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Selected applications of the use of supercritical fluids in coupled systems

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Abstract

The gas-like mass transfer and liquid-like solvating properties of supercritical fluids, together with considerations on automation, speed and cost, make supercritical fluids very attractive for application in coupled systems. A number of coupled systems in which supercritical fluids play a key role are described. Supercritical fluid extraction–capillary gas chromatography–mass spectrometry (SFE–cGC–MS) has been applied to the determination of pesticides in fruit juices. For the determination of polycyclic aromatic hydrocarbons, triazines, chlorophenols and phenoxy acids in water samples, the SFE–cGC–MS system was extended with a sample preparation station after SFE, allowing the addition of internal standards and derivatization of the acidic solutes after SF extraction. The potential of on-line solid-phase extraction–supercritical fluid chromatography–diode array detection is illustrated with the determination of xanthines in iced tea and of traces of pesticides in water samples.

1. Introduction

Whereas the process of extraction by supercritical fluids has been known for some time [1], e.g., the selective removal of caffeine from coffee, supercritical fluid extraction (SFE) has only recently been applied on an analytical scale, analysts rediscovering the technique as a powerful sample preparation tool for off-line and on-line combination with chromatographic techniques [2–15]. The most important characteristics of SFE are the high recovery rates attainable with relatively short extraction times (typically 30 min) and the high degree of selectivity that can be introduced into the sample preparation step [16]. Modern SFE instruments offer different tools to introduce selectivity, namely (a) the addition of polar or apolar modifiers to carbon dioxide, the supercritical medium used in routine analysis, (b) the control of supercritical fluid density and temperature during sample leaching, (c) the selecting of the packing material in the solid trap on which the extracted solutes are adsorbed, (d) the different solvent polarities to rinse the trap and (e) the possibility of adding an adsorbent in the extraction thimble to retain unwanted compounds. SFE is mostly used in the analysis of solid samples such as sediments, sludge, soil, solid waste, food and plant material, but in combination with the addition of an adsorbent to the extraction thimble or with solid-phase extraction on cartridges or membrane

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discs followed by SFE [17], the technique may also be used for preparing extracts from aqueous samples where the excellent selectivity of SFE applies equally well.

On the other hand, SFE lends itself to either off-line or on-line coupling with various separation techniques. There is still some controversy as to which approach requires the fewest compromises, but with the introduction of bridge arms to transfer SFE collection vials to GC, SFC and HPLC autosamplers there is in fact little difference between the off-line and on-line methods. In on-line coupling, the advantages are high sensitivity, no contamination and easy automation, while the disadvantages are memory effects, matrix effects, one-shot analysis (repeatibility?) too small sample sizes to be representative and difficulties encountered in focusing the solutes at the top of the chromatographic column during the SFE leaching process. For the off-line approach the following advantages can be advanced: larger sample sizes, different injections possible, different separation methods applicable, automation via bridge arms, operational simplicity and access to further clean-up and derivatization. The most important disadvantage is definitely lower sensitivity, although nowadays in all chromatographic modes large-volume injection is feasible.

In recent years, we have focused on the development of fully automated analytical system for aqueous samples, i.e., tap water, surface water, beverages, etc. Automatic systems improve precision, lead to reduced costs, increase the throughput and reduce the health hazards to laboratory personnel. In this paper, some selected applications on coupled off-line and on-line systems in which SFE plays a key role are described and discussed.

2. Instrumentation

2.1. Supercritical fluid extractor

SF extractions were performed on an HP 7680T extractor (Hewlett-Packard) equipped with a variable restrictor and solid trap. Carbon dioxide of SFE/SFC grade (Air Products) was used as the bulk extraction fluid in all SFEs.

2.2. Bridged SFE–capillary gas chromatographic (cGC)–mass spectrometric system

The SF extractor is positioned relative to the HP 5972 MSD cGC–MS system (Hewlett-Packard) such that the GC automatic sampler arm of an HP 7673B injector can provide and withdraw vials from the SFE instrument for injection into the cGC–MS system.

2.3. Bridged SFE–sample preparation station (PS)–cGC–MS system

A sample preparation unit, HP 7686 PrepStation (Hewlett-Packard), was installed between the HP 7680T extractor and the HP 5972 HP MSD cGC–MS system. The software to synchronize the three units was provided by Hewlett-Packard (Little Falls, DE, USA).

2.4. Supercritical fluid chromatograph

An HP SFC G1205A instrument (Hewlett-Packard) was used and operated in the downstream packed-column mode. The instrument was equipped with an HP 1050 diode-array detector with a 400 bar flow cell.

2.5. On-line SPE–SFC–diode-array detection (DAD)

The first set-up consisted of a six-way valve (Rheodyne Model 7010), a Varian Model 5000 HPLC pump and the supercritical fluid chromatograph. The external loop of the SFC valve was replaced with a cartridge precolumn (guard column) (Hewlett-Packard).

The second system was composed of a Gilson Model (Analis, Belgium) 233 XL sample preparation unit connected to the SFC instrument.

