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SUPERCRITICAL-FLUID EXTRACTION OF AQUEOUS SAMPLES AND ON-LINE COUPLING TO SUPERCRITICAL-FLUID CHROMATOGRAPHY

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SUMMARY

The potential of segmented-flow systems for on-line liquid/supercritical-fluid extraction was explored. The use of a phase separator (PS), its design and performance were investigated, utilizing phenol and 4-chlorophenol as the test compounds, water as the liquid phase and supercritical carbon dioxide as the extractant. On-line coupling of such a liquid/fluid extraction (SFE) system to supercritical fluid chromatography (SFC) was demonstrated, as was the feasibility of extracting phenol from an urine sample. The extraction efficiency for the test compounds was over 85%. The repeatability was about 8% relative standard deviation (R.S.D.) (n = 8) for the total SFE-PS–SFC system and 4% R.S.D. for both the SFE-PS and the SFC operation. The potential of coupling SFE to other chromatographic and detection principles is discussed.

INTRODUCTION

Supercritical fluids (SFs) possess physical properties intermediate between those of liquids and gases[1]. Their relatively high solvent strength, low viscosity and high solute diffusivity make them attractive for extraction because mass transfer is facilitated. Moreover, the extraction temperature can be low so that the risk of labile analyte degradation is reduced. As solvent strength is related to fluid density[2], selective extraction can be performed by varying the pressure and/or temperature of the fluid[3–5]. In addition, the recovery of the extracted analyte is simplified because many SFs are gases at room temperature and can be removed by decompression. Supercritical fluid extraction (SFE) has repeatedly been performed with solid matrices[6–20]. It has been coupled on-line with conventional packed column liquid chromatography (LC) for SFE–LC of *Radix valerianae*[15], with gas chromatography (GC) after cryofocusing for SFE–GC of flavours and fragrances[16] or polycyclic aromatic hydrocar-

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bons (PAHs)[3,5] or with supercritical fluid chromatography (SFC) for SFE–SFC[4,10,12,13,17–20].

In contrast, on-line SFE–SFC of aqueous samples has never been reported. These samples are not suitable for direct injection into SFC because of the low solubility of water in supercritical CO_2[21,22]. Moreover, water, like other polar modifiers is known strongly to affect solute retention, even with chemically bonded silica stationary phases[23,24]. Thus, the bulk of the water must be separated from the extraction fluid prior to SFC.

This paper describes a preliminary study of liquid/supercritical fluid extraction and its coupling with SFC by means of a phase separator (PS)[25] in order to remove the aqueous phase from the supercritical CO_2. Thus, after the extraction step the analytes are recovered in the supercritical CO_2 and can be directly switched into the SFC. Such a SFE-PS–SFC system is described for the determination of phenolic compounds in urine samples.

EXPERIMENTAL

Apparatus

Fig. 1 shows the SFE-PS–SFC apparatus. A single carbon dioxide cylinder with eductor tubing was connected to both the syringe pump used for extraction (Model Phoenix 20; Carlo-Erba, Milan, Italy) and the laboratory-made syringe pump used for SFC. The SFE was performed in a constant-pressure mode, and the SFC in a constant-flow mode, both at 40°C using a water-bath.

The reservoir of the extraction pump, maintained at 12°C by a water cooling jacket, was connected to the 1.3 m × 0.5 mm I.D. stainless-steel extraction coil after preheating of the CO_2. Initially, experiments were carried out with a water stream added to the CO_2 before the extraction coil via a T-piece (Fig. 1). The water was delivered by a reciprocating pump (Model 302; Gilson, Villiers-le-Bel, France). In the dual-pump set-up, the sample (5 μl) was directly introduced into the water stream via injection valve 1. In subsequent experiments, the water pump and the T-piece were removed. The samples were then injected as water plugs directly into the supercritical CO_2 using injection valve 2. The phase separator was connected to the extraction coil as depicted in Fig. 1; the upper outlet was connected either to the UV detector or to the SFC injection valve 3 and back-pressure maintained by means of restrictor 1. The lower outlet (waste) was connected to restrictor 2. For monitoring the liquid/supercritical fluid extraction, a single-wavelength UV detector (Knauer, Berlin, F.R.G.) was used. It was equipped with laboratory made 320 μm I.D. fused-silica capillary cell (*i.e.*, optical pathlength 320 μm). The restrictors were made of fused-silica capillaries (6 cm × 320 μm) packed with 5-μm Hypersil ODS particles (Shandon Southern, Runcorn, U.K.).

