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Solute collection after off-line supercritical fluid extraction into a moving liquid layer

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Abstract

A solute collection method after off-line supercritical fluid extraction using a moving layer of liquid organic solvent flowing down through a fused-silica capillary (0.5 mm I.D.) is proposed. Recoveries of over 90% were measured for selected polycyclic aromatic hydrocarbons (acenaphthene, phenanthrene, fluoranthene, pyrene and benzo[*a*]pyrene) and *s*-triazine herbicides when methanol was used as a trapping solvent.

1. Introduction

To establish supercritical fluid extraction (SFE) as a routine sample preparation method, three distinctive features need to be studied [1]. Whereas the extraction process itself is and will be unremittingly studied mainly from the viewpoint of new SFE applications, the problems connected with solute transport from the extractor and efficient analyte collection from the expanding supercritical fluid must be unambiguously solved. Transport of solutes from the extractor into a suitable collection device depends on restrictor selection and also on problems connected with restrictor plugging and its mechanical stability. Plugging is often encountered when real sample matrices contain large amounts of water or other co-extractable components. As pure or modified CO_2 is predominantly used in SFE, elimination and/or suppression of the Joule–Thompson cooling effect complicates solution of the plugging problem.

Three basic approaches have been proposed and used to collect solutes effectively from an expanding stream of gaseous fluid: direct trapping in a liquid solvent [2–8], use of a solid sorbent trap [9–15] and trapping on a cooled solid surface [16,17] (other methods such as solventless collection in an empty vial [18] are used more rarely).

The third method mentioned above is rarely used, owing to the properties of an expanding fluid mixture, where the formation of aerosols and solute clustering can occur. The use of a fused-silica capillary (0.5 mm I.D.) as a trapping element seems to be a promising method [19].

When solid sorbent traps are used, rinsing of adsorbed solutes by liquid solvent adds a further

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step to the overall analytical process, the recovery of which must be taken into account. Another problem is connected with a higher modifier concentration, which is a liquid under ambient conditions. Its partial condensation on the sorbent surface can lead to serious losses of solutes caused by their washing out from the sorbent trap [14].

The simplest and the most commonly used collection method consists of depressurizing a supercritical fluid into an organic solvent. The tip of the restrictor is simply immersed in liquid solvent in a vial. In spite of its obvious simplicity, this collection method offers a high degree of experimental variability and flexibility, especially from the point of view of method optimization.

Selection of the organic solvent is of primary importance. Langenfeld et al. [5] found the collection solvent polarity and temperature to be more important than the solvent volume and the height of its level in a vial. Based on the recoveries of 66 compounds, the best overall collection efficiency was found for methylene chloride and chloroform. Methanol failed to collect 35–50% of each test compound. The results for acetone were similar to those for methylene chloride; hexane exhibited the poorest collection capability for the most volatile species, but better than that of methanol for the less volatile components.

Thompson et al. [6] evaluated the collection efficiency of various solvents and solvent mixtures. A polarity test mixture consisting of acetophenone, N,N-dimethylaniline, naphthalene, decanoic acid, 2-naphthol and tetracosane (the same as in the work by Mulcahey and Taylor [14]) was added to a sand matrix and extracted with supercritical carbon dioxide. In addition to all the commonly used collection solvents, cyclohexane and perchloroethylene were used. The lowest recoveries and the highest R.S.D.s were found for decanoic acid and, in some solvents ($CHCl_3$, CH_2Cl_2 and perchloroethylene), for N,N-dimethylaniline. The recovery data indicate no correlation between any of the solvent physical property (i.e., boiling point, density, viscosity, surface tension and/or Hildebrand solubility parameter) and analyte recovery. Perchloroethylene, despite having the highest viscosity [8], exhibited the lowest overall trapping efficiency for all the analytes studied. Use of a multi-component collection solvent [hexane–trichloromethane–methanol (1:1:1)] increased all the individual analyte recoveries to above 92%.