3. Results and discussion

3.1. Off-line supercritical fluid extraction–capillary GC–MS (SFE–cGC–MS)

The application selected demonstrates first the possibilities of SFE as a selective sample preparation method and second that the technique can also be applied to the enrichment of trace analytes from aqueous samples. An orange juice sample spiked by an Italian food laboratory with the pesticides thionazin, methyl parathion, fenthion, methidation, pyrazophos, phosalone, vinclozolin and procymidone was sent to the laboratory to evaluate the possibilities of bridged SFE–cGC–MS, compared with the conventional method of liquid–liquid extraction followed by clean-up over silica–Florisil or by size-exclusion chromatography. The concentrations of the pesticides were communicated only after submission of the data. Initial attempts to extract the aqueous solution as such failed and mixing with an adsorbent was essential. A 2-g amount of Chromosorb W (60–80 mesh) (Alltech) was placed in a 7-ml extraction thimble and 2 ml of orange juice were added on top of the adsorbent followed by another 0.5 g of Chromosorb W. SFE was performed with a 1 ml/min flow of pure CO_2 at a density of 0.75 g/ml and an extraction temperature of 50°C during 5 min of static and 30 min of dynamic operation. The extracted solutes were collected on an ODS trap held at 20°C. The nozzle temperature was 45°C. After completion of the extraction, the trap was rinsed with 1 ml of chloroform and the vial containing the extract was automatically transferred via the vial transport arm of the HP 7673B autosampler to the GC–MS system operated in the full-scan mode. A 5-μl volume was injected in the splitless mode (45 s) using an increased inlet pressure at the time of injection (pressure pulse 300 kPa for 1 min) on a 30 m × 0.25 mm I.D. HP-5 MS column with a 0.25-μm film thickness. The temperature of the column was programmed from 50°C (1 min) to 150°C at 20°C/min and then to 260°C at 6°C/min. Helium was the carrier gas at 50 kPa in the constant-pressure mode.

Under the moderate extraction conditions applied, a relatively clean chromatogram was obtained with palmitic and oleic acid as the main peaks. The selectivity of SFE was fully exploited. The pesticides are soluble at the 0.75 g/ml density of neat CO_2 and an extraction temperature of 50°C, whereas both the adsorbent (silica) in the thimble and the collection (ODS) trap helped to retain unwanted matrix solutes, polar compounds such as the lower free fatty acids, and high-molecular-mass solutes, i.e., triglycerides, which could interfere as such or as the decomposition products formed in spitless injection, in the elucidation and dosage of the pesticides. The spiked pesticides could be identified in the elution window from 16 to 24 min by comparing the spectra with those listed in a pesticide spectral library. For quantification, the external standard method was applied, selecting a typical ion for each pesticide. Fig. 1 shows the extracted ion chromatograms for vinclozolin (m/z 285) and procymidone (m/z 283) in the fruit juice sample.

The data are summarized in Table 1, giving the quantification ion, the measured concentration (mean value of three experiments), the relative standard deviation (R.S.D.), the amount spiked in the sample, and the recovery. Compared with data obtained by conventional sample preparation methods, SFE proved to be an excellent technique to dose pesticides in fruit juices. Recoveries ranged between 85 and 100% with precisions lower than 5% for the total analytical procedure. Moreover, cGC–MS analysis in the selected-ion monitoring mode should allow determinations at the low ppb level. The total analysis time is approximately 1 h and both sample preparation and analysis are fully automated.

3.2. Off-line supercritical fluid extraction–sample preparation–capillary GC–MS (SFE–PS–cGC–MS)

Methods to determine organochlorine and organophosphorus pesticides in environmental samples are well developed. The determination of the more polar pesticides, i.e., triazines,

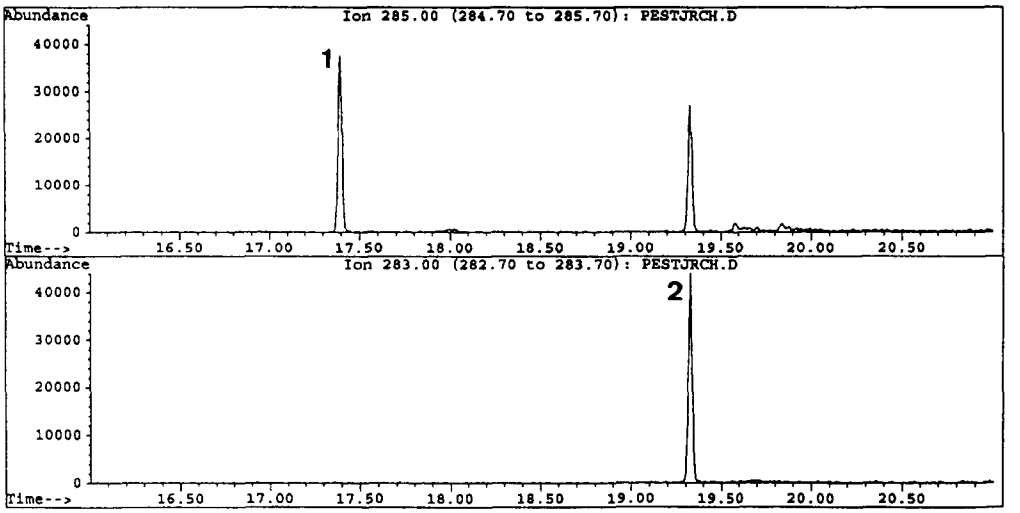

Fig. 1. Extracted chromatograms for vinclozolin (peak 1, *m/z* 285) and procymidone (peak 2, *m/z* 283). Time scale in min.

