Supercritical CO$_2$ assisted liquid extraction of nitroaromatic and polycyclic aromatic compounds in soil

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Abstract

In this work we present a supercritical fluid extraction method based on the technique of direct modifier addition. This supercritical CO$_2$ assisted liquid extraction method proves to be superior to conventional liquid extraction methods such as sonication or Soxhlet extraction. Furthermore we investigated the influences of polar additives in the modifier on extraction behaviour. Analytes of interest were polycyclic aromatic hydrocarbons and for the first time the extraction of nitroaromatic compounds and other explosive residues was attempted in real-world soil samples. Due to the use of an especially developed solid–liquid trapping system we were able to integrate a clean-up step (ICUS). Additionally, this trapping system allows quantitative recovery of even volatile nitroaromatics. © 1997 Elsevier Science B.V.

Keywords: Soil analysis; Nitroaromatic compounds; Polycyclic aromatic compounds; Carbon dioxide

1. Introduction

Supercritical fluid extraction (SFE) has become an attractive alternative to liquid solvent extraction (for example Soxhlet or ultrasonic extraction) of organic components from environmental matrices. However, in a great number of publications the authors reported poor SFE efficiencies of non-polar and moderate polar components such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) or polychlorinated dibenzo-*p*-dioxins (PCDDs) from real-world environmental samples [1]. All these substances have high bulk solubility in supercritical CO$_2$ but they are difficult to extract because of matrix influences [2].

The application of modifiers can overcome these limitations in many extraction procedures, but for a great number of real-world samples it is impossible to apply a SFE method working for one matrix to

every other sample. Furthermore SFE of matrices with high sorption capabilities is still difficult.

Recently it was discovered that SFE efficiency strongly depends on the way of modifier application. The spiking of the modifier directly onto the sample prior to extraction appeared to be much more effective than the use of premixed fluids [3]. Consequently the accelerated solvent extraction (ASE) [4], an extraction technique using pressurised subcritical solvents, was developed in 1995. This method has shown to be an adequate replacement for a number of Soxhlet extraction methods and found admission in methods from the US environmental protection agency (US-EPA).

Accordingly we developed an extraction method which benefits from the advantages of pressurised solvents, as well as of those of supercritical fluids. In former studies our method proved to be applicable for extraction of PAHs from different types of even highly sorptive matrices (e.g., fly ash) [5]. In this work it is evaluated whether the method can be

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applied to different kinds of analytes without major changes of the extraction parameters.

Therefore two groups of environmental contaminant substances are investigated. Due to the well known fact that sorption behaviour of naturally aged real-world samples cannot be satisfactorily simulated by laboratory spiking [6,7], only native soil samples have been used. On the one hand PAH contaminated soil was chosen. Due to the expanded π-electron system of the PAHs they tend to be adsorbed at a variety of active sites on the matrix surface, whereas their permanent dipole-moment is negligible. On the other hand a selection of nitroaromatic compounds present in explosive residues was examined. In contrast to the PAHs their functional groups (-nitro, -amino) are responsible for adsorption. Additionally their adsorption strength is distinctly lower. Therefore the most difficult step in the analysis of nitroaromatic substances is not the extraction itself, but due to their volatility the prevention of losses during SFE-trapping and the following sample preparation [8,9].

With these two groups of model substances a wide range of possible extraction and sorption characteristics is covered.

2. Experimental

2.1. Soil samples

For the evaluation of our SFE methods native soil samples were used. The PAH containing soil sample was taken from a polluted industrial site. Soil containing nitroaromatic compounds was sampled at a former ammunition plant. Before extraction the soil samples were freeze-dried for 10 h at 100 Pa, mortared and homogenised.

2.2. Reference methods

2.2.1. Soxhlet extraction of PAHs

Soxhlet extraction of the soil sample was performed according to DIN 38414, part 21 [10]. 5 g soil was transferred into a cellulose extraction thimble and extracted for 8 h with 150 ml n-hexane. The solvent volume was reduced to 50 ml with a rotary evaporator. A 1-ml aliquot was transferred to a

silica-SPE cartridge for clean-up. The column was eluted with 5 ml n-hexane–dichloromethane (1:1) and the solvent was removed under a gentle stream of nitrogen. 50 µl phthalic acid diethylester was added to prevent evaporation to dryness. 950 µl acetonitrile was added to the remaining residue and the solution was analysed by high-performance liquid chromatography (HPLC).

2.2.2. Ultrasonic extraction of nitroaromatic compounds

5 g soil was sonicated for 2 h in 10 ml toluene in a 320 W ultrasonic bath at room temperature. An aliquot of the extract was filtered through a 0.45 µm PTFE membrane filter disk and 1 µl was analysed without further clean-up.

2.3. Supercritical CO_2 assisted liquid extraction

PAHs were extracted using a Dionex SFE 703 and 703M extractor, at 40 MPa and 90°C oven temperature. The wafer-restrictors were heated to 150°C. The flow-rate was fixed to approximately 500 ml gas per min, due to the restrictor design. For all extraction procedures CO_2 was used as supercritical fluid and the following modifier solutions were applied: modifier 1: 1% triethylamine (TEA) in toluene; modifier 2: 1% trifluoroacetic acid (TFA) in toluene and modifier 3: 1% TEA plus 1% TFA in toluene.

Three different extraction methods were applied to evaluate the effects of the various modifiers: method PAH A: CO_2; 120 min dynamic; method PAH B: CO_2 premixed with 5% toluene; 120 min dynamic and method PAH C: modifier solutions were directly added into the extraction cell; 10 min static; 30 min dynamic SFE with CO_2; repeated four times.

The void-volume of the cells was filled with granulated copper to bind sulphur in the soil sample (see Fig. 1).

