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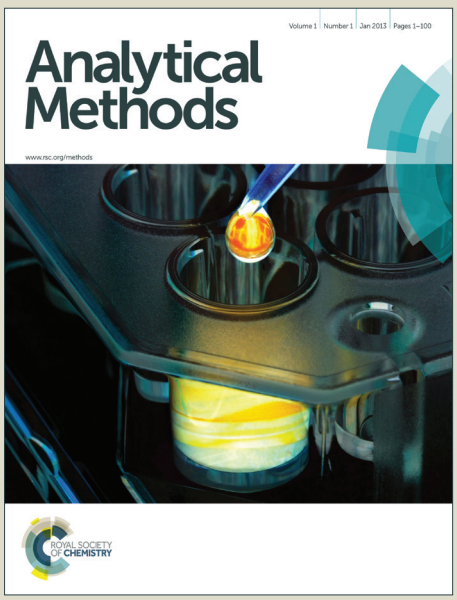

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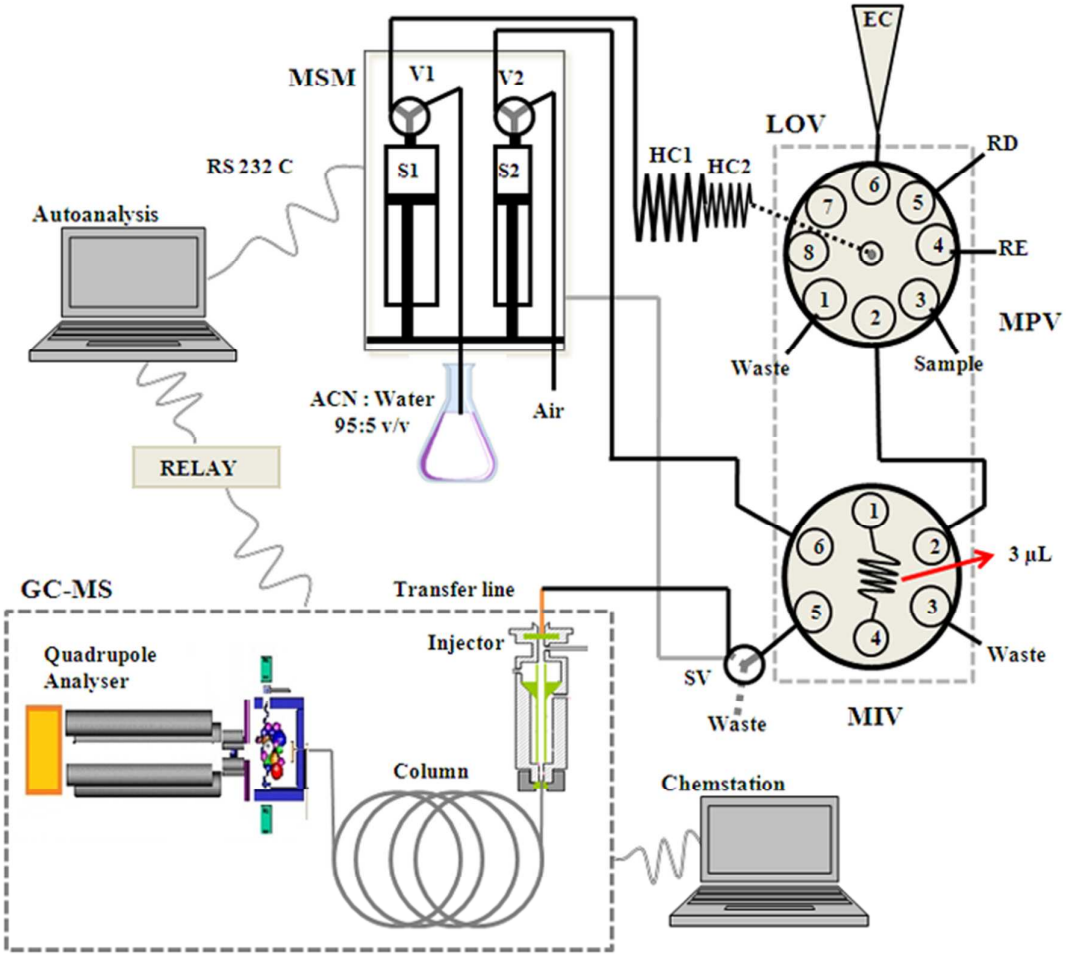
On line Gas Chromatography - Mass Spectrometry determination of priority PAHs in water



