Improved Analytical Extraction and Clean-up Techniques for the Determination of PAHs in Contaminated Soil Samples

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ABSTRACT: Development and optimisation of fast, efficient, quantitative, economic and environmentally friendly analytical extraction techniques for the extraction clean up, and pre-concentration in the quantification of 16 USEPA priority polycyclic aromatic hydrocarbons (PAHs) in contaminated soils have been carried out. Three different extraction methods (Soxhlet, ultra sonication and mechanical shaking) were investigated on a low-level PAH soil certified reference material (CRM131-100) and the results were compared to determine the technique with the highest extraction efficiency. The clean up and pre-concentration procedures were optimized using both the conventional method (i.e. column packing with silica gel) as well as the solid phase extraction (SPE). Chromatographic conditions for the separation of PAHs using High Performance Liquid Chromatography (HPLC) using UV-DAD and fluorimetric detection with programmed excitation and emission wavelengths were also optimized. Six different extraction solvents: acetone, cyclohexane, 2- propanol, methanol, acetonitrile and dichloromethane, were tested to select the most suitable solvent for the extraction of the 16 PAHs from the certified soil reference material. Acetonitrile, dichloromethane and tetrahydrofuran were also tested as eluants for the optimisation of SPE clean up. The optimized ultrasonic extraction procedure utilizing four 15 minutes extraction cycles at 50 °C and SPE clean up with tetrahydrofuran: acetonitrile (1:1) and subsequent separation by gradient reversed phase HPLC with fluorimetric detection extracted the PAHs from the certified reference material with recoveries ranging from 63.6 % to over 100 % .

Key words: PAHs, soil, Solid phase extraction, HPLC, Ultra sonication

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the atmosphere and water sources throughout the world. It is practically impossible for the human population to avoid exposure to trace amounts of these substances in routine life. The major sources of PAHs in the environment arise from various forms of insufficient combustion; instead of carbon being completely oxidised to carbon dioxide, hydrocarbon fragments are generated which can interact with each other to yield complex polycyclic structures (Sherma, 1993; Owabor *et al.,* 2010). The mechanisms through which this process occurs are complex and are still subjects of intensive investigation and have attracted the attention of environmental chemists and toxicologists.

PAHs are a group of highly lipophilic chemicals that are present ubiquitously in the environment as pollutants. PAHs originate from both natural and anthropogenic sources. The anthropogenic sources include combustion and pyrolysis of fossil fuels or wood (pyrolytic) and from release of petroleum products (petrogenic) (Kowalewska and Konat, 1997). Natural sources of PAHs include forest fires, natural petroleum seeps and post-depositional transformation of biogenic precursors (Lopez-Avila *et al.*, 1995). Due to the low water solubility and hydrophobicity of PAHs, they rapidly become associated with inorganic and organic suspended particles and subsequently deposited in sediments. Thus analysis of sedimentary mixture of contaminants such as PAHs can be used for assessment and interpretation of the impact of these anthropogenic pollutants on the aquatic environment.

There are a wide variety of solvent extraction techniques commonly used for extracting hydrocarbons from soils and sediments. Traditional extraction methods include Soxhlet (USEPA, 1996 ; Lopez-Avila *et al.*, 1998 ; Luque de Castro and García-Ayuso, 1998), ultrasonication (Eicemann *et al.*, 1980 ; Luque-Garcia and Luque de Castro, 2003 ; Sun *et al.*, 1998) mechanical shaking (Berset *et al.*, 1999) and reflux

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with methanolic KOH (Wong and Williams, 1980). Modern techniques include soxtec (automated soxhlet) (Lopez-Avila *et al.*, 1993) supercritical fluid extraction (SFE) (Hawthorne *et al.*, 1994 ; Hawthorne and Grabanski, 2000), microwave-assisted extraction (MAE) (Chee *et al.*, 1996 ; Camel, 2001), pressurised hot water extraction (PHWE) (Andersson *et al.*, 2002 ; Juhani *et al.*, 2004) pressurised liquid extraction (PLE) or accelerated solvent extraction (ASE) (Richter *et al.*, 1996 ; Saim *et al.*, 1998). The requirements of any extraction technique are that it can produce valid data, rapidly, with minimum operator involvement, be cost effective and satisfy safety considerations for both the operator, and other personnel and its location within the operating environment (Dean, 1998). Each technique has its own merits and the choice of extraction depends on several factors including capital cost, operating cost, sample matrix, simplicity of operation, sample throughput and the availability of a standardized method (Banjoo and Nelson, 2005).

Various methods of extraction and analysis of PAH have been proposed and several studies have been carried out to compare the traditional extraction methods of extraction with those of modern techniques (Luque de Castro and García-Ayuso, 1998 ; Berset *et al.*, 1999 ; Budzinski *et al.*, 1999 ; Lundstedt, 2003). Soxhlet extraction has been the standard and preferred method for extracting semi-volatile and non-volatile organics from solid matrices and is also a recommended method by the US Environmental Protection Agency (USEPA) as it is an easily standardized technique with high recoveries, compared to matrix dependent techniques such as MAE, PLE and SFE (Luque de Castro and García-Ayuso, 1998 ; Ramos *et al.*, 2000). However, the procedure is tedious as the extraction time is long, (16 hours or more may be needed); large volumes of organic solvents are required and can degrade thermally labile compounds (Luque de Castro and García-Ayuso, 1998). The requirements for reducing both the time and amount of organic solvents needed for the extraction of organic pollutants from solid samples have led to the recent development of a variety of new extraction approaches (Vallejo-Pecharromán *et al.*, 2001).

