122-36-7
74	2-Furanmethanol, 5-ethenyltetrahydro-à,à,5-trimethyl-, cis-	1032 , 3.336	769	59	70467	5989-33-3
75	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	1044 , 2.644	913	133	263374	118-65-0
76	à-Caryophyllene	1068 , 2.672	949	93	70097	6753-98-6
77	3-Buten-2-one, 4-(2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	1068 , 3.064	960	123	149134	23267-57-4
78	Benzenesulfonamide, N-butyl-	1068 , 3.160	830	141	22455	3622-84-2
79	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E)-	1072 , 2.944	932	177	59827	79-77-6
80	1,3-Cyclohexanedione, 2-(2-propenyl)-	1088 , 3.224	691	124	25632	42738-68-1
81	Cyclohexanone, 2-ethyl-	1088 , 3.848	713	98	40001	4423-94-3
82	Octanoic acid, 2-methylene-, methyl ester	1092 , 3.320	657	101	33809	3618-40-4
83	6-Methyl-1,5-heptadiene	1096 , 3.760	738	98	29398	7270-50-0
84	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	1096 , 3.808	889	180	26133	15356-74-8
85	2(1H)-Pyridinethione, 3-hydroxy-	1108 , 3.896	688	127	839174	23003-22-7
86	Lauric anhydride	1112 , 2.968	930	60	32726	645-66-9
87	1,2-Propanediol, 3-methoxy-	1128 , 1.784	661	75	87829	623-39-2
88	Diethyl Phthalate	1128 , 3.224	953	149	21347	84-66-2
89	Caryophyllene oxide	1152 , 2.992	920	79	968650	0-00-0
90	2H-Pyran-2-one, tetrahydro-4-(2-methyl-1-propen-3-yl)-	1164 , 3.032	685	99	33998	0-00-0
91	2-Cyclopenten-1-one, 4-hydroxy-3-methyl-2-(2-propenyl)-	1164 , 3.456	793	109	54817	551-45-1
92	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-3,5,5-trimethyl-, [r-[r*,R*-(E)]]-	1172 , 3.672	867	108	26938	52210-15-8
93	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1180 , 3.064	877	68	195480	102608-53-7
94	1,2-Epoxy-5,9-cyclododecadiene	1184 , 3.024	805	136	39005	943-93-1
95	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1184 , 3.072	863	134	6572	102608-53-7
96	Trifluoroacetyl-isopulegol	1188 , 2.688	812	81	39531	28587-54-4
97	3-Buten-2-one, 4-(4-hydroxy-2,2,6-trimethyl-7-oxabicyclo[4.1.0]hept-1-yl)-	1192 , 3.656	898	123	135132	38274-01-0

98	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	1196 , 3.520	754	193	32909	97371-50-1
99	Caryophyllene oxide	1204 , 3.040	856	131	29808	1139-30-6
100	11-Hexadecyn-1-ol	1208 , 3.368	812	96	94605	0-00-0
101	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1216 , 2.976	866	81	39907	102608-53-7
102	Benzenesulfonamide, N-butyl-	1232 , 0.400	953	170	1021929	3622-84-2
103	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	1240 , 0.800	706	111	121666	0-00-0
104	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1240 , 2.952	894	123	48388	102608-53-7
105	Tetradecanoic acid	1240 , 3.032	933	60	218396	544-63-8
106	7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane	1240 , 3.368	831	153	18403	96093-81-1
107	Dibutyl phthalate	1256 , 3.504	807	149	43850	84-74-2
108	Cholestan-3-ol, 2-methylene-, (3á,5à)-	1268 , 3.080	741	203	7863	22599-96-8
109	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	1280 , 3.000	743	139	34834	16778-27-1
110	Dibutyl phthalate	1284 , 3.336	804	149	47749	84-74-2
111	Caryophyllene oxide	1288 , 3.448	803	127	24120	0-00-0
112	Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	1292 , 3.296	840	149	56327	58422-92-7
113	Dibutyl phthalate	1292 , 3.720	938	149	415679	84-74-2
114	2-Undecanone, 6,10-dimethyl-	1296 , 2.880	876	250	26506	1604-34-8
115	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1300 , 2.624	867	68	271735	102608-53-7
116	Pentadecanoic acid	1300 , 3.072	877	60	66427	1002-84-2
117	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	1300 , 3.256	868	149	35086	84-69-5
118	Heptacosane	1304 , 1.648	941	57	210259	593-49-7
119	Hexadecanoic acid, methyl ester	1308 , 2.944	938	74	40269393	112-39-0
120	Dibutyl phthalate	1312 , 3.584	951	149	661536	84-74-2
121	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1316 , 2.656	873	82	116874	102608-53-7
122	1,2-Benzenedicarboxylic acid, diisooctyl ester	1324 , 0.520	917	149	107746	27554-26-3
123	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1324 , 2.664	874	123	35550	102608-53-7
124	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	1324 , 3.592	902	164	29406	0-00-0
125	6-Octenal, 3,7-dimethyl-	1328 , 1.944	701	341	4770	106-23-0
126	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-	1328 , 2.760	712	203	30185	102681-49-2
127	n-Hexadecanoic acid	1332 , 3.264	909	87	350768	57-10-3
128	Phthalic acid, butyl ester, ester with butyl glycolate	1332 , 3.472	920	149	451687	85-70-1
129	Eicosane	1336 , 2.592	916	268	815	112-95-8
130	Pentadecanoic acid, 14-methyl-, methyl ester	1336 , 2.920	890	124	11631	5129-60-2

131	5,9,13-Pentadecatrien-2-one, 6,10,14-trimethyl-, (E,E)-	1336 , 3.024	762	81	28702	1117-52-8
132	Heptacosane	1344 , 2.680	916	99	111566	593-49-7
133	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-	1344 , 2.792	714	203	154134	102681-49-2
134	Heptacosane	1348 , 2.680	919	239	5980	593-49-7
135	Dibutyl phthalate	1352 , 3.368	954	149	504730	84-74-2
136	6-Octenal, 3,7-dimethyl-	1356 , 2.816	788	137	54081	106-23-0
137	n-Hexadecanoic acid	1356 , 3.208	913	53	313471	57-10-3
138	Heptacosane	1360 , 2.648	927	239	6848	593-49-7
139	Bicyclo[5.1.0]octan-2-one, 4,6-diisopropylidene-8,8-dimethyl-	1360 , 2.864	784	105	14544	0-00-0
140	4-Chloro-3-n-hexyltetrahydropyrane	1360 , 3.400	776	70	150445	0-00-0
141	5,9-Tetradecadiyne	1364 , 2.976	732	106	29296	51255-61-9
142	n-Hexadecanoic acid	1364 , 3.128	908	41	2764269	57-10-3
143	10-Heneicosene (c,t)	1368 , 2.728	829	203	4632	95008-11-0
144	4-Chloro-3-n-hexyltetrahydropyrane	1368 , 3.368	776	70	80911	0-00-0
145	Trifluoroacetyl-isopulegol	1372 , 2.848	758	165	14888	28587-54-4
146	5,9-Undecadien-1-yne, 6,10-dimethyl-	1376 , 2.944	709	59	19224	100451-98-7
147	4-Chloro-3-n-hexyltetrahydropyrane	1376 , 3.328	777	215	1095	0-00-0
148	1-Eicosanol	1380 , 2.784	897	252	10376	629-96-9
149	Cyclohexanol, 2-methyl-5-(1-methylethenyl)-, (1à,2á,5à)-	1380 , 2.864	751	205	23088	38049-26-2
150	12,15-Octadecadiynoic acid, methyl ester	1384 , 2.904	618	61	4775	57156-95-3
151	4-Chloro-3-n-hexyltetrahydropyrane	1384 , 3.320	777	59	26920	0-00-0
152	4-Chloro-3-n-hexyltetrahydropyrane	1384 , 3.432	790	147	3520	0-00-0
153	Dodecane, 2,6,10-trimethyl-	1388 , 2.688	859	203	4844	3891-98-3
154	2-Methyl-E-7-hexadecene	1388 , 2.744	802	290	1403	0-00-0
155	2,6-Octadienal, 3,7-dimethyl-, (Z)-	1388 , 2.968	742	137	54936	106-26-3
156	4-Chloro-3-n-hexyltetrahydropyrane	1388 , 3.416	799	133	6108	0-00-0
157	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	1392 , 3.064	808	147	16236	4602-84-0
158	3-Trifluoroacetoxypentadecane	1392 , 3.248	766	139	3915	0-00-0
159	1-Docosene	1396 , 2.736	890	252	3611	1599-67-3
160	4aH-Cycloprop[e]azulen-4a-ol, decahydro-1,1,4,7-tetramethyl-, [1aR-(1aà,4á,4aá,7à,7aá,7bà)]-	1400 , 2.856	692	59	4910	5986-49-2
161	Oxirane, tetradecyl-	1408 , 2.808	850	35	341	7320-37-8
162	4-Chloro-3-n-hexyltetrahydropyrane	1408 , 3.328	795	41	2294400	0-00-0

