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Review

Coupling of supercritical fluid extraction with chromatographic techniques

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CONTENTS

1. INTRODUCTION

The growing interest in supercritical fluid extraction (SFE) is highlighted by the many monographs and reviews which have appeared in the literature since 1978[1–16]. The ability of a supercritical fluid (SF) to solubilize solids was first reported by Hannay and Hogarth[17] in 1879, when they noted that metal halides became soluble in supercritical ethanol as pressure was increased. Studies of solubilities in SFs continued during the following decades on a sporadic basis. An admirable review by Booth

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and Bidwell[18] covers this research up to 1949. About this time SFE was being scrutinized closely for its potential to reduce energy costs in process engineering, compared with conventional separation processes such as distillation or solvent extraction. Work by Kerr-McGee Refining (USA) led to the Residual Oil Supercritical Extraction (ROSE) process being developed in the 1950s for the removal of lighter products from the residue of the commercial distillation of crude oil[8]. Numerous other process applications were developed[2,10,15].In the 1970s, foodstuffs became the centre of focus for SFE. Many patents resulted from these early studies, covering the SFE of hops, coffee, tea, tobacco and spices. In 1979, Hag (F.R.G.) built the first large-scale production plant using SFE to remove caffeine from green coffee beans[13].

In the 1980s, increasing attention was given to the use of SFE as a means of sample preparation in analytical chemistry. The main advantages of SFE are (i) an improved efficiency, as extraction times, in comparison with Soxhlet extractions, are reduced from hours to minutes; (ii) the use of a non-toxic and cost-effective extraction solvent (carbon dioxide); (iii) the potential of extracting thermally labile compounds; (iv) the simplicity of controlling the extraction conditions; (v) the ease of separating the analytes from an SF; (vi) the possibility for direct analysis of complex matrices, thus reducing the risk of sample contamination; (vii) the potential of fractionation; (viii) the compatibility of the method with on-line methods; and (ix) the possibility of class-selective extraction by choosing the proper fluid polarity, density and/or entrainer. In general, the transport properties of the SFs, high diffusivity and low viscosity, favour high mass-transfer rates and the low fluid density facilitates phase separations in solid-fluid or liquid-fluid operations. Excellent qualitative and quantitative results have been documented, confirming the potential of SFE when coupled with various methods of analysis.

2. PRINCIPLES OF SFE

SFs possess physico-chemical properties intermediate between those of liquids and gases (Table 1)[19,20]. The density of an SF is typically 100-1000 times higher than that of a gas and comparable to that of a liquid. Consequently, molecular interactions can be strong owing to short intermolecular distances[21]. As a result, the solvation properties are similar to those of liquids, but with significantly lower viscosities and higher diffusion coefficients. The 10–100 times lower viscosities and the 10–100 times higher diffusion coefficients in SFs compared with liquids result in a significantly enhanced mass transfer of solutes in extractions with SFs than in extractions with liquids[1,15,22].

TABLE 1

PHYSICAL PROPERTIES OF SUPERCRITICAL FLUIDS, LIQUIDS AND GASES

Property	*Gas*	*Supercritical fluid*	*Liquid*
Density (g/ml)	10^{-3}	0.2 – 0.9	1.0
Viscosity (g/cm·s)	10^{-4}	10^{-4}–10^{-3}	10^{-2}
Diffusity (cm^2/s)	10^{-1}	10^{-3}–10^{-4}	$<10^{-5}$

The potential advantages of SFE accrue from the physico-chemical properties of the SFs[23], *viz.*, large changes in SF density (and hence solvating power) can be effected by small changes in pressure, because the compressibility of SFs is large just above the critical temperature (T_c)[9]. As the solvent strength of an SF is directly related to its density, the solvating ability of an SF towards a particular species can easily be modified by changing the extraction pressure (and, to a lesser extent, the temperature)[24]. SFs thus have "tunable" solvent strengths which make selective extraction possible. With the greatly enhanced mass-transfer properties in comparison with liquids, the use of SFs provides more rapid extraction rates and improved extraction efficiencies owing to better penetration of the matrix[25]. In addition, SFs do not have the surface-tension or wetting problems associated with liquid extraction".

Schneider' made the important point that the solvent power of an SF cannot exclusively be explained from its density increase, and Giddings et *al.*[26] elaborated this by stating that the solvent power of an SF has two facets, a "state effect" and a "chemical effect". The principal variable of the "state effect" is the density of the SF, whereas the "chemical effect" is unique to each solute and dependent on its polarity, acid-base properties and hydrogen-bonding properties. Some general rules regarding the observed relationship between the extractability with supercritical carbon dioxide and the chemical structure of model substances from various groups of naturally occurring materials have been formulated by Stahl[27].

In practice, the choice of the SF depends on the polarity of the analyte(s), the solvent strength and selectivity required, the thermal stability of the extracted compound at the necessary operating temperature and the instrumental limitations which are associated with the high critical pressures (P_c) of some of the SFs. Usually the SF is applied at a temperature higher than its critical value and at a pressure significantly higher than the critical pressure of the fluid'*. A considerable variety of SFs have been used in SFE covering a wide range of critical temperatures and pressures, molecular size and polarity[7,14]. Among these carbon dioxide is the fluid most frequently used. It has a moderate critical pressure (73.8 bar) and, with its low critical temperature (31.1°C), it is ideal for the extraction of many thermally labile compounds. However, carbon dioxide has its limitations, especially for the extraction of polar compounds.

A way to increase the polarity of an SF extraction solvent is to add small amounts of polar liquids (e.g., acetonitrile, methanol or water), which are referred to as entrainers in SFE[11,13,28] and as modifiers in SFC[19]. The effect of adding as little as 1 mol-% of entrainer to an SF can be dramatic and the solubility may be increased by several orders of magnitude. This increase appears to be restricted to solutes with a certain chemical functionality (polarity)[11]. It should be noted that the addition of entrainers will also change the critical properties of the mixture; these can be approximated by the equations used by Reed and Sherwood*':

$$T_c = X_a T_a + X_b T_b \quad (1)$$

$$P_c = X_a P_a + X_b P_b \quad (2)$$

Where T_c and P_c are the critical parameters for the mixed extraction solvent, X_a and X_b are the mole fractions of the solvent A and B, T_a and T_b are the critical temper-

ature of the solvents A and B, respectively and P_a and P_b are the corresponding critical pressures. More elaborate treatments are based on the methods of Cheuh and Prausnitz[30] for T_c and Kreglewski and Kay[31] for P_c. The use of different extraction pressures, entrainers and fluids with varying polarity is particularly valuable in allowing "class-selective" extraction methods to be developed[24].

To determine if an extraction process of interest is practically feasible, it is necessary to have an adequate quantitative representation of the phase equilibria for the extracted compound(s) and fluid(s) involved'. Without this information, process models cannot be made, and operating conditions, solvent flow-rates and extraction yields cannot be predicted[13]. A great deal of effort has been focused on correlating, and in some instances predicting, the solubility of various solutes in SFs using basic thermodynamics. In addition, extensive work has been carried out to depict the phase diagrams to allow a better understanding of the pressure-temperature domains which may be of interest in SFE[1,5,12,13,32–34].

Because SFE combines the processes that are involved in distillation and in liquid-liquid extraction, SFE has also been termed "destraction"[35]. Randall[4] preferred the term "dense gas" for SFs to emphasize the fact that the most important parameter in SFE is, in fact, neither the absolute pressure nor the absolute temperature, but the density.

3. SFE IN ANALYTICAL APPLICATIONS

3.1. *General aspects*

Until recently, the use of SFE has generally been confined to relatively large-scale chemical processing applications[7,8,10,11,14,15]. SFE is now also attracting increased attention for analytical purposes. SFs exhibit a large compressibility above their critical temperature, and small changes in pressure result in large changes in density and, therefore, in a variable solvating power of the SF. In addition, various SFs (or entrained SFs) that exhibit different specific chemical interactions can be used for selective extractions with efficiencies comparable to or better than those of conventional techniques. Other positive features of SFE in analytical applications are (i) its potential to reduce the sample preparation time, which results in faster analyses, reduced costs and greatly reduced sample and solvent consumption; (ii) the ability to analyse complex matrices directly and thus minimize contamination in work-up; (iii) the ease of separating an analyte from the SF; (iv) the possibility of fractionating during collection; (v) running multiple concurrent analyses from the same extraction or concentrate during decompression (focusing); and (vi) compatibility with on-line methods of analysis[13,24,25,36–38]. A logical extension of SFE is to combine the process with chromatographic techniques such as thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC) or supercritical fluid chromatography (SFC), so that sample preparation and analysis are instrumentally linked.

Safety must also be an important consideration. The complete SFE system, *i.e.*, the pump, extraction vessel, connecting tubing, inlet and outlet fittings, valves and in-line monitoring detectors, must be able to withstand the high pressures used in SFE. Saito *et al.*[39] recommend testing each item by pressurizing it prior to use with water at 1.5 times the maximum intended working pressure. They also provide useful

examples of calculating the minimum wall thicknesses of high-pressure vessels and tubing. Of course, one must also consider the nature of the SF (caustic, toxic, etc.) and any likely chemical reactions with the solutes. For example, nitrous oxide is a strong oxidant and for extractions involving large amounts of easily oxidized material, particularly at elevated temperatures, an explosion hazard exists[40] and ammonia is extremely toxic and corrosive[41].

3.2. *Closed- and open-loop systems*

Analytical SFE systems utilize either a closed- or an open-loop system. In the closed-loop system the SFE vessel is raised to the desired extraction pressure and a solubility equilibrium is allowed to be reached. This can be done in a static manner or by recirculating the SF through the closed-loop system. A sample can be diverted at any time to another analytical device by valve switching. The advantage of a closed-loop system is that parts of the extract of one sample can be taken for concurrent or consecutive analysis with virtually no difference in extraction profile. The use of a recirculating pump is likely to decrease the time required for the extraction equilibrium to be reached, although a disadvantage is that the whole system, including the pump, can become contaminated with the extract, necessitating extensive and time-consuming cleaning if another sample needs to be run. Another disadvantage of the closed-loop system is that only a fraction of the total extract is taken for analysis.

In the open-loop system, the SF passing through the sample is fed through a detector, usually an ultraviolet or flame-ionization detector and then led to waste. At periodic intervals the extract-laden SF can be led to other devices by means of valve switching (dynamic sampling). By utilizing an SFE density programme, selective fractions (dynamic fractionation) can be obtained or, alternatively, the entire extract can be analysed by connecting the extraction module with the analytical device right from the start of the SFE process, as is often done in SFE-GC, which will be discussed below. In trace analyses either a closed- or an open-loop system can be applied provided that some form of extract concentration is possible.

3.3. *Isolation of extracts*

In an SFE process, one or more components have to be removed from the sample matrix. The SF and sample are brought into intimate contact and compounds that are soluble in the SF are (selectively) extracted from the sample. After extraction, the extract can be separated from the SF in different ways[15]. Two methods are based on precipitating the components from the extract by reduction of the fluid density, i.e., via (i) temperature increase (isobaric method) or (ii) pressure reduction (isothermal method). The third technique is based on the adsorption of the solute on an appropriate stationary phase.