Nitroaromatic compounds were extracted using an Isco SFX 200 system with variable restrictors. Extraction was performed at 90°C and 40 MPa. The restrictors were heated to 60°C, and the dynamic flow was set to 1 ml supercritical fluid per min (about 500 ml gas per min). We used toluene, acetonitrile and methanol as modifiers for the SFE of the nitroaromatic compounds. Due to the polarity of the analytes the additive concentration was raised to
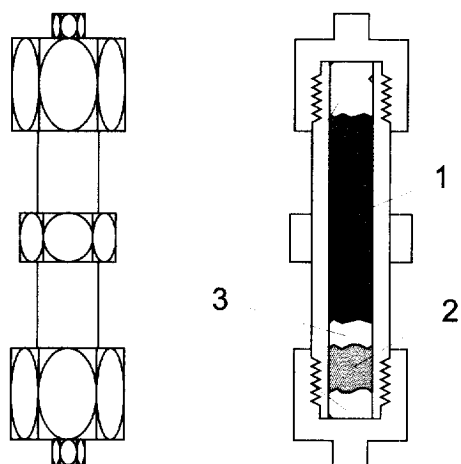
Fig. 1. SFE extraction cell. (1) Soil sample; (2) granulated copper; (3) glass wool.

Fig. 2. Combined solid–liquid trap; with integrated clean-up system (ICUS).

2% for the binary modifier solutions. As for the PAH extraction, we tested three different methods: method NC A: CO_2; 30 min dynamic; method NC B: CO_2 premixed with 5% toluene; 30 min dynamic and method NC C: direct addition of 2 ml modifier to the sample; 15 min static; 15 min dynamic.

2.4. Trapping system and sample preparation

Analyte collection was performed with a combined solid–liquid trap developed at our institute (see Fig. 2) [3,11]. This construction allows quantitative trapping of even volatile substances, like the mononitroaromatics. Furthermore the use of suitable solid phases can reduce the demand for sample clean-up. The trapping materials and the solvents for the elution of the solid phases are listed in Table 1.

For PAH analysis the solvent was removed as described in Section 2.3. Due to the volatile character of the nitroaromatics their extract was reduced to 1 ml using the Turbo Vap concentrator (Zymark); bath-temp: 25°C; N_2 pressure: 0.5 bar. A 100-μl aliquot was diluted to 1 ml with toluene and analysed without further clean-up.

2.5. HPLC method for PAH quantification

HPLC was performed on a HP 1050 LC system with fluorescence detection (FD). A common HPLC
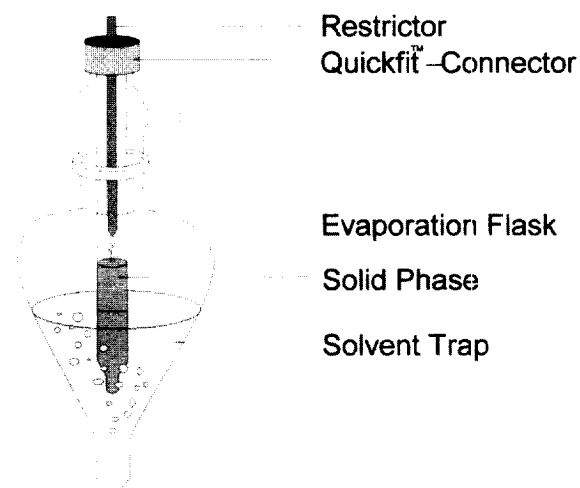
method was used [12]. Column: Bakerbond PAH 16-Plus 250 mm×3 mm I.D. (Mallinckrodt–Baker); mobile phase: acetonitrile–water gradient (see Table 2); flow-rate: 0.5 ml/min; temperature: 27°C; injection volume: 10 μl.

2.6. Quantification of nitroaromatic compounds

A HP 5890 gas chromatograph equipped with a programmed-temperature vaporisation injector (Gerstel KAS 3), a HT-8 column (SGE, Weiterstadt; 25 m×0.22 mm I.D., thickness 0.1 μm) and an electron capture detector (ECD) was used.

The injector was set to a split ratio of 1:30 for the first 2 min to fade out the solvent. The starting temperature was 80°C with a ramp of 2°C/s up to 140°C. After closing the split vent the injector was heated to 300°C at a rate of 12°C/s.

The column was held at 60°C for 4 min and then ramped to 170°C at 12°C/min. After 3 min it was finally heated at 12°C/min to 300°C and held for a total of 40 min. The used column contains m-carboran structure elements. Therefore retention is not only depending on the vapour pressure of the analytes, but also on steric interactions between their aromatic systems and the carboranes.

Fig. 3 shows the chromatogram obtained from a stock solution of seventeen compounds in toluene.

Table 1
Trapping conditions

	Solid phase	Liquid phase	Elution of the solid phase
PAHs	Silica-gel	15 ml n-hexane	4 ml toluene–petrolether (1:2)
Explosives	Lichrolut EN	10 ml toluene	5 ml methanol; 5 ml toluene

Table 2
Acetonitrile–water gradient

Time (min)	Acetonitrile–water (50:50) (%)	Acetonitrile (%)
0	100	0
6	100	0
35	2	98
51	2	98
52	100	0

The peaks marked with "s" result from impurities of the used standard substances.

3. Results and discussion

In this work we compare the efficiency of several

extraction methods using native soil samples. Since there are no certified values given for our matrices, Soxhlet extraction for PAHs in soil and ultrasonic extraction for soil contaminated with nitroaromatic compounds were used as reference. For tabulated data and as ordinate in the graphics the reference is set to 100%.

Commonly CO_2 is used as extraction fluid in SFE. Due to its linear structure, it has no permanent dipole moment and is therefore not efficient for the extraction of even moderate polar substances from highly adsorptive matrices [13]. Furthermore the delocalised π-electron system of the PAHs can cause strong interactions with the active sites of the matrix surface. Previous studies had shown that pure CO_2 is not capable to compensate for these effects [13].

