ELSEVIER

Journal of Chromatography A, 703 (1995) 523–536

JOURNAL OF CHROMATOGRAPHY A

Review

Applications of supercritical fluid extraction in multidimensional systems

T. Greibrokk

Department of Chemistry, University of Oslo, P.O. Box 1033 Blindern, 0315 Oslo, Norway

Abstract

Sample preparation by supercritical fluid extraction (SFE) has recently been demonstrated to have many advantages compared to traditional methods. This article contains a review of applications where SFE has been connected on-line to chromatography. Examples of instruments and interfaces have been included and the main parameters responsible for the quality of the analytical data are discussed briefly. Off-line extractions are not included, except as a part of robotic systems.

The applications include hydrocarbons in various matrices, polymer additives, pesticides and chlorinated compounds in environmental samples, natural products and drugs and miscellaneous applications.

The major benefits of SFE in multidimensional systems are improved selectivities, reduced extraction times and the reduced number of sample handling steps which can be obtained by highly automated procedures.

Contents

1. Introduction

Multidimensional separation methods can lead to exceptionally high peak resolution, particularly when directly orthogonal techniques are combined [1]. A good example is the combination of exclusion chromatography and electrophoresis of proteins in TLC, utilizing two widely different separation principles. However, the purpose of a multidimensional system is often to

0021-9673/95/$09.50
SSDI 0021-9673(94)01174-5

transfer only a selected part of the sample from one dimension to the next, while the constituents of no interest go to waste. Furthermore, multidimensional methods also combine sample preparation and analysis with extensively developed automation [2].

Supercritical fluid extraction (SFE) allows a part of a sample to be transferred to the next separation step, depending on the density of the fluid, the temperature and the inclusion of modifiers. Compared to extraction with solvents, SFE has the advantage of being more compatible to environmental requirements and of having a variable selectivity depending on the chosen physical parameters. Multiple fractions can also be obtained by multi-stage extractions [2,3].

Coupled to other separation methods (Fig. 1), SFE allows increased sample throughput by reducing the extraction time (compared to Soxhlet extractions) and by reducing the number of sample handling steps. The latter is an advantage of all coupled systems. Another advantage is that hands-free operations reduce the risk of contaminating the sample during sample handling, provided carry-over effects are absent in the connecting tubing, valves and collector.

The sample transfer from the supercritical state is more easily adaptable to coupled systems than from samples in a liquid state, due to the high volatility of the fluid at atmospheric pressure, particularly of carbon dioxide which is the most frequently used fluid. Milliliter volumes of supercritical carbon dioxide can be transferred to small traps and the analytes can be collected at a speed which is significantly higher compared to samples in liquid solvents. Thus, supercritical fluid extraction have an distinct advantage in multidimensional systems as the initial separation method, except in analysis of dilute aqueous samples, which usually require removal of water by solid-phase extraction as the first sample preparation step.

Since most SFE instruments are available with autosamplers, the level of automation which can be obtained in multidimensional systems is high, provided sufficient cleaning procedures are incorporated to avoid sample-to-sample overlap of components of low volatility or solubility.

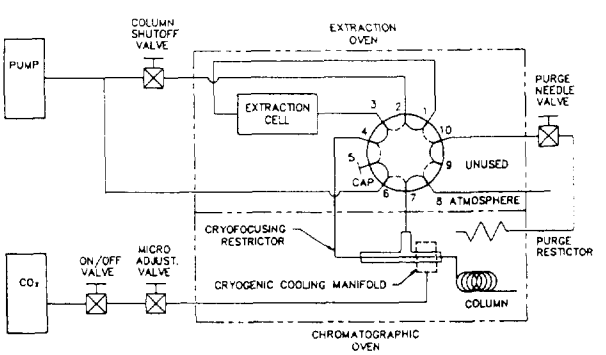

Fig. 1. A schematical representation of on-line SFE. From Ref. [60].

2. Extraction, solubility and modifiers

Carbon dioxide is usually chosen for SFE due to its many advantages, as outlined in a recent review article [4]. The solubility of polar compounds in carbon dioxide is, however, limited. With mixtures of CO_2 and organic solvents, more polar components can be extracted, but SFE with carbon dioxide can probably never be expected to become suitable for compounds of high water solubility. In pure CO_2 the solubility is a function of density and temperature, but in general non-polar to medium-polar components are extracted in good yields, although matrix effects may occasionally become more important than solubility alone. Based on determination of a series of common pollutants in sediments, the CO_2 extracts contained the compounds which were directly compatible with gas chromatography (GC) [5]. Thus, based on solubility, SFE can be coupled to GC, supercritical fluid chromatography (SFC) or liquid chromatography (LC) with expectations for good results. Samples containing large amounts of fats, such as in many polychlorinated biphenyl (PCB) determinations, need special attention to avoid ruining the column performance. Also very wet samples need drying agents in order to avoid plugging the restrictor with ice or filling the GC column with water.

Due more to matrix effects than to limited

solubility, the addition of modifiers may give higher extraction yields, even of hydrocarbons [6,7]. The modifier identity is often more important than the modifier concentration, since a major role of the modifier is to interact with the matrices to promote desorption into the fluid [8].

High temperatures can also be important for releasing analytes having strong interactions with sample matrices. Once the adequate pressure for analyte solubilization exists, temperature has been considered a more important variable than pressure [9]. Models describing the different processes involved in SFE are now available, bringing in a more systematic approach to the understanding of SFE [10].

In combinations of SFE and LC with more polar compounds, the need for modified CO_2 is obvious. In such cases higher concentrations of modifiers may be needed, requiring strict attention to the process of maintaining a reproducible and stable fluid mixture. Premixed fluids in cylinders can result in irreproducible modifier concentrations [11], and installation of an extra pump for modifier adds to the cost of the instrumentation. The simplest way of including a modifier is to add the solvent directly onto the sample in the extractor. In order to prevent that the modifier is swept too rapidly out of the extractor, static extraction followed by dynamic extraction appears to be a simple and efficient way of using modifiers in SFE. Thus, with 0.5 g sample sizes a combination of 5 min static and 10 min dynamic extraction gave high recoveries [8]. Combining static and dynamic extraction can be automated easily, requiring only the closing and opening of a valve.

The choice of extractor is related to the sample sizes and to the extent of automation, depending on the use of autosamplers. Most vessels are made of stainless steel. The use of a disposable polymeric vessel made from poly(ether ether ketone) has been reported to give a background [12], while a newer high-temperature polymer gave fewer contaminants [13].