In comparison, ultrasonication is an efficient technique, when compared to reflux methods for extracting trace organics from soils and sediments. Studies have shown that ultrasonic extraction techniques yield comparable or even greater quantities(Song *et al.*, 2002) of hydrocarbons than other techniques of extraction; although the method gave lower recoveries in some studies (Berset *et al.*, 1999). Depending on the contaminants and matrix, sonication can have the advantage of faster extraction

times. Optimisation of the ultrasonic extraction parameters, including solvent or solvent composition, extraction time, sample load, and water content are required many times for more efficient and reproducible extractions (Berset *et al.*, 1999). Ultrasonication techniques usually provide a relatively low cost method, using small volumes of organic solvent without the need of elaborate glassware and instrumentation.

Solid-phase extraction (SPE) is a sample treatment technique which passes a liquid sample through a sorbent. The analytes to be determined and/or the interferences of the samples are retained on the sorbent by different mechanism. The analytes in the sample are eluted in a small volume of a solvent and so, the analytes are cleaned up and concentrated (Marce and Borrul, 2000). Specifically, SPE aims at stripping the sample away from the analyte, putting the analyte into a small volume of a different solvent (Simpson, 2000). SPE is used alone or in combination with other techniques. For instance, for determining PAHs in solid samples such as soil, sludge, sediment or tissue, SPE has been used after Soxhlet extraction, ultrasonic extraction or accelerated solvent extraction (Berset *et al.*, 1999). Compared to the column chromatography clean up technique, SPE consumes fewer amounts of toxic solvents which is of a great advantage from the environmental sustainability standpoint as well as saving substantial amount of time.

The aim of this study was to use different extraction techniques and solvents for the determination of PAHs in low contaminated soils. The results were evaluated to study the efficiency of extraction method and solvent for optimal extraction. Furthermore sample purification was performed using the solid-phase extraction (SPE) clean-up technique employing different solvents as eluants for the optimisation of the SPE clean up and its influence with respect to the recovery of the PAHs was evaluated. Finally analytical determinations by HPLC and spectrofluorometric detection were applied in order to evaluate quantitation aspects.

MATERIALS & METHODS

A standard mixture of the USEPA 16 priority PAHs (2000μg/mL, dichloromethane: benzene): naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), Pyrene (Pyr), benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DaA), benzo[g,h,i]perylene (BgP) and indeno[123-cd]pyrene (IP) was obtained from SUPELCO, Bellefonte, PA, USA. Appropriate working dilutions of the standard solution with HPLC grade acetonitrile were made. All solvents used were of HPLC

grade and water was purified with a Millipore Milli-Q system.

A natural matrix soil certified reference material CRM131-100 (manufactured by R.T. Corporation Limited, Salisbury, United Kingdom) containing 15 PAHs with concentrations ranging from 0.744mg/kg to 6.36mg/kg was used to optimise the extraction procedures based on the recovery of the analytes as well as for quality control of each batch of extraction.

For each soil sample, 1 g of sample was accurately weighed into a pre-cleaned 25 mL amber glass bottle. To this was added 10 mL of extracting solvent (acetone, acetonitrile, 2-propanol, cyclohexane, methanol and dichloromethane) of HPLC grade. The bottle was sealed with a screw cap closure lined with a PTFE-faced silicone rubber septum facing the bottle contents. After sealing, the bottle was shaken vigorously to suspend the contents which were then sonicated in a high performance ultrasonic bath with microprocessor control for precision time and temperature controlled operation (Grant MXB14, Grant Instruments (Cambridge) Ltd, Cambridgeshire, UK) for 60 minutes at 50 ℃. During this period the sample bottle was occasionally inverted and shaken to continually re-suspend the sample. The extraction solution was later centrifuged and the supernatant decanted into an amber 4 mL vial (with a PTFE-faced screw cap closure). The vials were stored in the refrigerator until clean-up and analysis took place (Sun *et al.*, 1998 ; Banjoo and Nelson, 2005).

About 10 g of soil samples was weighed into Whatman extraction thimble that had been pre-extracted with dichloromethane (DCM). These were placed in a Soxhlet extractor and extracted with 250 mL DCM for 16 hours. The extract was carefully reduced to about 10 mL on a rotary evaporator and transferred into clean amber 4 mL vials and kept in the refrigerator for further clean up and analysis (Reimer and Suarez, 1995).

5 g of soil sample was weighed into conical flasks; 50 ml of the extracting solvent   was added and the flasks sealed with aluminium foil to prevent the loss of the solvent by evaporation. This was kept on an arm shaker at ambient temperature for a period of 2 hours. After the completion of the extraction, the solvent was centrifuged and the supernatant decanted. The volume of the extract was reduced by nitrogen blow down.

Several organic solvents ranging from moderately polar to non-polar solvents were tested for extraction of the PAHs from the sediment samples using ultra sonication method of extraction in order to compare their extraction efficiencies. The following solvents: acetone, acetonitrile, 2-propanol, cyclohexane, methanol and dichloromethane were tested for the optimal extraction solvent.

In contaminated soils and sediments, apart from the PAHs which are the target analytes, other components may interfere with the determination of the PAHs. In addition, the PAHs in the extraction solution from the soil cannot be determined directly because of their lower concentrations. As solid phase extraction (SPE) offers a faster, more cost-effective sample preparation method with dramatic time savings over many traditional liquid/liquid extraction techniques, 6 ml Supelco C_{18} SPE cartridges were used for the clean-up/preconcentration of the extracted PAHs.

