

*Full Length Research Paper*

# Comparison of extraction and clean-up techniques for the determination of polycyclic aromatic hydrocarbons in contaminated soil samples

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The development of a fast, efficient and quantitative technique for the extraction, clean-up, and pre-concentration of the 16 United States Environmental Protection Agency (USEPA) priority polycyclic aromatic hydrocarbons (PAHs) was carried out on contaminated soils. The effect of Soxhlet, ultrasonication and mechanical shaking used in the extraction of a low-level PAH soil certified reference material (CRM131-100) was investigated. 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. The results were compared to determine the method with the highest extraction efficiency. The clean up and pre-concentration procedures for the PAHs were optimised using the solid phase extraction (SPE). Acetonitrile, dichloromethane and tetrahydrofuran were tested as eluants for the optimisation of SPE clean up. 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 optimised. The optimised ultrasonic extraction procedure and SPE clean-up extracted the PAHs from the certified reference material with recoveries ranging from 63.6 % to over 100%.

**Key words:** High Performance Liquid Chromatography (HPLC), mechanical shaking, polycyclic aromatic hydrocarbons (PAHs), soil, solid phase extraction (SPE), soxhlet extraction, ultrasonication.

## INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are widely distributed in the atmosphere and water sources as a

result of combustion of biomass and fossil fuels (Golomb et al., 2001; Zhang et al., 2007). 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 (Valavanidis et al., 2008); instead of carbon being completely oxidised to

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carbon dioxide, hydrocarbon fragments are generated which can interact with each other to yield complex polycyclic structures (Sherma, 1993). The mechanism by which this process occurs is complex, a subject of intensive investigation and has 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 (Haritash and Kaushik, 2009). 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 (Yu et al., 2009). 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 (Eiceman et al., 1980; Eicemann et al., 1980; Sun et al., 1998; Luque-Garcia and Luque de Castro, 2003) mechanical shaking (Berset et al., 1999) and reflux 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). An efficient extraction technique should be such that it can produce good results within a short time with minimum operator involvement. It should also be cheap, and safe for both the analyst and the environment (Dean, 1998). Each of the techniques has its own advantages 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 modern techniques (Luque de Castro and García-Ayuso, 1998; Berset et al., 1999; Budzinski et al., 1999; Lundstedt, 2003). Soxhlet extraction is considered the standard and it is the preferred method for extracting semi-volatile and non-volatile organics from solid matrices. This method 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 h or more may be needed); large volumes of organic solvents are required and labile compounds can be degraded thermally (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-Pecharrómán et al., 2001).

Ultrasonication as an extraction method is an efficient technique when compared to reflux methods for extracting trace organics from soils and sediments. Ultrasonic extraction techniques proved to generate comparable or even greater quantities (Song et al., 2002) of hydrocarbons than other techniques of extraction; though the method gave lower recoveries in some studies (Berset et al., 1999). Depending on the type of contaminants and matrix, sonication can have the advantage of faster extraction times. Optimisation of the ultrasonic extraction parameters, including type of 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.

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. Solid phase extraction (SPE) offers a faster, more cost-effective sample preparation method with dramatic time savings over many traditional liquid/liquid extraction techniques. The analytes to be determined and/or the interferences of the samples are retained on the sorbent by different mechanism (Simpson, 2000). The analytes in the sample are eluted in a small volume of a solvent thereby the analytes are cleaned up and concentrated (Marce and Borrul, 2000). Specifically, SPE aims at stripping the analyte away from the sample, putting the analyte into a small volume of a different solvent (Simpson, 2000). SPE can be 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 lower volumes of toxic solvents and this is of a great advantage from the standpoint of environmental sustainability as well as saving substantial amount of time.

In this study, different extraction techniques and solvents for the extraction and pre-concentration of PAHs in low contaminated soils were used. The results were evaluated to study the efficiency of extraction technique and solvent for optimal extraction.