In the isobaric method, the SF and the dissolved material move from the extraction vessel to a heat exchanger where the SF is heated. The result is a decrease in density and the dissolved material precipitates and can be collected in a separation vessel.

The most common technique used for isolating the extract after SFE in analytical applications is depressurization. However, depending on the conditions, it is possible for analytes to nucleate and become entrained in the expanding gas to form an aerosol which can be easily lost into the atmosphere[25]. The effects of aerosol

TABLE 2

COMPARISON OF EXTRACTION RECOVERIES FROM XAD-2 RESIN USING DIFFERENT COLLECTION METHODS AND EXTRACTION CONDITIONS

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Analyte	*Recovery (%)*				
	Collection mode[a]		*Extraction condition*[b]		
	Open	*Closed*	*CO_2, (150°C)*	*CO_2, (50°C)*	*CO_2–CH_3OH (80:20 mol/mol) (150°C)*
Chrysene	2.2	75	2.2	25	60
Benzanthrene	2.5	79	2.5	28	62
1-Nitropyrene	1.8	83	1.8	24	29
Dibenzo[*a*,*i*]carbazole	6.3	95	6.3	10	65
Coronene	8.2	81	8.2	6.5	62
Rubrene	0	25			

[a] Extraction conditions: CO, at 150°C and 410 bar with ca. 250 ml of liquid.
[b] Open collection applied. Extraction performed at 410 bar with 250 ml of liquid.

formation on analyte losses during collection are illustrated in Table 2, where a comparison is made between the extraction recoveries from XAD-2 resin using open collection and sealed collection. Open collection was performed in a narrow-necked flask cooled to 0°C and for sealed collection a liquid nitrogen-cooled flask was applied. As the volatility of the analytes involved was very low at 0°C, the most probable explanation for the low recoveries is aerosol formation. By freezing the extraction effluent in a sealed vessel, analyte losses through aerosol formation can be eliminated. As at higher temperatures (i.e., 150°C) the expansion of carbon dioxide produces only a single gas phase and at lower temperatures (i.e., 50°C) a two-phase system of carbon dioxide (410 bar) is produced, the formation of small solute particles ($< 0.2\ \mu m$) will dominate at higher temperatures, and low collection efficiencies may be expected in this instance. The higher recoveries for the carbon dioxide-methanol fluid can be explained by the formation of larger liquid-methanol droplets during expansion, which have larger deposition efficiencies owing to their size and liquid character. As all recoveries with open collection are lower than obtained by the closed-loop collection method, the differences are attributed to an improved collection efficiency. Raynor et *al.*[42] also observed the loss of the more volatile components (e.g., naphthalene) during SF decompression and/or solute deposition in a microvalve loop. Hirata and Okamoto[43] noted that, in order to trap polymer additives after decompression of an extract-laden SF, the restrictor had to be connected to two trapping tubes in series, the first trap being empty and the second packed with silica. Most of the analytes were trapped efficiently in the first trap, but about one-third of the analytes were trapped on the silica. McNally and Wheeler[44] initially tried to adsorb sulphonylureas on two guard columns packed with C_{18} and silica. They observed that the major fraction of the analytes was deposited in the back-up collection flask, inserted after the guard columns, rather than in the columns themselves. Schneiderman *et al.*[45] found, in off-line SFE-HPLC studies, that the extraction recoveries of vitamin K_1 dropped

from 95.6% for a milk-based powder formula to less than a few per cent after SFE of a liquid formula. This was probably due to entrainment of water by supercritical carbon dioxide and, consequently, the silica becoming saturated with water and being rendered ineffective for trapping of vitamin K_1.

3.4. *Monitoring of extracts*

Coupling of SFE with analytical techniques can be performed in both the off-line and on-line modes. In order to optimize on-line SFE procedures, it has been recommended[46] that first the recoveries of the analytes should be measured by means of off-line SFE. In this way, parameters such as (i) pressure and temperature, (ii) polarity of the SF, (iii) volume of the SF per unit time, (iv) volume and dimensions of the extractor, (v) extraction time and (vi) amount of the sample can be optimized.

SFE can be coupled with a variety of detection and separation techniques. One of the oldest and, by modem standards, crudest techniques for determining the extraction yield is a gravimetric analysis, in which the mass of the extract and the mass of the sample are compared with each other[6,47]. Using this technique, Krukonis[6] illustrated the increased dissolving power of SFs at higher densities. SFE of ground ginger using carbon dioxide at 50°C produced a 9% yield at 335 bar, and only a 1% yield at 100 bar. The problem in using gravimetric analysis is that, intrinsically, it is an off-line process, which means extra sample manipulations and thus an increased analysis time and higher costs. A number of dedicated SFE instruments are now commercially available either as stand-alone devices or coupled to another analytical instrument whereby on-line monitoring of the SFE procedure is performed by means of ultraviolet (UV) or flame ionization detection (FID).

Monitoring by UV detection requires the presence of a chromophore in the extracted analytes. This illustrates one of the other advantages of using carbon dioxide as the SF as it is transparent down to *ca.* 190 nm. Wright *et al.*[48] used a closed-loop system with recirculation and on-line UV detection to monitor the effects of ultrasound during the extraction of chrysene from adsorbents or of caffeine from roasted coffee beans. They observed an enhanced extraction rate for caffeine, probably caused by inducing a convective flow through the pores of macroporous materials. Ultrasound with a frequency of 20 kHz did not improve the desorption of chrysene from the micropores of the adsorbents.

By using absorbance detection, in contrast to FID, spectra or functional group information can be obtained[49]. A fibre-optic monitor operating between 400 and 750 nm has been applied for the on-line detection of a blue dye in olive oil, solubilized by supercritical carbon dioxide. As fibre-optic monitor systems are also available for wavelengths in the UV and (near-)infrared range this technique may become increasingly important in coming years.

In addition to UV detection, mass spectrometric (MS) detection is a highly valuable alternative for obtaining structural information. The use of a direct fluid injection interface for MS detection was reported by Smith *et al.*[36] for both qualitative and quantitative solubility measurements of complex mycotoxins of the trichothene group in supercritical carbon dioxide or nitrous oxide. Kalinoski *et al.*[50] used on-line SFE with chemical ionization MS detection and collision-induced dissociation tandem MS (MS-MS) for the rapid identification of ppm levels of several trichothene mycotoxins with minimum sample handling. A limitation of SFE-MS is

the possible overloading of the mass spectrometer with co-extracted compounds when complex samples are analysed[50]. The result is that often sophisticated techniques such as tandem MS may be necessary to obtain the required selectivity and sensitivity. A cheaper and more attractive alternative is to perform some form of chromatography between extraction and detection. This coupling of SFE with various chromatographic techniques will be discussed extensively in the following sections.

4. COUPLING OF SFE WITH CHROMATOGRAPHIC TECHNIQUES

4.1. *SFE-TLC*

In 1976, Stahl and co-workers[27,51–55] developed a mini-extraction apparatus for the desorption of an SF extract on a moving thin-layer chromatographic (TLC) plate. Both carbon dioxide and nitrous oxide were used as SFs and a wide range of naturally occurring materials (e.g., coffee, dye mixtures, seeds, sage, leaves, ginger, flowers, pepper, chilies, hops, marijuana, vitamin oils and alkaloids) were studied. The apparatus (Fig. 1) consisted of a thermostated diaphragm compressor to attain the desired pressure for the SF[27]. Subsequently the SF flowed into a micro-extraction autoclave, the exit of which was sealed with a cut-off valve. After opening this valve the SF flowed via a narrow capillary onto the moving TLC plate, which was held horizontally at a distance of l-5 mm from the capillary tip. Extraction of the sample was started at 70 bar and a fixed volume of SF was allowed to flow through. After moving the plate, the pressure was increased stepwise by 5, 10 or 20 bar and the sample was again extracted with the same volume of SF. By comparing zone intensities it was possible to observe whether an increase in the pressure resulted in more or less extraction of an analyte (Fig. 2).

On-line SFE-TLC provides a rapid and simple insight into the extraction performance. Its strength is that the extract is deposited on a plate, which means that detection is a static process. Both one- and two-dimensional chromatography can be performed, ***i.e.,*** SFE can be combined with a development of the TLC plate in one or two directions, after which the components of interest can be detected on or isolated from the support material for further study (Fig. 2). Limitations of SFE-TLC are that quantification is difficult and that the stability of components on the support

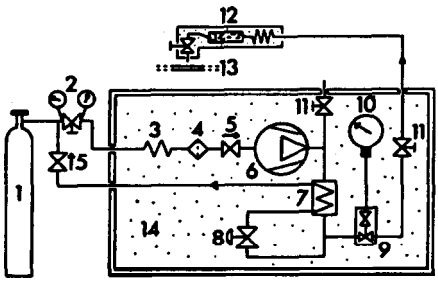

Fig. 1. Schematic diagram of the apparatus for fluid extraction coupled directly with TLC. 1 = Steel cylinder; 2 = reducing valve; 3 = pre-heating coil; 4 = filter; 5 = check valves; 6 = diaphragm compressor; 7 = heat exchanger; 8 = back-pressure regulator; 9 = damping parts; 10 = precision manometer; 11 = shut-off valves; 12 = micro-autoclave for extraction; 13 = TLC receiving layer; 14 = thermostatically controlled container. (Reprinted with permission from ref. 27.)

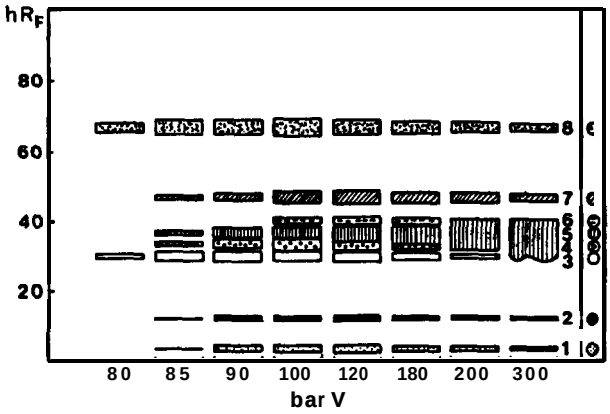

Fig. 2. Thin-layer chromatogram after fluid extraction of a vitamin oil mixture (200 μg each). 1 = Cholesterol; 2 = vitamin D_3; 3 = vitamin K_3; 4 = a-tocopherol; 5 = triglyceride; 6 = vitamin A acetate; 7 = cl-tocopheryl acetate; 8 = steryl ester. (Reprinted with permission from ref. 27.)

material or in the presence of oxygen may be a problem. Further, the resolution of TLC is low compared with that of HPLC, GC and SFC, and at high pressures (> 300 bar) problems are encountered such as stripping of the support material by the SF caused by the increased velocity of the expanding fluid.

