Journal of Hazardous Materials B113 (2004) 237–245

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Application of solid-phase extraction to determination of polycyclic aromatic hydrocarbons in sewage sludge extracts

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Received 16 March 2004; received in revised form 21 June 2004; accepted 23 June 2004
Available online 31 July 2004

Abstract

The study presents the efficiency of sewage sludge sample clean-up with the application of SPE columns with various types of adsorbents. Six columns were tested: C8-octyl, C18 PolarPlus, C18-octadecyl, silicagel (SG), phenyl, cyano. The highest efficiency of recovery was observed for C18-octadecyl. Then, using C18, the method was optimised by changing the following parameters: eluent type and volume, column drying and effect of washing of cartridge.

Keywords: Polycyclic aromatic hydrocarbons; Solid-phase extraction; Sewage sludge; HPLC

1. Introduction

The most effective way of utilising sewage sludge is its application in agriculture. This method is the preferred method in the European Union countries [1]. Moreover, sewage sludge can be subjected to controlled waste landfill site, incineration and subsequent discharge to the coastal systems [1,2].

Sewage origin and method of its purification influences the content of various toxic substances, both organic (phenols, polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls (PCBs) [3–8] and inorganic (e.g. heavy metals) [5,9]. There is a serious danger that a lot of very harmful pollutants (carcinogenic or mutagenic) can be introduced to the soil while fertilising it with sewage sludge [10–12]. Such pollutants can then undergo various changes in the soil depending on their properties and soil conditions. According to literature [13], pollutants that are very common in sewage sludge belong to the PAH type and are classed as one of the most harmful organic pollutants due to their mutagenic and carcinogenic properties [14,15].

Soil contamination with PAHs is dangerous as they can get from the soil to plants and then to the further stages of the food chain [16]. It is believed that PAHs get to the human organism mainly with food products.

The cleaning-up of the extracts is a very important step in determination of PAHs in sewage sludge samples. One of the techniques that is widely applied for the purification purposes to get rid of any interferences, is solid-phase extraction (SPE). SPE is a sample treatment technique which passes a liquid sample through a sorbent. The above technique can fulfil two functions. In the first case, the analytes are eluted in a small volume of a solvent and so, the analytes are concentrated; in the second case, the function of the solid-phase extraction is to clean the sample. One of the benefits of using this method is that only small volumes of the solvent are required and the purification time is short. It also allows for a wide variety of extraction conditions which may be used to achieve the desired separation and pre-concentration [17].

C18-octadecyl, C8-octyl, silicagel and florisil are most frequently used for the PAH determination to clean the extracts. Some of the works by other authors [18–20] quote very good recovery in the case of combining traditional C18

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doi:10.1016/j.jhazmat.2004.06.015

columns with the columns with medium-polar filling such as: amino (NH_2), cyano and phenyl. Some authors [13,21] gives also information on the immunosorbents (IS) based on the antigen–antibody interactions.

In the studies on the PAH analysis used for the SPE purification [19–23], there are often contradicting opinions as to the choice of columns and evaluation of their usability. Selection of a purification procedure for specific columns was based on the studies by various researchers [21,23,24].

2. Experimental

2.1. Materials and reagents

Acetonitrile, dichloromethane (DCM), methanol, cyclohexane (CH), *n*-hexane, tetrahydrofuran (THF) were of the high performance liquid chromatography (HPLC) grade. The standard mixture of PAHs in acetonitrile was obtained from Promochem. The concentration of each analyte was 100 μg/mL. A standard mixture contained 16 EPA priority PAH pollutant: naphthalene (Na), acenaphthylene (Ace), acenaphthene (Ac), fluorene (Fl), phenanthrene (Phen), anthracene (Ant), fluoranthene (Fluo), pyrene (Pyr), benzo[*a*]anthracene (BaA), chrysene (Ch), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenzo[*ah*]anthracene (DahA), benzo[*ghi*]perylene (BghiP) and indenol[1,2,3-*cd*]pyrene (Ind).

2.2. Sample preparation

Samples of sewage sludge were obtained from three urban sludge treatment plants and were collected at the end of the treatment cycle. Prior to extraction, the sewage sludge samples were air-dried in dark at room temperature (1 week). Next, 1 g samples were transferred into Erlenmeyer flasks (250 mL). A characteristic of sewage sludge samples are presented in Table 1. All the treatment plants, from which the sewage sludge samples were collected, were characterized by a similar method of sewage treatment (mechanical–biological systems). Any differences between these plants result from the size of the town, viz. the number of inhabitants and share of industrial sewage. The treated water is discharged by a population varying between 30,000 inhabitants, except for R-200 that treat the water of a population of 230,000 inhabitants. The share of industrial sewage in the plants from which sludge B-40, R-200 and J-190 originated, were, respectively: 3% (sewage from sawmill, furniture and knitting, fruits processing industry), 10% (sewage from food, clothing and pharmaceutical industry) and 35% (sewage from chemical and fruit-vegetables processing industry). Moreover, sludge J-190 originated from a site in which the petrochemical industry has been in operation for years. This industry can significantly influence any increase in PAH content in various elements of the natural environment, including sewage sludge.

