Composition of supercritical carbon dioxide derived extracts of *Chamaemelum nobile*

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ABSTRACT

The feasibility of extracting botanical substances from samples of *Cameamelum nobile* (Roman chamomile) with supercritical carbon dioxide (sc-CO_2) was investigated. The advantages of clean technology and the relevance of chamomile extracts to the fragrance, flavour, food, cosmetic and pharmaceutical industries served as motivation for the investigation.

Extractions were performed on selected dried plant material using a commercial laboratory-size supercritical fluid extractor. The extraction conditions (temperature, pressure, time) were optimised in terms of yield of extract using computer-assisted surface response analysis based on a statistical design. A maximum yield of 3 % (m/m) was obtained at optimum conditions (39 ^{0}C, 171 atm), in good agreement with steam distillation derived yields of 0.5 - 2 % (m/m) reported in the literature.

The dependence of yield of extract on the density of the fluid allowed conclusions to be drawn on the mechanism of extraction, and these could be supported by calculated values of a few activation parameters. It turned out that components are either desorbed from the plant matrix by sc-CO_2 at gas-like densities or dissolved in sc-CO_2 at liquid-like densities.

The extracts were analysed by GC/FID, GC/MS and GC-GC/TOF-MS. The three chromatographic techniques were complementary in identifying the major compounds present in the extracts, but the total of 462 substances identified by two-dimensional GC by far exceeded the identification output of the two other techniques. The results confirmed the acquisition of component-rich extracts with sc-CO_2, with many components also found in steam distillation extracts.

The study proved that sc-CO_2 extraction has advantages over steam distillation in terms of shorter extraction times, milder extraction temperatures and a wealth of components that may constitute different compositions by manipulating extraction conditions.

OPSOMMING

Die uitvoerbaarheid van die ekstraksie van plantaardige stowwe uit monsters van *Cameamelum nobile* (Romeinse kamille) met superkritieke koolstofdioksied (sc-CO_2) is ondersoek. Die voordele van skoon tegnologie en die relevansie van kamille-ekstrakte vir die reuk-, geur-, voedsel-, kosmetiese en farmaseutiese nywerheid het as motivering vir die ondersoek gedien.

Ekstraksies is op uitgesoekte gedroogde plantmateriaal uitgevoer deur 'n kommersiële laboratorium-grootte superkritieke-fluïed-ekstraktor te gebruik. Die ekstraksiekondisies (temperatuur, druk, tyd) is in terme van ekstraksie-opbrengs geoptimaliseer deur van rekenaargesteunde oppervlakresponsanalise gebaseer op 'n statistiese ontwerp gebruik te maak. 'n Maksimum opbrengs van 3 % (m/m) is by optimumkondisies (39 ^{0}C, 171 atm) verkry, in goeie ooreenstemming met stoomdistillasie-opbrengste van 0.5 - 2 % (m/m) wat in die literatuur gerapporteer word.

Die digtheidsafhanklikheid van die ekstrakopbrengs het gevolgtrekkings oor die ekstraksiemeganisme moontlik gemaak, en dit kon deur berekende waardes van enkele aktiveringsparameters ondersteun word. Dit het geblyk dat stowwe óf vanaf die plantmatrys deur sc-CO_2 met gassoortige digthede gedesorbeer of in sc-CO_2 met vloeistofagtige digthede opgelos word.

Die ekstrakte is met GC/FID, GC/MS en GC-GC/TOF-MS geanaliseer. Die drie chromatografiese tegnieke het mekaar ten opsigte van die identifikasie van die belangrikste stowwe in die ekstrakte gekomplementeer, maar die totaal van 462 stowwe wat met twee-dimensionele GC geïdentifiseer is, het verreweg die identifikasie-uitset van die ander twee tegnieke oortref. Die resultate bevestig dat komponentryke ekstrakte met sc-CO_2 verkry kan word en dat baie van die komponente ook in stoomdistillasie-ekstrakte gevind word.

Die studie het getoon dat sc-CO_2-ekstraksie voordele het bo stoomdistillasie in terme van korter ekstraksietye, matiger ekstraksietemperature en 'n magdom geëkstraheerde komponente wat verskillende ekstraksamestellings deur manipulasie van ekstraksie-kondisies tot gevolg kan hê.

CHAPTER 0

BIRD'S EYE VIEW OF PROJECT

A principal research topic of the supercritical technology group within Separation Science and Technology (SST) at the North-West University (Potchefstroom Campus) is botanical extraction. Extracts relevant to the food, flavour, pharmaceutical, medical and cosmetic industries are derived from locally cultivated plants while utilising the advantages of sc-CO_2 extraction over traditional steam distillation and solvent extraction.

In this study, which represents a further contribution in a series of botanical extractions[1-7], sc-CO_2 derived extracts of *Chamaemelum nobile* were investigated. Extracts of this plant have application potential in the food/fragrance industry, and for that reason sc-CO_2 was the preferred extracting agent as no solvent residues were left behind in the final product. The extraction by sc-CO_2 based clean technology is gaining increased interest for the production of natural products for the marketplace.

0.1 Specific goals

The specific goals of the project were

- to produce extracts of *Chamaemelum nobile* with sc-CO_2 on laboratory scale by using a benchtop supercritical fluid extractor and other available laboratory infrastructure;
- to investigate and implement suitable chromatographic techniques (GC-FID, GC-MS, GC-GC/TOF-MS) by virtue of which the composition of sc-CO_2 derived extracts could be analysed;
- to compare the composition of sc-CO_2 derived extracts with that of extracts obtained by traditional methods to establish any advantages of supercritical technology in terms of plant component selectivity;
- to identify process parameters and to vary these according to a statistical design using a suitable software programme (Statistica for Windows®) to establish optimum conditions in terms of yield of extract;
- to process the extraction data in such ways as to reveal the principal features of the extraction process as a means towards improved process control.

0.2 Other issues

In addition to these specific goals, the project also served the purpose to contribute to a lesser extent to the following relevant issues:

- The chemical substances derived from plants (volatile oils, waxes) are high-value products having significant commercial value.[8]
- The importance of clean technology for "green" or sustainable chemistry is increasingly emphasised.[9] *sc*-CO_2 is a non-hazardous solvent with which solvent-free extracts can be derived.
- There is academic interest in as well as financial support for the development of knowledge of indigenous plants[10]. The suitability of sc-CO_2 for the acquisition of substances which have been isolated for centuries by less favourable methods needs to be demonstrated.
- The application of supercritical fluid based processes in daily life creates science awareness since the replacement of natural products in ordinary household products (beer, shampoo)[11] captures the attention and imagination of the public.
- Finally, this investigation can help to convince industry to apply the technology despite the negative perceptions about extreme conditions and the high capital investment needed to set up the required infrastructure.

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6. G.A. Naudé, *Composition of Supercritical Carbon Dioxide Derived Extracts of Mentha piperita.* M.Sc. Dissertation, North-West University (Potchefstroom Campus), 2004, 60 pages.

7. A.A. Wessels, *Extraction of Helianthus annuus (Sunflower) Oil with Supercritical Carbon Dioxide*, M.Sc. Dissertation, North-West University (Potchefstroom Campus), 2004, 105 pages.

8. The price of harpogoside, for instance, is estimated at $120 for 10 mL of the pure substance.

9. ICS/UNIDO Workshop on *Cleaner Technologies for Sustainable Chemistry*, Cape Town, 9-11 December 2002.

10. The National Research Foundation (NRF) has identified knowledge of indigenous systems as one of its research focus areas and makes substantial funding available to prospective investigators.

11. In Bavaria (Germany) almost all hop extraction for the beer brewing industry is done by sc-CO_2. The company Wella® recently introduced a shampoo with a small amount of natural (instead of synthetical) wax obtained by sc-CO_2 extraction of apple skin which was well received by the consumer.

CHAPTER 1

ROMAN CHAMOMILE - AN OVERVIEW

sc-CO_2 extraction of material from samples of Roman chamomile was performed in this study. An overview of this plant is thus presented in this chapter. It mainly covers the types of constituents that can be extracted and the uses of such extracts in daily life.

1.1 Botanical description

Chamomile belongs to the *asteraceae* (or *compositae*) family, which also includes ragweed, echinacea and feverfew.[1] There are numerous chamomile species, but the most popular and widely cultivated are *Chamaemelum nobile* (Figure 1.1a), which is also known as Roman or English chamomile, and *Matricaria recutita* (Figure 1.1b), also known as German chamomile. These two species are commonly confused with each other, but they do differ in both morphology and chemical composition. Accurate identity of both species is hampered by the fact that their names have been applied to a number of species in the *asteraceae* (or *compositae*) family.

Chamaemelum nobile (Roman chamomile) is a creeping or trailing herb growing to a height of about 0.3 m. The aromatic plant is characterised by jointed and fibrous roots. The hairy stems are freely branching and are covered with leaves divided into thread-like segments. Its small flower heads grow at the ends of the shoot tips, and consist of a corona of white ligulae and many yellow tubular disk flowers at the center. The herb can be differentiated from other species by flowers with flattened corolla surrounding the receptacle on which yellow florets are situated. There are short and blunt scales among its florets. The whole plant is greyish-green in colour. There are two variants of *Chamaemelum nobile*, a double flowered variety *Flora Pleno* and a non-flowering *Trenague* commonly used for lawn or as an ornamental in flower gardens.[2]

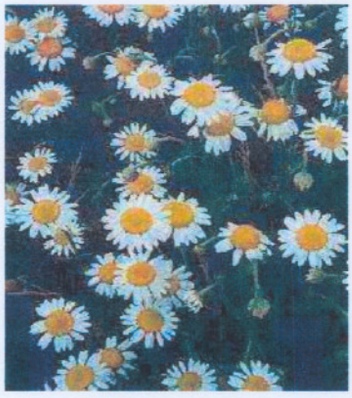


Figure 1.1a *Chamaemelum nobile* (Roman chamomile)

Figure 1.1b *Matricaria recutita* (German chamomile)

1.2 Cultivation and harvesting

Roman chamomile is cultivated in many countries including Belgium, France, England, Germany, Hungary, Bulgaria, Argentina and some African countries. It also occurs wild in certain areas. The plant prefers a sunny climate with temperatures ranging between 7 and 26 °C and it must be protected from the rigours of adverse weather.[3] The plant is set out in the fields in the first warm days of spring. Both *Chamaemelum nobile* species can propagate from seeds and cuttings. The non-flowering variety prefers dry sandy soil, while the double flowered variety requires a richer moist loam soil with a pH of 6.5-8.0.[3] Plowing is done in straight lines with spacing of about 50 cm between the plants and 60 cm between the rows. Application of fertilisers like super phosphate results in a maximum yield of flowers and oil. As the plant grows, it develops numerous clustered, carved stalks of about 30 cm high. The ends branch out and bear flowers, which are gathered during dry, clear weather. The plant blossoms in late spring through late summer and sometimes two or three harvests can be made in one season.[4] The flower heads are handpicked and usually the flowers of the second and third pickings contain the most volatile oil.

1.3 Constituents

The investigation of Roman chamomile oil was first undertaken more than a century ago and since then a host of chemical constituents have been identified.[4] The scope of this research is limited to the identification of compounds important on the basis of their therapeutic function and fragrance characteristics. The amount and quality of extract from plants depend on a wide range of variables, such as environmental factors, cultivation

practices, postharvest handling and plant age. The extracts of Roman chamomile cultivated in different areas vary in chemical composition, but there are compounds likely to be found in most extracts. The main constituents of Roman chamomile are flavonoids, terpenes and esters. Other constituents include coumarins, choline, phenolic and fatty acids.[5] Some of the previously identified compounds are directly derived from the source plant material, whereas others are artifacts of the extraction process. With careful extraction and handling, Roman chamomile extracts can have an aroma similar to the scent of the growing plant.

1.3.1 Flavonoids

Many flavonoids are easily recognised as flower pigments in most flowering plants. However, their occurrence is not restricted to flowers but found in all parts of the plant. Flavonoids play different roles in the ecology of the plant. Their attractive colours attract pollinating insects. A few have astringent properties and act as feeding repellants. Flavonoids are mostly found with their glycosides in plants, which complicates structural identification. The basic structure of flavonoids (Figure 1.2) consists of a 15-carbon skeleton to which hydroxyl, methoxyl or glycosyl groups are substituted at different positions on the three rings, resulting in various classes of flavonoids (Figure 1.3). These include flavones, flavonones, flavanols, flavonols, anthocyanins and isoflavones.

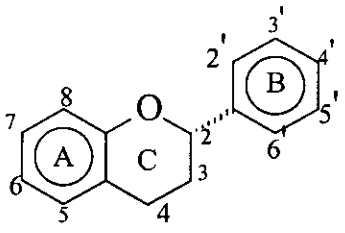


Figure 1.2 Basic structure of flavonoids

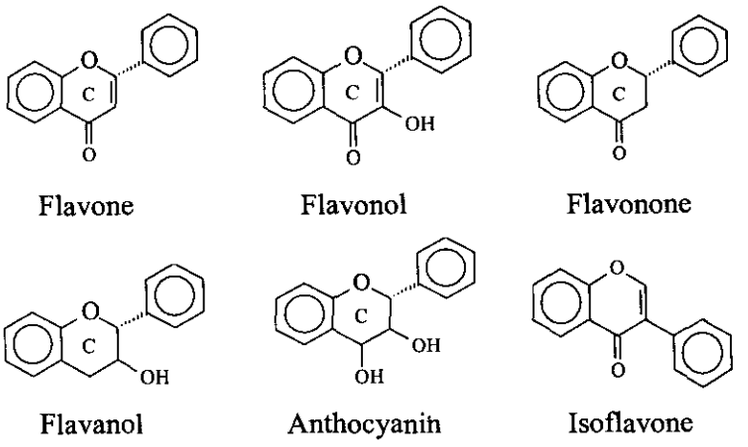


Figure 1.3 Classes of flavonoids

The flavonoid fraction of an aqueous extract of Roman chamomile includes the flavone apigenin and luteolin, the flavonol quercetin and their glycosides apigenin-7-apiosylglucoside, luteolin-7-glucoside and quercetin-3-rutin. A few major flavonoids found in extracts of Roman chamomile are presented in Figure 1.4

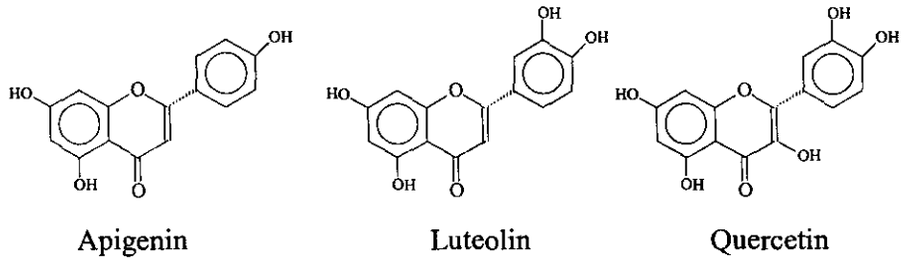


Figure 1.4 Flavonoids found in Roman chamomile

1.3.2 Volatile oil

The volatile oils extracted from plant material are byproducts of photosynthesis which consist of many organic compounds and generally smell like the botanicals from which they are derived. Although called an oil, they differ from the common vegetable oil as they are very light, non-greasy, quickly absorbed onto skin and readily evaporative. Freshly distilled Roman chamomile oil is colourless, but on prolonged standing and exposure to air and light it gradually changes to green and eventually to yellow. The odour

of the oil is strong, aromatic and characteristic of the flower. The principal constituents of a volatile oil fraction of Roman chamomile are terpenes, angelates and tiglates.[6]

1.3.3 Terpenes

Volatile oils are highly enriched in compounds based on a 5-carbon isoprene structure shown in Figure 1.5. The terpenes, with general formula $C_{10}H_{16}$, occur as diterpenes, triterpenes, tetraterpenes, hemiterpenes and sesquiterpenes. The classification of the terpenes is based on the number of 5-carbon units they contain.[7] When the compounds contain additional elements, usually oxygen, they are termed terpenoids. These are widely distributed in nature and are responsible for the characteristic scent of the plants in which they occur. They are considered to be safe and are frequently used as food additives or as fragrances. Table 1.1 lists the major terpenes found in Roman chamomile.

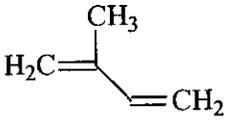
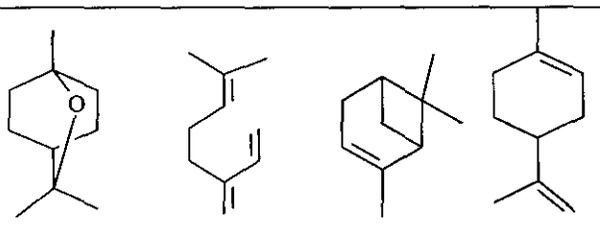
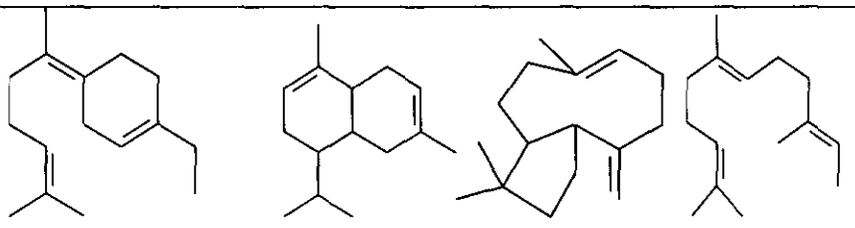


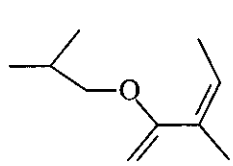
Fig 1.5: Isoprene structure

Table 1.1 Major terpenes of Roman chamomile

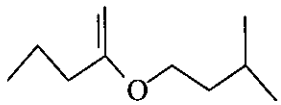
Classification	Examples of terpenes in Roman chamomile
Monoterpenes	 1,8-Cineole β-Myrcene α-Pinene Limonene
Sesquiterpenes	 Bisabolene Cadinene Caryophyllene Farnesene

1.3.4 Esters

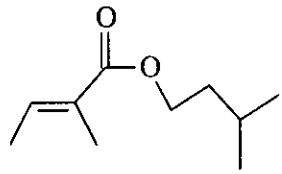
Esters are widely distributed in nature, mainly as volatiles in plants. Roman chamomile oil has a high content of esters (85%). More than 70 esters have been identified.[8] The ester constituents responsible for the fruitiness of the volatile oil are iso-amyl and iso-butyl esters of angelic and tiglic acids. Their structures are included in Figure 1.6



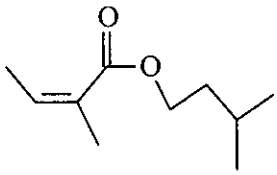
iso-butyl angelate



iso-amyl butyrate



iso-amyl angelate



iso-amyl tiglate

Fig 1.6 Esters of Roman chamomile

1.3.5 Other constituents

There is a host of other constituents in Roman chamomile extract including anthemic acid, phenolic and fatty acids, phytosterol, choline and inositol.