## MATERIALS AND METHODS

### Reagents and materials

A standard mixture of the USEPA 16 priority PAHs (2000 µg/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. Serial dilutions of the standard solution using HPLC grade acetonitrile were made. All solvents used were of HPLC grade and the 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 0.744 to 6.36 mg/kg was used for the optimisation the extraction

procedures. The recovery of the analytes and other quality control parameters were determined for each batch of extraction.

### Selection of method of extraction of polycyclic aromatic hydrocarbons (PAHs)

#### *Ultrasonication extraction*

1 g of each of the soil samples were accurately weighed into pre-cleaned 25 mL amber glass bottles. 10 mL of extraction solvents (acetone, acetonitrile, 2-propanol, cyclohexane, methanol and dichloromethane) were added respectively. The bottles were sealed with screw cap closure lined with a PTFE-faced silicone rubber septum facing the bottle contents and shaken vigorously to suspend the contents. The bottles 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, UK) for 60 min at 50°C. The sample bottles were intermittently inverted and shaken to continually re-suspend the samples. The extraction solutions were then centrifuged and the supernatant decanted into 4 mL amber vials and later stored in the refrigerator until when ready for clean-up and analysis (Sun et al., 1998; Banjoo and Nelson, 2005).

#### *Soxhlet extraction*

10 g each of soil samples were weighed into Whatman extraction thimbles (that had been pre-extracted with dichloromethane). The samples were extracted using the soxhlet extractor with 250 mL DCM for 16 h. The extracts were further reduced to 10 mL using a rotary evaporator and transferred into 4 mL amber vials and kept in the refrigerator for clean up and analysis (Reimer and Suarez, 1995).

#### *Mechanical shaking*

5 g each of soil samples were weighed into conical flasks and 50 ml of the extracting solvent added. The flasks were sealed with aluminium foil to prevent the loss of the solvent and shaken on an arm shaker at ambient temperature for 2 h. The extract was centrifuged and the supernatant decanted. The volume of the extract was reduced using nitrogen gas.

#### **Solid phase extraction (SPE) clean up of extracts**

SPE clean-up of the sediment extracts was carried out using a 12-port vacuum manifold from SUPELCO with 6 ml Supelco C18 SPE cartridges. The sorbents of SPE cartridges were conditioned with 5 ml of extracting solvents and 40 % of the extracting solvent in water respectively to prepare the column for the clean up process. The extraction solutions were each loaded and aspirated through the cartridge under gentle vacuum at a flow rate of less than 2 ml/min.

**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

**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

The cartridges were centrifuged for 15 min at 4500 rpm and then eluted by 3 x 1 ml of eluting solvents at a flow rate of 1 ml/min (sorbents were soaked for 10 min with the elution solution before each elution). The eluates were collected into 3 ml volumetric flasks and made up to volume with the elution solutions. Care was taken that the surface of the sorbent in the cartridges were not dry during conditioning and loading of the sample extracts (Marce and Borrul, 2000).

Six solvents, cyclohexane, 2-propanol, methanol, acetonitrile, dichloromethane and acetone, were investigated to select the best solvent for the SPE clean-up. For cyclohexane and dichloromethane which are non polar solvents, the procedure of extraction was the same as that described earlier except that the solvents were evaporated to near dryness by a gentle stream of nitrogen gas, and the residues were dissolved in 10 ml of 40 % methanol. When the polar solvents were tested as the extraction solvent, water was added to the extraction solution to make the solution contain 40% 2-propanol, methanol, acetonitrile and acetone, respectively.