phenoxy acids, phenylurea, chlorophenols, etc., which are widely used in agriculture, is still a challenging problem in environmental analysis and especially for determinations in aqueous samples. The low levels to be determined in the European Community, typically 1 μg/l (1 ppb) in surface water and 0.1 μg/l (0.1 ppb) in tap water require very sensitive analytical methods. The SFE–PS–cGC–MS combination is currently being evaluated for the unattended simultaneous determination of polycyclic aromatic hydrocarbons (PAHs), triazines, chlorophenols and phenoxy acids in water samples. This multi-residue monitoring method is based on SPE on 3M Empore discs (Varian) followed by supercritical fluid desorption. For the data reported here, 1 l of water was adjusted to pH 2 with phosphoric acid and then passed through the disc at a flow-rate of 20 ml/min [17]. Under these conditions, all solutes investigated are more than 85% retained. The disc was then transferred into the 7-ml extraction thimble of the SFE instrument and extraction was performed at 50°C with CO_2–MeOH (95:5) at a density of 0.75 g/ml, a flow-rate of 1 ml/min and an extraction time of 30 min. The extract was collected on an ODS trap and then eluted with 1.5 ml of acetone. Via the arm of the HP 7673B injector, the SFE extract

Table 1
Quantification data for pesticides spiked in orange juice and determined by SFE–cGC–MS

Pesticide	Quantification ion (*m/z*)	Measured concentration (ppb)	R.S.D. (%)[a]	Spiked (ppb)	Recovery (%)
Thionazin	97	17	3.5	20	85
Methyl parathion	263	107	2.9	101	103
Fenthion	168	92	4.1	103	89
Methidation	145	44	3.6	51	86
Pyrazophos	373	28	4.8	30	93
Phosalone	182	429	3.3	505	85
Vinclozolin	285	273	3.7	303	90
Procymidone	283	130	4.1	130	100

[a] $n = 3$.

was transferred to the PrepStation for further preparation. In the sample preparation unit, the sample is split into two parts. One part is used for the direct determination of the PAHs and the triazines, for which only internal standard addition is performed [18]. For the cGC analysis of the acidic herbicides, on the other hand, conversion into the less polar and more volatile pentafluorobenzyl derivatives is required. The following steps are automatically performed by the PrepStation: (1) evaporation of acetone under nitrogen, (2) addition of 250 μl of pentafluorobenzyl bromide solution (2.5% in acetone), (3) addition of 25 μl of saturated sodium carbonate solution, (4) mixing at medium speed for 30 s, (5) heating the sample at 60°C for 30 min, (6) mixing at medium speed for 30 s, (7) heating the sample at 60°C for 30 min, (8) dispensing 1 ml of water into the sample, (9) mixing at medium speed for 30 s, (10) dispensing 0.5 ml of isooctane into the sample, (11) mixing at medium speed for 1 min, (12) transferring 100 μl of the upper layer into an injection flask and (13) injection of 1 μl.

Fig. 2 shows the analysis for the eleven acidic pesticides listed in EPA method 515 spiked at the 1 ppb level in doubly distilled water and enriched and derivatized as described. cGC–MS was performed on a 30 m × 0.25 mm I.D. capillary column coated with a 0.25-μm film thickness of methylsilicone (HP-5 MS). Splitless injection was applied with the split valve closed for 45 s. From Fig. 2 it is clear that GC–MS in the full-scan mode can easily be applied to dose 0.1 ppb levels as required by the European Community regulations. Fig. 3A and B show the calibration graphs for 2,4-DB and bentazone, respectively. Both graphs are linear over the range 0.5–2.5 ppb spiked in water.

3.3. On-line solid-phase extraction–supercritical fluid extraction–supercritical fluid chromatography–diode-array detection (SPE–SFC–DAD)

For more polar pesticides and especially for phenylurea, HPLC is currently the method of choice and the trend is to enrich aqueous samples by means of SPE followed by HPLC analysis with UV diode-array detection [19–21]. Recently, different coupled systems combining SPE and HPLC–DAD have been described for the determination of target pesticides in water samples [22,23] and commercial systems have been

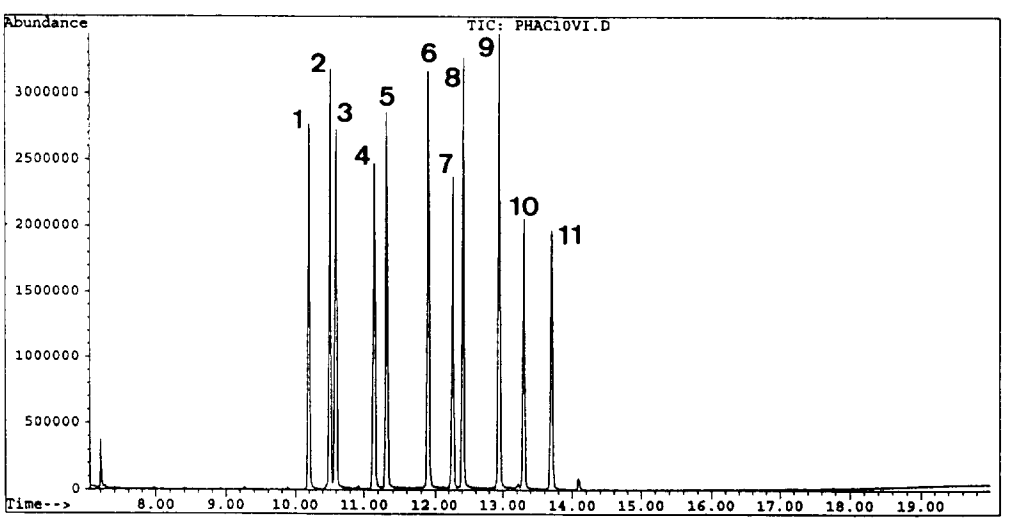

Fig. 2. SFE–PS–cGC–MS of acidic pesticides (EPA 515) as pentafluorobenzyl derivatives in water (1 ppb). Peaks: 1 = mecoprop; 2 = dicamba; 3 = MCPA; 4 = 2,4-D; 5 = bromoxynil; 6 = pentachlorphenol; 7 = bentazone; 8 = MCPB; 9 = 2,4-DB; 10 = ioxynil; 11 = benazolin. Time scale in min.

