Combined modifier/in situ derivatization effects on supercritical fluid extraction of polycyclic aromatic hydrocarbons from soil[1]

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Abstract

Supercritical fluid extraction (SFE) of polycyclic aromatic hydrocarbons (PAHs) from a real environmental soil sample containing a high content of humic acids was enhanced by using modifier and in situ derivatization. The addition of 10 mol% methanol increased the ability of the supercritical CO_2 (recovery set to 100%) to displace PAHs from matrix active sites up to an average value of 145%. In situ derivatization was performed under dynamic SFE conditions using boron trifluoride (BF_3) in methanol as modifier. Comparing the results of the SFE extraction with methanol and with BF_3 in methanol showed that the addition of BF_3 increased the PAH recoveries further. Increasing BF_3 concentration gave higher extraction yields of PAHs. A second SFE of the same sample with CO_2–methanol improved the average extraction yield for only 2%, whereas a second SFE of a CO_2–methanol extracted sample with CO_2–methanol–BF_3 increased the average yield for 18%. Especially the recoveries of the penta- and hexacyclic PAHs were increased by the addition of BF_3. The results indicate that the strong Lewis acid BF_3 cleave humic substances of the matrix releasing aromatic acids in the extract which were detected by HPLC–diode array detection. As a result of this PAHs associated to the matrix were also released in the extract. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Supercritical fluid extraction (SFE) of organic compounds from solid matrices requires three sequential steps: separation of the analyte from the sample into the extraction fluid, elution of the analyte from the extraction cell and collection of the extracted analyte in the trapping system. The first step, the desorption process, is mostly the step that limits the recovery of SFE for heterogeneous real environmental soil samples [1,2]. This was shown by the inability of SFE conditions developed on spiked samples to yield good recoveries of native analytes from real samples [3–5]. Native pollutants are in contact with the matrix for several years and have the potential to be associated with much stronger bonding sites than spiked compounds. The nature of the many various interactions between the analytes and matrix components is not well understood. The compounds may be associated with organic active sites, such as humic and fulvic acids, and/or inor-

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ganic sites, such as clay minerals. In addition, the analytes may be located in interstitial micropores of the soil particles or between clay plates, being covered by other organic material. Variation of extraction conditions like temperature, pressure, and modifier addition was done to overcome the interactions between the analyte and the matrix. The addition of modifiers strongly increases the ability of the supercritical fluid to extract analytes from matrix active sites [4,6–12]. Several studies demonstrate significant increases in SFE efficiencies by raising the extraction temperature [10,11,13,14]. The effect of pressure of the recoveries seems to be less important [13,14].

Derivatization of organic compounds before or during the SFE has been so far mainly used to make polar compounds easier to extract by the fluid CO_2. In situ chemical derivatization of chlorinated phenoxy acid herbicides [15,16] and chlorinated phenols [17] from soils and sediments has shown good recoveries. Trimethylphenylammonium hydroxide (TMPA) [15], pentafluorobenzyl bromide and triethylamine [16], acetic anhydride in the presence of triethylamine [17], a mixture of hexamethyldisilane and trimethylchlorosilane [18], and boron trifluoride (BF_3) in methanol [15] have been used so far as derivatization reagents. Apart from increasing the solubility of polar analytes in CO_2, derivatization can also improve the extraction of non-polar compounds by modification of the matrix active sites. Hills and Hill [18] found that with a mixture of silanes as derivatizing reagent the recoveries of four polycyclic aromatic hydrocarbons (PAHs) from an urban dust sample and a harbor sediment sample were greatly improved.

The aim of this study was to investigate the effect of the derivatization reagent BF_3 in methanol on the SFE of the 16 US Environmental Protection Agency (EPA) PAHs from a real environmental soil sample with a high content of humic acids. The high content of humic substances enables strong matrix–analyte interactions which we tried to overcome with the addition of BF_3 to the modifier methanol. For comparison, SFE using pure CO_2 and a mixture of CO_2–methanol as supercritical fluids was carried out, keeping all other important SFE parameters constant, i.e. pressure, temperature, and flow-rate.

2. Experimental

2.1. Characterization of the sample

The native soil sample LUT2 used in this study was obtained from a private garden. The sample was air-dried yielding a water content of 3% (w/w). After being ground using a mill (S1000, Retsch, Haan, Germany) the sample was homogenized. Water content was determined according to DIN 51718 (1978). The total organic carbon (TOC) analysis of the samples was performed using a LECO CR-12 carbon determinator. The content of humic acids was determined by gravimetric analyses after extraction with alkaline solution, reprecipitation of the soluted humic acids with hydrochloric acid and filtration. Soxhlet-extraction was performed by extracting 50 g of soil for 8 h with dichloromethane. The extracts were concentrated by rotary evaporation, 5 ml acetonitrile were added, and the extracts were subsequently evaporated to 5 ml using a gentle stream of clean nitrogen.