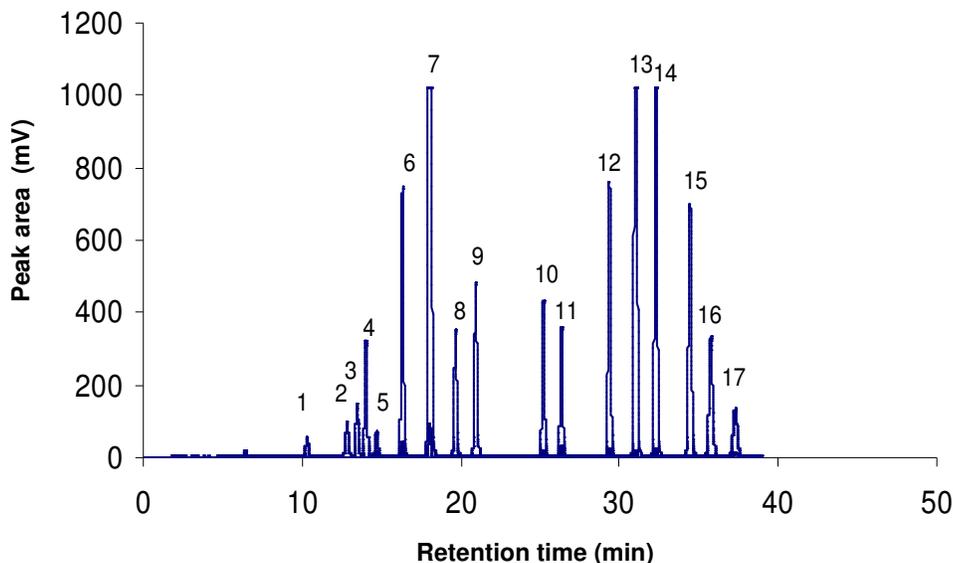
#### High Performance Liquid Chromatography (HPLC) analysis

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 2 cm x 4.6 mm i.d containing 5 µm particles and a guard column Supelguard LC-18, 2 µm. Baseline separations of the PAHs was achieved within 40 min by gradient programming. 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 were used as the mobile phase. All data for quantification of the PAHs were obtained by applying the gradient elution program shown in Table 1 at a flow rate of 1.5 ml/min and a controlled oven temperature of 25°C.

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). Figure 1 shows the HPLC chromatogram of the PAH standards.

The quantification of the PAHs was by external standards. A serial dilution of PAH standards were prepared with concentrations ranging from 0.001 to 5.0 ppm. PAH standards with lower sensitivities to the fluorescence detector were calibrated with higher concentrations. The linearity was evaluated at different concentrations using peak areas. They showed good linearity since the values of the correlation coefficients ranged from 0.9974 to



**Figure 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(flourene), 6(phenanthrene), 7(anthracene), 8(flouanthene), 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).

0.999 for all the PAHs investigated in this study.

#### Recovery of the PAHs in the certified reference material

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. As shown in Table 3, 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.

## RESULTS AND DISCUSSION

#### Selection of mode of extraction

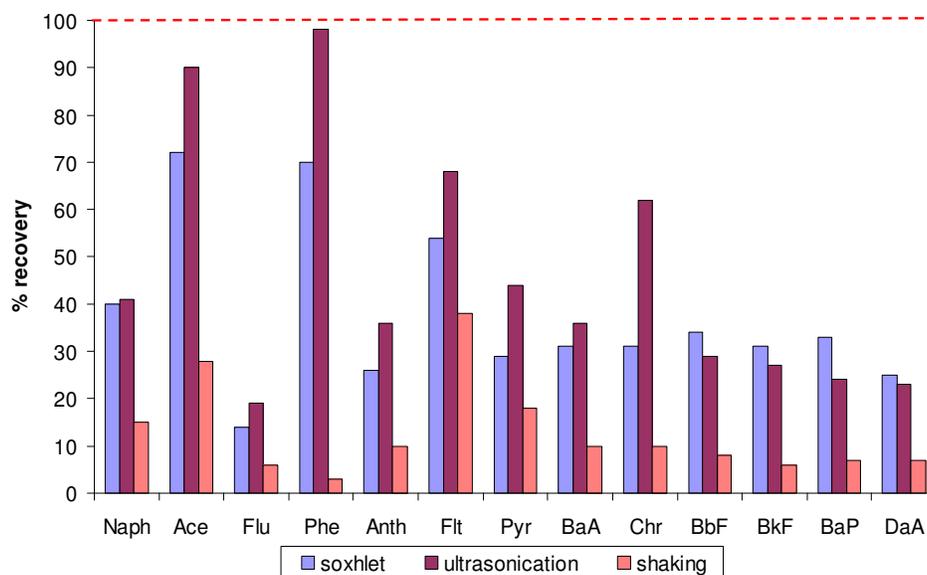
The results from the extraction of soils using different extraction methods (Soxhlet, ultrasonication and mechanical shaking) were investigated on the certified reference material and the results were compared to determine the method with the highest extraction

