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Determination of Polycyclic Aromatic Hydrocarbons in Biological Samples by Dispersive Liquid-Liquid Microextraction and Gas Chromatography

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ABSTRACT

This work presented determination of some polycyclic aromatic hydrocarbons such as naphthalene, phenanthrene and acenaphthene in biological samples by Dispersive Liquid-Liguid Microextraction (DLLME) and gas chromatography. In this method, the appropriate mixture of extraction solvent (dichloromethane) and disperser solvent (acetone) are injected rapidly into the aqueous sample by syringe. Therefore, cloudy solution is formed which consisted of fine particles of extraction solvent dispersed entirely into aqueous phase. The mixture was centrifuged and the extraction solvent is sedimented on the bottom of the conical test tube. Sediment phase injected into the GC. Some important parameters such as kind of extraction and disperser solvent and volume of them, extraction time were optimized.
Under optimum conditions, the LOD ($3S_b/m$) between 0.03-0.04 µg/l, LOQ, 0.1-0.12µg/l and (RSD = 1.14-2.08%, n = 5) for PAHs was obtained. Enrichment factor for naphthalene 228, for phenanthrene 235 and for acenaphthene 202 was obtained. The method was applied on real sample (urine) and the recovery of method was 85.41-91% for PAHs.
KEY WORDS: Dispersive liquid-liquid micro extraction (DLLME), Polycyclic aromatic hydrocarbons (PAHs), Gas Chromatography, Biological samples.

1. INTRODUCTION

Polycyclic aromatic hydrocarbons are a large group of over 200 different compounds containing two or more fuse aromatic rings made of carbon and hydrogen atoms [1]. PAHs containing up to four fused benzene rings are known as light PAHs and those containing more than four benzene rings are called heavy PAHs [2]. They are also hydrophobic compounds with very low water solubility and, therefore, they often occur at typically low levels in complicated and polluted aquatic environment [3]. Polycyclic aromatic hydrocarbons (PAHs) are important environmental pollutants which originating from a wide variety of natural and anthropogenic sources including fossil fuel combustion, oil spills and some industrial process [4-7]. They are highly toxic for plants as well as for animals in the form of both acute and chronic intoxication depending on time of exposure and concentration of PAHs. Intoxication of animals is connected with hormone imbalance, hepatotoxicity, anemia and changes in blood count [8]. Due to hazardous characteristics, it is necessary to develop suitable methods for the analysis of PAHs in the whole water sample [9].The main problem with PAHs monitoring is their low concentration and complexity of environmental matrices. As a result, pre concentration and separation are needed to achieve the required sensitivity and selectivity [9-14]. In recent years, the development of fast precise, accurate and sensitive methodologies has become an important issue. The extraction of PAHs from environmental water samples is usually performed using conventional liquid-liquid extraction (LLE) or solid phase extraction (SPE). However, this method require large amount of organic solvent that are often poisonous and hazardous and the procedure is time consuming and tedious. Efforts have focused on the miniaturization of the SPE or LLE extraction procedure by greatly reducing the amount of organic solvent required, leading to the development of solid phase microextraction (SPME) or liquid phase microextraction (LPME) [3], and cloud point extraction (CPE). DLLME is a new mode of LPME which introduced for pre concentration of trace amount of PAHs. The method is based on the appropriate mixture of extraction solvent and dispersive solvent. The advantages of DLLME method are simplicity of operation, rapidity, low cost, high recovery and enrichment factor.

The performance of DLLME for pre concentration of naphthalene, phenanthrene and acenaphthene in water and biological samples before determination by (GC-FID) was investigated.

The performance of various experimental parameters which affected the results such as kind of extraction and disperser solvent and volume of them, extraction time was investigated.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

All PAHs (naphthalene, acenaphthene and phenanthrene) were purchased from Merck (Germany). Ethanol, methanol, acetone and acetonitrile (suprasolv for gas chromatography) were purchased from Merck (Germany). Dichloromethane, tetrachloroethtylene, carbon tetrachloride, carbon disulfide, chloroform (GC grade) were obtained

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from Merck (Germany). Sodium bicarbonate, sodium sulphate and sodium chloride were obtained from Merck (Germany). Ultra pure water (Milli-Q plus system, Millipore, Bedford, MA, USA) was used throughout the work.

2.2. Instrumentation

A gas chromatography (Varian 380) with a split/splitless injector system equipped with flame ionization detector was used for separation and determination of PAHs. Ultra pure helium (99.9999%, Air products, UK) was made to pass through a molecular sieve trap and oxygen trap (Crs, USA) was used as the carrier gas. The injection port was held at 300^0C and used in the splitless mode with splitless time 1 min. The column temperature was held at 300^0C. Separation was carried out on a CP-sil 5, 50m, i.d: 0.32 mm. The oven temperature was programmed as follows: initial 60 ^{0}C for 3 min, from 230 ^{0}C (held 10/5 min) to 247 ^{0}C (held 2 min) at the rate of 20 ^{0}C/min, 253 ^{0}C (held 7 min) at the rate of 1 ^{0}C/min and 280 ^{0}C (held 1 min) at the rate of 40 ^{0}C/min. The total time for one GC run was 23.5 min.