Finally, the purity of the CO_2 gas may become the limiting factor in trace-level analysis, depending on the impurities present and the detector used [14].

3. Collection and transfer of extract

The interface between the extractor and the analyzer is extremely important for obtaining an instrument which can be used for routine purposes. Depending on the samples the interface may have to be optimized for the analytes and for the matrices. Some key words are: restrictor temperature and plugging, trap temperature, loss of solutes, trap dimensions, band broadening, peak focusing and memory effects.

In coupled systems the extracted material is deposited either at the inlet of the column, in a retention gap, in an external piece of tubing or on a solid sorbent, usually at reduced temperature, depending on the volatility of the analytes. A heated tubing at the inlet of the collector, or a heated restrictor, is required during dynamic extraction in order to avoid formation of plugs of solid CO_2 in the restrictor. In SFE–GC the heating can be supplied by the injector heater in a split injector or in an on-column injector. Restrictors made of fused-silica tubing (or metal), with an I.D. of 15–30 μm, produce gas flows of CO_2 of approximately 100–500 ml/min. Such high flow-rates combined with external cold traps in GC (where the gas is vented outside the GC column) can lead to low recoveries of volatiles. Thus, the choice of temperature of the cold trap can be a delicate balance between the need to collect the solutes at low temperature without simultaneously collecting water and plugging the restrictor. Restrictors with a fixed tubing or nozzle have a tendency to plug more often than heated variable restrictors.

In principle there are two different modes of coupling SFE to another separation technique. One is the on-line mode, connecting the two methods directly with tubing and valves. The other is the robotic mode, whereby the extract is transferred from the collector to the analytical column through robotic interaction [2]. Both modes have distinct, but different advantages. On-line coupling was first brought into use, particularly in SFE–GC, but both modes are available in commercial instruments. A simple interface for connecting SFE on-line to chromatographic techniques is shown in Fig. 1. The

supercritical fluid is pumped through the extraction cell, the extracted analytes are brought to the restrictor, precipitated at the lowered pressure and temperature and collected in the cold trap near the head of the chromatographic column. After extraction, the selection valve is switched to the column position, the trap is heated and the analytes are transferred by the mobile phase to the column, where band focusing is required if the band broadening in the trap becomes too high.

Care has to be taken in controlling memory effects, when components of low volatility/solubility are extracted. A short blank extraction between each sample may sometimes, but not always, be sufficient to clean tubing and valves. The whole extract is usually (but not necessarily, as in split SFE–GC) transferred to the chromatographic column. This is an advantage when analyzing small sample sizes with low concentrations of analytes.

Transferring the extract from the trap to the separation column requires thermal desorption in order to inject a sharp plug of sample and avoid band broadening [15,16]. If the extract can be collected in a narrow band in an open tubular cold trap, desorption is usually much more rapid than desorption from solid sorbents, with slow desorption kinetics. Thus, sufficient peak focusing can often be obtained simply by starting at low temperature in GC or at low pressure in SFC and thus maintaining high resolution in the chromatographic system, unless more efficient thermal desorption is needed [15,16]. Desorption with CO_2 of low density from a solid sorbent and focusing at the inlet of a packed SFC column was recently described, improving the detectability 1000-fold starting with solid-phase extraction, compared to direct injection [17].

In a robotic mode that has been implemented in one commercial instrument, the extract is deposited on a sorbent, flushed out with a solvent, collected in solution in vials and transferred to the autosampler of an analytical system with a robotic arm. The flushing with solvent which includes part of the connecting tubing reduces the risk for sample carry-over effects. Thus, the extract can be easily transferred to all analytical instruments equipped with an autosampler and multiple injections can be performed from each extract [2].

4. Hydrocarbons in soils, rocks, dust, sediments and tissues

Determination of total petroleum hydrocarbons (TPHs) has received significant attention recently. By conversion from the standard Freon extraction method to the US Environmental Protection Agency (EPA) draft method 3560, Lopez-Avila et al. [18] estimated that 30 000 l of Freon 113 could be saved per year in the USA. In an investigation of the determination of TPHs in a certified soil sample by SFE–GC, higher recovery, equally good or better reproducibility and more rapid procedures favored SFE–GC compared to Soxhlet extraction and determination by GC or IR [2].

Determination of volatile hydrocarbons with SFE–GC has previously suffered from some trapping problems [19]. Recently both soil samples and sediments were successfully analyzed by split SFE–GC using a thick film (5 μm) column at low temperature (-25°C) during trapping [20]. Gasoline to diesel range hydrocarbons (as low as *n*-pentane) could be determined. Wet sediments containing 25% water were analyzed by adding a drying agent (molecular sieve 3A). The total time of analysis was reduced from 18 h (with the Soxhlet method) to 80 min (with the SFE–GC method).

Polycyclic aromatic hydrocarbons (PAHs) have been extracted by SFE from a large variety of matrices. Most systematic studies have utilized off-line methods [8,21,22], although some early papers demonstrated the use of on-line coupling to GC [23–25]. Class fractionation according to ring size was obtained by extracting at 80, 125 and 200 atm [24] (1 atm = 101 325 Pa). In general there has been a tendency to obtain lower recoveries both at the low end and the high end of the PAH range. This is due to losses of the most volatile components in the collector and to low extraction yields of the large components. By more efficient trapping and by use of modifiers in a two-stage procedure, the recovery of PAH from soil samples by SFE in SFE–LC was

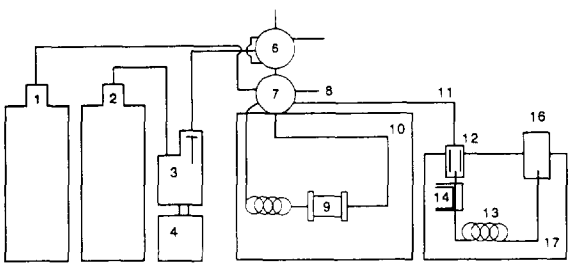

Fig. 2. Experimental set-up for the determination of high-molecular-mass hydrocarbons in natural gas using solid-phase preconcentration, SFE and on-line GC. 1 = Natural gas cylinder; 2 = CO_2 cylinder; 3 = SFE pump; 4 = cooling bath; 5 = on/off valve (not shown); 6 = switching valve; 7 = switching valve; 8 = natural gas flow restrictor; 9 = extraction cartridge; 10 = SFE oven; 11 = CO_2 flow restrictor; 12 = split injector; 13 = GC column; 14 = cold trap; 15 = CO_2 cylinder for cold trap (not shown); 16 = flame ionization detector; 17 = gas chromatograph. From Ref. [27].

recently shown to be significantly better than the recovery obtained by Soxhlet methods [2]. The two-membered ring compounds naphthalene and methylnaphthalenes are partially lost in the evaporation of solvent after Soxhlet extraction, but not by SFE [2]. GC is the most widely used method for determining PAH with up to four condensed rings, but HPLC is usually better suited for five- and six-membered rings and even for some of the smaller isomers. As in most coupled procedures with SFE, the total analysis time per sample by SFE–LC was approximately 1 h, which is a significant reduction compared to previous extraction and sample handling methods.

