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Determination of polycyclic aromatic hydrocarbons using lab on valve dispersive liquid–liquid microextraction coupled to high performance chromatography

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ABSTRACT

In this work, dispersive liquid–liquid microextraction (DLLME) method was applied for high performance liquid chromatography (HPLC) determination of 15 PAHs in aqueous matrices.The extraction procedure was automated using a system of multisyringe flow injection analysis coupled to HPLC instrument with fluorescence detector. Factors affecting the extraction process, such as type and volume of extraction and dispersive solvent, extraction time and centrifugation step were investigated thoroughly and optimized utilizing factorial design. The best recovery was achieved using 100 μL of trichloroethylene as the extraction solvent and 900 μL of acetonitrile as the dispersive solvent.The results showed that extraction time has no effect on the recovery of PAHs. The enrichment factors of PAHs were in the range of 86–95 with limits of detection of 0.02–0.6 μg L^{-1}. The linearity was 0.2–600 μg L^{-1} for different PAHs. The relative standard deviation (RSD) for intra- and inter-day of extraction of PAHs were in the range of 1.6–4.7 and 2.1–5.3, respectively, for five measurements.The developed method was used to assess the occurrence of 15 PAHs in tap water, rain waters and river surface waters samples.

1. Introduction

During the last decade the water quality has become one of the principal international concerns for the ecological fate of ecosystems and human health. Numerous pollutants need continuous monitoring, including polycyclic aromatic hydrocarbons (PAHs). These are a wide group of ubiquitous contaminants included to the European Union (EU) and United States Environmental Protection Agency (USEPA) priority pollutant list due to their toxicity and carcinogenic activity [1,2].

Their primary sources of contamination come from natural incomplete combustion processes and anthropogenic emissions, but these latter are generally considered to be the major source of these compounds input into the environment. They can reach surface waters in different ways, including atmospheric deposition, urban run-off, municipal and industrial effluents, oil spillage or leakage.

PAHs are hydrophobic compounds ($\log K_{ow}$=3–8) with very low water solubility [3]. As a result, it is necessary to incorporate a concentration step in the analytical procedure, prior to chromatographic determination in the environmental samples to improve the sensitivity of method. To resolve these problems, different techniques have been proposed. Therefore PAHs are generally extracted from water samples either by liquid–liquid extraction (LLE) [4,5] and solid-phase extraction (SPE) [6,7]. LLE is an effective technique for separation and preconcentration of analytes but requires large amounts of expensive and toxic solvents and uses multistep methods which lead to loss of analytes. SPE uses less solvent than LLE but the sample processing rates are slow and channeling reduces the capacity to retain analytes [8]. Another drawback in SPE, as in LLE, is the considerable amount of time needed and the manual operations involved [9].

Today, the attention is pointed towards the simplification of sample preparation with techniques that are environmental friendly by reduction of the amount of organic solvents. In this sense microextraction methods have attracted much attention in the recent years. Thus, methods such as solid-phase microextraction (SPME) [10,11], stir bar sorptive extraction (SBSE) [12,13], liquid-phase microextraction (LPME) [14,15], and more recently dispersive liquid–liquid microextraction (DLLME) [16,17] as developed as alternatives techniques for classic extraction procedures in the determination of PAHs.

Among these, the most simple, rapid and environmental friendly approach seems to be the DLLME procedure [18], introduced by Rezaee and co-workers [16]. In essence, DLLME is based to the rapid injection of an appropriate combination of solvents to an aqueous sample containing the analytes of interest in a conical test tube. The binary mixture of the solvents consists in a few microliters of a high density extraction solvent with very low water solubility and another, named disperser, with high miscibility in both extractant and water phases; in order to form a cloudy solution consisting of small droplets

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of extraction solvent which are dispersed throughout the aqueous phase.

DLLME is a low cost technique, with minimal organic solvents consumption, high recoveries and enrichment factor and very short extraction time (a few seconds).Their main limitation is its lack of selectivity due to the presence of interferences from matrix, especially in analysis of trace analytes in a complex sample [18]. So far, DLLME coupled with HPLC [11,19,20], supercritical fluid chromatography [21] and gas chromatography (GC) [10,16,22–24], in combination with various detectors are the most usefulness and popular methods for precise quantitative analysis of PAHs.

The main objective of this study was to develop and validate a DLLME–HPLC method for simultaneous determination of 15 PAHs in waters samples. Special attention was given on the optimization of the DLLME procedure by careful evaluation of the type and volume of extraction and dispersive solvents, as well as the effect of extraction time using experimental design. In addition, the optimal conditions of the solubility of PAHs in water were evaluated.

2. Experimental

2.1. Chemicals and reagents

HPLC grade methanol, acetonitrile, and acetone were obtained from Scharlau, (Spain), together with reagent grade methylene chloride and chloroform. GC–MS grade trichloroethylene was purchased from Sigma Aldrich Quimica SA, (Madrid, Spain). Millipore water obtained from a MilliQ Direct-8 purification system Millipore Iberica S.A.U., (Madrid, Spain) was employed to prepare the aqueous solutions after filtration through a 0.45-μm pore size cellulose filter.

