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Pressurized Liquid Extraction of Polycyclic Aromatic Hydrocarbons from Soil Samples

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ABSTRACT

A Pressurized Liquid Extraction (PLE) method was developed by using conventional High Performance Liquid Chromatography (HPLC). It was found that all of the PAHs have been successfully extracted with dichloromethane-acetone with high percentage recovery. A high temperature of 180°C gave the highest recovery for fluoranthene (94.4%). Meanwhile, fluorene showed the highest recovery at 150 bar, with 94.6% recovery. It is noted that there is no significant day-to-day difference in the efficiency of the developed method, with the R.S.D. values averaging at 0.02. The optimized conditions applied to the soil samples were analysed using the High Temperature High Performance Liquid Chromatography (HT-HPLC) with chromatographic conditions: Octadecylsilyl-silica (ODS-silica) column (100 mm × 4.6 mm I.D.); mobile phase acetonitrile:water 40:60 (v/v); flow rate 2.5 mL/min; temperature 70°C; UV absorbance 254 nm; injection volume 5µL.

Keywords: Polycyclic Aromatic Hydrocarbons (PAHs), pressurized liquid extraction, high temperature high performance liquid chromatography

INTRODUCTION

Accelerated solvent extraction (ASE), which is also known as pressurized liquid extraction (PLE), was first described by Richter *et al.* (1995). The PLE uses organic solvents at elevated temperatures and pressures to obtain a complete extraction of analytes from solid or semi-solid samples in shorter periods of time and with smaller quantities of solvents, as compared to conventional extraction procedures. According to Wan and Wong (1996), one of the major driving forces is the increasing demand from the regulatory bodies to reduce the large volumes of organic solvents consumed by the classical extraction methods such as Soxhlet.

In this study, the PLE method was developed using the conventional HPLC instrument for the analysis of the samples. The PLE has demonstrated advantages for automation, reduced extraction time and lower solvent use as compared to the conventional soxhlet extraction. Recently, the PLE with sub-critical heated water has been used to extract polar to moderately polar organic compounds from the sediment samples. The extraction of non-polar high molecular weight compounds, such as PAHs, PCBs, and brominated flame retardants at temperatures greater than 250ºC, has also been reported (Scott, 1984).

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Saim *et al.* (1997) discussed the effect of extraction solvents. A selection of organic solvents, single or in combination, was chosen to investigate their influence on the recovery of PAHs. The results indicate that the solvent has an effect on the recovery of PAHs, except for hexane. Meanwhile, poor recovery obtained for hexane was attributed to its lower polarity.

Richter *et al*. (1999) explained that temperature has a pronounced effect on the performance of PLE from the standpoint of solubility and mass-transfer. They studied the effect of temperature on the recovery of the total petroleum hydrocarbons (TPHs). The temperature was varied from room temperature, which was 27ºC to 100ºC. The researchers noted that using elevated temperatures and pressures, in combination with organic solvent, improved precision and recovery for the extraction of analytes from solid samples. In particular, the PLE gave a recovery that was comparable to those obtained from the soxhlet extraction and other techniques in use, while requiring only a fraction of the time and solvents needed for those techniques. Thus, the PLE has shown good potential for the recovery of volatile and semi-volatile compounds.

PAHs (Polycyclic Aromatic Hydrocarbons) are one of the typical persistent organic compounds (POPs) featured in regional and global cycling. PAHs are emitted mainly into the atmosphere and have been detected at long distances from their source. Compounds with five or more aromatic rings that exist are mainly adsorbed in to airborne particulate matter, such as fly ash and soot, due to their low vapour pressures. PAHs have been reported to have mutagenic and/or carcinogenic effects. The ability of polycyclic aromatic hydrocarbons (PAHs) to induce cancer has been documented by epidemiological studies of workers in coal tar, creosote, coal gas, coke, and cutting oil industries (Claessens and Straten, 2004).

MATERIALS AND METHODS

Reagents and Chemicals

HPLC grade solvent acetonitrile was obtained from Scharlau AC0340 (Barcelona, Spain). Meanwhile, methanol solvent was obtained from HyperSolv, BDH Laboratory, EC No. 200-659-6 (England). The polycyclic aromatic hydrocarbons (napthalene, fluorene (EC No. 2016955), phenanthrene (EC No. 2015815), and fluoranthene (EC No. 2059124) were obtained from Fluka Chemika, Sigma-Aldrich Chemic, Steinheim, (Switzerland). Double-distilled deionized water of at least 18 MΩ was purified using the Nano ultra pure water system (Barnstead, USA).

