Direct On-Line Coupling of Small Subcritical and Supercritical Fluid Extractors with Packed Column Supercritical Fluid Chromatography

K. R. Jahn/B. Wenclawiak*

Westfälische Wilhelms Universität, Analytische Chemie, Anorganisch Chemisches Institut, Wilhelm Klemm Str. 8, 4400 Münster, FRG

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Packed columns

Summary

A mini extractor of 85 μL void volume and a micro extractor of 3–4 μL void volume have been coupled directly with a packed column SFC and used under sub- and supercritical conditions. The mini extractor is suitable for holding adsorbates which can be on-line extracted and the extract chromatographed (direct SFE-SFC). The micro extractor can be used for direct sample introduction of liquid and solid materials under SF conditions. Thus any solvent interference with the sample and the chromatographic conditions is excluded. Standard samples of wood tar residue, engine oil, and metal organic compounds have been tested.

Introduction

In recent years supercritical fluid chromatography (SFC) has been recognized as a powerful separation technique supplementing high performance liquid chromatography (HPLC) and gas chromatography (GC). Both capillary and packed columns can be used in SFC. HPLC-type columns are most often used as packed SFC columns. The standard size for these columns is 4.6 or 4 mm inner diameter (ID) and a length of 250 mm. Usually detectors like a 254 nm ultraviolett (UV) absorbance detector are employed in connection with these columns as in HPLC. Detector cell volumes of a few μL are acceptable due to the large column volume.

Packed columns of 1 mm or less inner diameter with small particles have gained a wider interest as these microcolumns can be more easily connected with detectors, such as the flame ionization detector (FID). Capillary columns of less than 150 μm inner diameter are prefered for SFC, and columns of 100 μm ID or less are most often used. The slower diffusion in common mobile phases can be more easily overcome in columns of smaller diameter or in columns filled with small particles.

The volumes of injector, detector, and connecting capillaries have a large influence on the efficiency and need to be properly adjusted [1]. In this low volume range reproducible sample introduction into the column is of great concern. Theoretical calculations for capillary columns under LC conditions have shown that less than one nL injection volume is necessary in order to avoid an undesirable peak spreading for a 10 μm ID cloumn [2]. Peaden and Lee calculated for 100 μm ID capillary columns under supercritical conditions that injection volume plus detector volume have to be less than a few hundred nanoliters to prevent a significant loss in resolution [3]. Split injection or electronically-timed valving are therefore common means of sample introduction for this type of work [4–6].

In all cases these injection techniques require an analyte in a liquid solution. Solubility of the analyte is the limiting factor. The solvating properties of supercritical fluids strongly suggest their use in sample injection techniques. It has been reported that, for example, coronene is essentially insoluble in liquid n-pentane but is chromatographed easily with supercritical n-pentane [7]. For direct SFE–SFC it must also be considered that the equilibrium between the supercritical fluid and the analytes has to be reached in the extractor otherwise the sample is only partly extracted.

Besides the effect on column efficiency due to injection volume the disturbance of the chromatographic system by solvent effects must be considered [8, 9]. These problems become less important by using a direct coupled on-line supercritical fluid injector or an on-line supercritical fluid extractor (SFE).

So far all the systems described for SFE-SFC use a large volume extractor with or without a valve switching device, where only a part of the sample is introduced onto the column [10–13]. A system is described in this paper where SFE and SFC are coupled on-line. In addition the injection devices allow the introduction of solid sample material directly onto the column without any interference from solvent. This may be very important in cases where the sample material reacts (decomposes) with or in the solvent, or where the analyte is insoluble in other than the supercritical fluid.

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Theory

The maximum allowable volumes in a chromatographic system can be calculated from theoretical equations [1–3]. The volume of an on-line injector depends on column diameter and length, packing material and the column efficiency [14]. For some assumed efficiency losses (Θ^2) and other system constants the calculated maximum injection volumes are given in Table I.

The data show that V_{Imax} should be less than 20 μL with a negligible loss of efficiency for the column of our choice. The sample volume should be about 65 μL if a loss of 10% is tolerable.