2.3. DLLME Procedure

The sample solution (5 mL) containing the interest was placed in a 15 mL glass test tube with conical bottom. Acetone (0.5 ml) (as disperser solvent) containing 200 µL dicholoromethane (as extraction solvent) was rapidly injected into the sample solution using a 2.00 mL syringe. A cloudy solution was consequently formed. Then the mixture was gently shaken, in order to separate the phases, the mixture was centrifuged for 4 min at 3500 rpm/min. For aqueous standards, the extraction solvent (dichloromethane) was sedimented at the bottom of the conical test tube. Sedimented phase was dissolved by 0.2 mL of methanol. Then the extract solution was filtrated through a 0/45 µm filter to eliminate the white floccules and finally 1 µl of it injected into the GC for determination of PAHs.

3. RESULTS AND DISCUSSION

To obtain high extraction efficiency, it is necessary to investigate the effect of all parameters that can probably influence the extraction. In DLLME method, these parameters include the type and the volume of the extraction and the disperser solvent and effect of extraction time. The peak area of the analytes was used to evaluate the extraction efficiency under different conditions.

3.1. Selection of the extraction solvent

The type of extraction solvent in DLLME is an essential parameter. According to the DLLME principles, the selection of extraction solvent should demonstrate special characteristics including (a) extraction capability of interest compounds, (b) low solubility in water, (c) higher density than aqueous phase, (d) formation of tiny droplets in presence of a dispersive solvent, and (e) good chromatographic behavior. Based on these considerations, Dichloromethane displayed the highest extraction efficiency. It is may be because of high extraction capability of dichloromethane in a comparison with other solvents. Consequently, dichloromethane was selected as the extraction solvent. The result of different extraction solvent was presented in Fig 1.

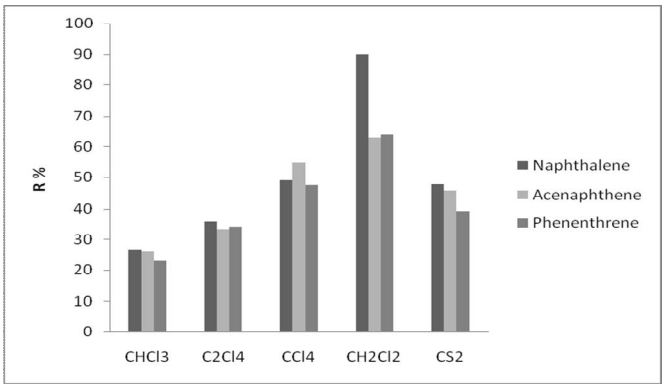

Fig. 1. Effect of different extraction solvent on the recovery of PAHs

3.2. Effect of extraction solvent volume

The influence of the extraction solvent volume on the extraction efficiency was investigated; appropriate amount of dispersive solvent (acetone) containing different volumes of dichloromethane in the range of 50-300 µL were subjected to the same DLLME procedure. It was observed that by increasing the extraction solvent volume, the sedimented phase volume increased linearly. Based on these observations, 200 µL of dichloromethane was chosen for further experiments. The result of different volumes of extraction solvent was presented in Fig 2.

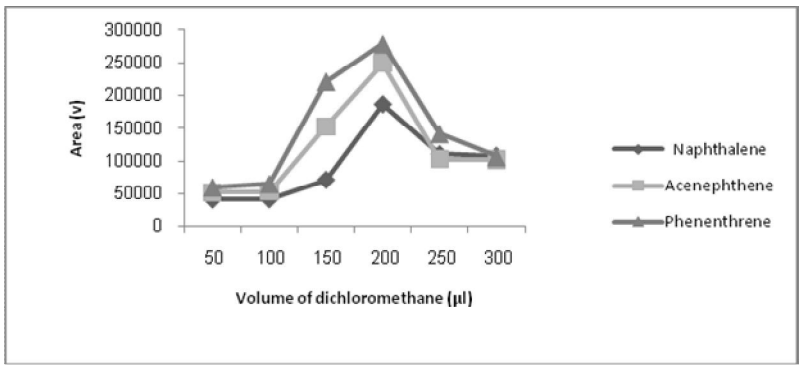

Fig. 2. Effect of different volume of dichloromethane on the extraction of PAHs

3.3. Selection of the disperser solvent

Selection of disperser solvent was based on miscibility of the disperser solvent with both aqueous phase (sample solution) and extraction solvent and capability of dispersing extraction solvent as very fine droplets in aqueous phase. Acetone, methanol, ethanol and acetonitrile were used to investigate the influence of these solvents on the DLLME performance. According to the results (Fig 3), the extraction efficiency was higher using acetone compared with the other solvents. This is probably due to its higher compatibility of acetone with aqueous solution than ethanol, methanol and acetonitrile. Hence, acetone was selected in subsequent studies.