163	9-Hexacosene	1412 , 2.776	823	73	22090	71502-22-2
164	9,12-Octadecadienoyl chloride, (Z,Z)-	1412 , 2.888	769	292	1929	7459-33-8
165	7-Pentadecyne	1420 , 2.912	738	249	4231	22089-89-0
166	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-	1420 , 3.008	782	203	41488	102681-49-2
167	Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(.+-.)-	1420 , 3.240	775	151	12085	129967-65-3
168	4-Chloro-3-n-hexyltetrahydropyrane	1420 , 3.400	787	55	87890	0-00-0
169	Oxirane, tetradecyl-	1428 , 2.880	833	280	3280	7320-37-8
170	9-Methyl-Z-10-tetradecen-1-ol acetate	1428 , 2.936	794	253	263	0-00-0
171	Hexadecen-1-ol, trans-9-	1428 , 3.128	955	111	145714	64437-47-4
172	7-Isopropyl-1,1,4a-trimethyl-1,2,3,4,4a,9,10,10a-octahydrophenanthrene	1428 , 3.240	814	143	21220	0-00-0
173	Geranyl isovalerate	1428 , 3.280	606	121	2242	109-20-6
174	ç Dodecalactone	1432 , 3.888	854	85	56415	5/7/2305
175	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	1436 , 3.008	805	203	3817	0-00-0
176	Phytol	1436 , 3.248	894	71	190096	150-86-7
177	10-Methoxy-nb-à-methylcorynantheol	1440 , 2.960	811	129	16231	55322-92-4
178	Bicyclo[3.1.0]hexane-6-methanol, 2-hydroxy-1,4,4-trimethyl-	1440 , 3.464	705	59	32201	58795-41-8
179	9,12-Octadecadienoyl chloride, (Z,Z)-	1444 , 3.032	814	109	86716	7459-33-8
180	Phytol	1444 , 3.248	929	86	139721	150-86-7
181	Bicyclo[3.3.1]nonan-9-one, 1,2,4-trimethyl-3-nitro-, (2-endo,3-exo,4-exo)-(.+-.)-	1444 , 3.560	662	199	7580	129967-65-3
182	n-Hexadecanoic acid	1448 , 2.960	907	73	41640	57-10-3
183	9,12-Octadecadienoic acid (Z,Z)-	1448 , 3.704	923	280	64719	60-33-3
184	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	1448 , 3.744	681	276	1342	515-00-4
185	9,12,15-Octadecatrienal	1448 , 3.784	892	278	9553	26537-71-3
186	2H-Pyran-2-one, 6-hexyltetrahydro-	1452 , 0.104	830	99	27105	710-04-3
187	2-Dodecen-1-yl(-)succinic anhydride	1452 , 3.080	803	97	250896	19780-11-1
188	2(3H)-Benzofuranone, hexahydro-4,4,7a-trimethyl-	1452 , 3.432	628	139	29453	16778-27-1
189	3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	1456 , 3.488	730	136	13006	5944-20-7
190	2,6-Dodecadien-1-ol, 3,7,11-trimethyl-, (Z,E)-	1460 , 3.512	801	84	180727	20576-58-3
191	Farnesol isomer a	1460 , 3.560	774	291	1717	0-00-0
192	Octadecanoic acid	1464 , 3.632	899	73	791952	57-11-4
193	6,6-Dimethyl-2-(3-oxobutyl)bicyclo[3.1.1]heptan-3-one	1464 , 3.664	779	291	7254	0-00-0
194	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1à,2á,5á)-	1464 , 3.880	703	123	60228	490-99-3
195	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	1468 , 3.160	816	111	7551	0-00-0

196	Cyclohexene, 4-(4-ethylcyclohexyl)-1-pentyl-	1468 , 3.232	781	71	71685	0-00-0
197	Nonanamide	1472 , 0.216	867	59	32517	1120-07-6
198	Pentacosane	1496 , 3.088	948	57	92558	629-99-2
199	6,6-Dimethyl-2-(3-oxobutyl)bicyclo[3.1.1]heptan-3-one	1496 , 3.928	741	139	112784	0-00-0
200	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	1500 , 3.456	833	123	30819	102608-53-7
201	2-Buten-1-one, 1-(2,2,5a-trimethylperhydro-1-benzoxiren-1-yl)	1512 , 0.584	724	151	45766	0-00-0
202	2,6-Octadienoic acid, 3,7-dimethyl-, ethyl ester	1520 , 0.720	740	151	20719	13058-12-3
203	1,6-Dioxaspiro[4.4]non-3-ene, 2-(2,4-hexadiynylidene)-	1524 , 0.536	655	200	13282	16863-61-9
204	Oleic Acid	1524 , 2.056	599	159	6098	112-80-1

4.6 References

[1] ADAMS, R. P. 2001. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Carol Stream : Allured publishing corporation. 456 p.

[2] NIST CHEMISTRY WEBBOOK. 2003. [Web :] http://webbook.nist.gov/chemistry/ [Date of access: 1 Spt. 2004].

[3] GORST-ALLMAN, P. (peter@lecoafrica.co.za) 2004. Sample analysis with Leco Pegasus 4D GC-GC/TOF-MS system at the NML, CSIR. [E-mail to:] Joubert, A. (cheaaj@puknet.puk.ac.za) Nov. 10.

Chapter 5 EVALUATION AND CONCLUSION

The extent to which the results presented in the previous chapter enabled realisation of the goals of the study is discussed in this final chapter. It is done by listing the successes and failures of the investigation against the goals outlined in the introductory chapter. Finally a perspective for future work is given.

5.1 Successes

This study showed that it is possible to extract substances from *Melissa officinalis* with sc-CO_2. The extraction conditions were optimised by surface response analysis. The extraction process was shown to depend primarily on the density of the fluid. Since density relates directly to solvent strength, the conclusion could be drawn that the mechanism of extraction is essentially chemical dissolution. Thermodynamic and kinetic quantities were determined to support this conclusion. The principal features of the extraction process could be revealed.

An attempt to mathematically describe the extraction by taking into account all possible contributing factors and combining these in an appropriate dimensionless grouping of variables turned out to be successful.

The GC-MS and GC-GC/TOF-MS analysis of the sc-CO_2 derived extract gave promising and exciting results. The majority of components found in these analyses is also reported by other authors.

5.2 Failures

One of the failures was the inability to prevent material loss through volatiles escaping at the restrictor and collection vial and through viscous/waxy residue staying behind in the flow line of the extractor. These problems were partly alleviated by using a specially designed collection/trapping vial and by rinsing the flow line repeatedly after each run in order to collect as much as possible of the extract.

The operation of the supercritical fluid extractor used in this study is based on a batch technique where for each extraction run a new sample of plant material is introduced. The amount of extract for each run is so small that the yield obtained by many "batches" need to be combined in order to accumulate a workable amount of extract. This poses a problem where larger amounts of extract are needed for sensory evaluation. A continuous extraction technique[1] could solve this problem, but no such equipment is available yet.

5.3 Future perspective

A necessity for the future is to improve the trapping vial to such an extent that more extract can be recovered. The problem of extract staying behind in the flow line should also be addressed.

There is worldwide interest in plant components exhibiting flavour, medicinal and pharmacological properties. It can be expected that knowledge will grow in this field and that skills will be developed. sc-CO_2 is not the only "solvent" that can be used to perform supercritical extractions. Different extraction solvents can result in new opportunities.

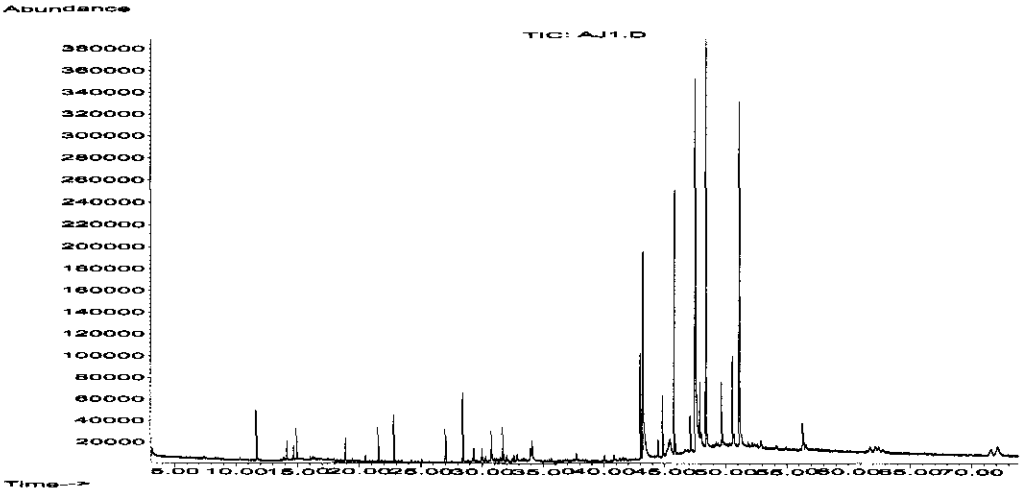
Finally, the issues of clean technology, green chemistry and sustainable development are well supported by the use of environmentally benign supercritical fluids. The development and application of supercritical technology need to be considered a priority for the future.