The SFC system was similar to that described elsewhere[23]. The laboratory-made syringe pump delivered the supercritical CO_2 with a flow-rate of 1.1 ml/min (20°C) and was connected via a preheating coil to injection valve 3, which was fitted with a 200-μl loop. For SFE-PS–SFC measurements, the 320-μm pathlength UV cell was used. A back-pressure regulator (Model 26-1721-24082; Tescom, Minneapolis, MN, U.S.A.) was used to ensure accurate and stable pressure control throughout the SFC system.

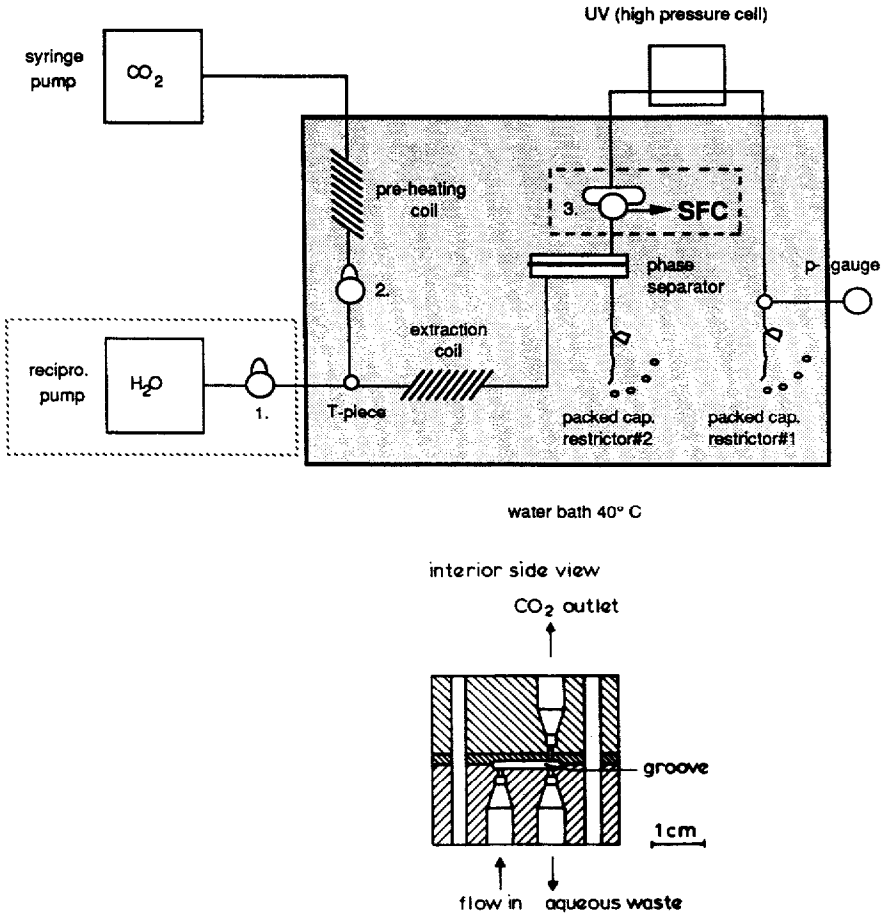

Fig. 1. Schematics of the SFE-PS–SFC apparatus. The single-pump (only CO_2) and dual-pump systems (CO_2 and water) are shown. Insert: expanded interior side view of phase separator.

The flow-rate of liquid CO_2 was regulated by the pumps. Supercritical fluid recovery, defined as the detector carbon dioxide flow-rate/total carbon dioxide flow-rate out of the phase separator, was determined by measuring the flow-rates of CO_2 after decompression using calibrated vessels filled with water, and placed above the outlets of the restrictors in the water-bath.

Chemicals

The 15 cm × 3.1 mm columns were laboratory-made using 5-μm LiChrosorb RP-18 (Merck, Darmstadt, F.R.G.) or 5-μm RSIL-CN (Alltech, Eke, Belgium) as the stationary phases. The CO_2 was technical grade (Hoekloos, Amsterdam, The Netherlands). Analytical grade phenol and 4-chlorophenol were chosen as the medium-polar test compounds to investigate the suitability of the system.

