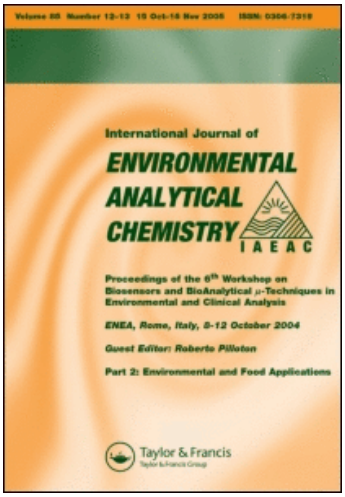


International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Extraction of polycyclic aromatic nitrogen heterocycles from spiked soil samples

Kamila Kočí[ab]; Hana Petrovská[c]; Zdeněk Šimek[a]; Elena VaraĎová[d]; Andrea Syslová[e]
[a] Research Centre for Environmental Chemistry and Ecotoxicology, Masaryk University Brno, 625 00 Brno, Czech Republic [b] Department of Environmental Chemistry and Technology, Brno University of Technology, CZ-612 00 Brno, Czech Republic [c] Tyco Electronics Czech, KAMP, 664 34 Kurim, Czech Republic [d] Czech Academy of Science, Institute of Analytical Chemistry, CZ-611 42 Brno, Czech Republic [e] Test-line Ltd, Clinical Diagnostics, CZ-612 00 Brno, Czech Republic

To cite this Article Kočí, Kamila , Petrovská, Hana , Šimek, Zdeněk , VaraĎová, Elena and Syslová, Andrea(2007) 'Extraction of polycyclic aromatic nitrogen heterocycles from spiked soil samples', International Journal of Environmental Analytical Chemistry, 87: 2, 111 — 123
To link to this Article: DOI: 10.1080/03067310600947276
URL: http://dx.doi.org/10.1080/03067310600947276

Intern. J. Environ. Anal. Chem.
Vol. 87, No. 2, 15 February 2007, 111–123

Extraction of polycyclic aromatic nitrogen heterocycles from spiked soil samples

KAMILA KOČÍ†‡, HANA PETROVSKÁ§, ZDENĚK ŠIMEK*†, ELENA VARAĎOVÁ¶ and ANDREA SYSLOVÁ‖

†Research Centre for Environmental Chemistry and Ecotoxicology, Masaryk University Brno, Faculty of Science, 126/3 Kamenice, 625 00 Brno, Czech Republic
‡Department of Environmental Chemistry and Technology, Brno University of Technology, Faculty of Chemistry, Purkynova 118, CZ-612 00 Brno, Czech Republic
§Tyco Electronics Czech, KAMP, 664 34 Kurim, Czech Republic
¶Institute of Analytical Chemistry, Czech Academy of Science, Veveri 97, CZ-611 42 Brno, Czech Republic
‖Test-line Ltd, Clinical Diagnostics, Krizikova 70, CZ-612 00 Brno, Czech Republic

(*Received 8 March 2006; in final form 4 August 2006*)

Extraction recovery of 10 selected polycyclic aromatic nitrogen heterocycles (PANHs), quinoline, 2-methylquinoline, 6-methylquinoline, 8-methylquinoline, acridine, benzo[*h*]quinoline, phenantridine, indole, 2-methylindole, and carbazole from spiked soil samples was tested. Four different extraction techniques, pressurized solvent extraction (PSE), supercritical fluid extraction (SFE), Soxhlet warm extraction (SOXW) and standard Soxhlet extraction (SOX), were applied and compared. The RP-HPLC technique with a silica-based octadecyl stationary phase was used for recovery determination of individual PANHs. Supercritical fluid extraction has been found to be the most effective method for the extraction of selected PANHs from soil. PSE and SOXW methods offered similar results with slightly lower extraction recoveries compared with SFE. On the contrary, SOX is a time-consuming method with a low recovery of target analytes and is not suitable for the extraction of PANHs from soils.

Keywords: Polycyclic aromatic nitrogen heterocycles; PSE; Soxhlet; SFE

1. Introduction

Polycyclic aromatic nitrogen heterocycles (PANHs), the N-heterocyclic analogous of PAHs, belong to the class of the biologically active environmental pollutants with both mutagenic and carcinogenic properties [1–3]. Their significance in the environmental pollution has been recognized during the past century when elevated concentrations in marine and freshwater sediments, aerosols of urban atmosphere, and sewage sludge have been found [3–8]. They are formed and released as the result of anthropogenic production related to industrial discharge and incomplete combustion processes [2–4].