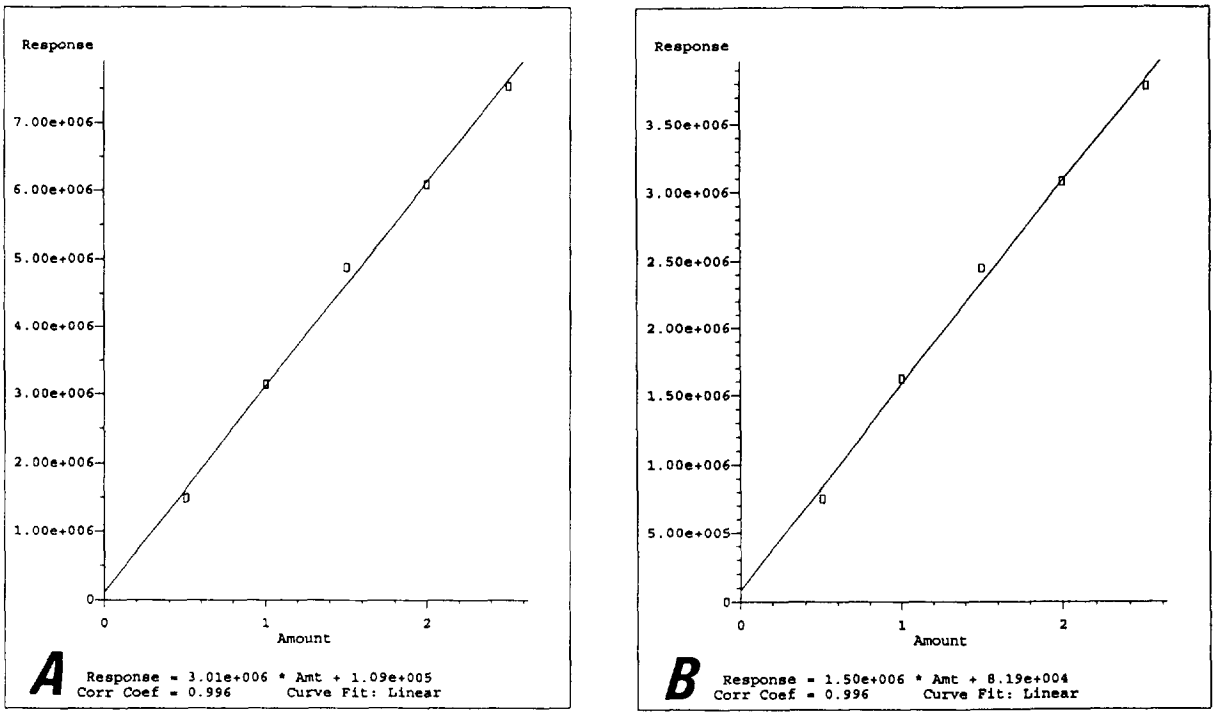

Fig. 3. Calibration graphs for (A) 2,4-DB and (B) bentazone in the 0.5–2.5 ppb range.

introduced [24,25]. The systems are based on SPE on small cartridges filled with octadecylsilica or polymeric material, followed by on-line liquid desorption and on-line HPLC analysis. Higher selectivity and sensitivity can be achieved by coupling LC with MS via a thermospray interface, whereas for the elucidation of unknown pesticides, the use of a particle beam interface has been described [26]. Impressive data have been reported using SPE–HPLC–DAD systems, and this method has been adopted by several water supply laboratories. Nevertheless this, it is worthwhile to evaluate SPE–SFC–DAD for two reasons: speed of analysis and desorption selectivity. Packed-column SFC has been evaluated for the determination of pesticides by Berger et al. [27,28]. Because the diffusion coefficients are higher than in liquids, SFC is inherently faster than LC. The possibilities for packed-column SFC in pesticides analysis are illustrated with the separation of the seventeen solutes listed in the German DIN norm 38407-F12 (Fig. 4) and with the determination of ten phenylureas (Fig. 5). Both separations were performed on a 25 cm × 4.6 mm I.D. LiChrospher silica 60 (5 μm) column (Keystone Scientific) operated at 40°C. Carbon dioxide with methanol as modifier at a flow-rate of 2 ml/min was programmed from 100 to 250 bar at 5 bar/min. The methanol concentration was raised from 1 to 15% at 0.5%/min. The concentration of the solutions was 20 ppm in methanol and an external loop of 5 μl was applied.

In Fig. 4, all solutes are separated except the pair atrazine (peak 4)–metazachlor (peak 5). However, the UV spectra of both compounds differ and elucidation of the identity in unknown samples can be achieved through these spectra. Compared with LC analysis of both mixtures, the analysis time is reduced by a factor of 3–4. Based on the good SFC separations, combined SPE–SFC was evaluated. The first system is based on solventless injection for preparative SFC [29]. The system consists of the SFC instrument, an extra six-way valve (Rheodyne Model 7010), an HPLC pump and a nitrogen gas supply

