Supercritical Fluid Extraction

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There are two interesting points of the phase diagram of a pure substance: the triple point, where solid, liquid and gas are all in equilibrium, and the critical point, at which liquid and gas phases cease to have separate existence. At temperatures and pressures beyond the critical point there exists only the supercritical phase, with properties between those of a gas and a liquid, varying with conditions. For example, at high pressures a supercritical fluid has solubility and density properties close to those of the liquid, but with greater diffusibility and lower viscosity. High diffusibility and low viscosity improve mass transfer and so help to decrease extraction time. All this would be of only academic or research interest, were it not that one substance, carbon dioxide, which is cheap, readily available in a pure state, and nontoxic, has a readily accessible critical point (31.1°C, and 72.9 atm, 73.8 bar, 1071 psi, or 7.38 MPa). The density of supercritical carbon dioxide at various values of temperature and pressure is given in **Figure 1**. Its vaporization on release of pressure avoids the step of concentrating a liquid solution after extraction.

The possibilities of using supercritical carbon dioxide as an extraction fluid were recognized first in industry in the 1950s and 1960s. It entered the laboratory in the 1980s at the same time pumps and control equipment were developed for supercritical fluid chromatography (SFC). Since then supercritical fluid extraction (SFE) has been explored with a wide variety of materials, and is generally recognized as a possible alternative to chlorinated or other toxic solvents for extraction of organic substances. There is no close competitor for carbon dioxide as a supercritical fluid for extraction. Other substances with easily accessible critical points are either too expensive (xenon), toxic (ammonia, nitrous oxide), flammable (ethane, pentane) or corrosive (ammonia). Extraction may be described as static (under pressure without flow of the supercritical fluid) or dynamic (the supercritical fluid flowing through the material to be extracted). Dynamic extraction is more common, but it can be preceded by a period of static extraction.

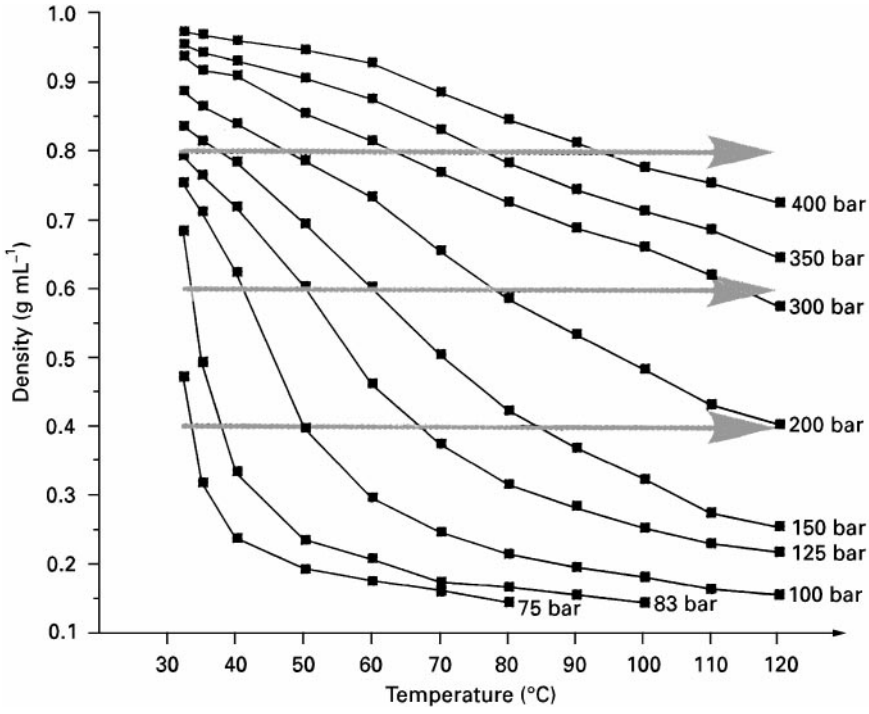


Figure 1 A plot of carbon dioxide density against pressure and temperature above the critical point. 10 bar = 1 MPa. Horizontal arrows represent constant densities of 0.4, 0.6 and 0.8. Fatty acids are extracted only above 0.4 g mL^{-1}, triglycerides above 0.6 g mL^{-1}. Reproduced with permission from Gere DR and Derrico EM (1994) *LC.GC International* 7: 325.