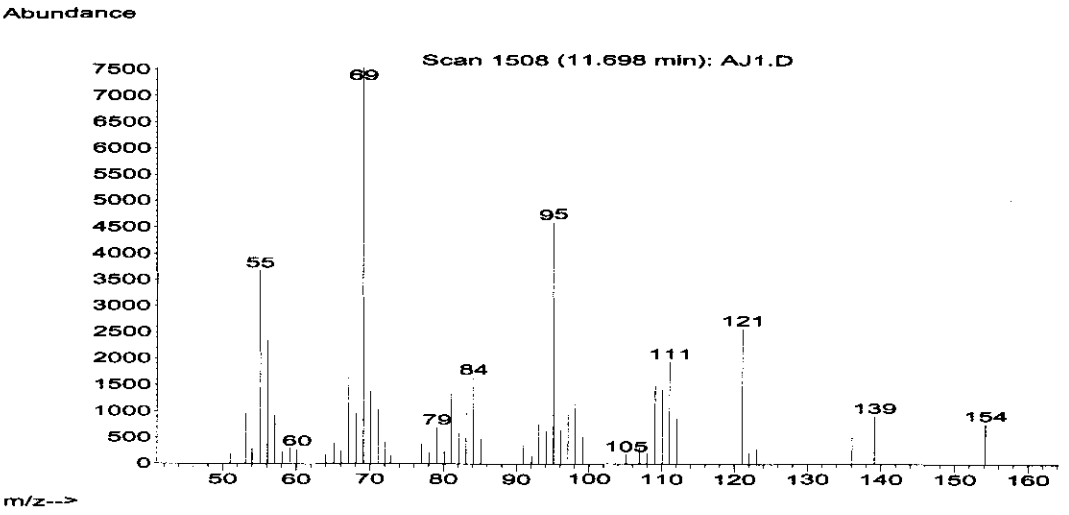
5.4 References

[1] VERSVELD, E. 2002. Extraction of harpagoside from secondary roots of devil's claw (*Harpagophytum procumbens)*. Potchefstroom : Potchefstroom University for Christian Higher Education. (Dissertation - M.Sc.) 63 p.

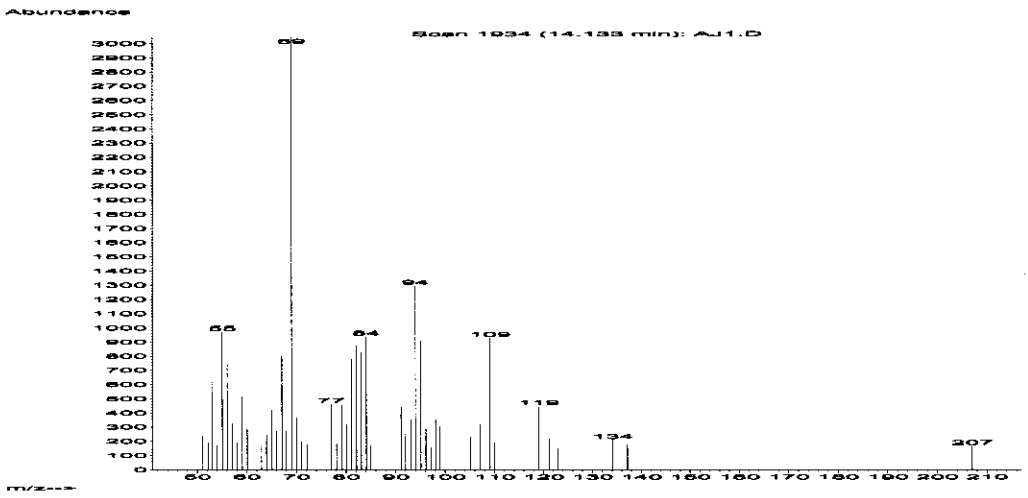
APPENDIX A

Appendix A includes a one-dimensional chromatogram for a typical extract of *Melissa officinalis* followed by mass spectra and best-match tables for a number of prominent peaks in the chromatogram. These results serve the purpose of informing the reader of the methodology followed to identify the components in a typical sc-CO_2 derived extract.

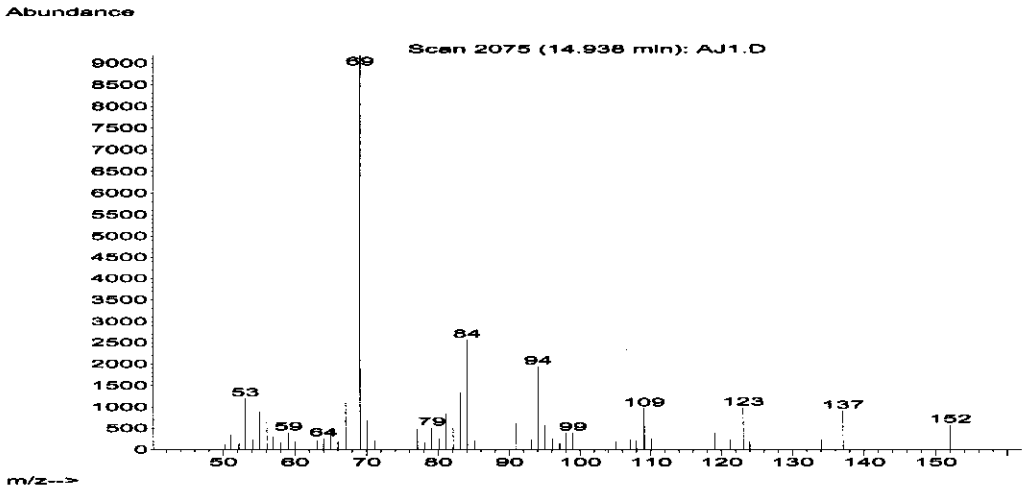




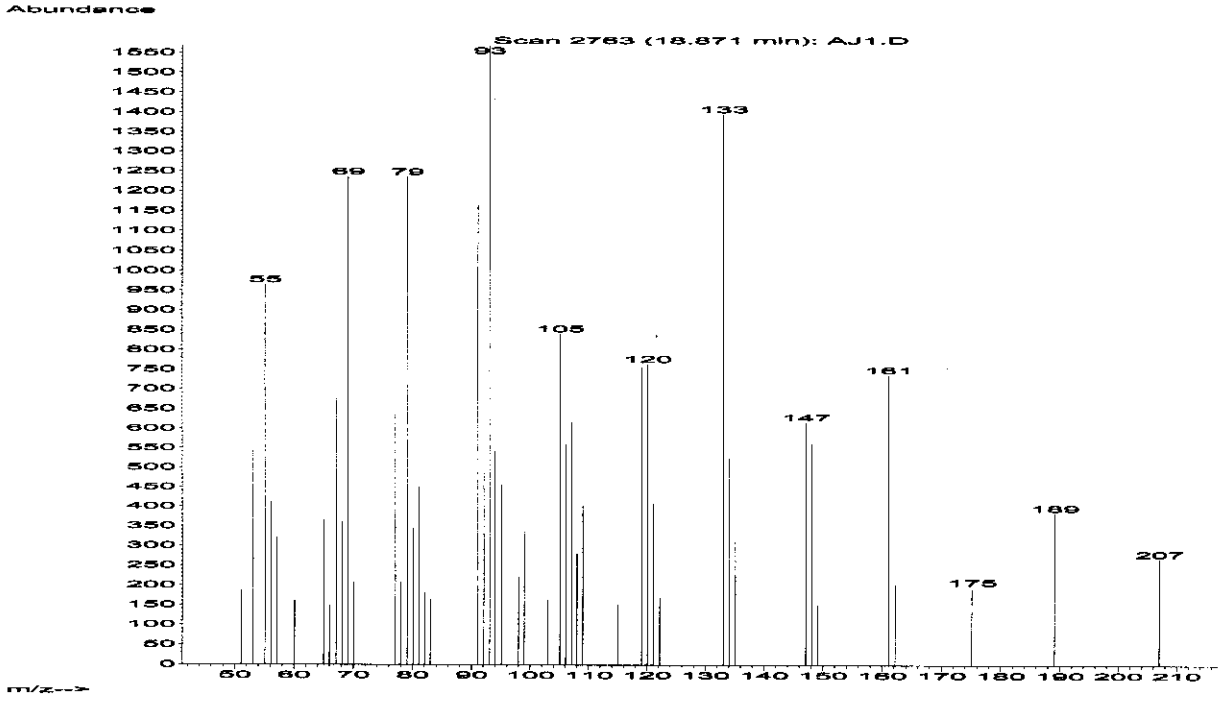
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	6-Octenal, 3,7-dimethyl-, (R)-	154	$C_{10}H_{18}O$	94	002385-77-5
2	6-Octenal, 3,7-dimethyl-	154	$C_{10}H_{18}O$	94	000106-23-0
3	6-Octenal, 3,7-dimethyl-	154	$C_{10}H_{18}O$	87	000106-23-0
4	7-Octenal, 3,7-dimethyl-	154	$C_{10}H_{18}O$	80	000141-26-4
5	Cyclohexanol, 5-methyl-2-(1-methylethenyl)-	154	$C_{10}H_{18}O$	64	007786-67-6