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ARTICLE TYPE

On line coupling lab on valve - dispersive liquid-liquid microextraction - multisyringe flow injection with Gas Chromatography - Mass Spectrometry for the determination of sixteen priority PAHs in water

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A novel approach exploiting lab on valve - dispersive liquid - liquid microextraction – multisyringe flow injection analysis (LOV-DLLME-MSFIA) coupled to gas chromatography - mass spectrometry (CG-MS) is presented. The method is based on the aspiration and mixing of the sample and all required reagents in the holding coil of a LOV-MSFIA system, delivering it into the miniaturized LOV platform equipped with a conical tube which is used as extraction chamber (EC), where the mixture of extraction solvent and disperser solvent is added at a high flow rate, resulting in the formation of a cloudy state and extraction of analytes of interest. The mixture of extraction and dispersive solvent used had a density significantly higher than water; consequently, the resulting fine droplets in the mixture, which contain the extracted analyte, are self-sedimented in thirty seconds, not requiring centrifugation for separation of the extraction phase. Afterwards, the extracted fraction was aspirated and transferred to a rotary micro-volume injection valve (MIV), where finally was introduced via an air stream into the injector of the GC, through a silica capillary transfer line with no stationary phase, used as interface. The potential of the devised LOV-DLLME-MSFIA/CG-MS assembly was demonstrated in the determination of polycyclic aromatic hydrocarbons (PAHs) in tap water, rain water, river surface water and raw landfill leachates. Under optimized conditions good enrichment factors (EFs) (27 - 38) and acceptable total DLLME yields (80 - 102%) were obtained. Calibration curves were linear with correlation coefficients higher than 0.996 in the working range level of 0.25 - 250 µg/L, and relative standard deviations (%RSD) were lower than 5% (n=5). Detection limits were within the range of 0.01 - 0.07 µg/L.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); Multisyringe Flow Injection Analysis (MSFIA); Lab on Valve (LOV), Gas Chromatography -Mass Spectrometry (GC/MS); Dispersive liquid-liquid microextraction (DLLME.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous semivolatile organic pollutants formed by the incomplete combustion of organic matter and also generated by fossil fuels or vegetation burning. The occurrence of these compounds in the environment is of justified concern, as these are known to be mutagenic and carcinogenic [1].

Environmental waters can be contaminated with PAHs from different sources, e.g. industrial and municipal wastewaters, runoff or rain water [2]. Due to their low solubility in water, PAHs are found at very low concentrations in aqueous media, i.e. a few parts per billion (ppb) or even less [3].

Monitoring of water samples in search for the presence of unknown pollutants at the trace-level requires fast, sensitive and selective methods, involving isolation of the compounds of interest and subsequent separation by means of a chromatographic technique [4]. The Environmental Protection Agency (EPA) prescribes liquid-liquid (LLE) and solid-phase extraction (SPE) as methods for preconcentrating PAHs from water samples [5]. The main disadvantage of LLE in ultra-trace analysis is the need of using large amounts of very clean solvents what involves their subsequent evaporation to obtain significant preconcentration factors. In this context, SPE seems to be better, as smaller amounts of organic solvents are usually employed. However, SPE cartridges are used only once in ultra - trace analysis; what makes analysis not only expensive but also generates a great deal of waste [6].

Efforts have been focused on the miniaturization of SPE and LLE extraction procedure to greatly reduce the amount of organic solvent required, leading to the development of solid-phase microextraction (SPME) [7] and liquid-liquid micro extraction (LLME) [8].

On the one hand, SPME has been widely applied for the determination of PAHs [9], however, it is expensive, its fibre is fragile, has a limited life time and sample carry over can be a

