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Integrated analysis of solid samples by on-line supercritical fluid extraction–gas chromatography

M. LOHLEIT* and K. BÄCHMANN
Technische Hochschule Darmstadt, Institut für Anorganische und Kernchemie, Hochschulstrasse 4, D-6100 Darmstadt (F.R.G.)

SUMMARY

A system for direct coupling of supercritical fluid extraction with carbon dioxide and capillary gas chromatography (GC) has been developed. Use of a double-oven gas chromatograph allows precise thermostating of all parts used for the extraction and sample transfer process. The valve position and the temperature are controlled by the GC panel. The system allows extraction chambers with dimensions from the micro to the semi-preparative scale. The modifications necessary for changing the vessel size and operation mode have been minimized. For small sample sizes, the whole extract is transferred to the analytical column and cryofocused there. The handling of larger amounts of sample or the performance of equilibration studies can be effected by using a "time-split" injection mode. The performance of the system was evaluated by the analysis of soil, plant material and smoke particles trapped on Tenax.

INTRODUCTION

The isolation of organic compounds from complex matrices is a limiting step in analysis because complete decomposition as in elemental analysis is not possible. Solvent extraction is time consuming and often requires large amounts of solvent. In addition, concentration and purification of the extracts are necessary in most instances. Thermal methods are very sensitive, but limited either by the thermal stability of the analyte or by the sorbents used for preconcentration.

A new approach to the solution of these problems is provided by supercritical fluid extraction (SFE). Although the basic principles of the solvation power of supercritical fluids had been known for a long time[1], it took almost a decade until Zosel *et al.*[2] introduced this technique for industrial-scale applications. Owing to the physicochemical properties of supercritical fluids, *i.e.*, a lower viscosity and higher diffusion coefficients than liquids, combined with higher solubility than in the vapour phase, they offer a number of advantages, *e.g.*, their use as a mobile phases in chromatography, as was first described in 1962[3]. A number of extraction processes on an analytical scale have been reported in recent years[4–23]. Extraction of environmental pollu-

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tants and pesticides from sorbent traps such as Tenax has been demonstrated[4–6]. Extraction of soil with supercritical methanol has been applied successfully to determine the amount of pesticide which is not extractable by liquids[7].

The liquids corresponding to supercritical fluids often have very low boiling points. Therefore, sample concentration, which is essential in trace analysis, can easily be achieved by reducing the pressure. The first application of a "solvent-free" micro-extraction and sample transfer to chromatographic analysis was demonstrated by Stahl and co-workers[8–10], who coupled SFE with thin-layer chromatography and studied the solubility behaviour of various compound classes in supercritical carbon dioxide. On-line coupling of SFE with high-performance liquid chromatography (HPLC)[11,12], packed[13,14] and capillary supercritical fluid chromatography (SFC)[15,16] has also been reported.

Although it seems that SFE–SFC is the most favourable system, because the dissolved extracts and the chromatographic carrier are in the same physical state, interfacing is difficult. Flow-rates and inner diameters of capillary SFC columns are small and only small amounts of fluid can be injected directly, because peak focusing, which is done by raising the column temperature, is successful only for high-boiling compounds. The use of external traps is possible, but their size and void volume have to be very small, thus also limiting this method to high-boiling compounds. Interfacing with packed-column SFC is similar to interfacing with HPLC, but in many instances the separation cannot be carried out by using pure carbon dioxide. With modifiers such as methanol, however, only HPLC detectors can be used, so that, compared with HPLC, SFC offers only the advantage of a higher resolving power.

Although gas chromatography (GC) is limited to thermally stable compounds, interfacing SFE with GC is a favourable approach that has also been reported[17–22]. The resolving power and detection sensitivity of GC are high. Also, extracts can be powerfully focused at the column head, so that there is no need for concentration loops, even if large amounts are transferred directly to the column. The solvation power of pure carbon dioxide is limited to apolar or slightly polar compounds. For the direct interfacing of SFE with GC this is not a general disadvantage. Compounds dissolved in supercritical carbon dioxide may be suitable for GC analysis. However, this is not expected for strongly polar compounds, which often tend to decompose at the temperatures required for GC analysis.