SPE clean-up of the sediment extracts was carried out using a 12-port vacuum manifold from SUPELCO. The sorbents of 6 ml Supelco LC-18 SPE cartridge were conditioned with 5 ml of extracting solvent and 40 % of the extracting solvent in water respectively to prepare the SPE column for the clean up process. The extraction solution was loaded and aspirated through the cartridge under gentle vacuum at <2 ml/min flow rate. The cartridge was centrifuged for 15 min at 4500 rpm, and then eluted by 3 x 1 ml of eluting solvent at 1 ml/min flow rate (each time the sorbents were soaked for 10 minutes with the elution solution before the elution). The eluates were collected into a 3 ml volumetric flask and made up to be 3 ml using the elution solution. Great care was taken so that the surface of the sorbent in the cartridge was not dry during the conditioning and loading of the sample extract (Marce and Borrul, 2000).

Different solvents, cyclohexane, 2-propanol, methanol, acetonitrile, dichloromethane and acetone, were tested to select the best solvent for the SPE clean-up after the extraction of the 16 PAHs from the soil. In the case of cyclohexane – a non polar solvent, the procedure of the extraction was the same as that described above except that the extraction solution was evaporated to near dryness by a gentle stream of nitrogen gas, and the residues were dissolved in 10 ml of 40 % methanol in water. 1 ml of acetonitrile was added to the extraction solution, and the subsequent steps were the same as that of cyclohexane when dichloromethane was used as the extraction solvent; water was added to the extraction solution to make the solution contain 40 % 2-propanol, methanol, acetonitrile and acetone, respectively, when 2-propanol, methanol, acetonitrile and acetone were tested as the extraction solvent. After the extraction, the SPE procedure was the same as that described above, except that the sorbents of the SPE cartridge were conditioned by 5 ml of the tested solvent and 40 % of the tested solvent in water, respectively.

PAHs were analysed with Agilent 1200 Series LC system with a programmable wavelength diode array detector, and a Jasco FP-1520 intelligent fluorescence detector. Separation of the 16 PAHs was performed on a monomeric type octadecyl silica column, SUPELCOSIL LC PAH 2cm x 4.6mm i.d containing 5μm particles and a guard column Supelguard LC-18, 2μm. Baseline separation of the PAHs is achieved within 40 minutes by gradient programming the eluent i.e. increasing its eluotropic strength with time according to a defined programme. Far-UV HPLC gradient grade acetonitrile (Fisher Scientific, Loughborough, UK) with a high eluotropic strength and HPLC grade water (Milli-Q) with low eluotropic strength are pumped by a gradient programme as the mobile phase. All data for quantification of the PAHs were obtained by applying the mobile phase gradient shown in Table 1 at a flow rate of 1.5 ml/min and at a controlled oven temperature of 25 °C.

Table 1. Mobile phase gradient programme

Time (min)	Acetonitrile (%)	Water (%)
0	40	60
5	55	45
10	60	40
15	70	30
20	80	20
25	90	10
30	100	0
40	100	0

The peaks were identified based on their retention times, and the appropriate times for wavelength switching for each PAH were optimised for different responses by changing the gain settings of the photomultiplier of the detector, to obtain the highest sensitivity. The optimal wavelengths for excitation and emission were found by peak scanning. Acenaphthylene could only be detected by the UV as it does not fluoresce and was not detected by the fluorescence detector (Table 2).

The quantification of the PAHs was by an external standard method, which relies on the reproducibility of the standard preparation. Different concentrations of PAH standards were prepared to check for linearity of the external calibration. The concentrations ranged from 0.001ppm to 5.0ppm. PAH standards that had lower sensitivities to the fluorescence detector were calibrated with higher concentrations while those that have very high sensitivities were calibrated at very low concentrations. Due to higher response from the fluorescence detector, the linearity was evaluated at different concentrations using the peak areas. They showed good linearity because the values of the regression coefficients ranged from 0.9974 to 0999 for all the PAHs.

Quality control was achieved by subjecting a PAH certified reference material (CRM 131-100) to the procedure used for the total PAH determination in the sediment samples and analysing it by the same method as was used for the samples. The average concentrations of the PAHs in the CRM had recoveries greater than 80% for all the PAHs, except for Ace and Anth with about 65% recovery and IP whose certified level was below the detection limit. (Table 3)

RESULTS & DISCUSSION

Three different extraction methods (Soxhlet, ultrasonication and mechanical shaking) were investigated on the certified reference material and the results were compared to determine the method with the highest extraction efficiency. The ratios of the ultrasonication method to the Soxhlet and mechanical shaking method for the extraction of the 15 PAHs from the soil certified reference material are shown in Fig. 2. The extraction efficiencies for most of the PAHs in the CRM by the sonication method were higher than Soxhlet, except for benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (a) pyrene and dibenzo(a,h) anthracene. Ultrasonication consumed less time and solvent (<1hour; 10 ml) than that the Soxhlet (16 hours; 250 ml). Mechanical shaking gave the worst recovery for all the PAHs. Therefore, ultrasonication extraction was used throughout this work.

This study has shown that ultrasonication is a more efficient technique, when compared to the other traditional methods for extracting trace organics from soils and sediments. Indeed this is in congruity with earlier studies that have shown that ultrasonic extraction yields comparable or even greater quantities (Garcia *et al.*, 1992 ; Sun *et al.*, 1998 ; Song *et al.*, 2002) of hydrocarbons than other techniques of extraction, although ultrasonication gave lower recoveries in some other studies (Lopez-Avila *et al.*, 1995 ; Berset *et al.*, 1999). The reproducibility obtained with ultrasonic extraction was higher (Banjoo and Nelson, 2005) or lower than those from Soxhlet extraction (Berset *et al.,* 1999).