2.2. Supercritical fluid extraction and in situ derivatization

The SFE experiments were performed using a Dionex SFE Model 723 consisting of an extractor and a co-solvent addition module (Dionex, Sunnyvale, CA, USA). The fluids used were pure SFE-grade carbon dioxide (Linde, Germany) or carbon dioxide with the co-solvent methanol (analytical-reagent grade, Merck, Darmstadt, Germany). The modifier was mixed dynamically during the extraction. The 10-ml cells were filled consecutively with clean sand (Aldrich, Steinheim, Germany), a 5-g sample of LUT2 mixed with 0.5 g hydromatrix (ICT-ASS-Chem, Germany), approximately 1 g copper granulate and, finally, clean sand again.

The extractions were started with a pressure of 10 MPa, which in the following was increased by 5-MPa steps, in 3-min intervals, up to 40 MPa. The final pressure was maintained for a further 22 min. The extraction cell temperature was maintained at 90°C, and the restrictor temperature was regulated at 180°C. The restrictors had a flow-rate of 500 ml/min CO_2 at 34 MPa. All extracts were collected in 12 ml

acetone in 30-ml vials which were cooled to 5°C. After extraction 1,1-binaphthyl (0.8 µg/ml) was added as internal standard prior to HPLC analysis. The extracts were filled up to a volume of 10 ml and filtrated through PTFE filters.

In situ derivatization was performed during the modifier-assisted SFE. BF_3 in methanol (Merck) was used as derivatization reagent and co-solvent during the extraction as well. Methanol (10 mol%) containing 1 or 4% (v/v) BF_3 was dynamically mixed with the fluid CO_2. The extracts were treated as described above.

Several extractions were performed for each condition and the data received were averaged out.

2.3. HPLC analysis

PAH analysis was performed using a Hewlett-Packard 1050 HPLC-system with Beckmann pumps (model 126, Beckmann, Munich, Germany). Separation was achieved on a 250 mm×3 mm I.D. RP-C_{18} column (particle size 5 µm) with a 12.5 mm×3 mm I.D. pre-column (MZ-Analytical, Germany). The temperature of the column was stabilized at 35°C with a peltier thermostat BFO-04 (Optilab, Berlin, Germany). An acetonitrile–water gradient, from 60 to 100% acetonitrile in 35 min, was used as mobile phase with a total flow-rate set to 0.6 ml/min. For detection, fluorescence and diode array detectors were connected in series. A time program was utilized to detect the different PAHs at optimal absorption and emission wavelengths. Quantitative analysis of the 16 EPA PAHs was performed using external standard calibration with a reference standard solution (Promochem, Wesel, Germany) diluted appropriately.

For the detection of polar aromatic compounds an acetonitrile–water gradient, from 30 to 100% acetonitrile within 35 min, was used.

2.4. Gas chromatography

Analysis of non-polar breakdown products of the humic acids in the extracts was carried out using a Hewlett-Packard 5890 GC system equipped with an HP-5-MS-capillary column (30 m×0.25 mm I.D.) coated with chemically bonded SE-54 (d_f=0.25 µm;

Hewlett-Packard, Germany). Helium (1.6 ml/min) was utilized as carrier gas. The analyses were performed using the following temperature program: 40°C held for 1 min isothermal, heating rate 4°C/min up to 310°C, final temperature held for 1.5 min. Injector and detector temperatures were 250 and 300°C, respectively; detection was achieved using a mass detector (Hewlett-Packard, 5872).

3. Results and discussion

3.1. Sample characterization

The native environmental soil sample LUT2 is contaminated with EPA PAHs to a minor extent. PAHs (5.97 mg/kg) were isolated by soxhlet extraction and measured by HPLC equipped with a fluorescence detector. The main EPA PAHs are naphthalene, fluoranthene and pyrene. The sample has a high TOC content (49.7 g/kg) with a high percentage of humic acids determined by the gravimetric procedure (63.4 g/kg).

3.2. Effect of modifier on the SFE

The addition of methanol at 10 mol% to CO_2 improved the extraction efficiency of the fluid. All of the PAHs extracted from the soil were found in higher amounts using CO_2–methanol compared to using pure CO_2 (Table 1). This is in accordance with data in the literature [4,7,12,19]. The results indicate that methanol, which has minor Brønsted acidity and is highly capable of building hydrogen bonds, breaks PAH–matrix interactions and substitutes PAHs on the active site of the matrix.