Table I Calculated maximum injection volume

dp μm	L mm	ID mm	N	Θ^2	V_{Imax} μL
5	300	4.6	6000	0.01	21
5	300	4.6	6000	0.05	46
5	300	4.6	6000	0.1	65
10	250	4.6	3800	0.01	22
10	250	4.6	3800	0.05	48
7	420	0.3	5000	0.01	0.13
7	420	0.3	5000	0.05	0.30

dp: particle size; L: column length; ID: inner diameter; N: plate number (measured under SF conditions): Θ^2: desired efficiency loss; V_{Imax}: resulting maximum allowable injection volume for a given Θ^2. Evaluated for a k'of 1 and a column porosity of 0,8.

Experimental

The home made SFC system has been described recently [15, 16]. Its main feature is a pressure PID-controler at the outlet of the chromatographic system [15]. Pressure gradients are performed with a personal computer (APPLE IIe) which also collects data and Adalab cards (Interactive Microware Inc., State College, PA). The detector signal was, in addition, recorded on a Shimadzu CR 1 A integrator/recorder (Shimadzu Deutschland, Düsseldorf). Pump heads were cooled with Peltier elements (Bahn Electronic, Düsseldorf, FRG).

The column used in this study was a standard HPLC column of 4.6 mm ID but of 300 mm length. The packing material was normal phase silica gel 5 μm particle size (Lichrosorb, SI 60, Merck, Darmstadt, FRG) slurry packed using a slightly modified balanced density method with isopropanol as pumping solvent [17].

The evaluation of the column efficiency was made with an internal loop injector (Valco CI-4W, 1 μL). For external loop (20 μL) and extractor injections a six-way switching valve (AT 3-C 6TX Valco, Houston, TX, USA) was used. The extractor replaces the external sample loop (Fig. 1). It is filled in the valve's Load position; switching to Inject transfers the dissolved sample onto the column. For injection under supercritical conditions the outlet B in Fig. 1 must be closed, but any additional dead volumes should be avoided. The plug can be replaced by a pressure sensor to measure the extractor pressure. In order to keep the memory effects as low as possible and to avoid a backflushing of sample in the reservoir line, the SF inlet of the extractor can be disconnected from the SF line by a micro valve (Capillary Needle Valve Chrompack, Netherlands) (A in Fig. 1). The extractors are individually temperature controlled by resistive heating.

Two extraction cells have been tested. One cell had a sample volume of about 3–4 μL (micro-extractor) the other one had a cell volume of about 85 μL (mini-extractor) (Fig. 2 and 3).

The micro-extractor (Fig. 2) was readily constructed from a solvent in-line filter (Rheodyne). Short connections (25 mm each) were made from 0.1 mm ID tubing and fittings (1/16''). The extractor can be installed after filling by connecting only one tube. The 5 μm frit in the extractor was compressed with a conical tool, generating a small

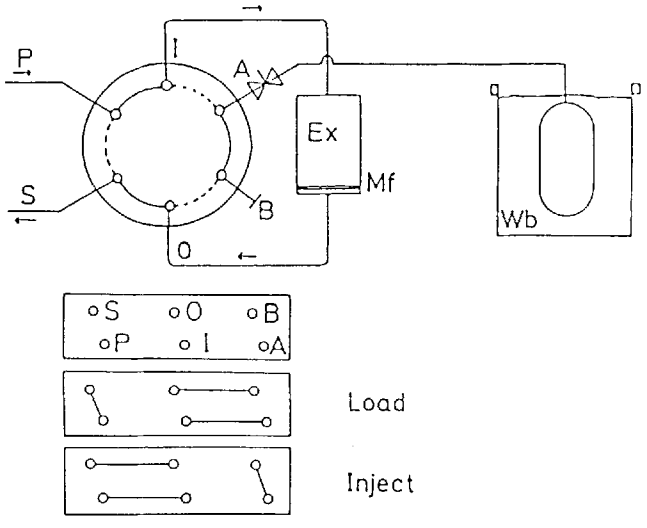

Fig. 1
Injection setup. Inject mode.
upper part
P: pump; S: Säule (column); I: extractor (Ex) in and out (0); Mf: membrane filter; Wb: waterbath with extracting fluid container (in this case CO_2).
lower part
switching schematic

hollow for the sample material. The frit remains permeable if it is not compressed too much.