Losses in PAHs in Soxhlet extraction was attributable to the use of high temperatures which resulted in losses of hydrocarbons due to volatilization and/or oxidation of highly volatile and thermally labile species. Although methods based on Soxhlet extraction

Table 2. Programme of excitation and emission wavelength pairs

Detected compound	Time (min)	Excitation wavelength (nm)	Emission wavelength (nm)	Gain setting
Nap, Ace, Flu, Phe	0	260	340	1000
Ant, Flt, Pyr	15	260	400	100
BaA, Chr	22	270	380	100
BbF, BkF, BaP	28	280	430	100
DaA, BgP	33	290	415	100
IP	36	300	500	1000

Fig. 1. HPLC fluorescence chromatogram of a PAH standard mix made up in acetonitrile.
Peaks: 1(naphthalene), 2(1-methylnaphthalene), 3(2-methylnaphthalene), 4 (acenaphthene), 5(fluorene), 6(phenanthrene), 7(anthracene), 8(fluoranthene), 9(pyrene), 10(benzo[a]anthracene), 11(chrysene), 12(benzo[b]fluoranthene), 13(benzo[k]fluoranthene), 14(benzo[a]pyrene), 15(dibenzo[a,h]anthracene, 16(benzo[g,h,i]perylene and 17(indeno[1,2,3-cd]pyrene

Table 3. Recoveries of PAHs in CRM 131-100

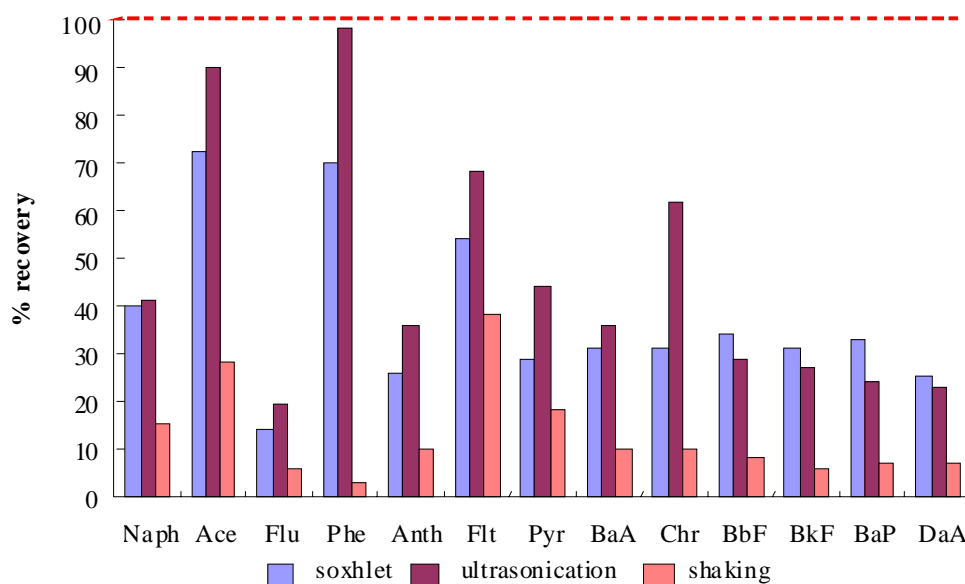| PAHs	Certified. I	Reference (µg/g) II	Material III	Average Concentration (µg/g)	Certified Level (µg/g)	% Recovery
Naph	3.61	3.72	3.58	3.64 ± 0.07	3.51 ± 0.43	103.60
Ace	1.48	1.64	1.76	1.63 ± 0.14	2.35 ± 0.28	69.24
Flu	5.25	5.49	5.38	5.37 ± 0.12	6.16 ± 0.70	87.24
Phe	3.46	3.40	3.19	3.35 ± 0.14	2.80 ± 0.24	119.71
Anth	2.70	2.84	2.93	2.82 ± 0.11	4.35 ± 0.33	64.90
Flt	2.19	2.12	2.17	2.16 ± 0.04	2.17 ± 0.22	99.55
Pyr	2.12	2.13	2.15	2.13 ± 0.01	2.30 ± 0.25	92.78
BaA	6.00	6.25	5.98	6.08 ± 0.15	5.16 ± 0.63	117.79
Chr	1.79	1.87	1.90	1.85 ± 0.06	2.02 ± 0.19	91.76
BbF	2.01	2.09	2.03	2.04 ± 0.04	2.10 ± 0.28	97.30
BkF	1.25	1.31	1.36	1.31 ± 0.06	1.39 ± 0.16	93.95
BaP	4.63	4.85	4.92	4.80 ± 0.15	5.16 ± 0.65	93.06
DaA	3.25	3.34	3.48	3.36 ± 0.12	3.57 ± 0.48	94.02
BgP	nd	nd	nd	nd	not certified	-
IP	nd	nd	nd	nd	0.77 ± 0.16	-

Fig. 2. Percentage recoveries of PAHs using different methods of extraction

have traditionally been used as references to assess the performance of methods based on other extraction methods such as that of ultrasonication. The result from this comparative analysis highlights the main advantages of ultrasound-assisted extraction over the conventional Soxhlet extraction. The extraction efficiency of ultrasonication was increased because cavitation increases the polarity of the system, including extractants, analytes and matrix. This can be similar to or greater than that of conventional Soxhlet extraction (Beard *et al.*, 1992). Also, ultrasound-assisted methods of extraction allows the addition of a co-extractant to increase further the polarity of the liquid phase (Luque-Garcia and Luque de Castro, 2003) and further allows the leaching of thermolabile analytes, which are altered under the working conditions of Soxhlet extraction. Obviously, the operating time of ultrasonication method was shorter than with Soxhlet extraction.