The subject roughly divides itself into two aspects: commercial-scale separation of valuable products (e.g. vitamins, drugs, flavours, fragrances, pigments) and laboratory-scale extraction for research or analysis of components. Bevan and Marshall have considered the design of large scale extractors. There are a number of commercial extractors available for small scale work. They usually have six or more chambers so that extractions can be carried out on several samples simultaneously or consecutively. Pressure can be maintained and controlled by an electronic valve or by use of a fixed restrictor, such as a length of silica capillary. After release of the pressure the extract can be collected on a solid adsorbant or the CO_2 bubbled through a solvent. Precautions have to be taken to prevent loss of material as an aerosol.

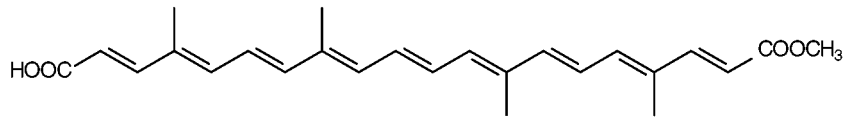
It is possible to build an extractor at modest cost. The chief needs are high pressure pumps (one for the CO_2, another for the modifier (if used, see below), a cooler for the CO_2, since it is pumped as a liquid, an old gas chromatography (GC) oven to maintain the desired temperature of the extraction, some empty high performance liquid chromatography (HPLC) columns for extraction chambers, a pressure gauge and some means of controlling and releasing the pressure. The simplest solution for this is a length of about 30–50 cm of silica capillary. Whatever the purpose of the extraction, it can be convenient to couple the equipment to a chromatographic system to monitor the course of extraction. Most convenient is on-line SFC. The composition of the extract can be sampled periodically by inserting a switching valve leading to the chromatograph. GC linked to the extraction is also much used, particularly for the extraction of essential oils and fragrances. The chromatograph can in turn be linked to a mass spectrometer. Now there is no end to the complexity of equipment that can be linked to the extraction. Albert has particularly explored the linking of SFE to nuclear magnetic resonance (NMR) spectroscopy, and gives examples that include natural products.

The solubility properties of supercritical CO_2 are close to those of hexane (polarity increases slightly with pressure), so its chief disadvantage is poor solubility for more polar substances. To extract lipids, the density needs to be above 0.4 g mL^{-1} (see Figure 1), for triglycerides it should be above 0.6 g mL^{-1}. The best way to improve solubility (if increasing pressure is not convenient) is to add a small proportion (5–10%) of a polar organic solvent, such as methanol. The organic solvent in this context is commonly called a modifier. The plant, animal or other material from which substances are being extracted is often called the matrix. The solubility of water in supercritical CO_2 is very low (0.4 mol% at 8.0 MPa, rising to 0.8 mol% of water at 20.0 MPa), but there are a number of examples where water has been used as a modifier for extraction of more polar compounds. The polarity of water drops remarkably as it approaches its critical point.

An early landmark in SFE was the commercial extraction of caffeine from coffee with CO_2. Caffeine is a relatively polar compound, so its extraction with the very apolar CO_2 is surprising. Hops and spices followed closely.

Lipids

Lipids from plant and animal sources are obvious targets for SFE, both on a commercial scale and as an analytical method in the laboratory. There are many reports of its use to extract fish oils (particularly to obtain concentrates of polyunsaturated acid glycerides), dairy products and seed oils. In many cases attempts are recorded to make selective extraction of valuable minor substances accompanying the oil, such as tocopherols, carotenes or sterols. The content of tocopherols is said to be significantly higher from rapeseed and soybeans by SFE. One can find examples of SFE of natural products in the reviews by Bevan and Marshall and by Jarvis and Morgan. The lipids of rapeseed and soybeans were extracted with CO_2 alone and with added propane or nitrous oxide. In another case, oil from a fungus *Mortierella ramanniane* was extracted with CO_2, N_2O, CHF_3 and SF_6. Extraction was best at 60°C and 157–295 bar with N_2O, followed by CO_2, CHF_3 and SF_6. Addition of 20% ethanol greatly increased the solubility of the oil and decreased its acidity. Some examples are listed in **Table 1** with some of the conditions used for the extraction, although in most cases a range of differing conditions were explored. Unfortunately, there is a scarcity of information directly comparing efficiency of SFE with solvent extraction.