Table 1
Characteristic of sewage sludge used in study

Characteristic	B-40	R-200	J-190
Properties of sewage sludge			
pH [KCl]	10.2	7.8	6.01
Total N (g/kg)	17.8	45.7	39.8
TOC (g/kg)	35.4	41.7	39.8
CEC (mmol/kg)	500.4	339.8	453.2
TEB (mmol/kg)	547.7	410.1	499.7
Available forms (mg/kg)			
P	652	154	169
K	192	154	169
Mg	120	99	133

TOC: total organic carbon; CEC: cation exchange capacity; TEB: the total of the exchangeable bases.

2.3. Extraction procedure

The choice of extraction method was based on the earlier studies by the same authors [25]. The flask covered with aluminium foil to prevent photodegradation, was placed in an ultrasonic bath. The sample was extracted dichloromethane in the ultrasonic bath (160 W) (Sonic-3, Polsonic, Poland) for 90 min with an occasional swirl to prevent its sticking to the bottom of the flask. The extraction solution was centrifuged and the supernatant was evaporated to dryness by a rotary vacuum evaporator at 40 °C. After that, the residues were re-dissolved in 4 mL of acetonitrile–water mixture (1:1, v/v) when bonded silica cartridges were applied for cleaning-up of the extracts or in 4 mL of cyclohexane when the samples were cleaned-up on silicagel (SG).

2.4. Clean-up procedure

The extract of sewage sludge was spiked with 16 PAHs (US EPA). PAHs recovery was determined at three different concentration levels. The following amounts of the standard mixtures were added to the individual extracts: 10, 25, and 50 μg (in each analyte). The spiked quantities correspond to 10, 25 and 50 mg/kg of dry matter sewage. The following columns were chosen for the present studies: C8-octyl(end-capped, 3 mL, 500 mg) C18-octadecyl(end-capped, 3 mL, 500 mg) C18 PP (PolarPlus, octadecyl, non end-capped, 3 mL, 500 mg), silicagel (SG), 3 mL, 500 mg), phenyl (C_6H_5, 3 mL, 500 mg), cyano (3 mL, 500 mg).

The same procedures of conditioning, washing and PAH elution was applied for all the columns except SG. The above procedure was first tried on the standard mixture. The recovery was at a level of 92–100%.

Bonded silica cartridges were conditioned with 1 × 3 mL of methanol, followed by two times 3 mL of water:2 propanol (9:1). The 4 mL sample solution was loaded onto the SPE cartridge. The columns were then dried in light vacuum (water aspirator) for 5 min. Then they were washed with 1 × 3 mL of methanol:water (1:1, v/v). The SPE cartridges were vacuum

dried (water aspirator) for 5 min. Then, the PAHs were slowly eluted by the application of 2 × 1.5 mL of acetonitrile.

SG cartridges were conditioned with *n*-hexane (2 × 1 mL). Four millilitre of the sewage sludge extract in cyclohexane was added into the column. The column was washed with 2 mL of *n*-hexane. The PAHs were then eluted (1 mL/min) by applying a mixture of dichloromethane:*n*-hexane (1:1, v/v) (2 × 1.5 mL). The elute was collected, evaporated to near dryness by a rotary vacuum evaporator at 30 °C and dissolved in 2 mL of acetonitrile.

2.5. HPLC analysis

The reversed-phase, high performance liquid chromatography system consisted of a Spectra Series P100 pump (Thermo Separation Products) coupled with a Spectra Series UV100 detector (Thermo Separation Products) and a computer PC. For the separation of 16 PAHs, an analytical Spherisorb S5 PAH (250 × 4.6 mm i.d., 5 μg by Schambeck SFD GmbH, Germany) column with chemically bound C18 phase, was used. The column was installed in a thermostated oven at 31 °C (LCO 101, ECOM, Czech Republic). Acetonitrile and water (80:20, v/v) were used as eluent solvents at a flow rate of 1 mL/min (in isocratic conditions). Detection was carried out at 254 nm. Elution of all PAH was carried out by 60 min. Data acquisition and analysis was performed using the Clarity Lite Chromatographic Station (DataApex, Czech Republic).