In this study, six different extraction solvents; acetone, cyclohexane, 2- propanol, methanol, acetonitrile and dichloromethane, were tested to select a solvent for the extraction of the 16 PAHs from the certified soil reference material. Large differences in the extraction steps and the solvents used for the extraction of PAHs from soil samples were observed. Acetone, cyclohexane, hexane, dichloromethane, toluene or mixtures of these solvents have been used for the extraction of PAHs from soil or sediment samples and differences have been observed in their extraction efficiencies. In most published works, the volatile PAHs with 2 or 3 aromatic rings, such as

naphthalene, acenaphthene, etc., were lost more because the solvent used for the extraction and the eluate in the SPE step was evaporated to almost dryness using a vacuum rotary evaporator or a gentle stream of nitrogen gas. Fig. 3 shows the recovery of PAHs in CRM using six different extraction solvents. The results presented in Fig.3 showed that the highest extraction efficiencies for most of the 15 PAHs were obtained by using methanol, except for naphthalene. The orders of the extraction efficiencies for most of the 15 PAHs from the certified reference material were as follows: Methanol > Acetonitrile > 2-propanol > Acetone > Dichloromethane >Cyclohexane.

In order to avoid loss of the more volatile PAHs and to increase the extraction efficiency, a selection of solvents to optimize the procedure of extraction and clean-up/ preconcentration of the 16 PAHs without the step for the evaporation of solvent is usually indispensable. However, since acetonitrile: water was used as the mobile phase, the solvent which can be miscible with the mobile phase was the best for the final injection solution. The first four solvents fit into this. Methanol was therefore selected as the extraction solvent because it could mix well with the mobile phase. This resulted in hardly any loss of the easily volatile PAHs because the step for the evaporation of the solvent to almost dryness was omitted since the sensitivity was enough for the determination of the PAHs by the method. Also it had the highest extraction efficiency for most of the 16 PAHs from soil in all the tested solvents. Fluorene and anthracene had the poorest recoveries for all the solvents investigated.
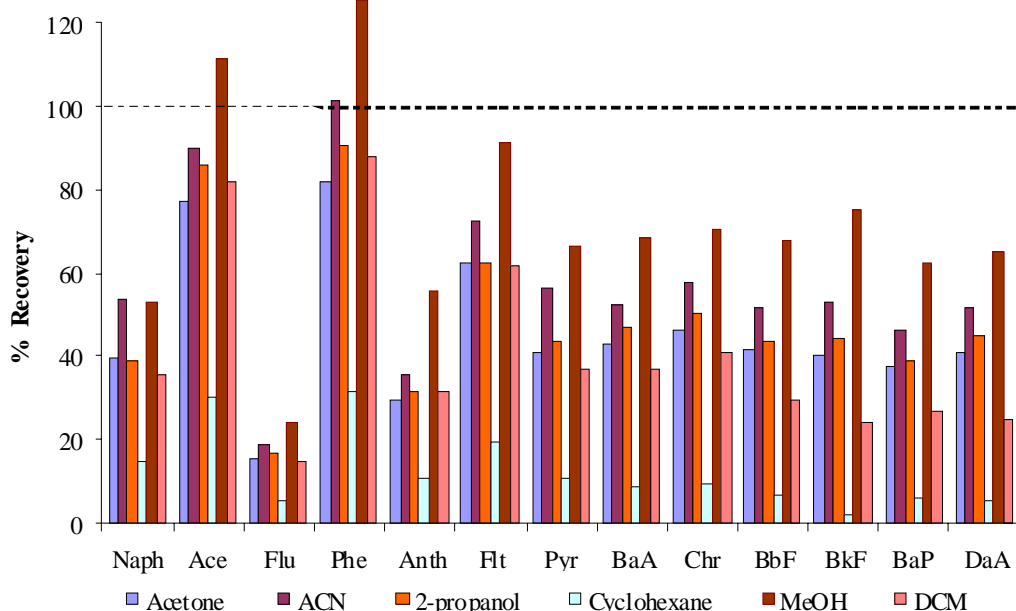
Fig. 3. Recovery of PAHs in CRM using different extraction solvents

Acetonitrile (ACN), dichloromethane (DCM) and tetrahydrofuran (THF) were used respectively as eluants for the optimisation of SPE clean up. In order to omit the step in which the easily volatile PAHs can be lost because of the evaporation of eluates to near dryness by the gentle stream of nitrogen gas, and make the operation easy and reproducible, these solvents were tested for the elution using different solvents for extraction as well.

Our results in this study showed that the recoveries of PAH spiked solution in these solvents are shown in Figs.4-6. The recoveries of the easily volatile PAHs, such as naphthalene, acenaphthene, acenaphthylene and fluorene, were lower when dichloromethane was used for the elution solution because of the evaporation of the eluates and exchange of the solvent to that compatible with HPLC analysis.
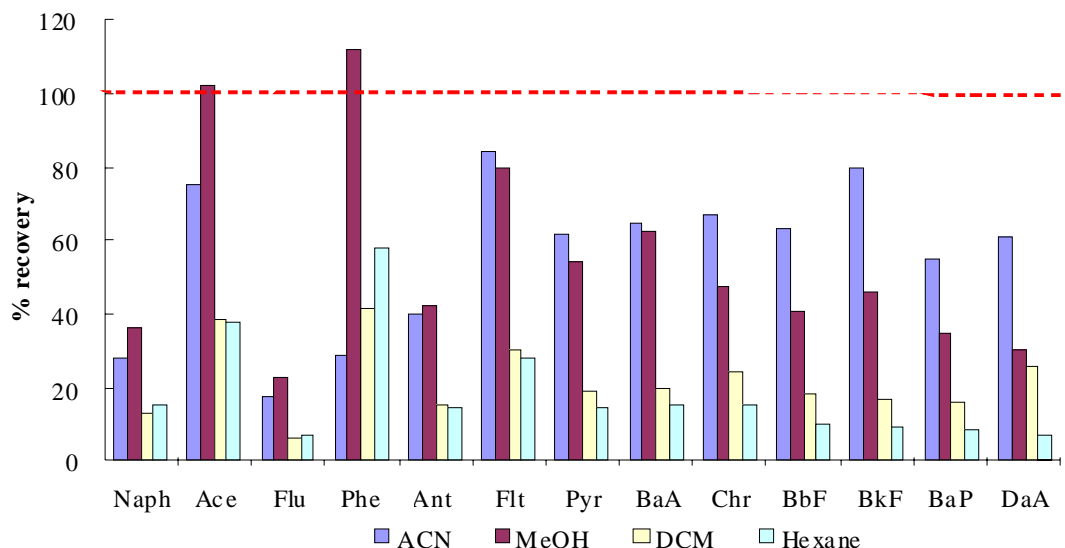
SPE using ACN as eluant