3.3. In situ derivatization during modifier-assisted SFE

To study the influence of a derivatizing reagent BF_3 was added in minor quantities to methanol (1%, 4%, v/v). These solutions (10 mol% methanol/1%, 4%, v/v, BF_3) were utilized as modifier in the SFE experiments. As shown in Table 1, the addition of BF_3 enhanced the extraction yields of PAHs in comparison to pure CO_2 and CO_2–methanol without

Table 1
PAH recoveries from soil LUT2 using SFE with modifier and derivatizing reagent

PAH	Pure CO_2	Recovery (%)±relative standard deviation[a] (R.S.D. %)		
	μg/kg±% R.S.D.	CO_2–10 mol% CH_3OH	CO_2–10 mol% CH_3OH with 1% BF_3	CO_2–10 mol% CH_3OH with 4% BF_3
Naphthalene	92±21	302±36	329±18	320±15
Acenaphthene	39±19	126±35	155±5	112±37
Fluorene	37±15	154±21	157±22	153±17
Phenanthrene	465±1	106±28	126±2	129±15
Anthracene	94±24	108±22	122±9	136±8
Fluoranthene	1022±4	100±15	118±3	123±6
Pyrene	1174±1	142±9	134±9	153±2
Benzoanthracene	369±1	105±7	132±10	138±12
Chrysene	393±6	126±7	131±3	141±4
Benzo[b]fluoranthene	328±3	126±6	160±14	201±33
Benzo[k]fluoranthene	121±0	110±6	116±17	140±12
Benzo[a]pyrene	250±8	119±20	94±16	109±38
Dibenzo[a,h]anthracene	23±21	175±32	198±32	229±11
Benzo[ghi]perylene	84±43	186±31	201±6	381±41
Indeno[1,2,3-cd]pyrene	99±5	193±33	214±32	307±45

[a] Statistical data are calculated from three experiments, respectively.

derivatizing reagent. Using 1% BF_3 in methanol the extraction efficiency was increased up to an average value of 159%, and using 4% BF_3 up to an average value of 185% compared to pure CO_2. Comparing Soxhlet extraction with dichloromethane versus SFE with 4% BF_3 in methanol, using SFE it was possible to recover 117% of the Soxhlet result. Four percent BF_3 in methanol gave higher extraction yields of the tetra-, penta- and hexacyclic PAHs than 1% BF_3. The amounts of the di- and tricyclic PAHs are similar for both concentrations. These results show that the reactive compound BF_3 is especially appropriate to extract PAHs with high organic carbon normalized partition coefficients (K_{oc}) which interact strongly with the active sites of the macromolecular organic or inorganic substances of the soil. The electrophilic reagent BF_3 attacks the soil matrix achieving in a breakdown of non-covalent hydrogen bondings, Van der Waals forces, and electron donor–acceptor complexes, as well as covalent ether and ester bondings [20].

The extracts of the SFE with BF_3 were colored yellow, indicating the extraction of matrix compounds like humic substances. This assumption is supported by the fact that the soil contains about 5% organic carbon. The HPLC–UV chromatograms of the extracts always showed a large peak at a retention time of 6.3 min, which could not be detected without BF_3. The elution before naphthalene (t_R=7.0 min) indicated that it is a polar compound or compound mixture. To yield an increasing separation of polar aromatic compounds another acetonitrile–water gradient was selected (see Section 2). Fig. 1 shows the UV chromatograms of extracts from SFE with methanol and methanol–BF_3 measured using this HPLC method. The addition of BF_3 to methanol resulted in several new peaks in the chromatogram. The UV spectrum of the peak at 7.4 min resembles the spectra of terephthalic acid, with an absorption maximum at 240 nm. The peak at 4.7 min and the reference compound benzoic acid with a retention time of 4.6 min show similar spectra with an absorption maximum at 225 nm. The benzoic acid skeleton is frequently found to be an essential part of humic acids. Thus the detected peaks indicate the breakdown of humic substances by BF_3 and the subsequent extraction of low molecular compounds from the humic matrix. As a result PAHs which are associated with humic active sites or covered by humic substances can be released from the matrix and extracted by the fluid.