Identification of PAH was accomplished by comparing retention times for standards and appropriate components identified in spiked and unspiked sewage sludge samples. The quantitative analysis of individual PAHs in the sewage sludge samples was carried out by comparing the peak areas of the individual PAHs with the peak areas of the PAHs in the standard mixture. Quantitative determination was performed using the external calibration curve method. Calibration was performed using standard solutions of PAHs in acetornitrile in the range 0.25–25 μg/L. The correlation coefficients of calibration functions (10 points) in the intervals of linearity were in the range 0.9982–0.9998 for individual PAHs. Precision was in the range of 2–6%. The detection limits calculated with a signal to noise ratio of three (IUPAC criterion), for 20 μL loop injection, were less than 0.05 μg/mL or 0.5 ng for all PAHs.

A method blank did not show reagent or equipment contamination with PAHs. All reported concentration of PAHs in sewage sludge are expressed on a dry-weight basis of soil (determined by drying the soils for 24 h at 105 °C) and are the average of triplicate extraction.

3. Result and discussion

Taking into consideration suggestions by various authors [13,17,19,21,26,27], process optimisation was based on the following criteria: choice of columns, selection of the eluent, effect of the eluent volume, column drying before PAH elution, effect of washing cartridge.

3.1. Choice of SPE cartridge

The application of columns with various fillings requires different analytical procedures which can have a bearing on the differences in the recovery of individual PAHs. This is especially true of SG fillings (solvent exchange), because it is necessary to exchange solvents. When selecting columns for comparative studies, we mainly considered how frequently they had been used by various researchers for PAH analysis in environmental matrices.

The tested columns are the ones most often used for the PAH determination in the soil samples, sediments, waters, etc. Opinions differ as to the efficiency of the individual types of filling. Kootstra et al. [23] obtained relatively poor results for the C18 columns (51–88%), and very good results for C8 (73–90%). Other authors reported similar, high results for the C18 columns [22]. Kootstra et al. [23] explained the above situation by the fact that the PAHs binding the C18 material was too strong for complete elution with a small amount of solvent. On the other hand, studies by Kiss et al. [21] and Fusheng et al. [22] pointed to a very high PAH recovery on the C18 column and advised its application.

The recovery of the individual PAHs when using columns with various types of adsorbents has been presented in Table 2. The highest level of recovery was observed on the columns filled with octadecyl-C18 (85%) and C18 PP (88%) (for the sum of PAH) (Table 2). Differences in the recovery levels of individual PAH were very small. A high recovery level on the C18 PP column (with a typical loading of 16.1% C, non end-capped), despite a lower contribution of carbon on the surface when compared to C18 (with a typical loading of 17% C, end-capped), is explained in literature [26,28] by a spherical orientation of the analyte (a flat PAH molecule) in a well-orientated surface structure of the stationary phase (C18 chains, especially those located in the pores).

Application of the remaining columns with bonded silica sorbents characterised by a moderate polarity – cyano solid-phase and phenyl solid-phase (C_6H_5) (a similar carbon content on the surface, i.e. 10.6 and 10.5%), yielded very poor results, i.e. 49 and 55%, respectively (for the sum of PAH) (Table 2). The recovery level when using the above columns was very close to the level reported by other authors [17]. Application of the C8 columns (with a typical loading of 14% C, end-capped), gave better results than in the case of cyano, however it was far from being satisfactory. The rule suggested by Kootstra et al. [23] was not confirmed. Most probably, higher recovery levels in this study resulted from the application of twice as much acetonitrile eluent (3 mL) as in the studies by Kootstra et al. [23].

When comparing results obtained by other authors [13,21,23], differences in the recovery of individual PAHs when using octadecyl sorbents, were noticed. In one of the studies [23], considerably higher recovery levels were