problem. LLME is more advantageous, since it is more economical, and in contrast to classical LLE the exposure to toxic organic solvents is minimal [10]. As a result of these advantages, recent research has been mainly focused on the development of microextraction techniques, based in LLME principles, such as dispersive liquid-liquid microextraction (DLLME), which has attracted much attention, given its outstanding features such as fast analysis, low consumption of organic solvents and simplicity. DLLME is a sample extraction procedure proposed by Assadi and co-workers in 2006 [11]. Essentially, DLLME is a fast microextraction technique based on the use of a small amount of an appropriate extractant solvent, immiscible with the aqueous sample and a ternary component, which is named the disperser solvent. The disperser solvent is miscible both in water and in the extractant solvent. The extractant and the disperser are mixed and injected rapidly into the sample, producing a turbulent mixture, due to the formation of small droplets of the extractant throughout the aqueous sample. The formation of small droplets enhances the effective surface area of the liquid-liquid extraction since the equilibrium is reached in a short time. The two phases are usually separated by centrifugation with a small volume of the extractant settled at the bottom of the centrifugation tube. Target compounds extracted in the recovered phase after DLLME are usually organic compounds subsequently analyzed using different techniques such as high-performance liquid chromatography (HPLC) [12], gas chromatography-flame ionization detection (GC-FID) [11] or GC-MS [13].

Most DLLME methods are carried out off line, probably because DLLME usually requires phase separation by centrifugation [14], leading to extraction and analysis being performed separately. Thus, automation of sample preparation is of great value in order to maximize throughput and minimize costs, time, and analyst risks due to chemicals exposure [15]. Since DLLME does not require solid supports for the extraction solvent such as hollow membranes or capillary tubes, its automation using flow techniques such as Flow Injection Analysis (FIA) [16], Sequential Injection Analysis (SIA) [17] and MSFIA [18] can be straightforward. This hyphenation, has already a history longer than 20 years [19] Main scopes for the automation of DLLME using these techniques has followed so far: (1) injection of the solvent mixture in a sample flow and collection of the extraction solvent droplets on a hydrophobic column with later elution[20], (2) injection of the solvent mixture into an extraction chamber [21] and (3) in-syringe extraction by filling the syringe pump with the solvent mixture followed by fast injection of the aqueous phase [22]. All these DLLME methods have been used with spectrophotometric detection, however these pretreatment systems might be hyphenated to a plethora of modern detection techniques/analytical instruments [23]. To the best of our knowledge, this is the first method dealing with automation of DLLME with GC for PAHs determination [24,24,26].

Therefore, the aim of this work is to measure the potential of DLLME with LOV-MSFIA [27] as front end of GC - MS, for the determination of PAHs in environmental matrixes, such as water and leachates. Optimization of different operating parameters affecting the DLLME sample treatment and GC injection was carried out. Different analytical parameters such as linearity, precision, and matrix effects were evaluated for the optimization of the LOV-DLLME-MSFIA - GC-MS analysis method.

2. Experimental section

2.1. Reagents

A 10 µg/mL 16 PAHs EPA Calibration Mix standard (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), indeno(1,2,3-cd)pyrene (IP), dibenzo(a,h)anthracene (DBA) and benzo(g,h,i)perylene (BghiP) in acetonitrile, and EPA 8270 semi volatile internal standard mix (acenaphthene-d10, chrysene-d12, 1,4-Dichlorobenzene-d4, naphthalene-d8, perylene-d12, phenanthrene-d10) 2000 µg/mL in methylene chloride, were purchased from Supelco (Bellefonte, PA, USA).

Chloroform (reactive grade), methylene chloride (reactive grade), acetone (HPLC grade), acetonitrile (HPLC grade) and methanol (HPLC grade) were purchased from Scharlau, (Spain). Trichloroethylene (GC-MS grade, 99.95%) was purchased from Sigma Aldrich Quimica SA, (Madrid, Spain). Distilled water was obtained from a MilliQ Direct-8 purification system (Millipore Iberica S.A.U., Madrid, Spain).

2.2. Instrumentation

2.2.1. GC-MS analysis

The analysis was performed using a Hewlett-Packard (Agilent Technologies, Palo Alto, CA, USA) HP 7890 series GC, equipped with a programmable-temperature vaporizer (PTV) injector, split/splitless and a HP 5973C mass selective detector system. The MS was operated at the electron impact (EI) mode (70 eV). Chromatographic data were recorded using a HP Chemstation controlled by Windows XP (Microsoft). Helium (99.999%) was employed as carrier gas at the flow rate of 1.5 mL/min. Analytes were separated on a 30m×0.32mm i.d.×0.25mm film thickness HP1 (100% methyl-silicone) gas chromatographic column (Agilent Technologies, Palo Alto, CA, USA) with the following oven temperature program: initially from 80 ºC (holding 1 min) at 20 ºC/min to 180 ºC (holding 5 min) then increasing at 20 ºC/min up to 280 ºC and holding for 8 min. The injection port was operated in pulsed splitless mode with an ultra inert commercial liner packed with glass wool (Agilent Technologies, Palo Alto, CA, USA), using the following temperature program: 100 ºC (holding 0.1 min) to 310 ºC increasing at 900 ºC/min (holding for 10 min), and finally returning to the initial temperature. The split valve was kept closed for 1 min to proceed with the transfer of analytes into the GC capillary column with a purge flow of 60 mL/min for later solvent elimination.