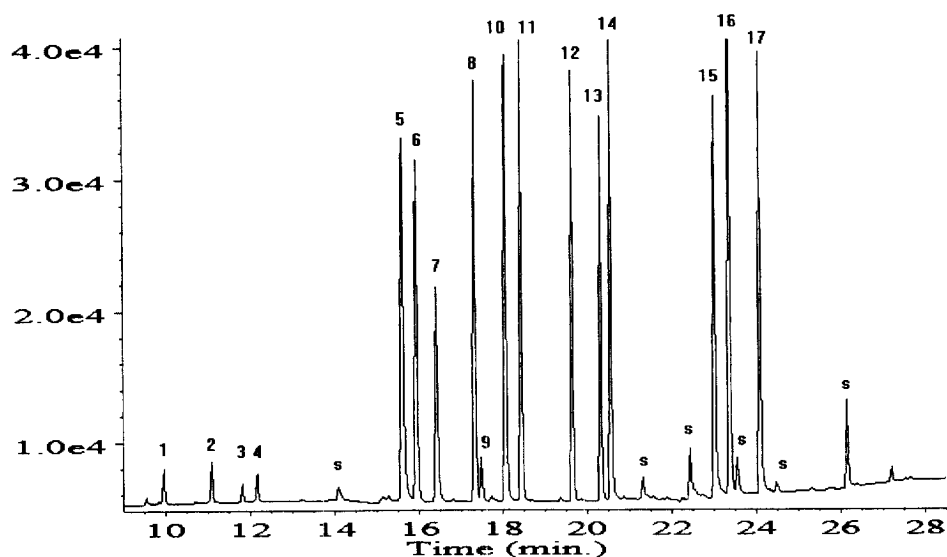Therefore the results of SFE with unmodified CO_2

Fig. 3. Chromatogram of a standard solution of nitroaromatic compounds. (1) Nitrobenzene (NB); (2) 2-nitrotoluene (2-NT); (3) 3-nitrotoluene (3-NT); (4) 4-nitrotoluene (4-NT); (5) 1,4-dinitrobenzene (1,4-DNB); (6) 2,6-dinitrobenzene (2,6-DNB); (7) 1,3-dinitrobenzene (1,3-DNB); (8) 1,2-dinitrobenzene (1,2-DNB); (9) 2-methyl-3-nitroaniline (2-M-3-NA); (10) 2,4-dinitrotoluene (2,4-DNT); (11) 2,3-dinitrotoluene (2,3-DNT); (12) 3,4-dinitrotoluene (3,4-DNT); (13) 2,4,6-trinitrotoluene (2,4,6-TNT); (14) 1,3,5-trinitrobenzene (1,3,5-TNB); (15) 4-amino-2,6-dinitrotoluene (4-A-2.6-DNT); (16) 1,5-dinitronaphthalene (1,5-DNN); (17) 2-amino-4,6-dinitrotoluene (2-A-4,6-DNT); (s) system immanent impurities.

Table 3

Extraction of a PAH containing soil sample using CO_2 or CO_2 modified with 5% toluene; Soxhlet extraction is assumed to 100% yield

Substance	Absolute concentration Soxhlet extract ($n=6$)			Extraction yield (% of Soxhlet extract)			
	Amount (mg/kg)	S.D. (mg/kg)	R.S.D. %	Without modifier ($n=9$)		With 5% toluene ($n=6$)	
				%	S.D.	%	S.D.
Fluorene (Fluo)	0.21	±0.02	±8.9	71.43	±26.1	119.05	±10.1
Phenanthrene (Phen)	2.54	±0.19	±7.5	78.84	±20.8	134.25	±9.6
Anthracene (Anth)	0.53	±0.03	±5.4	58.49	±23.6	105.66	±11.7
Fluoranthene (F)	4.34	±0.16	±3.6	60.37	±25.7	102.07	±13.9
Pyrene (Py)	3.00	±0.10	±3.4	52.67	±26.0	92.33	±15.5
Benz[a]anthracene (B[a]A)	2.02	±0.08	±4.0	28.71	±30.3	89.60	±22.8
Chrysene (Chry)	2.06	±0.09	±4.2	24.76	±30.6	79.61	±22.0
Benzo[b]fluoranthene (B[b]F)	2.81	±0.16	±5.6	11.39	±27.4	54.80	±29.2
Benzo[k]fluoranthene (B[k]F)	0.90	±0.04	±4.5	13.33	±29.0	66.67	±29.6
Benzo[a]pyrene (B[a]P)	1.67	±0.11	±6.8	7.78	±31.5	35.93	±34.1
Dibenz[a,h]anthracene (D[a,h]A)	0.25	±0.04	±14.0	8.00	±21.2	40.00	±36.5
Benzo[ghi]perylene (B[ghi]P)	1.01	±0.13	±12.9	3.96	±24.2	33.66	±49.2
Indeno[1,2,3-c,d]pyrene (I[c,d]P)	1.59	±0.25	±15.4	3.77	±27.2	38.36	±48.6

for the PAHs (Table 3) and for the nitroaromatic compounds (Table 4) are not unexpected. The data indicate that the yields are distinctly lower than those of the liquid extraction for nearly all components. Usually this problem is solved by using small amounts of organic solvents as modifiers. Therefore we performed the extraction according to methods PAH B and NC B. Surprisingly the use of modified CO_2 (Tables 3 and 4) does not enhance the extraction yield sufficiently. For PAH extraction there is an overall increase, but the results of the reference method are not reached for all compounds. In case of

the nitroaromatic compounds the extraction efficiencies for 1,3,5-trinitrobenzene and the aminodinitrotoluenes decrease.