Chromatographic Conditions

The high temperature HPLC systems consisted of a conventional HPLC system that is coupled with a column oven of a Shimadzu GC-8A Gas Chromatography (Shimadzu Kyoto, Japan). In this study, the HPLC separations were carried out using Waters 515 HPLC pump (Mildford, USA) for mobile phase delivery. The samples were injected into the system using a 25µL loop for sample introduction. The analyte peaks were detected using a Shimadzu SPD- 6A UV detector (Kyoto, Japan) and were recorded on a Waters 746 Data Module integrator (Mildford, USA).

Pressurized Liquid Extraction Method

Extractions were carried out using a JASCO PU 980 HPLC pump. The samples (7g) were accurately weighed into a 11 mL cell. The sample cell was then closed to finger tightness and placed in a Shimadzu GC 8A oven. *Fig. 1* shows a schematic diagram of the pressurized liquid extraction system. The extractions were carried out using dichloromethane-acetone. In addition, the extractions were performed using a preheated method. In this procedure, the sample cells were heated to the

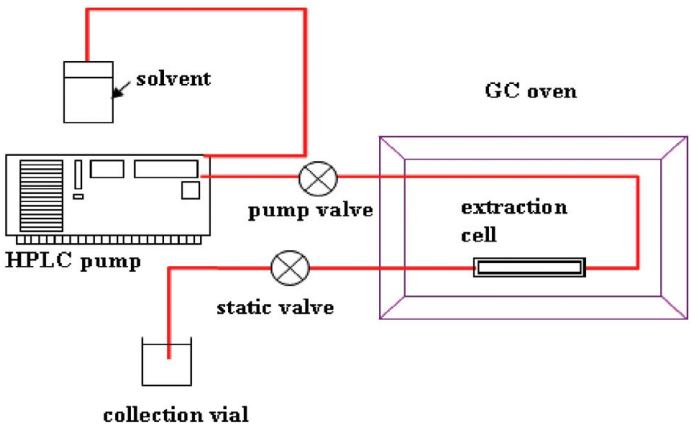


Fig. 1: Schematic diagram of pressurized liquid extraction system

extraction temperature (the range of 60°C to 250°C was tested). When a gas chromatograph oven was used, the cell was allowed to equilibrate for 10-15 min after the oven had reached the set point temperature. The static valve was opened during the preheating step.

The pump valve was opened after the heat-up time, whereas the solvent was introduced into the cell at 2.0 mL/min until about 1 mL was accumulated in the collection vial. At that point, the static valve was closed and the cell continued to pressurize to the set point (50-250 bar). The static period for these studies was 5 min. because longer periods did not show improvement in term of recovery. After the static period, the static valve was reopened, fresh solvent was introduced to flush the lines and cell, and the extract was collected in the vial. During this solvent flush step, 50-100% of the extraction cell volume of solvent was pumped into the cell.

Day to Day Experiments

The soil samples were analysed using the optimized method of the PLE to study the efficiency of the developed method.

Procedure

The samples of four PAHs, with a concentration of 20 ppm (napthalene, fluorine, phenanthrene, and fluoranthene), were dissolved in acetonitrile and kept in the freezer. Mixed solutions were prepared by mixing specific quantities of analytes from each stock solution. The prepared solution was injected in triplicates onto the column.

The separation was carried out using different compositions (acetonitrile-water: 70-30; 60-40; 50-50; 40-60, v/v). The eluent flow rate was 1.0 mL/min and the sample injection volume was 1µL. Meanwhile, the UV detection of analytes for the comparison study was at 254 nm.

RESULTS AND DISCUSSION

In this study, the optimized PLE conditions were investigated to be applied for the extraction of the PAHs from the soil samples. The extraction solvents, extraction pressure, and extraction temperature were optimized and the reproducibility of the method was also investigated. In the PLE, the soil was mixed with anhydrous sodium sulphate. The advantages of mixing the soil with anhydrous