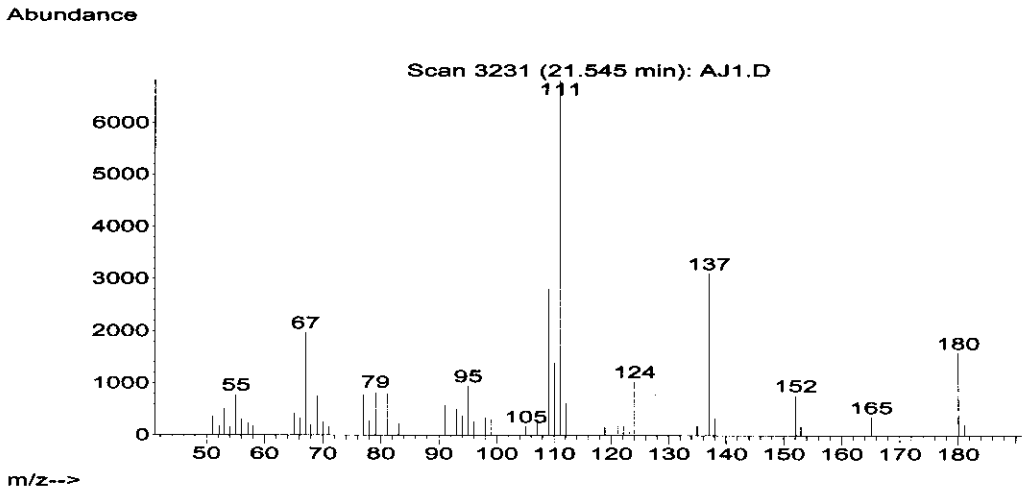
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	2,6-Octadienal, 3,7-dimethyl-, (Z)-	152	$C_{10}H_{16}O$	86	000106-26-3
2	2,6-Octadienal, 3,7-dimethyl-, (Z)-	152	$C_{10}H_{16}O$	64	000106-26-3
3	Cyclopropanemethanol, .alpha.,2-dimethyl-2-(4-methyl-3-pentenyl)-, [1.alpha.(R*),2.alpha.]-	182	$C_{12}H_{22}O$	47	121959-70-4
4	Cyclopropanemethanol, 2-methyl-2-(4-methyl-3-pentenyl)-	168	$C_{11}H_{20}O$	43	098678-70-7
5	6-Octen-1-ol, 3,7-dimethyl-	156	$C_{10}H_{20}O$	37	000106-22-9



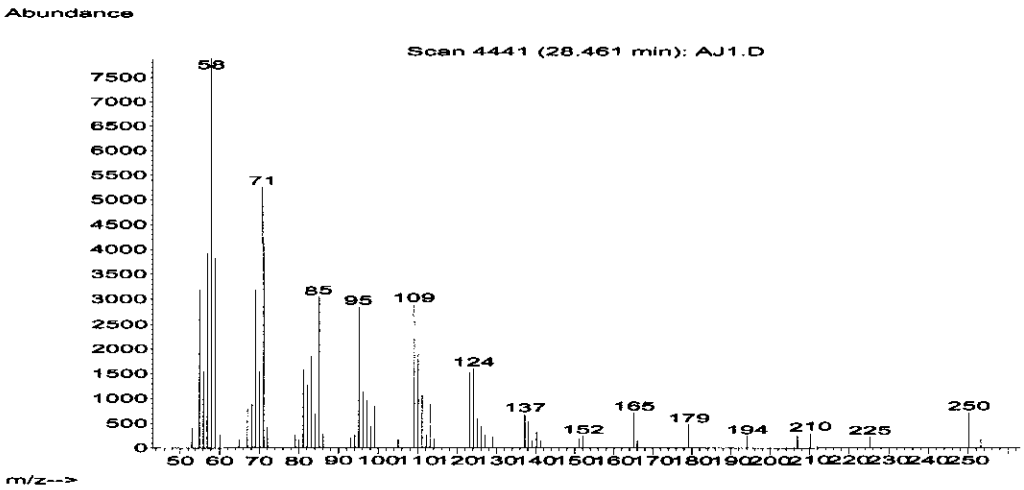
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	2,6-Octadienal, 3,7-dimethyl-	152	$C_{10}H_{16}O$	96	005392-40-5
2	2,6-Octadienal, 3,7-dimethyl-, (E)-	152	$C_{10}H_{16}O$	94	000141-27-5
3	2,6-Octadienal, 3,7-dimethyl-	152	$C_{10}H_{16}O$	94	005392-40-5
4	2,6-Octadienal, 3,7-dimethyl-, (E)-	152	$C_{10}H_{16}O$	93	000141-27-5
5	2,6-Octadienal, 3,7-dimethyl-	152	$C_{10}H_{16}O$	93	005392-40-5



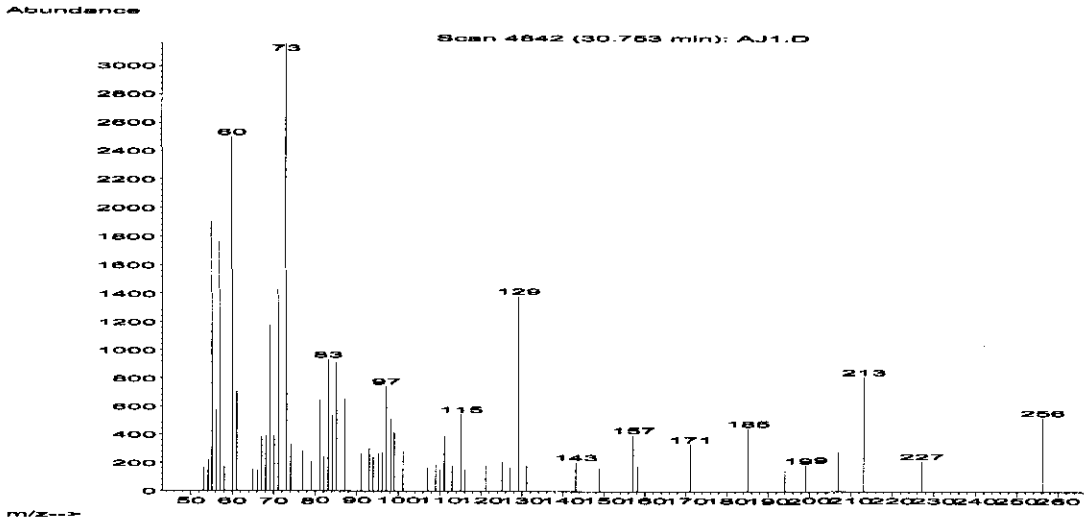
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Caryophyllene	204	$C_{15}H_{24}$	90	000087-44-5
2	Caryophyllene	204	$C_{15}H_{24}$	87	000087-44-5
3	Cyclohexane, 1,5-diethenyl-3-methyl-2-methylene-, (1.alpha.,3.alpha.,5.alpha.)-	162	$C_{12}H_{18}$	60	074742-35-1
4	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-,[1R-(1R*,4Z,9S*)]-	204	$C_{15}H_{24}$	53	000118-65-0
5	Bicyclo[5.2.0]nonane, 2-methylene-4,8,8-trimethyl-4-vinyl-	204	$C_{15}H_{24}$	52	242794-76-9



