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Review

Recent developments in the use of supercritical fluids in coupled systems

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ABSTRACT

Multi-dimensional systems utilizing supercritical fluids in either chromatography or on-line extraction are reviewed, with the main emphasis on phase transfer, reconcentration and selectivity.

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1. INTRODUCTION

Fractionation or purification of complex samples prior to analysis is usually performed either by extraction or by chromatographic methods. Off-line procedures are often chosen when multiple analyses are required on each sample, but automated on-line multi-dimensional techniques can reduce the analysis time and improve accuracy, reproducibility and detectability [1]. The requirements for multi-dimensional separations were defined by Giddings [2,3], demonstrating that multi-dimensional techniques can lead to extremely high peak resolution. An overview of interfacing methods, with particular emphasis on liquid chromatography–gas chromatography, was presented by Davies *et al.* [4].

The first fractionation step may consist of liquid extraction, solid-phase extraction, supercritical fluid extraction (SFE), chromatography with or without guard columns and other separation methods, such as dialysis, depending on the components of interest. Liquid samples from the first separation step can be transferred to the second mode of sep-

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aration, such as to gas chromatography (GC), supercritical fluid chromatography (SFC), liquid chromatography (LC), thin-layer chromatography or capillary zone electrophoreses, by narrow heart cuts via a sampling valve. An important question is whether the concentration of a narrow cut is high enough for analysis in the second step or if a concentration step is required. This paper discusses opportunities and problems involved with the use of supercritical fluids in multi-dimensional systems, with particular emphasis on phase transfer, reconcentration and selectivity, and demonstrates how some problems have been solved in recent developments.

2. PHASE TRANSFER OF LIQUID SAMPLES

Combining LC and GC is a fairly straightforward technique provided that suitable column dimensions are selected, with narrow-bore LC columns or split-flow systems.

The fraction to be transferred needs to be defined by on-line detection or by retention time. A detector will usually be inserted either directly in the flow line between the first column and the transfer valve (V1) or connected to the waste line (Fig. 1). The first alternative (a) requires an on-column flow-cell in order to limit peak broadening, but gives direct control of the fraction transferred. The second alternative (b) does not require on-column detection and

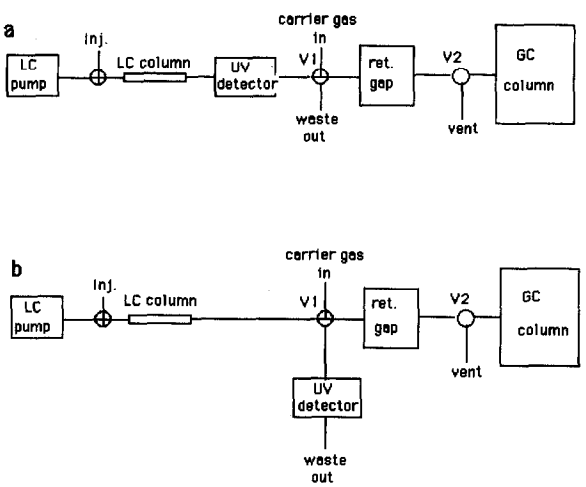

Fig. 1. Scheme of LC–GC with a UV detector (a) in the transfer line or (b) in the waste line.

gives no extra peak broadening but lacks the direct control of the transferred fraction.

After the collection time or volume has been determined, the fraction is eluted directly into a retention gap by the LC pump or is collected in a loop connected to the valve (V1).

With concurrent solvent evaporation [5], large volumes can be transferred to a heated retention gap, 2–20 m long and 0.3 mm I.D., with a pump rate of 20–200 μl/min, as the solvent evaporates as it enters the retention gap. With non-aqueous solvents, millilitre volumes can be transferred [5]. However, low-boiling solutes can only be reconcentrated with partial concurrent evaporation, using long retention gaps.