Table 2
Comparison of PAH recoveries for different SPE adsorbents

PAH	C8-2 mL		C8-3 mL		C18		C18 PP		SG		Cyano		Phenyl	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Naphthalene	55	16	54	13	99[a]	13	95[a]	10	49	13	30[a]	11	49	11
Acenaphthalene	74	12	71	9	104[a]	6	91[a]	9	55	11	45[a]	3	56	20
Acenaphtene	65	9	69[a]	9	64	6	66	6	40	13	56	12	61	9
Fluorene	49[a]	5	63	6	93[a]	7	96[a]	6	66	9	59	7	58	21
Phenanthrene	50[a]	6	55[a]	4	72	9	78[a]	7	71	10	64	11	61	10
Anthracene	68	3	75	4	82[a]	4	87[a]	9	66	9	50	11	53	16
Fluoranthene	48	3	66	3	81	4	95[a]	11	69	11	44[a]	16	49[a]	16
Pyrene	54[a]	4	69	3	90[a]	5	90[a]	5	70	8	57	20	55	19
Benz[*a*]anthracene	54	4	63	3	106[a]	5	99[a]	3	95	7	39[a]	18	43[a]	22
Chrysene	71	6	80	3	96	5	99[a]	4	102[a]	4	51[a]	12	59[a]	16
Benzo[*b*]fluoranthene	89	3	96	4	91	8	101[a]	4	90	5	46[a]	13	46[a]	8
Benzo[*k*]fluoranthene	65	3	73	5	87	9	88[a]	3	95[a]	5	61[a]	16	64	13
Benzo[*a*]pyrene	70	4	82	5	77	9	86[a]	3	79	5	40[a]	9	54[a]	19
Dibenz[*a,h*]anthracene	66	5	72	4	72	4	81	6	101[a]	6	45[a]	21	60	23
Benzo[*ghi*]perylene	49[a]	3	69	4	69	6	74	7	88[a]	6	48[a]	19	59	31
Indeno[1,2,3-*cd*]pyrene	55	3	62	3	81[a]	3	86[a]	6	81[a]	6	53[a]	23	55	27
Mean value of 16 PAH	61	6	70	5	85[a]	6	88[a]	6	76	8	49[a]	14	55	18

Relative standard deviation (R.S.D.) for $n = 9$.
[a] 95% confidence interval of the difference (Student *t*-test).

obtained for the 2–3-ring PAHs, and low recovery levels for the 4–6-ring PAHs. In the present studies, recovery of most PAHs was similar. Lower recovery values were only observed for acenaphthene, phenanthrene and benzo[*ghi*]perylene (Table 2).

Silicagel-adsorption phases require a non-polar solvent as an eluent. Further quantitative and qualitative PAH determination by the HPLC technique require higher polarity levels of the above solvent (achieved by evaporation) which in consequence results in losses in the recovery of volatile PAHs which can be clearly seen in Table 2. The application of the SG cartridges gave poor results in the PAHs recovery (naphthalene, acenaphthalene, acenaphthene) and despite high recovery levels of the remaining PAHs, was not satisfactory (Table 2).

Basing on the results obtained in this study (Table 2), the C18 column (which allowed for the highest recovery of the PAHs), was chosen for further studies on the optimisation of the SPE process.

3.2. Selection of the eluent

The solvent used for the PAH elution should be strong enough for the analyte elution leaving strongly bound pollutant. Literature reports [13,19,20,21,23] application of various eluents such as: acetonitrile, THF, acetone, methanol, 2-propanol and various mixtures of the above solvents. A well chosen solvent guarantees a significant increase of the recovery level. Kootstra et al. [23] reported an average increase of recovery by 20% when acetonitrile was changed for THF (C8). Kiss et al. [21] obtained very good recovery when using THF and dichloromethane. Similarly Kicinski et al. [19] obtained high recovery levels of 90% when using dichloromethane. Taking the above studies into consideration when comparing recovery levels with various eluents, the THF and acetonitrile were chosen for further studies.

Fig. 1 presents recovery of the individual PAHs when using THF and acetonitrile. As has been mentioned above, the solvents with low polarity are disadvantageous for the reversed-phase HPLC separation [17]. Their complete evaporation and dissolution in an appropriate solvent is required before they can be introduced into the chromatographic column. Such an analytic procedure results in considerable losses in the recovery of volatile PAHs [29]. It was observed both in the case of the silicagel columns (dichloromethane) (Table 2). Losses resulting from the solvent evaporation can be minimised under a gentle nitrogen flow. In our studies, water aspirator was used which influenced losses in the volatile PAHs (Table 2). Moreover, toxic characteristics of THF can limit its application. According to the results obtained, we should especially recommend acetonitrile. This facilitates better low molecular weight PAH recovery. THF ensures slightly higher recoveries of 5 and 6-ring PAHs in comparison to acetonitrile, but unfortunately THF is very toxic. Moreover, the application of THF requires the evaporation and dissolving

