
GC/MS Monitoring of Selected PAHs in Soil Samples Using Ultrasound-assisted QuEChERS in Tandem with Dispersive Liquid-Liquid Microextraction

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Abstract: Herein, a method consisting of ultrasound-assisted QuEChERS in tandem with dispersive liquid-liquid microextraction (DLLME) was developed for monitoring of selected polycyclic aromatic hydrocarbons (PAHs) in various soil samples using gas chromatography coupled to a single quadrupole mass spectrometry (GC/MS). Ultrasound-assisted QuEChERS was employed to extract the PAHs from 2.0 g of soil using 7 ml of initial extraction solvent (acetonitrile: water (5:2 v/v)) and the salt mixture. The resulting supernatant extract was cleaned through the addition of C₁₈, PSA and the mix salt followed by centrifugation, decantation and filtration. Of the clean organic phase, 1.0 ml was withdrawn and added with 12 μ l of C₂Cl₄ (disperser solvent). The resulting mixture was then injected rapidly into an aqueous sample (5.0 ml) by a syringe for further preconcentration. As a result, the cloudy solution consisting of fine particles of the extraction solvent dispersed into the aqueous phase was formed. After centrifuging, the fine particles were sedimented at the bottom of the conical test tube (5.0 \pm 0.5 μ l). Of which, 1.0 μ l was injected to the GC/MS for monitoring of the PAHs. Several influential parameters including ultrasound extraction time, initial extraction and disperser solvent and their respective volumes were all evaluated to achieve the optimal conditions. Under the optimal conditions, limits of quantification (2.5-4.0 ng/g) and linear ranges ($r^2 \geq 0.98$) were obtained for the PAHs. The method was then successfully applied for the extraction and monitoring of the PAHs in the real soil samples. Accuracy of the method was evaluated by the relative recovery experiments on spiked samples with the results ranging from 81 to 92%. In the mean time, the relative standard deviations (RSDs) were found to be in the range of 4.8–15.9%.

Keywords: PAHs, Ultrasound-Assisted QuEChERS, Dispersive Liquid-Liquid Microextraction, Soil Samples

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are found in environmental depositions, as a result of environmental pollution (e.g. oil spills) and can arise due to human activity (e.g. traffic, incineration, etc.) [1]. These pollutants have a high persistence in the environment, low bio-degradability and high lipophilicity and toxicity [2-4].

Soil is a complex and heterogeneous matrix, containing both inorganic and organic components [5] and is often subject to intense chemical pollution. When chemical compounds reach the soil, either via direct intentional application or as a result of accidental spillage [6, 7].

The amount of PAHs in soils is of importance because of their toxicity to humans and their effects on soil organisms

and plants [8]. As a result of their widespread presence, PAHs are generally introduced in monitoring programs [9] and are listed by the US Environmental Protection Agency (EPA) and the European Community as priority pollutants [10].

Due to the low concentration levels of soil pollutants, sample preparation step is needed to determine the type and quantity of pollutant present [11] and to avoid interferences and improve the sensitivity of the method. To extract contaminants from soil, a technique strong enough to extract bound residues is necessary [12].

Amongst the methods commonly applied for the extraction of pollutants from soil, QuEChERS is very flexible, modifiable, and is growing in popularity due to all the benefits described by its name: Quick, Easy, Cheap, Effective, Rugged and Safe. However, its effectiveness is dependent on the analyte properties, matrix composition, equipment, and analytical

technique available in the laboratory [13-15].

Although it is possible to detect PAHs at low levels by GC/MS analysis which follows QuEChERS method; however, the use of another extraction procedure for better clean-up and further preconcentration is needed to reduce the effects of soil's matrix as much as possible. For that reason, in this study a highly efficient preconcentration procedure known as dispersive liquid-liquid microextraction (DLLME) along with QuEChERS was employed [16-18].

The present study outlines a novel modified method in which the initial extraction of selected PAHs was carried out by ultrasound assisted QuEChERS treatment followed by a further preconcentration by DLLME procedure. Our aim was focused at studying the feasibility of DLLME procedure for further preconcentration along with ultrasound assisted QuEChERS treatment in soil's PAHs analysis. To the best of our knowledge, so far this has been the first report on the extraction and measurement of PAHs in soil samples based on the combination of ultrasound assisted QuEChERS treatment and DLLME procedure.