An example of large hydrocarbons which have been extracted by SFE is the fullerenes, which were separated by SFC in a coupled system [26].

By coupling solid-phase extraction to on-line SFE–GC, the higher hydrocarbons in natural gas (C_9–C_{20}) could be determined in an automated, completely unattended procedure [27], as shown in Fig. 2.

5. Polymer additives

Antioxidants, UV stabilizers and slip agents which are added to polymers can be determined by chromatographic methods after extraction or by infrared methods, if applicable [28]. A large number of additives are in use, depending on the properties of the polymer, and the concentrations need to be determined in the manufacturers' product control. Due to the slow mass transfer kinetics in polymers, polyolefin pellets must be sliced or ground to smaller particles in order to give acceptable yields with SFE [29,30]. Low-density polyethene has been extracted with good yields of some of the common additives [31], high-density polyethene was reported to be more efficiently extracted by Soxhlet extraction, while several compounded polymers gave high yields with SFE [30]. Extractions were performed at 90–120°C at 350 bar, with CO_2 or 5% of 2-propanol in CO_2. Higher pressures had no effect on the yields. By increasing the extraction time compared to the sample size, high-density polyethene can be extracted with good yields too. Soaking pellets in dichloromethane was used to increase the yields from polypropene [32]. Also utilizing SFE–SFC, polypropene film has been extracted at 350 bar and 40°C, with good yields, but restrictor plugging was mentioned as a problem with larger samples [33]. Effects from changes in CO_2 flow-rates on quantitative measurements were discussed [33]. Additives in poly(vinyl chloride) were determined using methanol as modifier [34].

Since capillary SFC has good chromatographic properties for most of the additives used, coupling SFE to SFC has become the most widely used two-dimensional technique in this field. Off-line SFE still dominates over on-line determinations of additives, an important reason being the need for representative sample sizes. However, small sample sizes have an advantage in avoiding the heavy constructions needed for large sample high pressure applications. Furthermore, small samples allow a high linear velocity in the extractor, reducing the extraction time, and also diminish the build-up of material from the polymers in restrictors and tubing. Thus, coupling SFE with either SFC or LC for determination of additives in polymers has a considerable growth potential, combined with improved sampling methods, smaller samples and automated equipment.

Extracting entrained volatiles is another qual-

ity test of some polymers. By combining SFE with GC–MS on-line, polybutylene terephthalate polymers were extracted at 200 bar and 55°C for determination of carbonic acid diphenyl ester and other volatiles [35].

6. Pesticides and chlorinated compounds in environmental samples

Determination of organic micropollutants in soil, sediments and similar matrices require little sample preparation, except possibly the removal of excess water, before SFE. Since "dirty" environmental samples contain many matrix components which are coextracted with the analytes, liquid extraction usually requires complex clean-up procedures. With the more selective SFE, extraction followed directly by chromatography is becoming increasingly important for environmental samples.

Even if low concentrations indicate the advantage of direct coupling to GC, SFC or LC, many applications so far utilizing SFE have been off-line methods [36] or static extraction of one sample [37]. This is not unexpected, since on-line techniques are still new and sometimes more complicated to set up. An example of a truly multidimensional method is the determination of ppb levels of the insecticide chlorpyrifos in grass samples by SFE–LC–GC. Off-line SFE and GC–electron-capture detection (ECD) gave too low selectivity and LC was incorporated to obtain a simpler chromatogram for better quantitation [38]. Another three-dimensional application is the determination of chlorinated pesticides and PCBs in fish fillets by SFE–SFC–GC [39]. The packed column SFC was incorporated to select a heartcut which was transferred to the cold trap and then to GC with ECD. The total analysis time was 2 h, which was one order of magnitude less than the procedure based on liquid extraction [39].

A combination of SFE and packed-column SFC was used to separate pesticides from the fat in soybean oil [40] and SFE–LC was used for the same purpose, with a size-exclusion column, but in corn oil [41]. Another combination of SFE–LC, with separation of pesticides from triglycerides on C_{18} cartridges, was used for analysis of cotton seed oil and corn oil (Table 1). With personal computer control, the method could be automated with a total sample preparation time of 15 min [42].

Table 1
Pesticide residues in crude cotton seed oil and corn oil using combined SFE–LC

Pesticides	Crude cotton oil (ng/g)	Corn oil (ng/g)
DDT	28.23	55.20
DDD	40.00	75.82
Benzene hexachloride	–	114.49
Heptachlor	–	72.16
Methyl parathion	165.07	–

From Ref. [42].

By using a thermal desorption modulator, extraction of pesticides can be followed on-line by incorporating high-speed GC with a radio-frequency plasma detector and a thermal energy analyzer for selective detection [43]. In the thermal desorption modulator the collected analytes are released by rapidly heating the collector by electrical pulses. The collector is coated by an electrically conductive paint.

Low concentrations of pesticides and other pollutants in aqueous samples need to be pre-concentrated, usually on solid-phase extractors, before starting the analytical procedures [44]. In order to transfer all the extracted analytes and to add another element of selectivity, the sorbents can be extracted by SFE. Depending on the properties of the analytes, combinations of SFE with GC, SFC or LC can be used. As shown in Fig. 3, organophosphorus pesticides were extracted from water on a C_{18} precolumn, the precolumn was dried with nitrogen and the analytes were extracted and transferred to the inlet of a packed SFC column with CO_2 at 150 bar [17]. With a thermionic detector, detection limits of between 0.1 ng/l and 1 μg/l were obtained, depending on the size of precolumn and the sample load on the precolumn. Organochlorine pesticides in water were also concen-