As observed in experiments with spiked matrices, the main source of error in SFE of nitroaromatic compounds is the trapping procedure [8,14], especially if a modifier is applied [9]. To avoid these problems we used the combined solid–liquid trap, as described in Section 2.4. The adsorbent Lichrolut EN (Merck) is an adsorbent based on a polystyrol divinylbenzene phase with an approximate specific surface of 1380 m^2/g (according to BET) and 0.76

Table 4

Extraction results of a nitroaromatic compound containing soil sample in percent compared to those obtained by ultrasonic extraction (set as reference=100%)

Substance	Absolute concentration, ultrasonic extract ($n=6$)			Extraction yield				Extraction yield with modifier toluene plus					
	Amount mg/kg	S.D. (mg/kg)	R.S.D. (%)	Without modifier ($n=2$)		With 5% toluene ($n=2$)		None ($n=2$)		TEA ($n=2$)		TFA ($n=2$)	
				%	S.D.	%	S.D.	%	S.D.	%	S.D.	%	S.D.
1,2-Dinitrobenzene (1,2-DNB)	0.04	±0.01	±8.2	111.1	±9.6	133.0	±6.0	137.6	±5.7	91.8	±2.2	87.3	±8.9
2,4-Dinitrotoluene (2,4-DNT)	1.99	±0.06	±3.1	100.7	±5.9	113.6	±4.8	119.4	±2.4	100.3	±0.2	93.5	±3.8
2,3-Dinitrotoluene (2,3-DNT)	0.14	±0.01	±3.5	101.5	±4.9	107.4	±5.0	114.0	±1.0	83.5	±0.7	81.2	±0.1
3,4-Dinitrotoluene (3,4-DNT)	0.17	±0.01	±3.2	100.6	±2.8	106.6	±4.9	125.0	±4.4	91.8	±0.2	92.8	±0.1
2,4,6-Trinitrotoluene (2,4,6-TNT)	3.94	±0.14	±3.4	83.4	±0.3	96.9	±3.4	122.9	±7.1	104.3	±0.3	89.8	±0.2
1,3,5-Trinitrobenzene (1,3,5-TNB)	0.23	±0.01	±5.6	44.1	±0.4	34.8	±6.8	120.4	±8.1	102.2	±0.2	75.4	±1.7
1,5-Dinitronaphthalene (1,5-DNN)	0.20	±0.01	±3.8	92.3	±6.9	98.6	±1.8	116.1	±4.6	105.7	±2.2	92.2	±1.0

ml/g pore volume [15]. It proved to be an effective SPE-phase in analysis of explosives from water samples [15,16].

In former studies we evaluated if it is important whether the modifier interacts with the sample either in the liquid or in the supercritical state [5]. It could be shown that modifiers have a much greater effect if applied as liquid. Therefore in the following investigations the modifiers were added directly onto the sample prior to extraction. Fig. 4 shows the results of this technique for the extraction of the nitroaromatic substances.

The use of toluene as well as methanol or acetonitrile as modifier enhances the extraction efficiency compared to ultrasonic extraction, especially for 1,3,5-trinitrobenzene and the aminodinitrotoluenes. It was noted that toluene and acetonitrile were the most suitable modifiers for the soil, whereas methanol was less effective. These results emphasize that direct addition of the modifier is useful for the extraction of nitroaromatics from our soil sample.

To increase the modifier polarity small amounts of highly polar substances (additives) were added. According to former studies [5] two additives were investigated. Trifluoroacetic acid (TFA) is a highly polar organic acid while triethylamine (TEA) is a good electron-pair donor.

The results of SFE of the nitroaromatics with different toluene–additive combinations are compared in Table 4. It is shown that the reproducibility could be enhanced if the additives were employed, whereas the yield decreased. This indicates that the extraction with pure modifiers is already quantitative. The same experiments have been performed with acetonitrile–additive and methanol–additive combinations with comparable results.

Due to the fact that the PAH extraction was carried out on a system which had to be adapted to allow direct addition of the modifier and to apply static extraction conditions, the method for PAH extraction was slightly altered [17]. After flushing the first amount of modifier out of the extraction cell with CO_2, it was refilled with modifier using a syringe and the whole procedure was repeated three times.
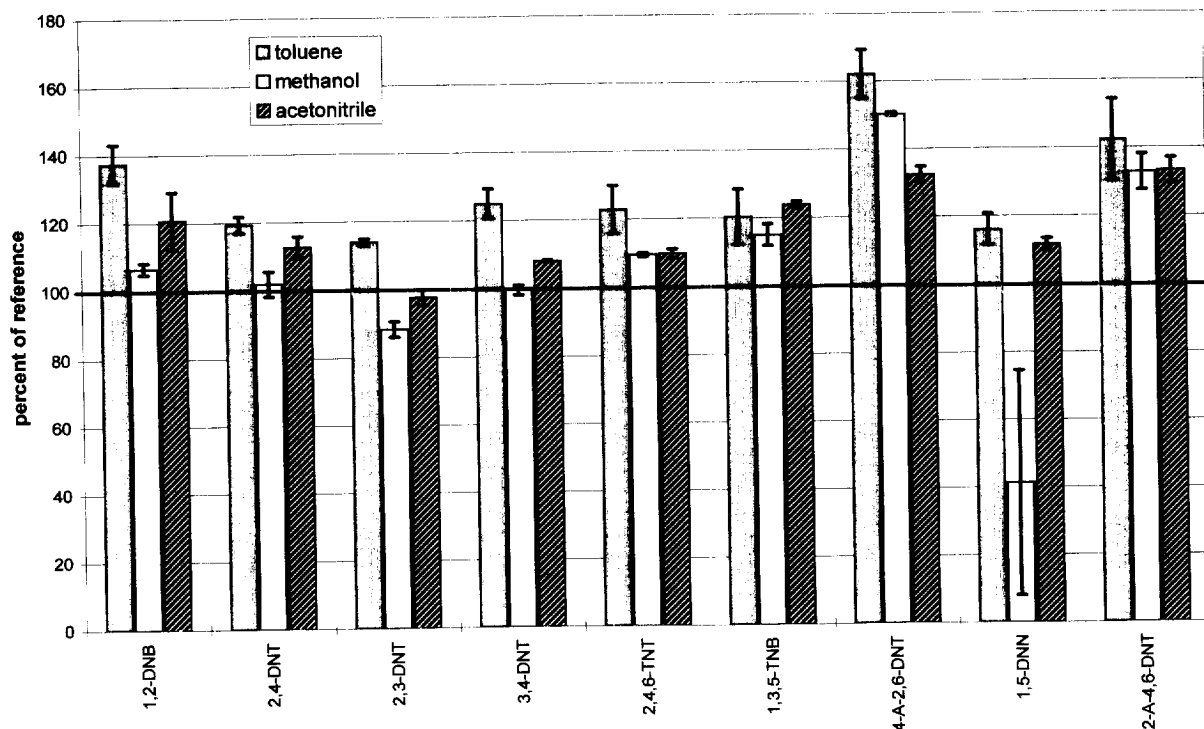