With the standard retention gap technique the solvent is not removed during the transfer step. With a 50 m × 0.32 mm I.D. retention gap, up to 300 μl of non-aqueous solvents can be injected, utilizing a thick-film column for better reconcentration [6]. Unfortunately, such high-volume injections are not permissible with aqueous solvents, as the surfaces of the retention gaps are not easily wetted by water and as the vapour volume of water is approximately four times higher than that of most non-aqueous solvents. As most LC applications utilize aqueous reverse-phase systems, the transferable volumes are drastically reduced.

With microbore (1 mm I.D.) LC columns, the peak volumes are usually in excess of the volumes that can be handled by conventional splitless or on-column GC injectors. With a 10 cm × 1 mm I.D. column, the peak volume at $k' = 3$ can be calculated to be 6.4 μl (Table 1) and with a 25 cm × 1 mm I.D. column to be 16 μl. However, as the components of interest often do not show up as visible peaks on the first column, but rather are hidden behind other components, the cut must be considerably wider than one calculated peak volume. With a buffer of two peak volumes on each side of the peak, to prevent losses, the corresponding volumes are 32 and 80 μl, respectively. If a relatively broad band ($k'_n - k'_m = 1$) is collected, the corresponding volumes are of the order of 64 and 160 μl (Table 1). The only way to concentrate such volumes is to use concurrent solvent evaporation with slow introduction into a heated retention gap. With packed microcolumns (0.1–0.3 mm I.D.), however, the effluent volumes can be handled with standard

TABLE 1

PEAK VOLUMES FROM PACKED COLUMNS, CALCULATED AS 4 STANDARD DEVIATIONS

Column length, 10 cm; N = 4000; linear flow = 0.14 cm/s.

Column I.D. (mm)	Peak volume (μl)			Flow-rate (μl/min)	Wide cut ($\Delta k'$ = 1)	
	k' = 1	k' = 3	k' = 7		μl	s
1	3.2	6.4	12.8	40	64	96
0.5	0.8	1.6	3.2	10	16	96
0.25	0.2	0.4	0.8	2.5	4	96
0.1	0.05	0.1	0.2	0.4	0.7	96

injection techniques, for example with a sampling valve with a 5-μl loop and a sampling time of 1–2 min.

3. LC–SFC

Compared with capillary GC, the column dimensions required to obtain similar efficiencies are significantly smaller in capillary SFC owing to the less favourable mass transfer properties of supercritical fluids compared with gases. Consequently, the volumes of the retention gaps that can be used in SFC need to be smaller than in GC, in order to avoid peak broadening. The required reduction in inner diameter is difficult to stipulate, as the reconcentration that can be obtained at the column inlet in SFC depends on the density gradient that can be applied.

In LC–SFC (Fig. 2), a selected fraction can be transferred and concentrated as above on a heated retention gap or the solvent can be removed with an inert gas (gas purging) or removed under supercritical conditions on a precolumn [7]. The latter procedure allows volumes up to 1–2 μl to be transferred to 50 μm I.D. columns. During solvent evaporation, the vent valve (V2) has been recommended to be closed towards the SFC column in order to maintain pressure on the column and avoid peak broadening [8].

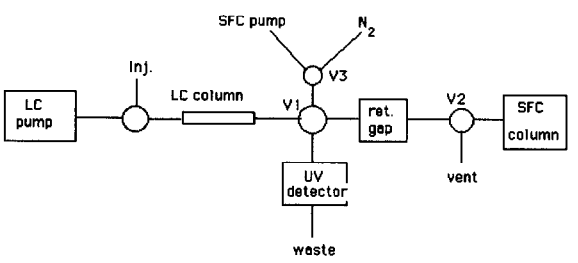

Fig. 2. Scheme of LC–SFC with a UV detector in the waste line and solvent venting valve (V2). The solvent can be removed by gas purging via the switching valve V3.

In a recent demonstration of LC–SFC, utilizing a packed capillary size-exclusion column in the first step, 3–7-μl samples were transferred, the solvent was evaporated with an inert gas on a long retention gap (13 m × 100 μm I.D.) and the components were focused at the inlet of the open-tubular SFC column [9]. Attempts to collect in coated open-tubular precolumns suffered from the disadvantage that solvent which was dissolved in the swollen film was released later, causing disturbances in the chromatograms.