*Corresponding author. Fax: +351-218464455/57. Email: simek@recetox.muni.cz

ISSN 0306-7319 print/ISSN 1029-0397 online © 2007 Taylor & Francis
DOI: 10.1080/03067310600947276

Their presence in the environment represents a risk, which has to be rigorously monitored.

The analytical methods for the determination of hetero-aromatic compounds have been improved during the last two decades [8]. Advances have been made in analysis, sample preparation, analytical separation and detection techniques. Different extraction procedures of PANHs from solid samples have been described in several articles involved in the determination of PANHs. Most of these approaches, applied either on environmental or on food samples, were based on the standard Soxhlet extraction technique. However, this method is time-consuming, requires large amounts of organic solvents, and is not very suitable for routine analysis of a large number of samples [9, 10].

Soil/compost mixture contaminated with PANHs was Soxhlet-extracted for 7.5 h with a mixture of dichloromethane and heptane (210 : 10, v/v) [11]. Sediments or suspended matter were Soxhlet-extracted for 18 h with dichloromethane [12]. Selected PANHs (azaarenes) were also Soxhlet-extracted from aerosol samples with dichloromethane [13]. Semi-volatile azaarenes in airborne and vapour phases were caught in PUF and extracted using Soxhlet and dichloromethane [14]. Soxhlet extraction with benzene for extraction of azaarenes and other basic compounds in fly ashes was used [15].

Galceran *et al*. [16] processed contaminated beef with acid and re-extracted this by shaking with cyclohexane after neutralization. Recovery of individual PANHs determined by spiking raw beef samples was 61.5–99.2%. Another possibility is pre-separation of alkaline beef extract on an Extrelut 20 diatomaceous earth column [17]. Ultrasonic desorption of PANHs from XAD-resin by dichloromethane was used for determination of basic nitrogen-containing polycyclic aromatic hydrocarbons formed during thermal degradation of polymers [4].

As can be seen, extraction of PANHs from solid samples is frequently done using Soxhlet extraction (SOX) and dichloromethane as the most extensive extraction solvent. However, data describing the extraction recovery of PANHs from investigated samples are not presented in most cases. In contrast to SOX, the extraction recovery of PANHs from spiked and/or real soil samples using either PSE or SFE has not been published.

The present paper was aimed at developing a method, simple sample handling, and comparison of given extraction techniques towards PANHs. The use of spiked soil samples was considered as a suitable approach to optimize the extraction conditions and evaluate the extraction efficiency of each technique. In experiments with spiked, clear soils, it is possible to eliminate possible analytical interferences of PANHs with PAHs, PASH, nitro-PAHs, and other PAH analogues usually present in real soil together with PANHs. Therefore, in the case of spiked sample treatment, the clean-up and pre-separation steps, which are sources of added errors, are not needed. Another advantage supporting the choice of spiked samples to minimize the recovery variations caused by inhomogeneities of native analytes in real contaminated soils.

In this study, freshly spiked and 48-h aged samples were used. On the basis of foregoing experiments, the period of 2 days was chosen as a suitable period for system equilibration and creation of 'matrix interactions'. Previously, it was found that after a period of 48 h, no significant changes in recovery appeared [18]. Besides this, a similar aging time (16 h) was used in another study [6].

Four extraction methods of PANHs from spiked soil samples have been compared. SOX is an example of traditional, non-instrumental techniques; Soxhlet warm

extraction (SOXW) is a modern variant enabling an increase in efficiency and speed in standard procedures; and pressurized solvent extraction (PSE) and supercritical fluid extraction (SFE) are very efficient and relatively new extraction techniques that are becoming routine. PSE combines elevated temperatures and pressures with liquid solvents to decrease extraction time and increase extraction efficiency. SFE uses a supercritical fluid as an extraction solvent. This leads to a greater selectivity, rapid mass transfer and higher flow rates compared with common extracting liquids.

Determination of extraction recovery of PANHs was carried out by RP-HPLC using octadecylsilica stationary phases.