1.4 Therapeutic function and other uses

As mentioned in the previous section, Roman chamomile possesses a complex arsenal of phytochemicals that may have significance for clinical trials and pharmacology. Despite the wide-spread use of the herb and the vast information on its chemical composition, there is limited pharmacological information available for Roman chamomile. Most clinical studies have been carried out on its German counterpart. These species have similar but not necessarily identical active constituents and, as such, many of the applications described for German chamomile are thought to be applicable to Roman chamomile.

Chamomile has many pharmacological properties. It is antispasmodic, anti-allergic, analgesic, antipyretic, antiseptic, antibacterial, antifungal and carminative.[9] In addition, Roman chamomile exhibits astringent, antimicrobial, analgesic and anesthetic therapeutic action.[10] The antispasmodic effect of the herb is mainly attributed to the flavonoids. The azulene components of the plant extract are reported to possess anti-allergic and anti-inflammatory properties. Oral administration of the azulenes has been reported to stimulate liver regeneration. Apigenin, luteolin and apigenin monoglucosides are smooth muscle relaxants. The coumarins and umbelliferone also have minor muscle relaxant activity.[11] 1,8-Cineole, a major terpenoid compound of Roman chamomile, is used in pharmaceutical preparations as a mild anesthetic and antiseptic.

The use of the extract and its byproducts is directly related to the properties of active constituents. It is believed that the therapeutic value of chamomile does not result from a single constituent but from a complex mixture of chemically different compounds. This aspect is common to many phytomedicines of which the activity cannot be assigned to specific constituents since many components may directly or indirectly contribute to or support the action of the active component. Each of the numerous active constituents of Roman chamomile listed in Section 1.3 comes to the fore under certain conditions and plays a supportive role in other situations.

Chamomile is used both internally and externally for treatment of an extensive list of conditions. For local applications extracts of the plant are used in the form of ointments and inhalations. Internally, it is mostly taken as tea, which represents the largest use of chamomile flowers in the marketplace. Its infusions are taken for poor appetite and indigestion.[12] By stimulating digestive secretions and relaxing the muscles of the gut, Roman chamomile helps normalise digestive function. It has been used to treat nausea, vomiting, heartburn and the discomfort associated with gingivitis. The mixture of the oil with flour is reported to be a remedy for indurations of the liver, stomach and spleen. It has also been used with rose oil in a poultice to help indurate tumours of parotid glands. Roman chamomile can decrease the pain associated with arthritis, sprains, inflamed joints, migraine and headaches. The herb has been reported useful in treating painful menstruation, insomnia and fevers.[13,14]

In addition to medicinal application, Roman chamomile is widely used in the food and cosmetic industries. The plant is known as a relaxing herbal tea which eases depression, anxiety and an overactive mind. The oil can be used as a flavouring agent in bitters, benedictine, vermouth, alcoholic and non-alcoholic beverages, baked goods, candy and pudding.[3] It has a sweet, fresh and fruity smell due to the high content of ketones and angelic acid esters. The oil has found extensive use in hair dyes, mouthwashes, shampoos, perfumes and sunscreens. Its use in hair preparations, particularly for blonde hair, is well known. Borneol, present in Roman chamomile, has a piney, camphoraceous odour and is used to perfume soaps and detergents.[9] The presence of iso-amyl esters of angelic and tiglic acid makes Roman chamomile oil one of a few to exhibit a non-citrus note for use in perfumery.[15]

Although Roman chamomile is considered to be generally safe, it should be taken with care. It contains active substances that may cause side-effects or interact with other herbs, supplements or medications. Because of its calming effect, chamomile cannot be taken in conjunction with sedative medication. The herb contains anthemic acid, which can induce vomiting if taken in high doses. The oil is a uterine stimulant and should not be used during pregnancy.[16] Roman chamomile should be avoided by individuals with a known hypersensitivity to any of members of the *asteraceae* (or *compositae*) family. It yields nobilin, a sesquiterpene lactone which is reported to be potentially allergenic.

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CHAPTER 2

SFE - AN IDEAL EXTRACTION PROCESS?

Several methods can be employed to extract plant components. The choice of an appropriate method depends on a number of factors. These include time, simplicity of method, cost, quantity and quality of yield. Even though progress has been made with classical extraction methods, development of an ideal extraction process remains a challenge, especially in view of the limitations of classical methods.

2.1 Supercritical technology

The discovery of the critical point at the beginning of the 19th century marked the use of solubility enhanced supercritical fluids.[1,2] The disappearance of the liquid/gas boundary by increasing the temperature of a material in a pressurised vessel was observed. A report at a meeting of the Royal Society (London) in 1879 remains the yardstick for the application of supercritical fluids. It highlighted the ability of supercritical fluids to dissolve solid material and to precipitate inorganic salts from ethanol by a pressure change at temperatures above the critical point.[3] Despite experiments substantiating the findings, there were many misconceptions about the pressure dependent solubility behaviour of supercritical fluids. There are scientists who believe that supercritical fluids might dissolve substances that generations of chemists had failed to solubilise.[4]

The solubility behaviour of supercritical fluids was not exploited until the second half of the 19th century. Researchers have since then reported on the solubility of different solutes in various supercritical fluids. During the last two decades, supercritical fluids have found application in many processes offering both technical and economic advantages. The ban on the use of organic solvents led to the development of supercritical fluid extraction (SFE) as an alternative method for the extraction of botanical components. One of the first commercial applications was the extraction of hop and the decaffeination of coffee.[5] Large scale SFE has since been extended to a variety of natural products, mainly for the phytopharmaceutical and food industries.

The unique characteristics of supercritical fluids make them attractive media for chemical reactions. Apart from replacing harmful conventional solvents, supercritical fluids

enhance many types of chemical processes. Reactions within supercritical solvents can be controlled with respect to product selectivity. Supercritical technology eliminates solvent residues and avoids degradation of low melting compounds. Dissolving the compound in a supercritical fluid and then lowering pressure to cause precipitation can accomplish the recrystallisation of waxy compounds.

In response to restrictive environmental legislation, supercritical technology has found promising application possibilities in the environment. The use of sc-CO_2 for the regeneration of activated carbon used to clean polluted effluent streams allows recycling of the adsorbent without a marked decline in adsorbing capacity.[5]

Another useful application of supercritical technology is the considerable reduction in water pollution from dyeing in the textile industry. Dyes are dissolved in a supercritical fluid and applied to the swelled textile. In comparison to conventional dyeing, the energy requirement is lower as there are no drying steps, and surplus dye can be recovered. Pressure controllable solubilities allow control of the dyeing process and the final colour intensity.

Even though there are many applications using GC or HPLC, a large number of applications exist where supercritical fluid chromatography (SFC) might be the method of choice.[5] These applications involve analysis of analytes that are difficult to separate by either GC or LC. SFC is used for analysis of samples that are thermally labile or non-volatile under normal GC conditions. In comparison to LC, SFC has higher separation efficiency. SFC can separate more complex mixtures than packed column LC.

2.2 Nature of supercritical state

A supercritical fluid is a substance heated beyond its critical temperature (T_c) and compressed beyond its critical pressure (p_c). T_c is the highest temperature at which a gas can be converted to a liquid by an increase in pressure, and p_c is the highest pressure at which a liquid can be converted to a gas by an increase in the liquid temperature. As shown in Figure 2.1, the critical region denoted by the shaded area marks the end of the vapour-liquid coexistence curve. Above the critical point there is no phase transition and one phase possesses properties of both a gas and a liquid. The critical point is characteristic for each substance as illustrated by the entries in Table 2.1.

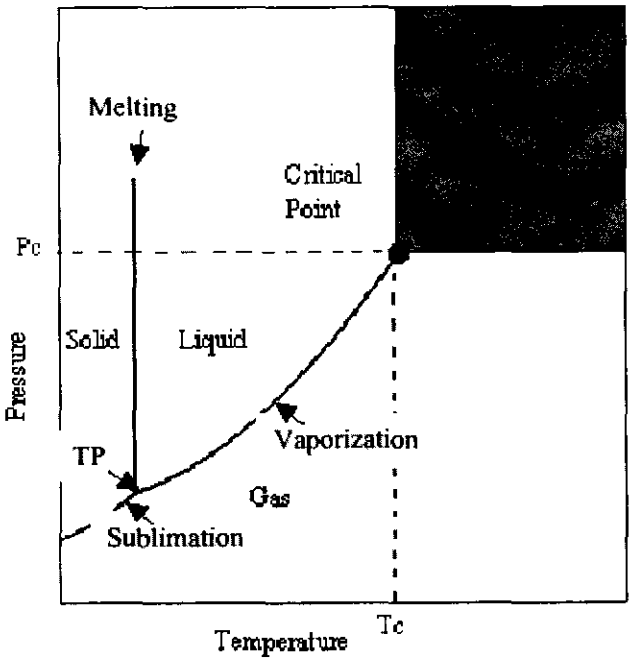

Figure 2.1 Generic pressure-temperature phase diagram

Table 2.1 Critical data of various solvents[6]

Solvents	T_c (°C)	p_c (bar)	ρ_c (g/mL)
Carbon dioxide	31.3	72	0.47
Nitrous oxide	36.5	70.6	0.45
Ammonia	132.5	109.8	0.23
Water	374.2	214.8	0.32
Sulfur hexafluoride	45.5	38	
Helium	-268	2.2	0.07
Xenon	17	56.9	1.11
Methane	-82	46	0.169
Propane	96.7	42.4	0.22
Ethylene	11	50.5	0.2
Benzene	288.9	98.7	0.302
Toluene	319	41.1	0.292
Methanol	239	78.9	0.27
Isopropyl alcohol	235.3	47.6	0.273
Ethyl methyl ether	164.7	47.6	0.272

Terahydrofuran	267	50.5	0.32
Trifluoromethane	26	46.9	0.52
Dichlorodifluoromethane	111.7	109.8	0.558
Chlorotrifluoromethane	28.8	214.8	0.58
Trichlorofluoromethane	196.6	28.9	0.554
Acetone	235	47.0	0.279
Acetonitrile	275	47	0.25

Although there are many substances which can be used as supercritical solvents, the choice of supercritical extractants has been limited to relatively few gases. The choice of a given supercritical solvent is determined by the solubility of the substance to be extracted, the chemical nature and properties of the components and the critical parameters of the particular solvent. CO_2 is the most commonly used solvent because of its practical advantages such as being non-toxic, non-flammable and chemically inert in addition to its moderate critical parameters as illustrated by comparison to other solvents in Table 2.1. To improve its affinity for polar molecules, sc-CO_2 is sometimes modified with polar cosolvents as will be discussed in Paragraph. 2.3.[7]

There are polar solvents that can be selected to extract polar compounds, but as mentioned above, their use is limited by other practical considerations. The most polar substances exhibit some of the lowest critical densities listed. sc-CH_3OH can be a good solvent, but its high critical temperature and its liquid state at ambient temperature makes it less attractive. sc-NH_3 has high solvent strength, but it is chemically reactive and difficult to pump. Nitrous oxide and chlorodifluoromethane have also been used for SFE of natural products.[7,8] Even though nitrous oxide is polar and has a moderate critical temperature, its application is limited by the risk of explosion. The use of chlorodifluoromethane has been seized because of its ozone depletion effect in the upper atmosphere. Extraction with sc-H_2O might have environmental advantages over solvent extraction and higher extraction ability for polar compounds than sc-CO_2, but it has less convenient critical parameters and may also give rise to corrosion problems.[7]

2.3 Solvent properties of supercritical fluids

The solubility of a substance in a given supercritical fluid is an important consideration when planning an extraction process. By understanding the parameters that are of prime importance in controlling the solvent strength of a supercritical fluid, one can predict the feasibility of an extraction or the initial extraction conditions. Certain properties of gases, liquids and supercritical fluids are compared in Table 2.2. Supercritical fluids exhibit physicochemical properties between those of liquids and gases. They have relatively high (compared to gases) liquid-like densities, which give them solvent strengths closer to those of liquids.

Table 2.2 Comparison of physical properties of supercritical fluids, gases and liquids.

	Density (g/cm^3)	Viscosity ($g/cm \cdot s$)	Diffusion coefficient (cm^2/s)
Gases	$(0.6 - 2)\ 10^{-3}$	$(1\text{-}3)\ 10^{-5}$	0.1- 1.0
Supercritical fluids	0.2-0.9	$(1\text{-}3)\ 10^{-4}$	$(0.1\text{-}5)\ 10^{-4}$
Liquids	0.6-1.6	$(0.2\text{-}3)\ 10^{-3}$	$(0.2\text{-}3)\ 10^{-5}$

The solvent strength of a supercritical fluid is a function of its density as it depends on both pressure and temperature.[9] Knowing how density changes with pressure and temperature, one can make a decisive choice of conditions for optimum solvent strength. It should be noted that controlling solubility during extraction cannot be based solely on density of the supercritical fluid. There are other factors, like the chemical nature of the solute, which governs the interaction with the supercritical fluid.

As Figure 2.2 depicts, the solvent strength of a supercritical fluid decreases with increasing temperature at low pressures but increases with temperature at high pressures. This occurs as density decreases with an increase in temperature at low pressures, whereas at high pressures, changes in temperature have less effect on density. There is a steady increase in density (and thus in solubility) with pressure at a constant temperature, but the increase is quite sharp near the critical point as illustrated in Figure 2.3

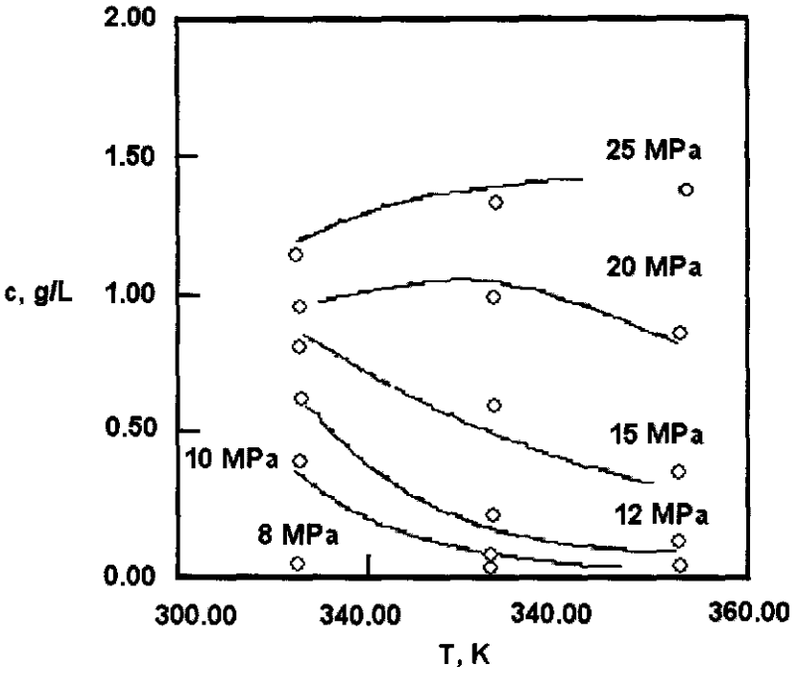

Figure 2.2 Solubility of tripalmitin in sc-CO_2 [10]

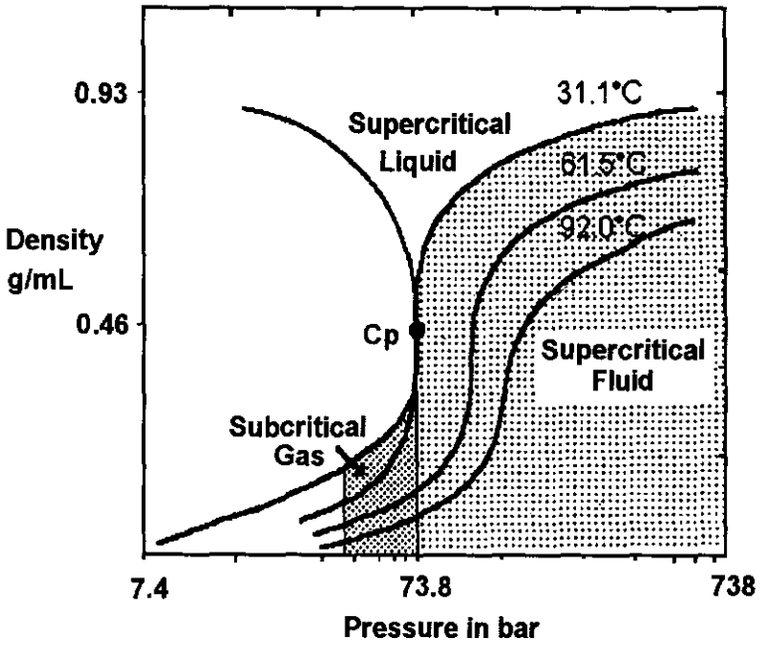

Figure 2.3 Density behaviour of CO_2

In addition to variable solvent strength, supercritical fluids possess gas-like diffusivity and viscosity.[11] These provide a means of fast and efficient extraction owing to rapid and complete penetration of the matrix and efficient transport of the extracted material. As illustrated by Figure 2.4 and Figure 2.5, both diffusivity and viscosity of supercritical fluids (like density) depend on temperature and pressure.