Such extensive studies reflect well the influence of the solvent physico-chemical properties only if all other parameters are kept constant (the same value in all experiments). The reproducibility of results obtained in another laboratory, especially if commercial devices are used [20], is questionable.

Our experience leads us to the opinion that direct bubbling into the bulk liquid is not as an efficient trapping process as is commonly believed. If the analyte recoveries are over 90%, usually relatively high volumes of organic liquid solvents are necessary, and the resulting sample volumes are sometimes the same as from Soxhlet or sonication methods.

We believe that it is necessary to focus on the development of more sophisticated (efficient) processes to facilitate analyte mass transfer from an expanding supercritical mixture into an organic liquid solvent. In a recent study, Burford et al. [7] used the constant delivery of organic solvent just after the restrictor tip. The stream of expanding supercritical fluid nebulized the added solvent, forming a fine mist. A glass tube (61 mm × 3 mm I.D.) was used to sweep it into the bulk of the collection solvent. The substantial increase in collection efficiency was attributed to enhanced solvent contact with the analytes in the depressurized extract. Although the method was proposed as one of the possibilities to remove restrictor plugging, and the authors found a certain solution, this method was unfortunately not investigated further.

Mulcahey and Taylor [14] found enhanced recoveries in the presence of liquid methanol in an ODS trap when 1–2% methanol-modified carbon dioxide was used. Also, Howard and Taylor [21] obtained nearly quantitative recoveries of sulphonylurea herbicides at 45°C on stainless steel beads when 2% methanol-modified carbon dioxide was employed. They con-

cluded that the presence of liquid methanol on the stainless-steel surface enhances analyte trapping. In both studies the main problem was to find conditions under which the modifier condensation is diminished and analytes are trapped only on the solid surface. Hence the conclusion that larger amounts of liquid modifier decrease the trapping efficiency is correct only from the viewpoint of the amount of analyte deposited on the solid sorbent.

As for analyte trapping in a liquid organic solvent, the above-mentioned facts indicate much more effective trapping than in the case of bubbling through a bulk liquid. A solid trap, where particles are covered with a dew of condensed modifier, resembles a multi-capillary with small internal diameters, coated with liquid solvent.

This mode of trapping was simulated using a capillary for the collection of flufenoxurone by Vejrosta et al. [22]. Carbon dioxide containing 10 vol.-% of methanol and a fused-silica capillary (30 cm × 0.5 mm I.D.) equipped with a cryofocusing unit as a trapping device were used for the SFE of a spike. The condensed methanol from the methanol-modified CO_2 was used as the trapping liquid. The amount of liquid methanol was controlled by the temperature of the cryofocuser. Recoveries of over 90% were found at a CO_2 flow-rate of ca. 100 ml/min (under ambient conditions).

It seems probable that in the above experiments the transfer of flufenoxurone into condensing methanol was enhanced by mutual clustering of flufenoxurone and methanol molecules during expansion of the methanol-modified supercritical CO_2.

Trapping of analytes leaving the flow restrictor combining flowing water with a solid sorbent (reversed C_{18} phase) has also been described [23].

In this work, for the SFE of a polycyclic aromatic hydrocarbon (PAH) mixture we used pure CO_2, and a continuous stream of methanol was pumped into the trapping capillary (fused silica, 0.5 mm I.D.) during extraction. Acenaphthene, phenanthrene, fluoranthene, pyrene and benzo[*a*]pyrene were selected because of their lower recoveries [5] when bubbling through liquid methanol was used as a collection method. A similar system was used for the collection of *s*-triazine herbicides. In addition, a new principle of liquid solvent recirculation through the trapping capillary was preliminarily tested.

2. Experimental

Samples for extraction of PAHs were prepared by spiking 20 μl of standard PAH solution (0.25 mg of each in 1 ml) in methanol–tetrachloromethane (1:1) into an inert glass bead bed (60–80 mesh).