2. Experimental

2.1 *Instrumentation*

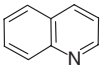
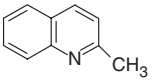
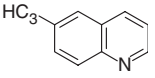
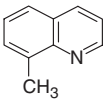
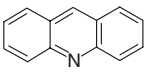
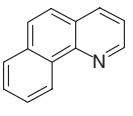
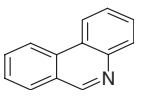
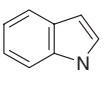
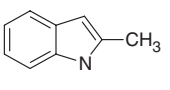
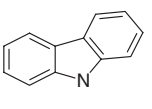
A Fastex extractor (Unikovo Brno, Czech Republic) with 11 cm^3 stainless steel extraction vessels was used for the pressurized solvent extraction (PSE). The temperature operating range of Fastex extractor was 50–150°C, and the maximum utilizable pressure was 15 MPa. The supercritical fluid extraction (SFE) was performed by means of the SE-1 extractor (SEKO-K Ltd, Czech Republic) with the 0.7–7.0 cm^3 stainless steel extraction vessels. The temperature-operating range of the SE-1 extractor was 30–150°C. The operating pressure varied between 7.0 and 40.0 MPa. The 200-cm^3 Soxhlet apparatus and 50-cm^3 paper cartridges were used for standard SOX. The B-811 extraction system (Büchi, Labortechnik AG, Switzerland) with 120-mL solvent vessel was used for SOXW.

The HPLC system consisted of a high-pressure linear pump HPP 5001 (Lab. pristroje Praha, Czech Republic), column thermostat LCO 101 (Ecom, Czech Republic), analytical injection valve with 5-μL injection loop (Ecom, Czech Republic), and UV photometric detector LCD 2082 (Ecom, Czech Republic) operating at 231 nm. A CSW data station (Data Apex, Czech Republic) was used for signal acquisition and data handling. The parallel HPLC system (Shimadzu LC A, Japan) consisted of a high-pressure Shimadzu LC-AT pump, column oven with three-port Shimadzu CT O-10A valve, 20-μL injection loop, spectrophotometric diode array Shimadzu SPD-M10AVP detector, and Shimadzu CLASS-LC10 software. Analyses of the extracts were carried out on two octadecyl silica stationary phases, a Hypersil BDS C18-5 μm (250 × 4,6 mm) (Shandon HPLC, UK) and a Biospher SiC 18–5 μm (250 × 4 mm) (Watrex, Czech Republic).

2.2 *Chemicals and materials*

The standard substances of tested PANHs were as follows: quinoline (>99%), benzo[*h*]quinoline (>99%), acridine (>97%), indole (>99%), 2-methylquinoline (>98%), 6-methylquinoline (>98%), 8-methylquinoline (>98%), phenantridine (>98%), 2-methylindole (>98%), and carbazole (>95%) (Fluka, Germany; Sigma Aldrich, St. Louis, MO) (table 1). The mixture of standards used for separation experiments, containing 0.01 mg/cm^3 of each PANH, was prepared by diluting standard stock solutions with acetonitrile. Standard stock solutions of individual PANHs were

Table 1. PANHs used in the study: physical–chemical properties [19][a].

	M_r (g/mol)	Structural formula	pK_a	Vapour pressure (mmHg at 25°C)	Log K_{OW}	Water solubility (mg/l at 25°C)
Quinoline	129.16		4.62	6.0×10^{-2}	2.03	6110
2-Methylquinoline	143.18		5.67	9.5×10^{-3}	2.59	499
6-Methylquinoline	143.18		4.97	6.4×10^{-3}	2.57	631
8-Methylquinoline	143.18		4.56	2.5×10^{-2}	2.60	489
Acridine	179.22		5.04	13.5×10^{-5}	3.40	38.4
Benzo[*h*]quinoline	179.22		4.28	21.8×10^{-5}	3.43	5.1
Phenantridine	179.22		4.66	2.08×10^{-5}	3.48	300
Indole	117.15		−2.40	1.22×10^{-2}	2.14	3560
2-Methylindole	131.17		−2.56	6.03×10^{-3}	2.53	628
Carbazole	167.40		−7 to 21	7.50×10^{-7}	3.72	1.8

[a] pK_a: dissociation constant of protonated forms ($PANH^+$). K_{ow}: distribution constant in the octanol–water system.

prepared by dissolution of standard substances in acetonitrile. The mixture of PANHs for the spiking procedure was prepared in the same way as the standard stock solutions. Acetonitrile, methanol, toluene, acetone, dichloromethane, *n*-heptane, and isopropanol (Merck, Germany; Sigma Aldrich, St. Louis, MO; Riedel de Hägen, Germany) were of HPLC-grade quality. Hexane (Riedel de Hägen, Germany) was of analytical-grade quality. The water used for HPLC analysis was prepared from deionized water by ultra-clear UV apparatus or redistilled in two steps (1: with $KMnO_4$; 2: in silica apparatus). Disodium hydrogen phosphate and potassium dihydrogen phosphate, forming a phosphate buffer in aqueous part of mobile phase, were of analytical-grade quality (Pliva Lachema, Czech Republic). The stock solution of buffer containing 66.7 mmol/dm^3 was prepared by dissolution of the aforementioned chemicals in the HPLC water. Other chemicals used were as follows: pelletized adsorbent Hydromatrix Celite (Varian, USA) and pure CO_2 (Siad TP, Italy). A list of tested PANHs is given in the table 1.