1 Bixin

Table 1 Examples of extraction of lipids with conditions used

Extracted	*Matrix*	*Temperature (°C)*	*Pressure (MPa)*	*Additional information*[a]
Bixin (**1**)	*Bixa orellana* (annatto)	40	60.62	4% Acetonitrile with 0.05% trifluoroacetic acid, yield 0.27%
Carotenes	*Daucus carota* (carrots)	40	60.6	5% $CHCl_3$ 92.7%, 1 h
Carotenes	*Mauritia flexuosa* (buriti fruit)	40–55	30	80%
Carotenes and lutein	Leaf protein concentrate	40	30	
Fatty acid methyl esters	Fish oil	40	8.0	20 min
γ-Linolenic acid	*Oenothera paradoxa* (evening primrose) seed	40–60	20–70	30 min, 95%
Phospholipids	Rape seed (Canola)	70	55.2	10% EtOH
Phytosterols	Seed, corn oil, margarine			
Sterols	Egg yolk	45	17.7	**a**[b], 1 h
Tocopherols	*Hordeum vulgare* (barley)	40	23.69	1 h, 4.38%, density 0.921
Triglycerides	Rapeseed, soybeans	20–40	25	**a**
Triglycerides	Ground rapeseed, linseed meal	90	34.3	
Triglycerides	Sunflower	42–80	15.2–35.4	20% EtOH

[a]High percentages refer to total content, usually compared to that obtainable by solvent extraction; low percentages refer to total extracted as a percentage of total mass.
[b]Yield comparable to solvent extraction.

Glycolipids and phospholipids are less easily extracted and usually require the addition of methanol as modifier. Sterols and sterol esters are more difficult to extract than triglycerides, but have also been explored. By stepwise increases of pressure, the concentration of phytosterols in soybean extract increased 30 times, from corn fibre by 12 times and from corn bran by 37 times. Methods are available for the analytical determination of sterols in animal skin, meat and fat. SFE is particularly useful for unstable compounds such as γ-linolenic acid and carotenes. The latter have been extracted from leaf protein concentrates and carrots (see Table 1). **Figure 2** shows a chromatogram of a carotene extract obtained from carrots. The content of tocopherols is greater in the oil of rapeseed and soybeans extracted by SFE than by solvent extraction. Fluorometric and electrochemical detectors with HPLC have been used to follow the extraction.

Lanolin has been extracted from wool using 20% acetone as modifier, giving comparable results to dichloromethane Soxhlet extraction, but it gave a cleaner product (less mineral salts and protein).

Essential Oils

Essential oils are an obvious target for SFE (**Table 2**), and this is probably the area that has received most attention in recent years, judging by the number of publications. Many compounds in essential oils are either highly unsaturated or subject to thermal or oxidative degradation, so that SFE offers a clear advantage for them. One complication is that many plant oils have been obtained by steam distillation, which extracts the monoterpenes but leaves most of the sesquiterpenes behind. SFE removes monoterpenes and sesquiterpenes together, so that the composition and odour of the SFE product may be distinctly different. When the price paid for a

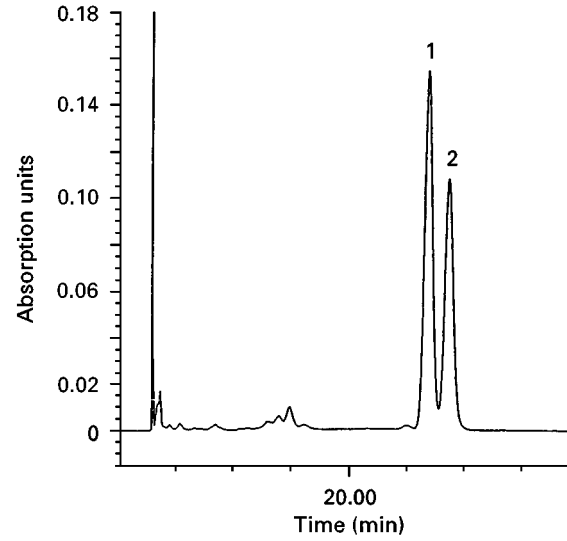


Figure 2 HPLC chromatogram of the SFE extract of carrots collected at 40°C and 50.5 MPa for 1 h with a flow rate of 600–750 mL min^{-1} of CO_2. 1, α-carotene; 2, β-carotene. (Reproduced with permission from Chandra A and Nair MG (1997) *Phytochemical Analysis* 8: 244. Copyright John Wiley & Sons Ltd.)