4. PHASE TRANSFER OF SAMPLES IN SUPERCRITICAL FLUIDS

In multi-dimensional systems starting with SFE or SFC, sample fractions in large (millilitre) volumes of carbon dioxide can be collected in a small cold-trap which usually consists of a cooled uncoated retention gap. Samples in carbon dioxide can also be adsorbed in an open-tubular coated precolumn or a packed precolumn. An advantage of SFE over liquid extraction is that a potentially higher selectivity can be obtained, as the density can be chosen at will. Although liquid extraction can be performed with solvents of different solubility, this is significantly more laborious than SFE, where multi-step extractions can be achieved by controlling the pressure and the temperature only, or by a

final step with batch-added solvent [10]. Further, owing to the lower viscosity and higher diffusivity and also the absence of phase separation problems, supercritical fluids often reduce extraction times considerably. Thus, based on these simple physical facts, utilizing a supercritical fluid in the first dimension will often be of significant advantage, whether this is SFE, capillary SFC or packed column SFC.

5. SFC–SFC

The first multi-dimensional SFC was performed on packed columns [11–13]. With valve switching and back-flushing, oil samples were class separated on three coupled microbore columns [11,13], utilizing a flame ionization detector (FID) for quantification. The columns gave good resolution of compound classes due to high selectivity, not high efficiency. The advantages of packed column SFC–SFC are speed and selectivity. Crude North Sea oils were separated into three fractions on three columns and determined within 10–15 min (Fig. 3). The flame ionization detector, however, sets limitations on the total flow into the detector [14]. With packed columns, current detectors require inner diameters not larger than *ca.* 0.5–1 mm for high-speed purposes.

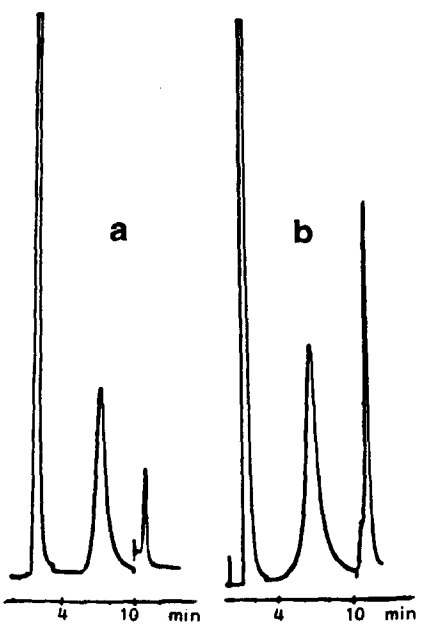

Fig. 3. SFC–SFC class separation of saturates, aromatics and resins in a crude light oil (a) and a crude medium heavy oil (b). From ref. 14, with permission.

For applications requiring high resolution, the last column will normally be a capillary column. In order to obtain high speed and good loadability, a packed narrow-bore column can be included as the first column [15]. The function of the packed column will often be class separation, as shown for coal tar samples [16]. With a rotary valve interface and a cold trap to focus the sample, overloading the capillary column can be avoided. The best resolution on the second column is obtained with a narrow cut from the first column, but there are practical limitations to very narrow heart cuts. Narrow time intervals are difficult to reproduce, and the risk of losing part of the material is considerable. With wider cuts, retention intervals are easier to control, assuming a focusing device is included to avoid overloading the capillary column.

Owing to the limited loading capacity, open-tubular SFC is not necessarily the first choice for the initial separation step. However, if sample concentration is not needed, SFC–SFC with two capillary columns has been demonstrated to give high resolution of narrow heart cuts from complex samples [16]. The total analysis time is increased with a capillary column in the front, although this can be reduced by using a short column at high flow-rates [7].

Sample concentration from the supercritical state is not completely without problems. Pressure reduction from several hundred bar to the lower levels that are needed to deposit the solutes in the retention gap requires a restrictor which is heated to avoid plugging (by solid carbon dioxide or by precipitated sample components), sufficient room for fluid–gas expansion and efficient trapping in the collector. Hence the construction of the restrictor–collector interface is vital to the practical utility of the system, particularly for applications including relatively high-molecular-weight compounds.