2.3 *Extraction procedures*

The four above-mentioned extraction methods were evaluated based on a comparison of percentage extraction recovery of individual PANHs from spiked soil samples. Each extraction experiment was carried out in triplicate. The processing of the extracts before the HPLC analysis differed according to both the extraction method and the extraction solvent applied. In general, the problematic (acetone) or non-compatible (dichloromethane) extraction solvents with RP-HPLC mobile phases had to be evaporated before the injection into the HPLC system. The concentrated extracts were either re-dissolved in a given amount of acetonitrile or directly analysed by RP-HPLC.

2.3.1 Pressurized solvent extraction (PSE). Extraction using a Fastex extractor (Unikovo Brno, Czech Republic) was performed as follows. The samples were loaded into a stainless steel extraction vessel and placed into the extraction oven. The oven was closed and solvent pumped into the vessel, which was heated and pressurized to the extraction conditions, applied in two ways: static and semi-dynamic mode. In the static mode, the set pressure was maintained during the static phase of each cycle. At the end of each cycle, the system was depressurized, and the entire liquid content of the extraction vessel was discharged into the collection vial. During the static phase of the semidynamic mode, the output valve was opened and closed periodically, causing the set pressure to fluctuate momentarily, and the vessel's content was partially discharged and refilled again.

Six grams of spiked soil sample with hydromatrix celite (mixed in a ratio of 2 : 1, w/w) was placed into an 11 cm^3 stainless steel cell and used for extraction. Raw or evaporated extracts were subsequently filled up to 25 cm^3 with extractant or pure acetonitrile and analysed.

During the extraction experiments, the extraction mode (static and semidynamic), time, composition of extractant, and temperature were tested. In the case of the extraction mode, the results from the static extraction performed in two 5-min cycles were compared with the semi-dynamic conditions realized in eight cycles, 1 min per cycle. The extraction time was probed only under the semi-dynamic mode. Four, eight,

and 10 cycles with two different extraction periods (1 and 2 min) per cycle were applied. The following solvents and solvent mixtures were used as the tested extractants: methanol, acetonitrile, isopropanol, dichloromethane, methanol–heptane (5% heptane), methanol–hexane (5% hexane), methanol–acetonitrile (90 : 10, 80 : 20, and 70 : 30, v/v) and acetone–hexane (1 : 1, v/v). The extraction experiments were carried out at 100 and 150°C, the extraction pressure was kept constant at 150 bar during all experiments.

2.3.2 Supercritical fluid extraction (SFE). Solid samples were held between frits in stainless-steel extraction cell, placed into a thermostated heating tube. During the static mode, the extraction cell was filled up with extracting solvent and left until equilibrium was established. All this time, the restrictor maintained the pressure inside the cell. As soon as the set period was over, the extract was discharged into a collection vial with acetonitrile.

A small amount (1.5 g) of soil was taken for each extraction. Extraction experiments were performed using either pure CO_2 or modified CO_2 as an extractant. The following modifiers of CO_2 were tested: toluene, water, and water–acetonitrile mixture (1 : 1, v/v). If modifier was used, 100 μL or 100 μL + 100 μL (in the case of mixed modifier) was applied immediately with a spiking solution. Extraction experiments were carried out in (a) one and in (b) two 20-min steps in the static mode. The extracts were collected in vials with acetonitrile of a given amount and subsequently analysed. The final volume of extracts ranged from 2 to 5 cm^3.

2.3.3 Soxhlet extraction. In the case of standard SOX, the soil sample was placed in a paper cartridge, and 50 g of spiked soil sample and 200 mL of extraction solvent were taken for each extraction. The final volume of extract was reduced using a vacuum evaporator and filled up to 5 cm^3 with the extractant or pure acetonitrile. The concentrated extract was filtered through a glass filter, and filter effluent was subsequently analysed. Different extraction times, 12, 15, 18, and 20 h, for each extraction mixture were used.

The basic principle of SOXW is the same as for SOX except that the extraction chamber of the apparatus is heated. The solubility of the analytes is increased by heating the condensed solvent in the extraction chamber. This dramatically reduces the duration of the entire extraction process.

Six grams of soil sample and 140 mL of extraction solvent were taken for each SOXW. First, the soil samples were extracted 40 min with warm solvent, followed by 20 min of cold solvent wash. Then, the extract thus obtained was concentrated under a stream of nitrogen to 1 mL for 20 min and analysed. Pure dichloromethane, methanol–acetonitrile (80 : 20, v/v), and acetone–hexane (50 : 50, v/v) mixtures were used as extraction solvents.