4.2. *SFE-HPLC*

4.2.1. Off-line. Various off-line- SFE-HPLC analyses have been reported[37,39,56–59]. The effect of different extraction parameters on the amount of caffeine extracted from roasted coffee beans using supercritical carbon dioxide was studied by Sugiyama *et al.*[56]. A closed-loop SFE system with recycling was applied. The trap column was packed with activated carbon and the trapped analytes were eluted, in the off-line mode, with methanol-water (55:45, v/v). Finally an aliquot was injected into the HPLC system. In Fig. 3 the effects of various parameters on the extraction yield are illustrated. The amounts of caffeine extracted are represented as percentages of the amount extracted with hot water, *i.e.*, as percentages of the caffeine level in drinking coffee. The recovery increased with increasing extraction pressure and time, and decreased rapidly with increasing temperature. Above 60°C caffeine was hardly extracted, owing to the diminished solubility of the analyte in carbon dioxide as a result of a decrease in density. Furthermore, reduction of the percentage of water resulted in a decreased recovery, which is in good agreement with other data suggesting that water is essential for the mass transfer of caffeine when carbon dioxide is used as the extraction fluid[11]. High recoveries were found when using a pressure of 200 bar, a temperature of 48°C, 20% of water in the SF and an extraction time of 60 min.

Schneiderman *et al.*[45] extracted vitamin K_1 (phylloquinone) from commercial soy protein-based and milk-based powdered infant formulas using supercritical carbon dioxide at 544 bar and 60°C. Quantitative extraction required only 15 min, whereafter the SF was depressurised, the extracted vitamin K_1 trapped in a short tube packed with silica and eluted off-line with a mixture of dichloromethane and acetone. After removal of the solvent, the residue was dissolved in the eluent and determined

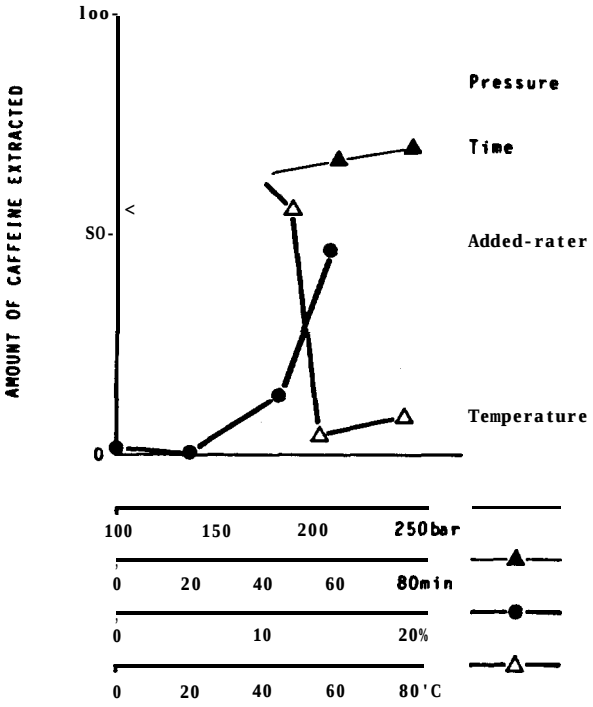

Fig. 3. Percentage of caffeine extracted from roasted coffee beans under various conditions with hot water. 0 = Various pressures with the added water, temperature, and extraction time constant at 20%, 48°C and 60 min, respectively; A = various extraction times with other parameters constant at 150 bar, 20% and 48°C; ● = various amounts of water added to coffee powder with other parameters constant at 150 bar, 48°C and 60 min; △ = various temperatures with other parameters constant at 150 bar, 60 min and 20%. The temperature and the amount of added water have significant effects on the extraction, as shown by the heavy lines. (Reprinted with permission from ref. 56.)

by reversed-phase HPLC with electrochemical detection[45]. The minimum detectable amount was 80 pg and the linear dynamic range was at least five orders of magnitude. The recovery of vitamin K_1 from a milk-based powder was 95.6% with a relative standard deviation (R.S.D.) of 7.4% and from a soy protein-based product 94.4% with an R.S.D. of 6.5%. The same group" used the same technique, with comparable results, for the determination of anthraquinone from Kraft paper and pine plywood sawdust, and vitamin K_3 in rat feed[58].

Hirata and Okamoto[43] extracted polymer additives from polyethylene and polypropylene using Supercritical carbon dioxide at 250 bar and 35°C. After decompression the analytes were collected in a microtrap filled with silica, held at 60–80°C to maintain a constant flow-rate, and subjected to microcolumn HPLC.

Ndiomu and Simpson" used SFE with carbon dioxide to isolate morphine and quinine from various plant materials. The recoveries were determined using off-line HPLC. The extraction was performed by heating of a sealed extractor which had been filled with a certain amount of dry-ice. The results compared favourably (higher recoveries in less time) with those obtained by extractions with subcritical methanol and tetrahydrofuran and organic Soxhlet extractions. Solid-phase extractions, e.g., of

blood samples spiked with 200 μg/ml of morphine, were compared with SFE in terms of percentage recovery. For ten replicate determinations with SFE, the average recovery was 96.7% (R.S.D. 3.2%), whereas the average recovery with solid-phase extraction was 92.2% (R.S.D. 4.0%). However, the time scale for the SFE analysis of the serum samples was excessive, because the aqueous nature of the serum samples first necessitated freeze-drying of the samples for 12 h. Supercritical carbon dioxide was not suitable for the efficient extraction of caffeine from kola nuts under the applied conditions.

Symmetrical triazine herbicides have been extracted from river sediment by supercritical carbon dioxide[60]. The extraction was performed in a 0.57-ml cartridge using a pressure of 230 bar and a temperature of 48°C. The extraction of 500 mg of sample was complete in about 30 min and the analytes were trapped via a capillary restrictor (30 cm x 25 μm I.D.) and analysed by reversed-phase HPLC using UV detection at 225 nm. The recoveries were in excess of 90% in the ppm-ppb" range.

Ehntholt *et al.*[61] studied the isolation and concentration of 23 compounds in the ppb range from aqueous samples with supercritical carbon dioxide. The analytes were dissolved in acetone and diluted with an aqueous solution containing sodium hydrogencarbonate, calcium sulphate and calcium chloride. The extractions were performed at 173 bar and 45°C and the extracts were analysed via off-line HPLC or GC. The recoveries for the various solutes were different. For biphenyl, a neutral and relatively non-polar solute, it was 23.4%; for methyl isobutyl ketone, as representative of aldehydes and ketones, 17.3%; for 2,4-dichlorophenol, an acidic phenol, 45.4%; for anthraquinone, an oxygen-containing heterocyclic, 84.6%; and inorganic sodium and calcium salts could not be extracted with this method. Finally, for caffeine, a nitrogen-containing heterocyclic, the recovery was 0%. Probably the low pH of the extraction medium (*ca.* 3) reduces the solubility of the nitrogen-containing solutes in the SF because of protonation. The last example shows that the pH of the extraction medium is an important parameter.

4.2.2. ***On-line.*** In 1983, Unger and Roumeliotis[62] described the first coupling device allowing on-line HPLC of SF extracts. The on-line system (Fig. 4) consisted of two high-pressure sample-injection valves connected in series. The first valve operated as a switching valve to the loop and controlled the pressure over a packed microbore column. Two short microbore columns packed with 5-μm LiChrosorb RP-18, positioned between the first and second valves, were used, respectively, to adsorb the analytes over a certain period of time and simultaneously to function as sample loop for the second valve which served as injector for the normal-phase HPLC column, packed with 5-μm LiChrosorb Si 100. The on-line SFE-HPLC system was used to monitor the extraction kinetics of valtrate from ***Radix valerianae***. Using an open-loop system with supercritical carbon dioxide at 40°C and 96 bar, an exponential decay was observed for the extracted amount of valtrate with time. The extraction was complete in 1 h.

Recently, Nair and Huber[63] described the on-line SFE-HPLC analysis of ground tablets for ibuprofen. The SFE unit consisted of a constant-pressure pump to transfer the carbon dioxide to a preheater, a heated vial containing the sample, a

[a] Throughout this article, the American billion (10^9) is meant.

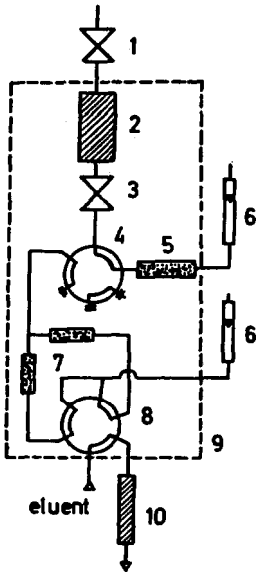

Fig. 4. Schematic diagram of the unit for coupling of SFE and HPLC. 1 = Back-pressure regulator; 2 = extraction vessel; 3 = high-pressure two-way angle valve; 4 = six-port external sample valve; 5 = packed microbore column for release and waste deposit; 6 = rotameter; 7 = microbore columns, serving for deposit and as loop; 8 = sample injector; 9 = thermostat; 10 = HPLC column. (Reprinted with permission from ref. 62.)

fixed-volume injection valve and finally an analytical column. The system mentioned was applied only for qualitative experiments. According to the authors, a fixed-volume recycle loop should be installed in order to obtain quantitative results.

4.3. *SFE-GC*

4.3.1. Off-line. Schantz and Chesler[22] extracted polychlorinated biphenyls (PCBs) from sediments and polynuclear aromatic hydrocarbons (PAHs) from transformer oil using supercritical carbon dioxide. The extract was trapped on a reversed-phase silica cartridge. Subsequently the trapped analytes were flushed off the cartridge with dichloromethane and, after partial evaporation of the solvent, the analytes were quantified by GC. The results of SFE were comparable to those of a Soxhlet extraction. Under the applied extraction conditions, ***i.e.,*** density 0.93 g/l, SFE of high-molecular-weight PAHs appeared to be more efficient than Soxhlet extraction (18 and 30% higher extraction values for benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene, respectively). SFE required only 30-60 min, whereas the dichloromethane extraction took 16 h. For more polar compounds the addition of an entrainer, such as methanol, to the SF appeared to be necessary[22].

Hawthorne and Miller[64] described an off-line SFE-GC method using supercritical nitrous oxide with 5% methanol as the entrainer for the extraction of PAHs from environmental solids. The extracted analytes were collected by inserting the outlet restrictor of the SFE system into a vial containing dichloromethane. Quantitative recovery of PAHs from urban dust and of deuterated PAHs from river sediment

and fly ash was obtained within *ca.* 30 min. The recovery of the deuterated PAHs was significantly better than that obtained after 4 h of sonication or 8 h of Soxhlet extraction with either benzene or dichloromethane. The same workers[24] described the rapid and quantitative recovery of PAHs from both solid samples and PAHs adsorbed on Tenax by means of off-line SFE–GC. The results of the class-specific extractions of alkanes and PAHs from diesel-exhaust particulates using different extraction pressures are given in Table 3.