RESULTS AND DISCUSSION

Dual-pump system

Phase separator. Phase separators with a sandwich design as described for liquid–liquid extraction[25] have been used in various areas in the past. The mechanism of separation is based mainly on the wettability of the stainless-steel and PTFE parts by an aqueous and water-immiscible extractant phase, respectively. In addition, density differences are considered. Hence, contrary to the situation normally encountered with liquid–liquid, *e.g.*, water–chlorinated hydrocarbon, mixtures for the system water–supercritical CO_2 ($d < 0.8$ g/ml) the phase separator had to be mounted upside down to function effectively (see Fig. 1). The dimensions and the groove volume (43 μl) were the same as previously reported[25].

The disc material functioning as the hydrophobic surface in the separation process had to be selected carefully to withstand the more rigorous pressure conditions of the supercritical system. The various materials tested for water–supercritical CO_2 separations are shown in Table I. PTFE cannot be used because it deforms at higher pressure and starts to leak. Kel-F changed its colour and shape as a result of the strong tightening which was necessary to withstand the SFE operating pressure. Good stability was obtained with Delrin and PVDF, which did not show any visible change after prolonged use (several months).

SFE system. The dual-pump system was the initial arrangement for study of the liquid/supercritical extraction system and can be seen in Fig. 1. Sample plugs were injected through injection valve 1 and the supercritical carbon dioxide stream was monitored by the UV detector.

As a first step, the existence of a water–supercritical CO_2 segmented system was tested by inserting the UV detector prior to the sandwich phase separator. As expected, this resulted in a very noise signal typical of the coexistence of two immiscible phases. This observation is in agreement with the binary phase diagram for water–supercritical CO_2[21]. Placing the UV detector after the phase separator yielded a low noise background with rather symmetrical signal peaks for the analyte, phenol. The sharp contrast between the system without and with the phase separator is nicely illustrated in Fig. 2. Even with the phase separator inserted, sharp random spikes occurred occasionally. They were caused by water being carried along by the supercritical fluid. The water spikes were never eliminated completely, but they posed no major problem since they were easily distinguished from the analyte signal. The influ-

TABLE I

EVALUATION OF DISC MATERIAL FOR THE PHASE SEPARATOR

Disc material	*PTFE*	*Kel-F*	*PVDF*	*Delrin*
Structure	$(CF_2)n$	$(-CFCl-CF_2)n$	$(CH_2-CF_2)n$	$(-CH_2-O)n$
Pressure stability (150 bar)	Leaks	Good	Good	Good
Lifetime	–	<2 weeks	>4 weeks	>4 weeks
Suitability for SFE-PS	–	±	+	+

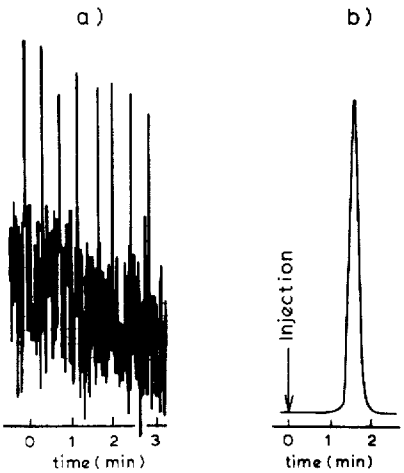

Fig. 2. Dual pump system (a) without and (b) with the phase separator inserted. Conditions: CO_2 at 120 bar, flow-rate 300 μl/min; water at 150 μl/min; temperature 40°C; UV detection at 254 nm (320-μm pathlength) at (a) 0.08 a.u.f.s. and (b) 0.01 a.u.f.s., respectively. Sample: 5 μl water containing 5 μg phenol. Note the difference in attenuation; phenol peak of b will not be visible in a.

ence of the supercritical fluid recovery on the size of the analyte signal and the background noise has not been studied in detail, because variable micro restrictors were not available. Obviously, for sensitivity reasons it is desirable to recover as much of the supercritical CO_2 as is possible. In practice, however, only 10–20% was recovered before spiking became problematical. The extraction efficiency was determined only in the SFE-PS–SFC system as described in that section. The extraction coil of 1.3 m × 0.5 mm I.D. was long enough to obtain distribution equilibrium.