2.4 *Sample preparation*

Uncontaminated samples of parabrown soil from farmland in the suburb of Brno (South Moravia, Czech Republic) were taken from a depth of 20–30 cm, air-dried at 25°C for 14 days, and ground in a grinding mortar after removing any impurities

(sticks, grits, etc.). Then, the samples were passed through a 2-mm sieve and stored in a dark glass bottle in a cold store room. The particle-size distribution identified the soil textural class as a clay loam with the following composition: 40% clay, 32% sand, and 28% silt. The organic carbon content of 3% was determined using a Shimadzu TOC analyser 5000 A (solid sample module, combustion at 900°C and acidification at 200°C). The soil humidity was 2.42%, and the pH value of moisturized soil was 6.9. A double pore Hamilton sensor was used for pH measurement of 1 : 1 soil : water suspension.

All soil samples were fortified with a standard solution of PANHs dissolved in acetonitrile, in the concentration range of 1.5–37.0 mg/kg. Two kinds of spiked soil samples were prepared: freshly spiked samples and 48-h aged spiked samples. In the case of the first type, the solution was applied right into the samples before the extraction started, and 48-h contaminated samples were left in contact with the solution for 2 days in a dark room to ensure that the system would reach equilibrium.

The possible recovery interference between spiked samples and naturally contaminated soil matrix was eliminated. Blank extractions of the representative natural soil samples were performed before the extraction experiments of spiked samples started.

2.5 *HPLC analysis*

To implement the quantitative analysis of extracts containing PANHs, the separation conditions were optimized. Good resolution and optimal capacity factor values ranging between 0.5 and 10 were achieved under the following isocratic elution conditions: acetonitrile-phosphate buffer ($c = 5$ mmol/L; pH = 7.2) mobile phase, flow rate = 0.5 mL/min, and temperature = 30°C. Detection of PANHs was carried out at 231 nm. The column hold-up volume was determined as the retention volume of uracil. Each analysis was performed three times. Quantitative determination of PANHs was based on an absolute calibration curve method, and all calculations were performed via a linear least-squares method. Ten concentrations of each PANH ranging from 1 to 30 μg/mL were injected into the HPLC system. The calibration curve for each standard was obtained by plotting the concentrations against the areas. The correlation coefficients of calibration functions in the interval of linearity were higher than 0.999 for all tested PANHs. The detection limit was calculated as the minimum amount of standard that produced a detector signal three times the peak height-to-noise ratio.

3. Results and discussion

3.1 *Extraction experiments*

3.1.1 PSE extraction. In general, the semi-dynamic mode gave 2–10% higher extraction recovery values compared with the static mode. Similarly, the extraction temperature of 100°C provided a slightly higher average recovery than extraction under 150°C (by 2%). The total extraction time of 4 min appeared to be sufficient to reach the maximum extractability for all tested solvents towards target PANHs. The most efficient PSE extraction agents among those tested were mixtures of methanol

and acetonitrile for both freshly spiked and 48-h aged samples. Recoveries obtained for 48-h aged samples were about 7% lower than recoveries for freshly spiked samples in most cases. The most significant decrease was found for indole and 2-methylindole. The failure in extraction of 2-methylindole was observed for all the tested extraction solvents and mixtures applied on 48-h aged samples. As one can see in table 2, the recovery of 2-methylindole never exceeded 11%. The comparison of the extraction yield for freshly spiked and 48-h aged samples using methanol–acetonitrile (80 : 20, v/v) mixture is given in table 3. For freshly spiked samples, the extraction yield varies between 63% and 96%, with an average recovery of 86%. The results for 48-h aged samples showed 77% of the average recovery but with the variation within 9% and 93%.

The extraction recovery of 63% from freshly spiked samples was shown to be highly suitable for the given extraction conditions (the temperature and physical–chemical properties of the extraction mixture) in removing 2-methylindole from soil. Accordingly, the loss in extraction recovery, in the case of 48-h aged samples, should be caused not only by strong 'matrix interactions' but also by other effects. A selective and strong 'matrix interaction' for 2-methylindole is not very likely, considering the

Table 2. PSE extraction recovery (%) of PANHs in the semi-dynamic mode extracted with different methanol–acetonitrile ratios ($T = 100°C$, $p = 150$ bar).