6. SFC–GC

By introducing GC in the last dimension, higher chromatographic efficiency is obtained, for applications that allow GC to be used. SFC–GC combinations have been reported for the analysis of fossil

fuel samples with packed SFC columns in the first step [17,18]. With the ability to transfer large effluent volumes to a cold-trap after density-related class separations, the potential of SFC–GC is probably far from being fully developed.

7. SFE–LC

SFE–LC utilizes the advantage of a selective extraction method, but does not always include sufficient resolution in the second dimension. Thus, pesticides in grass were determined at ppb levels with SFE–LC connected to GC with electron-capture detection in a three-dimensional system [19]. The phase transfer from the supercritical state was performed on a linear restrictor (12 μm) and a ceramic frit in a short (4 cm) 0.25 mm I.D. tube at the LC column inlet, venting the carbon dioxide gas to the atmosphere via a three-port connector. The recovery of the insecticide chlorpyrifos was virtually quantitative. Extraction of the same pesticide from wheat required 2% methanol in carbon dioxide for quantitative recoveries [20]. The LC–GC interface was recently shown to be able to transfer larger volumes of liquid, utilizing a modified version of concurrent solvent evaporation [21].

8. SFE–SFC

The most important feature of SFE–SFC is the ability to chromatograph the complete extract, after collection of large extraction volumes on a cold-