A properly cut filter was placed into the frit holder. The filter is needed to prevent the column from being plugged by solid material (3 in Fig. 2). PTFE and cellulose acetate (pore sizes 0.2 and 0.45 μm) are used as filter materials. They withstand even supercritical carbon dioxide at temperatures up to 80 °C. Higher pressure differences between the extractor and the SFC-system can rupture the filter. The micro-extractor was resistively heated (0.4 V, 180 W adjustable) via the capillary connectors (Fig. 2: 1a, 1b). The micro-extractor was used for highly viscous and solid samples which can be directly placed into the extractor, e.g., used engine oil and metal carbonyl compounds. Other applications and samples are not excluded. Solids and highly viscous samples were placed directly onto the frit and weighed with an analytical balance.

The mini-extractor was mainly used for extraction from adsorbents. Therefore a direct connect guard column

(Latek, Heidelberg, FRG) seemed to be properly suited. With an inner diameter of 2.1 mm and a length of 25 mm the volume is about 85 μL. The volume is sufficiently large to introduce different kinds of samples, e.g. soil, soot, or liquids deposited on adsorbates. By adding adsorbates or SF inert materials as sample carriers the void volume of the extractor is actually less than 85 μL. In the case of reversed phase C18 adsorbate the void volume is reduced by about 40%. The reduction required depends on the particle size and the filling procedure as has been pointed out for HPLC columns [18]. The mini-extractor was also fitted with a filter plate on the inner side of the replacable frit (3 in Fig. 3). After filling, the extractor was directly screwed into the valve body and the outlet side was connected via the capillary tubing.

This extractor was heated by applying a small aluminum clamp onto the extractor body (2 in Fig. 3). Two holes were drilled into the clamp. One hole housed the heater (GC detector heater, 70 W), the other one contained a Pt 100 resistive temperature detector (both Carlo Erba, Hofheim/Taunus, FRG) connected to a JUMO controller.

Fluorene standards were prepared by accurately weighing 17.68 mg (S1) or 23.24 mg (S2) of the solid into a 25 mL graduated flask and then filling to the mark with n-heptane. For the loop injections 10 μL of the standards were injected into a 20 μL loop. For the extractor injection 10 μL (micro liter syringe) of the standard were used to wet about 75 mg of C 18 material (taken from SEP-PAK cartridges, Waters Associates, Milford, MA, USA). After drying the solvent at room temperature for about 10 minutes, the extractor was closed. Removing the solvent by passing a small stream of air through the extractor leads to a significant loss of analyte. The extracted adsorbate could be easily removed (poured out) from the extractor and accurately weighed. Because of the high volatility of the solvent the standards could be used only for a very short time. Reversed phase adsorbate was selected for samples which might contain water (irreversible bonding to normal phase adsorbate), e.g., pollutants from the atmosphere or from water of waste dumps. With the fluorene standards two different modes of extraction were performed. One mode employed a significantly higher pressure (>5.0 MPa) in the extraction cell, the other mode used only a pressure slightly above (>0.5 MPa) the chromatography pressure.

For the preparation of the PAH standard naphthalene, biphenyl, fluorene, phenanthrene, anthracene, pyrene, p-terphenylene, gamma-phenylanthracene, benzo-a-pyrene were weighed and diluted so that 0.02 mg of each were contained in 1 mL n-heptane.

Metal carbonyls and metal complexes were prepared and kindly provided from students of the advanced inorganic chemistry laboratory.

Used engine oil was taken from an old passenger car (Opel Kadett built in 1978) and from engine oil of unknown origin from previous experiments. The viscous oil sample was directly introduced into the micro-extractor.