Fig. 4. Effects of different modifiers on the extraction of nitroaromatic compounds.

Fig. 5 shows the influence of the different binary modifier mixtures on PAH extraction. It is obvious that the results correlate well with those obtained by the reference method as extraction yields reach the results of Soxhlet extraction. Best results were gained by using toluene–(TFA–TEA).

To understand the influence of the additives on the extraction process, we have to refer to the kinetic model described by Pawliszyn [18]. Assuming that the matrix particles consist of an organic layer on an impermeable core, and the analyte is adsorbed onto the core surface (active site), the extraction process can be modelled by considering several basic steps. This is shown in Fig. 6. To remove the analyte from the extraction vessel, the component must firstly be desorbed from the active site; then it has to diffuse through the organic layer to reach the supercritical fluid, where it must be solvated. After diffusion through the static fluid present in the porous matrix it reaches the flowing bulk of fluid and exits from the extraction vessel [18].
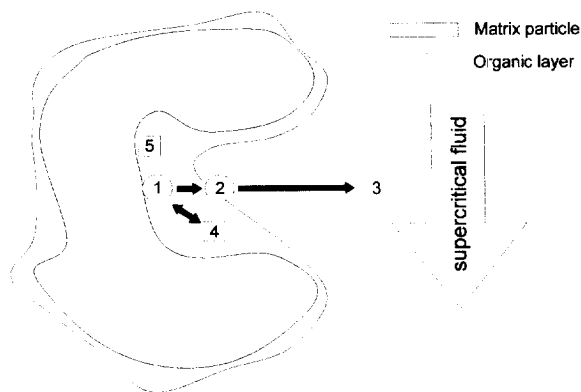
Fig. 6. Model of the SFE process (according to Pawliszyn [18]). (1) Adsorbed analyte; (2) analyte crosses border organic layer/fluid; (3) flush out; (4) interaction of the additive; (5) blocked site by additive.

We suppose the additives to be responsible for interaction with the matrix active sites and for blocking them after the desorption of the analyte molecule, due to their higher polarity or electron
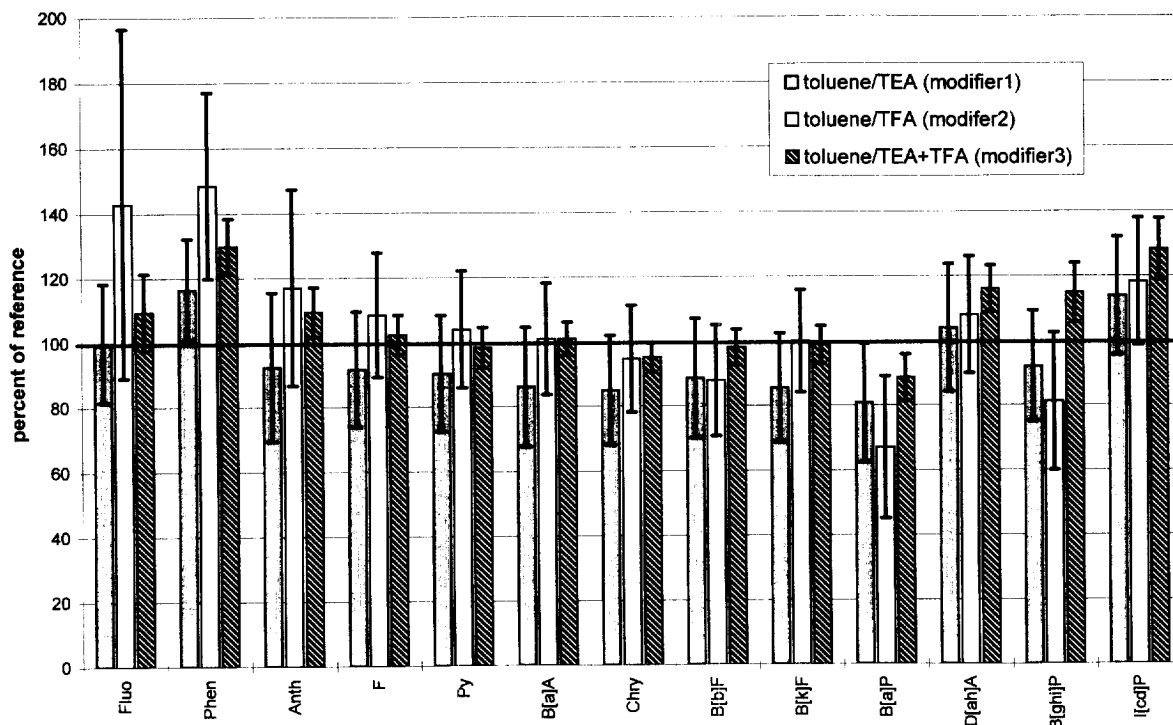