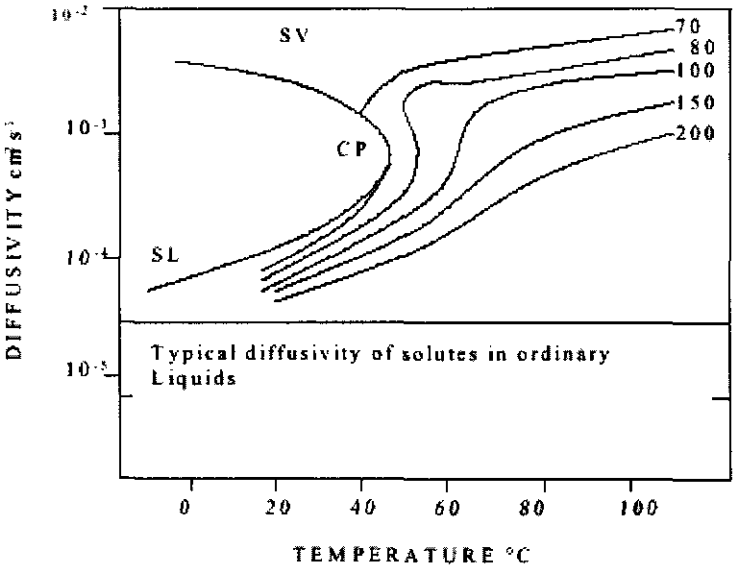

Figure 2.4 Variation of diffusivity of CO_2 with temperature at different pressures (CP = critical point, SV = saturated vapour, SL = saturated liquid) [6]

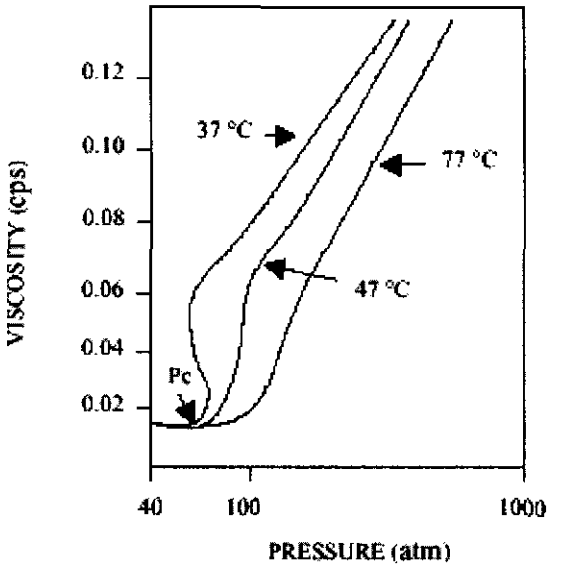

Figure 2.5 Variation of viscosity of CO_2 with pressure at different temperatures [6]

2.4 Basic principles of supercritical extraction

As mentioned earlier, SFE is rapidly gaining acceptance as a promising method of extraction of natural products. It is necessary to have a general understanding of the technology if imaginative application possibilities are explored. The remaining part of this chapter will therefore cover information on the fundamentals of this technology.

SFE is essentially the use of gases under supercritical conditions as solvents to extract desired substances from a given matrix. The compressed gas is continuously contacted with the sample to displace, desorp or dissolve the extractable components. This is followed by the expansion of the supercritical solution to separate the extracted components from the supercritical fluid.

The matrix is subjected to the supercritical fluid in a static or dynamic mode or a combination of both.

In the static mode the sample is soaked in the supercritical fluid and the system is allowed to reach equilibrium under the prevailing conditions. The fluid is transported out of the reactor by a short dynamic run and then depressurised to release the extract. This mode is mostly useful when the analyte cannot be readily removed from the matrix, especially from dense matrices.[12] It can be a slow process, as it is limited by the volume of the matrix. A static extraction may not be exhaustive if insufficient fluid has been used.

The dynamic mode of extraction differs from the static mode in that the supercritical fluid is continuously pumped through the sample. This mode is effective when the analytes are readily soluble and the matrix easily penetrable. Saturation of the extracting fluid is avoided, and hence better recoveries are obtained. One disadvantage of this extraction mode is the possibility of enhancing co-extraction of matrix components. The use of more supercritical fluid results in the removal of marginally extractable components.

In a combined mode a static extraction is performed for a certain period of time, followed by a dynamic extraction. This mode works best for the extraction of natural products.[13]

The selectivity for polar compounds can be enhanced by adding small quantities of a cosolvent (or modifier) to the fluid. Addition of large amounts are avoided as this may considerably change the critical parameters of the mixture. The nature of the modifier

depends on the nature of the solute to be extracted. It can be added dynamically by a modifier pump, fed from a premixed modifier/CO_2 cylinder or added directly to the matrix. Although addition of a modifier makes it possible to use milder processing conditions and decrease extraction time, it may complicate the system thermodynamics.[14]

2.5 Mechanism of extraction from plant matrix

The removal of extractable material from a plant matrix involves two essential processes, viz. dissolution of components in the supercritical fluid and/or desorption of components by the supercritical fluid. These processes may encompass various steps depending on the initial distribution of the extractable substances within the plant material. The substances may be adsorbed on the outer surface, present on the surface of pores or evenly distributed within the plant cells. The basic steps for extraction of soluble compounds include the following:

i) The plant matrix is exposed to the supercritical fluid during an extraction run.

ii) The solvent is transported to the solid particles by convection.

iii) The extractable compounds are dissolved and/or desorbed as a result of a larger affinity for and the higher concentration of solvent molecules.

iv) The compounds are transported to the outer surface of the solid particles by diffusive forces.

v) The compounds are transported from the surface layer through convection into the bulk of the supercritical solvent and eventually removed with the solvent from the bulk of the solid material.

2.6 Essentials of SFE apparatus

The essential components of an SFE apparatus are illustrated schematically in Figure 2.6. The pump supplies a fluid at a selected pressure to the extraction vessel in a temperature-controlled zone. Both syringe and reciprocating type pumps can be used as solvent delivery systems. If a modifier is required, it can be introduced by an additional pump or by addition directly to the sample matrix. The sample to be extracted is held in an extraction vessel (between frits) manufactured from material that can withstand high

pressure. The restrictor maintains the pressure within the extraction vessel and controls the depressurisation of the fluid for the release of extracted material. It is usually heated to offset Joule-Thompson cooling/freezing and thus prevent deposition of extracted material within the restrictor. The extracted material, which is completely separated from the fluid by a change in the system temperature and/or pressure, is trapped in a collecting device. The extract is either collected in a vial containing a small amount of solvent or trapped onto a solid material. Solid phase trapping requires an additional step, viz desorption of the analyte from the adsorbent with a small amount of solvent, prior to remote or on-line analysis.

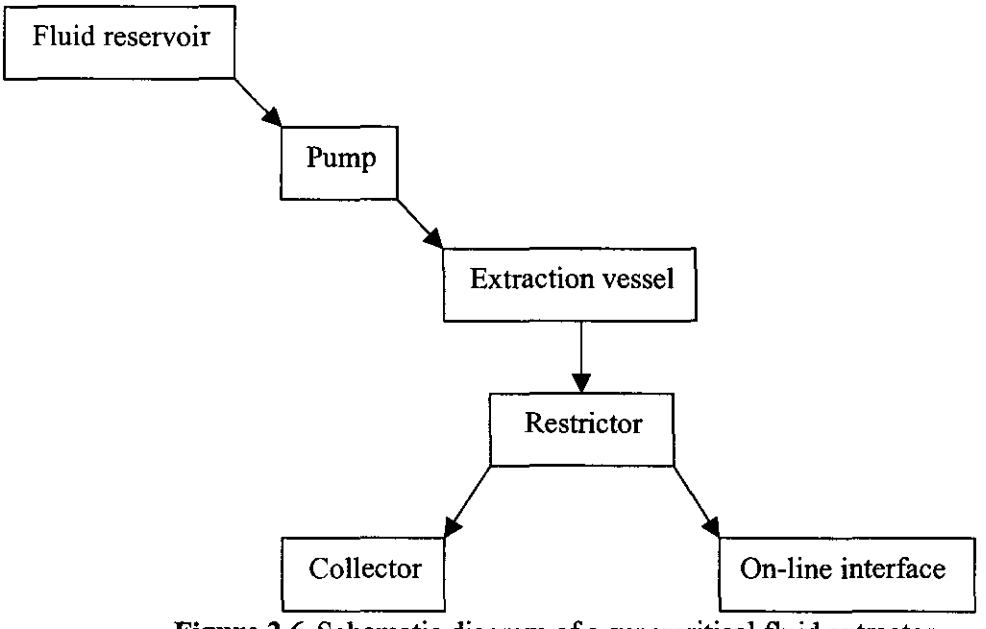

Figure 2.6 Schematic diagram of a supercritical fluid extractor

2.7 Why SFE for natural products?

SFE offers advantages for the extraction of a range of natural products. As discussed in Paragraph 2.3, the viscosity and diffusivity of supercritical fluids facilitate effective penetration of matrices and hence fast and efficient extraction. Supercritical fluids also offer selectivity through variable solvent strength by controlling pressure and/or temperature. A slight change in pressure/temperature can result in a significant change in solubility and thus in efficient extraction of components. In contrast to traditional methods, SFE cuts extraction time by reducing the number of preparation steps that are in most cases labour intensive and a major source of error in the laboratory.[14] These

preparation steps often involve organic solvents that lead to high solvent disposal cost. The use of supercritical fluids, which are gaseous at room temperature, affords total removal of solvent from the extract, an important consideration when products are to be used for human consumption. The automation of extraction using supercritical fluids contributes to the quality of the extract and acceleration of the extraction process.

Since extractions are carried out at low temperatures, SFE is especially suitable for thermolabile compounds. With traditional methods there is a risk that compounds may be altered during extraction. Certain volatile oils contain constituents that are slightly soluble in water and these may be lost to the distillation water. The comparatively low critical temperature and moderate critical pressure of CO_2 makes it an obvious choice for extraction of natural products since these may contain thermally labile material and thus restrict extraction conditions to ambient values.

SFE is not a panacea, however. It has its merits and disadvantages. Even though supercritical fluids are considered to be "super solvents", their solvent strengths are generally low compared to those of liquids used in conventional extraction processes. Attempts to improve their solvating abilities (by selecting suitable conditions) may sacrifice selectivity.

sc-CO_2 has the disadvantage of having rather low solvent strength for some compounds present in natural products, particularly polar and long-chained compounds, but because of its large quadrupole moment, it shows some affinity for polar solutes and can be a good extraction medium for moderately polar species like esters, alcohols, aldehydes and polyaromatic hydrocarbons.

Supercritical technology requires high initial investment costs. The equipment required to achieve and maintain high pressures is expensive. SFE is thus restricted mainly to extracts impossible to obtain by traditional methods. Apart from expensive equipment, plant material needs to be dried prior to the extraction process, which is an additional cost factor with a risk of losing volatile compounds.

References Chapter 2

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CHAPTER 3
EXPERIMENTAL DETAILS

In this chapter the experimental details of the investigation are presented. It covers aspects such as materials, methodology, equipment, procedures and data processing. These are presented in the same sequence as performed during the execution of the investigation.

3.1 Sample preparation

The plant material was collected for drying and storage before reaching full bloom. Air-drying, the easiest and most suitable method for drying flowers, was employed. Oven-drying is another common method for removing moisture from a plant matrix, but the risk of losing volatile or thermally labile analytes can be high. The flower heads were spread on the floor and left to dry in a well-ventilated place. The drying area was covered to protect the plant material against adverse weather conditions. Low moisture content is an important consideration if sc-CO_2 extraction is considered. Water can interfere with the extraction of polar analytes and can adversely affect trapping after extraction. Its presence can lead to either ice formation in the restrictor or the presence of water in the collection vessel. The presence of moisture in the matrix can also lead to problems if the analyte has more affinity for water than for carbon dioxide.

Figure 3.1 Commercial blender for grinding of plant material

The rate of extraction can be expedited by increasing the surface area or porosity of the matrix. Grinding the sample material is an obvious solution. After drying, the plant material was ground in a commercial blender shown in Figure 3.1 prior to extraction.

3.2 Supercritical fluid extractor

An ISCO SFX^{TM} 220 supercritical fluid extractor (Figure 3.2) was used for the extraction of plant material. It features a syringe pump and controller to set up and monitor the extraction conditions within a two-compartment extraction chamber and at twin capillary restrictors. The operation of the instrument is depicted by a flow diagram in Figure 3.3.

Figure 3.2 ISCO SFX^{TM}220 supercritical fluid extractor

CO_2 from the supply cylinder (C) is fed into the syringe pump (P) and pressurised to the desired level. Before entering the pump, the gas is passed through a cleanup column (cl) to remove any impurities. The pressurised gas moves via a T-inlet and supply valve (vs) to either or both extraction chambers. A check valve (vc1 or vc2) prevents the possibility of any crossover of fluid from one chamber to the other or back into the solvent delivery system. The check valves are linked to rupture discs (rd) which burst in the event of exceeding the rated pressure.

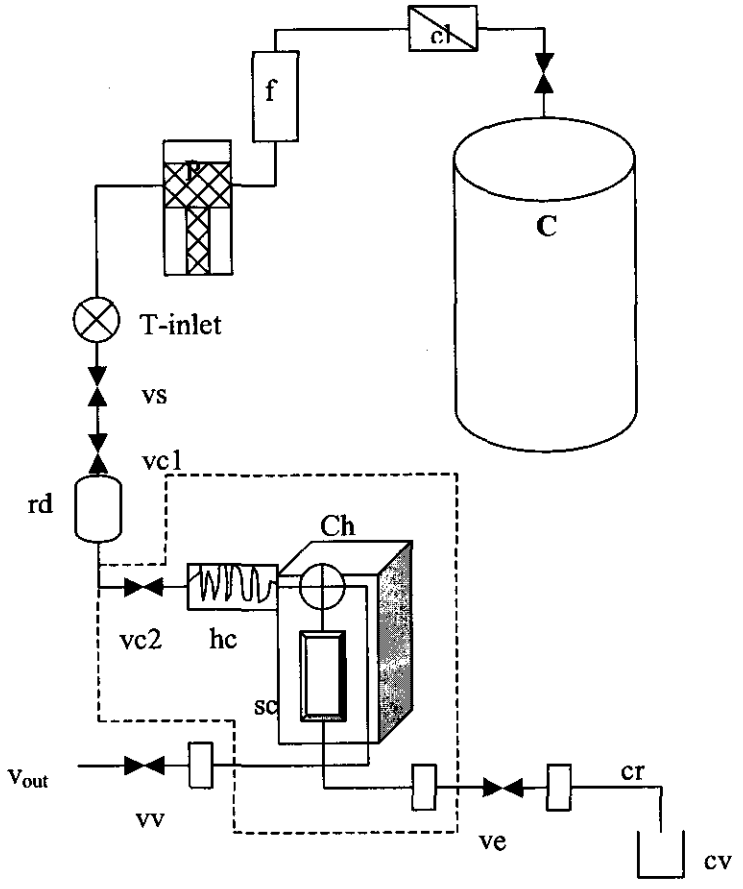

Figure 3.3 Simplified flow diagram for ISCO SFX 220 supercritical fluid extractor

A heating coil (hc) brings the temperature of the fluid to the set extraction temperature before it enters the extraction chamber (Ch). The chamber is housed in an aluminum block which acts as an effective heat transfer medium between two cartridge type heating elements. The fluid is splitted into the venting path, controlled by the vent valve (vv), and the extraction path, which is directed through the sample cartridge (sc) and is controlled by the extract valve (ve). The venting path allows rapid depressurisation of the extraction chamber. To prevent premature precipitation of the extract from the carrier solvent, the vent valve, extract valve and associated connecting tubing are kept at the operating temperature. A capillary restrictor (cr) linked to the extract valve maintains pressure within the extraction cell and controls depressurisation of the fluid for the release of extracted material into the collection vial (cv). When extraction is complete, the extract and supply valves are closed and the vent valve is opened.

3.3 Experimental design

The runs needed to be performed to establish optimum conditions in terms of yield of extract were determined by statistical design. Since the yield of extract depends on various factors, the relative importance of which is unknown, the influence of one independent factor can be monitored at a time, i.e. a monovariant experimental design can be implemented[1]. This can be time-consuming, and there is a risk of misinterpreting the results if important interactions between factors are present. A monovariant design can thus lead to an incomplete understanding and a lack of predictability of the behaviour of a system. The development of a central composite experimental design can be a solution to find the optimum settings for a selection of significant variables[2]. It is important to select the most significant factors to reduce the number of variables and to keep the number of experiments to a manageable number.

The time dependence of the extraction process was studied first to establish the required extraction time for the acquisition of an optimum yield at typical extraction conditions. A low, fixed flow rate was selected to ensure proper penetration of the extraction matrix. A cosolvent was not employed as it was important to acquire a natural extract free from any solvent residues. The independent variables influencing the yield of extract were thus restricted to temperature and pressure. To determine the relative influence of these two variables on the yield and to optimise the yield with respect to each of these two variables simultaneously, a (statistical) central composite design was employed. A reliable experimental design is expected to comply with two requirements, viz. orthogonality and rotatability[3]. For the two columns of the design matrix in Table 3.1 to be orthogonal, the sum of the products of the elements in the two rows should be equal to zero.

Table 3.1 Orthogonal design matrix

	Independent Variable1	Independent Variable 2
Run 1	1	1
Run 2	1	-1
Run 3	-1	1
Run 4	-1	-1

The second important requirement is that the design should be rotatable. This means that the design should yield the same amount of information in all directions of the fitted surface response.