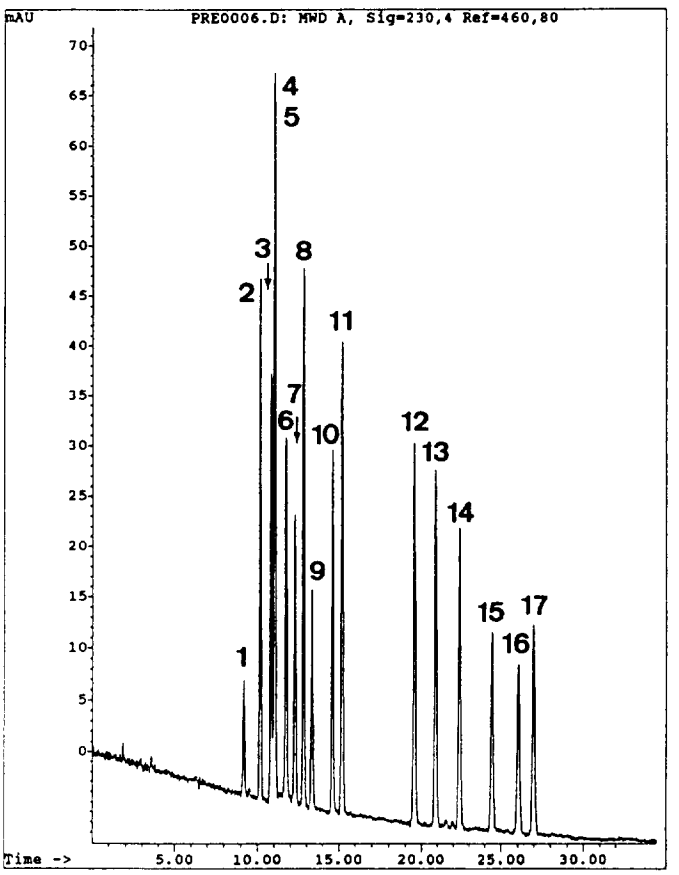

Fig. 4. Packed-column SFC of the seventeen pesticides enlisted in DIN norm 38407-F12. Peaks: 1 = metalochlor; 2 = terbutylazine; 3 = sebutylazine; 4 + 5 = atrazine + metazachlor; 6 = monolinuron; 7 = metabromuron; 8 = simazine; 9 = linuron; 10 = cyanazine; 11 = methabenzthiazuron; 12 = deisopropylatrazine, 13 = isoproturon; 14 = chlortoluron; 15 = diuron; 16 = hexazinon; 17 = metoxuron. Time scale in min.

with flow control (Fig. 6). The external loop of the injection valve of the SFC system was replaced with a 20×2.1 mm I.D. cartridge pre-column (guard column, Hewlett-Packard). The cartridges were dry packed with 20-μm ODS (Keystone Scientific) or 20-μm PLRP (Polymer Laboratories). The procedure is as follows (Fig. 6): sampling configuration, (a) the cartridge is conditioned with 5 ml of methanol and 5 ml of doubly distilled water at a flow-rate of 2 ml/min and (b) 10 ml of sample, followed by 2 ml of doubly distilled water are pumped through the cartridge at 2 ml/min; drying configuration, (c) the adsorbent is dried with a stream of nitrogen (250 ml/min) for 15 min; inject configuration, (d) desorption using 1% methanol in carbon dioxide at 100 bar and a flow-rate of 2 ml/min for 1 min. After the injection, the valves are repositioned to load a new sample and the SFC programme is started. As sampling and SFC analysis both take ca. 30 min, sampling and analysis run concurrently and an analysis can be performed every 30 min.

Critical in the procedure are the drying time, the supercritical fluid desorption time and the selection of ODS versus polymeric material. For complete and fast desorption of the pesticides from the adsorbent, a drying time of 15 min is necessary. This is illustrated in Fig. 7, showing the determination of phenylurea, spiked in distilled water in the 10 ppb range (5 μl of a 20 ppm solution in 10 ml of water) and analysed

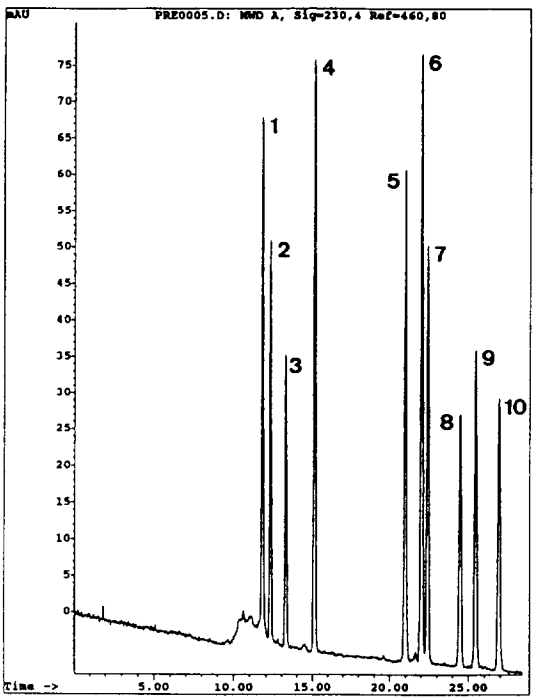

Fig. 5. Packed-column SFC of ten phenylurea pesticides. Peaks: 1 = monolinuron; 2 = metobromuron; 3 = linuron; 4 = methabenzthiazuron; 5 = isoproturon; 6 = fenuron; 7 = chlortoluron; 8 = diuron; 9 = chloroxuron; 10 = metoxuron. Time scale in min.

with (A) a 15 min drying time, (B) no drying and (C) incomplete (5 min) drying. Incomplete desorption and peak distortion and splitting are observed in (B) and (C). Desorption as a function of time was evaluated by changing the load–injection position of the SFC valve during 5, 10, 30, 60 and 120 s. Desorption times of 5, 10 and 30 s yielded too low recoveries whereas 60 and 120 s yielded high recoveries. A 60-s desorption time was adopted. For the solutes evaluated, i.e., the pesticides listed in the DIN norm 38407-F12 and the phenylureas, both the ODS and polymeric material yielded high recoveries, with the exception of desisopropylatrazine and chloroxuron. The very water-soluble metabolite of atrazine is hardly retained on ODS, whereas the recovery on the polymeric material is very good. This is in agreement with the observations of others [30]. Table 2 lists the recoveries for the seventeen pesticides spiked in water at 10 ppb on ODS and on polymeric material, together with the precision of the method for three experiments performed on new cartridges.

