Supercritical fluid extraction using a new restrictor design

F. Mellor[a], U. Just[b]*, Th. Strumpf[c]

[a]E. Merck, Frankfurter Strasse 250, D-64271 Darmstadt, Germany
[b]Bundesanstalt für Materialforschung und -prüfung (BAM), Unter den Eichen 87, D-12205 Berlin, Germany
[c]Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Königin-Luise-Strasse 19, D-14195 Berlin, Germany

Abstract

A simple SFE apparatus is presented. Particular emphasis is placed on the design of the restrictor, whereby the advantages of a special restrictor nozzle are discussed in relation to problems in conjunction with the use of capillary restrictors. The rubber additives, fullerenes and soil constituents used as example application underline the possibilities offered by the new restrictor presented here.

1. Introduction

Supercritical fluid extraction (SFE) is today frequently used as an alternative to Soxhlet extraction, because substance solubility and diffusion coefficients in supercritical fluids can be varied over a wide range [1] and, for example, biogenic and anthropogenic compounds can be extracted mildly from very different matrices. Currently a shift in interest from supercritical fluid chromatography (SFC) to SFE can be observed [2]. On the one hand, this is due to the fact that, in spite of all the advances made in the field of chromatography and spectroscopy, there are still crucial unsolved problems involved in specimen preparation procedures; on the other hand, particularly SFE can provide very promising initiation points for specimen preparation procedures in the future [3–7]. Particular emphasis is placed on on-line and off-line coupling of SFE with chromatographic and spectroscopic techniques.

Compared with conventional extraction methods (e.g. Soxhlet), SFE can provide a drastic reduction in the time required for the extraction and relocation of substances from a wide range of analytes. Furthermore, the solvent quantities required for extraction are considerably reduced.

A basic problem in SFE, which will continue to be a point of study in the future, is the strong matrix dependency of the extraction yield. In order to facilitate an easier detachment of the analytes from the matrix, modifiers such as alcohols, methylene chloride or carbon disulphide may be added to the supercritical carbon dioxide, as applicable from case to case. This eases the extraction of more polar compounds [8–12]. In our applications only pure, unmodified carbon dioxide was used.

The solvent characteristics of supercritical carbon dioxide (0.8–0.9 g/ml) have been compared with those of benzene [13] and toluene [14].

Besides the difficult methods involved in

---

* Corresponding author.

procedural development, on-line SFE methods have the disadvantage that only small amounts of specimen can be used. They are well suited for trace analysis in matrices that are not co-extracted [15]. The advantage of off-line extractions is that traces of substances are enriched and subsequently extracts can be analysed using the established analytical procedures HPLC and GC or GC–MS, respectively. Off-line extraction is thus particularly suited for instances where larger specimen amounts are available [15], and it was therefore used in our applications.

Although positioned at the end of the apparatus —as seen in the solvent flow direction— the restrictor unit is the crucial component of every SFE apparatus, and it will accordingly be dealt with in more detail here. However, in discussing the restrictor design, the problems involved in off-line extraction will also become evident [7,15–18]. As a result of the Joule–Thompson effect, the tip of fused-silica capillary restrictors commonly used in commercial extractors cools down when the carbon dioxide transits from the supercritical state to the gas phase. As a result, the extracted components fall out more easily and block the restrictor.

Burford et al. [18] present three different types of restrictors, with particular consideration being given to their heatability and the transfer of the extracted substances to different recipient vessels. The most effective arrangement was found to be with the capillary directly submerged in the recipient liquid and the upper part heated in a heater block.

For these reasons we led the restrictor nozzles directly into simple recipient vessels.

2. Experimental

HPLC columns were used as extraction cells. Stainless steel capillaries 1/16-inch (1.6 mm) O.D. having an internal diameter of 0.25 mm were used as connecting capillaries; the screw connections were based on the Knauer Dynaseal system. A column thermostat was used to provide the correct temperature for the extraction cells.