The 2-by-2 orthogonal design in Table 3.1 allows estimation of the main interaction effects. Center points (runs 5-6 in Table 3.2) can be added to the matrix to allow estimation of the errors and to provide a check on linearity. If the average response at the center points does not agree with the mean of the factorial points, non-linearity is indicated. To estimate the curvature, star points (runs 7-10 in Table 3.2) are added to the design. The star points (α) are given by $2^{k/4}$, where k is the number of factors. For a 2-factor experimental design $\alpha = 1.414$. A central composite design for a 2-factor system obtained by addition of center and star points to a simple 2-by-2 orthogonal design still complies with orthogonality and rotatability.

Table 3.2. Central composite design

	Run	Independent Variable 1	Independent Variable 2
Factorial points	1	1	1
	2	1	-1
	3	-1	1
	4	-1	-1
Center points	5	0	0
	6	0	0
Star points	7	-1.414	0
	8	1.414	0
	9	0	-1.414
	10	0	1.414

The temperature and pressure values calculated according to such an experimental design allow extraction runs to be performed and the yield of extract to be related to both these factors by virtue of a surface response graph as shown in Figure 3.4.

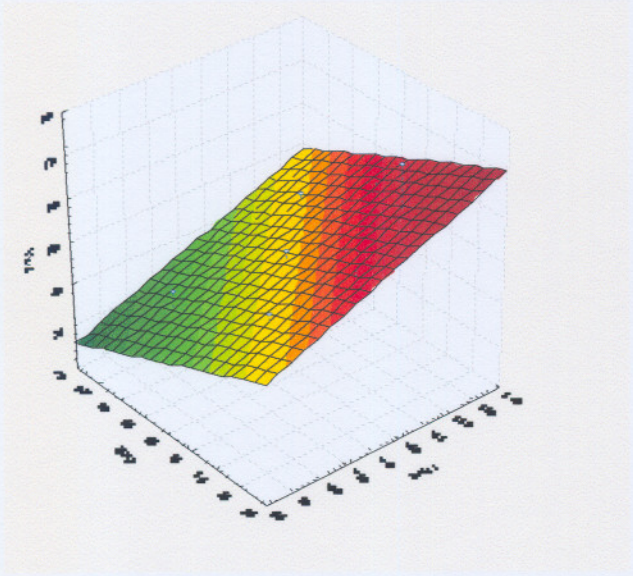

Figure 3.4a Response surface 1st-order model

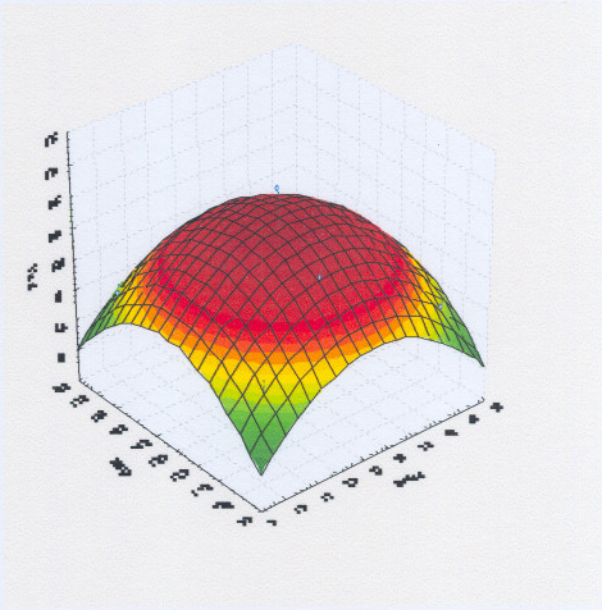

Figure 3.4b Response surface 2nd-order model

If the data constitutes a flat surface (Figure 3.4a), a first-order model applies. If a curvature occurs, the data fits a second-order model (Figure 3.4b). These surface response graphs can be used to predict the optimum value. If the predicted optimum does not fall in the region of experimentation, the shape of the surface can be analysed to indicate the direction in which further experiments should be performed. Once the optimum is located, the curvature in the neighbourhood of that point can be explored. Knowledge about the shape of the near-optimum response surface can be advantageous[4].

The certainty of a model fit needs to be validated so that predictions are sufficiently and verifiably accurate for the intended use. To determine the appropriateness of the model, it is essential to analyse the residuals (differences between actual and predicted values) of the regression model. If the model is reasonable, the residuals should average to zero, be normally distributed and occur randomly with respect to the values of the independent variables.

3.4 Extraction procedure

A set of experiments according to the statistical design discussed in Paragraph 3.3 was performed. Ground plant material of a mass sufficient to fill 90% of the 10 mL sample cartridge (Figure 3.5) was used. The space left in the cartridge was to accommodate the swelling of the matrix when the supercritical fluid was introduced. The tightly closed sample cartridge was put into the extraction chamber of the supercritical fluid extractor (Figure 3.2) and the extraction and collection methods entered via the keyboard of the controller unit.

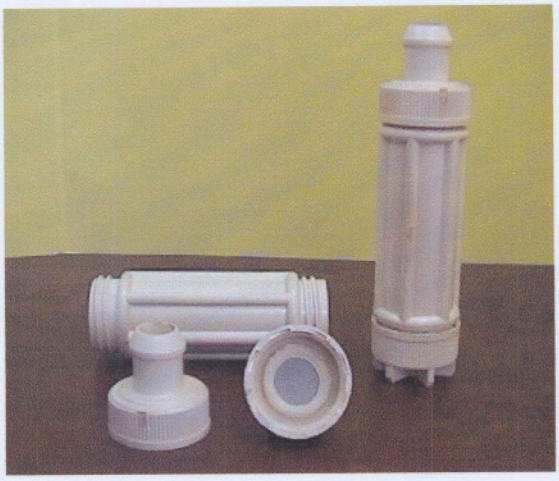

Figure 3.5 Sample cartridges

The extraction started once the set conditions were reached. The extractor automatically switched to static mode, keeping the extract and vent valves closed. As soon as the static extraction time expired, the instrument switched to dynamic mode by opening the extract valve and allowing the extract to be collected. By depressurisation of the fluid in the capillary restrictor the extracted components were released and deposited into the collection vial.

3.5 Methods of analysis

Gas chromatography is a suitable technique for the analysis of botanical extracts, providing qualitative and quantitative information on individual compounds present in a sample. The technique is limited to volatile samples which are sufficiently stable to pass through the column without thermal decomposition.

3.5.1 GC-FID

GC analysis was performed on a Hewlett Packard HP 6890 gas chromatograph equipped with a flame ionisation detector (FID) and fitted with a HP-5 fused silica capillary column (30 m x 0.32 mm x 0.25 μm film thickness). The GC was operated at conditions listed in Table 3.3.

Table 3.3 Protocol for GC-FID analysis of extract

Concentration	1% Solution
Solvent	Hexane
Volume injected	1 μL
Carrier gas flow	2.5 mL/min
Make up gas	N_2
Make up gas flow	11.7 mL/min
Oven temperature program	50 °C for 1 min, to 200 °C at 5 °C/min, hold for 5 min.
Detector temperature	300 °C
Hydrogen flow	33.8 mL/min
Synthetic air flow	337.5 mL/min
Injection mode	Split
Injector temperature	220 °C

Compounds were identified by comparison of their Kovats indices (KI) with those of standard substances available in the literature[5]. To obtain the Kovats indices the extracted sample was injected with a mixture of n-alkanes serving as internal standard. The KI values were calculated using the equation

$$KI = 100 \left(\log t_x - \log t_z / \log t_{z+1} - \log t_z \right) + 100z$$

where

x = compound to be measured

z = n-alkane eluting just before x

z+1 = n-alkane eluting just after x

t = retention time of compound

3.5.2 GC-MS

A mass spectrometer remains a preferred detector for GC based analysis of complex mixtures. A combination of the two techniques was employed to identify the constituents of an extract. The mass spectra for individual components were used to identify the compounds by matching them against those in the NIST (National Institute of Standards and Technology) reference library. These were recorded on a Micromass Autospec TOF-spectrometer coupled directly to the HP 6890 gas chromatograph fitted with an HP-5 fused silica capillary column (30 m x 0.32 mm x 0.25 µm film thickness). The GC operation conditions were the same as in Table 3.3.

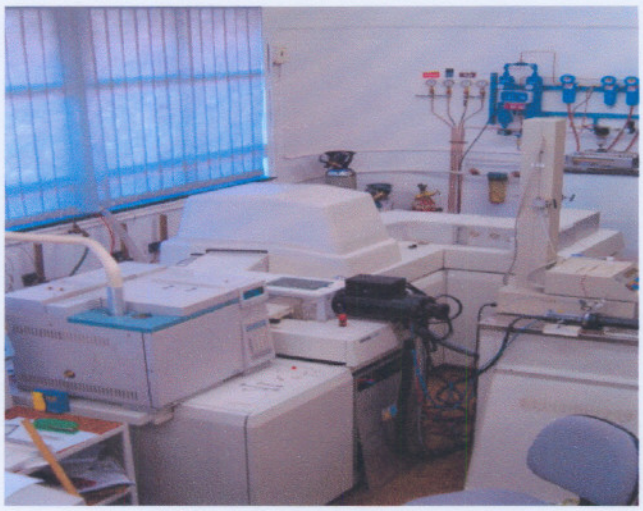

Figure 3.6 GC/MS system used for analysis of extract

3.5.3 GC-GC/TOF-MS

The limitation of GC-FID and GC-MS is that co-elution of components of the extract is likely to be observed even if conditions are carefully optimised. The number of components of the extract can be too large for complete separation on the basis of volatility alone. The resolution and detection sensitivity offered by two-dimensional GC were explored to identify compounds, which could not be identified by the other two techniques. GC-GC uses two separation mechanisms to separate complex sample mixtures. A non-polar capillary column is used first to separate samples on basis volatility (boiling point). The second column is shorter and separates selectively on the basis of polarity.

The extracts were analysed by a LECO Pegasus®4D two-dimensional gas chromatograph linked to a time-of-flight mass spectrometer as a detector. The conditions for data acquisition are listed in Table 3.4. The acquired data of each sample was processed with automated peak finding and spectral deconvolution software, followed by a NIST (National Institute of Standards and Technology) library search.

Table 3.4 Conditions for GC-GC/TOF-MS analysis of extract

Detector:	LECO Pegasus 4D Time-of-Flight Mass Spectrometer
Acquisition Rate:	150 spectra/sec
Stored Mass Range:	35 to 350 u
Transfer Line Temp:	240 °C
Source Temperature:	200 °C
Detector Voltage:	-1750 Volts
GC:	Hewlett Packard 6890N
Column 1:	VF-5MS, 30 m x 0.32 mm ID, 1 µm film thickness
Column 2:	DB-17, 2 m x 0.1 mm ID, 0.1 µm film thickness
Column 1 Oven:	50 °C for 1 min, to 150 °C at 10 °C/min, then to 290 °C at 5 °C/min, hold for 1 min.
Column 2 Oven	55 °C for 1 min, to 155 °C at 10 °C/min, then to 295 °C at 5 °C/min, hold for 1 min.
Second Dimension Separation Time:	3 s
Inlet	Split at 200 °C; split ratio 20:1
Injection:	0.2 µL
Carrier Gas:	Helium, 1.0 mL/min constant flow

3.6 Activation parameters

The activation energy of a process (e.g. extraction) can be described by the Arrhenius equation[6]. It expresses the temperature dependency of the rate constant of a reaction as

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

where E_a is the activation energy, R = 8.31 J K^{-1} mol^{-1} the gas constant, T the temperature in kelvin and A the pre-exponential factor. The equation can be rewritten in the form

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

which makes it possible to determine the activation energy from the gradient ($-E_a/R$) of a straight line obtained when ln k is plotted against 1/T. The rate constant k can be substituted by % yield of extract without changing the magnitude of the slope.

Likewise, from the empirical equation[7]

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

the volume of activation $\Delta V^{\neq}$ may be calculated from the slope ($-\Delta V^{\neq}/RT$) of a plot of ln k against p, where p is the pressure of the extracting fluid and k the rate constant which can be replaced by % yield of extract without changing the magnitude of the slope.

References Chapter 3

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CHAPTER 4

DATA PROCESSING AND INTERPRETATION

The experimental results obtained in this study are presented, processed, discussed and interpreted in this chapter. It covers aspects such as optimisation of extraction conditions, description of process characteristics, calculation of activation parameters, analysis of extracts and comparison of extract composition for different extraction methods.

4.1 Optimisation of extraction time

It was important to first determine the duration of an extraction run needed to obtain the maximum amount of extract at a typical set of extraction conditions. This optimum extraction time was established by performing runs of different duration at a fixed temperature (40 ^{0}C), pressure (100 bar) and flow rate (2 mL/min). The resulting curve of yield versus time is shown in Figure 4.1, with yield expressed as a percentage (m/m) of the total amount of extract obtained at infinite time.

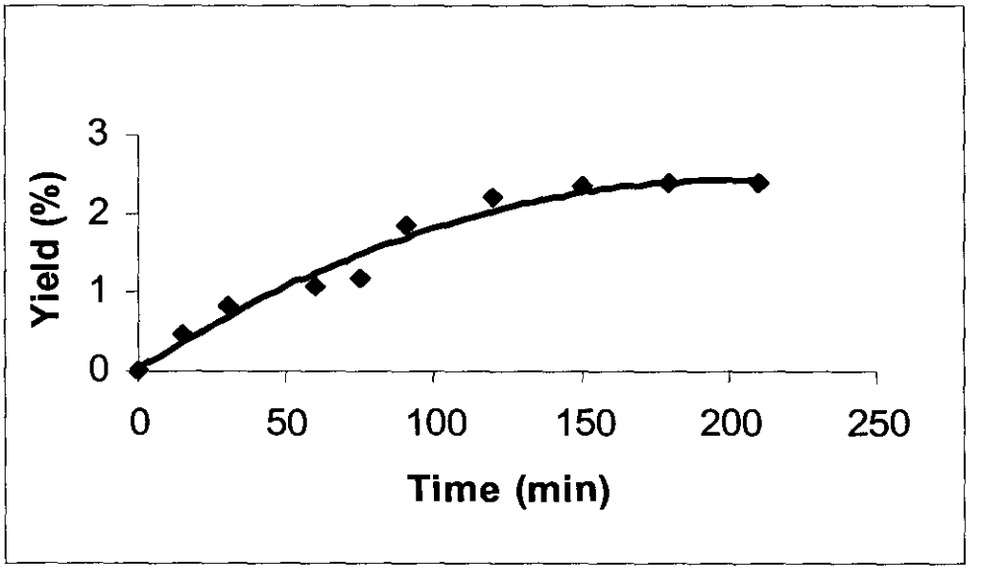

Figure 4.1 Yield versus time graph

The graph suggested that an extraction time of 150 min (dynamic mode) was sufficient to remove practically all extractable material from the sample. The extension of extraction time

did not result in any significant increase in yield. It was therefore decided that all experimental runs performed according to the statistical design discussed earlier (Chapter 3) should be of this fixed duration.

4.2 Statistical surface response analysis

One of the objectives of this study was to optimise the extraction conditions. The strategy followed to achieve this was to assign values to two variables, viz. temperature and pressure, according to a statistical design and within the limits of the available equipment, and to perform these suggested runs according to the procedures outlined earlier (Paragraph 3.4). The assigned values and corresponding yields are listed in Table 4.1 along with the densities resulting from the temperature/pressure combinations.

Table 4.1 Results of experimental design runs

Run	Temperature (°C)	Pressure (atm)	Density (g/mL)	Yield (%)
1	50	79	0.216	0.73
2	36	150	0.817	2.97
3	40	200	0.847	3.41
4	40	100	0.605	2.75
5	50	221	0.811	2.79
6	60	100	0.292	0.93
7	60	200	0.729	2.91
8	50	150	0.707	2.86
9	50	150	0.707	2.80
10	64	150	0.562	1.34

The surface response plot in Figure 4.2 illustrates that the yield of extract depends on both temperature and pressure but that pressure is the more decisive variable. At low pressures, the extraction yield decreases with an increase in temperature, whereas at high pressures the yield varies only slightly with temperature. These observations can be explained as follows: At low pressures the density is low and quite sensitive to changes in temperature. An increase in

temperature causes a decrease in density and therefore a decrease in the solvent strength as the fluid becomes more gas-like. The result is a decrease in yield. At high pressure the fluid attains more liquid-like densities, which result in increasing solvent strengths and higher yields, and which are less sensitive to variations in temperature as the almost constant yield over the entire temperature range at high pressures indeed shows.