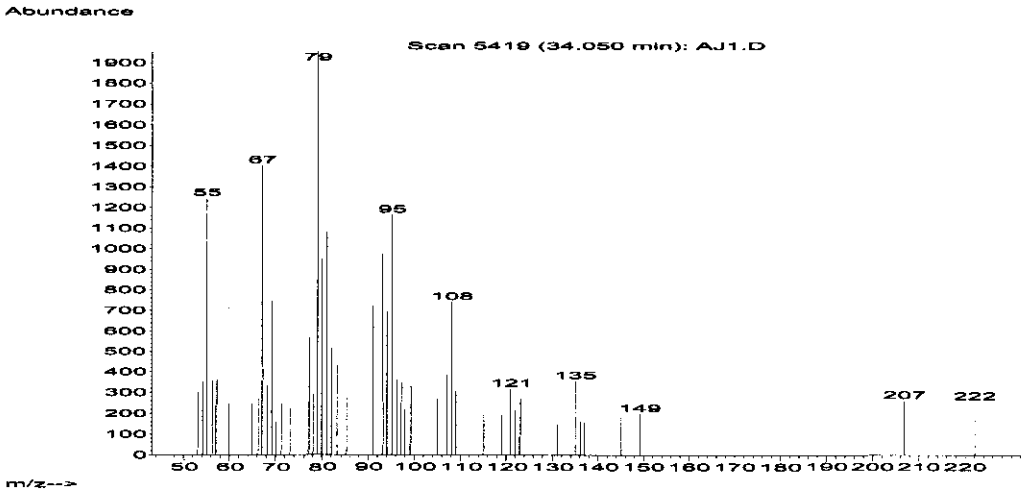
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	180	$C_{11}H_{16}O_2$	98	015356-74-8
2	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180	$C_{11}H_{16}O_2$	93	017092-92-1
3	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180	$C_{11}H_{16}O_2$	42	017092-92-1
4	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	180	$C_{11}H_{16}O_2$	38	017092-92-1
5	Phosphonic acid, methyl-, 2,2-dimethylcyclohexyl methyl ester	220	$C_{10}H_{21}O_3P$	38	100027-34-5



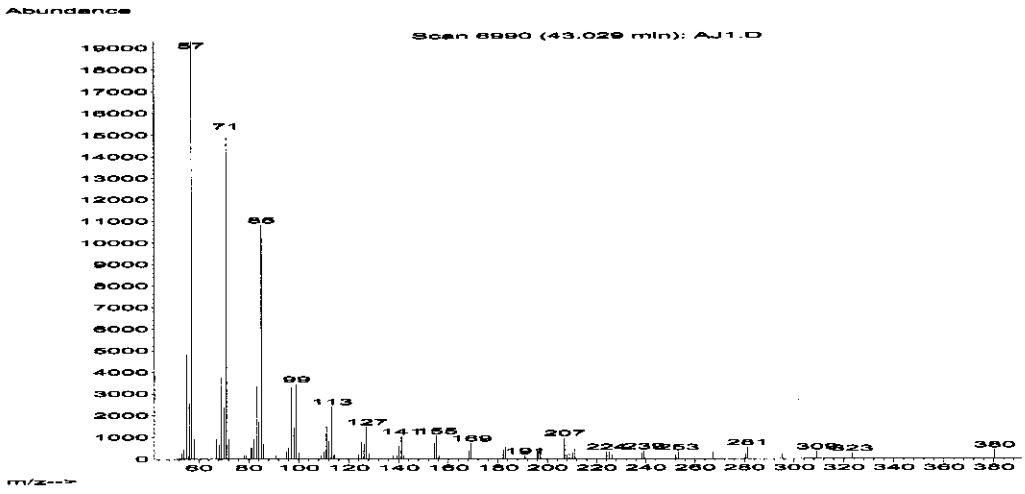
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	90	000502-69-2
2	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	86	000502-69-2
3	2-Pentadecanone, 6,10,14-trimethyl-	268	$C_{18}H_{36}O$	50	000502-69-2
4	5-Azulenemethanol, 1,2,3,3a,4,5,6,7-octahydro-.alpha.,.alpha.,3,8-tetramethyl-	222	$C_{15}H_{26}O$	38	055255-90-8
5	Cyclohexanol, 3,5-dimethoxy-, stereoisomer	160	$C_8H_{16}O_3$	35	030517-18-1



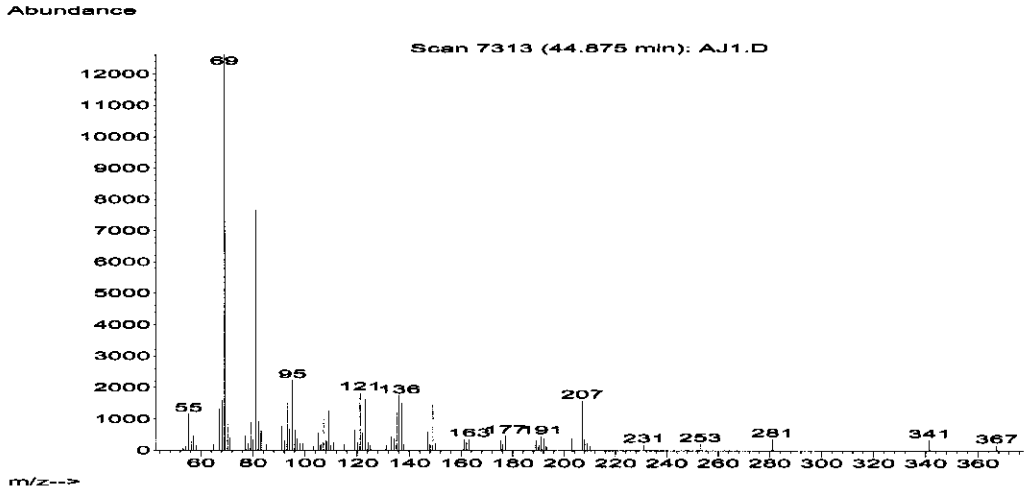
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	93	000057-10-3
2	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	91	000057-10-3
3	Pentadecanoic acid	242	$C_{15}H_{30}O_2$	72	001002-84-2
4	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	64	000544-63-8
5	n-Decanoic acid	172	$C_{10}H_{20}O_2$	64	000334-48-5



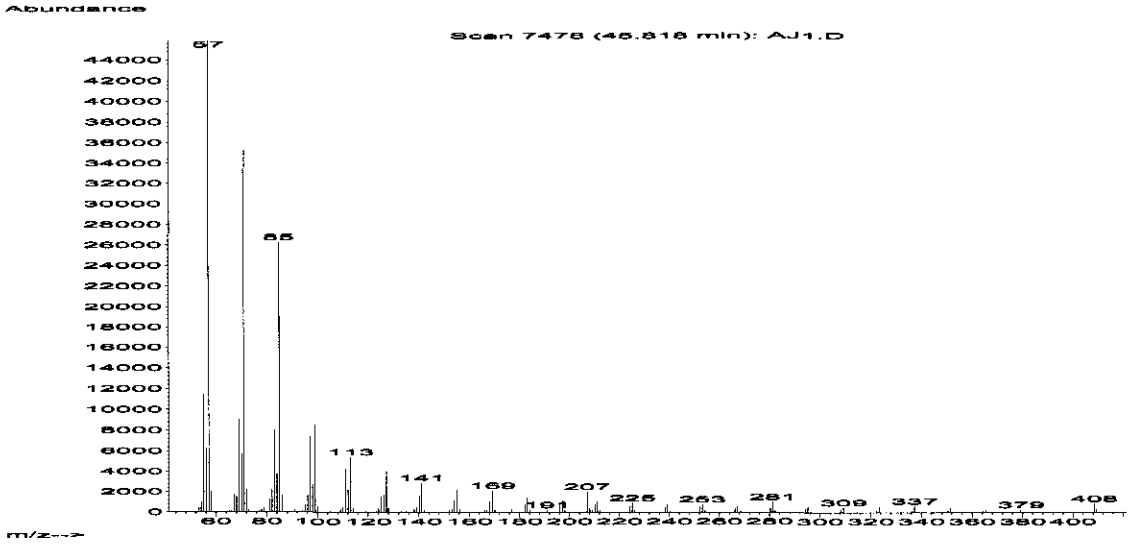
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	1,4-Cyclooctadiene, (Z,Z)-	108	C_8H_{12}	92	016327-22-3
2	11,14,17-Eicosatrienoic acid, methyl ester	320	$C_{21}H_{36}O_2$	80	055682-88-7
3	Methyl (Z)-5,11,14,17-eicosatetraenoate	318	$C_{21}H_{34}O_2$	80	059149-01-8
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	292	$C_{19}H_{32}O_2$	80	000301-00-8
5	1,3-Cyclooctadiene	108	C_8H_{12}	70	001700-10-3