A Knauer HHPN nozzle was used as a restrictor. This nozzle is normally part of the hydraulic high-pressure nebulizer system used for injecting specimens in atomic absorption spectrometry, and is suitable for pressures up to 40 MPa. It was not absolutely necessary to make changes in the nebulizer system, but it is planned to lengthen the nozzle mount in order to optimize the restrictor outlet. The o-ring is not used in SFE; instead of a polyether ether ketone (PEEK) capillary we used a stainless steel capillary.

To feed the liquid carbon dioxide, we used on the one hand the injection pumps used in commercial SFC units by Fisons and Dionex (density programming possible) and, on the other hand, the isocratic HPLC double-piston pump L-6000 by Merck. To provide a means of cooling the pump head of the Merck L-6000, this pump head was bored and threads were tapped from both sides to accommodate coolant tubes; the pump head was then integrated into a coolant circuit.

The Merck pump can be flow controlled as well as pressure controlled. The Teflon tube on the suction side was replaced by steel capillaries (0.5 mm diameter) and the valve mount on the suction side was replaced by a valve mount on the pressure side.

3. Results and discussion

The following solutions are apparent for preventing restrictor blockages, currently the main problem in off-line SFE [15,18]:
1. Minimising the pressure drop between the extraction cell and the restrictor outlet
2. Heating the restrictor
3. Using a "suitable" solvent in which the substances are dissolved when the supercritical fluid expands
4. Extracting smaller amounts of specimen
5. Using restrictors which afford a greater amount of flow

Fig. 1 shows the cross section of the entire nozzle. The nozzle plates are offered with laser boreholes of various sizes (one each). Nozzle plates (thickness 0.3 mm) having bore diameters of 5, 10 and 20 μm were tested for their

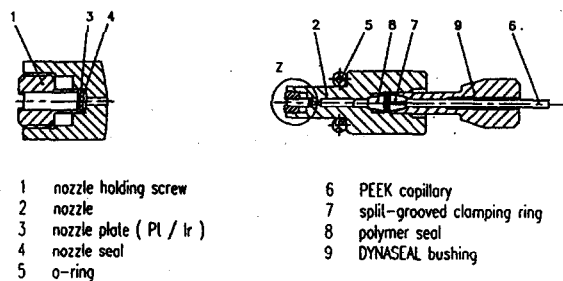| 1 | nozzle holding screw | 6 | PEEK capillary |
2	nozzle	7	split-grooved clamping ring
3	nozzle plate (Pt / Ir)	8	polymer seal
4	nozzle seal	9	DYNASEAL bushing
5	o-ring		

Fig. 1. Cross section of nebulizer nozzle (restrictor) and nozzle mount Z. (expanded plot). Reproduced with permission of Dr. Ing. H. Knauer GmbH.

suitability. Compared with fused-silica capillary restrictors, this nozzle is easier to handle in off-line operation because the nozzle plates can readily be replaced and blockages in the nozzle openings can easily be eliminated by turning the nozzle plate around.

Thus, once the nozzle plates have been acquired, when the task for which the equipment is being used is changed, it presents no problem to clean the nozzle plates and reuse them. On the other hand, capillary restrictors block up relatively quickly when in use, particularly in conjunction with soil extractions, and have then to be discarded and replaced with new ones [16].

A further advantage of the tested restrictor is that the supercritical medium only expands when it reaches the borehole in the plate; the expansion does not take place over a length of feed. This provides an exceptional solution for meeting the requirement stated under item 1.

Temperature control of the restrictor ($\sim 50°C$) may serve not only to prevent blockages but also to facilitate "fine-tuning" of the volume flow [18]. Since the nozzle mount is made of titanium, temperature control can be provided by wrapping heater wire around the upper part of the nozzle mount and the capillary connection, and then heating (item 2). The temperature can be measured at the upper part of the nozzle mount beside the Dynaseal bushing. That part of the nozzle mount that extends into the recipient liquid (20 ml of toluene at room temperature) is to be inserted in a PEEK or PTFE tube to avoid heat transition from the nozzle mount to the recipient liquid.