Single-pump system

Although the feasibility studies described above were done with a dual-pump system, injecting an aqueous sample into a water stream, with the subsequent creation of a two-phase system, it should be realized that the injection of an aqueous sample, a biological fluid or an aqueous environmental sample, directly into the supercritical carbon dioxide stream would be of real interest because of its simplicity and, probably, higher sensitivity. The system proposed is shown in Fig. 1, leaving out the reciprocating pump and injection valve 1, *i.e.*, injecting the sample via valve 2. The recovery of the supercritical CO_2 is now increased to *ca.* 50%. For identical analyte concentrations, the peak areas were some three-fold higher with the single-pump approach. This factor cannot easily be explained because not only is the supercritical fluid recovery different for the two systems, but also the ratio of the volumes of the two phases during the extraction and the flow-rate through the detector. Consequently, all further experiments were carried out using direct aqueous plug injections into the supercritical fluid stream, at a flow-rate of *ca.* 150 μl/min. The repeatability of the single-pump SFE-PS procedure was 4% R.S.D. (n = 5) with phenol as the analyte.

On-line coupling of SFE-PS with SFC

In the next stage, the single-pump extraction system was coupled via valve 3

(see Fig. 1) to a conventional SFC apparatus with a packed column, to study the on-line SFE-PS–SFC system. Having previously determined the experimentally observed peak volume after SFE-PS to be *ca.* 100 μl, valve 3 was fitted with a 200-μl loop to ensure total trapping of the compounds before switching to SFC. A plot of the peak height of the analyte *versus* the time of switching valve 3 after sample injection in valve 2 (Fig. 3) confirmed that the time of switching valve 3 was not critical with this size loop. Three runs were performed, each corresponding to a series of analyses performed during 1 h, without refilling of the 20-ml reservoir. The repeatability calculated for the mean peak height of the three runs was quite satisfactory (3% R.S.D.; $n = 3$).

For the on-line SFE-PS–SFC system, both a C_{18}- and a CN-bonded silica stationary phase were used for SFC of the phenolic compounds. On the more polar stationary phase, the capacity factors of the phenolic compounds are higher (see Table II). As a result, the pressure required to elute the analytes is also higher. Therefore, the C_{18} column was preferred for further work. It should briefly be remarked that, by employing a higher supercritical carbon dioxide density for SFC (130 bar) than SFE (118 bar), a volume compression will occur when the extracted compounds are switched into the SFC system via the large sample loop. For the present system, the calculated volume compression was only 8%.

Extraction yields were calculated by comparing the peak areas of the test compounds obtained by SFE-PS–SFC after aqueous or heptane injections, with 100% recovery being assumed for heptane. For 5-μl injections the SFE-PS–SFC system worked smoothly, with extraction yields of at least 85% for both compounds. Obviously, because only 50% of the supercritical CO_2 will go to valve 3 following SFE-PS, the actual quantity injected into the SFC is two times lower (42.5%). The peak height repeatability for the total system was 8% R.S.D. ($n = 8$). This value is quite satisfactory considering that the repeatability of both SFE-PS and SFC was about 4% R.S.D. ($n = 7$). In order to improve the detection limit of the system expressed as the

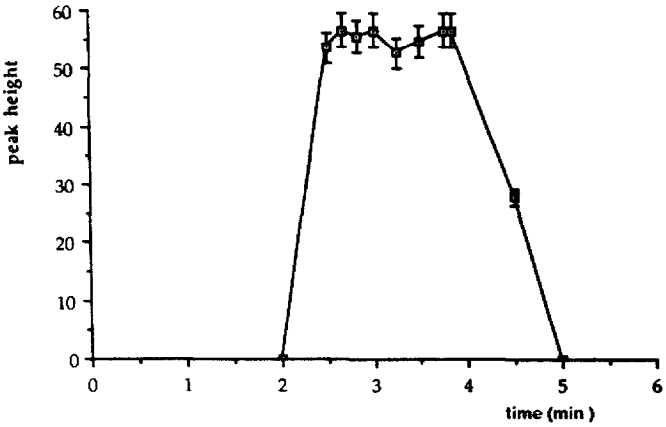

Fig. 3. Recovery of phenol *versus* the time valve 3 is switched after sample injection (in valve 2). SFE conditions: CO_2 at 40°C, pump pressure 118 bar, 150 μl/min. SFC conditions: CO_2 at 40°C and 150 bar (column inlet pressure); flow-rate 1.1 ml/min (20°C); 15 cm × 0.31 cm I.D. LiChrosorb RP-18, 5 μm; UV detection at 254 nm, 0.02 a.u.f.s. with 320-μm pathlength capillary cell. The bars indicate the maximum deviations for three 1-h series of analyses.