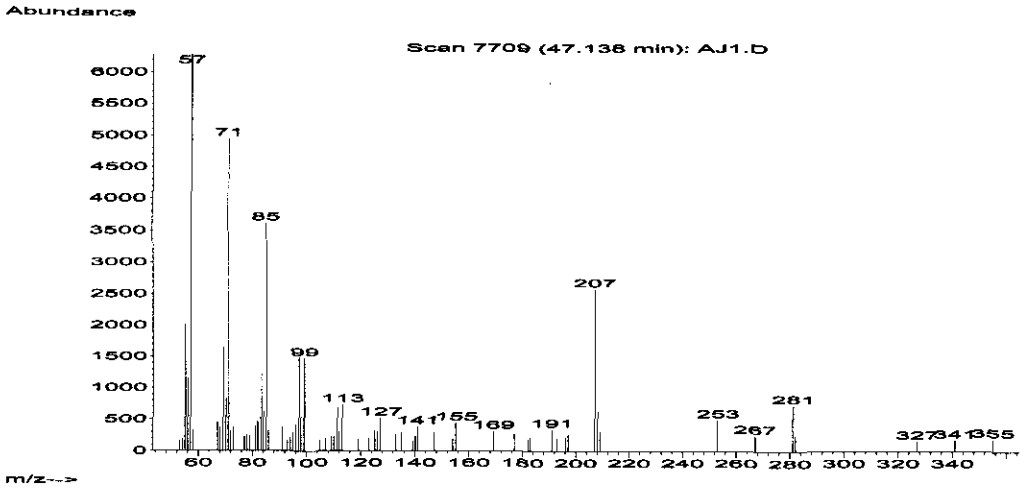
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Heptacosane	380	$C_{27}H_{56}$	99	000593-49-7
2	Tetratriacontane	479	$C_{34}H_{70}$	91	014167-59-0
3	Tetracosane	338	$C_{24}H_{50}$	91	000646-31-1
4	Tetratetracontane	619	$C_{44}H_{90}$	91	007098-22-8
5	Nonacosane	408	$C_{29}H_{60}$	91	000630-03-5



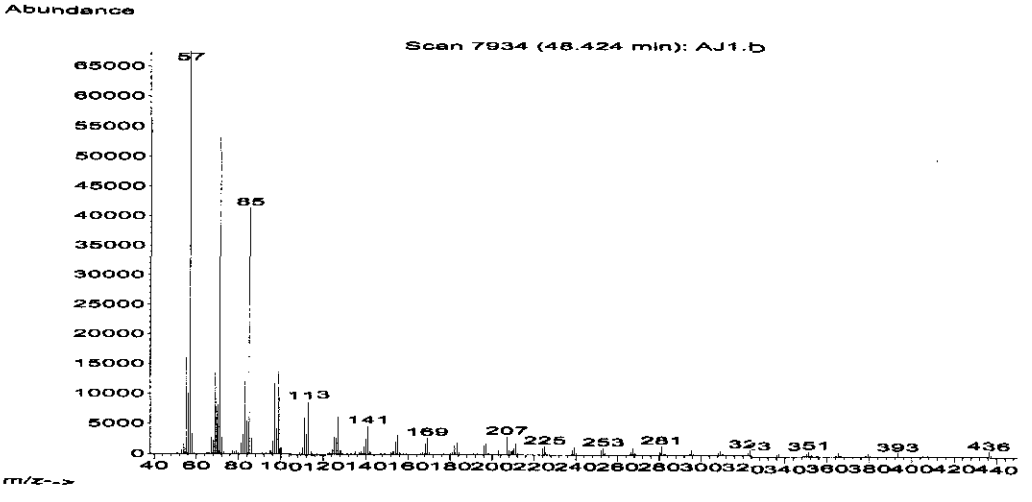
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	410	$C_{30}H_{50}$	94	000111-02-4
2	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	410	$C_{30}H_{50}$	91	000111-02-4
3	Squalene	410	$C_{30}H_{50}$	91	007683-64-9
4	Squalene	410	$C_{30}H_{50}$	80	007683-64-9
5	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene	342	$C_{25}H_{42}$	80	075581-03-2



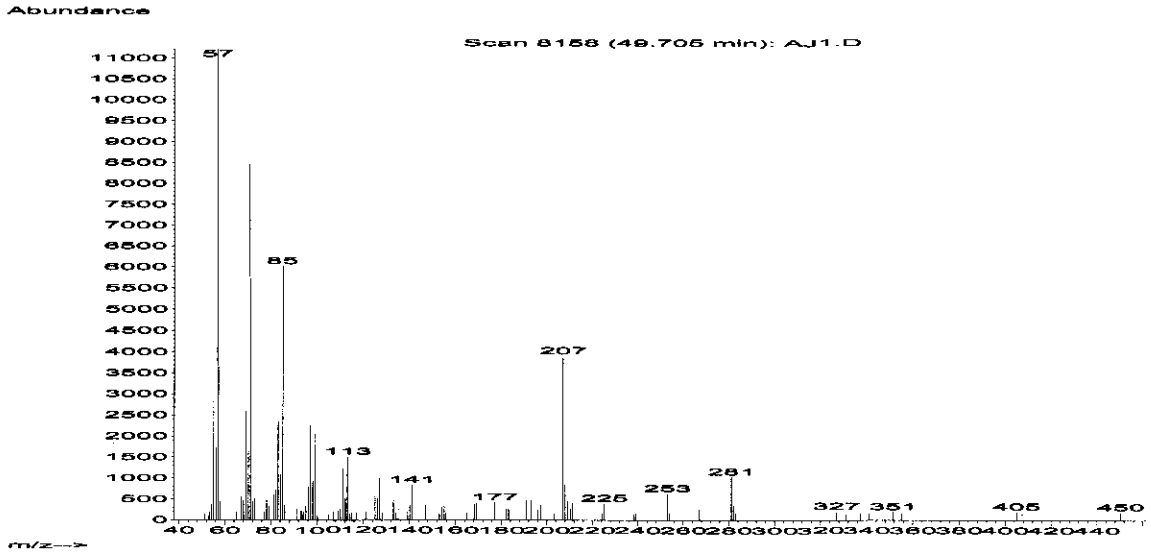
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Octadecane	254	$C_{18}H_{38}$	96	000593-45-3
2	Hexadecane, 1-iodo-	352	$C_{16}H_{33}I$	95	000544-77-4
3	Tetratriacontane	479	$C_{34}H_{70}$	94	014167-59-0
4	Heptadecane	240	$C_{17}H_{36}$	93	000629-78-7
5	Heptacosane	380	$C_{27}H_{56}$	91	000593-49-7



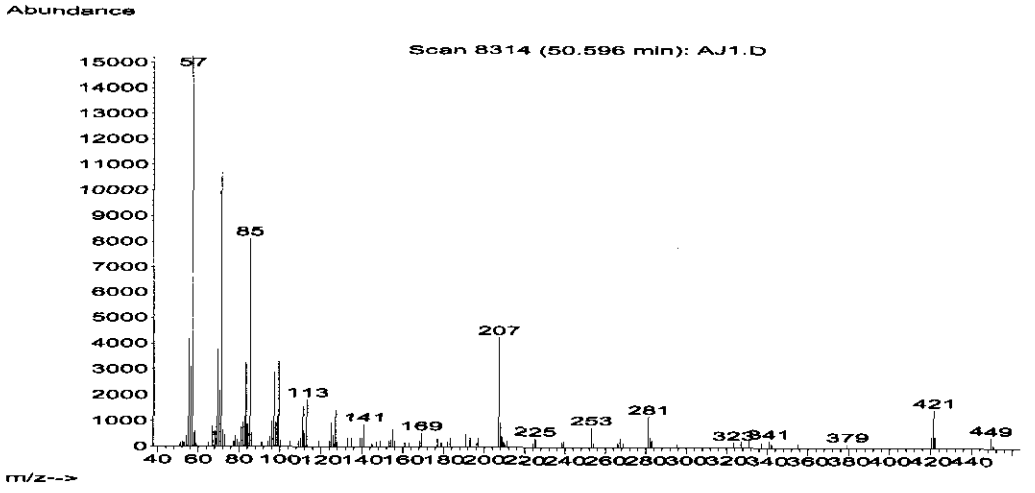
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Eicosane	282	$C_{20}H_{42}$	98	000112-95-8
2	Tetratriacontane	479	$C_{34}H_{70}$	64	014167-59-0
3	Eicosane	282	$C_{20}H_{42}$	60	000112-95-8
4	Hexadecane	226	$C_{16}H_{34}$	58	000544-76-3
5	Heptadecane	240	$C_{17}H_{36}$	58	000629-78-7