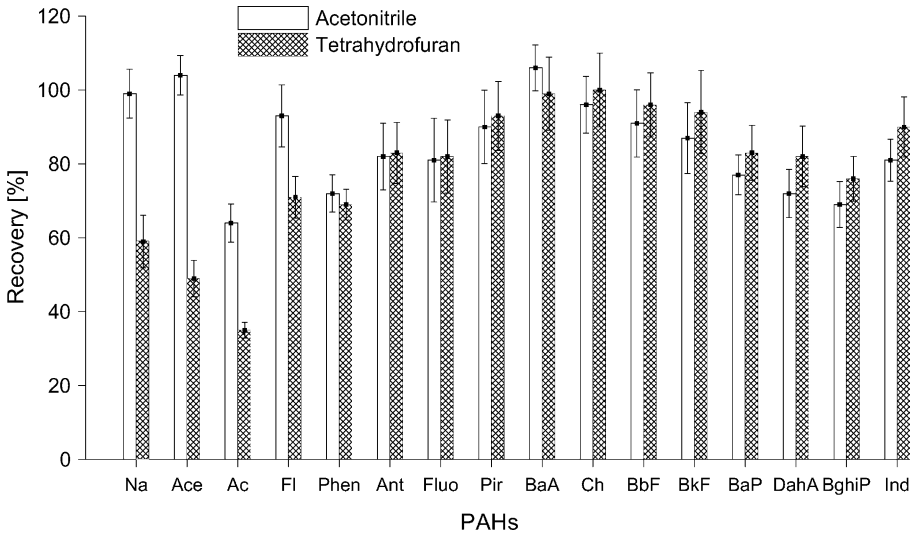

Fig. 1. The effect of the solvent on the recovery of PAHs from SPE cartridges (C18-octadecyl).

of the remains while still in motion before analysis with a chromatograph.

3.3. *Effect of drying cartridge*

Kiss et al. [21] drew attention to yet another problem of the influence of column drying after sample application. Baker [31] also advised to dry the sample both after it had been put in the SPE cartridge and rinsed. In the Kiss's work [21], the influence of drying by nitrogen flow or water aspirator, immediately after sample insertion, was tested. The recoveries obtained were then by 10–15% higher than without drying. Some of the authors [32] reported that application of light vacuum for complete column drying, can result in considerable losses in the recovery of volatile PAHs. However, there was no significant difference in the recovery of naphthalene and acenapthene when drying was applied for 4–5 min [21,32]. The above was confirmed by the present study (Table 3) in which complete column drying (after rinsing with water aspirator) resulted in losses in the recovery of volatile PAHs (10–20%), whereas a short drying for a 5 min increases the recovery level by 10% on average. It is interesting to notice that a complete column drying increased the recovery by 8 and 10%, respectively, in the case of benzo[*ah*]anthracene and benzo[*ghi*]perylene. Drying of the SPE cartridge after elution (methanol:water) is an important aspect when an apolar solvent is used for elution. Water should be removed from the cartridge before elution because if the cartridge is

Table 3
Effect of method of C18-octadecyl cartridge drying on the PAHs recovery

PAH	Without drying		Drying 5 min		Drying to dryness[a]	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Naphthalene	85	9	95	6	69	9
Acenaphthalene	80	6	91	8	73	9
Acenaphtene	61	7	66	7	58	9
Fluorene	84	6	96	6	91	8
Phenanthrene	69	6	78	6	77	8
Anthracene	77	5	87	6	87	6
Fluoranthene	88	3	95	4	95	8
Pyrene	85	3	90	5	91	5
Benz[*a*]anthracene	89	7	99	5	101	5
Chrysene	90	7	99	4	99	6
Benzo[*b*]fluoranthene	93	7	101	4	99	4
Benzo[*k*]fluoranthene	81	8	88	6	90	4
Benzo[*a*]pyrene	79	4	86	5	89	6
Dibenz[*a*,*h*]anthracene	75	4	81	5	90	3
Benzo[*ghi*]perylene	65	5	74	6	84	4
Indeno[1,2,3-*cd*]pyrene	77	6	86	7	92	4
Mean value of 16 PAHs	80	6	88	6	87	6

Relative standard deviation (R.S.D.) for $n = 9$.

[a] The cartridge was dried until a solid mass was obtained.

Table 4
Effect of solvent (methanol:water) ratio on recovery PAHs

PAH	40:60		50:50		55:45	
	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)	Recovery (%)	R.S.D. (%)
Naphthalene	96	11	94	9	87	16
Acenaphthalene	96	9	95	12	89	11
Acenaphtene	69	6	67	8	63	11
Fluorene	93	6	92	8	87	13
Phenanthrene	73	10	71	5	69	9
Anthracene	86	11	85	5	83	9
Fluoranthene	81	8	81	5	80	10
Pyrene	91	8	90	6	88	7
Benz[*a*]anthracene	100	8	101	7	99	5
Chrysene	96	4	96	6	96	5
Benzo[*b*]fluoranthene	90	4	92	5	93	6
Benzo[*k*]fluoranthene	87	6	88	3	90	3
Benzo[*a*]pyrene	75	9	77	3	77	7
Dibenz[*a*,*h*]anthracene	76	3	74	4	75	6
Benzo[*ghi*]perylene	72	3	71	4	73	4
Indeno[1,2,3-*cd*]pyrene	84	3	82	5	81	4
Mean value of 16 PAHs	85	7	85	6	83	8

Relative standard deviation (R.S.D.) for $n = 9$.

dried, the organic solvent is forced through the gravity, thus enabling complete elution of PAHs from all the pores of the stationary phase. However, if drying is neglected, the pores are filled with water and the eluting solvent cannot or can only slowly, penetrate into the pores because of miscibility and/or viscosity reasons [21].