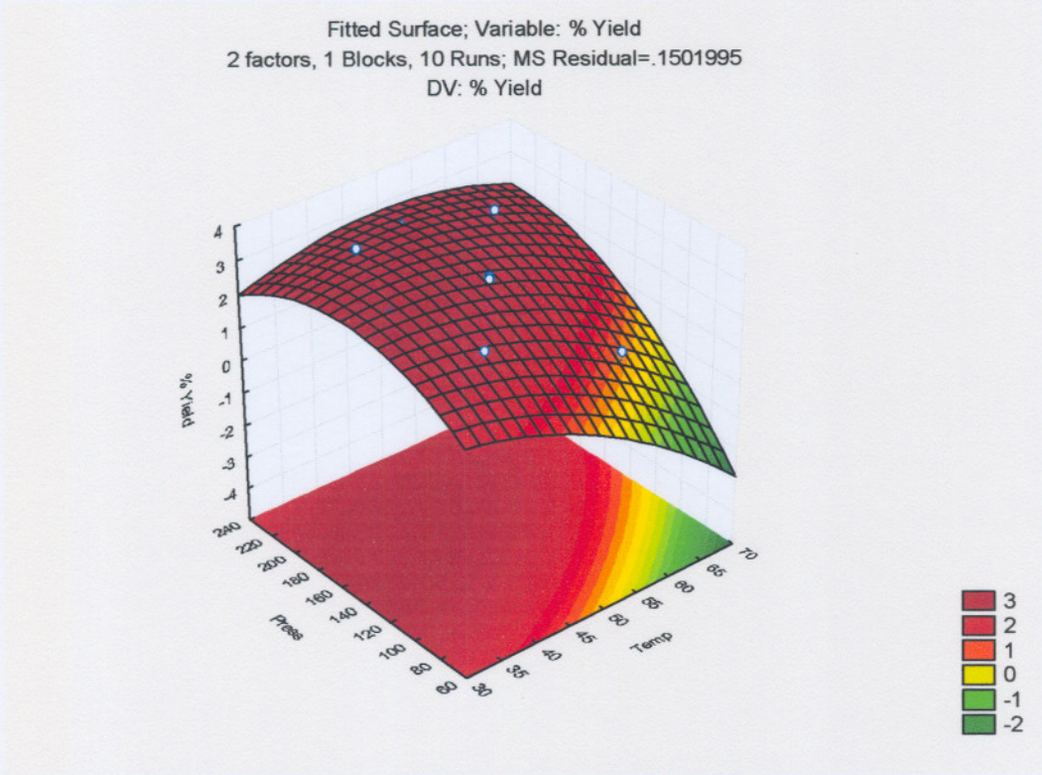

Figure 4.2 Effect of pressure and temperature on yield of sc-CO_2 extract

The statistical analysis underlying the surface plot in Figure 4.2 allowed determination of the experimental conditions at which a maximum yield of extract was obtained. These conditions turned out to be 39 °C and 171 atm. The corresponding maximum yield was 3 %, which means that from 1 g of plant material 30 mg of extract could be derived. The contour plot in Figure 4.3 illustrates the rotatability of the design, and close inspection of the plot enabled the optimum conditions and yield mentioned above to be read off instantly.

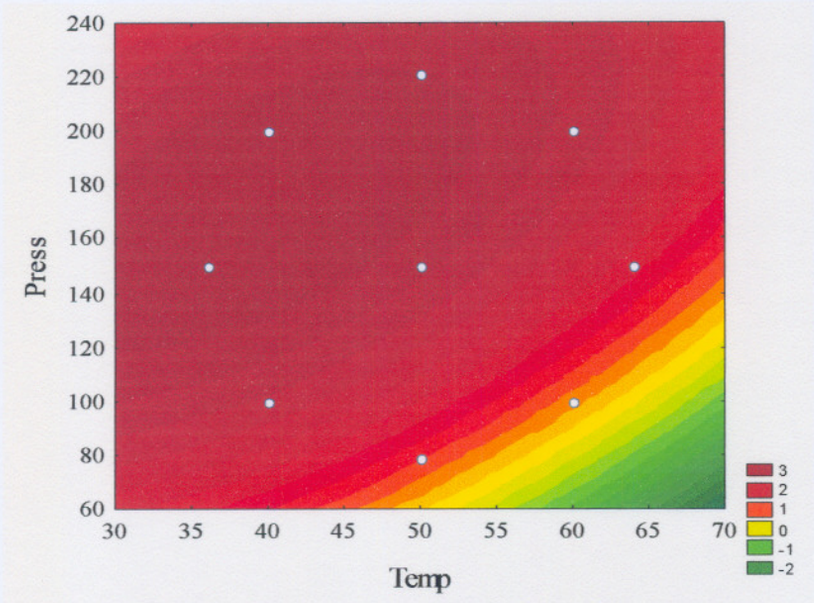

Figure 4.3 Contour plot showing rotatability of statistical design

Table 4.2 Yield at additional temperature/pressure or density values

Run	Temperature (°C)	Pressure (bar)	Density (g/ml)	% Yield
11	33	80	0.425	0.583
12	33	100	0.751	1.766
13	33	135	0.820	2.168
14	33	200	0.885	2.64
15	40	80	0.294	0.425
16	40	100	0.605	1.059
17	40	135	0.761	1.88
18	40	200	0.847	2.552
19	45	80	0.245	0.311
20	45	100	0.477	0.633
21	45	135	0.711	0.849
22	45	200	0.822	2.446

The 12 extraction runs listed in Table 4.2 were performed in addition to the 10 extraction runs in Table 4.1 based on the statistical design in order to give a more complete picture of the effect of density on the experimental yield in Figure 4.4. A principal feature of the plotted relationship is the almost exponential increase in yield as the density approaches liquid-like values ($0.7 < \rho < 0.9$ g/mL) at which sc-CO_2 acts as a solvent capable of dissolving material from the plant matrix. This had also been observed for the sc-CO_2 extraction of other plant material, the best known case being the extraction of caffeine from green coffee beans[1]. At gas-like densities ($0.2 < \rho < 0.7$ g/mL) the corresponding yield is not zero but has a finite value, indicating that not all material is extracted from the plant matrix by dissolution in sc-CO_2 but that some material (probably volatile substances) is removed by another mechanism (physical desorption, mechanical displacement, bulk diffusion) from the sample. The cuticular waxes are located on the surface of the plant material and can probably be extracted by simple leaching at all extraction conditions, whereas the essential oil components are located in the internal part of the material and may only be extracted if internal mass-transfer resistance is overcome.

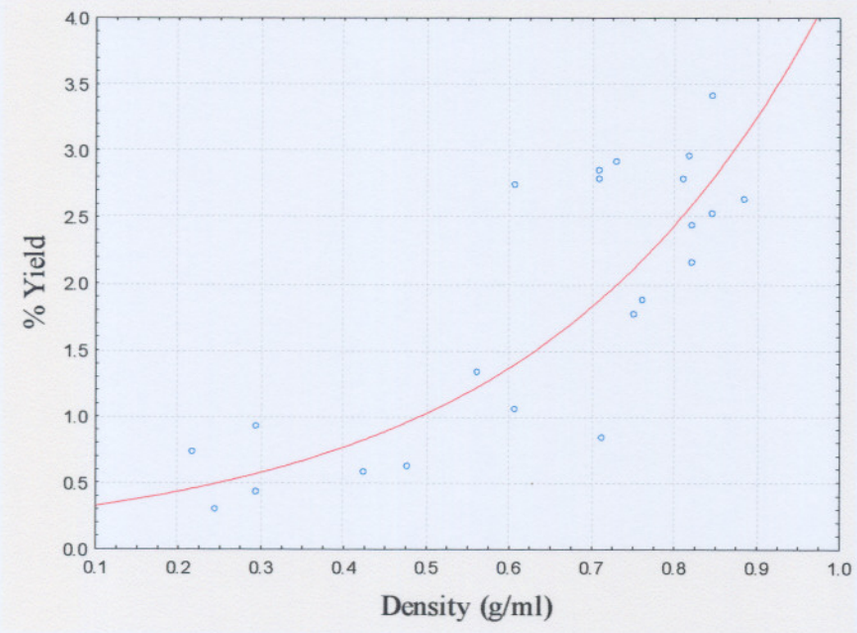

Figure 4.4 Dependence of yield of extract on solvent density

The scatter in the data presented in Figure 4.4 can be attributed to several factors. When ambient conditions were restored at the end of an extraction run, part of the extracted material (especially volatile components) was either released with CO_2 into the atmosphere or swept out of the collecting vessel by the prevailing flow of CO_2. The yield of extract was also affected by the deposition of waxy material in the flow line of the extractor and by blockage of the capillary restrictor as a result of Joule-Thompson cooling/freezing caused by the rapidly expanding CO_2. It was realised that efforts should be made to improve reproducibility if the benefits of sc-CO_2 for plant extraction were not to be compromised. Such efforts could include bubbling of the fluid through a trapping solvent at a low flow rate or controlling the temperature of the restrictor to cater for both volatile and viscous materials.

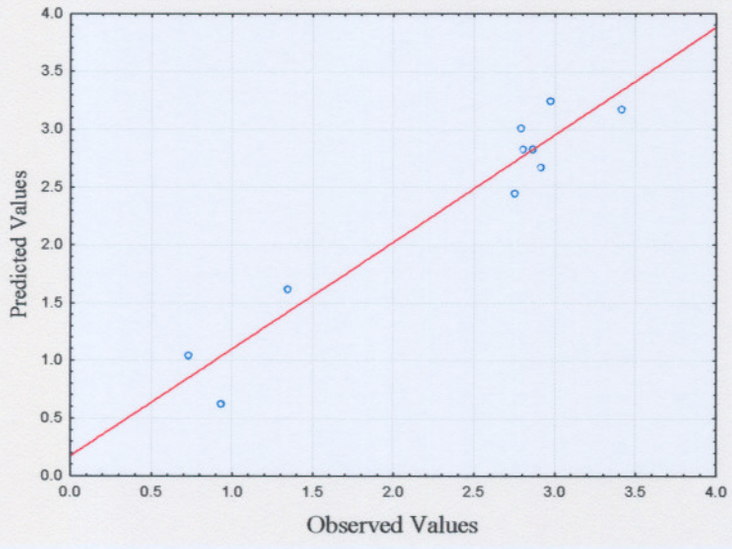

Figure 4.5 Accuracy of the model

The reliability of the model employed to calculate the surface response graph in Figure 4.2 was proven by the satisfactory agreement between predicted and measured values shown in Figure 4.5. The response surface graph thus describes the relationship of yield with temperature and pressure within the experimental range well.

4.3 Activation parameters

The energy of activation E_a for extraction over the density range in Figure 4.4 ($0.2 < \rho < 0.9$ g/mL) was determined by plotting ln (yield) vs 1/T according to the Arrhenius equation cited in physical chemistry textbooks.[2] The sign and magnitude of the activation energy E_a is an indicator of the mechanism of extraction, but the available data was insufficient to estimate separate values for the two different mechanisms operating in the two different density ranges shown in Figure 4.4. The value $E_a \sim -24$ kJ/mol estimated over the entire density range is numerically an average of a higher value expected for a mechanism of chemical nature (viz. dissolution) and a lower value anticipated for a mechanism of physical nature (viz. desorption). For a chemical event (dissolution with collapse of structure), values of $E_a \sim 50$ kJ/mol or more are expected. A value of $E_a \sim 10$ kJ/mol signifies a diffusion controlled process (desorption limited by film and pore diffusion). The negative sign of the estimated energy of activation reflects the dominance of chemical dissolution by sc-CO_2 as mechanism of extraction since this will be adversely affected when an increase in temperature lowers the density (solvent strength) of the fluid and thus the yield of extract.

The empirical equation[3] $\ln k = -(\Delta V^{\#}/RT)p + \text{constant}$ could be used to determine the volume of activation $\Delta V^{\#}$ by plotting ln (yield) versus p. The volume of activation is also a mechanistic indicator as it relates to changes in volume in the transition state, and its sign (positive or negative) specifically indicates whether bonds are ruptured or formed and/or solvational changes occur during the extraction process. The limited data did not allow calculation of separate values for the two density ranges in Figure 4.4, but the average value $\Delta V^{\#} \sim -250$ mL/mol calculated over the entire density range is consistent with the expected significant volume collapses associated with the desorption and dissolution of plant material by and its solvation within the highly compressed sc-CO_2.

4.4 Extract analysis

The sc-CO_2 derived extracts were examined sensorially and investigated analytically. Analysis was done by capillary gas chromatography coupled to different detectors to take advantage of the separation and identification capability of these techniques.

4.4.1 Extract description

A sc-CO_2 derived extract of Roman chamomile has the characteristic smell of the flower. It is a mixture of volatile oil and other substances trapped within a waxy material. Its viscosity is different from the volatile oil obtained by steam distillation of the same batch of plant material. It was impossible to obtain a wax-free extract under any of the extraction conditions. The yellow colour of the sc-CO_2 extract differs from that of a steam distilled extract, which varies from pale blue (grey) to clear blue[4-6]. The colour formation occurs by heat catalysed cyclisation of precursors in the plant to an 8-membered aromatic ring containing substance chemazulene. It does not necessarily influence the sensory characteristics of the extract, but it does affect its visual characteristics. On prolonged standing and exposure to air, the sc-CO_2 extract loses its colour due to loss of volatile components and leaves behind white cuticular waxes.

4.4.2 GC-FID/GC-MS

A few components of a typical sc-CO_2 extract were identified using the chromatogram in Figure 4.6. The major peaks in the chromatogram were identified as 2-methyl-2-butenoic acid (RT = 7:12 min) and 5-ethyl-(5H)-furan-2-one (RT = 7:38 min). These substances had been previously reported as constituents of Roman chamomile[7]. The remaining peaks were mainly those of esters, and these had also been identified. It was impossible, however, to identify some of the minor components since co-elution reduced the spectrum match factor during the automated target compound identification.

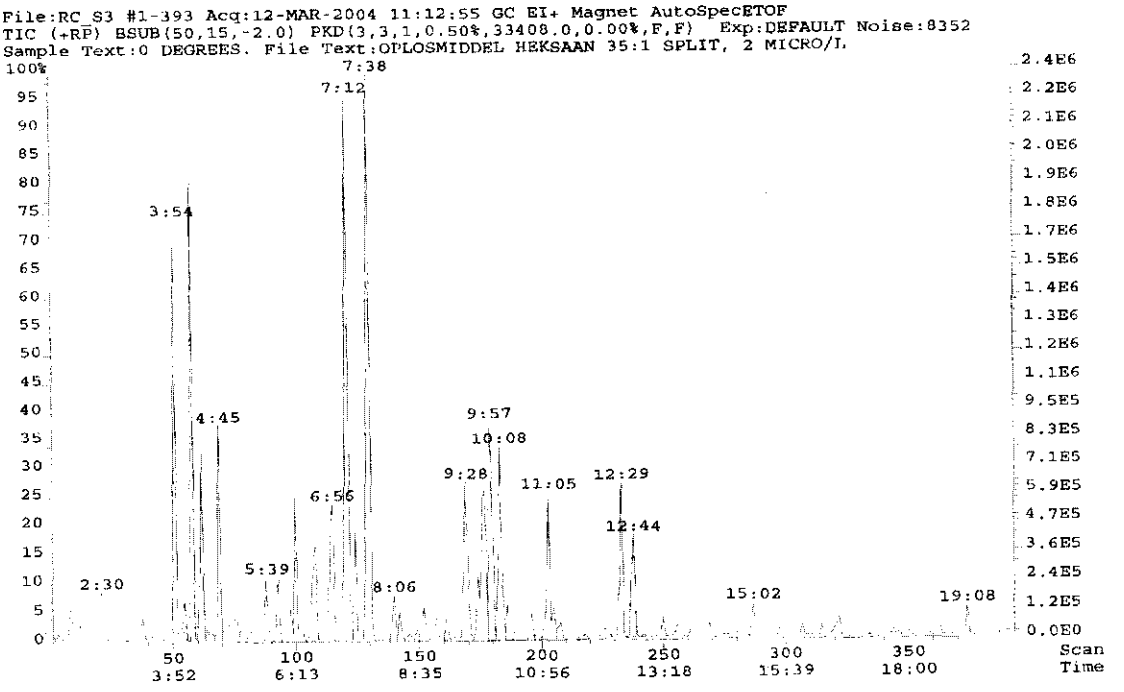

Figure 4.6 GC chromatogram of sc-CO_2 extract of Roman chamomile.

Table 4.3 lists a number of compounds in the sc-CO_2 extract identified with GC/FID and GC-MS that were also found in extracts of Roman chamomile obtained by other methods[7-9]. Many compounds could not be identified by virtue of their Kovats indices (KI value) measured in this study as no published indices for such compounds could be found in the literature. Propyl tiglate was the only compound identified by its KI value. Its experimental KI value agrees fairly well with the published KI value.[10]

Table 4.3 GC/MS and GC/FID analysis results

GC-MS Data		GC Analysis of Sample		
Published Retention Index	GC-MS Compounds	Qualitative Match (%)	Retention Time (min)	Experimental Retention Index
	Propanoic acid, 2-methyl, 2methyl-propyl ester	91.6	3.9	915
	2-bromo-2-methyl Butane	56.7	4.23	932
	Butyl crotonate	78.9	4.43	942
	Caratan	80.6	4.75	957
	Propanoic acid, 2-methyl, 2-methyl butyl ester	62	6.22	1009
	Amyl Methacrylate	48.8	6.93	1026
1038	Propyl tiglate	73.3	7.20	1032
	5-ethyl-(5H)-furan-2-one	82	7.63	1134
	2-Butenoic acid, 2-methyl	44.7	9.85	1297
	2-Butenoic acid, 3-methyl	56.2	9.95	1305
	Bicyclo[3.1.1] hepta-3-one	56	10.13	1319

Attempts were made to identify more compounds by using capillary gas chromatography linked to fourier-transform infrared spectroscopy (CGC-FTIR). This was, however, not successful because of the poor sensitivity and resolution of the technique. CGC-FTIR had been used previously to identify components in Roman chamomile extracts[8].

4.4.3 GC-GC/TOF-MS

In view of the shortcomings of GC/FID and GC/MS analysis, GC-GC/TOF-MS was explored. It is a fairly new technique suitable for volatile oil analysis.

The results of GC-GC/TOF-MS analysis presented in Table 4.4 showed that sc-CO_2 extracts of Roman chamomile contains a large variety of compounds. A total of 462 compounds were identified as opposed to a few identified by GC/FID and GC/MS. The extract contained a number of cuticular waxes including dodecane, hexadecane, pentadecane, heptadecane, hexadecene, tetracosane and nonadecane. Figure 4.7 shows the total ion chromatogram of the extract presented as a surface plot.

