The Malaysian Journal of Analytical Sciences, Vol 13 No 1 (2009): 141 - 145

COMPARISON OF PRESSURIZED LIQUID EXTRACTION WITH SOXHLET EXTRACTION IN THE DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN SOIL

(Perbandingan Pengekstrakan Cecair Prestasi Tinggi dengan Pengekstrakan Soxhlet dalam Menentukan Polisiklik Aromatik Hidrokarbon di dalam Tanah)

Noorashikin Md Saleh[1]*, M. Marsin Sanagi[2]

[1]*Department of Chemical Sciences, Faculty of Science and Technology,*
Universiti Malaysia Terengganu, 21030 Kuala Terengganu, Terengganu, Malaysia.
[2]*Department of Chemistry, Faculty of Science,*
Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia.

**Corresponding author: noorashikin@umt.edu.my*

Abstract

Pressurized liquid extraction (PLE) method was developed by using conventional high performance liquid chromatography instrument and the performance of the PLE system was tested using environmental soil samples were collected at different places from FELDA palm oil plantation at Serting, Negeri Sembilan. PLE method showed good reproducibility for the majority of PAHs tested. Fluoranthene showed the highest recovery (94.4%) followed by fluorene (92.3%), phenanthrene (88.5%) and naphthalene (81.5%). High temperature at 180°C along with polar solvent dichloromethane-acetone was found to be necessary to extract the PAHs quantitatively from soils. The PAHs detected in the samples are phenanthrene (4.1 to 49.4 µg/mL), fluorene (10.1 µg/mL and 11.6 µg/mL) and fluoranthene (2.2-60.7 µg/mL. Naphthalene was not detected in the soil samples. The results obtained indicate that the developed method can be applied to the analysis of PAHs in soils.

Keywords: Pressurized liquid extraction (PLE), Polycyclic Aromatic Hydrocarbons (PAHs), high temperature high performance liquid chromatography (HT- HPLC).

Abstrak

Kaedah pengekstrakan cecair tekanan tinggi telah dibangunkan dengan menggunakan kromatografi cecair prestasi tinggi dan pelaksanaannya diuji dengan sampel tanah persekitaran. Kaedah PLE menunjukkan perolehan yang baik untuk semua PAH yang diuji. Fluorantena menunjukkan dapatan semula yang paling tinggi (94.4%) diikuti dengan fluorena (92.3%), fenantrena (88.5%) dan naftalena (81.5%). Suhu tinggi pada 180°C dengan pelarut berkutub diklorometana-aseton didapati sesuai untuk mengekstrak PAH secara kuantitatif daripada tanah. Lima sampel tanah digunakan dalam eksperimen diambil daripada tempat berlainan dari ladang kelapa sawit FELDA di Serting, Negeri Sembilan. PAH yang dikesan dalam sampel adalah fenantrena (4.1-49.4 µg/mL), fluorena (10.1 µg/mL dan 11.6 µg/mL) dan fluorantena (2.2-60.7 µg/mL). Walau bagaimanapun, naftalena tidak dikesan di dalam sampel tanah itu. Keputusan yang diperolehi menunjukkan kaedah yang dibangunkan boleh diaplikasi untuk analisis PAH dalam tanah.

Kata kunci: Pengekstrakan cecair tekanan tinggi (PLE), Polisiklik aromatik hidrokarbon (PAH), kromatografi cecair prestasi tinggi bersuhu tinggi (HT - HPLC).

Introduction

Pressurized liquid extraction (PLE) is a sample preparation technique for the extraction of analytes from solid materials. According to Wan and Wong [1] one of the major driving forces is the increasing demand from authorities to reduce the large volumes of organic solvents consumed by classical extraction methods such as Soxhlet. Another contributing factor for the rapid acceptance of PLE is that the method development is rather straight-forward. In this study, a PLE method was developed by using conventional high performance liquid chromatography instrument and the performance of the PLE system was tested using environmental soil samples. Since PLE was demonstrated to be very efficient, the technique was rapidly accepted by the US Environmental Protection Agency (EPA) as a method for evaluation of solid wastes [2]. Often the organic solvent or combination of solvents utilized in existing Soxhlet method can simply be adopted by the PLE method. Consequently, the year after the first publication dealing with persistent organic pollutants (POPs) in soils and sediments were presented, the number of publications dealing with PLE of POPs has increased, where the main focused on PAHs [3]. In this experiment, we did a comparison between pressurized liquid extraction with Soxhlet extraction in determination of PAHs in soil samples.