3.4. Effect of washing of SPE columns

Column clean-up with an appropriate mixture (e.g. methanol:water) is to rinse interfering contaminants that may disturb the course of quantitative and qualitative evaluation. It is important especially in the case of the 2–4-ring PAHs. Some authors [33] found identification of these PAHs difficult in a rich matrix such as sewage sludge (e.g. naphthalene and phenanthrene). Taking into consideration the multitude of contaminants in sewage sludge, the stage of rinsing is worth further elaboration. In the present study, washing with a mixture of methanol:water in three ratios (v/v), i.e. 50:50 (as advised by Baker [31], 60:40, and 65:35 was applied. The results obtained (Table 5) showed that the optimum concentration (methanol:water) was (50:50, v/v). An increase in the methanol concentration together with eluotropic strength, resulted in losses in the recovery levels of some PAHs as expected (Table 4).

3.5. Effect of the eluent volume

Studies by various authors [23,27,30] showed that the eluent amount that guarantees the optimum recovery is 1–3 mL, which has been confirmed in the present study. When the amount of eluent is increased from 2 to 3 mL, an increase in

Table 5
The recovery, limit of determination (LOD), linearity (regression coefficent) and precision (R.S.D.) for the determination of 16 PAHs in sewage sludge sample (B-40)

PAHs	LOD (mg/kg)	Regression coefficient	Recovery (%)	R.S.D. (%)
Naphthalene	3.0	0.998	63	14
Acenaphthalene	0.3	0.998	81	10
Acenaphtene	0.3	0.998	79	11
Fluorene	0.5	0.998	82	12
Phenanthrene	0.1	0.999	95	8
Anthracene	0.1	0.999	97	11
Fluoranthene	0.5	0.998	101	12
Pyrene	0.5	0.999	94	8
Benz[*a*]anthracene	0.08	0.999	91	9
Chrysene	0.3	0.998	96	11
Benzo[*b*]fluoranthene	0.1	0.998	92	7
Benzo[*k*]fluoranthene	0.09	0.998	89	6
Benzo[*a*]pyrene	0.3	0.999	95	7
Dibenz[*a*,*h*]anthracene	0.6	0.998	91	9
Benzo[*ghi*]perylene	0.07	0.999	88	5
Indeno[1,2,3-*cd*]pyrene	0.1	0.999	87	6

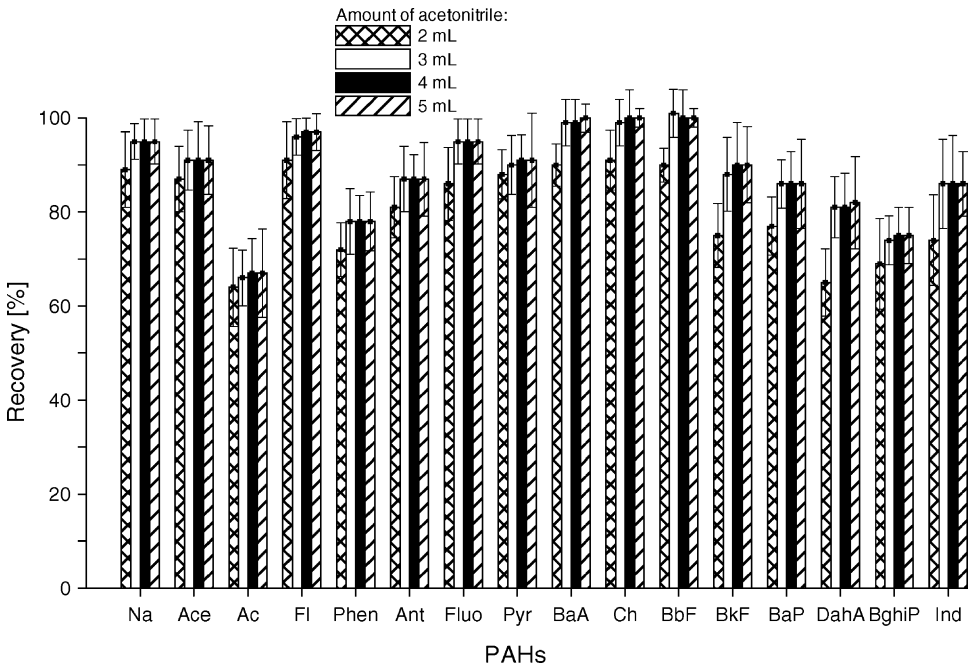

Fig. 2. Effect of solvent (acetonitrile) volume on the recovery of PAHs form C18-octadecyl cartridge.