Toluene was used as the recipient liquid since it proved to be a universally applicable solvent for compounds having aromatic structure components (item 3).

Blockages only occurred with soil extractions, not with fullerenes or rubber additives, using 5-μm nozzles without heating. In our applications with soil extractions, heating of the nozzle was not necessary when nozzle plates with a larger bore diameter, e.g. 20 μm were used (item 5); this resulted in a higher consumption of liquid carbon dioxide and an occurrence of bigger gas bubbles in the recipient vessel.

Table 1 shows the experimental conditions of three applications using SFE with this restrictor.

Extractions of rubber additives are chosen because they are less frequently described in the literature.

The main components of the extracted addi-

Table 1
SFE conditions

	Rubber additives	Fullerenes	Soil extract
Extraction cell	Empty column 30 × 4 mm I.D.	30 × 4 mm I.D.	60 × 4 mm I.D.
Weighed specimen	Finely ground rubber, 0.05 g	Soot, 0.04 g	Air-dried soil, 1 g
Oven temperature	70°C	40°C	40°C
Pressure of CO_2	41.5 MPa	25 MPa	30.5 MPa
Density of CO_2	0.87 g/ml	0.88 g/ml	0.92 g/ml
Volume flow	2 ml/min liquid CO_2	2.4 ml/min	1 ml/min
Nozzle aperture	20 μm	20 μm	10 μm
Extraction time	30 min	30 min	30 min
Recipient liquid	Toluene	Toluene	Toluene

tive of the rubber specimen in our example are N-(1,3-dimethyl-butyl)-N'-phenyl-p-phenylene-diamine, alkylated phenols and alkylphenylic sulfonates.

Fig. 2 shows the SFC chromatograms created on the Dionex SFC unit of the extract on SB-Biphenyl-30 and, for comparison, the following components: N-(1,3-dimethyl-butyl)-N'-phenyl-p-phenylene-diamine, octylated diphenylamine, alkylphenylic sulfonates and stearic acid.

A total of 9.4% extractable components [approximately 90% of the amount of extractable components that can be attained with Soxhlet extraction (1 h) using acetone] was found.

A further example of the possibilities offered by SFE using pure carbon dioxide is shown with the extraction of fullerenes.

Fig. 3, lower trace, shows the HPLC sepa-

Fig. 3. HPLC chromatogram of fullerenes C_{60} and C_{70} following their SFE with pure carbon dioxide (lower trace) and with carbon dioxide/10% acetone (upper trace). HPLC conditions: Column, Inertsil ODS 2, 150 A, 5 μm; flow: 1.0 ml/min; dimensions, 250 × 4.6 mm I.D.; injection, 20 μl; eluent, CH_2CL_2–MeOH (60:40, v/v); detector, UV, 260 nm.
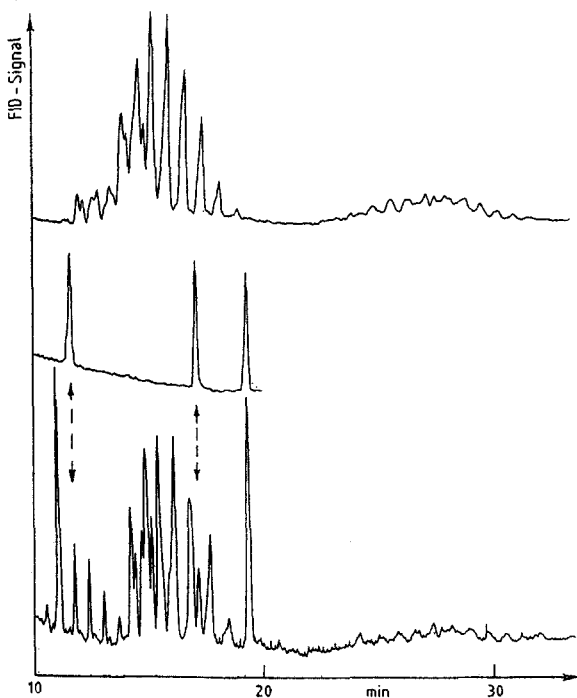