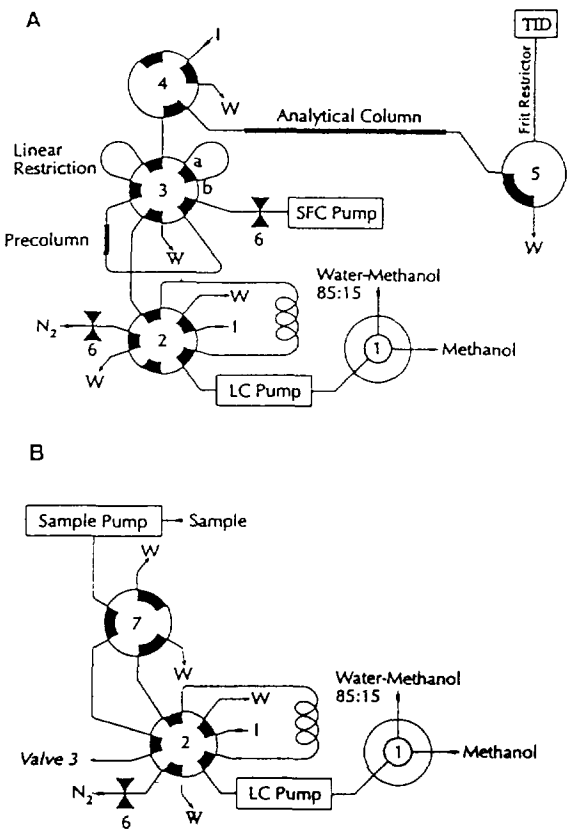

Fig. 3. (A) Instrumentation combining solid-phase extraction, SFE and SFC, with thermoionic detection for determination of organophosphorus pesticides. 1 = Solvent selection valve; 2, 3 = ten-port switching valves; 4 = injection valve with 100-nl loop; 5 = three-port switching valve; 6 = on/off valve. The desorption from the solid-phase extractor was performed with methanol-modified CO_2 at 150 bar for 5 min at 60 μl/min. (B) Set-up for the introduction of large volumes with a sample pump. Valve 7 is a six-port switching valve. (B) is connected to (A) via valve 3. From Ref. [17].

trated on C_{18} cartridges and determined by GC–ECD [45].

PCBs as well as polychlorinated dioxins, dibenzofurans and phenols have been extracted largely by off-line SFE. The applications with coupled SFE are much fewer and are mainly concerned with PCBs. In addition to the SFE–SFC–GC method mentioned above [39], SFE–GC has been used to determine PCBs in human milk and in blood plasma, both after solid-phase extraction [46]. The extraction of PCBs from samples with high fat content, without coextracting large amounts of fat, has been acknowledged as a problem [47], although by finetuning SFE conditions the yields of extracted fat can be reduced [36,48]. At a CO_2 density of 0.6 g/ml at 60°C, 75% of the PCBs and 2% of the fat from cod liver oil was extracted within 15 min. At a density of 0.8 g/ml, 88% of the PCBs but also 72% of the fat was extracted [48].

In a study of PCBs in cow's milk, SFE–SFC was utilized to separate the PCBs from fat. In this case SFE alone was reported not to give sufficient selectivity, even after Simplex optimisation of the procedure [49]. In fat fish with more than approximately 8% fat, additional cleaning procedures or splitless injection were recommended to avoid overloading the GC columns with fat [47], even if the collection on solid sorbents introduced another possibility for increased selectivity. Ground lyophilized fish samples were mixed with anhydrous sodium sulfate and extracted with CO_2 at 218 bar and 60°C. Dual GC columns were selected to increase the separation of PCBs from organochlorine pesticides [47]. Good recoveries were obtained compared to traditional Soxhlet extraction (Table 2).

In order to increase the robustness of extracting PCBs in the presence of fat, the addition of deactivated neutral alumina [50] or active basic alumina [51] helps retain the fat in the extractor. Extraction was performed with CO_2 at a pressure of 145 bar and a temperature of 60°C from human milk, blood serum and crab tissue [51]. The aqueous samples were concentrated on solid-phase extractors. Due to the high number of PCB congeners, capillary GC–ECD is generally chosen for quantitation. However, since a few of the PCBs, the "coplanar" congeners with no *ortho* substituents, are much more toxic than the other congeners, and since the concentrations of the "coplanars" are much lower, coupling SFE to LC (Fig. 4) has been used to separate the PCBs in three fractions [52]. Each fraction was analyzed by GC–ECD or GC–MS (Fig. 5).

Sulfonylurea herbicides and their metabolites have been determined with SFE–SFC [53].

Chlorinated phenols have been extracted from

Table 2
Comparison of SFE with Soxhlet extraction on PCBs in lyophilized fish (bleak)

PCB No.	Soxhlet, mean ± S.D. ($n = 2$) (ng/g)	SFE, mean ± S.D. ($n = 4$) (ng/g)	SFE recovery relative to Soxhlet (%)
28	4.0 ± 0.2	4.6 ± 0.1	114
52	12.6 ± 0.9	15.3 ± 0.8	122
101	30.7 ± 1.8	37.0 ± 1.2	120
105	7.1 ± 0.3	8.1 ± 0.4	114
118	24.3 ± 0.9	27.4 ± 1.0	113
128	5.3 ± 0.3	6.0 ± 0.3	113
138	34.7 ± 0.3	38.4 ± 0.8	111
149	28.0 ± 1.9	33.8 ± 1.5	121
153	44.9 ± 3.1	51.7 ± 1.8	115
156	2.7 ± 0.1	3.2 ± 0.2	118
170	4.9 ± 0.4	5.4 ± 0.2	111
180	21.4 ± 0.6	23.2 ± 0.7	108
DDE	82.5 ± 4.6	89.5 ± 2.8	108
DDD	12.0 ± 0.8	13.1 ± 0.8	108
DDT	3.6 ± 0.2	4.0 ± 0.2	110

From Ref. [47].

wood samples with 5% methanol in CO_2 using SFE–LC. The extraction was static, allowing aliquots to be transferred to the LC–UV for analysis. Good selectivity was obtained [54].

On-line systems accumulate contaminants from the CO_2 as well as the analytes. With ECD the background caused by such contaminants was found to increase the detection limits of pesticides and PCBs by at least a factor of 10, demonstrating the need for high-purity CO_2 [14].