For the phenylureas (Fig. 7A), spiked at the 10 ppb level, the recoveries were of the same order except for the more polar chloroxuron, exhibiting recoveries of 72% (R.S.D. 6.8%) on ODS and 77% (R.S.D. 5.8%) on polymeric material. The cartridges can be re-used several times as cross-contamination was not observed. It was also noted that on-line SF desorption compared with conventional liquid SFC injection resulted in sharper peaks. In Table 2, the peak widths for the same amounts of the seventeen

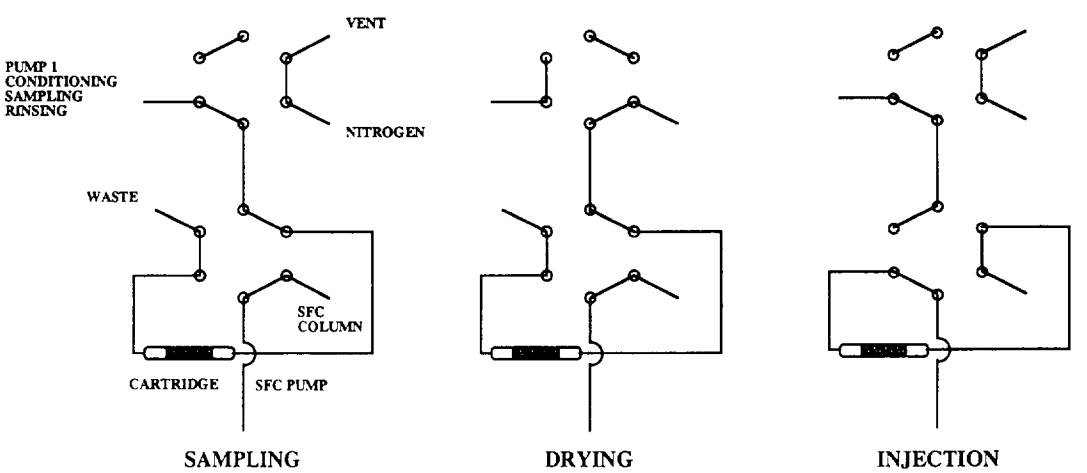

Fig. 6. Valve configurations in SPE–SFC–DAD.