SFE was performed using the same device as described in previous papers [19,22]. Instead of a syringe, a linear sampling pump was connected to three-port union allowing a continuous flow of liquid solvent into the trapping capillary. In all experiments with SFE of PAHs the flow-rate of methanol fed into the trapping capillary was 50 μl/min. A fused-silica capillary (15 cm × 25 μm I.D.) was used as a restrictor and the last 4-cm length of the restrictor on the outlet edge was heated to 100°C. In some instances (SFE of *s*-triazine herbicides) the restictor tip (17 and/or 25 μm I.D. restrictor) was not heated because methanol acted as an antifreeze, preventing blocking of the restrictor. Because of the volatility of methanol, the final sample volume deposited in a microvial at the outlet of the trapping capillary was ca. 0.3–0.75 ml (the volume depends on the restrictor parameters and the resulting flow-rate of gaseous CO_2). The time of SFE of PAHs was 15 min in all experiments (temperature 60°C and pressure 25 MPa). More than half of the methanol volume pumped into the trapping capillary evaporated during SFE. The flow-rate of gaseous CO_2 was 170 ml/min ± 10%, measured under ambient conditions.

For all analyses a Chrom 5 gas chromatograph equipped with an OV-101 column and a flame ionization detector (Laboratory Devices, Prague, Czech Republic) was used.

For all experiments with *s*-triazines, a techni-

cal mixture of *s*-triazines (Zymazin herbicide) was used for recovery measurements. Zymazin is a product containing 93% of atrazine, 3.5% of simazine and 3.5% of propazine. As only the trapping efficiency after SFE was tested, a simple and inert matrix was used, Gas Chrom Q silanized support for gas chromatography (Alltech). The absolute amount of Zymazin spiked into the cartridge package was 40 μg. Zymazin was spiked in 20 μl of methanolic solution and allowed to dry before SFE. As *s*-triazines may not be extracted effectively by pure carbon dioxide [24], modifier (20 μl of methanol or acetonitrile) was added to the extraction cartridge before SFE of the spiked matrix. SFE using CO_2 with modifier was also tested in a recent study of the extraction of triazines from spiked soil and other environmental solids using a commercial device [25].

We did not heat the restrictor tip in this instance. Methanol delivered into the trapping capillary acted as an antifreeze and also transported a sufficient amount of heat to prevent its blocking by solid CO_2.

s-Triazine herbicides were determined by high-performance liquid chromatography (HPLC). For all analyses an HP 1050 liquid chromatograph (Hewlett-Packard) equipped with 200 × 4.6 mm I.D. column (Hypersil ODS, 5 μm) and a multiple-wavelength detector (MWD) was used. The signal of the MWD was monitored at 225 and 254 nm and peak identities were checked by measuring UV spectra. The mobile phase was acetonitrile–water (50:50) at a flow rate of 1 ml/min. Acenaphthene was used as an internal standard (added to the solution obtained after SFE). The capacity factors of the analytes were as follows: propazine 0.84, atrazine 1.16, simazine 1.80 and acenaphthene 6.32. The recovery of the analytes was calculated by comparison of peak areas obtained from the SFE extract and a reference "100% recovery" solution.

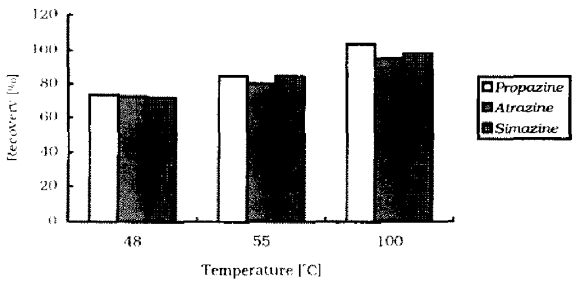

Fig. 1. Recoveries of *s*-triazines at different temperatures of extraction cartridge. Pressure of carbon dioxide, 23 MPa; restrictor, 15 cm × 20 μm I.D.; time of SFE, 10 min; 20 μl of methanol added to the cartridge before SFE (time of SFE 25 min and 100 μl of methanol added to the cartridge for extraction temperature 48°C).