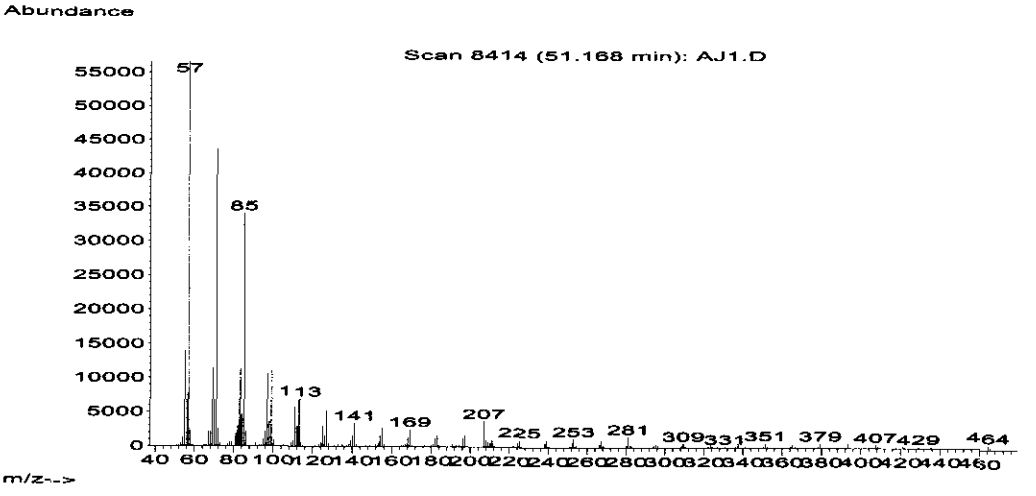
Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Octadecane	254	$C_{18}H_{38}$	97	000593-45-3
2	Heneicosane	296	$C_{21}H_{44}$	96	000629-94-7
3	Eicosane	282	$C_{20}H_{42}$	95	000112-95-8
4	Hexadecane	226	$C_{16}H_{34}$	94	000544-76-3
5	Pentacosane	352	$C_{25}H_{52}$	93	000629-99-2



Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Eicosane	282	$C_{20}H_{42}$	98	000112-95-8
2	Octadecane	254	$C_{18}H_{38}$	96	000593-45-3
3	Octadecane	254	$C_{18}H_{38}$	95	000593-45-3
4	Tetratetracontane	619	$C_{44}H_{90}$	76	007098-22-8
5	Tritetracontane	605	$C_{43}H_{88}$	76	007098-21-7



Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Triacontane	422	$C_{30}H_{62}$	97	000638-68-6
2	Eicosane	282	$C_{20}H_{42}$	95	000112-95-8
3	Dotriacontane	451	$C_{32}H_{66}$	93	000544-85-4
4	Octadecane	254	$C_{18}H_{38}$	91	000593-45-3
5	Eicosane	282	$C_{20}H_{42}$	78	000112-95-8



Best Match	Component Name	Molar Mass	Molar Formula	Accuracy %	CAS Number
1	Octadecane	254	$C_{18}H_{38}$	97	000593-45-3
2	Heneicosane	296	$C_{21}H_{44}$	95	000629-94-7
3	Octacosane	394	$C_{28}H_{58}$	95	000630-02-4
4	Tetratriacontane	479	$C_{34}H_{70}$	91	014167-59-0
5	Tetratetracontane	619	$C_{44}H_{90}$	91	007098-22-8

APPENDIX B

Appendix B covers the common, IUPAC and synonym names of components found in the analysis presented in Appendix A.

Citronellal : 3,7-Dimethyl-6-octenal/2,3-dihydrocitral/rhodinal β-Citronellal; Rhodinal;Levo-citronellal; Citronellel; Citronellol,(d); 2,3-Dihydrocitral

Citronellol : β-Citronellol ; Cephrol ; Rodinol ; 3,7-Dimethyl-6-octen-1-ol; Elenol; RHODINOL; 2,6-Dimethyl-2-octen-8-ol ; 2,3-Dihydrogeraniol ; R-(+)-β-Citronellol; R-(+)-3,7-Dimethyl-6-octen-1-ol / 3,7-Dimethyl-6-octen-1-ol/dimethyl-6-octen-1-ol

1-octen-3-ol : Vinyl pentyl carbinol / Pentyl vinyl carbinol; Amyl vinyl carbinol; Oct-1-en-3-ol; Vinyl amyl carbinol; 3-Hydroxy-1-octene; Matsutake alcohol; 1-Okten-3-ol; n-Oct-1-en-3-ol; 1-Octene-3-ol; 3-Octenol; Flowtron mosquito attractant; Matsuka alcohol; Morillol; Mushroom alcohol; Octen-3-ol; Vinyl hexanol

3-octanol/octan-3-ol : n-Octan-3-ol; Ethylamylcarbinol; Amylethylcarbinol; Ethyl-n-amylcarbinol; Octanol-3; D-n-Octanol; Ethyl vinyl carbinol

3-octanone : n-Octanone-3; Amyl ethyl ketone; Ethyl amyl ketone; Ethyl n-amyl ketone; Ethyl pentyl ketone; EAK; Octan-3-One; Ethyl n-pentyl ketone; Ethyl pentyl ketone; n-Amyl ethyl ketone

Caffeic-acid : 2-Propenoic acid, 3-(3,4-dihydroxyphenyl)-; 3-(3,4-Dihydroxy phenyl)-2-propenoic acid; 3-(3,4-Dihydroxyphenyl)propenoic acid; 3,4-Dihydroxybenzeneacrylic acid; 3,4-Dihydroxycinnamic acid; 4-(2-Carboxyethenyl)-1,2-dihydroxybenzene; 4-(2'-Carboxyvinyl)-1,2-dihydroxybenzene; (2E)-3-(3,4-Dihydroxyphenyl)-2-propenoic acid; 3,4-Dihydroxycinnamic acid

Alpha-cubebene : α-Cubebene; 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, 3a,3b,4,5,6,7-hexahydro-3,7-dimethyl-4-(1-methylethyl)-, [3aS-(3aα,3bβ,4β,7α,7aS*)-(-)-]; 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, 3aα,3bα,4,5,6,7-hexahydro-4α-isopropyl-3,7β-dimethyl-, (-)-; (-)-α-Cubebene; 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, 3a,3b,4,5,6,7-hexahydro-3,7-dimethyl-4-(1-methylethyl)-, *3aS-(3aα,3bβ,4β,7α,7aS*)-(-)-; 1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, 3a,3b,4,5,6,7-hexahydro-3,7-dimethyl-4-(1-methylethyl)-, (3aα,3bα,4α,7β,7aR*)-(-)-; 4-Isopropyl-3,7-dimethyl-3a,3b,4,5,6,7-hexahydro-1H-cyclopenta[2,3]cyclopropa[1,2-a]benzene ; 3a,3b,4,5,6,7-hexahydro-3,7-dimethyl-4-(1-methylethyl)-, (3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*))-1 cyclopenta(1,3)cyclopropa(1,2)benzene

Alpha-humulene : 2,6,6,9-tetramethyl-1,4,8-cycloundecatriene/alpha caryopyllene

Beta-bourbonene

Beta-caryphyllene/Caryophyllene (E) :

Caryophyllene; Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]-; Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, (E)-(1R,9S)-(-)-; β-Caryophyllen; β-Caryophyllene; trans-Caryophyllene; L-Caryophyllene; Bicyclo(7.2.0)undec-4-ene, 8-methylene-4,11,11-trimethyl-, (E)-(1R,9S)-(-)-; CARYOPHYLLENE ,α + β MIXT.; 8-Methylene-4,11,11-(trimethyl)bicyclo(7.2.0)undec-4-ene; 4,11,11-Trimethyl-8-methylenebicyclo[7.2.0]undec-4-ene;4,11,11-trimethyl-8-methylenebicyclo(7.2.0)undec-4-ene

Beta-caryphyllene oxide

Caryophyllene-oxide

Chlorogenic acid : Chlorogenic acid

Catechin

Cis-3-hexenol : (Z)-Hex-3-en-1-ol; cis-3-Hexen-1-ol; cis-3-Hexene-1-ol; cis-3-Hexenol;
Blatteralkohol (German); Leaf alcohol; Z-3-Hexenol; 3-(Z)-Hexenol; β,γ-
Hexenol; Blatteralkohol; HEXEN-30L-1; 3-Hexen-1-ol; 3-Hexen-1-ol, cis-; 3-Hexenol, cis-; (3Z)-3-Hexen-1-ol;cis-3-hexen-1-ol

Cis-ocimene : 1,3,6-Octatriene, 3,7-dimethyl-, (Z)-; β-cis-Ocimene; cis-β-Ocimene; cis-
3,7-Dimethyl-1,3,6-Octatriene; Ocimene, cis-β-; Z-Ocimene; β-Ocimene,
Z;-; (3Z)-3,7-Dimethyl-1,3,6-octatriene

Citral : Citral/2,6-Octadienal, 3,7-dimethyl-; 3,7-Dimethyl-2,6-octadienal; Citral,c&t; cis,trans-Citral; Geranial; NCI-C56348; 3,7-Dimethyl-1,2,6-octadienal; cis-Citral; Citral (cis and trans); Citral acis-3,7-dimethyl-2,6-octadienal; Lemarome n; Neral; Z-3,7-Dimethyl-2,6-octadiene-1-al; (2E)-3,7-Dimethyl-2,6-octadienal;1,1-dimethoxy-3,7-dimethyl-, (Z)-2,6-octadiene/dimethoxy nerol