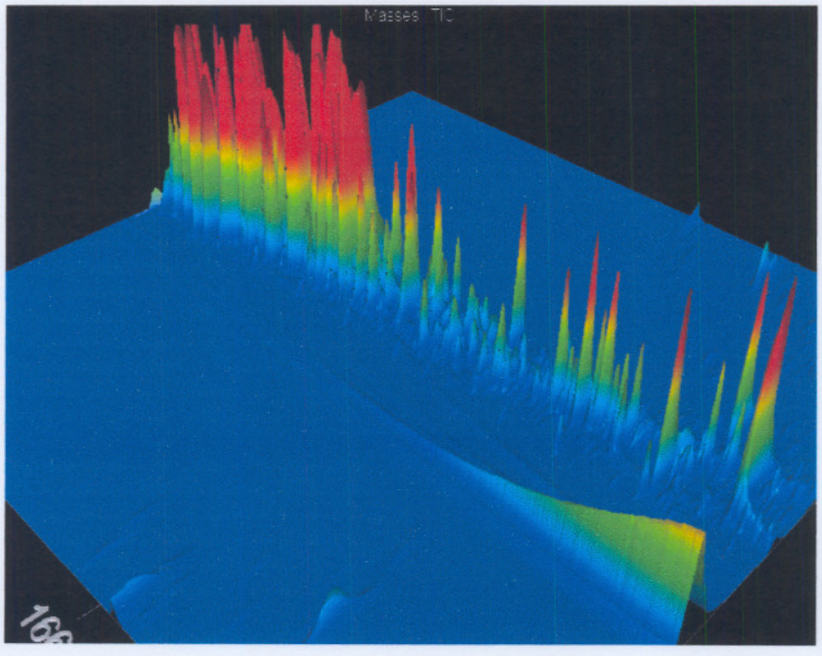

Figure 4.7 Total ion chromatogram of Roman chamomile extract

Table 4.4 lists the compounds corresponding to the first library hit from the 10 best library matches for each peak in the chromatogram. The table also reflects the quality of the spectral match with the database spectrum (expressed as a number up to 1000) and the abundance (area %) of the identified compounds in the extract. A few peaks were associated with more than one compound as a result of a similarity in structure and therefore similar mass spectra.

Such cases were eliminated by editing the list against previous studies on the same plant. This was not easy though, as the possibility of a wrong assignment was highly probable due to the inability of the technique to differentiate between isomers.

A high resolution contour plot of the total ion chromatogram is presented in Figure 4.8. The most intense peaks are shown in red and the positions of very low level peaks are indicated by black dots. The x-axis shows the separation on the VF-5 MS column, where separation occurs on the basis of boiling point. The y-axis shows the separation on the DB-17 column, which functions on the basis of polarity. By projecting the retention times of the second column, one realises that using only one column would have resulted in co-elution. The use of the second column allowed the analytes to separate.

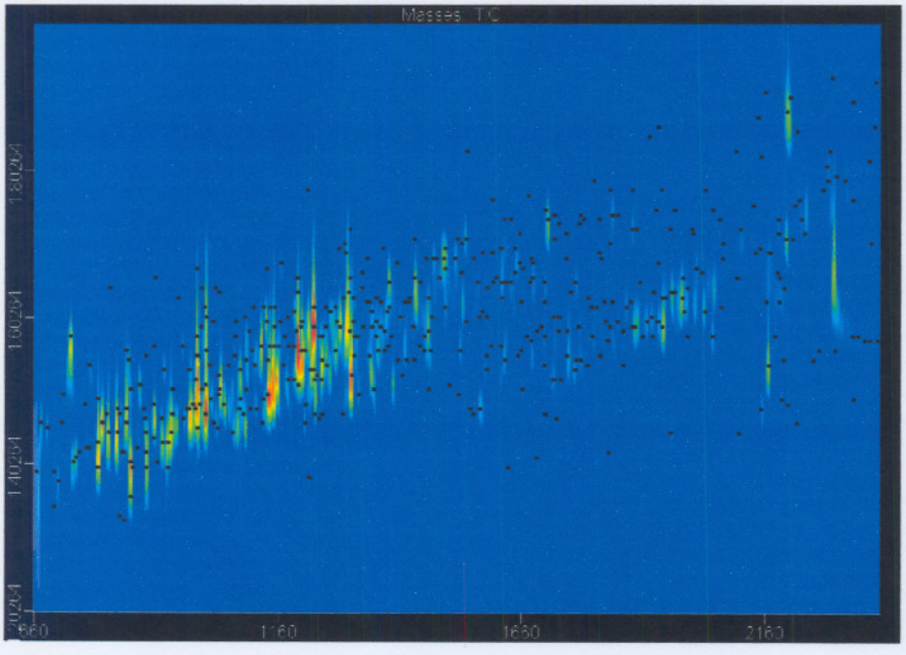

Figure 4.8 Contour plot of total ion chromatogram of Roman chamomile extract

It is difficult to compare the composition of the sc-CO_2 extract with the work of other researchers as the chemical make-up differs among chemotypes and among plants grown in different areas. However, sc-CO_2 extracts of Roman chamomile contain most analytes reported as major constituents of the plant. The compounds marked with * in Table 4.4 were also identified in a

Roman chamomile extract obtained by steam distillation[11]. There are compounds listed in the table that are not reported in the literature. A conclusion that these compounds cannot be extracted by steam distillation cannot be made unless an extract obtained by steam distillation is also subjected to GC-GC/TOF-MS analysis. Also, one has to take into consideration that the information in the literature on the composition of a steam distillation extract may be incomplete.

The sc-CO_2 extract is rich in esters, with more than 70 esters identified. These comprise more than 60% of the extract. A number of terpenes reported to form part of the extracts obtained by classical methods appear in the list of analytes in Table 4.4. 1,8-Cineole (eucalyptol), α-pinene, camphene and caryophyllene are some of the major terpenes identified in the sc-CO_2 extract. The flavonoid fraction of Roman chamomile previously extracted by traditional methods could not be identified in the sc-CO_2 extract. The solvent strength of sc-CO_2 is insufficient to extract polar compounds like flavonoids, as was shown previously for sc-CO_2 extracts of rooibos tea[12]. Addition of a modifier might have increased the extraction efficiency for flavonoids.

Table 4.4 Compounds in sc-CO_2 Roman chamomile extract identified by GC-GC/TOF-MS

Peak #	Name	Area %	R.T. (s)	Similarity
1	Butanoic acid, 3-methyl-	0.039	665.993 , 1.393	838
2	Butanoic acid, 2-methyl-	0.186	674.982 , 1.459	942
3	Butane, 2-bromo-2-methyl-	0.071	686.968 , 1.452	864
4	Butanoic acid, 2-methyl-, ethyl ester	0.018	701.95 , 1.346	950
5	1-Pentanol, 3-methyl-	0.031	701.95 , 1.393	916
6	Propanoic acid, 2-methyl-, propyl ester	0.113	710.939 , 1.379	915
7	Pentanoic acid	0.042	716.932 , 1.459	949
8	2,3-Butanedione	0.012	719.928 , 1.498	894
9	2-Butenoic acid, 2-methyl-, (E)-	1.122	734.91 , 1.577	950
10	1-Butanol, 2-methyl-, acetate	0.203	740.903 , 1.406	887
11	2-Propenoic acid, 2-methyl-, propyl ester	0.137	746.896 , 1.412	925
12	1-Butanol, 2-methylene-, acetate	0.008	752.888 , 1.419	837
13	Propanoic acid, 2-methyl-	0.004	764.874 , 1.485	677
14	2-Butenoic acid, 3-methyl-, ethyl ester	0.048	767.87 , 1.426	789
15	Cyclopropylmethanol acetate	0.007	773.863 , 1.426	859
16	2-Butenoic acid, 2-methyl-, (E)-	0.077	773.863 , 1.538	947
*17	Propanoic acid, 2-methyl-, 2-methylpropyl ester	0.592	791.842 , 1.399	908
18	Propanoic acid, 2-methyl-, 2-methylpropyl ester	2.069	791.842 , 1.432	911
19	Propanoic acid, 2-methyl-, 2-methylpropyl ester	0.048	791.842 , 1.492	888
20	2-Buten-1-ol, 3-methyl-, acetate	0.777	800.831 , 1.459	868
21	Butane, 2-bromo-2-methyl-	0.474	809.82 , 1.472	810
22	Butane, 2-bromo-2-methyl-	0.655	812.816 , 1.445	849
23	Butyrolactone	0.008	815.813 , 1.643	967
24	2-Propenoic acid, 2-methyl-, 2-methylpropyl ester	2.796	830.795 , 1.445	937
25	2-Propenoic acid, 2-methyl-, 2-methylpropyl ester	0.094	830.795 , 1.478	925

26	Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv.	0.009	836.788 , 1.333	910
27	Butane, 2-methyl-	0.091	836.788 , 1.472	846
28	Butanoic acid, 2-methyl-, propyl ester	0.045	839.784 , 1.419	888
29	Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-	0.004	845.777 , 1.327	918
30	Propanoic acid, 2-methyl-, butyl ester	0.057	845.777 , 1.419	890
31	Cyclopropanecarboxylic acid, 2-pentyl ester	0.604	848.773 , 1.459	691
32	Methacrylic anhydride	1.415	848.773 , 1.478	832
33	3-Methyl-3-nitrobut-1-ene	0.096	848.773 , 1.558	797
34	Hexanoic acid	0.112	854.766 , 1.544	944
*35	1S-à-Pinene	0.300	857.762 , 1.360	901
*36	1R-à-Pinene	0.489	857.762 , 1.406	944
37	Hexanoic acid	0.124	857.762 , 1.505	812
38	Pentane, 2-nitro-	0.008	863.755 , 1.399	864
39	Propanoic acid, pentyl ester	0.052	872.744 , 1.426	920
40	2-Furanmethanol, tetrahydro-, acetate	0.194	875.741 , 1.511	802
41	Ethanone, 1-cyclohexyl-	0.017	884.73 , 1.478	768
42	Cyclopropanecarboxylic acid, cyclohexyl ester	0.040	887.726 , 1.465	790
*43	Camphene	0.384	890.723 , 1.399	943
44	2,6-Octadien-1-ol, 2,7-dimethyl-	0.117	890.723 , 1.419	696
45	4-Methyl-2-pentyl acetate	0.139	890.723 , 1.445	767
46	Carbamic acid, phenyl ester	0.012	890.723 , 1.551	768
47	2-Butenoic acid, 2-methyl-	0.293	905.705 , 1.439	821
48	2-Butenoic acid, 2-methyl-	0.189	905.705 , 1.485	816
49	Benzaldehyde	0.020	905.705 , 1.564	820
*50	2(5H)-Furanone, 3-methyl-	0.027	908.701 , 1.657	881
51	Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	0.038	911.698 , 1.406	928
52	1-Penten-3-ol, 3-methyl-	1.073	920.687 , 1.465	790
*53	Butanoic acid, 2-methyl-, 2-methylpropyl ester	0.497	923.683 , 1.432	939
54	3-Hexanone, 2-methyl-	0.309	926.68 , 1.531	884
*55	á-Pinene	0.145	935.669 , 1.399	890
*56	Propanoic acid, 2-methyl-, 3-methylbutyl ester	0.164	935.669 , 1.432	909
*57	Propanoic acid, 2-methyl-, 2-methylbutyl ester	0.556	941.662 , 1.445	915
58	Butanal, 3,3-dimethyl-2-oxo-, hemihydrate	0.063	944.658 , 1.472	814
59	1-Hydroxy-3-methyl-2-butanone	0.011	944.658 , 1.505	733
60	1H-Pyrrole-2-carboxaldehyde	0.003	953.647 , 1.630	892
*61	Vinyl crotonate	0.107	962.636 , 1.564	730
62	2-Buten-1-ol, 3-methyl-, acetate	0.025	965.633 , 1.465	854
63	2-Butenoic acid, 2-methyl-, propyl ester, (E)-	0.052	971.626 , 1.478	778
64	Cyclopropanecarboxylic acid,3-methylbutyl ester	1.485	977.618 , 1.478	862
65	2-Propenoic acid, 2-methyl-, 1-methylbutyl ester	0.012	977.618 , 1.531	842
66	Benzene, 1-methyl-3-(1-methylethyl)-	0.597	986.608 , 1.485	935
67	3-Methyl-2-butenoic acid, octyl ester	25.939	989.604 , 1.478	670
*68	2-Butenoic acid, 3-methyl-, pentyl ester	0.324	989.604 , 1.518	590
*69	3-Methyl-2-butenoic acid, heptyl ester	1.577	992.6 , 1.551	808
70	3-Methyl-2-butenoic acid, heptyl ester	0.095	992.6 , 1.577	855
71	3-Methyl-2-butenoic acid, heptyl ester	0.041	992.6 , 1.670	836
72	Butanoic acid, 3,7-dimethyl-2,6-octadienyl ester, (E)-	0.129	995.597 , 1.452	739
73	2-Buten-1-ol, 3-methyl-, acetate	0.071	995.597 , 1.551	770
74	Butanoic acid, 2-pentenyl ester, (Z)-	1.008	998.593 , 1.485	801
75	Benzyl Alcohol	0.067	1001.59 , 1.591	881
*76	2(5H)-Furanone, 5-ethyl-	0.002	1001.59 , 1.643	875

77	Butanoic acid, anhydride	0.147	1004.59 , 1.505	748
78	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	0.251	1007.58 , 1.617	909
*79	Eucalyptol	0.488	1010.58 , 1.472	882
*80	Eucalyptol	0.185	1010.58 , 1.511	777
81	1-Hexene, 6-bromo-	0.657	1013.58 , 1.558	704
82	Cyclobutanecarboxylic acid, 2-bromo-4-fluorophenyl ester	1.003	1013.58 , 1.604	830
83	Hydroperoxide, 1-methylhexyl	0.008	1019.57 , 1.538	736
84	Butane, 2-bromo-2-methyl-	0.206	1022.56 , 1.492	890
85	Benzeneacetaldehyde	0.031	1025.56 , 1.597	952
86	Propanoic acid, hexyl ester	0.070	1028.56 , 1.459	801
87	2(3H)-Furanone, 5-ethyldihydro-	0.003	1031.55 , 1.643	927
88	1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	0.198	1034.55 , 1.452	789
89	Cyclopentane, nitro-	0.021	1037.55 , 1.498	797
90	3,5-Octadien-2-one, (E,E)-	0.304	1037.55 , 1.525	788
91	Ethanone, 1-(1H-pyrrol-2-yl)-	0.016	1037.55 , 1.637	890
92	Cyclopentane, nitro-	0.280	1040.54 , 1.505	743
93	2-Butenoic acid, 2-methyl-, (E)-	0.571	1049.53 , 1.485	822
94	3-Mercaptohexyl acetate	0.083	1058.52 , 1.538	706
95	Butanoic acid, 2-methyl-, 3-methylbutyl ester	0.045	1067.51 , 1.445	852
96	2-Methylbutanoic anhydride	0.274	1070.51 , 1.551	778
97	1,5-Dimethyl-6-oxa-bicyclo[3.1.0]hexane	0.012	1070.51 , 1.577	719
98	Ethanone, 2,2-dihydroxy-1-phenyl-	0.002	1073.5 , 1.597	921
99	1,6-Octadien-3-ol, 3,7-dimethyl-	0.303	1079.5 , 1.478	816
100	Camphenol, 6-	0.024	1082.49 , 1.492	771
101	2-Furanmethanol, tetrahydro-, acetate	0.101	1082.49 , 1.531	839
102	2-Nonen-1-ol, (E)-	0.391	1088.49 , 1.472	788
103	Ethanone, 1-cyclopentyl-	0.101	1091.48 , 1.604	706
104	à-L-Mannopyranoside, methyl 6-deoxy-2,4-di-O-methyl-, acetate	0.171	1094.48 , 1.505	669
105	Propanoic acid, 2-methyl-, ethyl ester	0.024	1094.48 , 1.544	662
106	Benzoic acid, methyl ester	0.024	1100.47 , 1.584	903
107	Hexanoic acid, 2-pentenyl ester, (Z)-	0.014	1103.47 , 1.478	762
108	Bicyclo[2.2.1]heptan-2-one, 3,3-dimethyl-	0.014	1106.46 , 1.558	775
109	3-Methylene-2,6-heptanedione	0.044	1109.46 , 1.617	935
110	Isopentyl 3-hydroxy-2-methylenebutanoate	0.060	1112.46 , 1.538	702
111	Terpineol, cis-á-	0.030	1115.45 , 1.478	890
112	Trifluoroacetic acid, 4-methylpentyl ester	1.916	1124.44 , 1.564	813
113	2-Propenoic acid, 2-methyl-, hexyl ester	0.042	1127.44 , 1.459	792
114	2-Penten-1-ol, 2-methyl-, (Z)-	0.013	1130.43 , 1.492	794
115	Phenylethyl Alcohol	0.005	1130.43 , 1.617	903
116	1-Azabicyclo[3.1.0]hexane	1.805	1136.43 , 1.597	780
117	Cyclohexane, 1,2,3-trimethyl-, (1à,2á,3à)-	0.009	1136.43 , 1.670	794
118	3-Methyl-2-butenoic acid, dodecyl ester	0.321	1139.42 , 1.498	769
119	1-Tetradecanamine	0.162	1139.42 , 1.544	672
120	3-Methyl-2-butenoic acid, pentadecyl ester	0.068	1142.42 , 1.564	744
121	2-Butenoic acid, 3-methyl-, 3-methylbutyl ester	0.031	1142.42 , 1.617	768
122	2-Butenoic acid, 3-methyl-, 3-methylbutyl ester	0.785	1151.41 , 1.564	826
123	2-Butenoic acid, 3-methyl-, pentyl ester	0.101	1151.41 , 1.591	829
124	Bicyclo[3.1.0]hex-3-en-2-one, 5-(1-methylethyl)-	0.040	1151.41 , 1.617	732
125	Vinyl butyrate	0.051	1160.4 , 1.564	815
126	Bicyclo[4.1.0]heptane, 7-(1-methylethylidene)-	0.065	1163.4 , 1.597	764
127	Benzoic Acid	0.113	1166.39 , 1.676	952
128	Propanoic acid, 2,3-dihydroxy-2-methyl-	0.048	1169.39 , 1.538	684