2. Instruments

The gas chromatographic system consisted of an Agilent (Centerville Road, Wilmington, USA) series 7890A GC coupled to an Agilent MSD 5975C quadrupole mass spectrometer. The GC was fitted with HP-5 MS capillary column (30 m \times 0.25 mm i.d., 0.25- μ m film thickness) from Agilent J&W Scientific (Folsom, CA, USA). Helium (99.999%) was used as the carrier gas at the flow rate of 1.0 ml/min. The following temperature program was employed for the separation: 80 °C for 1 min, increased to 280 °C at 8 °C/min, and held for 6 min; finally increased to 300 °C at 50 °C/min and held for 3 min. The MS quadrupole and the MS source temperatures were set at 150 and 230 °C, respectively. Data acquisition was performed in the full scan mode (m/z in the range of 50 - 400) to confirm the retention times of analytes and in selected ion monitoring (SIM) mode (see Table 1) for quantitative determination of PAHs. A dwell time of 100 ms was used for each mass operated at SIM mode with high resolution. The filament delay time was set at 3 min. A vortex-homogenizer was applied to homogenize both the blank and real dried soil samples prior to each extraction. An ultrasound probe (UP-500 ultrasound homogenizer) from ECHROM Company (Avagene, Taiwan) was used for the extraction of the test PAHs from dried soil matrices.

3. Experimental

3.1. Samples and Reagents

The test PAHs (acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene and chrysene) was purchased from Aldrich (Milwaukee, WI, USA). Analytical grade acetonitril (ACN), tetrachloroethylene (C₂Cl₄), Acetone, anhydride magnesium sulfate (MgSO₄) and sodium chloride (NaCl) were supplied by Merck Company (Darmstadt,

Germany). Primary secondary amine (PSA) and octadecylsilane (C₁₈) sorbents were obtained from Varian (Varian, Harbor City, CA). Pure water was provided by a home-made deionizer. Stock standard solutions (500 mg/L) of PAHs were prepared in acetonitrile and then they were diluted with acetone (as working solutions) and then all stored at -20°C. A number of soil samples were collected twice a week for one month in order to obtain the representative samples over this period of time. Upon their arrival at the laboratory, the samples were dried and sieved in order to obtain a fraction of <1 mm and maintained at 4°C until the time of analysis. For constructing the calibration curve, ten standard solutions in the range of 2.0–100 ng/g were prepared by spiking the working solution into the blank samples- later confirmed to be the PAHs free. The standard solutions were stored at -20°C until final analysis. Meanwhile, the suspected soil samples (hereafter, called real samples) were taken from three different parts of Tehran (Tehran, Iran).

3.2. Sample Preparation

3.2.1. Extraction Procedure

1) 2.0 g of the treated soil sample (already dried, filtered and spiked/non-spiked with the PAHs) was placed in a 50-mL ultrasound probe.

2) 7.0 mL of ACN:Water (5:2, v/v) was added to the probe and it was vibrated using ultrasound energy for 3 min.

3) The mix salt (MgSO₄:NaCl; 4:1 g) was added to the above solution.

4) The resulting mixture was immediately shaken vigorously for 2 min and centrifuged at 3000 rpm for 5 min.

3.2.2. Clean-Up

5) The upper organic phase was removed and transferred to an Eppendorf vial. Then it was added with 50, 50 and 150 mg of C₁₈, PSA and MgSO₄, respectively.

6) The final extract (the supernatant) was filtered through a 0.22 μ m PTFE syringe filter to obtain ~ 1ml of ACN.

3.2.3. Preconcentration

7) 12 μ l of tetrachloroethylene (C₂Cl₄) was added to the ACN and the resulting mixture was rapidly injected to a 5.0 ml of pure water for further preconcentration.

8) The sedimented organic phase (5 \pm 0.5 μ l) was immediately formed and withdrawn. Of which, 1.0 μ l was injected to a GC/MS for identification and measurement of the PAHs.

4. Evaluation of Analytical Parameters

The evaluated analytical parameters include: Selectivity, relative recovery, precision (RSD), linearity and limit of quantification (LOQ). They were all evaluated for each PAH using spiked and non-spiked blank soil samples.

4.1. Selectivity

The selectivity of the method was demonstrated by injecting the diluted working solution of the test PAHs. In addition, six blank samples were analyzed to check if there

were any interfering peaks in the chromatogram that could potentially complicate the analysis.

4.2. Relative Recovery and Precision

Relative recovery was determined using the following equation:

$$\% \text{ Relative recovery} = 100 \times (C_{\text{found}} - C_{\text{real}}) / C_{\text{added}}$$

Where, C_{found} and C_{added} are the concentration of the concerning PAH in the real/blank sample after and before the addition of known amounts of the working solution, respectively. Meanwhile, precision (RSD%) of the method was investigated on three replicate experiments without any internal standard.