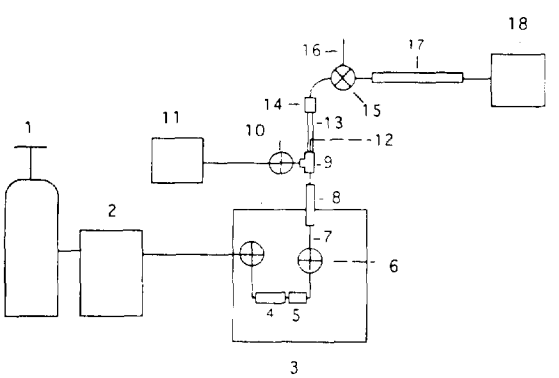

Fig. 4. Instrumentation for on-line SFE–LC for class separation of PCBs. 1 = CO_2 gas reservoir; 2 = SFE pump; 3 = extractor oven; 4 = extractor cell; 5 = alumina column; 6 = switching valve; 7 = restrictor; 8 = restrictor heater; 9 = T-coupling; 10 = switching valve; 11 = HPLC pump; 12 = restrictor tip; 13 = steel tubing; 14 = union; 15 = switching valve; 16 = fused-silica capillary; 17 = Pye columns; 18 = UV detector. From Ref. [52].

7. Natural products and drugs

7.1. Flavors and fragrances

Many applications with SFE in flavors and fragrances are off-line methods, since samples often are collected for multiple uses. The first time SFE was coupled to another separation technique (i.e. SFC) was in extracting caffeine from roasted coffee beans [55]. SFE–GC with mass spectrometry (MS) or flame ionization detection (FID) has been demonstrated for flavor compounds in spices, chewing gum, orange peel, spruce needles and cedar wood [56], from lime, lime peel, eucalyptus [57,58], basil [57,59], grapefruit oil [19,60], thyme [59,61], orange juice [58] and chamomile [59]. Many of the earlier applications were of a qualitative nature. In a comparison between SFE–GC and SFE–SFC, the latter was given an

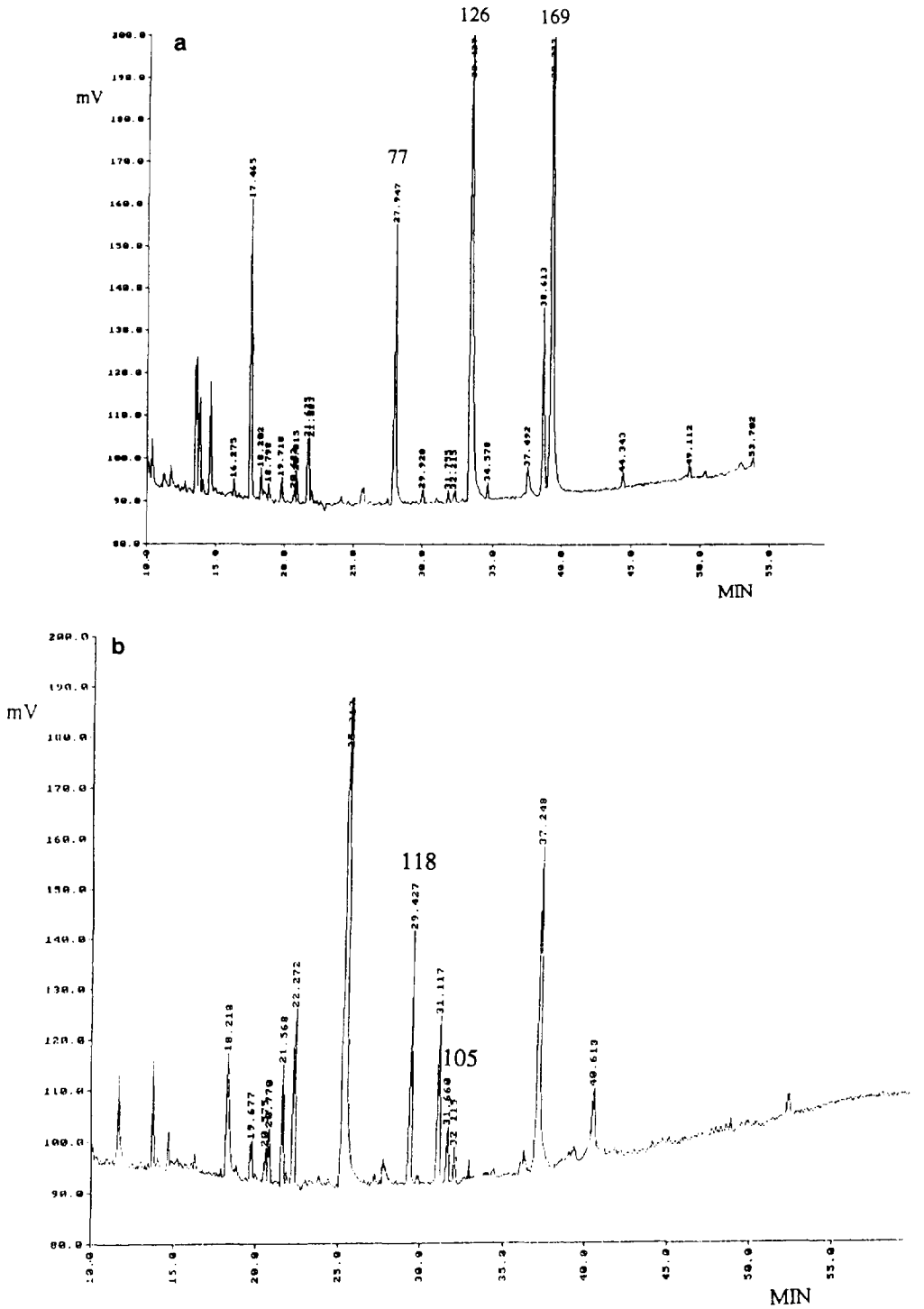

Fig. 5 (continued on p. 532).