Fig. 2. SFC chromatograms of the rubber extract (lower trace), of stearic acid, octylated diphenylamine and N-(1,3-dimethyl-butyl)-N'-phenyl-p-phenylene- diamine (in this order, middle trace) and of alkylphenylic sulfonates (upper trace). SFC conditions: Column, SB-Biphenyl-30 (ID 50 μm; 10 m); temperature, 120°C. Density program: initial density 0.2 g/ml, increase to 0.6 g/ml at 0.025 g/ml/min, then increase to 0.65 g/ml at 0.002 g/ml/min.
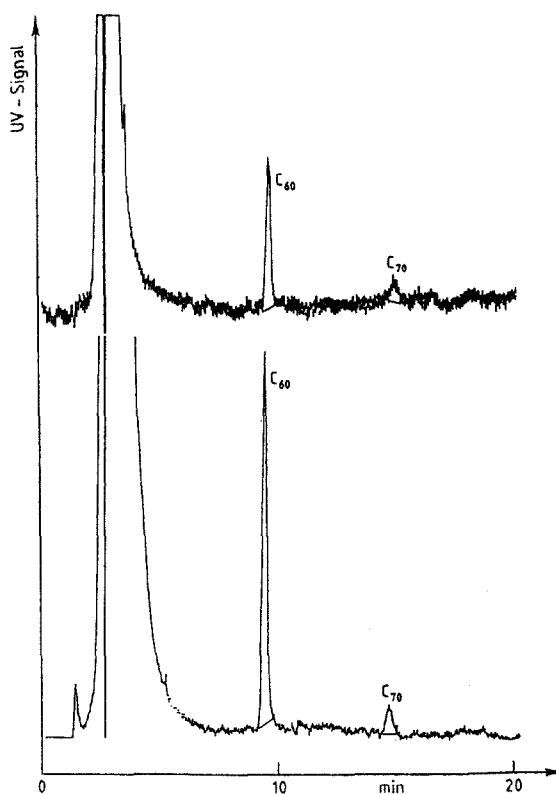
ration of C_{60} and C_{70} out of the SFE recipient in accordance with the described example in Table 1. The extraction using supercritical carbon dioxide yielded 6% extractable components. The fraction of C_{60} and C_{70} is unknown [19].

Fig. 3, upper trace, shows the result of the SFE for C_{60} and C_{70} using supercritical carbon dioxide and 10% acetone as a modifier in accordance with Ref. 20. A lower yield was obtained under similar SFE conditions using carbon dioxide–10% acetone.

As investigations by Jinno et al. [21] show, the yield can be increased through the addition of toluene as a modifier.