Experimental

Reagents and chemicals

HPLC grade solvent acetonitrile was obtained from Scharlau (Barcelona, Spain). Methanol solvent was obtained from HyperSolv (BDH Laboratory, U.K.). The polycyclic aromatic hydrocarbons (napthalene (70211), fluorene (46880), phenanthrene (77470), fluoranthene (46530) and internal standard (pyrene (82648) were obtained from Fluka Chemika, Sigma-Aldrich Chemic, Steinheim, (Switzerland). Double-distilled deionized water of at least 18 MΩ was purified by Nano ultra pure water system (Barnstead, USA).

Soil Sample Preparation

The land soils were sampled from a known site (FELDA palm oil plantation, Serting, Negeri Sembilan) and transported to the laboratory. The samples were air-dried for 24 hours and sieved through <7 mm sieve. The fine powdered soils were then stored in air-tight containers at room temperature for analysis.

Pressurized Liquid Extraction

Extraction were done using JASCO PU 980 HPLC pump. Samples (7 g) 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. Extractions were carried out using dichloromethane-acetone. Extractions were performed using preheated method. In this procedure, the sample cell was heated to a selected extraction temperature (the range of 60°C to 250°C). The cell was allowed to equilibrate for 10-15 min after the oven has reached the set point temperature. The static valve was opened during the preheat step. The pump valve was opened after the heat-up time and solvent was introduced into the cell at 2.0 mL/min until about 1 mL had 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 the extraction was 5 min, as longer periods did not show improvement in the recoveries. 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.

Soxhlet Extraction

The Soxhlet extraction method was based on EPA method 3540. In summary, 30 g of soil were mixed with 30 g anhydrous sodium sulfate to adsorb moisture. Four PAHs standards were spiked in a soil sample and transfer into a cellulose extraction thimble and inserted into a Soxhlet assembly fitted with a 250 mL flask. The solvents used were dichloromethane-acetone was heated for 20 hours. One third of the extract was concentrated to 10 mL on a rotary evaporator and evaporated under a gentle flow of nitrogen gas. Subsequently, 1 mL of solvent extract and 50 µL of internal standard were added to sample and aliquots of 2 µL of the extracts were injected into HPLC for analysis.

High Temperature Reversed Phase Liquid Chromatography

The high temperature HPLC system consisted of a conventional HPLC system coupled with a column oven of a Shimadzu GC-8A Gas Chromatography (Shimadzu Kyoto, Japan). HPLC separations were carried out using JASCO PU-980 HPLC pump for mobile phase delivery. Samples were injected into the system using a 25 µL loop for sample introduction. 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). A 30 cm x length of stainless-steel tubing was placed in the oven between the injection valve and the column as pre-heating coil. The column used in this research was packed with silica (Hypersil ODS, 5µm)(4.6mm I.D. x 100mm, Zorbax HPLC column, Agilent Technologies). The column and the preheating coils were placed together in the oven. A thermometer (Zecol, England) with temperature range of 20ºC-360ºC was placed inside the oven to measure the exact oven temperature. mobile phase: acetonitrile-water: 40-60 v/v, flow rate: 2.5 mL/min, UV wavelength at 254 nm.

Results and Discussion

Comparison of PLE with Soxhlet Extraction

In this study, PLE and Soxhlet extraction were carried out to determine whether the PLE method developed was in good agreement with conventional Soxhlet extraction method. The extraction efficiencies of PLE employing optimum extraction conditions were compared with the efficiencies of Soxhlet extraction for the extraction of PAHs from soil samples. PLE was completed in about 40 min for each extraction condition with 5 min for static extraction time whereas Soxhlet extraction was carried out for 20 h (follow EPA Method 3540). In this study, 5 min of static time was used because no difference in recovery was observed when longer static extraction time was used.

The quantities of representative PAHs extracted by each method tested at spiked 20 ppm PAHs in soil sample is shown in Table 1. PLE method showed good reproducibility for the majority of PAHs. Fluoranthene showed the highest recovery (94.4 %) followed by fluorene (92.3 %), phenanthrene (88.5 %) and naphthalene (81.5 %). Based on data gathered, all PAHs are successfully extracted using PLE with minimum percentage recovery 81.47 %. High temperature at 180 °C along with polar solvent dichloromethane-acetone was found to be necessary to extract the PAHs quantitatively from soils.

Table 1: Comparison of extraction efficiencies of spiked 20 ppm PAHs in soil samples between Soxhlet and PLE

	% Recovery (R.S.D.)	
Compound	**Soxhlet**	**PLE (180 °C/150 bar)**
Naphthalene	34.31(8.3)	81.5(1.0)
Fluorene	82.2(2.7)	92.3(1.1)
Phenanthrene	90.1(6.1)	88.5(1.8)
Fluoranthene	73.6(1.2)	94.4(0.2)

*Soxhlet extraction conditions: solvent, 150 mL dichloromethane-acetone (50:50, v/v) heating 20 hours

Higher recoveries are shown for naphthalene using PLE with 81.5 % compared with Soxhlet extraction 34.31 %. It was probably because of increased solvent penetration from increased swelling of soil particles [4]. Similar result has been reported by Burkhardt *et al*. [4] whereby the extraction of PAHs compounds such as naphthalene and fluorene are more efficient using PLE compare to Soxhlet extraction. Saim *et al.* [5] also reported that fluoranthene show the highest recovery using Soxhlet extraction compared with pressurized liquid extraction. In comparison to the established methods such as sonication and Soxhlet, PLE offers the advantage of faster extraction times and lower solvent consumption.