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 as shown in Figure 2. The extraction efficiencies for most of the PAHs in the CRM using the sonication method were higher than Soxhlet, except for benzo (b) fluoranthene (Soxhlet 34%, Sonication 29%), benzo (k) fluoranthene (Soxhlet 31%, Sonication 27%), benzo (a) pyrene (Soxhlet 33%, Sonication 24%) and dibenzo (a,h) anthracene (Soxhlet 25%, Sonication 23%). The time spent for ultrasonication was less than 1 h while soxhlet took about 16 h. Also, ultrasonication consumed solvent (10 ml) than Soxhlet which required about 250 ml. Mechanical shaking gave the worst recovery for all the PAHs, the recovery of Phe was 3%, Flt was 38%, while for sonication the recoveries of these PAHs were 100 and 70%, respectively. Therefore, ultrasonication extraction was used throughout this work.

This study has shown that ultrasonication is a more efficient technique when compared to other traditional methods for extracting trace organics from soil and

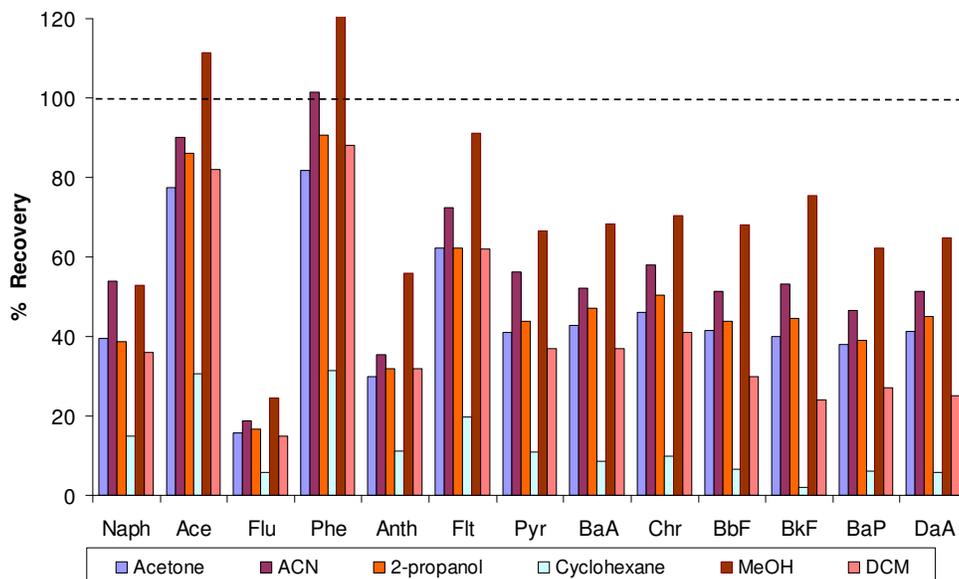
**Table 3.** Recoveries of PAHs in CRM 131-100.