Citral (mixture of cis & trans) : 3,7-Dimethyl-2,6-octadienal

Copaene

Delta-cadinene

Geranial/Citral a : α-Citral; (E)-Citral; trans-Citral; trans-3,7-Dimethyl-2,6-octadienal; Citral a; Geranaldehyde; Geranial; Citral α; (E)-3,7-Dimethyl-2,6- octadienal; (2E)-3,7-Dimethyl-2,6-octadienal; 3,7-dimethyl, (E)-2,6-octadienal/E-citral

Geraniol : trans-Geraniol; Guaniol; Lemonol; Nerol; Neryl Alcohol; Geraniol; trans-3,7-Dimethyl-2,6-octadien-1-ol; Geraniol alcohol; Geraniol extra; Geranyl alcohol; 2,6-Dimethyl-trans-2,6-octadien-8-ol; 2,6-Octadien-1-ol, 3,7-dimethyl-, trans-; 3,7-Dimethyl-trans-2,6-octadien-1-ol; (E)-3,7-Dimethyl-2,6-octadien-1-ol; Meranol; trans-3,7-Dimethyl octa-2,6-dien-1-ol; (2E)-3,7-Dimethyl-2,6-octadien-1-ol

Geranyl-acetate : 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate; Geraniol acetate; Geranyl acetate; (2E)-3,7-Dimethyl-2,6-octadienyl acetate; Acetic acid, geraniol ester; Bay pine (oyster) oil; Geraniol acetate; Geranyl acetate; trans-3,7-Dimethyl-2,6-octadien-1-ol, acetate; NCI-C54728; 2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, trans-; 3,7-Dimethyl-2-trans, 6-octadienyl acetate; trans-3,7-Dimethyl-2,6-octadien-1-yl acetate; trans-2,6-Dimethyl-2,6-octadien-8-yl ethanoate; Geranyl ethanoate; (E)-3,7-Dimethyl-2,6-octadien-1-yl acetate; Meraneine; (2E)-3,7-Dimethyl-2,6-octadienyl acetate

Germacrene-D

Isogeranial

Linalool : 1,6-Octadien-3-ol, 3,7-dimethyl-; β-Linalool; Linalol; Linalool; Linalyl alcohol; 2,6-Dimethyl-2,7-octadien-6-ol; allo-Ocimenol; p-Linalool; LINOLOOL (D); 2,6-Dimethyl-2,7-octadiene-6-ol; 3,7-Dimethyl-1,6-octadien-3-ol; 3,7-Dimethylocta-1,6-dien-3-ol; Linolool; b-Linalool; Linanool; dl-3,7-Dimethyl-3-hydroxy-1,6-octadiene; Linalool ex bois de rose oil; Linalool ex ho oil; Linalool ex orange oil; Phantol; Linaloyl oxide;3,7-dimethyl-1,6-octadien-3-ol

Luteolin-7-glucoside

Methyl-heptenone

Neral acetate

Neral/Citral b : 2,6-Octadienal, 3,7-dimethyl-; 3,7-Dimethyl-2,6-octadienal; Citral,c&t; cis,trans-Citral; Geranial; NCI-C56348; 3,7-Dimethyl-1,2,6-octadienal; cis-Citral; Citral (cis and trans); Citral acis-3,7-dimethyl-2,6-octadienal; Lemarome n; Neral; Z-3,7-Dimethyl-2,6-octadiene-1-al; (2E)-3,7-Dimethyl-2,6-octadienal; Z-citral/3,7-dimethyl-, (Z)-2,6-octadinal

Nerol : trans-Geraniol; Guaniol; Lemonol; Nerol; Neryl Alcohol; Geraniol; trans-3,7-Dimethyl-2,6-octadien-1-ol; Geraniol alcohol; Geraniol extra; Geranyl alcohol; 2,6-Dimethyl-trans-2,6-octadien-8-ol; 2,6-Octadien-1-ol, 3,7-dimethyl-, trans-; 3,7-Dimethyl-trans-2,6-octadien-1-ol; (E)-3,7-Dimethyl-2,6-octadien-1-ol; Meranol; trans-3,7-Dimethyl octa-2,6-dien-1-ol; (2E)-3,7-Dimethyl-2,6-octadien-1-ol; cis-Geraniol; cis-3,7-Dimethyl-2,6-octadien-1-ol; Nerol; Neryl alcohol; 2-cis-3,7-Dimethyl-2,6-octadien-1-ol; 2,6-Dimethyl-2,6-octadien-8-ol; 3,7-Dimethyl-2,6-octadien-1-ol; Vernol; (2Z)-3,7-Dimethyl-2,6-octadien-1-ol

Octyl-benzoate : n-Octyl benzoate; Octyl benzoate; Benzoic acid, octyl ester

Oleanolic-acid

Pomolic-acid

Protocatechuic-acid : 3,4-dihydroxybenzoic acid

Rhamnazin

Rosmaric-acid

Rosmarinic-acid

Stachyose

Succinic-acid : Butanedioic acid; Succinic acid; Amber acid; Asuccin; Bernsteinsaure; Dihydrofumaric acid; Katasuccin; Wormwood acid; 1,2-Ethanedicarboxylic acid; Ethanedicarboxylic acid; Wormwood; Kyselina Jantarova; Acid of amber; Dicarboxylic acid; Ethylene succinic acid; Sal succini; Salt of amber; Succinellite

Thymol : 5-Methyl-2-(1-methylethyl)phenol;1-Methyl-3-hydroxy-4-isopropylbenzene; Thymol; p-Cymen-3-ol; Thyme camphor; 2-Isopropyl-5-methylphenol; 3-Hydroxy-p-cymene; 3-Methyl-6-isopropylphenol; 5-Methyl-2-isopropylphenol; 6-Isopropyl-m-cresol; 6-Isopropyl-3-methylphenol; m-Cresol, 6-isopropyl-; p-Cymene, 3-hydroxy-; Isopropyl cresol; Phenol, 2-isopropyl-5-methyl-; Thymic acid; 1-Hydroxy-5-methyl 2-isopropylbenzene; 1-Methyl-3-hydroxy-4-isopropylbenzene; 3-p-Cymenol; 3-Hydroxy-1-methyl-4-isopropylbenzene; 5-Methyl-2-isopropyl-1-phenol; 5-Methyl-2-(1-methylethyl)phenol; Isopropyl-m-cresol; m-Thymol

Trans-ocmene : 1,3,6-Octatriene, 3,7-dimethyl-, (E)-; β-trans-Ocimene; trans-β-Ocimene; trans-3,7-Dimethyl-1,3,6-Octatriene; Ocimene, trans-β-; (E)-Ocimene; β-Ocimene, E;-; (3E)-3,7-Dimethyl-1,3,6-octatriene

Ursolic-acid

Furulic acid : 4-Hydroxy-3-methoxycinnamic acid

Quercitrin

Rhamnocitrin

Apigenin

Quercetin : 3,3',4',5,7-Pentahydroxyflavone / Sophoretin / Meletin
2-(3',4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one /

Kaempferol

***α*-Pinene**

cis-ß-ocimene

trans-ß-ocimene

6-methyl-5-heptene-2-one : 6-Methyl-5-heptene-2-one; 6-Methyl-5-hepten-2-one; 2-Methyl-2-hepten-6-one; -Methyl hept-5-en-2-one

cis-linolool oxide

Eugenol acetate : Phenol, 4-allyl-2-methoxy-, acetate; Aceteugenol; Acetyleugenol; Eugenol acetate; Eugenyl acetate; 1,3,4-Eugenol acetate; Aceto eugenol;1-Acetoxy-2-methoxy-4-allylbenzene; 4-Allyl-2-methoxyphenol acetate; 4-Allyl-2-methoxyphenyl acetate

Eugenol : Phenol, 2-methoxy-4-(2-propenyl)-; Phenol, 4-allyl-2-methoxy-; p-Allylguaiacol; p-Eugenol; Caryophyllic acid; Engenol; Eugenic acid; 2-Methoxy-1-hydroxy-4-allylbenzene; 2-Methoxy-4-allylphenol; 4-Allyl-2-methoxyphenol; 4-Allylguaiacol; 4-Hydroxy-3-methoxyallylbenzene; NCI-C50453; 1-Hydroxy-2-methoxy-4-allylbenzene; 1-Hydroxy-2-methoxy-4-prop-2-enylbenzene; 2-Methoxy-4-(2-propenyl)phenol; 2-Methoxy-4-prop-2-enylphenol; 4-Allyl-1-hydroxy-2-methoxybenzene; 4-Allylcatechol-2-methyl ether; 1,3,4-Eugenol; FA 100; FEMA No. 2467; 2-Metoksy-4-allilofenol; 2-Hydroxy-5-allylanisole; 2-Methoxy-4-(2-propenyl)phenyl; Allylguaiacol Para-menth-3-en-8-ol

Linolenic acid : Methyl all-cis-9,12,15-octadecatrienoate; Methyl linolenate; Methyl (9Z,12Z,15Z)-9,12,15-octadecatrienoate