Fig. 4. Recoveries of PAHs in acetonitrile, methanol, dichloromethane and hexane extraction solvents using acetonitrile as eluant for SPE clean up

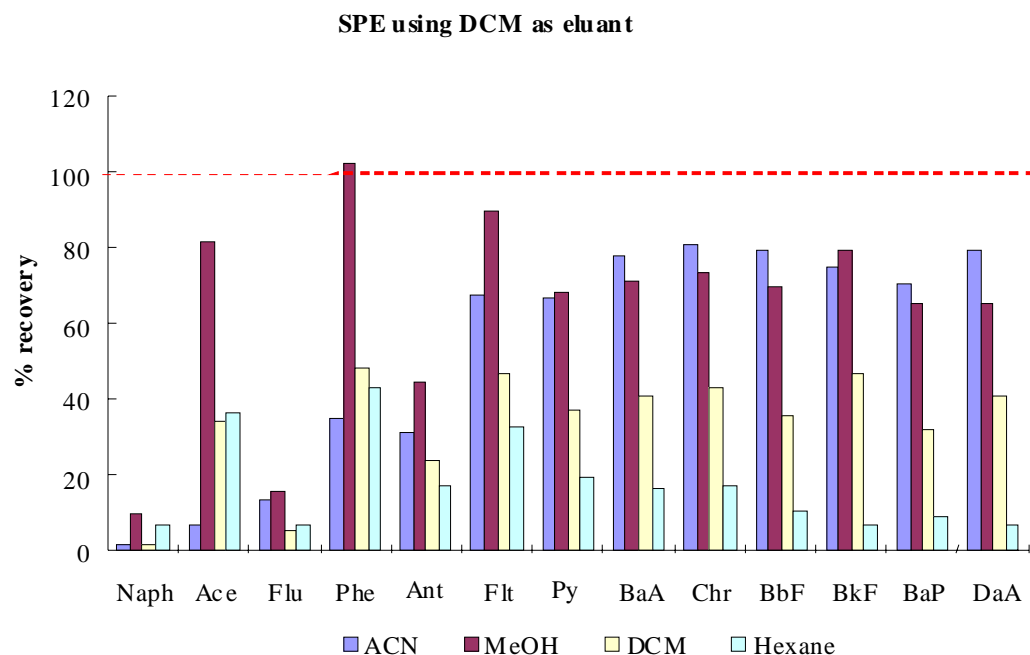**SPE using DCM as eluant**

Fig. 5. Recoveries of PAHs in acetonitrile, methanol, dichloromethane and hexane extraction solvents using dichloromethane as eluant for SPE clean up
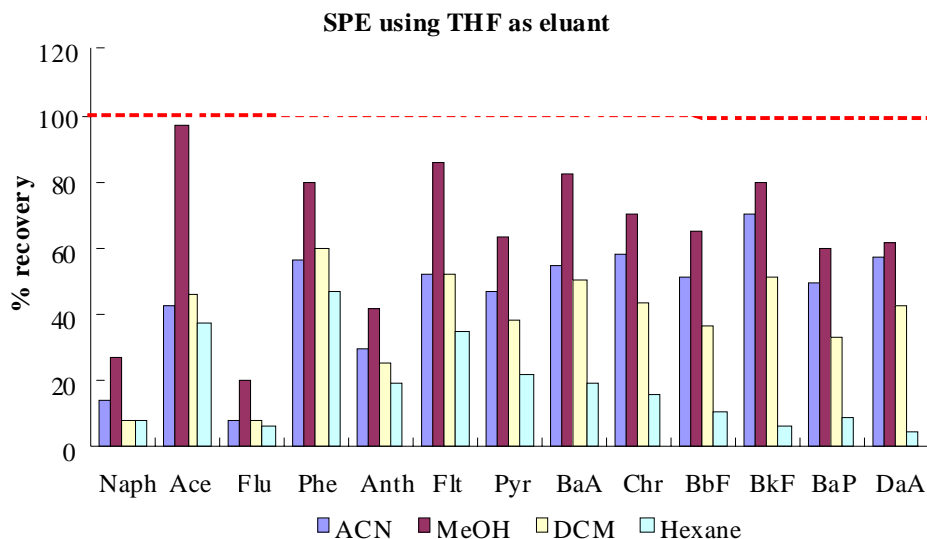
SPE using THF as eluant

Fig. 6. Recoveries of PAHs in acetonitrile, methanol, dichloromethane and hexane extraction solvents using tetrahydrofuran as eluant for SPE clean up

As shown in Fig. 5, recoveries of LMW PAHs were lower when dichloromethane was used due to the evaporation of the eluates prior to chromatographic separation. Recoveries for most of the PAHs with 4-6 aromatic rings, such as BaA, Chr, BbF, BaP, DaA, IP, were lower when acetonitrile was used, and this may be due to the fact that the polarity of acetonitrile is higher than dichloromethane but the polarities of the PAHs with 4-6 aromatic rings are very low or almost zero. Tetrahydrofuran was tried as eluant because the

polarity of tetrahydrofuran is much lower than that of acetonitrile, and it can also be easily miscible with the mobile phase, thereby omitting the step of evaporation of eluates to near dryness. Tetrahydrofuran had higher recoveries than acetonitrile and dichloromethane with the PAHs but very poor for naphthalene and fluorene (Fig.6).