A PAH calibration mix standard of naphthalene (Nap), acenaphthylene (Acpy), acenaphthene (Acp), fluorene (Flu), phenanthrene (PA), anthracene (Ant), fluoranthene (FL), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbFl), benzo[k]fluoranthene (BkFl), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DBA), benzo[g,h,i]perylene (BghiP) and indeno[1,2,3-cd]pyrene (IP), at 10 mg L^{-1} in acetonitrile were bought from Supelco (Bellefonte, PA, USA), as a reagent kit containing all 16 priority PAHs listed by the United States Environmental Protection Agency.

2.2. Sample preparation

A stock solution, containing 10 μg mL^{-1} of each analyte, was prepared by suitable dilution of standard PAH kit with acetonitrile and was stored at 4 °C. Water samples were prepared daily by spiking millipore water with analytes at known concentrations to study the extraction procedure under different conditions in order to optimize the method.

To validate the propose method for the separation and preconcentration of PAHs, different types of waters were analyzed. Tap water (TW) was sampled in the laboratory, rain water (RW) was collected in our university campus (University of Balearic Islands, Spain) and streamwater (SR) (Santa Margalida–Mallorca), was collected in October 2012 in glass bottles and stored refrigerated.

Before analysis, aqueous samples were diluted with 5% (*v*/*v*) acetonitrile as organic modifier, and stirred during 24 h to prevent adsorption of PAHs on the PTFE tubing of the flow manifold in order to obtain quantitative recoveries and prevent the cross-contamination between samples. All solutions were filtered through a 0.45 μm cellulose filter.

2.3. Extraction procedure

For the DLLME procedure, an aliquot (4.00 mL) of an aqueous solution containing the analytes was analyzed. A mixture of 900 μL of acetonitrile as dispersive solvent and 100 μL of trichloroethylene as extracting solvent was used.

A cloudy solution was formed. Then the fine droplets of the extraction phase were settled at the bottom of tube. Then an aliquot of 20 μL of the separated phase was aspirated into an injection loop for further analysis.

2.4. Liquid chromatographic analysis

The analysis of the sample were performed by HPLC using a Jasco system constituted by an autosampler (AS-2055 Plus), two pumps (PU-2080 Plus), and a multi-wavelength fluorescence detector (FP 2020 Plus). Separations were carried out on a Vydac RP-18e (250 mm × 4.6 mm) column. The analytical signal was monitored and integrated using ChromNav software.

Gradient elution, (see Table 1), was programmed for total separation of 15 PAHs in 20 min (Fig. 1). The injection volume was 20 μL and the mobile phase was composed of solvent A: acetonitrile–water (60:40) and solvent B: 100% acetonitrile at a flow rate of 1.5 mL min^{-1}.

Detection was performed at different selected fluorescence wavelengths programmed to obtain the better sensitivity and minimal interference for each compound. The excitation/emission wavelengths pairs (nm) were monitored as shown in Table 2.

2.5. Instrumentation

A scheme of the MSFIA system used for the extraction and injection of PAHs in HPLC column is shown in Fig. 2. The manifold was constructed with PTFE tubing of 0.8 mm i.d., except for a two long holding coil 500 cm HC1 and 300 cm HC2, made of 1.5 and 0.5 mm i.d., respectively. Supply tubes for syringe loading and waste discharge were made of PTFE tubing of 1.5 mm i.d.

A multisyringe burette module, a valve module with one rotary eight-port multiposition valve and a rotary six-port micro-injection valve (loop volume 20 μL) from Crison SL (Alella, Barcelona) were used to distribute the liquid through the system. The multisyringe module was equipped with one glass syringe (Hamilton, Bonaduz, GR, Switzerland) of 5 mL, which had a three-way solenoid valve (N-Research, Caldway, NJ) at its head (V).

A Lab on valve (LOV) microconduit (Sciware Systems, Spain) mounted atopthe eight-port multiposition selection valve, fabricated with KelF® (polychlorotrifluoroethylene) and encompassing eight integrated microchannels (0.5 mm i.d./14.0 mm length) avoided the dispersion of solvent and promoted propelling to the extraction chamber (EC).The extraction process occurred at position 6 of LOV in a 5 mL commercial pipette tip adapted through a connector. The HPLC pump and the chromatography column were connected directly to the inject valve to perform the separation analysis. The operations sequence followed for the determination of PAHs is detailed in Table 3. It is very important the order in which the sample and solvent are introduced in the EC, obtaining the best results when the extraction solvent is dispensed followed by the dispersive solvent and sample at the end.

Table 1
Optimized HPLC solvent gradient program of PAH analytes.

Time (min)	Solvent composition	
	% solvent A	% solvent B
0	100	0
6	100	0
14	0	100
20	0	100
26	100	0