	Percentage of methanol in methanol–acetonitrile extraction mixture			
	100	90	80	70
Quinoline	78	85	77	79
2-Methylquinoline	78	86	82	86
6-Methylquinoline	84	87	88	89
8-Methylquinoline	91	97	93	95
Acridine	59	74	73	77
Benzo[*h*]quinoline	81	85	83	85
Phenantridine	86	89	88	88
Indole	80	83	81	80
2-Methylindole	11	10	9	7
Carbazole	88	89	90	89
Average recovery	74	79	76	78

Table 3. Comparison of extraction recovery of PANHs using PSE for freshly spiked and 48-h aged soil samples.

	Percentage recovery freshly spiked soil	Percentage recovery 48-h aged soil
Quinoline	80	77
2-Methylquinoline	85	82
6-Methylquinoline	92	88
8-Methylquinoline	96	93
Acridine	77	74
Benzo[*h*]quinoline	87	83
Phenantridine	92	88
Indole	93	81
2-Methylindole	63	9
Carbazole	93	90
Average recovery	86	77

similar physical–chemical properties of all tested PANHs (table 1). A combination of the 'matrix interaction' with decomposition processes may be possible. The time dependence of the extractable portion for two most problematic analytes (indole and 2- methylindole) from spiked samples during 48 h is shown in figure 1.

Pure methanol with the average recovery of 74% seemed to be efficient for most of the PANHs. However, the small change in polarity between pure methanol and methanol–acetonitrile (90 : 10, v/v) mixture appeared to be more suitable for the majority of PANHs tested. Besides the slight positive effect on the PANHs average recovery (74% for pure methanol vs. 79% for above-mentioned mixture), one should notice the significance in the case of acridine extraction yield which increased from 59% up to 74% by 15%. At the same time, there was no effect on the extraction yield of 2-methylindole which remained lower than 11%. Similar results for three different methanol–acetonitrile mixtures (90 : 10, 80 : 20, and 70 : 30, v/v) with average recoveries of 76–79% were achieved (table 2). The most consistent results with the smallest difference between the lowest and highest recovery value (except for 2-methylindole) and an average value of 76% (85% except of 2-methylindole) were obtained using an 80 : 20 (v/v) methanol–acetonitrile mixture under the following conditions: semidynamic mode, $T = 100^{\circ}C$, $p = 150$ bar, four cycles each lasting 1 min.

Using pure acetonitrile as an extraction solvent, the extraction recovery ranged from 77 to 89%. The extraction recovery obtained with a mixture of methanol and hexane or heptane (95 : 5 v/v) was significantly lower (15 or 20%, respectively) than with methanol–acetonitrile mixtures.

A mixture of acetone and hexane in a volume ratio of 1 : 1 (v/v) was used as a frequently recommended solvent for the PSE extraction mixture of polar and non-polar compounds. In most cases, we obtained slightly lower values compared with

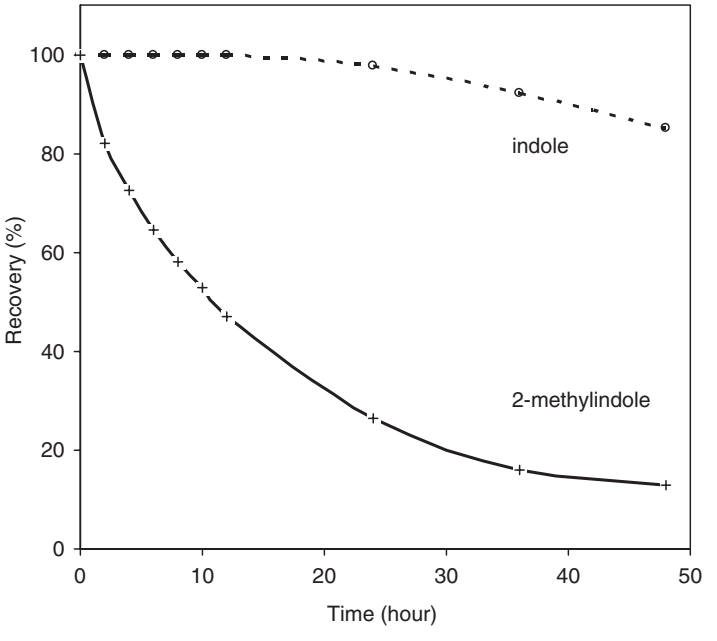


Figure 1. Decrease in amount of indole and 2-methylindole extracted from soil during 48 h.

values obtained using a methanol–acetonitrile (80 : 20, v/v) mixture, especially for carbazole, phenantridine, and 8-methylquinoline (70–90%). A significantly lower extraction recovery was obtained for acridine (56%) and 2-methylquinoline (64%). Moreover, the extraction mixture with acetone was limited in application when the above-mentioned RP-HPLC chromatographic conditions were used for analysis of extracts. Evaporation of acetone from the extract has to be done every time when compounds of interest elute in lower retention times than acetone zone does.