3. Results and discussion

The results of ten repeated spike experiments with the PAH mixture are summarized in Table 1. Almost quantitative trapping was achieved for all individual PAHs with reasonable R.S.D. values. Because the input of methanol was placed above the heated restrictor zone, a great deal of methanol was evaporated and condensed again after restrictor tip. This means that the nebulization effect should be taken into account.

At the start of experiments with the SFE of triazines, the conditions were optimized. This was done using trapping of analytes at the outlet of the restrictor in 2 ml of methanol. The outlet of the flow restrictor was immersed directly in the methanol. All extractions were performed at 23 MPa. The influence of the temperature of the extraction cartridge is shown in Fig. 1. It can be

Table 1
Recoveries with R.S.D.s of selected PAHs (n = 10)

	Acenaphthene	Phenanthrene	Fluoranthene	Pyrene	Benzo[*a*]pyrene
Recovery (%)	90.5	91.0	91.2	93.3	93.0
R.S.D. (%)	9.3	4.8	6.5	7.5	6.1

seen that the best recoveries were obtained at 100°C. Therefore, all further SFE with trapping of analytes into the continuously rinsed capillary was carried out at this cartridge temperature. An SFE time of 10 min was sufficient for removing of triazines from the cartridge at 100°C. The use of acetonitrile instead of methanol as a polarity modifier of carbon dioxide had no effect on the *s*-triazines recovery.

The positive influence of elevated temperature on recovery suggests that the SFE efficiency was limited by the vapour pressure of *s*-triazines and kinetic phenomena rather than by solubility in a less dense fluid (the higher the temperature of a fluid at constant pressure, the lower are its density and solvation power).

The recoveries of *s*-triazines using a trapping capillary into which methanol was continuously pumped during SFE are given in Table 2. The flow-rate of methanol during the SFE was 0.05 ml/min. This means that the whole volume of methanol in the microvial after finishing the 15-min SFE is less than 0.75 ml (a portion of methanol evaporates during SFE owing to bubbling of gaseous CO_2 through the contents of the vial). Recoveries of SFE with direct trapping of *s*-triazines in 2 ml of methanol are also given in Table 2 for comparison. It can be seen that the recovery of the analytes is the same with comparable standard deviations. However, the concentration factor is at least three times higher when using trapping in the capillary owing to the lower final volume of the solution obtained after SFE. On the other hand, in our experimental set-up, a flow-rate of methanol through the trapping capillary of 0.05 ml/min was the minimum that prevented blocking of the restrictor unless the tip of restrictor needed to be heated. When lower flow-rates of methanol were used, the amounts of methanol and heat transported by methanol to the restrictor tip were insufficient.

A question is how to compare the efficiencies of different collection modes using liquid solvents. It might be possible to trap analytes quantitatively if greater volumes of solvent and longer paths of bubbles were used.

As a very rough parameter for mutual comparison of different solvent trapping methods, the trapping efficiency coefficient (TEC), defined as follows, can be introduced:

$$\mathrm{TEC} = \frac{V_{\mathrm{fr}}}{V_{\mathrm{l}}} \cdot \frac{R(\%)}{100} \qquad (1)$$

where V_{fr} is volume flow-rate of depressurized fluid mixture measured under ambient conditions, V_{l} the resulting volume of trapping solvent and R the recovery.