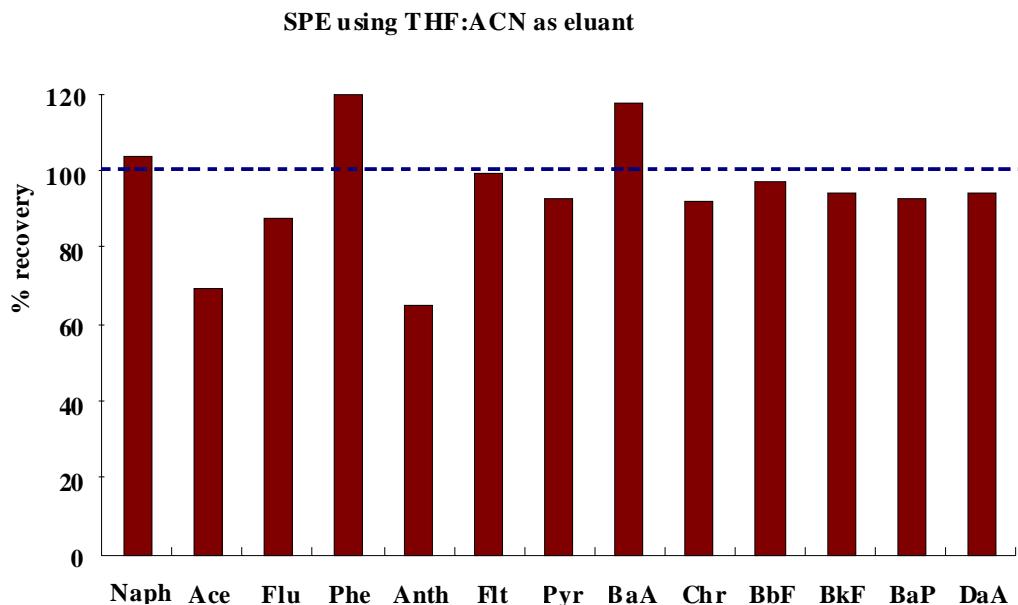Due to lower recoveries of the LMW PAHs using THF alone as eluant, a 1:1 mixed solution of THF: ACN

SPE using THF:ACN as eluant

Fig. 7. Recoveries of PAHs in CRM using THF: ACN as eluant for SPE clean up

was tested because the polarity of THF is much lower than that of acetonitrile, and it can also be easily miscible with the mobile phase (acetonitrile:water), omitting the step of the evaporation of eluates to near dryness. The recoveries of the PAHs were satisfactory; they were between 85.0% and 108% except for Ace and Anth which were 70% and 65% respectively as shown in Fig.7.

CONCLUSION

This study has shown that the 16 US EPA PAHs can be separated and determined by reversed phase HPLC with fluorescence detector using ultrasonic extraction and SPE clean up. Our results have affirmed that ultrasonic extraction was better than the Soxhlet extraction for the extraction of the 16 PAHs from soils because it was not only higher in the extraction efficiency, but also economic and easily operated. Methanol was the best solvent among the six solvents tested for the extraction of the 16 PAHs from the soil, and the order of the extraction efficiencies for most of the 16 PAHs from the soil by the six solvents were as follows: methanol> acetonitrile > 2-propanol> acetone> dichloromethane > cyclohexane. 6 ml Supelco LC-18 SPE cartridge was suitable for the preconcentration/ clean-up of the soil extraction solution, and acetonitrile: THF (1:1) was the best elution solution among the solutions tested for the elution of the 16 PAHs from the cartridge because the recoveries for most of the 16 PAHs were higher than those obtained by the other SPE procedures. This simple analytical procedure developed which was not time-consuming was successfully applied in determining the concentration

levels of 16 PAHs in real sediment samples, good results and excellent recoveries were obtained.

REFERENCES

Andersson, T., Hartonen, K. T. H. and Riekkola, M. L. (2002). Pressurised hot water extraction and thermal desorption of polycyclic aromatic hydrocarbons from sediment with use of a novel extraction vessel. Anal Chim Acta, **446,** 93-100.

Banjoo, D. R. and Nelson, P. K. (2005). Improved ultrasonic extraction procedure for the determination of polycyclic aromatic hydrocarbons in sediments. Journal of Chromatography A, **1066,** 9-18.

Beard, A., Naikwadi, K. and Karasek, F. W. (1992). Comparison of extraction methods for polychlorinated dibenzo-p-dioxins and dibenzofurans in fly ash using gas chromatography—mass spectrometry J. Chromatography A, 589, 265-270.

Berset, J. D., Ejem, M., Holzer, R. and Lischer, P. (1999). Comparison of Different Drying, Extraction and Detection Techniques for the Determination of Priority Polycyclic Aromatic Hydrocarbons in Background Contaminated Soil Samples. Anal. Chim. Acta, **383,** 263-275.

Budzinski, H., Letellier, M., Garrigues, P. and Le Menach, K. (1999). Optimisation of the microwave assisted extraction in open cell of Polycyclic Aromatic Hydrocarbons from soils and sediments. Study of moisture effect. Journal of Chromatography A, **837,** 187-200.