Using a comparable system agrochemicals (e.g., atrazine) and corresponding metabolites in soil samples were analysed[65]. In this study the solubilities of the various analytes in carbon dioxide were calculated using the Peng-Robinson equation of state.

The desorption characteristics of various materials were investigated by Wright *et al.*[25] and Raymer and co-workers[66,67] using off-line SFE-GC. Adsorbents such as XAD-2, polyurethane foam, Spherocarb and Tenax were systematically studied. Wright *et al.*[25] used a modified HPLC pump to pressurize and deliver the extraction fluids. The pump head and check valves were cooled by circulating an ethylene glycol-water mixture (− 15°C) through a cooling-jacket. The pressurized fluid was transferred to the high-pressure extraction vessel using 1/16-in. stainless-steel tubing. The extraction vessel allowed operation at pressures of over 400 bar and temperatures higher than 200°C (Fig. 5). The extraction vessel was maintained at elevated temperatures in a GC oven. The transfer line was extended to the bottom of the extraction cell to allow the fluid to move through the sample from the bottom to the top and then to exit the extraction vessel. A stainless-steel frit (0.5-2.0 μm) was placed in the exit port of the extraction vessel to prevent the sample from being flushed out. The

TABLE 3

FRACTIONATION OF ALKANES AND PAHs DURING EXTRACTION WITH SUPERCRITICAL CARBON DIOXIDE OF DIESEL-EXHAUST PARTICULATES

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Species	*Proportion in fraction 1 (%)*[a] *(75 atm CO_2)*	*Proportion in fraction 2 (%)*[a] *(300 atm CO_2)*
n-Alkanes		
Nonadecane (C_{19})	86	14
Eicosane (C_{20})	84	16
Heneicosane (C_{21})	86	14
Docosane (C_{22})	85	15
Hexacosane (C_{26})	85	15
PAHs		
Phenanthrene	28	72
Fluoranthene	9	91
Pyrene	7	93
Benz[a]anthracene	ND[b]	> 90
Chrysene	9	91
Benzo[*ghi*]perylene	ND	> 90

[a] Relative standard deviations were generally < 10% for the alkanes and < 5% for the PAHs.
[b] Not detected

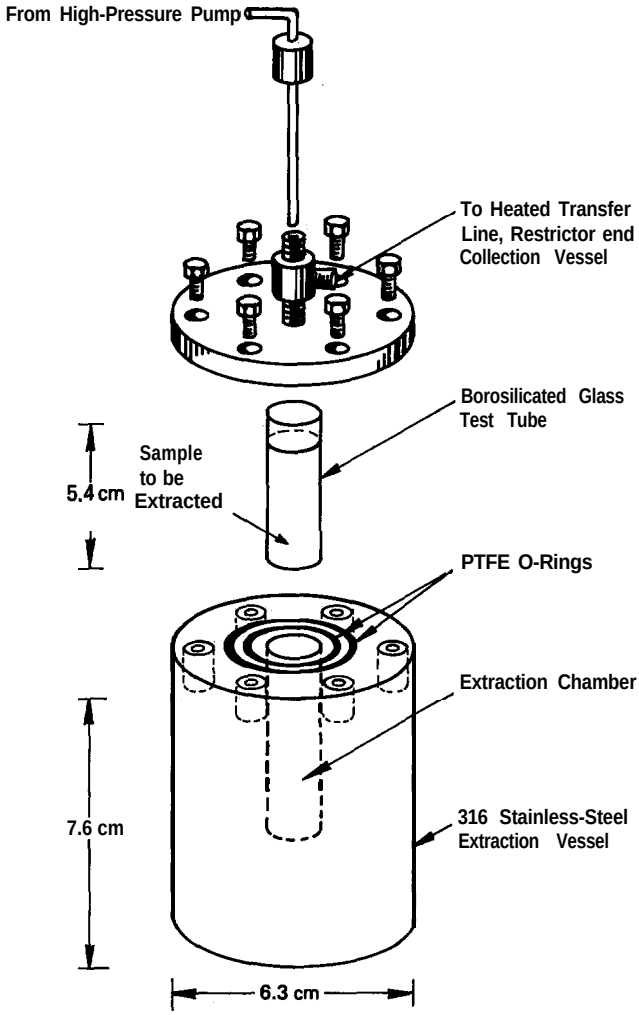

Fig. 5. Design of SFE vessel. (Reprinted with permission from ref. 25.)

extraction effluents were collected by freezing them in a sealed flask cooled in a liquid nitrogen bath. When pure carbon dioxide was used, the 50-μm stainless-steel restrictor tubing was heated by an electrical current to prevent it from freezing during the fluid expansion process in the cooled collection flask[25]. Extraction data for spiked XAD-2 resin obtained by Soxhlet extraction with dichloromethane and by SFE with various fluids are presented in Table 4. With Soxhlet extraction high recoveries were obtained for all model compounds. Similar recoveries were achieved with SFE for the low-molecular-weight compounds, but for high molecular weights, the recoveries diminished progressively. This behaviour was explained by the lower solubility of the higher molecular weight analytes in the SF and the relatively high temperatures and pressures used in this study. SFE with isobutane or with methanol-entrained carbon dioxide provided better overall extraction efficiency than with pure carbon dioxide.

TABLE 4

COMPARISON OF EXTRACTION OF XAD-2 RESIN USING SOXHLET EXTRACTION AND VARIOUS SUPERCRITICAL FLUIDS

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Compound	*Recovery (%)*						
	Soxhlet only[a]	CO_2[b]		*Isobutane*[d]		CO_2–CH_3OH[e]	
		SFE	*Soxhlet'*	*SFE*	*Soxhlet'*	*SFE*	*Soxhlet'*
Chrysene	79	84	0	86	5	88	3
Benzanthrone	86	98	0	110	0	88	0
1-Nitropyrene	93	81	0	70	0	98	0
Dibenzo[*a*,*i*]carbazole	88	54	22	96	6	97	0
Coronene	81	46	63	93	9	90	5
Decacyclene	85	6	88				

[a] Dichloromethane for 16 h.
[b] Carbon dioxide at 125°C and 400 bar with *ca. 200* ml of liquid.
[c] Extraction with dichloromethane of the same sample after SFE.
[d] Isobutane at 150°C and 185 bar with *ca. 300* ml of liquid.
[e] 20 mol-% methanol in carbon dioxide at 130°C and 400 bar with *ca.* 210 ml of liquid.

When entrainers were used, the transfer line was maintained at the same temperature as the oven. These SFEs were accomplished in ca. 30–45 min compared with 16 h for the Soxhlet extractions.

In another off-line study, Raymer and co-workers found that SFE was superior to thermal desorption techniques when applying supercritical carbon dioxide for the desorption of hexachlorocyclohexane, a hexachlorobiphenyl, anthracene and parathion from Tenax[66] and polyimide sorbents[67]. All compounds showed recoveries of over 90% from Tenax by SFE, whereas thermal desorption resulted in only a 13% recovery for hexachlorobiphenyl and parathion.

Off-line GC was used by Sugiyama and Saito[47] to compare quantitatively the amounts of components of lemon peel oil obtained by SFE and by cold-pressing. A photographic representation of the lemon peel before and after SFE was also included. Before extraction the oil-containing cells were clearly visible, but after extraction the oil had been drawn out of the cells, which now looked like craters. Obviously, the oil was not simply squeezed out by the pressure of the carbon dioxide, but carbon dioxide had diffused into the oil-containing cells, dissolving the oil and drawing it out of the cells, *i.e.*, the oil was extracted.

4.3.2. *On-line.* Hawthorne and Miller[24] were the first to couple SFE directly with on-line GC, when they successfully performed a qualitative analysis of automobile-exhaust organics collected on Tenax. Since then, the number of publications involving on-line SFE-GC has continued to increase[20,23,40,48,68–70]. Within this methodology Wright *et al.*[23] reported that several modes of operation are possible, such as quantitative extraction of analytes from a sample matrix, quantitative extraction and concentration of trace analytes and selective extraction at various solvating powers to obtain specific fractions by pressure or density programming.

A modular-design open-loop on-line SFE-GC system was described by Mapelli *et al.*[71]. The system consisted of an oven with an air recirculation system in which the extraction cell and two high-pressure valves were placed. The extraction cell had a volume of 0.4 or 1.5 ml. The coupling was achieved by transferring the contents of the loop to a capillary column by means of a splitting system. The interface was controlled by a heated transfer line, fixed at one end in the top of the extraction module. A fused-silica capillary passed through the heated interface, so that the restrictor penetrated inside. A make-up gas flow was supplied around the restrictor to dilute the decompressed fluid. In this way analyte losses were minimized and even the reconcentration of volatile analytes was possible.

The usefulness of on-line SFE-GC was well demonstrated for the extraction and selective fractionation of PAH standard mixtures[23]. The instrumentation is shown in Fig. 6. It consisted mainly of four sections, viz., a high-pressure pump and extraction cell, a switching valve and interface region, GC with FID and an appropriate microcomputer for complete system automation. A PAH mixture, adsorbed on glass beads, was extracted for 1 min at three progressively increasing pressures and the effluent of each fraction was analysed by temperature-programmed capillary GC prior to the next extraction (Fig. 7). During each GC analysis, the extraction process was continued (cu. 75 min) with the effluent being vented to the collection reservoir. In this way, essentially all the material which was soluble at each pressure was extracted from the matrix prior to the next extraction step. Carbon dioxide was used as the extraction fluid. The extractions were performed at 50°C and at densities of 0.23, 0.62 and 0.78 g/ml. The extraction effluent was collected and concentrated on-column using a retention gap (deactivated fused silica, 30 cm × 0.53 mm I.D.) at 30°C, which proved to be adequate to focus the solute injection bands. Collection at higher temperatures or without the retention gap resulted in peak broadening and decreased resolution. Examination of the chromatograms showed that high-resolution separations of three essentially unique fractions of material were obtained. As expected, progressively higher molecular weight material was extracted at higher densities of

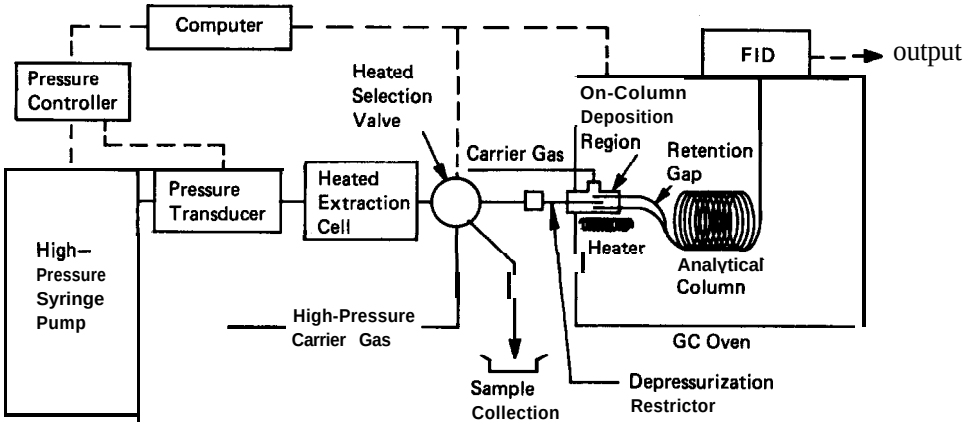

Fig. 6. Schematic of on-line SFE–capillary GC instrumentation. (Reprinted with permission from ref. 23.)