129	5-Hepten-2-one, 6-methyl-	0.021	1172.38 , 1.577	681
130	2,6,6-Trimethyl-2-cyclohexene-1,4-dione	0.087	1178.38 , 1.604	804
131	1-Cyclohexyl-2-nitropropane-1,3-diol	0.004	1181.37 , 1.518	656
132	2-Pentanone, 4-mercapto-4-methyl-	0.021	1181.37 , 1.564	681
133	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1à,2á,5à)]-	0.804	1193.36 , 1.518	928
134	3-Trifluoroacetoxypentadecane	0.044	1193.36 , 1.564	674
135	Acetic acid, phenylmethyl ester	0.150	1193.36 , 1.610	938
136	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	0.410	1196.36 , 1.584	824
137	Bicyclo[3.1.1]heptan-2-one, 6,6-dimethyl-, (1R)-	0.259	1199.35 , 1.637	926
138	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1à,3à,5à)]-	0.117	1199.35 , 1.657	850
139	2-Butenoic acid, 3-methyl-, pentyl ester	1.145	1202.35 , 1.511	782
140	Ethanone, 1-cyclopropyl-	0.016	1205.34 , 1.591	756
141	3-Methyl-2-butenoic acid, cyclopentyl ester	0.767	1208.34 , 1.558	744
142	3-Methyl-2-butenoic acid, 3-tridecyl ester	0.143	1208.34 , 1.597	754
143	Butanoic acid, heptyl ester	0.072	1214.33 , 1.459	845
144	Bicyclo[2.2.1]heptan-2-one, 1,7,7-trimethyl-, (1S)-	0.203	1214.33 , 1.558	953
145	Butanoic acid, 2-methylcyclohexyl ester, trans-	0.006	1217.33 , 1.485	797
146	Dehydromevalonic lactone	0.014	1220.33 , 1.775	916
147	Dodecane	0.023	1223.32 , 1.386	946
148	Bicyclo[2.2.1]heptan-2-ol, 2,3,3-trimethyl-	0.179	1226.32 , 1.531	765
149	Bicyclo[2.2.1]heptan-2-ol, 2,3,3-trimethyl-	0.088	1232.31 , 1.531	770
150	2(10)-Pinen-3-one, (ñ)-	0.627	1232.31 , 1.591	861
151	2(10)-Pinen-3-one, (ñ)-	1.822	1232.31 , 1.617	838
152	2,2-Dimethylpropionic acid, 4-methylpentyl ester	0.097	1235.31 , 1.472	812
153	Cyclohexanol, 5-methyl-2-(1-methylethyl)-, (1à,2á,5à)-(ñ)-	0.724	1235.31 , 1.518	957
*154	Isoborneol	0.027	1238.31 , 1.525	899
155	Hexanoic acid, 3-tetradecyl ester	0.029	1244.3 , 1.465	791
156	Benzenemethanol, à,à,4-trimethyl-	0.081	1244.3 , 1.577	923
157	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-	0.566	1247.29 , 1.518	831
158	Propanoic acid, 2-methyl-, heptyl ester	0.016	1250.29 , 1.472	882
159	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	0.399	1250.29 , 1.544	891
160	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-	0.114	1250.29 , 1.577	867
161	2-Pentene, 3-methyl-, (Z)-	0.070	1256.28 , 1.591	729
162	Ethanone, 1-(4-methylphenyl)-	0.054	1256.28 , 1.617	903
163	2-Hexen-1-ol, 2-ethyl-	0.164	1259.28 , 1.538	657
164	4-Nonene, 2,3,3-trimethyl-, (E)-	0.457	1265.27 , 1.610	695
165	Bicyclo[3.1.1]hept-2-ene-2-methanol, 6,6-dimethyl-	0.233	1277.26 , 1.558	923
166	1-Butanol, 2,3-dimethyl-	0.070	1277.26 , 1.617	781
167	à-D-Xylofuranoside, methyl 3-O-methyl-	0.003	1280.25 , 1.597	503
168	1,2,4,5-Tetroxane, 3,3,6,6-tetramethyl-	0.002	1283.25 , 1.696	709
169	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6,6-dimethyl-	0.506	1286.25 , 1.591	942
170	Propanoic acid, 2-methyl-, heptyl ester	0.022	1292.24 , 1.472	875
171	2-Butenoic acid, 3-methyl-, ethyl ester	0.189	1292.24 , 1.630	687
172	2-Butanone, 3-methyl-	0.261	1295.24 , 1.703	773
173	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	0.101	1298.23 , 1.690	674
174	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-	2.248	1304.23 , 1.637	783

175	Nonanoic acid	0.259	1307.22 , 1.584	834
176	2(3H)-Furanone, 3-acetyldihydro-	0.031	1307.22 , 1.657	741
177	3-Oxo-4-phenylbutyronitrile	0.006	1307.22 , 1.723	742
178	3-Methyl-2-butenoic acid, 4-methylpentyl ester	1.347	1310.22 , 1.525	783
179	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	0.070	1313.22 , 1.597	670
180	2-Butenoic acid, 3-methyl-, pentyl ester	0.019	1313.22 , 1.630	684
181	1,5,7-Octatrien-3-ol, 2,6-dimethyl-	0.010	1316.21 , 1.551	676
182	(1R,2R,3S,5R)-(-)-2,3-Pinanediol	0.034	1316.21 , 1.577	740
183	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-	0.045	1322.2 , 1.577	825
184	Furan, 2-butyltetrahydro-	0.067	1325.2 , 1.498	755
185	Ether, 3-butenyl pentyl	0.012	1331.19 , 1.643	804
186	2-Butanone, 4-cyclopentylidene-	0.040	1331.19 , 1.663	747
187	2-Butenoic acid, 2-methyl-	0.039	1334.19 , 1.591	749
188	Acetic acid, 2-phenylethyl ester	0.078	1343.18 , 1.624	841
189	Benzaldehyde, 2-methoxy-	0.020	1343.18 , 1.683	807
*190	(-)-trans-Pinocarvyl acetate	0.293	1346.18 , 1.551	826
191	3-Cyclopentylpropionic acid, 2-phenylethyl ester	0.240	1346.18 , 1.624	689
192	Benzaldehyde, 4-(1-methylethyl)-	0.024	1352.17 , 1.591	759
193	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-	0.056	1352.17 , 1.610	727
194	2-Butenoic acid, 3-methyl-, hexyl ester	0.059	1355.16 , 1.505	815
195	2-Cyclohexen-1-one, 2-methyl-5-(1-methylethyl)-, (S)-	0.048	1361.16 , 1.577	734
196	Cyclopropanecarboxylic acid, 2-ethylhexyl ester	0.140	1361.16 , 1.597	804
197	Acetate, 4-hydroxy-3-methyl-2-butenyl-	0.014	1361.16 , 1.630	789
198	Isopentyl 3-hydroxy-2-methylenebutanoate	0.280	1364.15 , 1.558	912
199	Tetradecane, 1-iodo-	0.031	1370.15 , 1.591	727
200	3-Cyclopenten-1-one, 2-hydroxy-3-(3-methyl-2-butenyl)-	0.017	1370.15 , 1.624	767
201	Acetic acid, 5-[3-(4-methoxyphenyl)oxaziridin-2-yl]pentyl ester	0.016	1373.14 , 1.696	764
202	Decanoic acid, 3-hydroxy-, methyl ester	0.011	1379.14 , 1.558	683
203	Bicyclo[5.1.0]octane, 8-(1-methylethylidene)-	0.099	1379.14 , 1.584	690
204	5,5-Dimethyl-cyclohex-3-en-1-ol	0.265	1382.13 , 1.610	702
205	1-Hydroxy-3-methyl-2-butanone	0.015	1385.13 , 1.564	799
206	(1R,2R,3S,5R)-(-)-2,3-Pinanediol	0.118	1388.13 , 1.617	757
207	(1R,2R,3S,5R)-(-)-2,3-Pinanediol	0.460	1388.13 , 1.650	773
208	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	0.009	1391.12 , 1.577	769
209	Benzylidenemalonaldehyde	0.087	1403.11 , 1.676	778
210	Bornyl acetate	0.011	1406.1 , 1.544	834
211	Propanoic acid, 2-methyl-, phenylmethyl ester	0.071	1409.1 , 1.604	827
212	Cyclopentaneundecanoic acid	0.112	1415.09 , 1.525	719
213	Benzenemethanol, 4-(1-methylethyl)-	0.073	1418.09 , 1.624	851
214	Propanoic acid, 2-methyl-, ethyl ester	0.021	1424.08 , 1.544	660
215	Cyclobutanecarboxylic acid, 2-pentadecyl ester	0.062	1427.08 , 1.624	708
216	Cinnamaldehyde, á-methyl-	0.051	1430.07 , 1.630	739
217	1-Cyclohexene-1-methanol, 4-(1-methylethenyl)-	0.167	1436.07 , 1.591	817
218	Furan, 2-hexyl-	0.003	1439.06 , 1.544	720
219	3-Acetonylcyclopentanone	0.582	1439.06 , 1.657	713
220	1,3-Propanediol, 2-ethyl-2-(hydroxymethyl)-	0.008	1439.06 , 1.709	748
221	2-Octanone, 1-nitro-	0.064	1451.05 , 1.564	707
222	4-Chloro-3-n-hexyltetrahydropyran	0.021	1451.05 , 1.650	775
*223	(-)-Myrtenyl acetate	0.043	1460.04 , 1.558	795

*224	Isopinocarveol	0.016	1460.04 , 1.577	786
225	4-Hydroxy-3-methylacetophenone	0.002	1463.04 , 1.683	752
226	3-Methyl-2-butenoic acid, heptyl ester	0.342	1469.03 , 1.505	799
227	n-Decanoic acid	0.206	1469.03 , 1.584	945
*228	Benzyl methacrylate	0.201	1469.03 , 1.630	777
229	n-Decanoic acid	0.042	1472.02 , 1.558	834
230	2-Nitrohept-2-en-1-ol	0.009	1475.02 , 1.604	722
231	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)-	0.088	1478.02 , 1.683	755
232	Ethanol, 2-(3,3-dimethylbicyclo[2.2.1]hept-2-ylidene)-	0.044	1484.01 , 1.683	778
233	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	0.307	1490 , 1.657	737
234	3-Hexanone, 2,5-dimethyl-4-nitro-	0.343	1498.99 , 1.683	872
235	Methyl cis-2-methyl-2-butenoate	0.028	1501.99 , 1.617	766
236	3-Hexanone, 2,5-dimethyl-4-nitro-	0.204	1501.99 , 1.670	825
237	Cyclopropanecarboxaldehyde, 2-methyl-2-(4-methyl-3-pentenyl)-, trans-(.+-.)-	0.116	1501.99 , 1.696	733
238	3-Methyl-2-butenoic acid, pentadecyl ester	0.022	1510.98 , 1.511	829
239	Cyclopropane, 1-bromo-2-butyl-, trans-	0.259	1522.96 , 1.683	684
240	Acetophenone, 4'-methoxy-	0.007	1525.96 , 1.709	923
241	6,8-Nonadien-2-one, 8-methyl-5-(1-methylethyl)-, (E)-	0.022	1528.96 , 1.498	810
242	Hexadecane	0.145	1531.95 , 1.558	705
243	5-Octen-2-yn-4-ol	0.125	1534.95 , 1.663	762
244	Bicyclo[3.2.0]heptan-2-one, 5-formylmethyl-6-hydroxy-3,3-dimethyl-6-vinyl-	0.048	1534.95 , 1.703	683
245	2,2-Dimethyl-4-octenal	0.056	1543.94 , 1.597	737
246	2-Propenoic acid, 2-methyl-, oxiranylmethyl ester	0.164	1543.94 , 1.709	838
247	1(3H)-Isobenzofuranone	0.017	1546.93 , 1.828	946
248	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1S-(1à,4à,7à)]-	0.011	1552.93 , 1.478	855
249	Propanoic acid, 2-methyl-, decyl ester	0.006	1558.92 , 1.472	786
250	8-Oxabicyclo[3.2.1]oct-6-en-2-one, 1,4,4-trimethyl-	0.005	1558.92 , 1.683	603
251	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-	0.177	1567.91 , 1.650	808
252	Hexanoic acid, 2-phenylethyl ester	0.015	1570.91 , 1.604	687
253	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1à,4aà,8aà)-	0.242	1573.9 , 1.478	918
254	Cyclobutanecarboxylic acid, 3-tridecyl ester	0.006	1576.9 , 1.610	755
255	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	0.059	1576.9 , 1.690	795
256	Bicyclo[3.1.1]hept-2-en-4-ol, 2,6,6-trimethyl-, acetate	0.125	1585.89 , 1.531	803
257	Propanoic acid, 2-methyl-, 2-ethyl-3-hydroxyhexyl ester	0.006	1585.89 , 1.551	681
258	5-Octen-2-one, 3,6-dimethyl-	0.062	1585.89 , 1.604	739
259	Benzeneacetic acid, 2-hexenyl ester, (E)-	0.026	1585.89 , 1.643	744
260	Cyclopropanemethanol, à-butyl-	0.018	1594.88 , 1.564	707
261	2-Propen-1-ol, 2-bromo-, acetate	0.058	1597.87 , 1.690	675
262	Vanillin	0.028	1597.87 , 1.762	905
263	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	0.013	1600.87 , 1.650	761

264	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	0.013	1606.86 , 1.657	784
265	Butanoic acid, 2-hexenyl ester, (E)-	0.007	1615.85 , 1.564	707
266	cis-Pinonic acid	0.123	1615.85 , 1.696	803
267	Cyclopropanecarboxylic acid, 2-phenylethyl ester	0.049	1618.85 , 1.630	727
268	Cyclohexane, 1,1,3,5-tetramethyl-, cis-	0.127	1618.85 , 1.650	782
269	4H-Imidazol-4-one, 2-amino-1,5-dihydro-	0.065	1621.84 , 1.736	780
*270	(-)-trans-Pinocarvyl acetate	0.159	1624.84 , 1.544	795
271	5-Octen-2-one, 3,6-dimethyl-	0.011	1627.84 , 1.650	748
272	Heptadecane, 2,6,10,14-tetramethyl-	0.006	1630.83 , 1.399	898
*273	Benzyl tiglate	0.114	1636.83 , 1.650	843
274	3-Methylene-2,6-heptanedione	0.047	1636.83 , 1.736	737
275	2,2,6,6-Tetramethylheptane	0.110	1645.82 , 1.663	783
276	2H-Pyran, 2-(1,1-dimethylethoxy)tetrahydro-	0.014	1645.82 , 1.696	727
277	1,5,7-Octatrien-3-ol, 2,6-dimethyl-	0.006	1651.81 , 1.551	710
278	3-Cyclopentene-1-acetaldehyde, 2,2,3-trimethyl-	0.060	1654.8 , 1.676	871
279	3,5-Heptadien-2-ol, 2,6-dimethyl-	0.083	1654.8 , 1.716	774
280	Furan, 2,5-dihydro-2,2-dimethyl-5-(1-methylethenyl)-3-(1-methylethyl)-	0.012	1657.8 , 1.558	702
281	Cyclobutanecarboxylic acid, 4-pentadecyl ester	0.012	1657.8 , 1.624	765
*282	Caryophyllene	0.026	1660.8 , 1.505	951
283	7-Methyl-Z-tetradecen-1-ol acetate	0.009	1660.8 , 1.670	709
284	Cyclopropanemethanol, à-butyl-	0.018	1669.79 , 1.577	769
285	Butanoic acid, 3-bromo-3-methyl-	0.107	1672.78 , 1.769	677
286	Naphthalene, 2,6-dimethyl-	0.069	1675.78 , 1.650	689
287	(-)-trans-Pinocarvyl acetate	0.049	1678.78 , 1.571	812
288	4,4-Dimethyl-1-hexene	0.020	1678.78 , 1.683	822
289	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	0.007	1681.77 , 1.657	697
290	Tetrahydrofuran, 2-propyl-	0.041	1681.77 , 1.676	837
291	Pentadecane	0.017	1690.76 , 1.412	952
292	4-Hexen-3-ol	0.008	1690.76 , 1.584	817
293	9,12-Octadecadienoic acid (Z,Z)-	0.433	1699.75 , 0.079	890
294	Butyric acid, 4-pentadecyl ester	0.029	1699.75 , 1.591	680
295	Propanoic acid, 2-methyl-, 3,7-dimethyl-2,6-octadienyl ester, (E)-	0.038	1705.74 , 1.538	874
296	3-Methyl-2-butenoic acid, 2-pentyl ester	0.023	1705.74 , 1.577	673
*297	Carveol (fr.1)	0.080	1705.74 , 1.624	738
298	7-Isopropenyl-4,4,8,8-tetramethylbicyclo[4.2.0]octane-1,5-diol	0.024	1705.74 , 1.650	766
299	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-methylethyl)-, [s-(E,E)]-	0.011	1708.74 , 1.472	871
300	Octahydro-1-oxa-cyclopropa[c]indene	0.061	1708.74 , 1.749	744
301	2,6-Dimethyl-6-nitro-2-hepten-4-one	0.456	1711.74 , 1.736	802
302	Cyclohexene, 1,5,5-trimethyl-6-acetylmethyl-	0.068	1723.72 , 1.604	761
303	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)-	0.169	1726.72 , 1.518	910
304	Methacrylic anhydride	0.033	1726.72 , 1.709	765
305	10-Dodecyn-1-ol	0.011	1726.72 , 1.742	758
306	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3aà,3bá,4á,7à,7aS*)]-	0.050	1729.71 , 1.465	893
307	Butylated Hydroxytoluene	0.047	1735.71 , 1.584	919
308	Benzenepropanoic acid, dodecyl ester	0.021	1735.71 , 1.604	770