Application of the Developed PLE Method to the Analysis of Soil Samples

PLE of soil samples were performed at 180°C and pressure of 150 bar using dichloromethane-acetone 50:50 (v/v) with single 5 min extraction step. Sample 1-2 were collected near palm oil trees were black loamy sand and Sample 3-5 were obtained near a river were clay sand. All soils were sieved through a 2 mm sieve. Results of the HT-HPLC analysis are shown in Table 2. The PAHs detected in the samples are 2.2-60.7 µg/mL. Phenanthrene (4.1 to 49.4 µg/mL) fluorene (10.1 µg/mL and 11.6 µg/mL) fluoranthene (2.2-60.7 µg/mL). However, naphthalene was not detected in the soil samples.

Table 2: PAHs concentrations in five soil samples using developed PLE method followed by HTLC determination

	Compound	**Concentrations (ppm)**	**R.S.D.**
Sample 1	Phenanthrene	8.9	0.66
	Fluoranthene	60.7	0.42
Sample 2	Phenanthrene	49.4	0.73
	Fluoranthene	23.7	0.60
Sample 3	Fluorene	11.6	0.81
	Fluoranthene	2.4	0.70
Sample 4	Fluorene	10.1	0.54
	Phenanthrene	4.1	1.00
	Fluoranthene	2.2	0.50
Sample 5	Phenanthrene	38.7	0.26
	Fluoranthene	42.8	0.60

Figure 2 shows HT-HPLC chromatograms of the PAHs extracted from five soil samples. Based on the chromatogram of HT-HPLC, phenanthrene and fluoranthene peaks were observed in all samples except sample 3. High amount of phenanthrene was detected in Sample 2 (49.4 µg/mL) and Sample 5 (38.7 µg/mL). Fluoranthene were detected at high concentrations in Sample 1 (60.7 µg/mL), followed by Sample 5 (42.8 µg/mL) and Sample 2 (23.7 µg/mL). Fluorene were detected in Sample 3 and 4 with concentrations of 11.6 µg/mL and 10.1 µg/mL, respectively. Lower concentration of fluoranthene were obtained in Sample 4 (2.2 µg/mL) and Sample 3 (2.4 µg/mL). All peaks were identified by comparing the retention time of standard PAHs and samples. The results indicated that the differences in PAH content determined in different type of soil samples depend closely on the interactions with the solid and liquid matrix components, interferences of the macromolecular compounds of the matrix and owing to their various volatility [6].

The results obtained indicate that the developed method can be applied to the analysis of PAHs in soils. Popp *et al.* [7] reported a similar study explained that phenanthrene (581 ng/g) and fluoranthene (823 ng/g) were mostly detected in contaminated soil from river Mulde, German. The higher recovery obtained in that experiment because finest size particle of soils was analysed caused increased permeability of PAHs in the soils therefore it were easier to extract the PAHs. Filipkowska *et al.* [6] show the average recoveries of PAHs from raw sediment samples was approximately 62% and phenanthrene and anthracene was observed in lower recovery, 47% and 21% respectively. All these are due to diverse a physical property which includes sorption, solubility and volatility of the compounds studied.