Unsatisfactory results were obtained using isopropanole as an extraction solvent. The PANH's recovery ranged, in the majority of analytes, between 60 and 80%, and the recovery of acridine and quinoline was lower than 30%.

Dichloromethane was applied as estimated the most recommended solvent for PANHs extraction from solid samples, but unusual results have been found. In contrast to a number of references presenting dichloromethane as a suitable extractant for a majority of organic pollutants including PANHs, we found dichloromethane ineffective. For the majority of PANHs investigated, the average extraction yield was below 12%. The only two exceptions were found within the 'carbazole' subgroup of PANHs: indole reached 48% and carbazole 60% extraction recovery.

3.1.2 Supercritical fluid extraction. The lowest extraction recovery within the range of 0 and 10% gave pure CO_2 as an extraction agent. Maximum recovery between 72 and 98% provided modified CO_2 with water and acetonitrile mixed in a ratio of 1 : 1 (v/v). Less recovery (60–89%) was reached with pure water as a modifier. Extraction recovery obtained with toluene as a CO_2 modifier ranged between 0 and 65%. These conditions were not suitable for quinoline, benzoquinoline, and acridine with an extraction yield below 3%. As one can see, the extractability of PANHs is strongly dependent on the presence and character of the CO_2 modifier. The character of the CO_2 modifier, affecting the polarity of the supercritical fluid, is a crucial factor for SFE of PANHs. SFE with two 20-min cycles was shown to be more efficient than the extraction process with one cycle.

3.1.3 Soxhlet extraction. Soxhlet warm extraction (SOXW) was chosen as an efficient modification of standard Soxhlet extraction. Two solvent mixtures (acetone–hexane 1 : 1, v/v and methanol–acetonitrile 80 : 20, v/v) and pure dichloromethane were applied and compared with results obtained from PSE in a separate experiment. An acetone–hexane mixture (1 : 1, v/v) is the recommended extractant for polar and non-polar compounds. Methanol–acetonitrile (80 : 20, v/v) is the most effective PSE extractant for PANHs resulting from this study, and pure dichloromethane is the most popular extractant according to references in the literature.

A lower extraction efficiency compared to PSE results was obtained for the majority of selected PANHs. However, in individual cases, one can obtain better results for SOXW using both solvent mixtures depending on the solvent mixture composition. This holds especially for 2-methylquinoline and acridine using an acetone–hexane (1 : 1, v/v) mixture and/or for phenantridine and carbazole using a methanol–acetonitrile mixture (80 : 20, v/v; see table 4).

The extraction recovery using dichloromethane was less than 5% for tested acridines. In the case of the 'carbazole' sub-group, recovery of carbazole reached 86%,

Table 4. Comparison of extraction recovery of PANHs using Soxhlet warm extraction and PSE with the mixtures of acetone–hexane (1 : 1, v/v) and/or methanol–acetonitrile (80 : 20, v/v).

	Acetone–hexane (1 : 1, v/v)		Methanol–acetonitrile (80 : 20, v/v)	
	SOXW	PSE	SOXW	PSE
Quinoline	69	68	69	78
2-Methylquinoline	73	64	76	88
6-Methylquinoline	62	79	87	87
8-Methylquinoline	87	90	79	93
Acridine	64	56	75	78
Benzo[*h*]quinoline	68	79	75	85
Phenantridine	72	86	99	88
Indole	n.i.[a]	n.i.	81	81
2-Methylindole	n.i.	n.i.	7	9
Carbazole	75	88	93	90
Average recovery	57	61	74	76

[a] n.i.: not identified.

indole 60%, 2-methylindole 12%, and phenantridine 5%. 6-Methylindole was not identified in the extract.

The SOX method showed a very small recovery in the same extraction environment compared with PSE and SOXW methods. Using the standard Soxhlet extraction method, we were able to remove a maximum of 62% of the amount of individual analytes added, during a 20-h extraction. Extraction for 12 h yields a recovery of 25–45%.

4. Conclusion

The polarity of the extraction agent affected by either the composition of extraction solvent or supercritical fluid proved to be a crucial factor for the extraction of PANHs. However, the extraction temperature and mode of the extraction process (especially in the case of PSE) also affect extraction efficiency.

As to extraction recovery, supercritical fluid-modified CO_2 with a mixture of acetonitrile and water (1 : 1, v/v) was, in general, the most efficient method for the majority of tested PANHs. However, the most consistent results have been found using PSE with a mixture of acetonitrile and methanol (80 : 20, v/v) as an extraction solvent. On the contrary, dichloromethane did not prove to be suitable for the extraction of selected PANHs. Neither result from PSE or from SOXW reached an average extraction recovery above 50% when dichloromethane was used. The physical–chemical properties of this solvent do not seem to fit a group of two- and three-ring PANHs that were selected for this study.