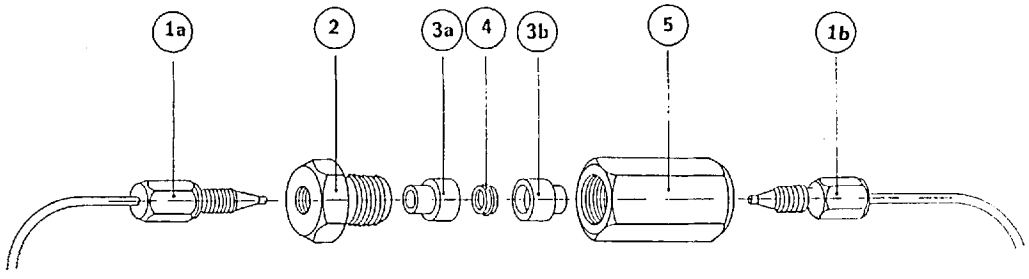

Fig. 2
Micro-extractor.
1a, b: 1/16" male nuts with ferrules; **2, 5:** casing; **3a, b:** female connection and frit holder; **4:** frit.

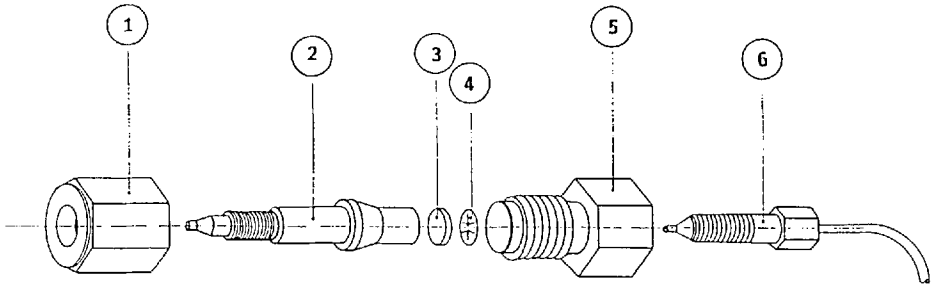

Fig. 3
Mini-extractor.
1: nut; **2:** extraction cell; **3:** frit; **4:** stream distributor; **5:** bolt; **6:** extra long 1/16" bushing.

Applications, Results, and Discussion

For evaluation of injection efficiency the mini-extractor was filled with adsorbent. A small quantity of toluene was directly applied to the adsorbent in the open extractor. The extractor temperature was kept at 0 °C and then pressurized with CO_2. The chromatogram (Fig. 4) also shows repeated injections (indicated by the second and third narrow "peak"). The second and third valve switching (after SF loading) during the same run showed that no sample was left in the extractor. This means that under the conditions used the whole sample was transferred onto the column during the first injection.

At higher pressure differences (> 0.5 MPa) between extractor and column a detector signal (sharp and narrow peak) appears regularly in the chromatogram. The height is about proportional to the pressure difference, but not related to the dead time (t_0) which is about 3.9 min. – The pressure controller wich was adjusted for the pressure stroke caused by the "injection" keeps the column conditions suitably constant as data from pressure control recordings show –.

An example of the use of the micro-extractor is the injection of benzene, toluene, and naphthalene (Fig. 5). To reduce the retention time for naphthalene a pressure gradient at constant flow was applied. The liquids and the solid were directly deposited onto the frit.

To evaluate the on-line extraction and chromatography under SF conditions a sample of fluorene was either injected or extracted (see experimental) onto the column (Fig. 6). The relative peak areas for two independent injections are compared in Table II.

The absolute peak areas are smaller for the extractor injections but the deviations are within the same range.

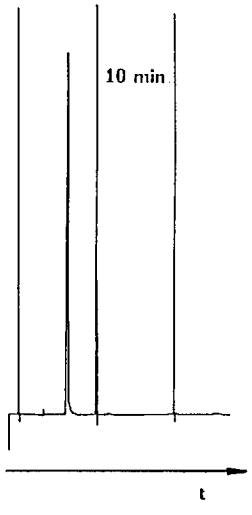

Fig. 4
Toluene injection through mini extractor from adsorbant.
Conditions:
Extractor: mini extractor, 10.0 MPa, 0 °C, valve switching time 0.02 min.
Column: 8.0 MPa, 48 °C, 1 mL/min CO_2.