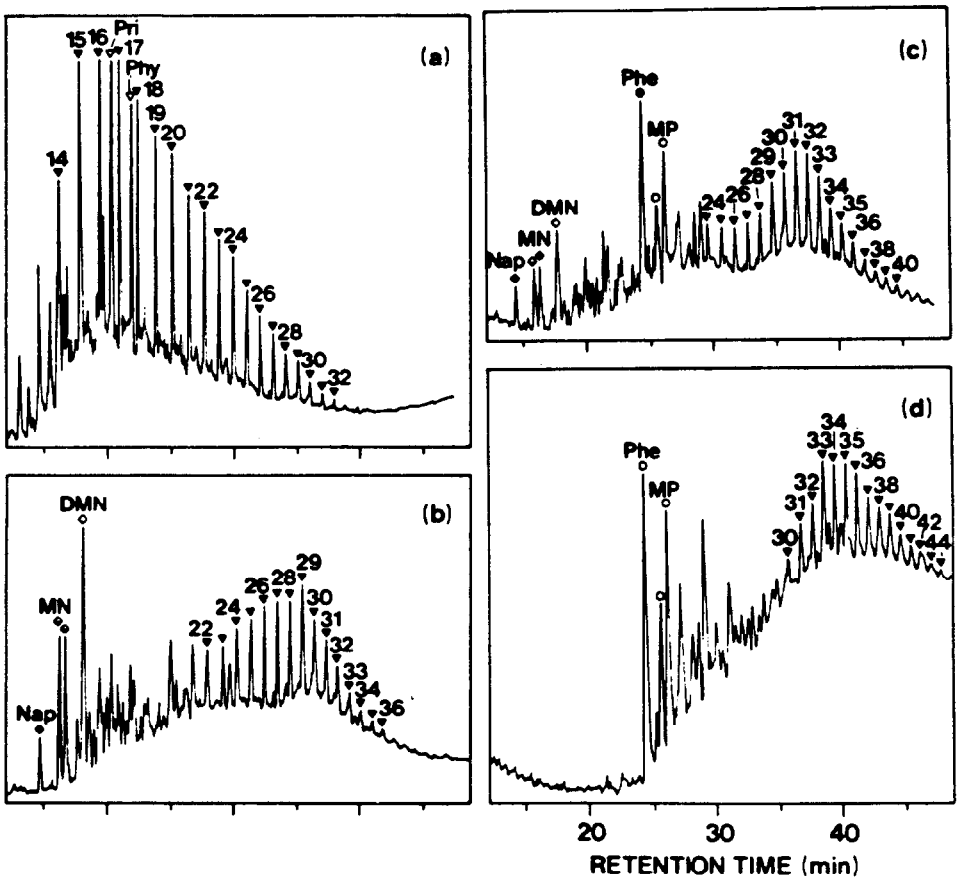

Fig. 4. Capillary SFC of four-stage extraction of Kimmeridge Clay shales with carbon dioxide at (a) 25 MPa and 110°C, (b) 40 MPa and 110°C, (c) 40 MPa and 40°C and (d) 40 MPa, 40°C and CS_2. Peaks: Pri = pristane, Phy = phytane, Nap = naphthalene, MN = methylnaphthalenes, DMN = dimethylnaphthalenes, Phe = phenanthrene, MP = methylphenanthrenes; numbers refer to *n*-alkanes. From ref. 10, with permission.

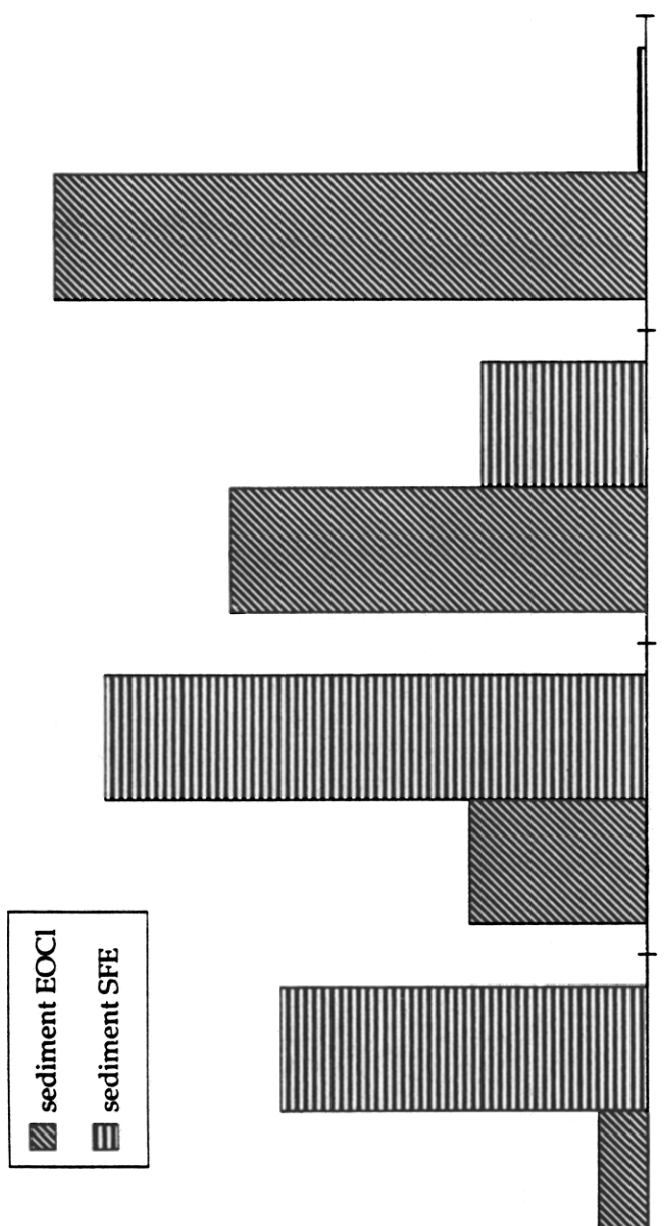

Fig. 5. Polarity distribution of extracts of marine sediments with supercritical CO_2 (sediment SFE) compared with extraction with cyclohexane–isopropanol (sediment EOCl) by elution on silica with the effluents A–D. A = toluene–cyclohexane (1:3); B = diethyl ether–cyclohexane (14:86); C = diethyl ether; D = methanol. From ref. 35, with permission.

trap. Applications utilizing SFE–SFC have been demonstrated in a substantial number of papers with cryotrapping as the most common collection method [22–28], but also with sorbent adsorption as an alternative [27,29–32]. With polar solutes, sorbent trapping may give rise to mass transfer problems, owing to lower desorption rates of polar solutes from adsorbents [27].