4.3. Linearity and Evaluation of LOQ

Linearity of the method was tested by spiking the blank samples at ten concentration levels over the range of 2.0-100.0 ng/g for the test PAHs. Calibration curves were constructed by plotting the PAH signal obtained, which was the average of three measurements against the respective concentration of the test PAH. The LOQs were determined based on the following definition: The lowest concentration at which the error falls between -20% and +20% with the maximum RSD of 20% obtained under three measurements.

5. Results and Discussion

5.1. Optimization of Ultrasound Assisted QuEChERS

To find the optimum conditions for the test PAHs extraction in the soil samples, initially sonication time was tested in the range of 1–5 min. The result revealed that the recovery of the PAHs slightly enhanced, whilst the ultrasonic treatment was increased from 1 to 4 min. However, the recovery remained almost unchanged beyond 4 min (date not shown). Therefore, 4 min was taken as the optimum sonication time. Further on, the variation in the volume ratio of ACN:Water was made in such a way that the extracted upper layer after the ultrasound assisted QuEChERS treatment was 2 ml right before the clean-up process. Accordingly, the ratio of ACN:Water 5:2 ml was applied for the initial extraction of the PAHs from the soils. Other factors such as the amount of sample (2.0 g), the sonication parameters (50 KHz, 110 W), and the mix salt amounts ($\text{MgSO}_4:\text{NaCl}$; 4:1 g) and the clean-up materials (50, 50 and 150 mg of C_{18} , PSA and anh. MgSO_4) were maintained invariable due to being reported in the literature [7, 19].

5.2. Optimization of the Influential Parameters in DLLME

To the clear supernatant (~1 ml of ACN) obtained from the clean-up process, different amounts of C_2Cl_4 , CCl_4 , CHCl_3 were added in order to find out the optimum conditions. It should be noted that the sedimented phase, resulting from the three above-mentioned solvents later must fall in the range of $5 \pm 0.5 \mu\text{l}$. Accordingly, the optimal conditions of DLLME

was found out to be $12 \mu\text{l}$ of C_2Cl_4 as reported elsewhere [16] (see Figure 1,2). It is noted that the PAHs recovery from the spiked soil samples at 10 ng/g level was taken as the response of the optimization experiments.

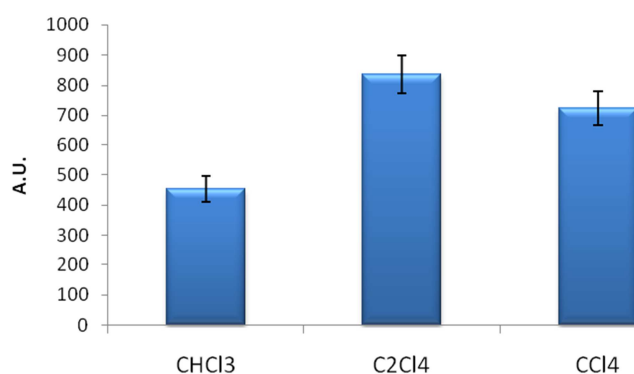


Figure 1. The effect of type of extraction solvent used in DLLME procedure.

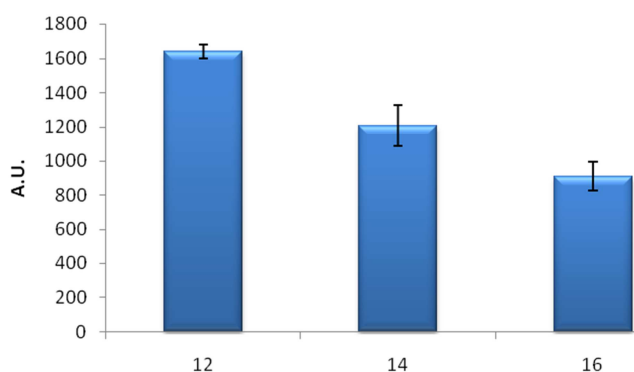


Figure 2. The effect of volume of C_2Cl_4 (μl) on the extraction efficiency of the method.

5.3. Results Regarding the Analytical Parameters

According to the results obtained, there were no interfering peaks, originating from the blank sample matrix or the chemicals and reagents used, at the same retention time of the test PAHs in any of the six blank samples studied in the selectivity experiments (Figure 3B).

The results also demonstrated a fair linearity for the test PAHs within the mentioned-above test range with the minimum correlation coefficient of 0.98. The LOQs were found to be in the range of 2.5-4.0 ng/g based on the following definition: The lowest concentration at which the error falls between -20% and +20% with the maximum RSD of 20% obtained under three measurements (see Table 1).