The aim of this investigation was to establish a multi-purpose SFE–GC system for research studies. Coupling of a continuous extraction chamber with the GC column provides the highest possible sensitivity. Quantification is easy to accomplish, because the recoveries of extracted compounds are usually very high. This is favourable for the ultra-trace analysis of aerosol particles or of airborne trace compounds collected on sorbent tubes in remote areas.

The volume of the extraction cell is limited to 200–300 μl in this method. Larger cell dimensions require either extremely long extraction times or high flow-rates of carbon dioxide. Both result in a poor trapping efficiency and, therefore, low resolution and bad peak shapes.

On the other hand, the high sensitivity of this method can easily cause overloading of the analytical column. Reduction of the sample size creates problems with regard to the homogeneity of the material. No representative quantification can be achieved by using sample sizes of 1 mg. For these reasons, a versatile SFE–GC system must be carefully designed for coping with these different requirements.

EXPERIMENTAL

Supercritical fluid supply

The supercritical fluid was supplied either by a programmable, computer-controlled high-pressure syringe pump (Lee Scientific, Salt Lake City, UT, U.S.A.; Model 600) or a constant-pressure HPLC pump with slow speed drive equipped with commercially available cooling jackets (Milton Roy, Riviera Beach, FL, U.S.A.; CP 3000). The syringe pump was operated at 8°C and the HPLC pump heads were cooled to 0°C with an external cryostat.

Carbon dioxide was of SFC grade (Scott Gases, Plumsteadville, PA, U.S.A.) and used without further purification, except for passing it through a 2-μm inlet filter.

SFE–GC instrumentation

The separation and extraction were performed with a double-oven gas chromatograph (Siemens, Karlsruhe, F.R.G.; Sichromat II), equipped with flame ionization and electron-capture detectors and suitable for cryogenic operation. Data analysis was performed by using an integrator (Shimadzu, Kyoto, Japan; CR 2-A). A schematic diagram of the system is shown in Fig. 1.

The columns used were 50 m × 0.32 m I.D. coated with either SE-30 (film thickness 0.5 μm) or SE-52 (0.25 μm) (Macherey, Nagel & Co., Düren, F.R.G.). The columns were connected with a retention gap (1–2 m × 0.32 mm I.D.) to promote cryogenic concentration and to protect the column. Direct connection of the column to the outlet restrictor of the extraction cell resulted in visible damage to the stationary phase at the column entrance after a few extraction cycles. Moreover, the fused

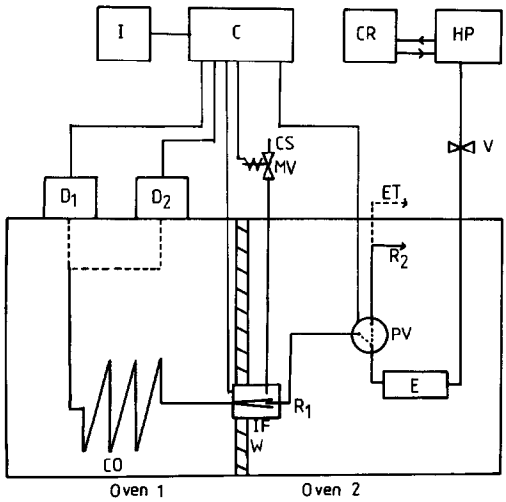

Fig. 1. Schematic diagram of the SFE–GC system. HP = high-pressure pump; CR = cryostat; V = high-pressure shut-off valve; E = extraction cell; PV = air-actuated three-way valve; IF = thermostated SFE–GC coupling unit (for details, see Fig. 2); W = oven separation wall; CO = capillary column; D_1, D_2 = electron-capture and flame ionization detectors, respectively; R_1 = restrictor for on-column deposition; R_2 = waste restrictor; ET = external trap; C = GC control unit; CS = carrier gas supply; MV = magnetic carrier shut-off valve; I = integrator; thin lines, control circuits; thick lines, transfer lines.

silica became fragile. For this reason part of the retention gap (*ca.* 15 cm) was removed after 10–20 extraction cycles. Supercritical conditions were maintained in the extraction cell with straight restrictors made of fused silica (10–20 cm × 15 μm I.D.; Lee Scientific), resulting in flow-rates of gaseous carbon dioxide of about 30–80 ml/min.