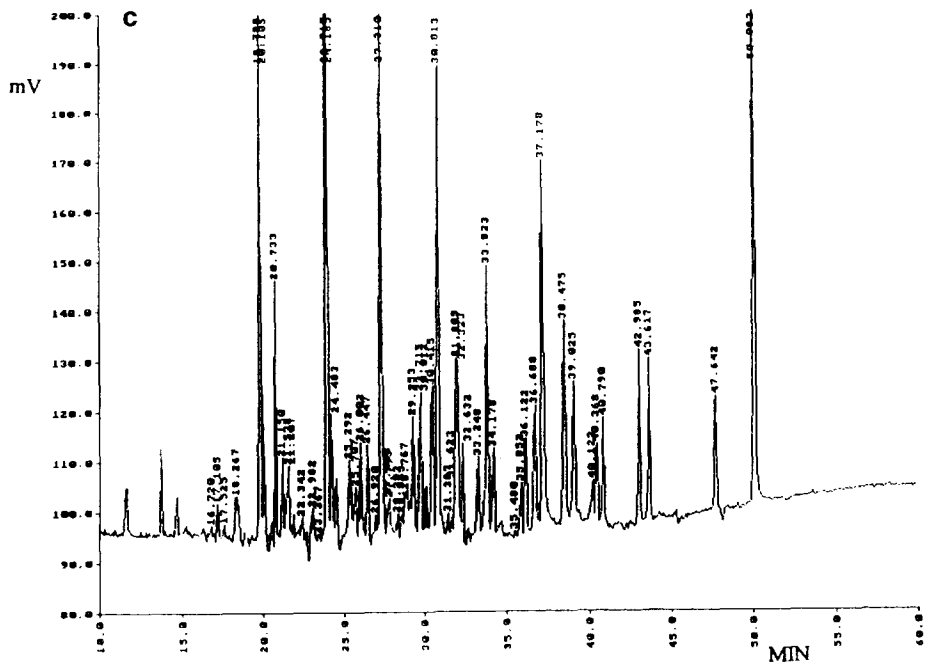

Fig. 5. (a) Non-*ortho*-substituted PCBs (congeners 77, 126, 169), separated from (b) mono-*ortho*-substituted PCBs (congeners 105, 118) and (c) poly-*ortho* congeners, determined in crab hepatopancreas after on-line SFE–LC. From Ref. [52].

advantage due to higher yields of oxygenated terpenes and no need for derivatization [59].

7.2. Lipids

Thanks to good solubility of many lipids in supercritical fluids, combinations of SFE and SFC are the most common coupled techniques, although off-line methods are often preferred due to the need for larger sample amounts for various uses [62]. SFE results agree very well with Soxhlet extractions in determination of total oil content in foods [63]. Fatty acids in glycerides have been determined by in situ transesterifications in the extractor, either by chemical methods and coupled to GC [64] or by immobilized enzymes and coupled to SFC [65]. The coupling, the packing of the reactor/extractor and a chromatogram is shown in Figs. 6–8.

Retinol palmitate and tocopherol acetate were determined in ointment by SFE–SFC [66]. A mixture of fatty acids, esters and glycerides was involved in a study of phase transfer and memory effects in SFE–SFC after sample application on sorbents [67].

Freeze-dried hamster feces were analyzed by SFE–SFC in order to measure fatty acids and sterols in dietary relations [68]. The extraction was performed at 400 bar and 40°C.

Oleoresins such as turmerons and curcuminoids have been extracted from turmeric, a

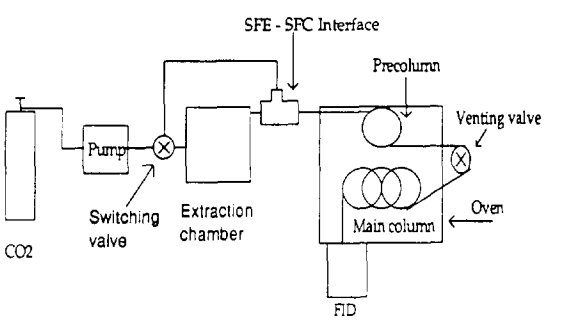

Fig. 6. Instrumentation for on-line SFE–SFC. From Ref. [65].

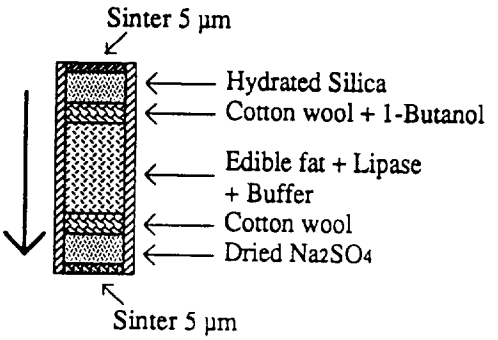

Fig. 7. Extraction cell with immobilized enzyme for on-line transesterification of edible fat. From Ref. [65].

widely used spice and additive in foods and beverages [69]. The extraction and the chromatography (SFC) were performed with 20% methanol in CO_2.

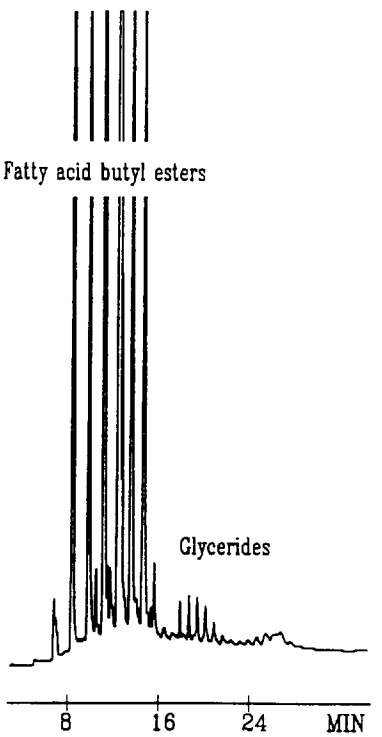

Fig. 8. On-line transesterification–SFE–SFC of edible fat to fatty acid butyl esters. From Ref. [65].

7.3. Drugs

Prostaglandins have been extracted from drugs [70] as well as from aqueous solutions [71]. A glycoside, ouabain, was used as a model compound to study the coupling of SFE and SFC, combined with fraction collection, allowing determination of the biological activity of the collected fractions [72]. A cytostatic, mitomycin C, has been determined in plasma by SFE–SFC after application on a XAD-2 sorbent. After drying the sorbent, the drug was extracted and chromatographed with 12% methanol in CO_2 [73].

By combining SFE–SFC–SFC with MS, a "digitalis like factor" was looked for in peritoneal dialysates [74].

SFE has also been coupled to flow injection analysis for determination of the antibiotics chloramphenicol and penicillin G [75].

In general the drugs must not be too water soluble, if extraction with CO_2 or modified CO_2 can be performed successfully. Since levels of drugs in body fluids normally are low, concentration on sorbents will almost always be required. This process should be automated, as well as washing and drying of the sorbent. Efficient drying is a requirement for avoiding problems with the restrictor and rapid, efficient desorption is a requirement for high yields and narrow bands.

8. Miscellaneous

Organotin compounds have been determined in soil with SFE–GC [76]. Dialkyltin compounds in poly(vinyl chloride) were determined by SFE–SFC after CO_2 modification with acetic acid [77].