The pressure of carrier gas was reduced to 0.1 psi during 0.1 min just before the eluate was transferred into the injector, using the pressure pulse option of the GC instrument, ensuring the quantitative and reproducible sample introduction into the injector. Later, the inlet pressure was automatically increased up to a constant flow of 1.5 mL/min.

The EI ion source, quadrupole mass analyzer and the interface temperature were maintained at 230, 150 and 280 ◦C,

respectively. The MS was tuned to m/z 69, 219 and 502 for the EI corresponding to perfluorotributylamine (PFTBA). It was equipped with the mass spectral library NIST MS search 2.0, which was used to compare the obtained experimental spectra. The MS was operated on the total ion current mode (TIC), scanning from m/z 50 to 550 with identification purposes. To gain the highest possible sensitivity, the acquisition was performed at the selected ion monitoring mode (SIM), based on the selection of some mass peaks of the highest intensity for each compound. The quantitative ion for each analyte was set as follows: Nap 128, Acpy 152, Acp 154, Flu 166, PA and Ant 178, FL and Pyr 202, BaA and Chr 228, BbFl; BkFl and BaP 252, IP and BghiP 276 and DBA 278 (m/z).

2.2.2. LOV-DLLME-MSFIA system

The hyphenated LOV-DLLME-MSFIA system used for the extraction and injection of PAHs is shown in Figure 1.

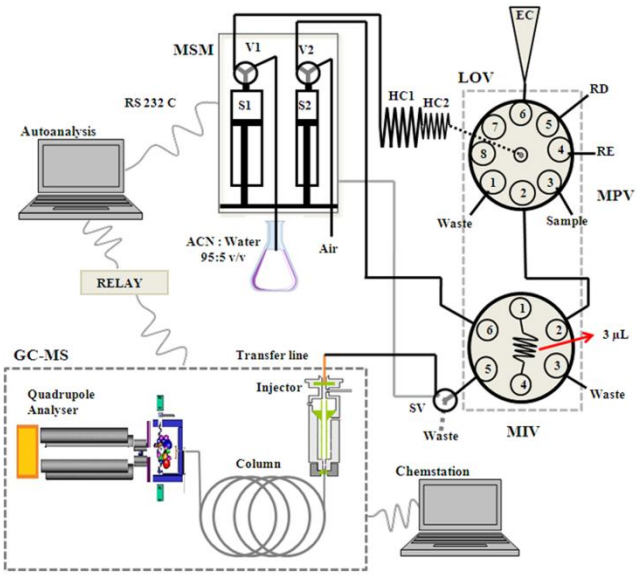

Fig 1. Schematic illustration of the LOV-DLLME-MSFIA system hyphenated to GC-MS for preconcentration and determination of PAHs in water and leachate samples: MPV: multiposition valve, MSM: multisyringe module, MIV: microinjection valve, RD: reagent dispersant (acetonitrile), RE: reagent extractive (trichloroethylene), HC 1 -2: holding coil 1 - 2, SV: solenoid valve, S 1 - 2: syringe pump 1 - 2, V 1 - 2: valve 1 - 2 and EC: extraction chamber.

PTFE tubing of 0.8 mm i.d. was used for the flow network, excepting the 500 cm long holding coil (HC1) and 300 cm holding coil (HC2) which were made from 1.5 and 0.5 mm i.d. respectively. Supply tubes for syringe refilling and waste discharge were made of PTFE tubing of 1.5 mm i.d.

A multisyringe burette module (MSM) and a valve module with one rotary 8-port multiposition valve (MPV) and one rotary 6-port micro injection valve (loop volume 3 microliters) (MIV) from Crison SL (Alella, Barcelona) were used for liquid handling and distribution. The MSM was equipped with 2 glass syringes (Hamilton, Bonaduz, GR, Switzerland) denoted as S1 and S2 of 5 mL and 1 mL, respectively. Each syringe had a three-way solenoid valve (N-Research, Caldway, NJ) at its head (V). The MSM also controlled an additional external commutation solenoid valve (N-Research).