The carrier gas supply (helium at about 40 cm/s) and the restrictor were connected to the retention gap with a custom-made T-piece (Fig. 2). Heating of this interface reduced clogging of the restrictor and was performed by a heating block operated at 150°C, which was fixed into the separating wall of the gas chromatograph. During the extraction, the carrier gas was shut off, because the high backpressure of the GC column caused transfer of carbon dioxide to the carrier gas supply, resulting in long equilibration times after the extraction. An additional magnetic valve was used to flush the carrier gas supply, if necessary. The flow-rates of gaseous carbon dioxide caused problems with the flame of the flame ionization detector and therefore ignition before starting the analysis was necessary and was performed by using the GC time programme. The operation of the electron-capture detector was unaffected by the large amounts of carbon dioxide and the baseline was stable after flushing the column with carrier gas. Moreover, this detector responsed linearly to carbon dioxide, so that the extraction could easily be monitored.

Extraction cells could easily be constructed of empty HPLC columns equipped with sintered-steel frits of 2 mm × 2 μm pore size and standard reducing fittings. For small amounts of solid samples, an extraction cell made from modified standard fittings was used (see Fig. 3).

Sample transfer from the extraction cell to the column or waste was accomplished by using an air-actuated three-way valve (Valco C3W; VICI, Schenkon, Switzerland). The valve position was switched using the time programme of the GC controller. The transfer lines were made out of 1/16 in. × 0.007 in. or 1/16 in. × 0.25 mm I.D. stainless-steel tubing.

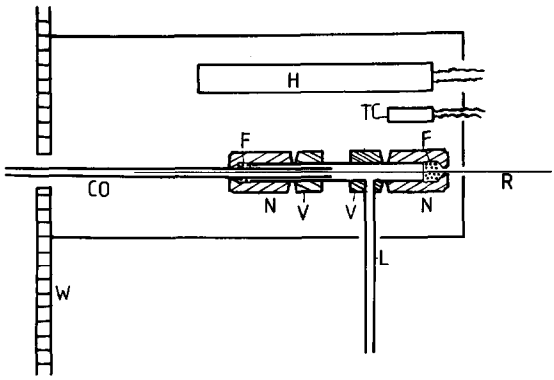

Fig. 2. Interface for on-line SFE–GC coupling. CO = retention gap, 0.32 m I.D.; R = Restrictor; N = 1/16 in. SGE nuts; F = Vespel ferrules; V = 1/16 in. Valco fittings; L = 1/16 in. × 0.5 m I.D. stainless-steel tube from carrier supply; H = heating unit; TC = thermocouple; W = separation wall of gas chromatograph.

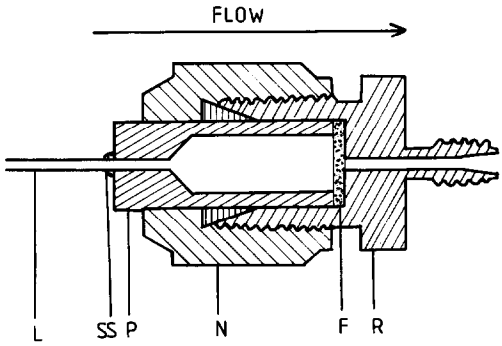

Fig. 3. Micro extraction cell for solid samples. P = 1/4 in. plug; L = 1/16 in. × 0.007 in. I.D. stainless steel line; R = 1/4 in. × 1/16 in. zero volume reducer; F = 2 mm x 2 μm porous stainless steel frit; SS = silver soldering.

Operation modes

The waste outlet of the valve was either equipped with an additional restrictor (R_2) or closed with a Vespel ferrule, depending on the operation mode used.