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

**Figure 2.** Percentage recoveries of PAHs using different methods of extraction.

sediments. This is in congruity with earlier studies that showed ultrasonic extraction yields comparable or higher

quantities in extraction of hydrocarbons than other techniques of extraction (Garcia et al., 1992; Sun et al.,



**Figure 3.** Recovery of PAHs in CRM using different extraction solvents.

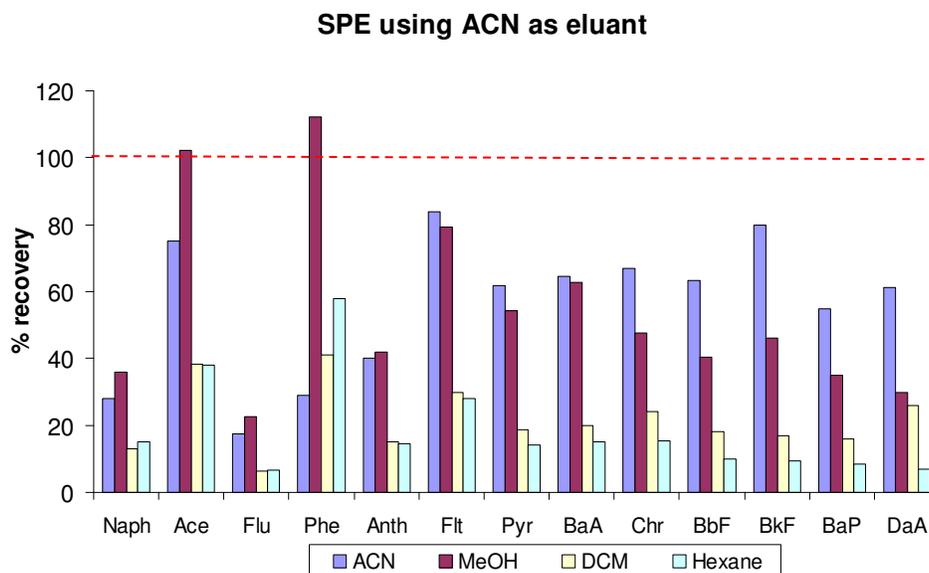
1998; Song et al., 2002). However, some studies showed that ultrasonication gave lower recoveries (Lopez-Avila et al., 1995; Berset et al., 1999). The reproducibility obtained with ultrasonic extraction was shown to be higher (Banjoo and Nelson, 2005) or lower than those from Soxhlet extraction (Berset et al., 1999).

Losses of PAHs in Soxhlet extraction can be attributable to high temperatures used which can result in losses of hydrocarbons due to volatilization and/or oxidation of highly volatile and thermally labile species. Methods based on Soxhlet extraction have traditionally been used as references to assess the performance of other extraction methods; the result from this comparative analysis highlights the superiority of ultrasound-assisted extraction over the conventional Soxhlet extraction. The extraction efficiency of ultrasonication may have increased because cavitation increases the polarity of the system including extractants, analytes and matrix thereby making the extraction efficiency similar to or greater than that of conventional Soxhlet extraction (Beard et al., 1992). Also, ultrasound-assisted methods of extraction allow the addition of a co-extractant to further increase the polarity of the liquid phase (Luque-Garcia and Claque de Castro, 2003). It also allows the leaching of

thermolabile analytes which are altered under the working conditions of Soxhlet extraction. The operating time of ultrasonication method was shorter than with Soxhlet extraction.

#### **Selection of solvent for extraction of polycyclic aromatic hydrocarbons (PAHs)**

In this experiment, six different extraction solvents; acetone, cyclohexane, 2-propanol, methanol, acetonitrile and dichloromethane were tried to select a solvent for the extraction of the 16 PAHs in the certified soil reference material using ultrasonication extraction. Figure 3 shows the recovery of PAHs in CRM using six different extraction solvents. The results in Figure 3 showed that the highest extraction efficiencies for most of the 15 PAHs were obtained by using methanol (Ace and Phe were 111.5 and 125%, respectively), except for fluorene (24.5%). Fluorene had the poorest recovery for all the solvents used – acetone: 15.7%, acetonitrile: 18.8%, 2-propanol: 16.7%, cyclohexane: 5.7% and DCM: 15%. For all the solvents investigated in this experiment, cyclohexane had the poorest recoveries ranging from 2.1



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

(BkF) to 31.4 % (Phe). 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.

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.