Table 2 Examples of extraction of essential oils, flavours and fragrances with conditions

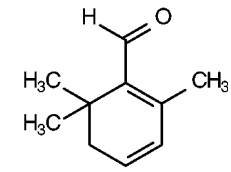
Extracted	*Matrix*	*Temperature (°C)*	*Pressure (MPa)*	*Additional information*
Onion flavour	*Allium cepa* (onion)	37	24.5	Flow 0.5 L min^{-1}, yield improved by EtOH modifier
Organo–sulfur compounds, cepaenes, allicin	*Allium tricoccum* (ramp)			
Root oil (118 compounds identified)	*Angelica archangelica*	40	12.0	1 h static, 2 h dynamic
Alkylpyrazines	*Arachis hypogaea* (roasted peanuts)	50	9.6	Density 0.35, lipids not extracted
Carvone, limonene	*Carum carvi* (caraway seed)	32	12.5	Time and flow rate affected yield
Capsacinoids	*Capsicum frutescens* (chili), *Capsicum annum* (paprika)	80		Density 0.75, H_2O, yield lower or equal to solvent extraction
Essential oil	*Cuminum cyminum* (cumin seed)	40	10.0	
Curcumin (**3**)	*Curcuma longa* (turmeric)	60	28.0	20% MeOH, 2 mL min^{-1}
Neral, geranial, geraniol, nerolic and geranic acids	*Cymbopogon citratus* (lemongrass)			
β-Phellandrene, *p*-cymene, cryptone, spathulenol and 86 others	*Eucalyptus camaldulensis*			
Limonene, fenchone, methylchavicol, anethole	*Foeniculum vulgare* (fennel)	31–35	8.0–8.4	10.0%
Anethole	*Illicium verum* (star anis)	80		Density 0.35, 90% pure
Olive oil aroma (hexanal, 2-hexenal, hexanol, 3-hexenol and others)	Olive oil and olives	40–45	7.7–11.5	Static 1–5 min, dynamic 30 min
Essential oil (limonene)	Orange peel	20–50	8–28	Optimum for limonene (99.5%) 35°, 12.5 MPa; optimum for linalool, 35°, 8.0 MPa
Kavain, yangonin, methysticin and derivatives	*Piper methysticum* (kava)			
Essential oil	*Piper nigrum* (black pepper)	30–50	15–30	
Carnosic acid	*Rosmarinus officinalis* (rosemary)	37–47	10–16	
Eugenol, eugenol acetate, α- and β-caryophyllenes	*Syzygium aromaticum* (clove buds)	50	24	
Tanshinone IIA	*Salvia militiorrhiza* (bunge)	60	24.5	0–10% MeOH
Pyrazines	*Theobromo cacao* (cocoa beans)	60	20.0	2% MeOH, 20 min

product depends upon odour, or content, this is important. Cedarwood oil obtained by SFE is closer in aroma to the original wood than steam-distilled oil. A disadvantage of SFE is that, at higher pressure, leaf waxes, which are not extracted by steam, are extracted as well. Freshly cut peppermint and spearmint plants extracted with supercritical and subcritical CO_2 (temperature 24–43°C) gave oils similar to that from steam distillation. A comparative study of essential oils and wax from lavender showed that the SFE extract contained three times as much linalyl acetate as the steam distillate; presumably the lower content was caused by hydrolysis. In the citrus industry it is reported that removing terpenes from citrus oil avoids oxidation to undesired products, while an SFE product can be used to re-blend to give new flavour. SFE of rosemary leaves for 10 min gave similar yield to that of 4 h sonication with CH_2Cl_2 (Table 2). There is interest in the antioxidants obtainable from rosemary and sage, obtainable by a two-stage SFE extraction of the essential oil followed by the antioxidants. In a study of extraction of orange peel, the dried peel should be reduced to 2 mm particles for rapid extraction. For particles of 0.3 mm, 75% of the total oil was extracted with a ratio of 6 kg of CO_2 per kg of orange peel. In a study of effect of different modifiers on the SFE of lemongrass oil, GC–mass spectrometry (GC-MS) indicated a different profile of monoterpenes depending upon the modifier.

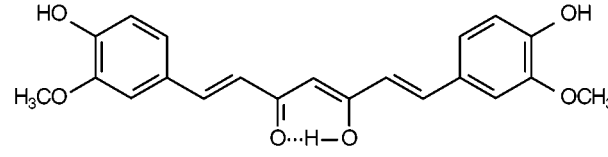
Flavours and Fragrances

The subject of flavours and fragrances overlaps with essential oils (Table 2). The mild conditions used for SFE with CO_2 can provide an accurate representation of the taste, colour and odour of natural substances found in herbs, spices, beverages and foods. Many studies have been of an analytical nature, to compare products obtained by different processes, to compare plant materials for quality, or to find the essential source of the desirable odour, as for example in the cases of coffee and olive oil. A brewed coffee aroma as similar as possible to the original brewed coffee has been obtained by SFE, monitored by smelling the product. Attempts have been made also to extract the aroma of virgin olive oil, from the oil and the olives, trap it on Tenax and analyse the product by GC-MS. SFE of cocoa beans with GC-MS analysis of the extract was used to assess the effect of storage on quality of the beans. Some alkylpyrazines were reduced after storage. There have been a number of studies of clove oil, none of which have reported a distinctly different yield between SFE and distillation methods. In many reports the yield is slightly lower by SFE. Hops have been the subject of study to produce bitter extracts for the brewing industry. The optimum conditions for an extract for the beer bitterness and aroma have been developed. Extraction of the leaves of hops gave no bitter extract. By adjusting the conditions the flavour of roasted peanuts could be collected without extracting the oil (Table 2).