Sorbent cartridges were analysed with an additional 5 cm × 15 μm restrictor placed inside oven II (see Fig. 1). After transferring the extract to the column and switching the valve to the waste position, the sorbent traps were cleaned for the next use by raising the pressure to 40 MPa and venting the solutes to R_2 during the analysis. Additionally, the waste restrictor could be used to flush all lines with supercritical carbon dioxide before analysis.

Fractionation was carried out in a similar way except that the waste restrictor was replaced with a vespel ferrule. To avoid possible losses of extracts, the carbon dioxide supply was closed during the analysis.

Time-split injections were performed with a closed waste outlet and with the valve switched to the waste position during the equilibration period (10–20 min). Sample injection was carried out by switching the valve to the column restrictor. The amount sample deposited on the column could easily be varied by changing the time of the sample transfer. Transfer of the extracts to an external trap is also possible (ET, dashed line in Fig. 1).

RESULTS AND DISCUSSION

Different kinds of samples were chosen for evaluation of the performance of the described SFE–GC system. Fir needles (*Abies alba*) were analysed using the 'time-split' injection mode after a 12-min equilibration period. The chromatogram obtained by this method is shown in Fig. 4. Most compounds detected in the samples are terpenes and sesquiterpene hydrocarbons, *i.e.*, C_{10}, C_{15} and oxygenated C_{10} hydrocarbons.

The volume of the extraction cell was 1 ml. There is no limitation to the size of the extraction cell in this method. Amounts of sample large enough to represent true bulk properties can be analysed. Owing to the short injection time, the peak shape was good even for volatile compounds and using thin-film columns. Exact quantifica-

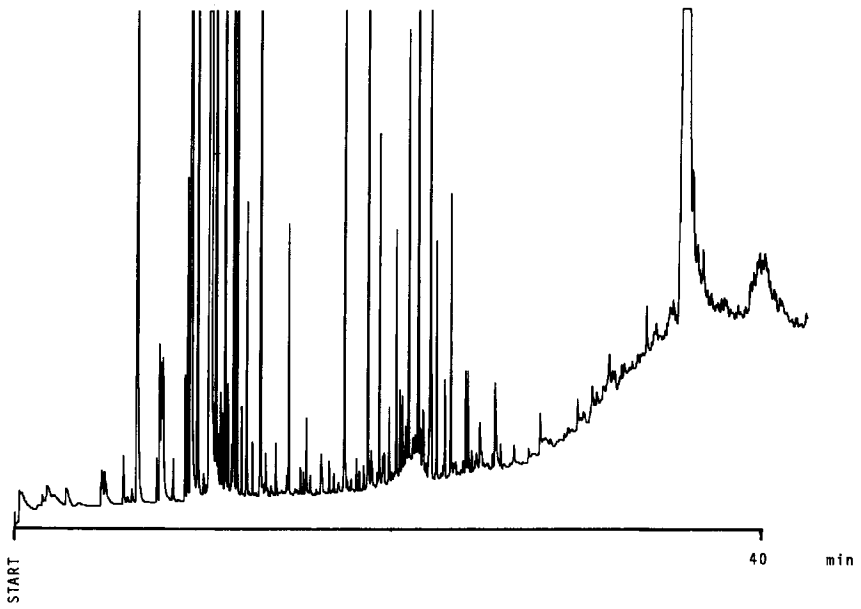

Fig. 4. SFE–GC in the time-split injection mode. Sample: *Abies alba* needles, 680 mg; extraction cell, 1 ml; 12-min equilibration at 30 MPa, injection for 3 s at 0°C; column, 50 m × 0.32 mm I.D.; stationary phase, SE-52, d_f 0.25 μm; flame ionization detection; temperature programme, 2 min at 0°C, 15°C/min to 80°C, 8°C/min to 280°C.