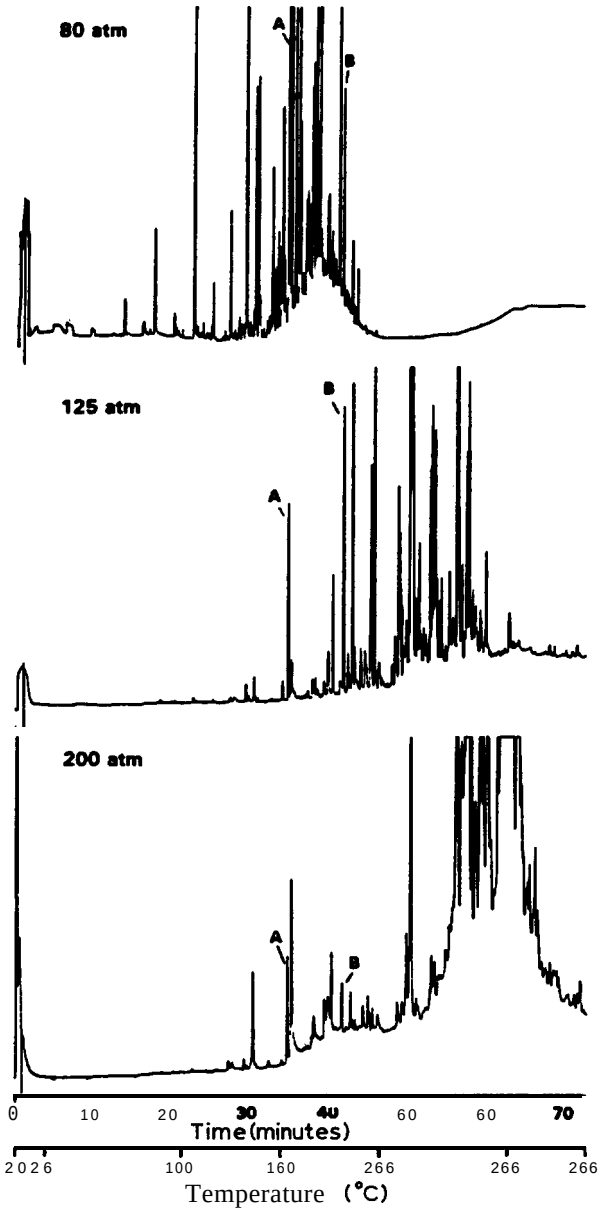

Fig. 7. Capillary gas chromatograms of PAH fractions obtained from supercritical carbon dioxide extraction of a complex matrix at various pressures. Compounds A and B are arbitrarily marked in each fraction to facilitate comparison. (Reprinted with permission from ref. 23.)

the extraction fluid. Although some overlap of components occured in the various fractions, the example demonstrates the potential of SFC-SFE for efficient on-line fractionation.

The same instrumentation was used for the quantitative analysis of orange peel at two different extraction pressures[48] and the potential of the technique was shown by determining PAHs adsorbed on XAD-2 resin.

A similar method for the direct coupling of SFE with GC was used by Hawthorne and Miller[40] for the analysis of environmental samples for PAHs and PCBs. Quantitative studies were performed using FID, electron-capture detection (ECD) and MS. The direct coupling of the SFE vessel with the GC column was accomplished by inserting the SFE outlet restrictor capillary (15 cm × 15-30 μm I.D. × 150 μm O.D.) into the GC column using an on-column injector port. The GC oven was cooled during the extraction to allow thermal focusing of the extracted analytes inside the GC column at the outlet of the SFE restrictor. Restrictors with larger internal diameters (e.g., 30 μm) yielded higher extraction efficiencies in shorter times than restrictors with smaller internal diameters, but internal diameters larger than 30 μm were not practical, because the resulting flow-rates were too high for the pumping system. This meant that a compromise had to be found, because the internal diameter of the restrictor also affected the efficiency of the cryogenic trapping. Nitrous oxide was chosen as the SF in this study, because it is a gas at temperatures which are normally used for cryogenic trapping of organic species in GC columns and because it provided better extraction efficiencies for PAHs than carbon dioxide and ethane[64]. The feasibility of the direct coupling of SFE with GC-FID was confirmed by the analysis of 10 mg cigarette ash, which was extracted for 10 min with supercritical nitrous oxide at 45°C and 300 bar. The extracted species were collected in a wide-bore fused-silica capillary GC column (30 m × 0.32 mm I.D., 1 μm thick film of DB-5), by inserting the outlet restrictor of the extraction cell directly into the GC column via the on-column injector. The GC oven was held at 5°C during the extraction, allowing cryogenic focusing of the analytes at the top of the column. Next, the oven was rapidly heated to 50°C and the GC separation was performed using a temperature programme of 8°C/min to 320°C. Good agreement with the certified values for PAHs in urban dust from the National Bureau of Standards was found (Table 5). The values found for fluoranthene, benz[*a*]anthracene and benzo[*a*]pyrene were slightly higher than the certified values. As the certified values are based on 48 h extractions in a Soxhlet apparatus [both methylene chloride and benzene-methanol (1: 1) were used as

TABLE 5

CONCENTRATION OF SELECTED PAHs IN NBS SRM 1649 URBAN DUST

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Compound	*Concentration (μg/g)*[a]	
	Certified	*SFE*
Fluoranthene	7.1 ± 0.5	8.0 ± 0.6
Benz[*a*]anthracene	2.6 ± 0.3	2.9 ± 0.5
Benzo[a]pyrene	2.9 ± 0.5	3.2 ± 0.3
Benzo[*ghi*]perylene	4.5 ± 1.1	4.4 ± 0.3
Indeno[1,2,3-*cd*]pyrene	3.3 ± 0.5	3.1 ± 0.2

[a] Data are given as average values ± standard deviations ***(n = 3).***

solvents], the higher values obtained from 60-min SFE may be the result of an increased extraction efficiency.

In more recent on-line SFE-GC studies, Hawthorne *et al.*[68] noted that within certain limits, the influence of the internal diameter of the restrictor is less important than the cryogenic trapping temperature in the chromatographic oven. The chromatograms obtained by on-line SFE-GC analysis of spices, chewing gum, orange peel, spruce needles and cedar wood showed good peak shapes comparable to those obtained by using standard on-column injections. In Fig. 8 the GC-FID trace generated by on-line SFE-GC of rosemary herb is compared with a standard on-column injection of a dichloromethane extract. The on-line SFE-GC took 40 min per sample whereas sonication, concentration and off-line GC took *ca.* 5 h[68].

Recently, on-line SFE-GC was successfully applied to the extraction of PAHs from treated wood, urban dust and river sediment, phenolic species from wood smoke particulates, nicotine from tobacco, biological markers from coal, flavour components from food products[70] and PCBs and PAHs from polyurethane foam sorbents[72]. In general, recoveries of over 95% were achieved in 10–20 min.

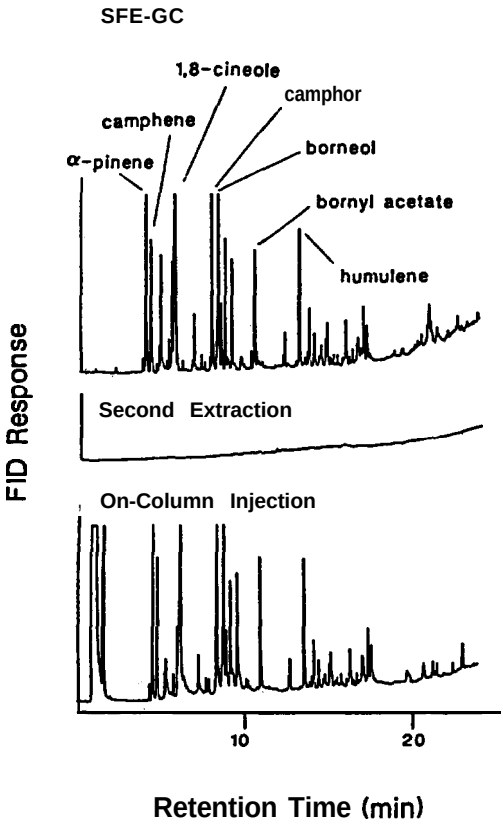

Fig. 8. Comparison of chromatograms generated by using SFE–GC–FID analysis of rosemary herb and standard on-column injection of a dichloromethane extract. The middle chromatogram shows the result of a second SFE–GC–FID analysis of the same sample. (Reprinted with permission from ref. 68.)

Hawthorne *et al.*[23,48,64] have found that a limitation with an on-line SFE-GC interface consisting of a linear fused-silica restrictor, directly inserted into a capillary GC system via an on-column injector or via a T-piece onto a retention gap, is that the restrictor becomes fragile after a few extractions, especially when nitrous oxide is used as the SF. In addition, all of the expanding SF passes through the chromatographic column and all co-extracted components are deposited in the column (or retention gap). Nielen *et al.*[69] described an alternative on-line SFE-GC system that addresses these problems. The transfer line from SFE to GC was an electrically heated (300°C) linear fused-silica capillary that also functioned as a restrictor for the SFE. Injection into the GC column was performed in the split or in the splitless mode. The system was robust and compatible with existing thermal desorption–cold trap equipment. Further, there was no restriction on the choice of GC columns or detectors, as the expanding carbon dioxide could be vented via the GC splitter, while the analytes were trapped in the desorption unit. Its potential for environmental trace analyses was demonstrated by the analysis of Tenax spiked with PCBs at the picogram level. The recoveries were satisfactory (52–63%) and the detection limit for the individual PCBs was 30 pg. Improvement of the detection limits was limited by the presence of background interferences in the GC-ECD system, resulting from the concentration of trace impurities in the 99.999% pure carbon dioxide during SF decompression (cryogenic focusing). The limitations of the technique are that thermally labile compounds cannot be analysed because of the high temperature of the SFE-GC transfer line and that problems are encountered in trapping more volatile compounds. The temperature in the transfer line should be relatively high as the pressure is reduced gradually along the entire length of the column.

An on-line SFE-GC-ECD system for the determination of PCBs was described by Onuska and Terry[73]. In this study the dynamic and static extraction modes were compared. In the dynamic mode the sample was transferred into the extractor, heated and pressurized. When the required pressure had been attained, a valve was switched and the extraction process run according to a previously determined time interval. In the static mode extracts were provided under conditions reaching equilibrium between the analytes in the fluid and in the sample. In order to determine PCBs at trace levels, static extraction was considered advantageous as in this instance the total amount of carbon dioxide passing through the extractor was smaller, resulting in less contamination with impurities.