Extraction of fennel seeds gave a higher yield by SFE (10.0%) than steam distillation (3.0%), about the same as extraction with hexane (10.6%), and less than ethanol extraction (15.4%), but the SFE and distilled products had a much more intense odour and taste than the solvent extracts. Cumin seed oil by SFE contained valuable components which would be thermally degraded by steam distillation. In the case of onion flavour oil SFE and liquid CO_2 extracts had the flavour of fresh onions, while the steam distillation solvent-extracted oil had a cooked onion flavour. The flavour of Emmentaler cheese during ripening has been followed by SFE and GC-MS, but further fractionation was needed because of the dominance of fatty acids, in order to analyse the less abundant alcohol, carbonyl and lactone aroma compounds. Guaca or quemadora (*Splilanthes americana*), with a slightly burning and numbing taste, is used in South American cooking. Comparison of steam-istilled and SFE extracts of leaves, stem and flowers showed significant differences (**Figure 3**). Eighty-eight compounds were identified in the extracts.



2 Safranal

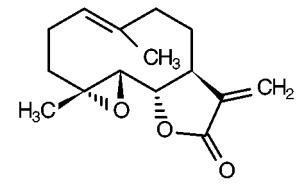


3 Curcumin

Saffron is such as expensive spice, that it is very liable to fraudulent imitation. The most important aroma compound, safranal (**2**), has been studied by isotope analysis. Synthetic safranal can easily be distinguished from the natural by ^{13}C-isotope content. SFE gave a cleaner and faster method of obtaining an extract of volatiles than solvent extraction, but there was some isotopic fractionation depending upon extraction yield.

Medicinal Compounds and Alkaloids

There are many medicinal compounds in plants that are targets for SFE, but many of these are more polar substances and are therefore more difficult to extract efficiently. The conditions must be explored for each example at our present state of knowledge (**Table 3**). Feverfew (*Tanacetum parthenium*) is much in demand as a herbal remedy and other worthless dried plants of similar appearance are frequently sold as feverfew. The value of the plant can be checked by SFE with CO_2 for the content of parthenolide (**4**), the active ingredient. Artemisinin (**5**) is an antimalarial present in *Artemisia annua*. Texanes and baccatins have been extracted from ground needles and seeds of *Taxus* spp. with 3% ethanol modifier; ethyl acetate, methanol, dichloromethane and diethyl ether have all been used as modifier for this purpose. In all cases waxy materials are co-extracted, so hexane solvent extraction was used first