Explosives and propellants have been determined by SFE–SFC in residues for forensic investigations [78]. With ECD, 100 pg amounts could be determined.

Flame retardants in polyurethane foams were determined by SFE–SFC [79].

By coupling SFE directly to Fourier transform IR, different polymer fibers were extracted and the fiber finishes ranging from polysiloxanes to

surfactants, fatty acid esters and antioxidants were determined [80].

9. Conclusions

Most of the initial coupling of SFE to chromatographic methods was obtained with GC. Since there is a high compatibility between solubility in CO_2 and volatility in GC, this trend is likely to continue to be developed into highly automated systems. Robotic systems are certain to be of high interest when fraction collection or multiple uses are required, in routine analysis.

Combinations of SFE and SFC are to some extent limited by the much smaller group of chromatographers familiar with SFC, compared to GC. Also the application area for SFC is not as clearly defined as that for GC. At the moment lipids and polymer additives are probably the two groups of compounds with most success in using SFE–SFC. However, many more areas have large development potentials, provided sufficient time is spent on designing the methods for handling practical problems, such as plugging and carry-over effects.

The main challenge lies in combining SFE with SFC or LC for the more polar compounds, particularly in determinations of drugs in body fluids and tissues. Within the limits set by the solubility in modified CO_2, this area is a wide and so far largely unexplored field.

References

[1] J.C. Giddings, *Anal. Chem.*, 56 (1984) 1258A.
[2] D.R. Gere, C.R. Knipe, P. Castelli, J. Hedrick, L.G. Randall Frank, H. Schulenberg-Schell, R. Schuster and L. Doherty, *J. Chromatogr. Sci.*, 31 (1993) 246.
[3] T. Greibrokk, M. Radke, M. Skurdal and H. Willsch, *Org. Geochem.*, 18 (1992) 447.
[4] T.L. Chester, J.D. Pinkston and D.E. Raynie, *Anal. Chem.*, 66 (1994) 106R.
[5] A.L. Kvernheim, K. Martinsen, G.E. Carlberg, B.E. Berg, M. Fresvig and T. Greibrokk, *Chemosphere*, 27 (1993) 733.
[6] J.M. Levy, L. Dolata, R.M. Ravey, E. Storozynsky and K.A. Holowczak, *J. High Resolut. Chromatogr.*, 16 (1993) 368.
[7] T.M. Fahmy, M.E. Paulaitis, D.M. Johnson and M.E.P. McNally, *Anal. Chem.*, 65 (1993) 1462.
[8] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller and J. Pawliszyn, *Anal. Chem.*, 66 (1994) 909.
[9] J.J. Langenfeld, S.B. Hawthorne, D.J. Miller and J. Pawliszyn, *Anal. Chem.*, 65 (1993) 338.
[10] K.D. Bartle, A.A. Clifford, S.B. Hawthorne and D.F.G. Walker, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 248.
[11] F.K. Schweighardt and P.M. Mathias, *J. Chromatogr. Sci.*, 31 (1993) 207.
[12] B.E. Berg, E.M. Hansen, S. Gjørven and T. Greibrokk, *J. High Resolut. Chromatogr.*, 16 (1993) 358.
[13] P.B. Liescheski, H.L. Walters and J. Tehrani, *Natl. Meet. —Am. Chem. Soc. Div. Environ. Chem.*, 33 (1993) 355.
[14] M.W.F. Nielen, J.A. Stäb, H. Lingeman and U.A.Th. Brinkman, *Chromatographia*, 32 (1991) 543.
[15] Z. Liu, P.B. Farnsworth and M.L. Lee, *J. Microcol. Sep.*, 4 (1992) 199.
[16] M. Wu, Z. Liu, P.B. Farnsworth and M.L. Lee, *Anal. Chem.*, 65 (1993) 2185.
[17] B.N. Zegers, H.-J. de Geus, S.H.J. Wildenburg, H. Lingeman and U.A.Th. Brinkman, *J. Chromatogr. A*, 677 (1994) 141.
[18] V. Lopez-Avila, J. Benedicto, N.S. Dodhiwala, R. Young and W.F. Beckert, *J. Chromatogr. Sci.*, 30 (1992) 335.
[19] M. Ashraf-Khorassani, M.L. Kumar, D.J. Koebler and G.P. Williams, *J. Chromatogr. Sci.*, 28 (1990) 599.
[20] M.D. Burford, S.B. Hawthorne and D.J. Miller, *J. Chromatogr.*, in press.
[21] S.B. Hawthorne, S. Eckert-Tilotta, K. Hegvik, D.J. Miller, M.D. Burford, J.J. Langenfeld and J. Field, *Natl. Meet. —Am. Chem. Soc. Div. Environ. Chem.*, 33 (1993) 331.
[22] V. Lopez-Avila, R. Young, J. Tehrani, J. Damian, S. Hawthorne, W.F. Beckert, J. Dankers and C. van der Heiden, *J. Chromatogr.*, in press.
[23] S.B. Hawthorne and D.J. Miller, *J. Chromatogr. Sci.*, 24 (1986) 258.
[24] B.W. Wright, S.R. Frye, D.G. McMinn and R.D. Smith, *Anal. Chem.*, 59 (1987) 640.
[25] S.B. Hawthorne and D.J. Miller, *J. Chromatogr.*, 403 (1987) 63.
[26] K. Jinno, H. Nagashima, K. Itoh, M. Saito and M. Buonoshita, *Fresenius' J. Anal. Chem.*, 344 (1992) 435.
[27] H.-G. Janssen, C.A. Cramers, L. van der Meulen-Kuijk and A.L.C. Smit, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritial Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 148.
[28] M.W. Raynor, K.D. Bartle, I.L. Davis, A. Williams, A.A. Clifford, J.M. Chalmers and B.W. Cook, *Anal. Chem.*, 60 (1988) 427.