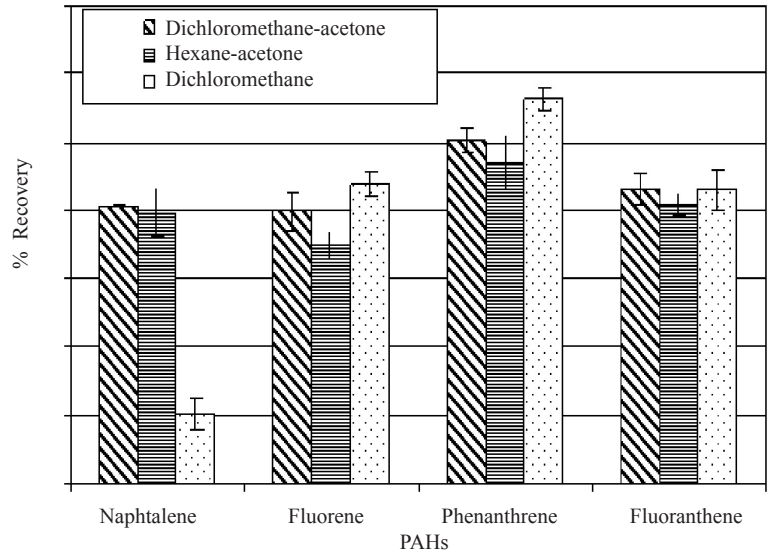


Fig. 2: Average percentage recoveries of spiked 50 ppm PAHs in soil samples using different extraction solvent

sodium sulphate include trapping of water through binding to sodium sulphate, minimized soil exposure to laboratory air, stopping the microbial activity due to the high salt concentration, and a longer storing period for the sample that was treated in this way.

Liquid chromatographic analysis of the extracts that were obtained from the PLE of spiked soil samples was also carried out. It was found that different extraction solvents gave different recovery percentages (*Fig. 2*). The recovery of PAHs ranged from 80% to 101% for dichloromethane-acetone, 70% to 94% for hexane-acetone and 21% to 113% for dichloromethane as extractant. The highest recovery percentages were observed for phenanthrene (113%) and fluorene (88%) using dichloromethane as the extraction solvent. All relative standard deviations for the spiked standard PAHs were acceptable (R.S.D. 0.3-9.3). Low recovery was obtained for naphthalene with dichloromethane as single solvent extractant, indicating that the solvent was not suitable for naphthalene as compared to other analytes. Similar results have also been reported by Burkhardt *et al.* (2005) who obtained a mean recovery of 67% for naphthalene, as compared to phenanthrene (102%), and fluoranthene (111%), using water-isopropanol as extraction solvents. Meanwhile, Fisher *et al.* (1994) found that spiking in more polar solvent resulted in a higher recovery. Similar results were also obtained by Eschenbach *et al.* (1994) who explained that acetone showed a greater efficiency than toluene or dichloromethane.

Temperature has a significant influence on the diffusion coefficient of solvents; hence, the kinetics of an extraction process and its overall efficiency is strongly dependent on this parameter. In this work, the recovery of PAHs from the spiked samples was determined using different extraction temperatures, in the range of 100°C-180°C, with the mixture of dichloromethane-acetone 50:50 (v/v) as the extraction solvent.

In general, higher recovery of all the PAHs studied was obtained at higher temperatures (*see* Table 1). For example, at 100°C and 180°C, the recovery of phenanthrene was found to be 83% and 89%, respectively. A high temperature of 180°C gave the highest recovery for fluoranthene (94%), followed by fluorene (92%), phenanthrene (89%), and naphthalene (82%). The higher

TABLE 1
The recovery percentages of the spiked standard PAHs at 20 ppm in 7g of the soil samples at different extraction temperatures

Compound	% Recovery (R.S.D.)		
	Temperature		
	100°C	150°C	180°C
Naphthalene	42(±2.5)	68(±1.0)	82(±1.0)
Fluorene	87(±1.3)	92(±1.4)	92(±1.1)
Phenanthrene	83(±1.7)	88(±7.7)	89(±1.8)
Fluoranthene	87(±1.1)	87(±1.5)	94(±0.2)

(R.S.D. %) was based on triplicate injections

recovery for the more hydrophobic compounds (phenanthrene and fluoranthene) can be explained because these compounds are generally more thermally stable. Hawthorne *et al*., 2000 reported that increasing the extraction temperature gave a higher recovery for higher molecular weight of the PAH extracted. Meanwhile, PAHs with high-molecular weight may have stronger adsorption and formation of non-extractable residues (Hawthorne *et al.*, 2000). Hence, fluoranthene with the highest molecular weight shows the highest recovery and naphthalene with the lowest molecular weight reveals the lowest recovery.

The high recovery at 180°C for the PAHs studied can be explained by the fact that at higher temperatures, there is a decrease in the viscosity of liquid solvents, which allow a better penetration of the matrix particles and enhance extraction. Thus, an increase in the temperature can disrupt the strong solute-matrix interactions caused by the van der Waals forces, hydrogen bonding, and dipole interactions of the solute molecules and active sites on the matrix. In addition, increased temperatures can also decrease the surface tension of the solvent that facilitates a better contact of the solvent with the analytes and enhance extraction (Bruce, 2000).