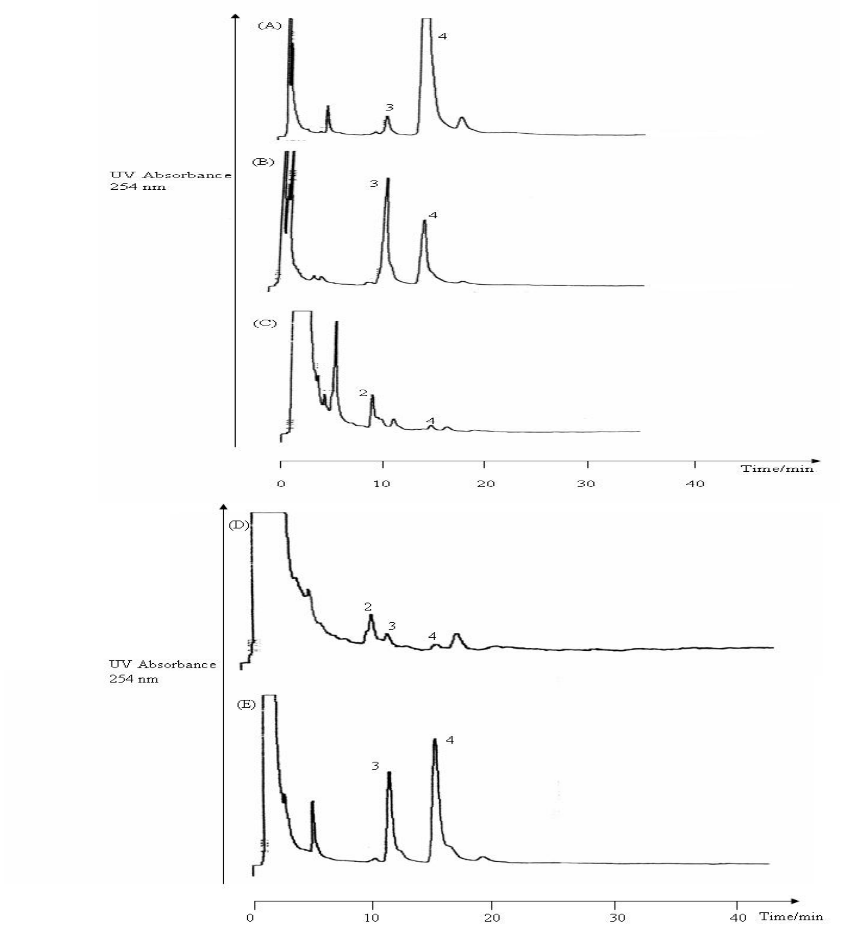

Figure 2: Chromatograms of PAHs extracted from soil samples using HTLC. (A) Sample 1; (B) Sample 2; (C) Sample 3; (D) Sample 4; (E) Sample 5. Chromatographic conditions: ODS-silica column (100 mm × 4.6 mm I.D.); mobile phase 40:60 (v/v); flow rate 2.5 mL/min; temperature 70°C; UV absorbance 254 nm; injection volume 5µL. Peaks: (2) fluorene; (3) phenanthrene; (4) fluoranthene.

Conclusions

PLE showed excellent efficiency in term of total extraction time, total solvent usage as well as the method reproducibility. PLE method demonstrated an outstanding performance with dichloromethane-acetone 50:50 (v/v) as solvent extraction, pressure 150 bar and extraction temperature 180 °C.

PLE have shown to be more powerful techniques compared to Soxhlet extraction for soil samples. Saving in time, solvent usages are possible due to the high extraction efficiencies at high temperature /pressure conditions. This PLE method of soils sample preparation has advantages over conventional Soxhlet extraction for sample automation, reduced extraction time and reduced solvent volume.

Acknowledgement

We thank Universiti Teknologi Malaysia and The Ministry of Science, Technology and Innovation, Malaysia (MOSTI) for financial supports through IRPA Project 09-02-06-0074 EA211 Vote No. 74255.

References

1. Wan, H.B. and Wong, M.K. 1996. Minimization of Solvent Consumption in Pesticide Residue Analysis. J. Chromatogr. A. 754:43-47.
2. Bjorklund, E., Bowardt, S., Nilsson, T. and Mathiasson, L. 1999. Pressurized Fluid Extraction of Polychlorinated Biphenyls and Polycyclic Aromatic Hydrocarbons in Solid Environmental Samples. J. Chromatogr A. 836:285-293.
3. Song, Y.F., 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.
4. Burkhardt, M.R., Revello, R.C., Smith, S.G. and Zaugg, S.D. 2005. Pressurized Liquid Extraction Using Water/Isopropanol Coupled with Solid-Phase Extraction Cleanup for Industrial and Anthropogenic Waste-Indicator Compounds in Sediment. Anal. Chim. Acta. 534:89-100.
5. Saim, N., Dean, J.R., Abdullah, M.F. and Zakaria, Z. 1997. Extraction of Polycyclic Aromatic Hydrocarbons From Contaminated Soils using Soxhlet Extraction, Pressurized and Atmospheric Microwave-assisted Extraction, Supercritical Fluid Extraction and Accelerated Solvent Extraction. J. Chromatogr. A. 791:361-366.
6. Filipkowska, A., Lubecki, L. and Kowalewska, G. 2005. Polycyclic Aromatic Hydrocarbons Analysis in Different Matrices of the Marine Environment. Anal. Chim. Acta. 547:243-254.
7. Popp, P., Keil, P., Moder, M., Paschke, A. and Thuss, U. 1997. Application of Accelerated Solvent Extraction Follwed By Gas Chromatography, High Performance Liquid Chromatography and Gas Chromatography-Mass Spectrometry For The Determination of Polycyclic Aromatic Hydrocarbons, Chlorinated Pesticides and Polychlorinated Dibenzo-p-dioxin and Dibenzofuran in Soild Wates. J. Chromatogr. A, 774:203-211.