Due to the derivatizing reagent methylethers or methylesters of the phenols or aromatic acids of the humic acids could be expected in the extracts.
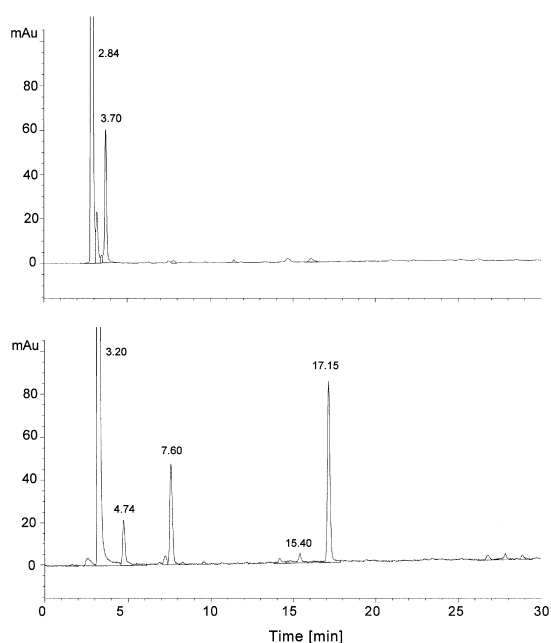
Fig. 1. HPLC–UV chromatograms (230 nm) of soil extracts received by SFE with CO_2–10 mol% methanol (top) and CO_2–10 mol% methanol with 1% BF_3 (bottom). HPLC conditions: RP-C_{18} column with pre-column (250 mm×3 mm and 12.5 mm×3 mm I.D., particle size 5 μm); acetonitrile–water gradient, from 30 to 100% acetonitrile within 35 min; flow-rate of 0.6 ml/min.

However, the GC–MS chromatograms of the extracts do not show characteristic peaks corresponding to esters or ethers, probably because derivatization with BF_3/methanol is less ambiguous. Hawthorne et al. [15] compared GC–electron-capture detection (ECD) chromatograms of extracts of soil using TMPA–methanol and BF_3–methanol reagents. The latter extract contained significant fewer ECD-responsive organics than the TMPA extract.

3.4. Consecutive SFE on the same sample

For studying the effect of BF_3 in more detail two consecutive SFE of the same soil sample were performed. The first extractions were carried out with 10 mol% methanol as modifier. The second extractions were performed with either 10 mol% methanol again or 10 mol% methanol–1% BF_3. The second extraction with methanol improved the average yield of the PAHs by only 2%, whereas with methanol–BF_3 the improvement was 18% (Table 2). This shows clearly the great increase of extraction efficiency achieved by the derivatizing reagent combined with methanol. The extraction yields of the second extractions with methanol–BF_3 of the highly volatile PAHs, such as naphthalene, acenaphthene

Table 2
PAH recoveries of the second SFE from soil LUT2 using methanol or methanol–BF_3

	Recovery[a] (%)±R.S.D.[b] (%) after a second SFE extraction	
	10 mol% CH_3OH	10 mol% CH_3OH with 1% BF_3
Naphthalene	<1	2.6±38
Acenaphthene	<2	6.5±45
Fluorene	<1	8.0±90
Phenanthrene	1.4±4	8.1±19
Anthracene	0.7±21	15.3±46
Fluoranthene	1.6±25	8.2±19
Pyrene	2.0±9	21.6±27
Benzoanthracene	0.5±62	10.8±2
Chrysene	0.7±9	13.5±0
Benzo[b]fluoranthene	2.1±11	21.7±3
Benzo[k]fluoranthene	3.6±12	27.9±9
Benzo[a]pyrene	0.3±78	22.6±19
Dibenzo[a,h]anthracene	<2	25.5±31
Benzo[ghi]perylene	3.7±8	55.2±16
Indeno[1,2,3-cd]pyrene	1.6±24	50.6±12
Average	1.6±8	18.1±1

[a] Recovery normalized on the first SFE extraction with 10 mol% methanol as modifier.
[b] Statistical data are calculated from two experiments, respectively.

and fluorene, were increased only by 3–8%. In contrast, the second extractions with methanol–BF_3 are clearly beneficial to the recovery of penta- and hexacyclic PAHs. The extraction results of benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene were improved by about 50%. Hills and Hill [18] described an even higher extraction efficiency of PAHs by the addition of hexamethyldisilane–trimethylchlorosilane; however, they only investigated four tri- and tetracyclic PAHs.

4. Conclusions

Addition of a polar modifier like methanol to supercritical CO_2 enhanced the recoveries of PAHs in minor contaminated soils with a high content of humic substances compared to those obtained using pure CO_2. Further addition of a derivatizing reagent, such as BF_3, to the modifier can improve the extraction efficiency further. Based on these results the modifier and derivatizing reagent appear to act by different extraction mechanisms. The strong Lewis acid BF_3 seems to cleave humic substances of the matrix, releasing aromatic acids in the extract. Due to this, PAHs associated with humic active sites or covered by organic material can be released from the matrix. Polar compounds bound to the matrix can be released and derivatized to esters or ethers which can be extracted by the fluid. The results shown in this study indicate that using supercritical CO_2 with reactive compounds as modifier can be a efficient SFE method for increasing the extraction yields of nonreactive analytes, such as PAHs, from soils with high contents of humic substances.

Acknowledgements

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