Fig. 5. Effects of different binary modifier mixtures on PAH extraction; binary modifiers: toluene–TEA (modifier 1), toluene–TFA (modifier 2), toluene–TEA–TFA (modifier 3).
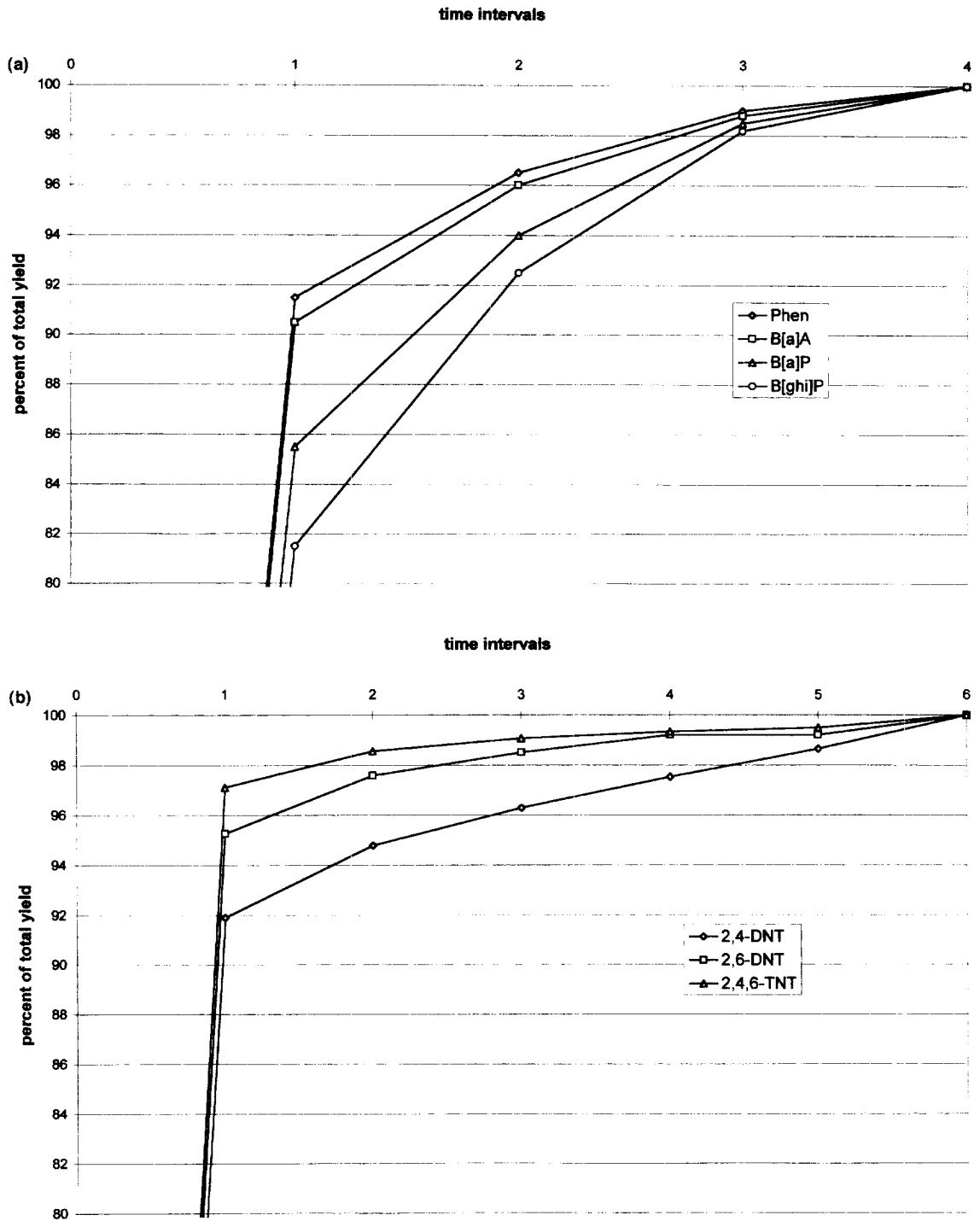
time intervals

time intervals

Fig. 7. (a) Fractionated extraction of PAHs showing four selected PAHs; one time interval is 30 min; graph crosses origin. (b) Fractionated extraction of nitroaromatic compounds showing three selected nitroaromatics; one time interval is 4 min; graph crosses origin.

donor potential. Because of this a re-adsorption of the analyte molecules will be suppressed. Furthermore we suggest that the liquid modifier will soak the matrix, and the analytes only have to reach the media present in the pore to get flushed out in the dynamic step.

In order to investigate the latter presumption we performed fractionated extraction. The trap was changed after extraction intervals of 4 min for the nitroaromatic compounds, and 30 min for PAH extraction. The results of this fractionated extraction of the PAHs shown in Fig. 7a are exemplary for three components. It can be seen that more than 80% of the total yield was extracted within the first 30 min.

Similar results are obtained for the extraction of the nitroaromatic compounds (Fig. 7b). In this diagram the time interval between the changes of the trap is 4 min. In this period only 4 ml supercritical CO_2 have passed the vessel. Because of the high amount of analytes in the first fraction the shorter diffusion distance into the liquid modifier seems to

have a great effect on the supercritical CO_2 assisted liquid extraction of the nitroaromatic compounds. Due to their lower sorption potential, compared to the PAHs, they are rapidly solvated in the subcritical modifier and the CO_2 only has to flush them out of the extraction vessel. These assumptions correlate well with our model of the modifier and additive effects.