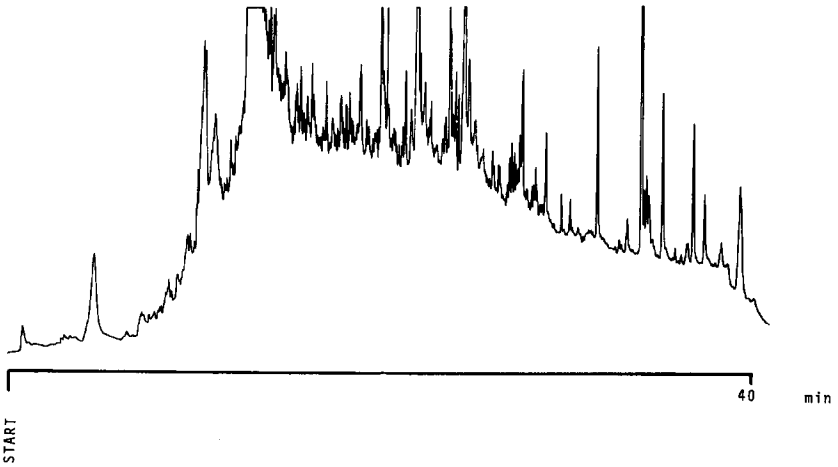

Fig. 5. SFE–GC of cigarette-smoke particles trapped on Tenax. Extraction at 25 MPa and 50°C for 12 min, trapping at 0°C; extraction cell, 200 μl; column 50 m × 0.32 mm I.D.; stationary phase, SE-52, d_f 0.25 μm; flame ionization detection; temperature programme, 1 min at 0°C, 15°C/min to 100°C, 10°C/min to 300°C.

tion can be achieved in a similar way as for headspace analysis, if the equilibration concentrations are known. Measurement of these concentrations can easily be performed by this system, but complications are expected owing to matrix effects, especially for biological samples with variable water content.