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/ pre-concentration 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,

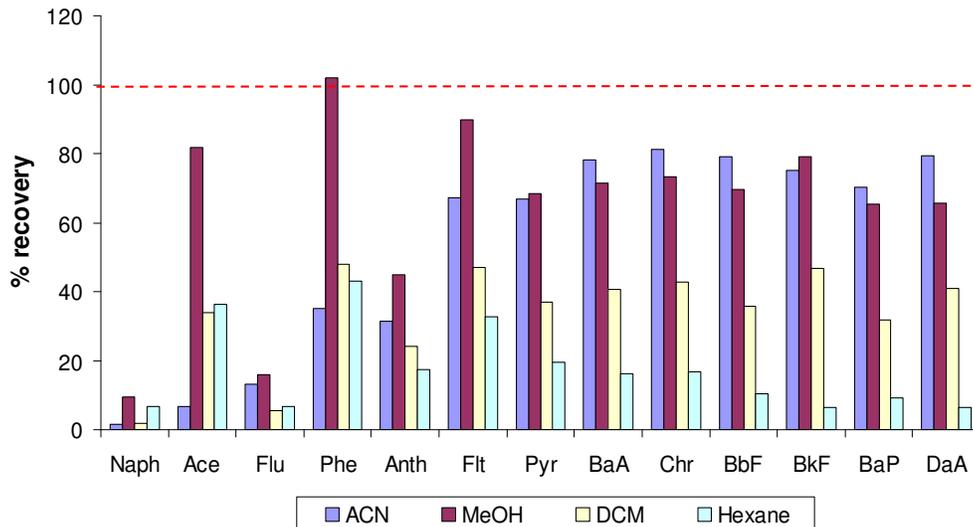
the solvent which can be miscible with the mobile phase was the best for the final injection solution. The first four solvents fit into this. Methanol was therefore selected as the extraction solvent because it could mix well with the mobile phase. This resulted in hardly any loss of the easily volatile 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.

#### **Selection of elution solution for solid phase extraction (SPE)**

Acetonitrile (ACN), dichloromethane (DCM) and tetrahydrofuran (THF) were used 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 and to achieve reproducible results, these three solvents were tried.

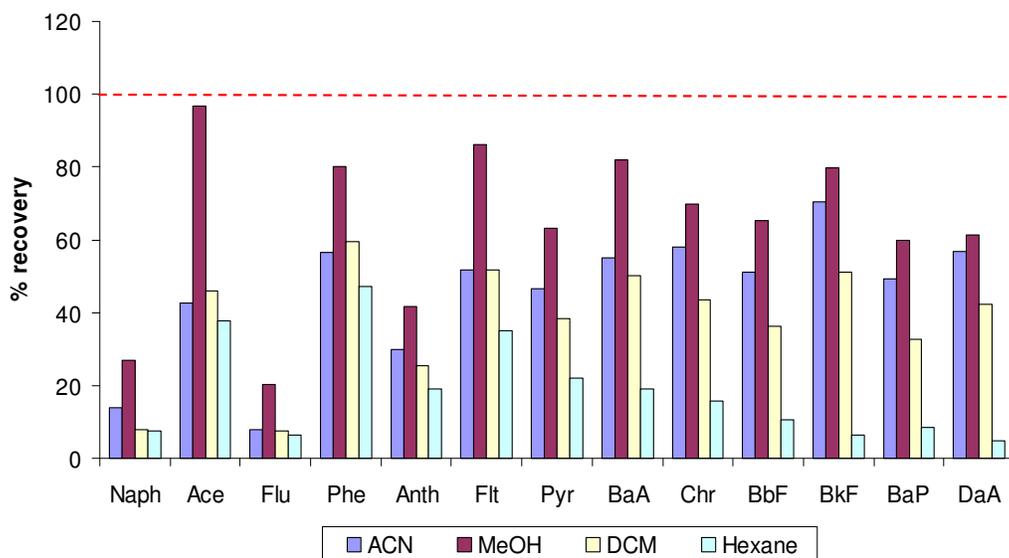
The results for the recoveries of PAH spiked solution in these solvents are shown in Figures 4 to 6. The recoveries