After examining the influence of modifier solutions we evaluated the influence of pressure on extraction efficiency of PAHs. Therefore we extracted the soil sample according to method PAH C with toluene–TFA–TEA as modifier at different pressures (Fig. 8). As shown in this figure, lower pressure, and therefore a decrease of solvation power, has no negative influence on the extraction efficiency. The yield of extraction at lower pressure is even slightly higher and the repeatability could be distinctly increased. This might be caused by the restrictors design. The Dionex extractor is equipped with non-adjustable restrictors and the flow-rate is determined by their inner diameter and the given
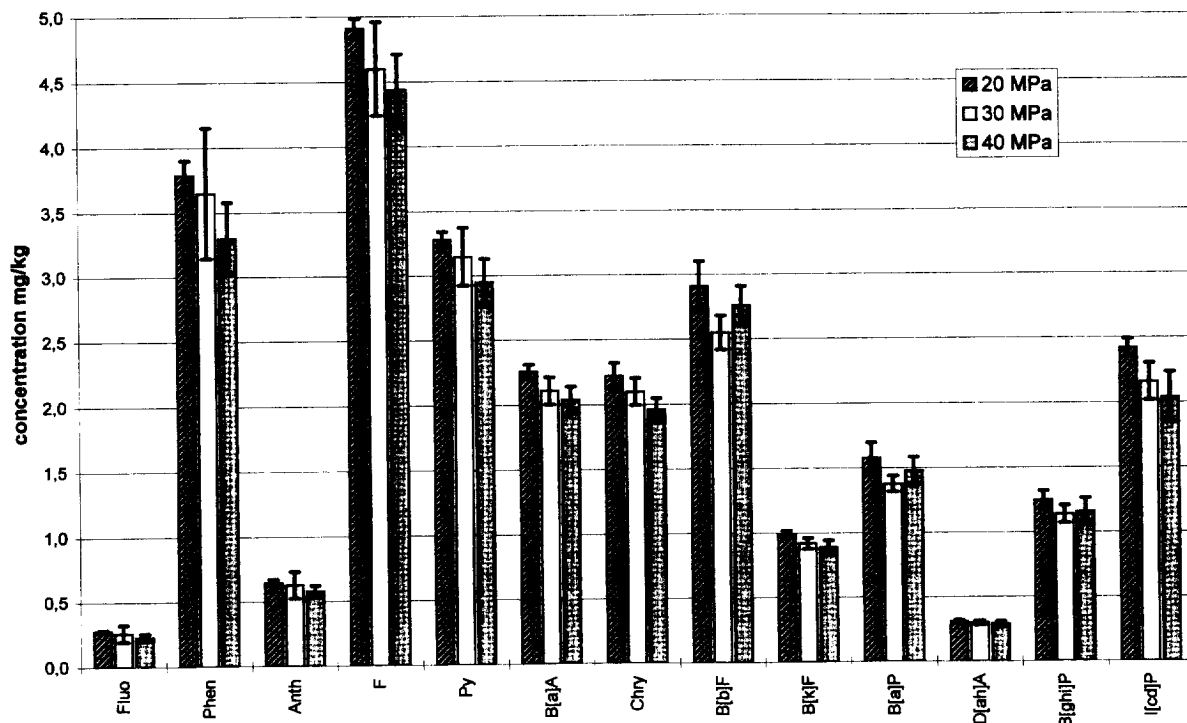

Fig. 8. Influence of pressure on PAH extraction using method PAH C and modifier 3.

pressure. Increasing pressure results in an increase of absolute CO_2 volume passed through the trap. Therefore losses due to vaporisation and especially aerosol forming of solvent causes collected analytes to be carried out of the trap [11].

During the investigation concerning the extraction of the explosives an interesting phenomenon could be observed. As described in Table 3, the application of binary modifiers containing TFA yielded high recoveries for the nitroaromatic compounds. Surprisingly for substances containing amino-groups (2-amino-4,6,-dinitrotoluene, 4-amino-2,6-dinitrotoluene) poor extraction efficiencies were obtained. This effect is outlined in Fig. 9. The graphic shows that the decrease of recovery for toluene and acetonitrile is enormous, whereas there are no losses in recovery using methanol as solvent. Therefore the protic character of a solvent may be responsible for this effect.

To investigate whether low extraction power or the occurrence of chemical reactions are responsible for these results, a second extraction step was

performed with the samples. Therefore after extraction according to method NC C using toluene–TFA the second extraction procedure was performed with methanol–TEA under the same conditions (Fig. 10). It is remarkable that only a small amount of aminodinitrotoluenes could be detected in the extract of the second extraction. Therefore we assume that a chemical reaction has taken place. The reaction products have not been investigated yet, but we suppose ion-pairing or formation of acid–base pairs. Further investigation on this task will be made to ascertain the reaction products.

4. Conclusions

A very effective SFE method could be developed. It was shown to be superior to conventional extraction techniques like sonication or Soxhlet extraction.