309	1'-Acetonaphthone	0.221	1735.71 , 1.729	715
310	Aromadendrene	0.011	1738.7 , 1.518	870
311	2-Cyclohexyl-hex-5-en-2-ol	0.049	1750.69 , 1.683	700
312	2,6R-Diethyl-3,5S-dimethyl-3,4-dihydro-2H-pyran	0.055	1750.69 , 1.716	668
313	á-Vatirenene	0.046	1753.69 , 1.551	806
314	3-Methyl-1-[(1H)-1,2,4-triazol-1-yl]butan-2-one	0.014	1753.69 , 1.584	685
315	Benzeneethanol, á-butyl-	0.008	1759.68 , 1.637	716
316	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2à,4aà,8aá)]-	0.060	1768.67 , 1.531	929
317	2,6-Nonadienal, 3,7-dimethyl-	0.182	1768.67 , 1.650	756
318	12,15-Octadecadiynoic acid, methyl ester	0.022	1771.66 , 1.544	722
319	Oleic Acid	0.033	1774.66 , 1.591	743
320	1H-Indene, 1-ethylideneoctahydro-7a-methyl-, cis-	0.073	1777.66 , 1.729	809
321	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	0.031	1780.65 , 1.544	898
322	Mint furanone	0.015	1780.65 , 1.742	865
323	Cycloundecene, 1-methyl-	0.036	1786.65 , 1.736	788
324	1-Hydroxy-1,7-dimethyl-4-isopropyl-2,7-cyclodecadiene	0.020	1792.64 , 1.538	832
325	2,5-Octadecadiynoic acid, methyl ester	0.006	1795.64 , 1.630	709
326	Oxiranemethanol, 3-methyl-3-(4-methyl-3-pentenyl)-	0.061	1801.63 , 1.617	723
*327	Carveol (fr.1)	0.021	1804.62 , 1.577	776
328	2(3H)-Furanone, dihydro-5,5-dimethyl-4-(3-oxobutyl)-	0.053	1807.62 , 1.789	777
329	11-Dodecen-2-one, 7,7-dimethyl-	0.032	1813.61 , 1.617	759
330	Propylphosphonic acid, fluoroanhydride, decyl ester	0.081	1816.61 , 1.690	726
331	3,4-Methylenedioxypropiophenone	0.013	1816.61 , 1.736	754
332	Bicyclo[3.1.1]hept-3-en-2-ol, 4,6,6-trimethyl-, [1S-(1à,2á,5à)]-	0.008	1819.61 , 1.551	803
333	7-Methyl-Z-tetradecen-1-ol acetate	0.005	1822.6 , 1.683	724
334	Allyl 2-ethyl butyrate	0.066	1828.6 , 1.624	770
335	1,2,3,4,6,7,8,8a-Octahydronaphthalene-6,7-diol, 5,8a-dimethyl-3-isopropenyl-, cyclic carbonate, trans-	0.005	1831.59 , 1.597	624
*336	Caryophyllene oxide	0.025	1834.59 , 1.571	757
337	Heptadecane	0.017	1837.59 , 1.419	952
*338	trans-Pinocarveol	0.036	1840.58 , 1.591	777
339	3-Methylene-2,6-heptanedione	0.109	1840.58 , 1.703	701
340	1-Cyclooctene-1-carboxylic acid	0.057	1840.58 , 1.775	739
341	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	0.069	1843.58 , 1.624	751
342	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	0.053	1843.58 , 1.742	896
343	cis-3-Hexenyl iso-butyrate	0.023	1855.56 , 1.597	693
344	Diethyl Phthalate	0.040	1855.56 , 1.742	952
345	Calarene epoxide	0.005	1867.55 , 1.538	809
*346	Caryophyllene oxide	0.048	1867.55 , 1.584	804
347	3-Oxatricyclo[4.1.1.0(2,4)]octane, 2,7,7-trimethyl-	0.089	1873.54 , 1.617	696
348	1,6-Octadiene, 3-ethoxy-3,7-dimethyl-	0.404	1882.53 , 1.624	730

349	á-Vatirenene	0.010	1885.53 , 1.591	784
350	Cyclohexane, octyl-	0.075	1885.53 , 1.723	826
351	Hexanoic acid, 2-hexenyl ester, (E)-	0.003	1885.53 , 1.775	695
352	Ledene oxide-(II)	0.117	1891.52 , 1.591	817
353	3-Amino-4-methoxybenzoic acid	0.040	1894.52 , 1.723	689
354	6-Nonenal, 3,7-dimethyl-	0.020	1900.51 , 1.650	752
355	Geranyl vinyl ether	0.023	1906.5 , 1.577	820
*356	Caryophyllene oxide	0.118	1915.49 , 1.597	888
357	Dihydrojasmone	0.018	1921.48 , 1.848	740
358	1-Cyclohexene-1-acetaldehyde, à,2-dimethyl-	0.116	1924.48 , 1.604	813
359	Cubenol	0.016	1927.48 , 1.577	785
360	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3-yl ester	0.013	1930.47 , 1.637	767
361	Nona-3,5-dien-2-ol	0.085	1933.47 , 1.749	688
362	Caryophyllene oxide	0.103	1939.46 , 1.597	833
363	(3-Oxo-2-pent-2-enylcyclopentyl)acetic acid, methyl ester	0.043	1939.46 , 1.676	916
364	Bicyclo[2.2.1]heptan-2-one, 3-(2-oxopropyl)-, exo-	0.013	1939.46 , 1.861	681
365	Propiophenone, 3',4'-dimethoxy-	0.235	1945.46 , 1.775	932
366	Cubenol	0.567	1948.45 , 1.591	829
367	Isoaromadendrene epoxide	0.036	1948.45 , 1.630	833
368	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-methyloxiranyl)-	0.050	1954.44 , 1.630	753
369	Oxacycloheptadec-8-en-2-one	0.315	1954.44 , 2.779	928
370	9,12,15-Octadecatrien-1-ol, (Z,Z,Z)-	0.113	1960.44 , 2.851	853
371	1(2H)-Naphthalenone, 2-furfurylidene-3,4,4a,5,6,7,8,8aá-octahydro-	0.316	1963.43 , 0.436	733
372	2-Cyclohexen-1-one, 3,5,5-trimethyl-4-(3-oxo-1-butenyl)-	0.045	1963.43 , 1.723	827
373	1-Hexadecene	0.035	1966.43 , 1.445	957
374	2H-Cyclopropa[g]benzofuran, 4,5,5a,6,6a,6b-hexahydro-4,4,6b-trimethyl-2-(1-methylethenyl)-	0.106	1966.43 , 1.637	803
375	1(2H)-Naphthalenone, 2-furfurylidene-3,4,4a,5,6,7,8,8aá-octahydro-	0.119	1972.42 , 0.389	729
376	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.043	1972.42 , 1.637	771
377	2-Hydroxy-4,5-methylenedioxypropiophenone	0.227	1972.42 , 1.749	808
378	Diepicedrene-1-oxide	0.353	1987.41 , 1.630	837
379	2-Butyloxycarbonyloxy-1,1,10-trimethyl-6,9-epidioxydecalin	0.008	1990.4 , 1.657	637
380	à-Cadinol	0.092	1993.4 , 1.610	828
381	Cubenol	0.007	1999.39 , 1.577	822
382	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.013	1999.39 , 1.643	777
383	4,6,10,10-Tetramethyl-5-oxatricyclo[4.4.0.0(1,4)]dec-2-en-7-ol	0.183	2008.38 , 1.723	800
384	Spirio-10-(2,11-dioxabicyclo[4.4.1]undeca-3,5-diene)-2'-(oxirane), 1,3,7,7-tetramethyl-	0.009	2011.38 , 1.716	749
385	1H-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis-	0.074	2017.37 , 1.670	753
386	Aromadendrene oxide-(2)	0.012	2029.35 , 1.663	784
387	Globulol	0.498	2035.35 , 1.610	795
388	Pentadec-7-ene, 7-bromomethyl-	0.019	2035.35 , 1.775	703
389	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-	0.015	2041.34 , 2.706	757

390	Tetradecanoic acid	0.144	2050.33 , 1.577	942
391	Ppropiolic acid, 3-(1-hydroxy-2-isopropyl-5-methylcyclohexyl)-	0.061	2050.33 , 1.749	715
392	Cyclopentaneacetaldehyde, 2-formyl-3-methyl-à-methylene-	0.115	2065.31 , 1.736	762
393	Cyclohexane, 1,2-diethenyl-4-(1-methylethylidene)-, cis-	0.005	2068.31 , 1.617	741
394	Bicyclo[2.2.1]heptane-2,5-diol, 1,7,7-trimethyl-, (2-endo,5-exo)-	0.008	2071.3 , 1.683	787
*395	cis-Z-à-Bisabolene epoxide	0.014	2071.3 , 1.703	765
396	Oxirane, tetradecyl-	0.008	2074.3 , 2.112	747
397	Heptylcyclohexane	0.123	2098.27 , 1.828	729
*398	Caryophyllene oxide	0.028	2101.27 , 1.657	839
399	Heptadecane	0.012	2104.26 , 1.445	936
400	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, cis-	0.041	2107.26 , 1.723	717
401	2,6-Octadiene, 2,4-dimethyl-	0.040	2122.24 , 2.792	692
402	Ledol	0.025	2134.23 , 1.756	761
403	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.037	2137.23 , 1.690	810
404	2H-1-Benzopyran-2-one, 3,4,4a,5,6,7-hexahydro-4a-methyl-	0.024	2143.22 , 1.874	689
405	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9-(prop-1-en-3-ol-2-yl)-	0.107	2146.21 , 1.696	832
406	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.010	2149.21 , 1.478	860
407	2-Cyclohexen-1-one, 4-hydroxy-3,5,6-trimethyl-4-(3-oxo-1-butenyl)-	0.008	2149.21 , 1.822	863
408	5-Isopropyl-6-methyl-hepta-3,5-dien-2-ol	0.029	2158.2 , 1.907	702
409	12-Methyl-E,E-2,13-octadecadien-1-ol	0.014	2161.2 , 1.624	753
410	Dimethyl(chloromethyl)silyloxycyclohexane	0.008	2161.2 , 2.376	623
411	2-Pentadecanone, 6,10,14-trimethyl-	0.488	2164.19 , 1.538	907
412	5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)-	0.138	2164.19 , 1.690	813
413	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.028	2170.19 , 1.690	809
414	Pentadecanoic acid	0.054	2179.17 , 1.577	858
415	Oleic Acid	0.010	2182.17 , 1.663	784
416	Limonene-1,2-epoxide(fr.1)	0.124	2185.17 , 1.716	825
417	4,4-Dimethyl-3-(3-methylbut-2-enylidene)octane-2,7-dione	0.118	2185.17 , 1.736	758
418	3,6-Octadien-1-ol, 3,7-dimethyl-, (Z)-	0.185	2188.16 , 1.624	768
419	Oleic Acid	0.009	2188.16 , 1.657	773
420	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.018	2191.16 , 1.492	835
421	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	0.016	2197.15 , 1.544	778
422	9,12-Octadecadienoic acid (Z,Z)-	0.012	2197.15 , 1.643	751
423	Cholestane, 4,5-epoxy-, (4à,5à)-	0.341	2200.15 , 1.709	751
424	1-Decanol, 2-hexyl-	0.033	2200.15 , 2.053	887
425	Tetracosane	0.872	2203.15 , 1.881	968
426	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.035	2206.14 , 1.485	898
427	Tetracosane	0.030	2209.14 , 1.901	952
*428	Caryophyllene oxide	0.017	2215.13 , 1.709	784
429	Gitoxigenin	0.034	2218.13 , 1.756	746
430	Nonadecane	0.010	2224.12 , 1.459	897
431	Phenanthrene	0.056	2224.12 , 1.855	868
432	2-Dodecen-1-yl(-)succinic anhydride	0.046	2227.12 , 1.762	798

433	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a-tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	0.136	2233.11 , 1.736	771
434	Isoaromadendrene epoxide	0.133	2239.1 , 1.716	793
435	Hexadecanoic acid, methyl ester	0.069	2257.08 , 1.544	924
436	Benzene, (1-methyldodecyl)-	0.013	2269.07 , 1.558	797
437	Acetic acid, 7-hydroxy-1,3,4,5,6,7-hexahydro-2H-naphthalen-4a-ylmethyl ester	0.010	2278.06 , 1.775	746
438	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	1.626	2284.05 , 1.808	750
439	7-Hydroxy-6,9a-dimethyl-3-methylene-decahydro-azuleno[4,5-b]furan-2,9-dione	0.044	2287.05 , 1.789	783
440	1,2-Dihexylcyclopropene-3-carboxylic acid	0.416	2290.04 , 1.828	789
441	Oxazole, 4-phenyl-	0.194	2296.03 , 1.927	556
442	4-Butyl(dimethyl)silyloxypentadecane	0.019	2302.03 , 1.558	694
443	Cholestan-3-ol, 2-methylene-, (3á,5à)-	0.049	2305.02 , 1.795	787
444	Isoaromadendrene epoxide	0.124	2323 , 1.789	798
445	n-Hexadecanoic acid	0.071	2337.98 , 1.577	897
446	Isoaromadendrene epoxide	0.047	2337.98 , 1.762	787
447	2(3H)-Furanone, 4,5-dihydro-4-(2-methyl-3-methylene-1-buten-4-yl)-	0.030	2337.98 , 1.894	702
448	Tetracosane	0.009	2340.98 , 1.492	943
449	N-Isobutyl-(2E,4Z,8Z,10E)-dodecatetraenamide	0.070	2340.98 , 2.303	701
450	n-Hexadecanoic acid	0.066	2349.97 , 1.577	918
451	n-Hexadecanoic acid	0.048	2361.96 , 1.571	930
452	Thiophene, 3-methyl-2-pentadecyl-	0.043	2361.96 , 2.244	746
453	4,8,12,16-Tetramethylheptadecan-4-olide	0.005	2364.95 , 2.178	908
454	Dodecane, 2,6,10-trimethyl-	0.108	2370.94 , 1.815	791
455	4,8,12,16-Tetramethylheptadecan-4-olide	0.040	2370.94 , 2.185	955
456	n-Hexadecanoic acid	0.012	2373.94 , 1.571	917
457	4,4-Dimethyl-3-(3-methylbut-3-enylidene)-2-methylenebicyclo[4.1.0]heptane	0.008	2376.94 , 1.703	765
458	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one	0.062	2382.93 , 1.861	796
459	Eudesma-5,11(13)-dien-8,12-olide	0.028	2385.93 , 1.921	781
460	n-Hexadecanoic acid	0.034	2388.92 , 1.571	915
461	1,6-Dibromo-2-cyclohexylpentane	0.137	2391.92 , 2.515	741
462	2-Butenoic acid, 2-methyl-, dodecahydro-8-hydroxy-8a-methyl-3,5-bis(methylene)-2-oxonaphtho[2,3-b]furan-4-yl ester, [3ar-[3aà,4à(Z),4aà,8á,8aá,9aá]]-	0.003	2394.92 , 2.581	744

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CHAPTER 5
EVALUATION AND FUTURE PERSPECTIVE

In this chapter the project is evaluated by considering the extent to which the objectives could be achieved. This is done by reflecting on the successes and shortcomings of the study. A few future opportunities related to the study are also presented.

5.1 Successes and shortcomings

The study demonstrated the feasibility of obtaining a component-rich sc-CO_2 derived extract of Roman chamomile. The extraction conditions (temperature, pressure, time) were optimised by statistical design and surface response analysis. The dependence of the yield of extract on various process variables enabled conclusions to be drawn about the mechanism of extraction, and these could be supported by a few calculated activation parameters. A typical yield of sc-CO_2 derived extract of Roman chamomile amounted to 3% (m/m), which compared favourably with yields of 0.5 - 2 % (m/m) reported in the literature for steam distillation.

The analysis of sc-CO_2 extracts performed by GC-FID, GC-MS and GC-GC/TOF-MS complemented one another in terms of the identification of constituents of Roman chamomile, though the 462 compounds identified by GC-GC/TOF-MS by far exceeded the 11 compounds identified by GC-MS. The results obtained by two-dimensional GC proved the capability of sc-CO_2 to extract a multiple of different compounds from Roman chamomile, and many of the identified compounds have also been reported by other authors or for other extraction methods.

One cannot categorically conclude that sc-CO_2 extraction is superior to or can replace steam distillation to obtain extracts of Roman chamomile, but the study proved that sc-CO_2 extraction has advantages over steam distillation such as shorter extraction times and milder conditions while still yielding component-rich extracts comparable to those obtained by steam distillation.

The collection of the entire amount of sc-CO_2 extracted material proved to be difficult to achieve. The waxy nature of the extract caused some of the material to stay behind in the flow line of the extractor, resulting in a lack of reproducibility of the extraction data. Collection or trapping systems used during the study proved to be inefficient to fully prevent loss of volatile material. Efforts were made to minimise the loss by working at low flow rates and temperatures, even at the expense of extended extraction times. A possible solution could be to implement a solvent trapping system, but this was not desirable as the intention was to obtain extracts as natural and as solvent-free as possible.

Another shortcoming was the inability to extract flavonoids, which are pharmaceutically important constituents of Roman chamomile. The solubility of the flavonoids in sc-CO_2 could have been improved by the addition of a suitable cosolvent or polarity modifier, but with the effort to get a "clean" extract, this option was not pursued.

5.2 Further research

The collection of extracted material remains an important area for improvement. The problem of extract staying behind in the flow line needs to be addressed. At none of the extraction conditions a wax-free extract could be produced, and it thus remains a challenge to find a way of separating the undesirable cuticular waxes from the volatile oil.

It is essential to perform GC-GC/TOF-MS analysis on both steam distilled and sc-CO_2 derived extracts in order to really compare the composition of the extracts obtained by the two techniques. It could be an objective of a follow-up study to apply two-dimensional GC to both types of extract for comparison purposes.

There is a need to submit sc-CO_2 derived extracts of Roman chamomile for organoleptic analysis as this can assist in producing suitable/desired compositions for the cosmetic, food and fragrance industries. This might require optimisation of extraction conditions not only in terms of yield but also with regard to extract composition and compound selectivity.

The current *sc*-CO_2 extraction methodology entails a batch process whereby many consecutive extraction runs need to be performed to acquire sufficient amounts of extract for

sensory evaluation. A valuable future development would be a continuous method of extraction whereby plant material is continuously fed into a reactor at supercritical conditions by virtue of an extruder and extracted material is continuously collected from the reactor without perturbing the supercritical conditions. There are already claims in the literature of extruder designs[1,2] that could possibly address the problem of batch versus continuous extraction modes.

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