TABLE II

DATA FOR SFC OF THE TEST COMPOUNDS ON COLUMNS WITH A C_{18}- OR A CN-CHEMICALLY BONDED PHASE

15 cm × 0.31 cm I.D. columns with 5-μm LiChrosorb RP-18 or 5-μm RSIL-CN; CO_2 at 40°C and indicated inlet pressure.

Compound	*Capacity factor on*	
	C_{18}	*CN*
Phenol	0.2	6.7
4-Chlorphenol	0.8	8.9
Pressure (bar)	130	270

injected concentration, preliminary experiments were done with 50-μl instead of 5-μl injections. Although the expected increase of sensitivity was indeed observed with the larger sample volume, water spikes in the detector signal increased dramatically. Optimization of the phase separator design may well help to eliminate this problem.

Urine sample- Liquid/supercritical-fluid extraction in combination with phase separation can be regarded as a clean-up procedure, and should serve a useful purpose with complex matrices such as urine. Fig. 4a shows a chromatogram obtained upon direct injection into the SFC system of a 5-μl urine sample spiked with 4-chlorophenol as a model compound. Fig. 4b was obtained when injecting the same sample into the SFE-PS–SFC system, and Fig. 4c after the injection of a blank urine sample into this system. These chromatograms show the potential of the SFE system for the analysis of aqueous samples. The sensitivity can, of course, be considerably increased by using a longer-pathlength high-pressure flow-cell.

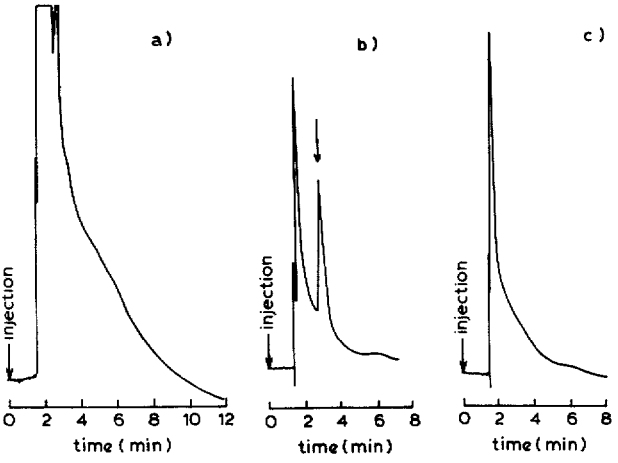

Fig. 4. Comparison of SFC and SFE-PS–SFC of an urine sample spiked with 4-chlorophenol. (a) Direct injection of a spiked (120 μg/ml) urine sample into the SFC. (b) SFE-PS–SFC of the same spiked urine. (c) SFE-PS–SFC of an urine blank. SFE conditions as in Fig. 3. SFC conditions as in Fig. 3 except 130 bar and 0.005 a.u.f.s.

It should be remarked that problems started to occur after some ten injections of urine samples. The restrictors became plugged and, consequently, the extraction flow-rate decreased. In addition, the background noise level increased. A series of methanol injections served to solve these problems.

CONCLUSIONS

The on-line coupling of a continuous liquid/supercritical carbon dioxide extraction system with SFC is reported for the first time. Preliminary results demonstrate the feasibility of the procedure by using phase separation between SFE and SFC. This seems to be a promising on-line sample pretreatment technique for either SFC or other separation techniques.

In the future, attention should be devoted to the optimization of the extraction process (pressure, temperature, flow-rates, phase separator), and to improving the detection limit of the set-up by increasing the injection volume. In addition, other model solutes will have to be chosen in order to investigate the efficiency and selectivity of SFE and to determine the limits of SFE with regard to solute polarity. This should include the use of modifiers.

As for the potential of SFE, besides the possibility of directly analysing aqueous samples by means of on-line coupling to SFC or for that matter, GC or LC, there is also the possibility of an on-line post-column phase switch. In the latter case, a reversed-phase LC separation can be followed by on-line liquid/supercritical fluid extraction and coupling to a mass spectrometer, GC-type detectors, a Fourier-transform IR spectrometer or other detectors that are not easily compatible with current LC mobile phases. As an alternative, after the LC separation, the analytes of interest can be trapped on a suitably selected trapping column, desorbed by means of a supercritical fluid and monitored by the same types of detectors.

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