[29] M. Ashraf-Khorassani, D.S. Boyer and J.M. Levy, *J. Chromatogr. Sci.*, 29 (1991) 517.
[30] S. Ourén, T. Greibrokk, D.R. Hegna and K. Kleveland, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 308.
[31] M. Ashraf-Khorassani and J.M. Levy, *J. High Resolut. Chromatogr.*, 13 (1990) 742.
[32] H. Daimon and Y. Hirata, *Chromatographia*, 32 (1991).
[33] L. Baner, T. Bücherl, J. Ewender and R. Franz, *J. Supercrit. Fluids*, 5 (1992) 213.
[34] T.P. Hunt, C.J. Dowle and G. Greenway, *Analyst (London)*, 116 (1991) 1299.
[35] S. Schmidt, L. Blomberg and T. Wännman, *Chromatographia*, 28 (1989) 400.
[36] F. David, M. Verschuere and P. Sandra, *Fresenius' J. Anal. Chem.*, 344 (1992) 479.
[37] H. Engelhard and A. Gross, *J. High Resolut. Chromatogr.*, 11 (1988) 726.
[38] H.J. Cortes, L.S. Green and R.M. Campbell, *Anal. Chem.*, 63 (1991) 2719.
[39] K.S. Nam, S. Kapila, A.F. Yanders and R.K. Puri, *Chemosphere*, 23 (1991) 1109.
[40] B. Murugaverl and K. Voorhees, *J. Microcol. Sep.*, 3 (1991) 11.
[41] D.L. Stalling, S. Saim, K.C. Kuo and J.J. Stunkel, *J. Chromatogr. Sci.*, 30 (1992) 486.
[42] M. Ahmad, A.J. Berry and E.D. Ramsey, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 282.
[43] M. Wu, E.S. Francis, Z. Liu, P.B. Farnsworth and M.L. Lee, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 255.
[44] J.B. Pawliszyn and N. Alexandrou, *Water Pollut. Res. J. Can.*, 24 (1989) 207.
[45] M. Vershuere, F. David and P. Sandra, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 291.
[46] H.R. Johansen, C. Thorstensen, T. Greibrokk and G. Becher, *J. High Resolut. Chromatogr.*, 16 (1993) 148.
[47] S. Bøvadt, B. Johansson, P. Fruekilde, M. Hansen, D. Zilli, B. Larsen and J. de Boer, *J. Chromatogr. A*, 675 (1994) 189.
[48] F. David, A. Kot, E. Vanluchene, E. Sippola and P. Sandra, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 10.
[49] A.G. Mills and T.M. Jefferies, *J. Chromatogr.*, 643 (1993) 409.
[50] J.E. France, J.W. King and J.M. Snyder, *J. Agric. Food Chem.*, 39 (1991) 1871.
[51] H.R. Johansen, G. Becher and T. Greibrokk, *Fresenius' J. Anal. Chem.*, 344 (1992) 486.
[52] H.R. Johansen, G. Becher and T. Greibrokk, *Anal. Chem.*, in press.
[53] M.E.P. McNally and J.R. Wheeler, *J. Chromatogr.*, 435 (1988) 63.
[54] M.H. Liu, S. Kapila, K.S. Nam and A.A. Elseewi, *J. Chromatogr.*, 639 (1993) 151.
[55] K. Sugiyama, M. Saito, T. Hondo and M. Senda, *J. Chromatogr.*, 332 (1985) 107.
[56] S.B. Hawthorne, M.S. Krieger and D.J. Miller, *Anal. Chem.*, 60 (1988) 472.
[57] S.B. Hawthorne, D.J. Miller and M.S. Krieger, *J. Chromatogr. Sci.*, 27 (1989) 347.
[58] J.L. Levy and M. Ashraf-Khorassani, in K. Jinno (Editor), *Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction (Journal of Chromatography Library*, Vol. 53), Elsevier, Amsterdam, 1992, p. 197.
[59] M.-L. Riekkola, P. Manninen and K. Hartonen, in K. Jinno (Editor), *Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction (Journal of Chromatography Library*, Vol. 53), Elsevier, Amsterdam, 1992, p. 275.
[60] M.R. Andersen, J.T. Swanson, N.L. Porter and B.E. Richter, *J. Chromatogr. Sci.*, 27 (1989) 371.
[61] K. Hartonen, M. Jussila, P. Manninen and M.-L. Riekkola, *J. Microcol. Sep.*, 4 (1992) 3.
[62] M.T.G. Hierro and G. Santa-Maria, *Food Chem.*, 45 (1992) 189.
[63] S.L. Taylor, J.W. King and G.R. List, *J. Am. Oil Chem. Soc.*, 70 (1993) 437.
[64] J.W. King, J.E. France and J.M. Snyder, *Fresenius' J. Anal. Chem.*, 344 (1992) 474.
[65] B.E. Berg, E.M. Hansen, S. Gjørven and T. Greibrokk, *J. High Resolut. Chromatogr.*, 16 (1993) 358.
[66] M. Masuda, S. Koike, M. Handa, K. Sagara and T. Mizutani, *Anal. Sci.*, 9 (1993) 29.
[67] U. Ullsten and K.E. Markides, *J. Microcol. Sep.*, 6 (1994) 385.
[68] J.D. Pinkston, T.E. Delaney, D.J. Bowling and T.L Chester, *J. High Resolut. Chromatogr.*, 14 (1991) 401.
[69] M. Marsin Sanagi, U.K. Ahmad and R.M. Smith, *J. Chromatogr. Sci.*, 31 (1993) 20.
[70] D.A. Roston, *Drug Dev. Ind. Pharm.*, 18 (1992) 245.
[71] I.J. Koski, B.A. Jansson, K.E. Markides and M.L. Lee, *J. Pharm. Biomed. Anal.*, 9 (1991) 281.
[72] Q.L. Xie, K.E. Markides and M.L. Lee, *J. Chromatogr. Sci.*, 27 (1991) 365.
[73] W.M.A. Niessen, P.J.M. Bergers, U.R. Tjaden and J. van der Greef, *J. Chromatogr.*, 454 (1988) 243.
[74] R.X. Moulder, L.N. Tyrefors and K.E. Markides, in P. Sandra and K. Markides (Editors), *The 2nd European Symposium on Analytical Supercritical Chromatography and Extraction, Riva del Garda, 1993*, Huethig, Heidelberg, 1993, p. 210.

[75] J.D. Brewster, R.J. Maxwell and J.W. Hampson, *Anal. Chem.*, 65 (1993) 2137.
[76] Y. Liu, V. Lopez-Avila, M. Alcaraz and W.F. Beckert, *J. High Resolut. Chromatogr.*, 16 (1993) 106.
[77] J.W. Oudsema and C.F. Poole, *J. High Resolut. Chromatogr.*, 16 (1993) 198.
[78] A. Munder, R.G. Christensen and S.A. Wise, *J. Microcol. Sep.*, 3 (1991) 127.
[79] G.A. MacKay and R.M. Smith, *Anal. Proc.*, 29 (1992) 463.
[80] C.H. Kirschner, S.L. Jordan, L.T. Taylor and P.D. Seemuth, *Anal. Chem.*, 66 (1994).