An example of the selectivity of SFE was demonstrated by multi-stage extraction of oil shales at different densities (Fig. 4).

9. SFE–GC

SFE–GC, which probably is the most widely applied multi-dimensional technique involving supercritical fluids, was first described by Hawthorne and Miller [33] in 1986. One reason for the instant popularity is that the SFE effluent could simply be depressurized inside the conventional split/splitless injection port, without additional heated transfer lines [33,34]. The advantages of the SFE–GC combination are that the requirements for small retention gaps are less demanding in GC, allowing standard GC dimensions to be used, and that capillary GC in general gives the highest resolution of all the chromatographic techniques. The limits of the SFE–GC combination are usually determined by the limitations set by the chromatographic technique, but often the extraction properties of supercritical carbon dioxide are highly compatible with analytes suitable for GC. As an example, the total content of organic chlorine extracted from marine sediments with plain carbon dioxide was much smaller than that with liquid extraction, particularly of the polar components (Fig. 5). The amount of chlorinated compounds that could be chromatographed by GC, however, was independent of the extraction method [35], demonstrating the selectivity of the extraction and the advantage of not filling up the injector with "dirt".

10. COLD-TRAPS AND SAMPLE TREATMENT

The cold-traps used in SFC–SFC, SFC–GC, SFE–SFC and SFE–GC commonly utilize temperatures of −10 to −50°C. If too high temperatures are chosen, volatile components are lost. If too low temperatures are chosen, water or carbon dioxide may become collected in addition to the solutes, ruining the chromatography after desorption. In order to avoid filling the cold-trap with ice, moist samples should be dried, by freeze-drying or by treating the sample with hydrate formers, such as anhydrous sodium sulphate. Freeze-drying is not compatible with volatile analytes. Hydrate formers have been used on various occasions, but so far no systematic studies have appeared on their ability to withhold the crystalline water under supercritical conditions.

Samples of biological origin and food-related samples often contain considerable amounts of fats, which also have a tendency to fill the cold-trap and overload the column after desorption. The selective extraction of analytes without the fat by adjusting the carbon dioxide density alone is a very tricky procedure and often not possible. A far more practical approach is to add a sorbent with a high fat affinity to the system. Thus, in the extraction of polychlorinated biphenyls (PCBs) from crab hepatopancreas, containing 9% fat [36], the PCBs were virtually selectively extracted by including basic aluminium oxide in a separate vessel in the flow-line after the extractor (Table 2). Thus, treating the sample to increase the adsorption of unwanted sample components introduces the opportunity to improve the selectivity by simple methods. In the future, combinations of SFE and adsorbents are likely to be of value for many applications where high selectivity is required.

TABLE 2

EXTRACTION OF FAT FROM CRAB HEPATOPANCREAS (CONTAINING 9% FAT) WITH CARBON DIOXIDE AT 60°C

The samples were treated with anhydrous sodium sulphate (1:3, w/w), with or without an equal amount of basic alumina. The PCBs were extracted quantitatively at 14.5 MPa. From ref. 36.

Pressure (MPa)	Extracted fat (% of total mass)	
	Na_2SO_4	$Na_2SO_4 + Al_2O_3$
14.5	0.90	<0.01
20	–	0.31
25	2.2	0.44
30	–	0.55
35	4.5	0.60

11. CONCLUSIONS

Automated on-line multi-dimensional separation techniques can reduce analysis times of multi-component samples and improve accuracy, reproducibility and detectability. The use of supercritical fluids in coupled systems is favoured by the lower viscosity and the higher diffusivity of supercritical fluids compared with liquids. Rapid, selective single-stage or multistage extractions can be performed, particularly in combination with the use of selective adsorbents, and coupled to LC, SFC or GC. The consumption of toxic or environmentally hazardous solvents is reduced. Packed column or open-tubular column SFC is coupled to LC or GC depending on solute solubility, selectivity and the efficiency required. Recently developed solvent venting techniques allow increasingly larger volumes of liquid fractions to be transferred to capillary columns. Fractions in supercritical fluids can be transferred to high-resolution columns, either directly or after on-line concentration.

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