4 Parthenolide

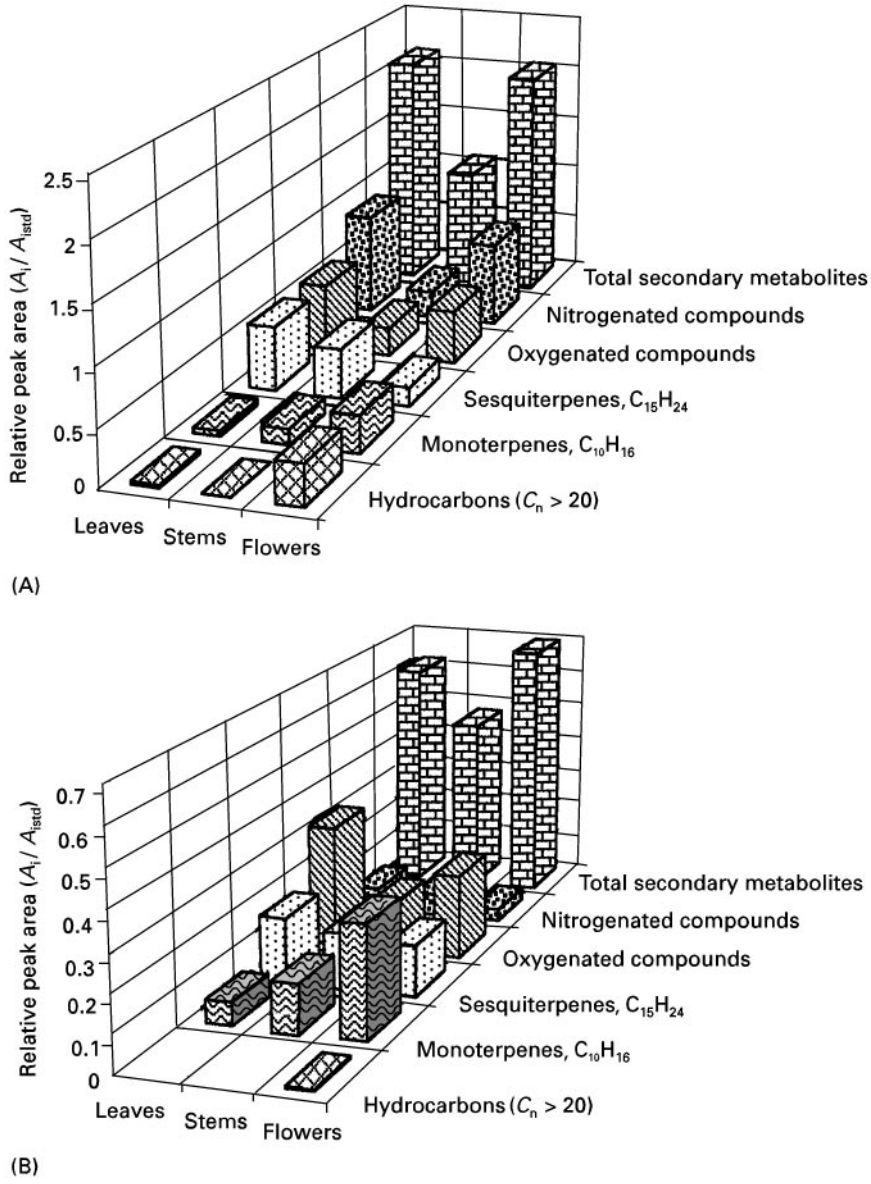
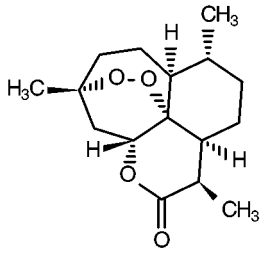


Figure 3 The composition of extracts obtained from different parts of the plant *Spilanthes americana* by (A) SFE and (B) simultaneous steam distillation–extraction. (Reproduced from Stashenko EE, Puertas MA and Combariza MY (1996) *Journal of Chromatography A* 752: 223 with permission from Elsevier Science.)



5 Artemisinin

to remove the waxes and then SFE was applied for the precursors of paclitaxel (taxol). Ginkgolides and bilobalides are extractable from *Ginkgo biloba* with 10% methanol added. There is a report of SFE of medicinal plants being directly coupled to a uterotonic bioassay on abdominal muscle to discover possible substances to induce uterine contraction.

The bark of *Magnolia officinalis* is used in Chinese medicine for a number of purposes. A major active compound is magnolol (**6**), a neolignin. SFE was compared with solvent extraction with phytosols, a series of new nonchlorinated fluorocarbon

Table 3 Examples of extraction of medicinal compounds, alkaloids and polar compounds with conditions used

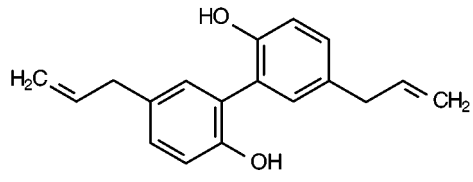
Extracted	*Matrix*	*Temperature (°C)*	*Pressure (MPa)*	*Additional information*
Atractylon (**8**)	*Atractyloides* rhizomes	40	10	2 mL min^{-1}, 20 s
Bile acids	Bovine bile	70	22	15% MeOH, 20 min, 88% recovery
Cedrelone (**9**)	*Cedrela toona*	40	40.0	30 min static, 40 min dynamic, 0.6 g sample wetted with 40 μL MeOH
Pyrethrins	*Chrysanthemum cinerariaefolium*	40	8.3	Most extracted in 3 h
Uterine contractants	*Clivia miniata, Ekebergia capensis, Grewia occidentalis, Asclepias fruticosa*		20–40	
Podophyllotoxin (**6**)	*Dysosma pleiantha* roots	40–80	13.6–34.0	With MeOH added, yield 95%
Phenols	Olive leaves	100	33.4	CO_2 density 0.70, 10% MeOH, total 2 mL min^{-1}, 140 min
Glycosylated flavonoids	*Passiflora edulis* (passion fruit leaves)	75	10.1	15% MeOH, 5 min, 1.75%
Schisandrols, schisandrins (lignans)	*Schisandra chinensis*	40–80	13.6–34.0	80% of that from MeOH extraction
Flavonoids	*Scutellariae radix*	50	20.0	CO_2–MeOH–H_2O 20 : 2 : 0.9
Isoflavones	Soybean products	50	60	20% EtOH, 1 h, 93%
Theobromine, caffeine, cocoa butter	*Theobromo cacao* (cocoa)	40–90	8.0–30	EtOH modifier