Our third application example involves a soil extraction. Before a soil–humus complex is char-
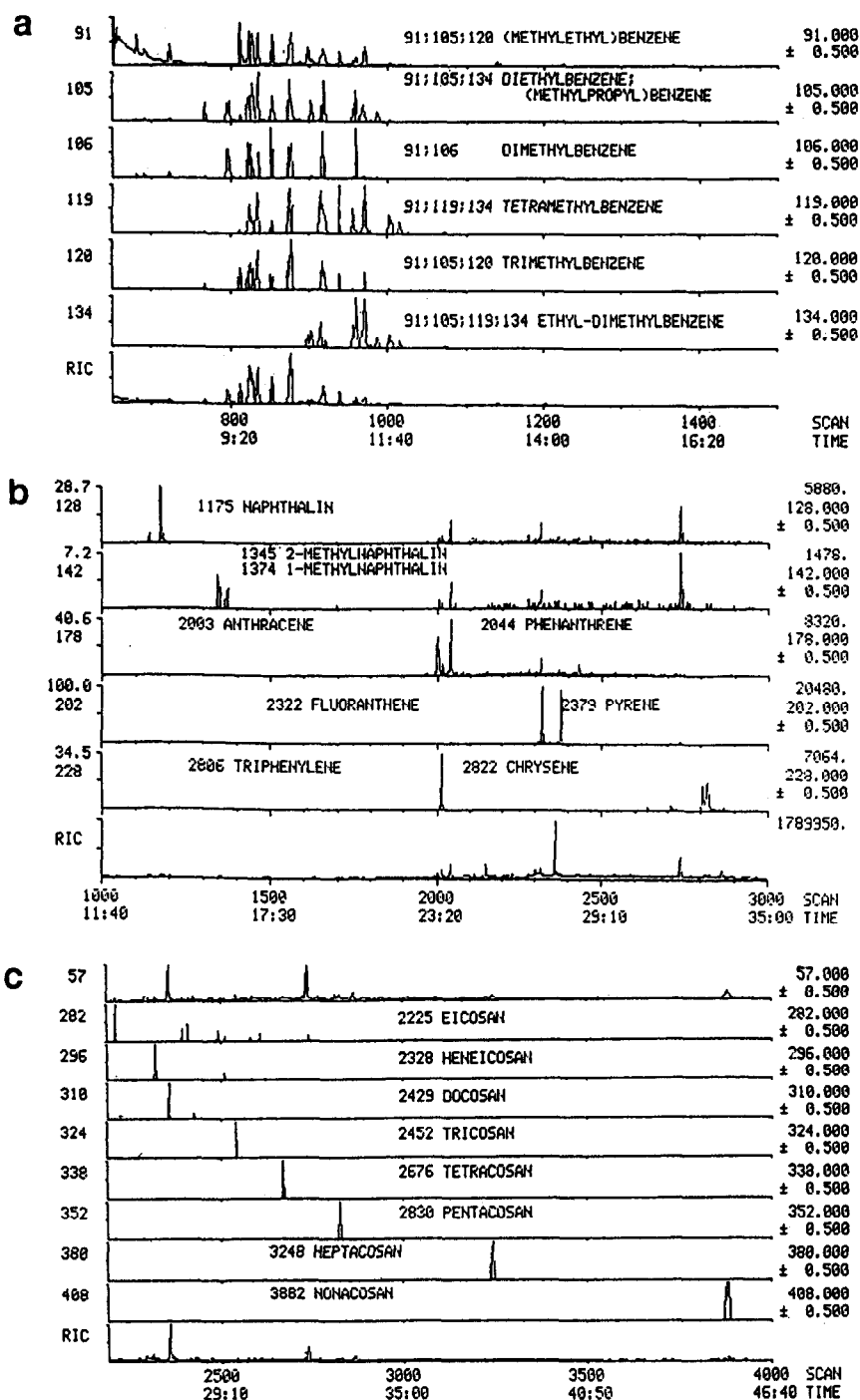
Fig. 4. GC–MS chromatogram of an SFE extracted soil in the Dahlem test field, Plot 21, soil horizon 1 . . . 30 cm. (a) alkylated benzenes; (b) polyaromatic hydrocarbons; (c) hydrocarbons (eluting in this order out of the column). GC conditions: column, DB1 (30 m × 0.25 mm I.D., 0.25 μm film thickness); injection 2 μl; injector, 270°C. Temperature program: initial temperature 50°C, hold 3 min, then increase to 100°C at 15°C/min, then increase to 280°C at 9°C/min, hold 20 min.

acterized, it is necessary to separate from it any unincorporated organic components, and to do so in such a way that its primary structure is not considerably denaturalised, so that subsequent fractionation of the humin substances into fulvic acids, humic acids and humin can provide a realistic interpretation of the structural composition of the individual fractions. This can be achieved through the mild SFE process, whereby, if necessary, modifiers may be added.