Table 1. Analytical data of the method.

PAHs	SIM	LOQ (ng/g)
Acenaphthylene	152	4.0
Acenaphthene	153	3.5
Fluorene	166	2.5
phenanthrene	178	2.0
Anthracene	178	2.0
Fluoranthene	202	2.0
Pyrene	202	2.5
Chrysene	228	3.0

5.4. Analysis of Real Samples

Application of ultra-assisted QuEChERS along with DLLME to the soil samples from three different soil samples was assessed. Of the three suspected samples analyzed, none were found to be contaminated with the test PAHs. The method was successfully applied to the analysis of the test PAHs in the above-mentioned samples spiked at 5.0 ng/g level to investigate the extraction efficiency of the method.

The RSD% results based on three similar measurements

were within the range of 4.8–15.9%, as presented in Table 2. The Table's data also demonstrated good relative recoveries in the range of 81–92%, indicating that the applied ultrasound QuEChERS combined with DLLME is highly efficient for the measurement of the test PAHs in various soil samples. The GC/MS chromatograms relating to A) the standard solution sample and B) the blank sample are shown in Figure 3. As can be seen and deduced, the chromatograms are almost free of matrix effects.

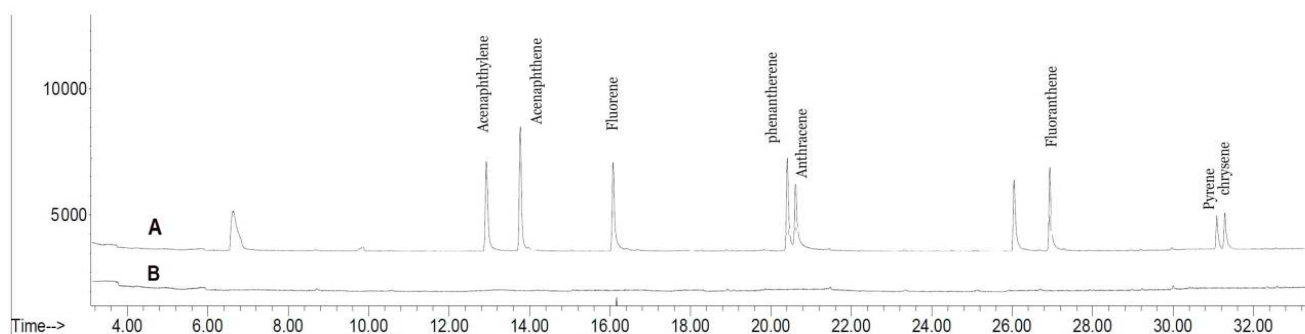


Figure 3. The GC/MS chromatograms relating to A) the standard solution and B) the blank sample.

Table 2. Analysis of real samples.

PAHs	1			2			3		
	^a RC	Found ^b	RR (%)	RC	Found	RR (%)	RC	Found	RR (%)
Acenaphthylene	ND	4.0 ± 0.6	81	ND	4.3 ± 0.6	86	ND	4.3 ± 0.4	85
Acenaphthene	ND	4.2 ± 0.5	84	ND	4.0 ± 0.4	81	ND	4.2 ± 0.6	84
Fluorene	ND	4.6 ± 0.4	92	ND	4.0 ± 0.3	81	ND	4.1 ± 0.5	82
Phenanthrene	ND	4.4 ± 0.7	85	ND	4.2 ± 0.5	83	ND	4.6 ± 0.6	91
Anthracene	ND	4.6 ± 0.5	91	ND	4.1 ± 0.6	82	ND	4.6 ± 0.5	92
Fluoranthene	ND	4.1 ± 0.2	82	ND	4.2 ± 0.6	84	ND	4.5 ± 0.5	90
Pyrene	ND	4.2 ± 0.6	83	ND	4.6 ± 0.5	91	ND	4.3 ± 0.6	86
Chrysene	ND	4.4 ± 0.5	88	ND	4.5 ± 0.4	89	ND	4.6 ± 0.4	92

a: real concentration; b: mean ± sd (n=3) ; c: non-detected

6. Conclusion

The present study centers on a novel modified method in which the initial extraction of selected PAHs is carried out by ultrasound assisted QuEChERS treatment followed by a further preconcentration by DLLME procedure. Several influential parameters including ultrasound extraction time, initial extraction and disperser solvent and their respective volumes were all evaluated to achieve the optimal conditions. Afterwards, the method was successfully applied to the analysis of the test PAHs in real soil samples. Finally, the method seems to be simple, low cost and highly efficient for the analysis of PAHs in soil samples.

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