Sorbents used for preconcentration of airborne pollutants can easily be ana-

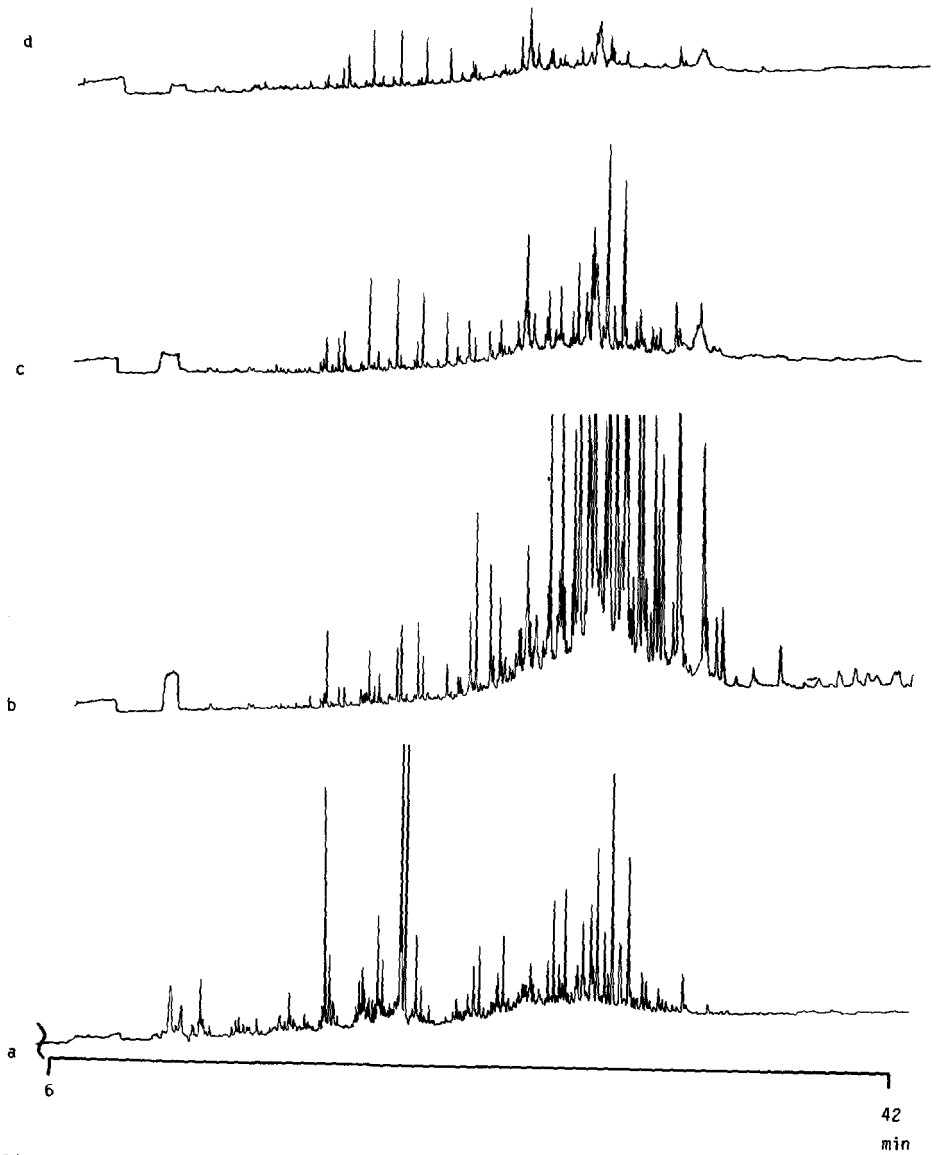

Fig. 6. Fractionation by SFE. Sample 20 mg of soil; extraction cell as in Fig. 3. (a) Extraction at 8 MPa for 12 min; (b) extraction at 16 MPa for 12 min; (c) extraction at 26 MPa for 12 min; (d)) system blank at 16 MPa for 12 min. Trapping at 0°C; column 50 m × 0.32 mm I.D.; stationary phase, SE-30, d_f 0.52 μm; electron-capture detection; temperature programme, 3 min at 0°C, 10°C/min to 280°C.

lysed by this system. Fig. 5 shows the SFE–GC of cigarette smoke particles (phenolic and N-heterocyclic compounds) trapped on Tenax.

Extraction and fractionation of compounds sensitive to the electron-capture detector from a soil sample that had been exposed to laboratory air for several years is shown in Fig. 6a–c. Volatile compounds are quantitatively recovered in the first fraction, in which only trace amounts of less volatile solutes are detectable. Extraction at higher pressures yielded these less volatile solutes and additional amounts of intermediate volatile compounds. A third fraction extracted at 26 MPa yielded only traces of additional material and indicated that the recoveries of the compounds were almost quantitative. A number of peaks that are present in all fractions are due to the blank. A chromatogram obtained under similar conditions without sample is shown in Fig. 6d.

Blank values obtained with the SFC-grade carbon dioxide were acceptable for trace analysis using electron-capture detection, but for flame ionization detection, however, the blank values are higher and can interfere with analytes of low concentration. This fact has not been mentioned by other workers studying upper ppm levels[17–21,23] or using lower flow-rates of carbon dioxide[22]. It seems that additional contamination is emitted by new seals of the pump and valves. The syringe pump used for delivery of carbon dioxide for more than 1 year resulted in a lower blank value than an HPLC pump that had not been used very long.

No problems concerning the stability of the restrictor mentioned by others[17–20] were observed. Therefore, the interface described seems to be more favourable than inserting and removing the restrictor through an on-column injector.

Although, as reported[18], low temperatures resulted in better peak shapes for the early eluting compounds, problems caused by clogging of the column (not the restrictor) were observed below 0°C. This problem appears to be related to the expansion of the carbon dioxide and the small amounts of water contained in the samples. No clogging of the column was observed after trapping of the extracts of double the amount of similar samples (soil) at −196°C obtained by the thermal desorption method.

CONCLUSION

Supercritical fluid extraction is a powerful technique for isolating organic compounds from complex matrices. On-line coupling with GC minimizes the time consumption and avoids contamination or sample losses. The present SFE–GC system combines the advantages of different operation modes with precise and simple control of the extraction and analysis parameters. The potential of the system has been successfully demonstrated with different kinds of samples. Quantification aspects, especially for the time-split injection mode, and reduction of blank values will be the aim of further research.

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