Camel, V. (2001). Recent extraction techniques for solid matrices-Supercritical Fluid Extraction, Pressurized Fluid Extraction and Microwave Assisted Extraction: their potential and pitfalls. Analyst, **126,** 1182-1193.

Chee, K. K., Wong, M. K. and Lee, H. K. (1996). Optimization of microwave-assisted solvent extraction of polycyclic aromatic hydrocarbons in marine sediments using a microwave extraction system with high-performance liquid chromatography-fluorescence detection and gas chromatography-mass spectrometry J. Chromatography A, **723,** 259.

Dean, J. R. (1998). Extraction methods for Environmental Analysis, England, John Wiley and Sons Ltd.

Eicemann, G. A., Viu, A. C. and Karasek, F. W. (1980). Anal Chem., **52,** 1492.

Garcia, A. L., Gonzalez-Ayuso, E. B., Alonso, J. I. G. and Mendel, A. S. (1992). Chromatographia, **33,** 225.

Hawthorne, S. B. and Grabanski, C. B. (2000). Vaporization of polycyclic aromatic hydrocarbons (PAHs) from sediments at ambient conditions. Environ. Sci Technol, **34,** 4348–4353.

Hawthorne, S. B., Yang, Y. and Miller, D. J. (1994). Extraction of organic pollutants from environmental solids with sub and supercritical water. Anal. Chem., **66,** 2912–2920.

Juhani, K., Kettunen, J., Hartonen, K. and Marja-Lissa, R. (2004). Pressurised hot water extraction of n- Alkanes and PAHs in soil and sediment from the oil shale industry district in Estonia. J. Soils Sediments, 1-8.

Kowalewska, G. and Konat, J. (1997). Distribution of polynuclear aromatic hydrocarbons (PAHs) in sediments of the southern Baltic Sea. Oceanologia, **39,** 83–104.

Lopez-Avila, V., Benedicto, J. and Bauer, K. M. (1998). J. AOAC. Int, **81,** 1224-1228.

Lopez-Avila, V., Dodhiwala, N. S. and Beckert, W. F. (1993). Developments in the supercritical-fluid extraction of chloropenoxy acid herbicides from soil samples. J. Agric. Food Chem., **41,** 2038–2044.

Lopez-Avila, V., Young, R., Benedicto, J., Ho, P., Kim, R. and Beckert, W. F. (1995). Extraction of Organic Pollutants from Solid Samples Using Microwave Energy. Anal. Chem., **67,** 2096-2102.

Lundstedt, S. (2003). Analysis of PAHs and their transformation products in contaminated soil and remedial processes, Ph.D Thesis. Department of Environmental Chemistry. Umeå, Umeå University SWEDEN.

Luque-Garcia, J. L. and Luque de Castro, M. D. (2003). Ultrasound: a powerful tool for leaching. Trends in Analytical Chemistry, **22,** 41-47.

Luque de Castro, M. D. and García-Ayuso, L. E. (1998). Soxhlet extraction of solid materials: an outdated technique with a promising innovative future. Analytica Chimica Acta, **369,** 1-10.

Marce, R. M. and Borrul, F. (2000). Solid-phase extraction of Polycyclic Aromatic Compounds. J. Chromatography A, **885,** 273–290.

Owabor, C. N., Ogbeide, S. E. and Susu, A. A. (2010). Model Simulation of Biodegradation of Polycyclic aromatic Hydrocarbon in a Microcosm, Int. J. Environ. Res., **4 (4),** 807-816

Ramos, L., Vreuls, J. J. and Brinkman, U. A. T. (2000). Miniaturised pressurised liquid extraction of polycyclic aromatic hydrocarbons from soil and sediment with subsequent large-volume injection–gas chromatography J. Chromatogr. A, **891,** 275-286.

Reimer, G. and Suarez, A. (1995). Comparison of supercritical fluid extraction and Soxhlet extraction for the analysis of native polycyclic aromatic hydrocarbons in soils. J. Chromatogr. A, **699,** 253–263.

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L., Avdalovic, N. and Pohl, C. (1996). Anal. Chem., **68,** 1033-1039.

Saim, N., Dean, J. R., Abdullah, M. P. and Zakaria, Z. (1998). Anal. Chem, **70,** 420.

Sherma, J. (1993) Liquid Chromatography of Polycyclic Aromatic Hydrocarbons, Boca Raton, CRC Press.

Simpson, N. J. K. (2000). Solid-Phase Extraction: Principles, Techniques and Applications, New York, Marcel Dekker, Inc.

Song, Y. F. X., Jing, X., Fleischmann, S. and Wilke, B.-M. (2002). Comparative study of extraction methods for the determination of PAHs from contaminated soils and sediments. Chemosphere, **48,** 993–1001.

Sun, F., Littlejohn, D. and Gibson, M. D. (1998). Ultrasonication Extraction and Solid Phase Extraction Clean-up for Determination of US EPA 16 Priority Pollutant Polycyclic Aromatic Hydrocarbons in Soils by Reversed phase Liquid Chromatography with Ultraviolet Absorption Detection. Anal. Chim. Acta, 364 1.

USEPA, (1996). Method 3650, Revision 2, December 1996, http://www.epa.gov/epaoswer/hazwaste/test/main.html.

Vallejo-Pecharromán, B., García Ayuso, L. E. and Luque de Castro, M. D. (2001). Chromatographia, **53,** 5-10.

Wong, M. K. and Williams, P. J. L. (1980). A study of three extraction methods for hydrocarbons in marine sediment. Marine Chemistry, **9,** 183-190.