All parameters, usually studied without interrelation among different workers, such as solvent nature, trapping temperature, flow-rate of expanded CO_2 and system geometry, can be compared with respect to the TEC values. In this work, for example, the TEC values were approximately 560 min^{-1} and in the work of Howard

Table 2
Recovery of *s*-triazines by SFE and trapping in a continuously rinsed capillary (0.05 ml/min of methanol) and by SFE with direct trapping into 2 ml of methanol

s-Triazine	Trapping system			
	Capillary		2 ml of methanol	
	Recovery (%)	R.S.D. (%) ($n = 5$)	Recovery (%)	R.S.D. (%) ($n = 5$)
Propazine	90.9	2.5	91.5	1.8
Atrazine	90.0	2.1	90.8	1.6
Simazine	91.7	2.6	91.2	2.2

SFE conditions: temperature of extraction cartridge, 100°C; time of SFE, 15 min; restrictor, 15 cm × 17 μm I.D.; pressure, 23 MPa; 20 μl of methanol added to the extraction cartridge before commencing SFE.

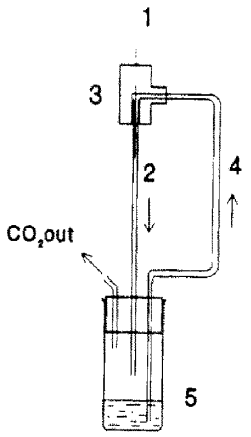

Fig. 2. Scheme of solvent trapping system with solvent recirculation. 1 = Restrictor; 2 = trapping capillary; 3 = three-port union; 4 = connecting line for solution recirculation; 5 = vial with solvent.

and Taylor [21] 83 min^{-1}. It is clear that higher values reflect more efficient collection.

One of common objections against the nebulization [7] of an expanding supercritical stream is the necessity to use another pump (a low pressure pump is sufficient) for solvent addition. In our arrangement, where the restrictor is placed in the trapping capillary (0.5 mm I.D. or less), the trapping system resembles and behaves like a water vacuum pump and a pressure lower than atmospheric is generated in the connection union above the restrictor tip. One of possibilities to utilize this suction effect is shown schematically in Fig. 2. In this arrangement, liquid solvent recirculates permanently along the restrictor and through the trapping capillary. Preliminary results are presented in Table 3. All the extraction conditions were the same as in previous experiments with PAHs. The resulting recoveries suffer from the short length of trapping capillary (10 cm), which was the maximum possible length at which solvent recirculation functioned. No heating of the restrictor tip is necessary because of the sufficient heat flow from liquid methanol passing around the flow restrictor and its antifreeze properties.

As the device used was not specially designed for this trapping method, dead volumes and also the use of PTFE tubing for the recirculation line could be sources of possible analyte losses. A more suitable device for utilization in this trapping method is under development.

4. Conclusions

Solvent trapping of analytes after off-line SFE can be a source of potential analyte losses that may be incorrectly interpreted as a poor SFE efficiency. In addition to parameters usually studied such as solvent nature, temperature, volume and height, the trapping efficiency depends strongly on the method of mutual treatment of both phases. From this point of view, bubbling through the bulk liquid seems to be the process with the lowest efficiency. When the average distance from the precipitated analyte molecules to the liquid surface is shortened and/or the expanding mixture is nebulized with solvent, the trapping efficiency increases substantially. As a result, quantitative trapping can be obtained with lower solvent volumes.

This was demonstrated by means of the proposed trapping mode, where analyte transfer into the liquid solvent is realized in a capillary (0.5 mm I.D.) and a liquid layer flows down along the inner capillary wall.

For solvent supply to the trapping capillary, which can simultaneously compensate for the losses of trapping liquid solvent, the suction

Table 3
Recoveries with R.S.D.s of selected PAHs (experiment with methanol recirculation; $n = 5$)

	Acenaphthene	Phenanthrene	Fluoranthene	Pyrene	Benzo[*a*]pyrene
Recovery (%)	79.5	83.0	85.8	81.1	89.7
R.S.D. (%)	4.6	1.9	1.4	2.0	19.2

effect resulting from the restrictor location in a narrow capillary can be employed.

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