4.4 *SFE-SFC*

An obvious advantage of SFE is that it is an ideal way to introduce a sample into an SFC system[21,38,74,75]. Because the injection solvent is the same as the mobile phase[76], the criteria for a successful coupling of different techniques are fulfilled", i.e., the output characteristics from the first instrument and the input characteristics of the second instrument are compatible. An additional advantage of on-line SFE–SFC over on-line SFE-HPLC and SFE-GC is that it is unlikely that sample constituents which are insoluble in the mobile phase will be introduced into the column'*.

SFE can be combined with several forms of SFC, *i.e.*, with conventional packed columns (1–4.6 mm I.D.; packed-column SFC), with capillary columns (10–250 μm I.D.; capillary SFC) and, as has been done more recently, with packed capillary columns (200-530 μm I.D., 3-10 μm particles; packed capillary SFC).

4.4.1. On-line SFE-packed-column SFC. Directly coupled laboratory-scale SFE-packed-column SFC was introduced in 1985 by Sugiyama *et al.*[56]. Qualitative on-line SFE-packed-column SFC of powdered coffee beans was performed and monitored by multi-wavelength UV detection, using a high-pressure cell. The separation was performed without any sample pretreatment. Fig. 9 shows a scheme of the SFE–packed-column SFC apparatus. The flow direction during the extraction mode is indicated by the solid line in Fig. 9a. Valve 9/9′ was set in the non-connecting position to make a dead end for the extraction line and at the same time to maintain the pressure over the pre-pressurized columns. Once equilibrium had been reached at the desired extraction pressure, valve 7 was switched to fill sample loop 8. The flow direction during the chromatography is indicated by the solid line in Fig. 9b.

Skelton *et al.*[78] also used a valve-switching scheme to extract solid samples with supercritical carbon dioxide and they introduced the analytes directly onto the SFC column. The viability of the method was demonstrated by the on-line SFE-packed column SFC of paprika. Qualitative comparisons were made between on-line SFE–packed column SFC with UV detection and conventional off-line dichloromethane extraction-packed column SFC for coal and coffee samples. The on-line procedure provides easy sampling, as there is no introduction of solvent into the SFC system because the SF is used both for extraction and as the eluent.

Coupling SFE and packed-column SFC on-line was also described by McNally and Wheeler[44,79]. They applied this configuration for the determination of sulphonylurea herbicides and their metabolites in soil, plant material and cell culture media. Methanol-entrained supercritical carbon dioxide was necessary for the extraction and the separation of the analytes studied. Increasing the flow-rates and the entrainer concentration improved the extraction recoveries. No quatitative data were given for the system. The relatively low temperatures used in the system prevented decomposition of thermolabile compounds and this was a significant advantage over GC methods. A limitation of the open-loop SFE system used was the long equilibration time required if the flow-rate or the concentration of the entrainers had to be adjusted. This is especially disadvantageous in coupled SFE-packed-column SFC systems where the extraction should be carried out at much higher flow-rates and entrainer concentrations than are suitable for packed-column SFC of the polar metabolites. Hence, a compromise has to be chosen and 100% extraction efficiencies should not be expected.

Engelhardt and Gross[80] performed on-line SFE-packed-column SFC-FID. A single-piston reciprocating HPLC pump was used to supply the carbon dioxide for both SFE and packed-column SFC via a T-joint. Finely ground drugs and food were dry-packed into standard HPLC columns of appropriate size. The extractor was then filled with supercritical carbon dioxide and the closed loop allowed a static equilibrium to be reached. After a certain time, the sample loop was filled with the extract by opening a valve. The sample loop could be filled repeatedly with the same extract via the applied closed-loop system. Alternatively, the system could be operated in an open-loop configuration or in a stepwise extraction process, in which the first extract could be vented to waste, and the extraction column refilled with supercritical carbon dioxide and the extraction repeated. In this way the kinetics of the extraction were studied using sample sizes of 1-2 g. The concentration of the extracted solutes reached constant values after 10–15 min. In a similar way. the yield of the extraction was

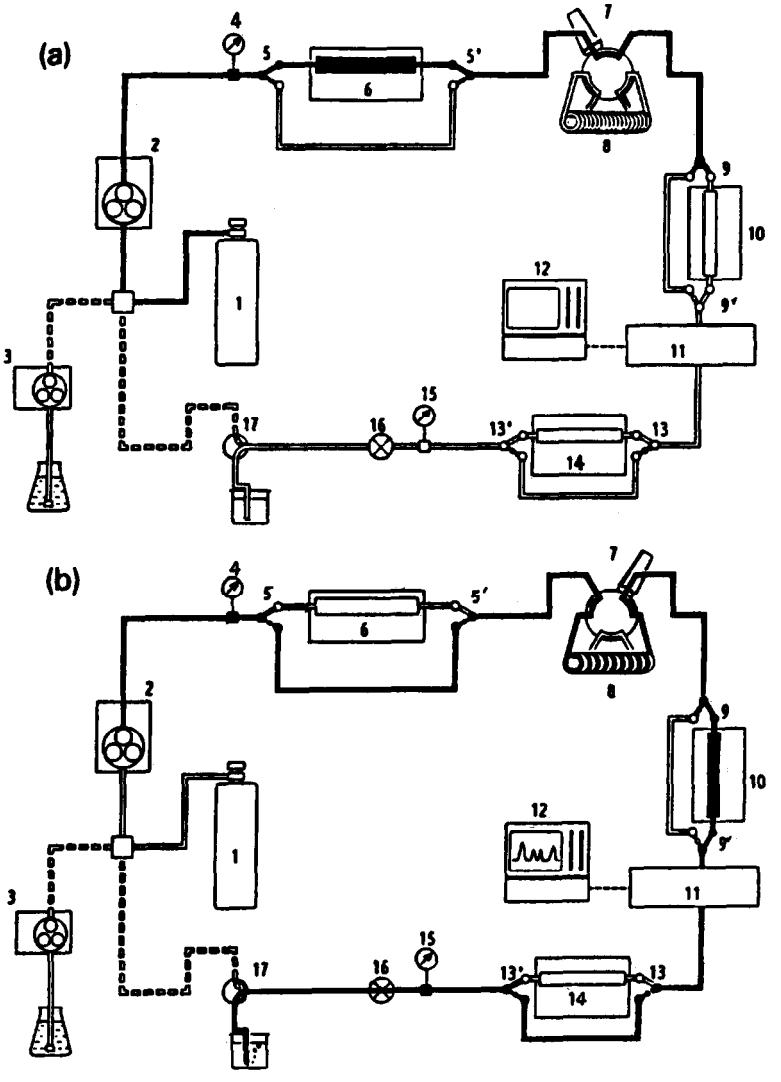

Fig. 9. Hydraulics of directly coupled SFE-SFC for extraction. 1 = Carbon dioxide cylinder: 2 = pump for delivering liquefied carbon dioxide; 3 = pump for delivering modifier solvent; 4 = pressure gauge; 5/5′ = six-way valve: 6 = extraction cartridge, thermostatted in oven; 7 = injector valve; 8 = extract trap loop; 9/9′ = six-way valve; 10 = chromatographic separation column in oven; 11 = highly sensitive multi-wavelength UV detector; 12 = data processor for 11; 13/13′ = six-way valve; 14 = extract trap column in oven; 15 = pressure gauge for monitoring back-pressure; 16 = pressure regulator; 17 = three-way valve. (a) After SFE, the injector (7) is switched to load the extra trap loop (8) with the extract. The injector is then switched back to by-pass the loop for pre-pressurization and equilibration of the separation column (10), while the loop holds the extract. (b) After pre-pressurization and equilibration of the separation column, the injector is switched to inject the extract held in the trap loop into the column. The injector valve in this figure is shown in the position for injection. (Reprinted with permission from ref. 56.)

studied and the amount of carbon dioxide for optimum extraction was determined. The extraction vessel (containing 1.6 g of caraway seed) was filled with supercritical carbon dioxide. A 20-μl aliquot was transferred to the packed-column SFC system and after refilling the extractor, the process was repeated. A plot of the concentration of the residual solutes extracted against the volume of carbon dioxide used in this stepwise extraction process yielded a decreasing curve. The rates of decrease for the various analytes differed significantly. The on-line SFE-packed-column SFC system allowed easy monitoring and control of the kinetics and the yield of the extraction process. SFE-packed-column SFC of ***Radix valeriana*** was also performed (Fig. 10). Further, a comparison was made between SFE-packed-column SFC and steam distillation-packed-column SFC of curry leaves.

Using the same set-up, Engelhardt and Gross[81] have also shown that non-polar pesticides such as lindane, aldrin and DDT can be selectively extracted from spiked soil (10 ppm of each pesticide) by supercritical carbon dioxide at 138 bar using stepwise extraction. After an equilibration time of 15 min, a 20-μl aliquot was switched to the SFC column with FID. No interfering substances were extracted from the soil. The minimum detectable concentration was about 1 ppm.

On-line SFE-packed-column SFC has been compared with dichloromethane extraction followed by packed-column SFC for double-base propellants by Ashraf-Khorassani and Taylor[82], using both FID and FT-IR detection. SFE with supercritical carbon dioxide (275 bar, 60°C) was performed for 12 h using 100 mg of propellant and a recirculating closed-loop system, while Soxhlet extraction with dichloromethane was performed with 2 g of propellant for 72 h. More than twice as many components were detected via packed-column SFC-FID coupled to SFE as with dichloromethane extraction. The conclusion was that either the SFE process dissolved a larger number of components or that the SFE extract was more concentrated than the dichloromethane extract. Some quantitative experiments should have been performed to study which of these explanations was true.

Ramsey ***et al.***[83] evaluated the SFE-packed-column SFC combination for the detection of a small group of veterinary drugs in freeze-dried pig's kidney. During extraction with supercritical carbon dioxide, the drugs were retained by the amino-

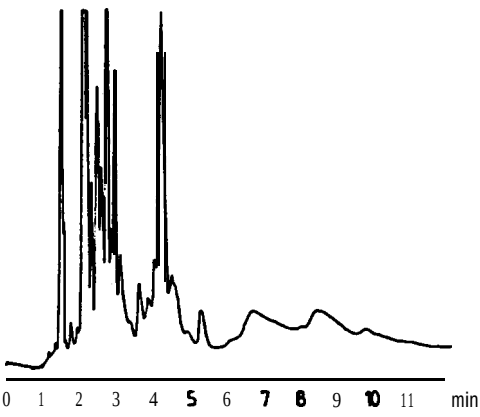

Fig. 10. SFE-SFC of ***Radix valeriana***. (Reprinted with permission from ref. 80.)

bonded SFC column, whereas non-polar endogenous material was not retained and passed to waste. The extraction cell was then switched out of the carbon dioxide flow, the mobile phase composition was altered by the addition of methanol and the drugs were eluted. Initial optimization of the SFE-packed-column SFC system with UV detection did not afford sufficient resolution and selectivity to allow detection of the drugs spiked at the 10 mg/kg level. In experiments using SFE-packed-column SFC–MS the intense background of the co-eluting components hindered the analysis. SFE-packed-column SFC-MS-MS was necessary to provide daughter ion spectra virtually free from interferences and to permit the unambiguous detection of drugs at the 10 mg/kg level. Unfortunately, quantitative comparisons between SFE and liquid extraction of both analytes and extraneous material extracted from these biological specimens were not made.