solvents of varying polarity. SFE with 10% added methanol gave the highest yield of magnolol (1.86%), and phytosol A gave the lowest (0.78%). Digoxin can be obtained from *Digitalis lanata* leaves by SFE, but the process is not very selective and various strategies have been tried to improve selectivity, including use of trifluoromethane and tetrafluoroethane as extractives, but no one alternative had a clear advantage.

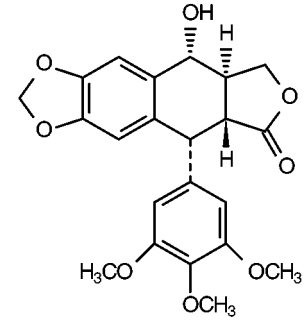
The antiviral compound podophyllotoxin (7) from *Dysosma pleiantha* and atractylon (8) from the oriental drug *Atractyloides* rhizome illustrate further the types of separations that have achieved. Atractylon, an oxidatively unstable compound, was extracted analytically in 20 s with a recovery 30% higher than by solvent extraction. The phototoxic furocoumarins (psoralen and derivatives) were extracted analytically from the vegetable celariac (*Apium graveolens*) by SFE, Soxhlet extraction with ethanol and sonication with chloroform. SFE gave higher extractions (**Figure 4**).

Polar Compounds

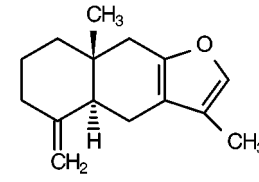
The wide range of polar compounds of interest as natural products is a greater challenge to the power of



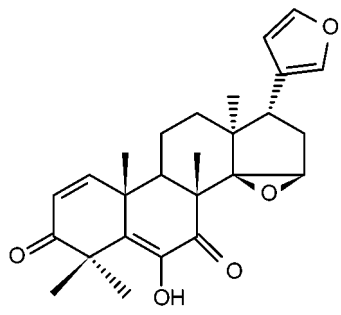
6 Magnolol



7 Podophyllotoxin



8 Atractylon



9 Cedrolone

SFE. The success obtained varies considerably, and our understanding of the physical process of diffusion, cell wall penetration, rate of dissolution and solubility are too rudimentary to make predictions. Attempts to extract the tetranortrieterpenoid azadirachtin ($C_{35}H_{44}O_{16}$) from neem (*Azadirachta indica*) seeds with or without added methanol did not give as high a yield as methanol solvent extraction. Attempts to remove triglyceride oil from the seeds first by selecting extraction conditions were not successful. Trichothecene mycotoxins at p.p.m. levels in wheat have been extracted for analytical purposes and determined online by chemical ionization–mass spectrometry (CI-MS).

McHugh and Krukonis have discussed the decaffeination of coffee extracts. The removal of nicotine from tobacco and snuff has also been achieved, chiefly for analysis. Some lignans and coumarins with lower numbers of hydroxyl groups can be extracted. Hydroxypinoresorcinol was extracted from *Fraxinus japonica* and other *Fraxinus* species using CO_2 with water modifier. The extraction of lignans by SFE from *Forsythia* species was as good as solvent extraction with refluxing hexane or ethanol. SFE gave better extraction of flavanones and xanthones from the root bark of the Osage orange (*Maclura pomifera*), provided 20% methanol was used as modifier, than liquid extraction, and in much shorter time. An unusual example is the determination of the alkaloids berberine and palmatine in *Phellodendri* cortex using ion pair SFE. The ion-pairing agent was dioctyl sodium sulfosuccinate, with 10% methanol as the modifier. The extraction required only 10 min. Microcystins, toxic peptides, have been extracted from cyanobacteria with a ternary mixture of 90% CO_2, 9.5% acetic acid and 0.5% water. Even the particulate material from hardwood smoke has been examined (the principal substances identified by GC-MS were guiacol and syringol derivatives).

Where cost is mentioned, SFE is in many cases admitted to cost more at present than Soxhlet extraction, but some studies suggest that costs can be reduced with proper experiment design, even for poorly soluble natural products.