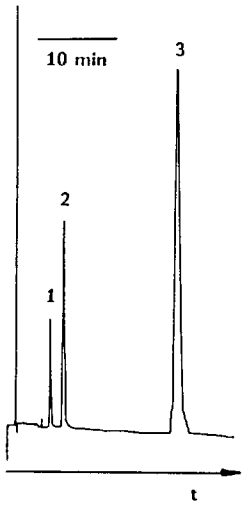

Fig. 5
Extractor injection of benzene (1), toluene (2), naphthalene (3).
Conditions:
Extractor: micro extractor, benzene, toluene as liquids naphthalene as solid directly introduced into micro extractor, valve switching time 0.02 min, 10.0 MPa, 50 °C.
Column: Pressure gradient from 8.0 MPa to 11.2 MPa in 50 min, 48 °C, 1 mL/min CO_2.

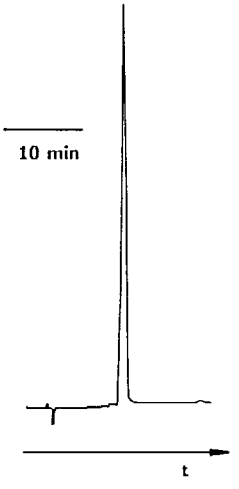

Fig. 6
Fluorene from adsorbant.
Conditions:
Extraction: mini extractor, 13.0 MPa, 40 °C, valve switching time 0.02 min.
Column: 12.5 MPa, 48 °C, 1 mL/min CO_2.

Table II Comparison loop and extractor injection of fluorene.
Conditions: Extractor at 40 °C, 13.0 MPa, needle valve (A in Fig. 1) closed, column at 48 °C, 12.5 MPa.

	loop (20 µL)	S1+	S2+
sample vol µL	10	10	10
	10	10	5
peak areas	372.7	315.3	313.6*
abs. dev.	+-2.3	+-1.9	+-10.0
rel. dev.%	0.8 %	0.6 %	3.2 %

+ standards 1 and 2 as described in the experimental section
* corrected for higher concentration and smaller sample volume of 5 µL

A tar residue from pyrolized wood was directly introduced into the micro-extractor. The extractor was then pressurized (13.0 MPa) and the temperature adjusted to 50 °C. These conditions were held for about a minute before injection. A pressure gradient was used for the chromatographic separation (Fig. 7a). Phenols interfere and cause the humps in the baseline. For comparison a PAH standard is shown in Fig. 7b. A comparison with GC data shows that the tar residue contained acenaphthenes also.

The coupling of SFE-SFC with UV detection demonstrates the desired selectivity of this system. Compounds with no UV absorbance give no signal and do not interfere with the recorded chromatogram. With CO_2 as eluent UV detection at 190 nm is possible, but is not shown here. Other detectors like MS or FID would reveal different information about the extract.

A sample of used engine oil and a sample of used engine oil which was suspected to be improperly contaminated by organic solvents was used for SFE-SFC experiments (Fig. 8a, b). The chromatographic conditions for the contaminated sample were optimized for the BTX (benzene, toluene, xylene) fraction. For HPLC analysis of used oils a sophisticated filtering technique is usually necessary to remove any suspended particles. With SFE-SFC no further sample preparation is required.

Many metal carbonyl and metal carbonyl complexes react with solvents and some are also thermally unstable (For information see [19]). HPLC or GC separation is then excluded but on-line SFE-SFC can be used. The solid analyte is introduced into the chromatographic system without the necessity of dissolution in an additional solvent. Also solvent molecules are not integrated into the coordination sphere of the complex which could easily result in non-reproducible chromatography. A chromatogram of Cr, Mo, and W mesitylene carbonyl compounds is shown here (Fig. 9) as an example. The first eluted peak shows the non-retained metal carbonyl starting materials. These compounds decompose easily in pure methanol but did not do so during the chromatographic separation with CO_2/10% MeOH. Fraction collection afterwards was still not possible because the CO_2 volatilized and MeOH remained in the fraction vials causing the decomposition of the compounds.