the recovery of some PAHs by as much as about 10% was observed (Fig. 2). Further increase (to 4 and 5 mL) resulted in a slight increase (by 1–2%) in the case of PAHs with high molecular weight.

3.6. Quality control

The precision (R.S.D.), linearity and limit of determination obtained with the method described above, were verified by a calibration procedure. The calibration was performed using low polluted sewage sludge sample (B-40) with PAHs at six concentration levels in the range 50–600 μg/kg of single PAH. The sample was analyzed and the results were calculated. The results of the analysis are shown in Table 5. After substraction of the amount of sample derived PAHs, the coefficient of variation (according to ISO 8466-1) and recovery (expressed as mean of single recoveries from each concentration) were calculated (Table 5). The curves were linear in

Table 6
Concentration of 16 PAHs in sewage sludge samples form municipal sewage treatment plants

PAHs	Sewage sludge					
	B-40		R-200		J-190	
	Concentration [μg/kg]	R.S.D. (%)	Concentration (μg/kg)	R.S.D. (%)	Concentration (μg/kg)	R.S.D. (%)
Naphthalene	n.d.	–	n.d.	–	n.d.	20
Acenaphthalene	n.d.	–	4510.3	18	7105.1	19
Acenaphtene	n.d.	–	n.d.	14	n.d.	–
Fluorene	n.d.	–	n.d.	16	974.2	18
Phenanthrene	547.6	22	1052.2	15	1149.2	17
Anthracene	153.1	18	425.5	19	317.6	9
Fluoranthene	1470.1	19	2827.2	21	5399.9	15
Pyrene	1057.5	15	n.d.	–	5050.5	10
Benz[*a*]anthracene	818.2	19	613.2	12	2579.3	12
Chrysene	281.7	14	414.4	16	1869.1	14
Benzo[*b*]fluoranthene	724.5	17	3512.3	18	7572.5	16
Benzo[*k*]fluoranthene	n.d.	–	n.d.	–	n.d.	–
Benzo[*a*]pyrene	430.4	13	370.1	17	1786.0	21
Dibenz[*a*,*h*]anthracene	n.d.	–	n.d.	–	405.7	15
Benzo[*ghi*]perylene	83.2	18	260.9	19	835.1	9
Indeno[1,2,3-*cd*]pyrene	188.2	17	315.6	13	1395.6	12
Sum of 16 PAHs	5754.5	17.2	14301.7	16.5	36439.8	15

±: Relative standard deviation (R.S.D.) for $n = 3$; n.d.: not detected.

the range studied and regression coefficients were >0.998 for all PAHs studied. Limit of determination (Table 5) was comparable to data presented by other authors [33]. Recoveries of the analysis of PAH in sewage sludge were between 81 and 101% (with the exception of naphthalene) and the relative standard deviation was between 5 and 14 (Table 5). The higher volatility of naphthalene compound caused poorer recovery and precision.

3.7. Total PAH concentration in sewage sludges

The method presented in this article had been used to measure 16 PAHs in the samples of sludge from three Polish municipal waste treatment plants. The results obtained are presented in Table 6 and Fig. 3. The chromatograms contained many different peaks (Fig. 3), but only the 16 PAHs of the US-EPA list were specifically quantified. Comparison of UV absorbance spectra with standards was used to confirm identity and purity of solutes. The results obtained are higher or near the data reported by other authors [3,5,6,11]. PAH content was clearly depended on amount of processed sewage, as well as on its character. High content of PAH in sewage sludge J-190, without any doubt resulted from presence of refinery operating in this area. However, neither in sludge J-190 nor in the remaining sludges, allowed by European Union limits [33] for fluoranthene (5 mg/kg), benzo[*a*]pyrene (2.5 mg/kg) and benzo[*b*]fluoranthene (2 mg/kg) were not exceeded. In the Australian norms the maximum concentration of 6 PAHs (Fluo, BbF, BkF, BaP, BghiP, Ind) cannot exceed 9.7 mg/kg of dry sludge. In this case investigated sewage sludges (beside J-190) remained within required limits (Table 6).