The results obtained are in good agreement with those reported by Jaska *et al.* (2004) who suggested that in order to enhance the efficiency of recovery, extractions need to be performed at an elevated temperature within the range of 50-180°C, depending on the boiling point of the analytes studied. In the PLE, the samples were extracted at the temperatures well above the boiling point of the extractant used. The kinetics of the mass transfer can greatly be improved at elevated temperatures. Therefore, it is highly possible that a poor extraction solvent in a conventional extraction method, such as Soxhlet extraction, can be a good solvent in the PLE at high temperatures.

Meanwhile, the use of pressure facilitates the extraction of analytes from the samples, in which the analytes are trapped in the matrix pores. In this case, an elevated pressure, along with an elevated temperature, reduces the solvent surface tension that helps force the solvent into the sample pores to contact the analytes. In this study, the following pressures were used to determine the effects of pressure on the extraction efficiency: 50 bar, 100 bar, and 150 bar. However, higher pressure was not attempted in this study because only modest pressure in the range of 100-150 bars (10-15MPa) is usually needed to maintain the solvents as liquid in the PLE (Jaska, 2004). The results show that the recovery percentage of the analytes improves with the increase in pressure (Table 2). All the analytes studied showed the highest recovery at 150 bar. In particular, fluorene showed the highest recovery percentage (95%), followed by phenanthrene (94%), fluoranthene (87%), and naphthalene (52%). These results confirm the hypothesis that the increased pressures would allow the analytes

TABLE 2
The recovery percentage of the analyte studied at different extraction pressures (temperature: 180°C)

Compound	% Recovery (R.S.D.)		
	Pressure (bar)		
	50	100	150
Naphthalene	45 (±5.8)	49 (±0.20)	52 (±0.04)
Fluorene	83 (±4.2)	88 (±0.03)	95 (±0.08)
Phenanthrene	79 (±1.1)	83 (±0.03)	94 (±0.03)
Fluoranthene	77 (±2.6)	82 (±0.20)	87 (±0.04)

TABLE 3
Relative recovery percentages and standard deviation of the analytes studied on daily basis

	% Recovery			Mean % recovery	% R.S.D.
Compound	Day 1	Day 2	Day 3		
Naphthalene	88	63	68	73	$\pm 0.1_1$
Fluorene	95	97	91	94	$\pm 0.0_4$
Phenanthrene	90	93	89	91	$\pm 0.0_2$
Fluoranthene	91	89	88	89	$\pm 0.0_1$

which were found in pores effectively blocked by solvent to be more rapidly extracted than what is possible at the room temperature and atmospheric pressure.

From the results obtained (Table 3), it is noted that there are no significant day-to-day differences in the efficiency of the developed method, while the day-to-day R.S.D. values were lower. The highest value was obtained for fluorene (95%) at Day 1, while the lowest recovery was naphthalene (63%) at Day 2. The result showed that the three-ring PAHs (fluorene, phenanthrene) appeared to be more efficiently extracted by the PLE as compared to the two-ring PAH (naphthalene), and this was probably due to its higher volatility of naphthalene which had caused more analyte loss during extraction.

The results indicate that the developed method for the PLE of PAHs has good reproducibility. The RSDs were good, i.e. ranging from 0.0_1 to 0.1_1. Therefore, the PLE method is proven to be readily applicable as a standard method for the extraction and analysis of PAHs. Currently available US EPA methods for the extraction of PAHs from contaminated soil include Method 3540 for Soxhlet extraction, Method 3541 for SFE and Method 3545 for PLE. Conte *et al*. (1997) reported that the PLE is advantageous because the parameters involved include the ease of total automation that adds to better reproducibility, reduced time direct contact between operator and solvent vapour, reduced consumption of organic solvents, the subsequent storage and disposal.

CONCLUSIONS

A method for the determination of PAHs has been developed using a homemade pressurized liquid extraction (PLE) system and elevated temperature RPLC. In particular, the PLE has shown an excellent efficiency in term of the total extraction time, total solvent usage, and reproducibility. In fact, the PLE method has demonstrated an outstanding performance with dichloromethane-acetone 50:50 (v/v) as solvent extraction, pressure 150 bar, and extraction temperature of 180°C. Data showing the method R.S.D. and reproducibility have proven the suitability of the method for routine monitoring of the compounds of interest in samples of soil. Although not all classes of environmentally-important compounds have been covered, the PLE of representative semi-volatile compounds in the soil matrixes indicate this technique as a viable technique for extraction, among the new regime of rapid method. In short, recovery with the use of the PLE in all cases was found to be satisfactory, offering a good agreement with the established data reported.

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