Looking Ahead

SFE is firmly established as an industrial process for the isolation of a small number of natural products. Patents exist for a larger number of examples. As new industrial plant is bought into operation, and old

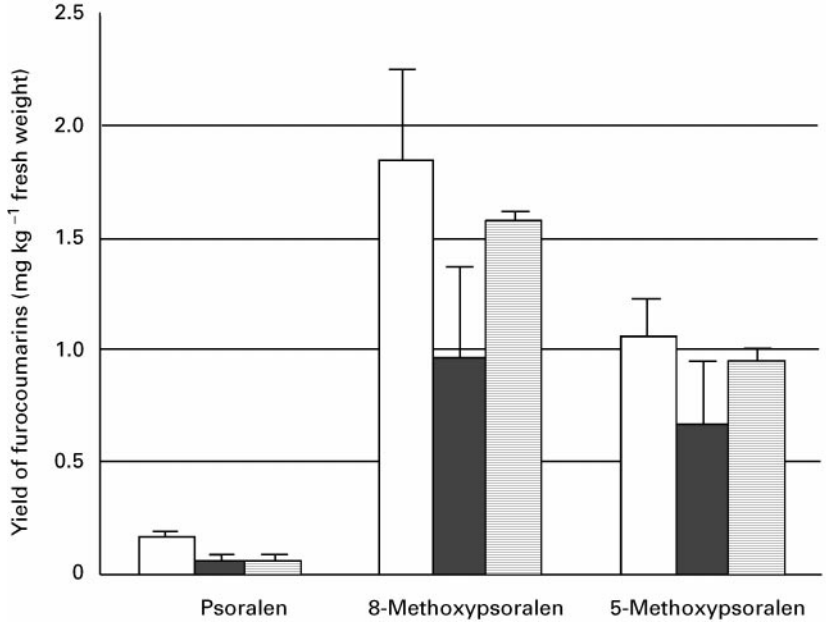


Figure 4 Comparison of extraction of three phototoxic furocoumarins from celeriac (*Apium graveolens*) by SFE (open columns), Soxhlet extraction (ethanol: filled columns) and sonication with chloroform (hatched columns). (Reproduced with permission from Järvenpää EP, Jestoi MN and Huopalahti R (1997) *Phytochemical Analysis* 8: 250. Copyright John Wiley & Sons Ltd.)

equipment removed, the number of examples will increase. Essential oils are going that way quickly. For polar substances, the published work shows that each material has to be examined to find the best conditions. It is difficult to assess how many natural products are being routinely extracted in this way, but the steady output of papers indicates continuing interest. As the chlorinated solvents are withdrawn, we can expect to see the use of SFE increase strongly. There is a ready market for some cheaper and less elaborate extraction equipment to meet this growing demand.

See also: **II/Chromatography: Supercritical Fluid:** Theory of Supercritical Fluid Chromatography. **Extraction:** Supercritical Fluid Extraction. **III/Supercritical Fluid Extraction-Supercritical Fluid Chromatography.**

Further Reading

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Thin-Layer (Planar) Chromatography

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Introduction

The use of thin-layer chromatography (TLC) for the analysis of plant extracts began in the 1960s, with the work of Stahl and Randerath. The continuing use, and further development of TLC in plant analysis is justified because of its rapidity and because of the availability of a number of different sorbents. TLC is also useful in plant analysis because it is possible to work on crude extracts, which is not the case with other analytical methods. In plant analysis, derivatization methods serve to increase sensitivity and selectivity in addition to providing evidence concerning the quality of the separation. A selection of the most important derivatization/detection methods are given in **Table 1**. As well as these advantages, TLC is very economical and can be employed for routine use because the consumption of solvent is very low, and it is possible to analyse numerous samples on the same plate. The literature on plant analysis by TLC is very extensive, with the most important contributions being those of Stahl, Randerath, Fried and Sherma, Harbone, and Wagner, who is our main reference on this topic. Most TLC studies are listed in the different pharmacopoeias of the world, as reported by Wagner. The classes of plant compounds separated by TLC covered in this article are as follows:

- Alkaloids
- Glycosides: flavonoids, coumarins, anthocyanins, ginkgolides, anthraquinone glycosides, cardiac glycosides
- Saponins
- Essential oils
- Cannabinoids
- Valepotriates
- Bitter principles

Alkaloids

Most plant alkaloids are tertiary amines; others contain primary, secondary, or quaternary nitrogen (**Figures 1–4**). The basicity of individual alkaloids varies considerably; depending on which of the four types is represented. The pK_b values lie in a range 10–12 for weak bases like purines to 3–7 for stronger bases like opium alkaloids. These factors must be borne in mind for extraction, and also for derivatization. The sample sizes applied to the TLC plate must be calculated, according to the average alkaloid content of the specific extracts. The majority of workers