DLLME was carried out in the EC using the propelling action of S1 in solvent mixture, while S2 was used for propelling the extract through the interface line from the injection valve to the gas chromatograph. Since all syringes move simultaneously, the 3-way solenoid head-valves were connected to their respective liquid reservoirs in OFF position and to the manifold in ON position, for sequential aspiration of the various constituents for the DLLME process, via the central communication channel (CC) for S1.

A LOV microconduit (Sciware Systems, Spain) mounted atop of an eight-port multiposition selection valve, fabricated with Kel-F® (polychlorotriflouroethylene) with chemical resistance to a wide range of organic solvents and encompassing eight integrated microchannels (0.5 mm i.d./14.0 mm length) avoided the dispersion of solvent and promoted the propelling to the extraction chamber (EC).The LOV channels configuration was as follows: waste (1), sample (3), trichloroethylene (4) and acetonitrile (5). At channel 6, was the EC, constituted by a 5 mL commercial pipette tip, linked to the LOV through a small PTFE connector, with the adequate form and size for holding it. The EC was used for improve the mixing among solvents. Its conical shape allows the reduction of the dead volume in extract aspiration by S1. Channel 2 of the LOV was linked via a short PTFE tube directly to the port 2 of the injection valve for pushing the organic extract into the 3 µL eluate loop. S2 is connected to port 6 of the MIV and in the inject position pushes the organic extract to the GC-MS.

The 25 cm long silica capillary transfer line (0.32 mm i.d.) with no stationary phase (Análisis Vínicos, Ciudad Real, Spain) was permanently mounted in the GC injector. A discrete solenoid valve (SV) was implemented within the transfer line to facilitate the rinsing of the transfer line between assays. Instrument control was performed using the software package AutoAnalysis 5.0 (Sciware Systems). The distinctive feature of developed software based on dynamic link libraries (DLLs) at 32 bits, provides the possibility of using a single and versatile application without further modification for whatever instrumentation and detection system needed [28]. The synchronism of the GC-MS with the LOV-DLLME-MSFIA system was performed through the digital output of the multisyringe burette. One of the digital outputs is connected to a relay, which is utilized for activation of GC via the AutoAnalysis 5.0 software when the eluate is injected into the interface GC-MSFIA.

2.3. Analytical Procedure

The complete operational sequence for DLLME of PAHs from water and leachates with further on-line chromatographic separation and detection is given as Supporting Information (Table S-1), and summarized as follows: first of all, channel 3 of the LOV is cleaned twice with 0.5 mL of sample by aspiration from the selection valve at 1 mL/min and dispense to waste (channel 1) with head valve position (V1) ON. By this, sample carry-over is minimized and better reproducibility is achieved. Then, 4 mL of sample, 0.9 mL of acetonitrile and 0.1 mL of trichloroethylene are loaded into HC at 1.0, 0.8 and 0.5 mL/min respectively, aspirated by S1, prior to being pumped and mixed by their propelling at 15 mL/min into the EC (LOV channel 6).

This, results in a cloudy state, where analytes are extracted into fine droplets formed during DLLME. The mixture of solvents and the sample is then sedimented at the bottom of the EC. After waiting 30 s the mayor part of the organic phase, (~90 ± 10 µL measured with a 250 µL glass syringe) containing the extracted PAHs is aspirated segmentally at 0.5 mL/min by S1. In order to prevent the introduction of aqueous mixtures into the GC transfer line, the eluent zone was divided: first an amount of 50 µL corresponding to the dead volume of the LOV channel and EC connection is first aspirated into HC and delivered to waste (channel 1 LOV). Then, a well-defined organic eluent volume (20 µL) is aspirated into the HC and the rotary injection valve is automatically activated to the load position and the eluate is transported to the valve micro loop (3 µL). Afterwards, the injection valve is switched to inject position, and the eluate is forthwith delivered to the GC by a gentle stream of air provided by S2. The GC is at this moment activated (triggered by the relay), and the temperature programs of the injector and column oven are initiated.

The operational sequence for the injection (step 3) is repeated three times for each sample, in order to measure the reproducibility of DLLME for a particular sample. Finally all syringes are refilled from their respective reservoirs.