On-line SFE-packed-column SFC has also been described by Niessen et *al.*[84] using a phase-switching system. Plasma samples containing the thermolabile and pH-sensitive cytostatic drug mitomycin C (MMC) were injected onto a short precolumn. After washing with water and drying the precolumn with a stream of nitrogen, the compound of interest was desorbed using 12% methanol in supercritical carbon dioxide and analysed directly by packed-column SFC using the same mobile phase composition. Up to 1 ml of plasma containing 20 ng of MMC was analysed, with typical recoveries of 70% (Fig. 11). The on-line technique was far less time consuming and labour-intensive than its off-line counterpart. Drying of the precolumn appeared to be the rate-determining step. This was necessary, because water becomes entrained

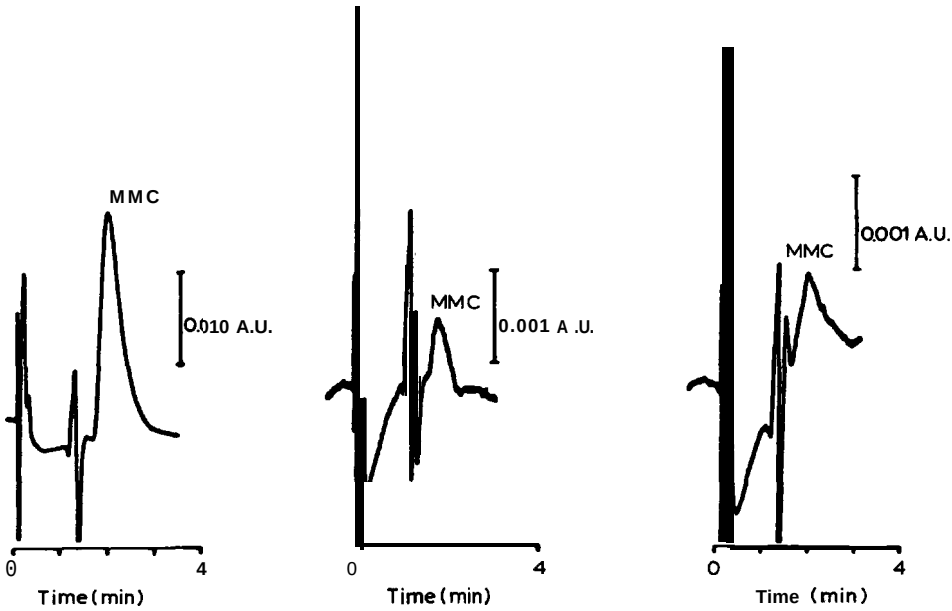

Fig. 11. Chromatograms obtained after on-line liquid-solid extraction of mitomycin C (MMC) from plasma samples. Left, sampling of 20 μl of plasma containing 200 ng of MMC; centre, sampling of 20 μl of plasma containing 20 ng of MMC; right, sampling of 1 ml of plasma containing 20 ng of MMC. (Reprinted with permission from ref. 84.)

in supercritical carbon dioxide and as a result deactivates the SFC column. At room temperature drying times of up to 25 min were not sufficient to remove all the water completely. Raising the temperature to 60°C for 10 min resulted in a significant decrease in the chromatographic signal, probably owing to thermal degradation of MMC. As a compromise 50°C was employed.

Direct SFE of aqueous samples and on-line coupling to packed-column SFC has been performed by Thiebaut *et al.*[85] using a dynamic open-loop system. Aqueous samples were injected directly into a supercritical carbon dioxide stream and extracted in a coil of appropriate length. Water and supercritical carbon dioxide are immiscible and therefore must be separated before detection. The extract-laden SF was separated from the water by means of a phase separator; the effect on the UV signal of SFE with and without phase separation is illustrated in Fig. 12. By trapping the extract-laden SF in a downstream sample loop, it could be diverted to a packed-column SFC system by switching the valve. Phenol and 4-chlorophenol were used as medium-polarity test compounds. The clean-up and extraction of 4-chlorophenol from urine was also shown. The extraction efficiency for the test compounds was over 85%, and the repeatability was 8% (R.S.D.) for the total SFE-phase switching–packed-column SFC system and 4% for both the SFE-phase switching and packed-column SFC systems separately.

Jahn and Wenclawiak[86] described an on-line system using a mini-extractor (85 μl) and a micro-extractor (3–4 μl), which could be coupled on-line with packed-column SFC and used under sub- or supercritical conditions. The mini-extractor was used for on-line SFE-packed-column SFC and the micro-extractor was applied for direct sample introduction.

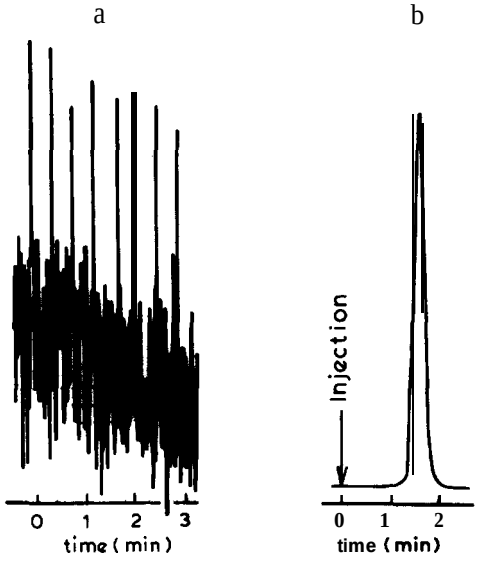

Fig. 12. Dual pump SFE-phase switching-SFC system, (a) without and (b) with phase separator inserted. Sample: 5 μl of water containing 5 μg of phenol. Note that there is a difference in attenuation; the phenol peak in (b) is not visible in (a). (Reprinted with permission from ref. 85.)

4.4.2. On-line SFE-capillary SFC. The direct coupling of SFE to capillary SFC was systematically investigated by Gmür *et al.*[87–89]. The optimization of some important instrumental parameters such as internal diameter and length of the capillary, pressure drop along the column, linear velocity and injection volume was studied. The coupled system was used to analyse natural products such as cheese, butter, coffee, tobacco and camomile. For cheese analyses the coupled technique allowed the simultaneous determination of volatile methyl ketones and non-volatile fatty acids, without any additional sample pretreatment.

An elegant way to ensure the solubility of the analytes in the mobile phase, before they are introduced into an SFC system, is to use the same fluid both as the eluent and as the injection fluid. A sample introduction system for capillary SFC

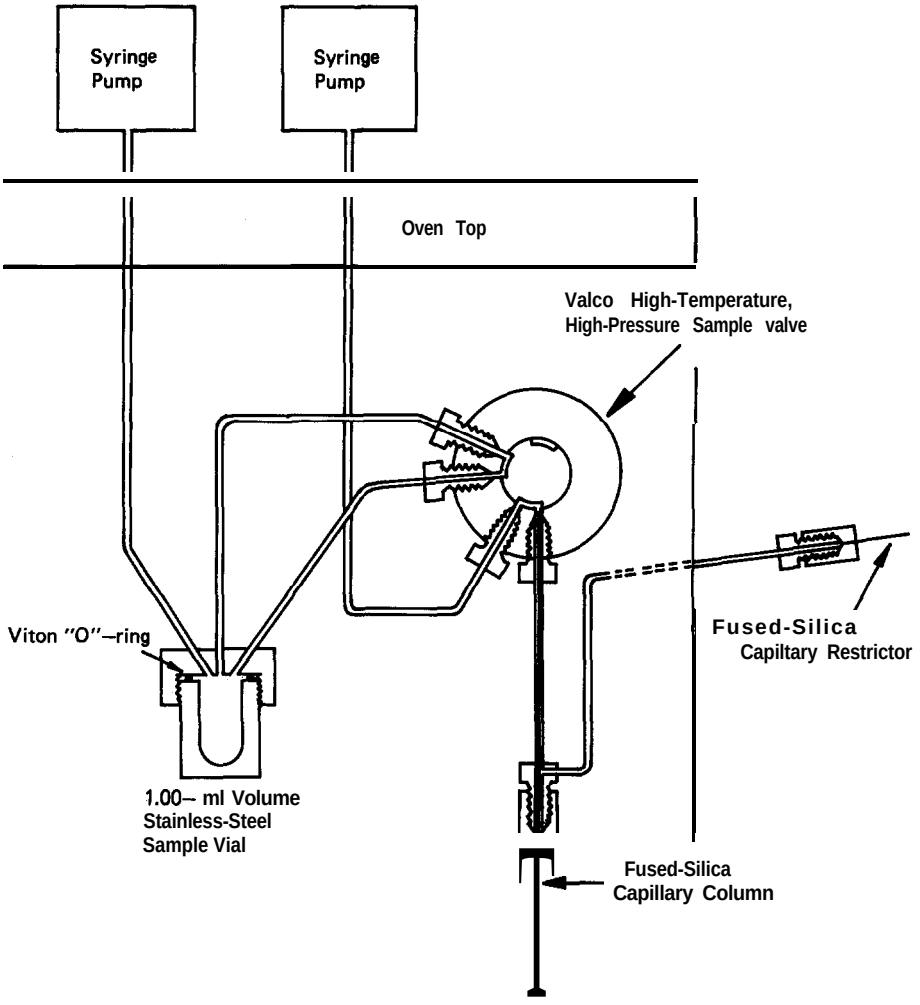

Fig. 13. Schematic diagram of a supercritical fluid injector for SFE–capillary SFC. (Reprinted with permission from ref. 76.)

allowing the dissolution of the sample in the SF before it is introduced into the column was constructed and evaluated by Jackson et *al.*[76]. It consisted of a closed-loop SFE system and injection of the SF was accomplished using a high-temperature, high-pressure sample-loop valve. The valve was mounted inside the oven, with the stand-off handle extending through the oven wall (Fig. 13). A l.O-ml volume high-pressure stainless-steel sample vial was used as extraction vessel and was connected via two tubes to the valve. A third tube was connected to the syringe pump which served to pressurize the SF in the injector. The analytical column was connected to the valve by means of a glass-lined splitter. The feasibility of SF injection was examined by comparing the results of injections using liquids and fluids[76]. Split injections of the SF solution were found to be more reproducible than split injections of liquids. Further, the solvating capacity of supercritical n-pentane as injection solvent was studied by comparing the SF injection of the high-molecular-weight PAH ovalene with injections in two different liquids. The results indicated that supercritical *n*-pentane solvated high-molecular-weight PAHs more rapidly than a few common liquids (Table 6). SF injection introduced over sixteen times as much ovalene into the SFC system as a solution in dichloromethane, and nearly twice as much as a solution in 1,2,4-trichlorobenzene.