### SPE using DCM as eluant

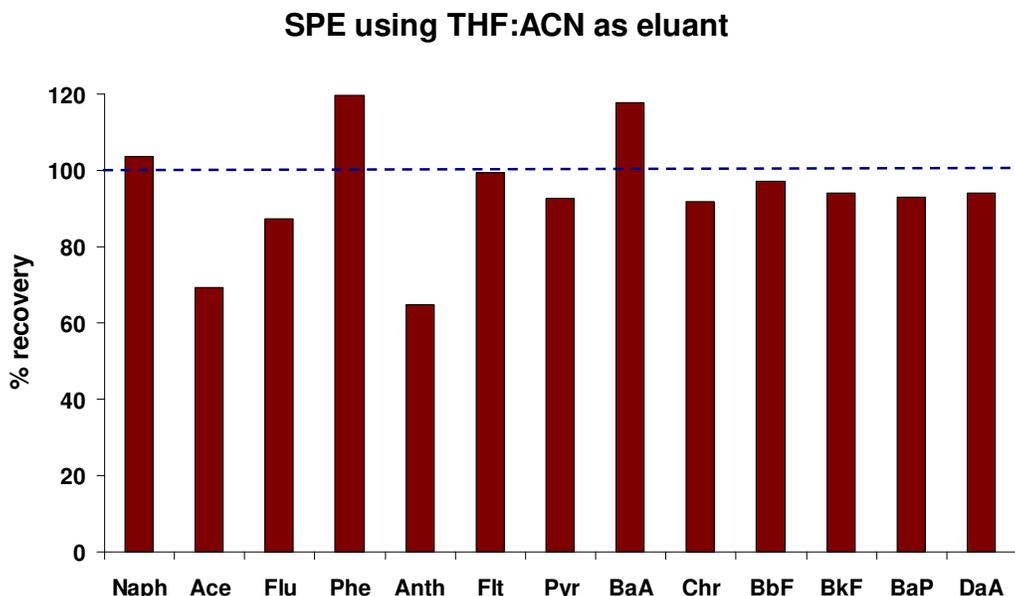


**Figure 5.** Recoveries of PAHs in acetonitrile, methanol, dichloromethane and hexane extraction solvents using dichloromethane as eluant for SPE clean up.

### SPE using THF as eluant



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



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

of the easily volatile PAHs, such as naphthalene, acenaphthene, acenaphthylene and fluorene were lower when dichloromethane was used for the elution solution. This could be as a result of the evaporation of the eluates and exchange of the solvent to that compatible with HPLC analysis.

From Figure 5, recoveries of LMW PAHs were lower when dichloromethane was used due to the evaporation of the eluates prior to chromatographic analysis. Recoveries for most of the PAHs with 4-6 aromatic rings, that is, BaA, Chr, BbF, BaP, DaA, IP, were lower when acetonitrile was used. This may be because 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 (Figure 6).

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

tried since the polarity of THF is much lower than acetonitrile. Also the mixture can 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 (Figure 7).

## Conclusions

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. The results revealed that ultrasonic extraction was better than the Soxhlet extraction for the extraction of the 16 PAHs from the soil samples because it was not only higher in the extraction efficiency, but also economic and easy to use. Methanol was the best among the six solvents tried for the extraction of the 16 PAHs from the soil. 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. Using a 6-ml

Supelco LC-18 SPE cartridge for the pre-concentration/clean-up of the soil extraction solution and using acetonitrile: THF (1:1) was the best elution solution among the solutions tested for the elution of the 16 PAHs from the cartridge as the recoveries for most of the 16 PAHs were higher any 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 with good results and excellent recoveries.

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**Abbreviations:** **HPLC**, High Performance Liquid Chromatography; **PAHs**, polycyclic aromatic hydrocarbons; **SPE**, solid phase extraction; **ASE**, accelerated solvent extraction; **SFE**, supercritical fluid extraction; **MAE**, microwave-assisted extraction; **PLE**, pressurised liquid extraction.

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