Conclusion

The direct on-line extraction of adsorbed compounds from solid materials is possible. This might be of great interest for studies of pollutants in air or water and for adsorption equilibria studies. The on-line extractors can be used also for solid sample introduction avoiding any interference from solvents. This is important for analytes

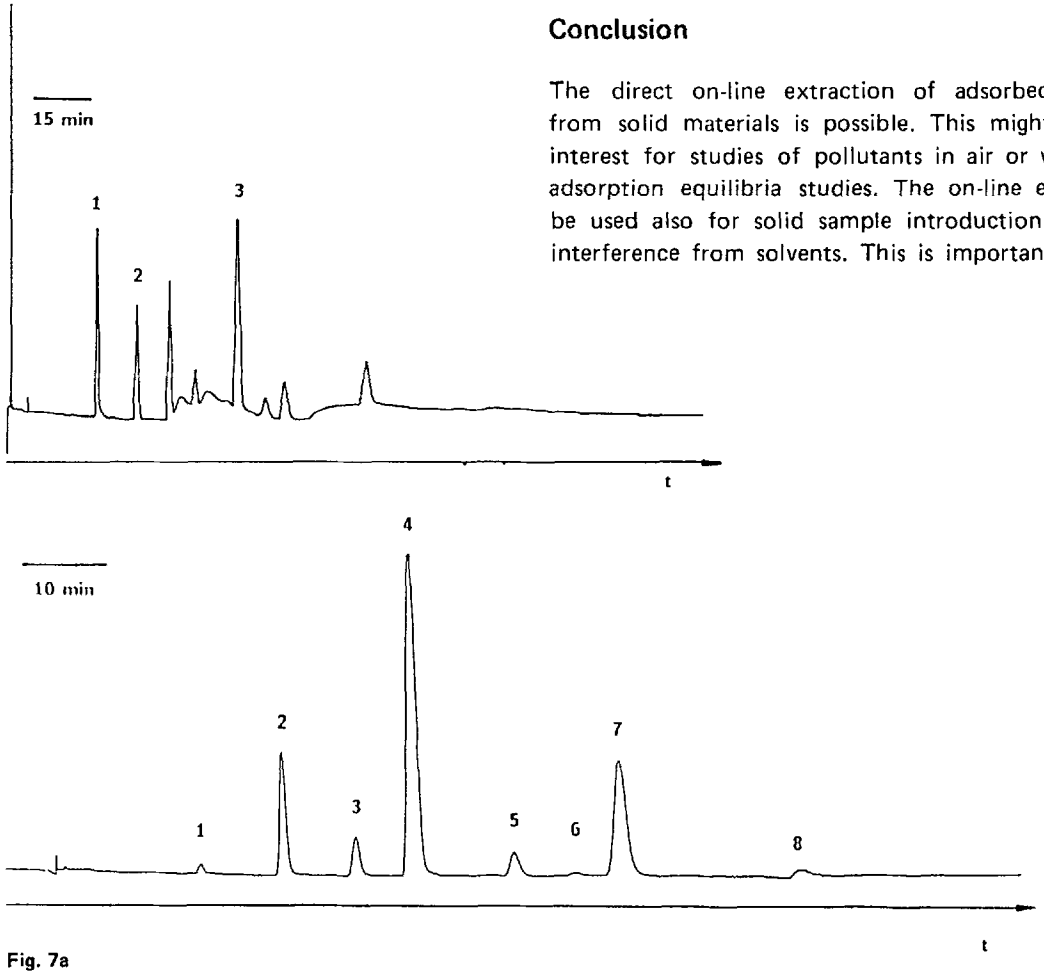

Fig. 7a
Wood tar residue (**1**, naphthalene; **2**, biphenylene; **3**, phenanthrene/anthracene); **7b**: PAH standard: (**1**, naphthalene; **2**, biphenylene; **3**, fluorene; **4**, phenanthrene/anthracene; **5**, pyrene; **6**, p-terphenylene; **7**, γ-phenyl-anthracene; **8**, benzo(a)pyrene).
Conditions:
a) Extractor: micro extractor 13.0 MPa, 50 °C, valve switching time 0.01 min.
b) 1 μL loop injection of standard.
Column: programmed from 8.0 to 11.2 MPa in 50 min, to 14.2 in another 50 min then constant, 1 mL/min CO_2, detector at 246 nm.