Three extraction methods with sufficient efficiency for nine from 10 tested PANHs can be recommended as the suitable base for use with real soil samples:

- PSE with an average extraction recovery of 76% (85% except 2-methylindole) and extraction time of about 15 min (semi-dynamic mode, $T = 100°C$, $p = 150$ bar, acetonitrile–methanol 80 : 20, v/v).

- Soxhlet warm method with an average extraction yield of 74% (82% except 2-methyl indole) and extraction time of 40 min + 20 min cold wash (acetonitrile–methanol 80 : 20, v/v).
- SFE with an extraction time of about 40 min and an average recovery of 85% (static mode, CO_2 modified by a mixture of acetonitrile–water 1 : 1, v/v).

SFE provided the highest average recovery of PANHs, but PSE requires the lowest extraction time when compared with other methods. The Soxhlet warm method is cheap, but PSE can be automated better for routine analysis of a large number of samples.

SOX is a time-consuming method with low recovery rates for target analytes, ranging between 0 and 62%. According to these results, we cannot recommend the SOX method as being suitable for the extraction of PANHs from soil samples.

None of the extraction conditions applied on 48-h aged soil samples were suitable for the extraction of 2-methylindole (the extraction recovery never exceeded 11%). This low extractability can be caused by a strong matrix interaction or by any decomposition processes. Information on degradation, transformation, and the fate of indolic class chemicals (including indole, 1-methylindole, 2-methylindole, and 3-methylindole) is extremely limited. Further studies are needed to help understand this better and to describe the fate of 2-methylindole in soil.

Acknowledgements

This work was carried out under the project of the Ministry of Education, Youth and Sports Czech Republic MSM 0021622412. The authors gratefully acknowledge Jiri Vejrosta for providing hardware devices for pressurized solvent extraction and supercritical fluid extraction. We would also like to thank Lea Lojkova, Pavel Karasek, and Anna Ciglerova, for their kind help during implementation of the experiments.

References

[1] H. Carlsson, C. Östman. *J. Chromatogr. A*, **715**, 31 (1995).
[2] H. Carlsson, C. Östman. *J. Chromatogr. A*, **790**, 73 (1997).
[3] L. Warzecha, B. Janoszka, C. Dobosz. *Chem. Anal. (Warsaw)*, **677**, 44 (1999).
[4] M. Wilhelm, G. Matuschek, A. Kettrup. *J. Chromatogr. A*, **878**, 171 (2000).
[5] H. Chen, M.R. Preston. *Environ. Sci. Technol.*, **32**, 577 (1998).
[6] S. Meyer, S. Cartellieri, H. Steinhart. *Anal. Chem.*, **71**, 4023 (1999).
[7] S. Nito, S. Ishizaki. *Chemosphere*, **35**, 1755 (1997).
[8] F.I. Onuska, K.A. Terry. *J. High Res. Chromatogr.*, **12**, 362 (1989).
[9] S.B. Hawthorne, C.B. Grabanski, E. Martin, D.J. Miller. *J. Chromatogr. A*, **892**, 421 (2000).
[10] J.R. Dean, G. Xiong. *Trends Anal. Chem.*, **16**, 553 (2000).
[11] S. Meyer, H. Steinhart. *Chemosphere*, **40**, 359 (2000).
[12] P.J. Osborne, M.R. Preston, H.-Y. Chen. *Anal. Chim. Acta*, **501**, 71 (2004).
[13] H.-Y. Che, M.R. Preston. *Atmos. Environ.*, **38**, 1023 (2004).
[14] H.-Y. Chen, M. Preston. *Anal. Chim. Acta*, **501**, 71 (2004).
[15] S. Nito, S. Ishizaki. *Chemosphere*, **35**, 1755 (1997).

[16] M.T. Galceran, M.J. Curto, L. Puignou, E. Moyano. *Anal. Chim. Acta*, **295**, 307 (1994).
[17] L. Rivera, M.J.C. Curto, P. Pais, M.T. Galceran, L. Puignou. *J. Chromatogr. A*, **731**, 85 (1996).
[18] K. Koci. Extrakce a stanoveni polycyklickych aromatickych dusikatych heterocyklu. Diploma thesis, Brno University of Technology (2001).
[19] Syracuse Research Corporation. Interactive PhysProp Database Demo. Available online at: http://www.syrres.com/esc/datalog.htm (accessed 22 May 2006).