In our example, a soil from an exposed position on the Dahlem test field of the Federal Biological Research Centre for Agriculture and Forestry (BBA) was selected for presenting the results. The components were extracted from 1 g of soil each ranging from 1 ... 30 cm soil horizon, and were determined using GC–MS (Finnigan MAT).

Fig. 4a, b and c are parts of the same GC–MS chromatogram. Fig. 4a shows alkylated benzenes eluting first out of the column (8 to 12 min elution time), then polyaromatic hydrocarbons (Fig. 4b) and hydrocarbons up to 46 min elution time (Fig. 4c). Evidence of approximately 20 compounds of the classes alkylated benzenes (Fig. 4a) and hydrocarbons (4c), and approximately 10 compounds of the class polyaromatic hydrocarbons (Fig. 4b) was found in the soil extract.

4. Summary

The point of emphasis in the above paper is the presentation of a new restrictor design as part of a simple SFE apparatus. The advantages of the restrictor nozzle are discussed in comparison with capillary restrictors.

Handling of the presented restrictor nozzle proved to be good, as shown by a number of selected examples in SFE practice.

The apparatus is suitable for extraction with supercritical carbon dioxide as well as with mixtures of carbon dioxide and modifiers. In order to set solvent gradients for SFE, it is necessary to use 2 pumps and a mixing chamber.

References

[1] St. Küppers, Chromatographia, 33 (1992) 434.
[2] J.M. Levy, M. Ashraf-Khorassani, A.C. Rosselli, D. Boyer, L.A. Dolota, R.M. Ravey and E. Storoznyski, SFE Applications, Suprex Corporation, Pittsburgh, PA, 1989.
[3] R.E. Majors, LC·GC, 4 No. 2 (1991) 10.
[4] R.E. Majors, LC·GC, 4 No. 3 (1991) 10.
[5] W. Pipkin, LC·GC, 5 No. 1 (1992) 8.
[6] F. Höfler, Labor-Praxis, 16 (1992) 350.
[7] J.W. King and M.L. Hopper, J. Assoc. Off. Anal. Chem., 75 (1992) 375.
[8] E. Stahl and K.W. Quirin, Naturwissenschaften, 71 (1984) 181.
[9] W.M.A. Niessen, P.J.M. Bergers, U.R. Tjaden and J. van der Greef, J. Chromatogr., 454 (1988) 243.
[10] J. Doehl, A. Farbrot, T. Greibrokk and B. Iversen, J. Chromatogr., 392 (1987) 175.
[11] M. Ashraf-Khorassani and L.T. Taylor, Anal. Chem., 61 (1989) 145.
[12] S.B. Hawthorne, D.J. Miller and J.J. Langenfeld, in P. Sandra (Editor), Proceedings of the 13th International Symposium on Capillary Chromatography, Riva del Garda, May 1991, Hüthig, Heidelberg 1991.
[13] V. Janda, G. Steenbeke and P. Sandra, J. Chromatogr., 479 (1989) 200.
[14] J.A. Hyatt, J. Org. Chem., 49 (1984) 5097.
[15] S.B. Hawthorne, D.J. Miller and J.J. Langenfeld, in K. Jinno (Editor), Hyphenated Techniques in Supercritical Fluid Chromatography and Extraction (Journal of Chromatography Library, Vol. 53), 1992, p. 225.
[16] Y.Y. Wigfield and M. Lauouette, J. Agric. Food Chem., 41 (1993) 84.
[17] H. Engelhardt, J. Zapp and P. Kolla, Chromatographia, 32 (1991) 527.
[18] M.D. Burford, S.B. Hawthorne, D.J. Miller and T. Braggins, J. Chromatogr., 609 (1992) 321.
[19] H.J. Möckel, Hahn-Meitner-Institut GmbH, Abt. S3, Berlin, personal communication.
[20] F. Höfler, SFE-Applikation, Dionex GmbH, Idstein, 1992.
[21] K. Jinno, H. Nagashima, K. Itoh, M. Saito and M. Buonoshita, Fresenius' J. Anal. Chem., 344 (1992) 435.