The method proved to be applicable for two completely different types of analytes. It was shown
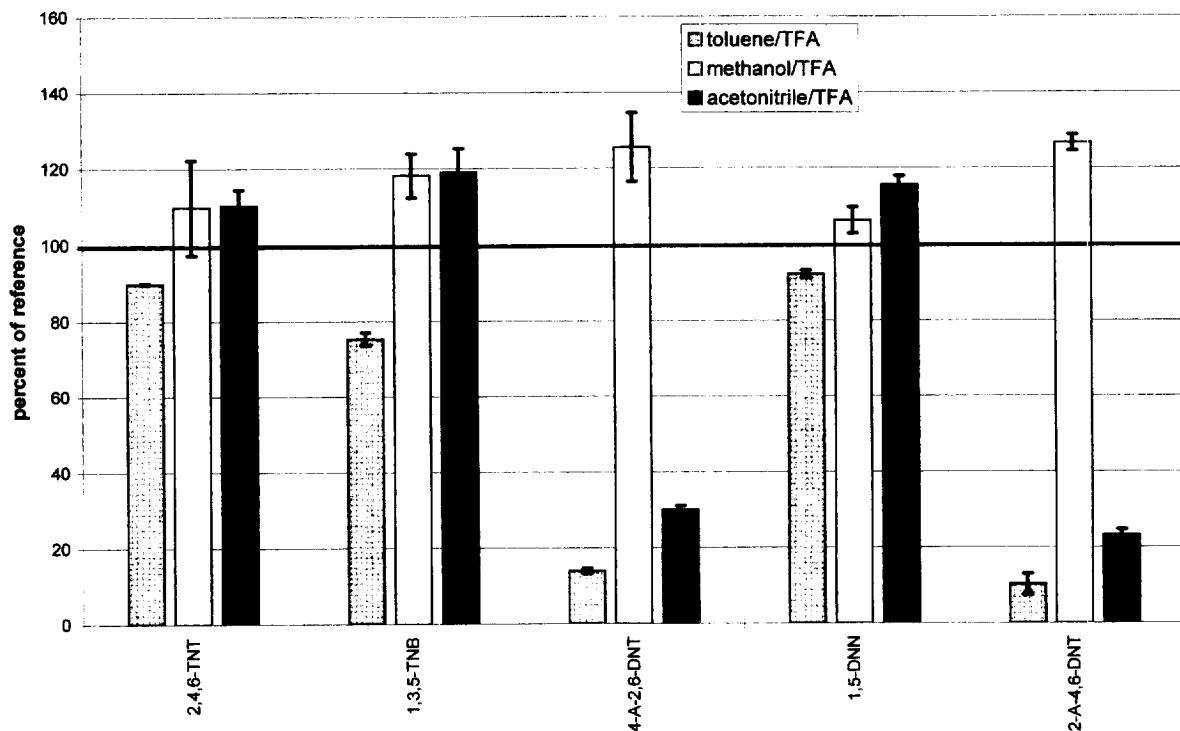

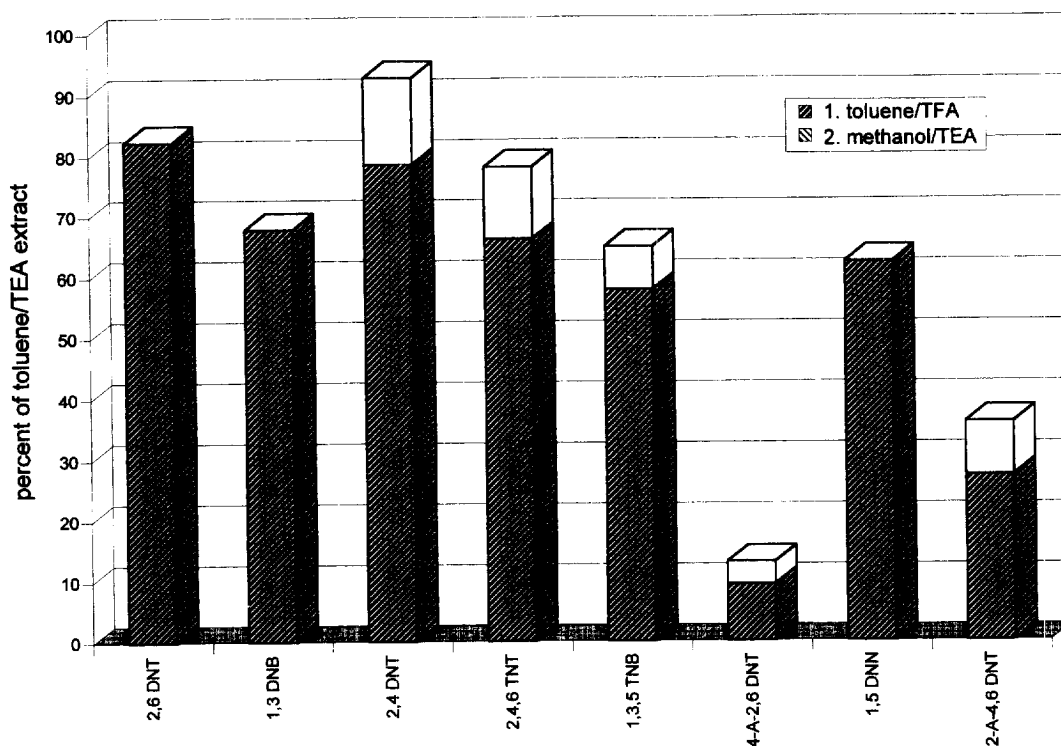Fig. 9. Extraction behaviour of the amino-dinitrotoluenes using different modifier–TFA combinations.

Fig. 10. Extraction yields after two extraction steps of the same soil sample; after extraction according to method NC C using toluene–TFA a second extraction was performed using methanol–TEA under the same conditions; yields obtained by extraction according to method NC C using toluene–TEA as modifier are set to 100%.

that efficiencies of SFE could be seriously enhanced by the presented technique of direct modifier addition and the application of binary modifiers. Furthermore we were able to integrate a simple clean-up step into the analyte collection device of the SFE. With this integrated clean-up system further sample preparation could be reduced and, furthermore, losses during the analyte collection could be avoided even for volatile components.

We showed that if using an appropriate modifier the pressure has only little influence on the supercritical fluid extraction efficiency. This leads us to some assumptions concerning the function of supercritical fluid and modifier. Supercritical CO_2 itself is only insignificantly involved in the desorption process, but it works well as reaction medium and is efficient for the transport of the dissolved analytes out of the extraction vessel. The modifiers and the way of their addition are the parameters determining efficiency of SFE. However, extraction yield can be

raised if pressurised liquid modifiers are employed. Overall the direct addition of modifiers and the use of polar additives can enhance both reproducibility and extraction efficiency.

Acknowledgements

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