The development of an on-line SFE-capillary SFC system with off-line FT-IR detection was recently reported by Raynor et *al.*[42] for the separation and identification of PAHs in coal pitch. An open-loop system was used. The SF extract was decompressed by means of a frit restrictor into the sample cavity of a cooled micro-valve injector, thus depositing the analytes and concentrating them, while the carbon dioxide escaped through the other valve opening. Subsequently, the contents of the loop were switched in-line with the mobile phase of the coupled capillary SFC. Several of the separated analytes were collected on a potassium bromide disc and, after solvent elimination, FT-IR analysis using a microscope accessory was performed (Fig. 14). The spectra obtained showed the power of this detection technique for distinguishing isomers. During on-line SFE-capillary SFC pressure programming was applied to fractionate coal pitch selectively during SFE and to transfer these fractions to the capillary SFC system[42]. The injection valve had to be kept above the critical temperature of the mobile phase, otherwise solutes deposited in the valve after SFE would not be redissolved. Another important aspect was that most samples analysed by capillary SFC were injected using low mobile phase densities. Conse-

TABLE 6

QUANTITATIVE COMPARISON OF LIQUID AND SUPERCRITICAL FLUID INJECTIONS FOR THE DETERMINATION OF OVALENE BY SFC WITH FLUORESCENCE DETECTION

Reprinted with permission from ref. 76.

Solvent	*Peak area*[a]
Dichloromethane (room temperature)	73 ± 13
1,2,4-Trichlorobenzene (room temperature)	674 ± 42
Supercritical n-pentane (210°C, 180 p.s.i.)	1177 ± 137

[a] Average of four injections ± standard deviation.

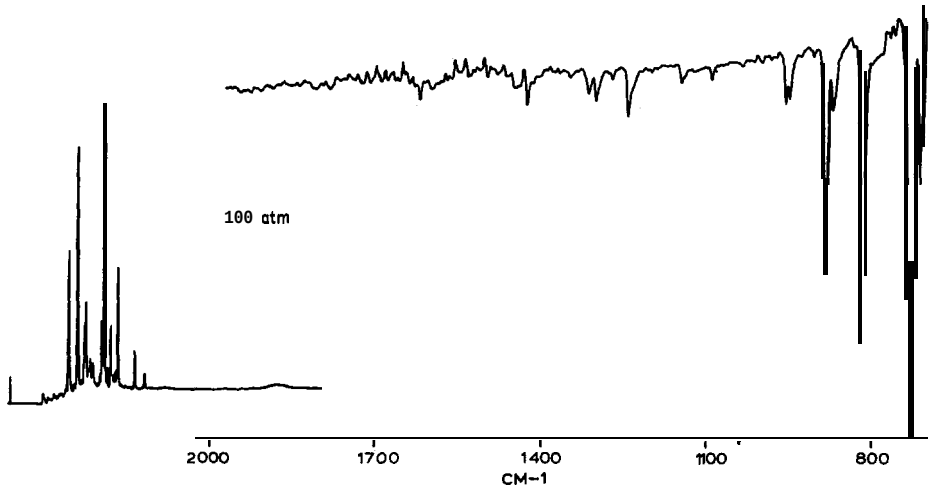

Fig. 14. Left, SFC of coal tar pitch extracted at 100 atm. Right, IR spectrum of peak a indicating the presence of phenanthrene and anthracene which have been co-eluted. (Reprinted with permission from ref. 42.)

quently, certain compounds which were soluble in carbon dioxide at high extraction densities may not have been introduced into the column at the low mobile-phase densities used during injection. An interesting feature is that a difference in density between the SFE and SFC allows samples to be focused on top of the analytical column[90].

With an open-loop system, Anton *et al.*[91] performed rapid qualitative studies of complex materials such as plastics, coffee powder and PAH-contaminated soil using analytical-scale SFE with concurrent capillary SFC. The deposition process did not cause peak broadening.

An SFE-capillary SFC fraction-collection system was developed to perform on-line extraction, separation and fraction collection of biologically important drugs (e.g., ouabain)[92]. The SF extract was decompressed via a linear restrictor and deposited in a deactivated capillary concentrator within a cryogenic trap. The internal diameter of the restrictor is important because larger internal diameters provide higher extraction efficiencies, but lower cryogenic trapping efficiencies. A 25-μm I.D. restrictor seemed to be a good compromise. The internal diameter of the concentrator (150 μm) was slightly larger than the outer diameter of the restrictor (148 μm), allowing tight insertion of the restrictor into the concentrator. This resulted in good chromatographic peak shapes, Fractions of the SFC effluent were collected from a frit restrictor at the column outlet in vials containing a preselected solvent, such as dichloromethane or methanol.

4.4.3. ***On-line SFE-packed-capillary SFC.*** On-line SFE-packed-capillary SFC is an interesting development in comparison with SFE–capillary SFC, because of a higher loadability and shorter analysis times. In comparison with SFE-packed column SFC the advantages are a lower pressure drop, higher efficiency (theoretical number of plates) and lower flow-rates, resulting in an easier interfacing with FID or MS instruments.

The use of on-line SFE-packed-capillary SFC was described by Hirata et *al.*[75]. Polyethylene film was extracted with supercritical carbon dioxide and the analytes were trapped on an uncoated fused-silica tubing (15 cm length). By coupling 5-cm sections of this tubing to a packed capillary column and using direct injection, they were able to confirm that the extracts were efficiently trapped in the first 5-cm section, even at an extraction temperature of 65°C. The feasibility of extending the technique to quantitative studies was also demonstrated. Improvements will probably centre on analysing narrower sections of the extracts by controlling the trap temperature and on using coated tubing or even a packed column.

5. CONCLUSIONS

The coupling of SFE with an analytical technique provides the potential for combined sample preparation and analysis. In addition to completely automated operation, rapid analyses and high recoveries can be achieved. Extraction rates often increase by more than an order of magnitude in comparison with Soxhlet extractions and, in general, better extraction recoveries are obtained compared with Soxhlet and thermal desorption techniques. Further, SFE is capable of processing thermolabile compounds, which cannot be desorbed by thermal desorption. Selectivity can be manipulated easily by the wide range of solvent powers available with SFs providing the potential for fractionation of complex samples and isolation of apolar to relatively polar analytes from a variety of matrices. In addition, SFE offers the possibility of sample concentration by decompression of the fluid prior to chromatographic analysis.

Although SFE has a number of advantages over the more classical methods, there are some limitations and problems. For instance, mainly solid samples are handled in SFE systems coupled to separation techniques. However, it will be obvious that for this type of sample this is often the only technique for on-line extraction. The problems associated with extracting aqueous samples have only recently been addressed, and considerable work is still needed in this respect, especially as in a considerable number of studies the samples are still freeze-dried to overcome problems with water. Commercial SFE apparatus is also focusing on the handling of solid samples. Further, mainly qualitative data are available, almost no systematic studies have been performed and the detectabilities are at the ppm-ppb levels.

In comparison with Soxhlet extractions a more complicated set-up is necessary. However, off-line SFE allows the collection of the extracted analytes in a vial containing a suitable organic solvent. In this way the use of complicated interfaces, such as those needed in on-line systems, can be avoided. The major problems in SFE are probably the loss of volatile analytes and blocking of the capillaries due to either precipitation of the extracted analytes or cryogenic cooling of the expanding SF if open collection is performed.

The potential strength of SFE in analytical chemistry is its coupling to other separation techniques such as TLC, HPLC, GC and SFC. SFE-TLC is an elegant and relatively cheap technique when only qualitative data are required. Separation can be improved by applying two-dimensional chromatography. Off-line SFE-HPLC has been applied for the determination of various compounds obtained from solid matrices or liquid matrices after freeze-drying. On-line SFE-HPLC has until now

only been applied to monitor extraction efficiencies. The use of column-switching systems for removing unwanted components in the extraction fluids will allow (semi)-quantitative analyses in the future.

So far, on-line SFE-GC is the most often applied combination. Extractions can be performed at relatively low temperatures and no sample handling or concentration procedures are required between extraction and GC analysis, thus reducing the possibilities for degradation and loss of analyte. The extracted analytes are quantitatively collected, which means that maximum sensitivity can be obtained and hence that the amount of sample needed can be reduced. Class-selective extractions can be achieved by performing multiple SFE-GC analyses at different extraction pressures. Further, on-line SFE-GC requires no modification of the gas chromatograph and there are good possibilities for focusing the extracted analytes at the top of the column. As a result, several companies are now providing SFE units with on-line GC interfaces.

The fastest growing technique in this field is on-line SFE-SFC, because the number of sample manipulations is limited by using the SFE fluid also as the eluent in the subsequent analysis, and because a wide variety of detection devices can be applied.

In general, it may be stated that for uncharged relatively apolar compounds, which can be dissolved in supercritical carbon dioxide, SFE shows several advantages over liquid extraction techniques. However, for more polar analytes the extraction efficiency depends strongly on the extraction conditions (i.e., pressure and temperature) and the addition of an entrainer is often required. The addition of a suitable entrainer or the use of a more suitable SF (e.g., ammonia or nitrous oxide) and the modification of the matrix (e.g., pH) should provide more favourable extraction conditions. However, this makes the interfacing to other techniques, such as GC, more difficult.

Future trends in SFE will probably be the processing of various kinds of matrices by using different SFs and various entrainers, the development of new interfaces for the coupling of techniques, the use of column-switching systems to remove the extraction fluid before the extracted compound is introduced to the actual chromatographic system, miniaturization of SFE systems if small samples have to be analysed and the development of efficient extraction systems allowing larger samples to be extracted and, hence, diminishing the problems in trace analysis caused by impurities present in SFs.

6. SUMMARY

After a brief description of the basic principles of supercritical fluid extraction (SFE), this review extensively discusses the application of SFE via its off-line and on-line coupling to chromatographic techniques, such as thin-layer, high-performance liquid, gas and supercritical fluid chromatography. Aspects such as speed, selectivity, sensitivity, potential for automation and possibilities of fractionation of the supercritical extract are discussed. Further, SFE liquid-liquid and liquid-solid extraction procedures are compared. Until now, SFE has been applied almost exclusively to the extraction of apolar compounds from solid samples, but the method seems also to be attractive for liquid samples. Generally, SFE is more efficient (in terms of extraction times and recoveries) than Soxhlet extractions and more suitable for ther-

molabile compounds. Furthermore, efficient coupling to chromatographic techniques is possible, although much work still has to be done to optimize the necessary interfaces. The extraction of relatively polar compounds is possible only if high densities are used or if modifiers are added to the supercritical fluid. The interfacing with separation techniques is then less simple.

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