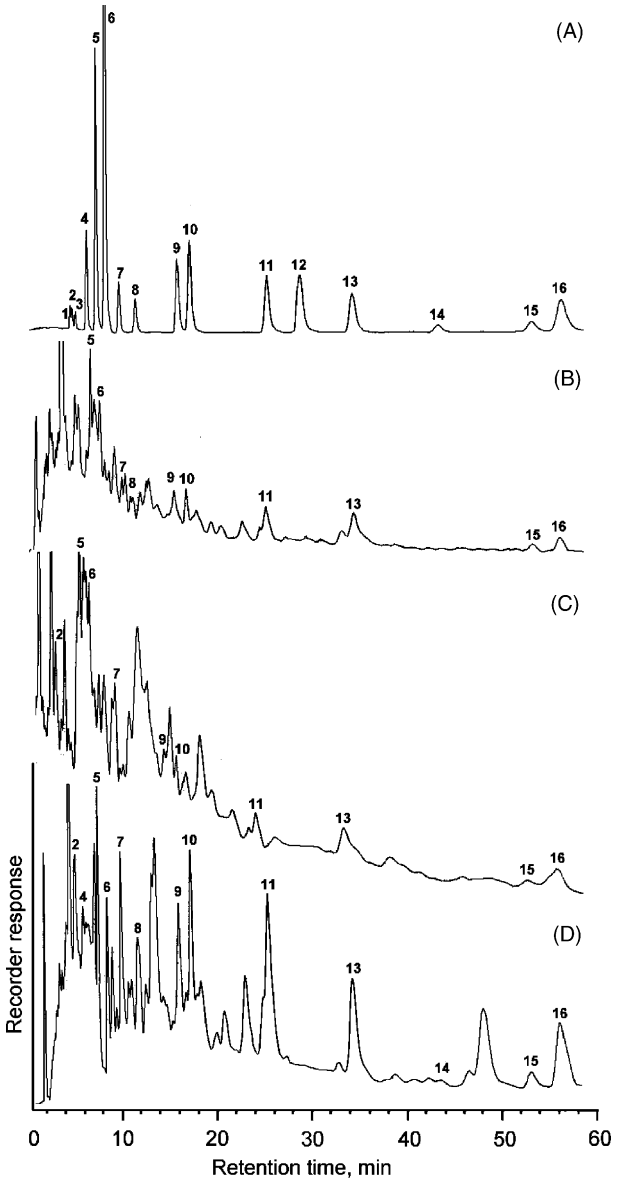

Fig. 3. HPLC–UV chromatograms of standard mixture (A) and real sample sewage sludges (B–D). (A) Standard mixture, (B) B-40, (C) R–200, (D) J–190. SPE condition: dried 5 min, washed-off mixture methanol:water (1:1) and elueted with acetonitrile (3 mL).

4. Conclusions

The preliminary studies with the view of selecting an appropriate column for further optimisation processes showed that the highest recovery rate (88%) with a simultaneous high reproducibility (R.S.D. = 3–9%, only naphthalene = 13%) was achieved on the columns with octadecyl filling C18. Also on the C18 PP columns, reproducibility was high (R.S.D. = 3–11%).

Optimisation of the individual phases of the processes influenced mainly the changes in the recovery of low molecular weight (LMW) PAHs (2–4-ring). Recovery in the case of high molecular weight (HMW) PAHs (5–6-ring) was on a stable level. However, some cases (drying), an increase in the HMW PAHs was also observed. The conditions applied (evaporation to the dry state by means of an water aspirator) influenced the level of LMW PAH losses.

The problem of the purification of samples before chromatographic analysis is, at the moment, one of the most important stages of the analytical process; quite simply, the requirements for filling the columns are ever more stringent. In addition to the high degree of recovery, sample concentration and purification, attention is also paid to the possibility of using one type of filling for the concentration of the various pollutants during one specific, analytical process. Basically a comparison between different phase material has been published by other authors as well, though not expressively aimed at PAH analysis. Simultaneous solid-phase extraction of acidic, neutral and basic pharmaceuticals from aqueous samples performed by Weigel et al. [34]. The results obtained by the authors quoted show that the fillings used by them (styrene–methacrylate and styrene-*N*-vinylpyrrolidone co-polymers) can be applied to contaminants which differ significantly in their chemical structure during the course of a single extraction. Styrene–methacrylate fillings, then, are worth considering when studying other pollutants, for example PAHs and their derivatives.

Acknowledgements

Financial support from the State Committee for Scientific Research (KBN, Warsaw, Poland), project No. P06H 06220 is gratefully acknowledged. P. Oleszczuk is granted by the Foundation for Polish Science.

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