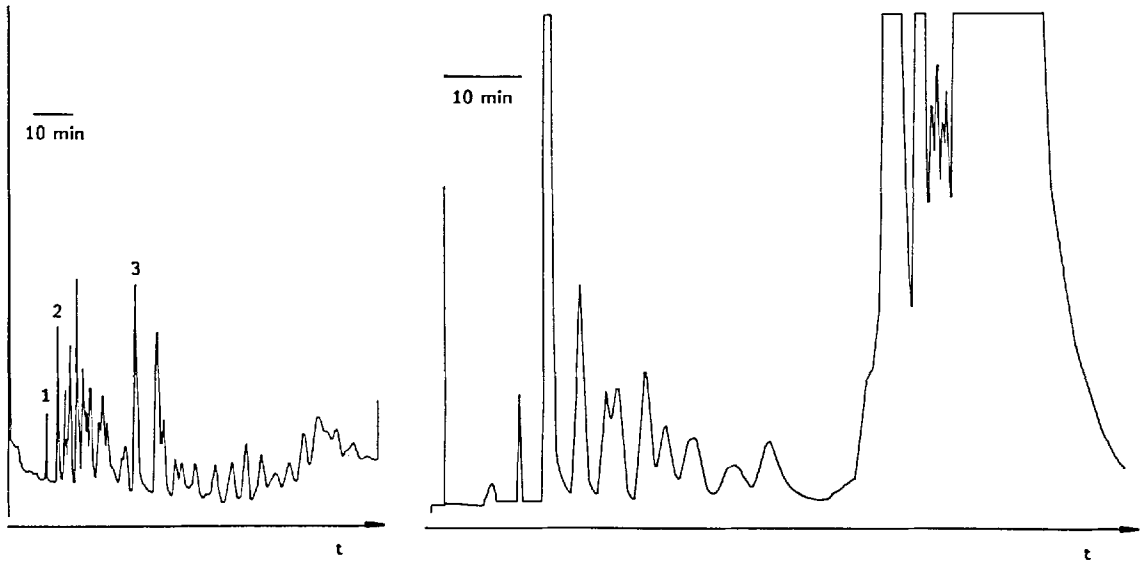

Fig. 8a
Used engine oil (**1**, benzene; **2**, toluene; **3**, naphthalene); **8b:** contaminated used engine oil (optimized for BTX fraction).
Conditions:
Extractor: micro extractor, 10.0 MPa, 50 °C, valve switching time 0.01 min.
Column:
a) pressure gradient 8.0–12.0 MPa in 100 min, 48 °C, 1 mL/min CO_2.
b) isoconfertic 7.5 MPa for 30 min, pressure gradient 7.5–11.2 MPa in 50 min, 44 °C, 0.8 mL/min CO_2.

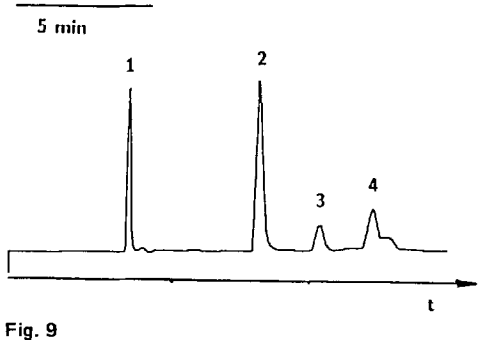

Fig. 9
Solid sample injection Cr, Mo, W carbonyls (**1**) and Cr, Mo, W mesitylene carbonyl compounds (**2** Cr-, **3** Mo-, **4** W-mesitylene tricarbonyl).
Conditions:
Extractor: 5.2 MPa, ambient temperature (conditions not sufficient to dissolve analytes), valve switching time 0.01 min.
Column: 10.5 MPa, 39 °C, 1 mL/min CO_2 with 10 % MeOH (eluent dissolves analytes).

which are solvent sensitive. In addition SFC offers the advantage of low thermal burden on the analytes. High viscosity materials can be directly transferred into the chromatographic system without the necessity of dilution and all the linked problems. Direct on-line SFE-SFC is best suited if the analytes are easily extracted and the fluid can also be used for the following chromatography. It has been shown that both extractors are well suited for packed column SFC.

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