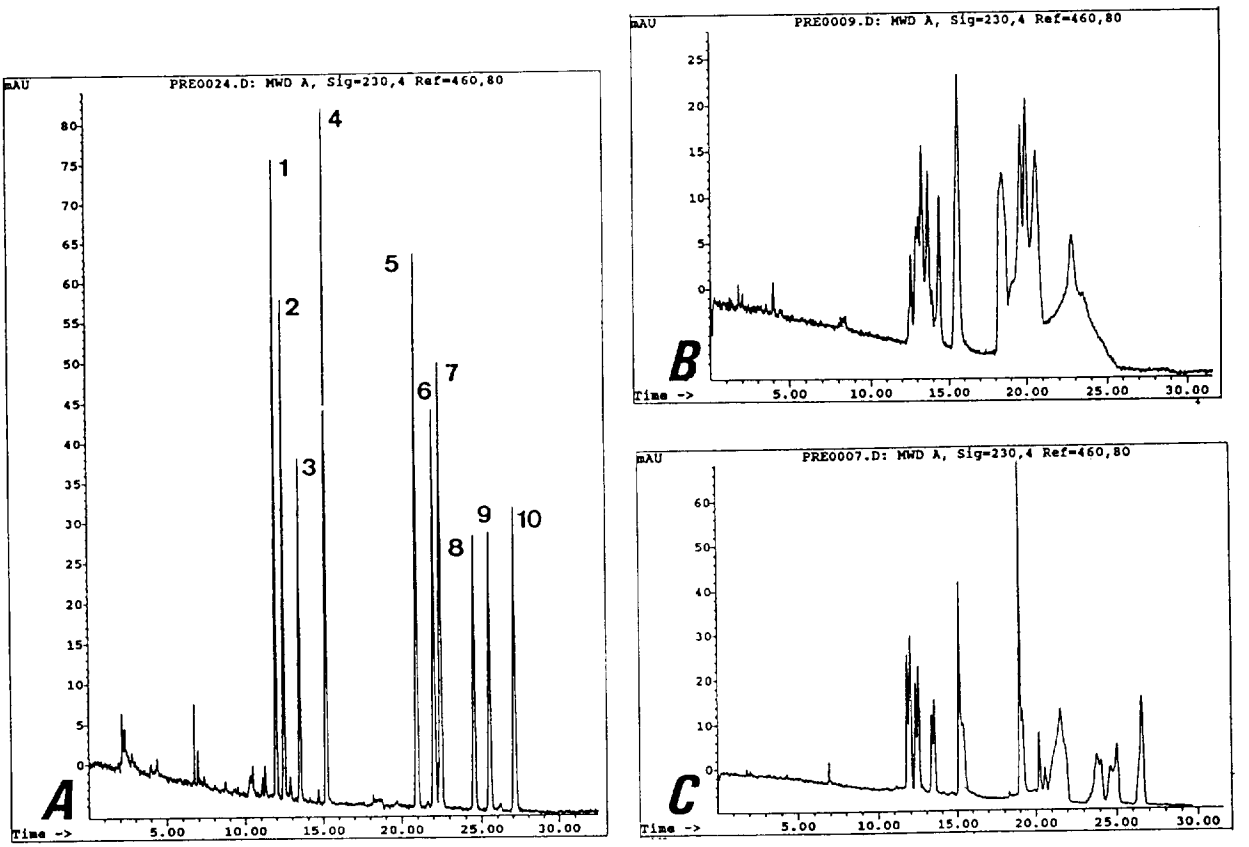

Fig. 7. Influence of the drying time on the SFC of phenylureas. (A) SPE–SFC–DAD analysis of spiked water (10 ppb) with 15 min of drying; (B) without drying; (C) with 5 min of drying. Time scales in min.

pesticides determined directly as methanol solutions (5 μl) and after spiking in water and SPE–SF desorption (10 ml) are given.

A practical application of SPE–SFC–DAD is shown in Fig. 8. A 10-ml volume of filtered river water (river Leie, Belgium) was analysed under the conditions described. The concentration of atrazine is 0.54 ppb (R.S.D. 9.3%) and that of isoproturon is 2.42 ppb (R.S.D. 4.9%) ($n = 3$). The latter value could not be taken into account as, although the DAD peak purity was 977, the library fit was only 530.

For larger sample sizes, breakthrough of the bed was observed. Subsequently, the amount of sample pumped through precolumns of 20 × 2.1 mm I.D. was limited to 10 ml.

In an attempt to automate the system further, a Gilson Model 233 XL sample preparation unit was coupled to the SFC instrument. This column-switching system consists of two high-pressure valves and a dispensing unit, allowing one automatically to condition, load, dry, desorb and recondition the solid phase cartridge. The valves were configured as in Fig. 6. The cartridge holder was equipped with cartridges of 20 mm × 4.6 mm I.D. and, based on the previous data, filled with PLRP (20 μm). The diluter can be equipped with 1- or 10-ml syringes and corresponding loops.

In the first instance, the system was evaluated for the SFC analysis of beverages, i.e., coffee, decaffeinated coffee, cola, beer, iced tea, etc.

Table 2
Recoveries (%) by SPE–SFC–DAD for seventeen pesticides (DIN norm 38407-F12) on octadecylsilica (ODS) and PLRP

Peak	Compound	ODS[a]	POL[a]	PW (5 μl)[b]	PW (SPE)[b]
1	Metalochlor	94 (6.2)	96 (5.6)	0.108	0.085
2	Terbutylazine	91 (6.3)	93 (6.2)	0.097	0.068
3	Sebutylazine	93 (5.4)	99 (4.5)	0.100	0.073
4 + 5	Atrazine + Metazachlor	94 (4.8)	92 (3.8)	0.108	0.083
6	Monolinuron	99 (4.2)	102 (4.0)	0.114	0.094
7	Metabromuron	97 (5.2)	100 (4.8)	0.122	0.096
8	Simazine	92 (4.2)	95 (3.8)	0.108	0.092
9	Linuron	90 (6.4)	94 (5.8)	0.117	0.105
10	Cyanazine	93 (4.0)	98 (3.2)	0.108	0.118
11	Methabenzthiazuron	87 (6.8)	89 (4.7)	0.118	0.107
12	Desisopropylatrazine	42 (9.5)	99 (3.1)	0.145	0.130
13	Isoproturon	99 (3.9)	98 (4.2)	0.142	0.130
14	Chlortoluron	95 (5.7)	92 (3.7)	0.145	0.129
15	Diuron	92 (5.6)	93 (4.9)	0.147	0.126
16	Hexazinon	91 (4.2)	98 (3.1)	0.166	0.149
17	Metoxuron	95 (4.1)	96 (3.4)	0.168	0.150

[a] R.S.D.s (%) for $n = 3$.
[b] Peak widths for direct injection [PW (5 μl)] and SPE-SF desorption [PW (SPE)].

[31]. As an illustration, the SPE–SFC–DAD of iced tea is presented. Iced tea from a can was diluted 100-fold with water and 0.5 ml was pumped through the cartridge. After rinsing with 0.5 ml of water and drying for 15 min with a nitrogen flow, desorption was performed with