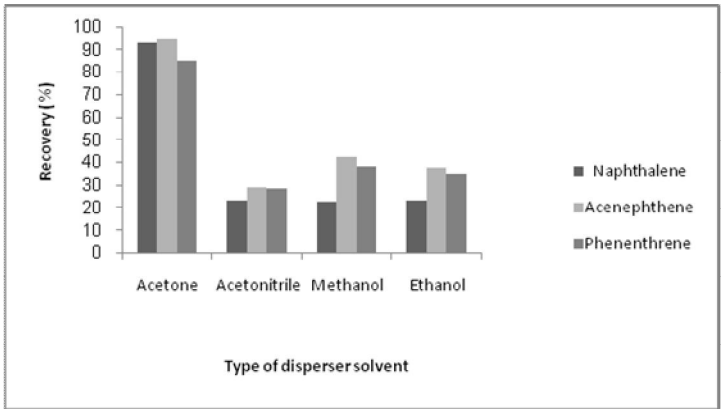

Fig. 3. Effect of different disperser solvent on the recovery of PAHs

3.4. Effect of disperser solvent volume

For optimization of dispersive solvent volume, different volumes of acetone containing 0.25, 0.5, 1, 1.5, 2 and 2.5 ml were used with the constant volume of dichloromethane. It was observed that the peak area of the PAHs increased with increasing dispersive solvent volume. This may be attributed to the fact that at first, acetone cannot disperse dichloromethane properly and tiny droplet formation may not be effective, while at high volumes, the solubility of the PAHs in water was increased and thereby, the extraction efficiency was decreased. Subsequently, the volume of 0.5 mL was chosen as the optimum volume of the dispersive solvent. The result of different volumes of disperser solvent was presented in Fig 4.

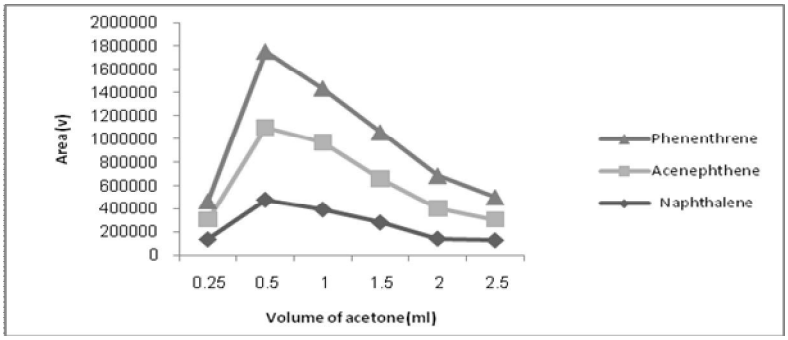

Fig. 4. Effect of different volume of acetone on the extraction of PAHs

3.5. Effect of extraction time

In DLLME, extraction time is defined as interval time between the injection of the mixture of disperser solvent (acetone) and extraction solvent (dichloromethane), before starting to centrifuge. The effect of extraction time was examined in the range of 2 to 5 min under constant experimental conditions. The obtained results showed that the extraction time had no significant effect on the peak area of PAHs. Therefore, the DLLME method was time independent, which was the most important advantage of this technique. In this method, the most time-consuming step was the centrifuging of sample solution in the extraction procedure, which took about 3 min. The result of different extraction time was presented in Fig 5.

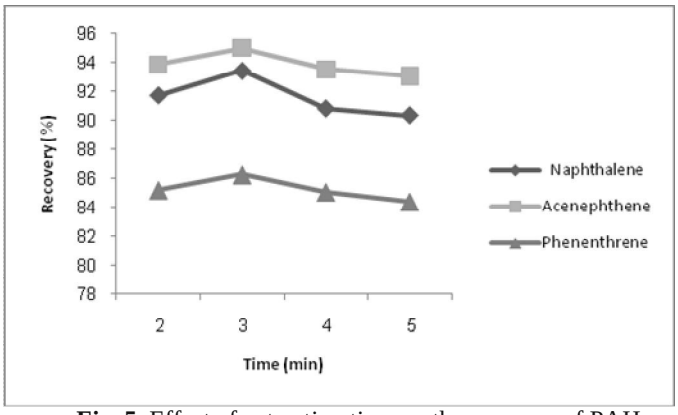

Fig. 5. Effect of extraction time on the recovery of PAHs

3.6. Analytical figure of merit

The characteristic of calibration curves was obtained under optimized conditions. LOD of the method was obtained between 0.03-0.04µg/l for ($3S_b/m$), LOQ ($10S_b/m$) was 0.1-0.12 µg/l and (RSD = 1.14-2.08%, n=5) was achieved. The method was applied on real sample (urine). The recovery of method for water sample is 89.14-95.02%, for urine sample is 85.41-91%.

4. Conclusions

This paper described a DLLME–GC–FID method for the analysis of Polycyclic aromatic hydrocarbons in biological and water samples. In this method, sample preparation times as well as consumption of toxic organic solvents have been minimized without affecting the sensitivity of the method, compared to other extraction methods such as SPE and SPME, the presented method has much shorter extraction time (3 min). This method is also convenient, cost effective and sensitive, which can be used for the determination of PAHs in biological samples and water samples.

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