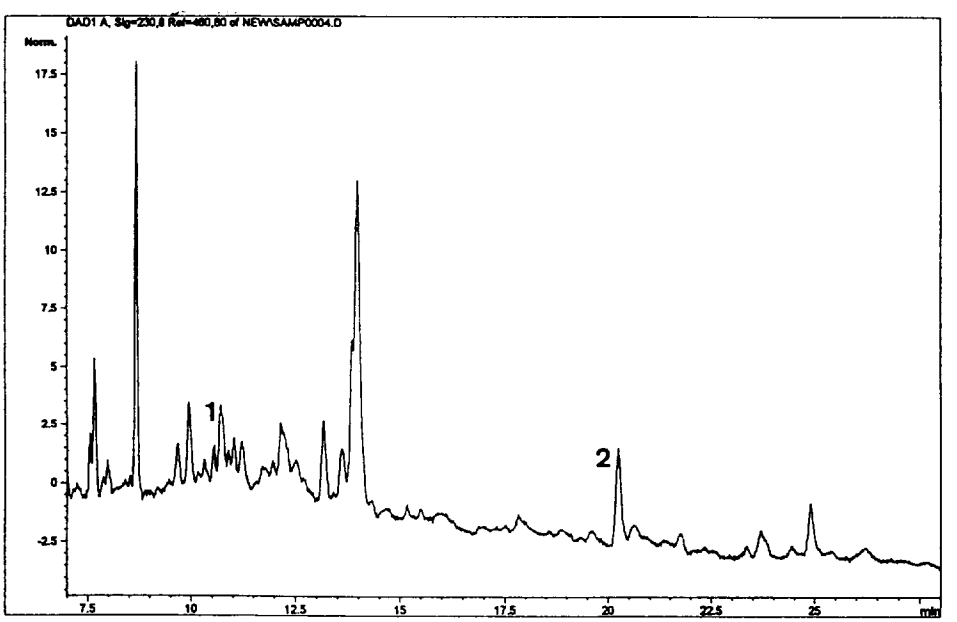

Fig. 8. Determination of pesticides in water from the river Leie by SPE–SFC–DAD. Peaks: 1 = atrazine; 2 = isoproturon.

carbon dioxide containing methanol in the concentration sequence 24% for 15 s and 4% for 45 s; 24% was needed to desorb the solutes quickly, but this must be followed by a concentration of 4% to focus the solutes in the inlet section of the column to avoid band broadening. The SFC analysis was carried out on a 250 × 4.6 mm I.D. 5-μm aminopropylsilica column (Bio-Rad) at 100 bar and 40°C and with a flow-rate of 2 ml/min of carbon dioxide containing 12% of methanol. Fig. 9A shows the determination of caffeine, theophylline and theobromine spiked in water at 1.62, 1.65 and 1.47 ppm, respectively. The procedure was repeated ten times and the R.S.D.s were 0.8, 0.5 and 0.5%, respectively. The analysis of iced tea is depicted in Fig. 9B and the measured concentrations are 127 ppm (R.S.D. 1.47%) for caffeine and 13 ppm (R.S.D.

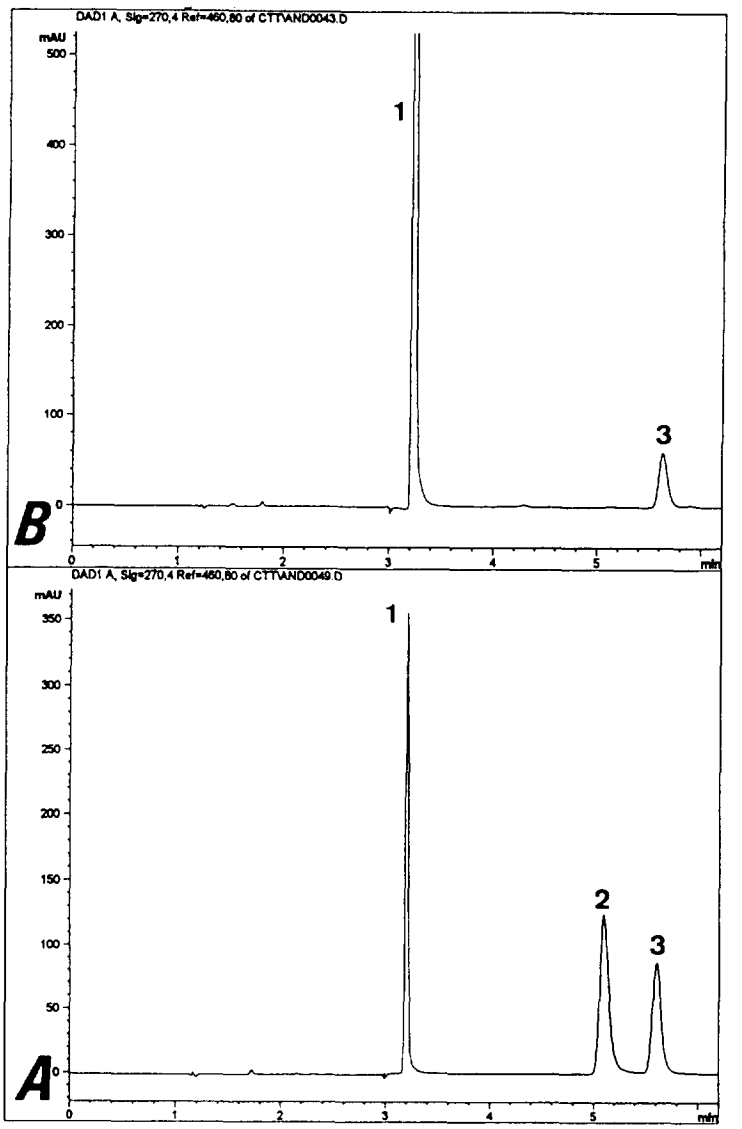

Fig. 9. Determination of xanthines in iced tea by SPE–SFC–DAD. (A) Standard solution of 1.62 ppm of caffeine (peak 1), 1.65 ppm of theophylline (peak 2) and 1.47 ppm of theobromine (peak 2). (B) Iced tea.

2.6%) for theobromine ($n = 3$). The concentration of theophylline is below 0.1 ppm.

The analyses of the pesticide samples reported earlier were repeated on the fully automated system. The recoveries were of the same order but the R.S.D.s dropped below 2.8% for all solutes.

4. Conclusions

Owing to the reduced use of organic solvents and increased sample throughput, interest in and the application of supercritical fluid extraction continue to grow. The off- and on-line combination of SFE with chromatographic techniques such as cGC and SFC seems a straightforward procedure, especially at the present time where productivity and automation are key aspects in laboratories. The applications selected illustrate that the robustness, as characterized by accuracy, repeatability and reproducibility, of coupled methods based on supercritical fluids is excellent.

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