At this point, the liquid contained in the EC is back flushed into the HC and then discharged to waste reservoir (channel 1). The EC is rinsed by first loading 2 mL of acetonitrile:water (95:5 v/v) from the carrier reservoir (S1); secondly, discharging this volume to waste; and thirdly, loading again 5 mL of carrier, with all steps carried out at a flow rate of 5 mL/min. The transfer line to GC is cleaned with an air-segmented volume of acetonitrile, which is dispensed to waste via the additional solenoid valve integrated into the manifold. Hence, the system is ready to initiate a new analysis eliminating any possibility of cross-contamination between consecutive runs.

The GC separation is actually synchronized with the LOV-DLLME-MSFIA procedure, i.e. a sample is analyzed, while the ensuing one is being processed in the flow system. Total sample preparation time and transportation of eluate into GC - MS takes ca. 8 min for the first run. However, the overlap of both the chromatographic run and column/injector re-equilibration to the initial conditions increases the overall sample throughput.

2.4. Configuration of the LOV-DLLME-MSFIA-GC-MS system

Online coupling of GC methods is a great challenge because of reliable transfer of eluate into the GC injector has to be ensured, requiring the use of special interfaces to prevent injection port and column overloading [25]. Most common approaches are based on the use syringe pumps as liquid drivers for the accurate handling of the eluent [29] and simultaneous injection techniques such as on-column, loop-type interface, and programmable temperature vaporizer (PTV), being the latter the most frequently employed for the determination of PAHs in environmental samples [30].

In this novel approach, a micro injection loop of 3 µL volume was used prior to on line transfer into the PTV interface, for an accurate delivery of discrete volumes of eluate from the LOV-DLLME-MSFIA system, making the on line transfer simpler and more reproducible.

Another element that must be taken into account when coupling online GC, is the use of a solvent stream as carrier, because it might increase the dispersion of eluate in the transfer line causing irreproducibility on the injection step. Moreover, direct air segmentation into the transfer line has been reported inappropriate as a consequence of the build-up of back-pressure which compressed the air segments leading to an undue solvent transfer [29]. Thus, the injection valve was implemented into the flow network to feed the injector with the eluted analytes via a pressurized air stream provided by S2 (see Figure 1).

2.5. Samples and sample pre-treatment

Working organic solutions were made by direct dilution in trichloroethylene.

A 10 µg/mL single stock solution of custom mix EPA 8270 was prepared in acetonitrile as instrument control standard (ICS) and added to work standard solutions with a final concentration of 10 µg/L. Stock and working standard solutions were stored at 4 ºC in the refrigerator.

Daily, aqueous working solutions were prepared adding to the stock solution 5 % (v/v) of acetonitrile by magnetic stirring during 24 h, which acts as organic modifier assuring the good solubility of PAHs and preventing their adsorption on the PTFE tubing of the flow manifold and glassware; otherwise PAHs could not be recovered quantitatively, and cross-contamination might occur.

Nine concentration levels (0.25, 0.5, 1, 5, 10, 25, 50, 150 and 250 µg/L) were prepared from stock mix solutions.

Genuine tap water from our laboratory (TW) and rain water from our campus university (RW) were collected in November 2012 (University of Balearic Islands Mallorca, Balearic Islands, Spain). Solid waste landfill leachate sample (SW) (Santa Margalida – Mallorca), the leachate sample from a landfill of bulky and construction equipment (SC) (Manacor - Mallorca), rain water (SP) collected from the streets around a waste treatment plant (Santa Margalida - Mallorca), and river water (SR) (Santa Margalida - Mallorca), were collected in October 2012 in glass bottles and stored refrigerated.

Before analysis, samples were filtered through a 0.45 µm cellulose filter modified with 5% (v/v) acetonitrile

3. Results and discussion

3.1. Optimization of DLLME-LOV-MSFIA-GC-MS system

Optimized extraction conditions and high extraction efficiency was accomplished by means of the evaluation of several factors including namely: type and volume of the extraction and dispersive solvents, extraction time, sample volume, aspiration sequence and mixing flow rate. All variables were optimized using the “single factor at time”. Extraction recovery (ER) was employed as a response to the optimization procedure and was calculated using the following equation:

$$ER = ((C_0 * V_0)/ (C_{aq} * V_{aq})) \times 100 \quad (1)$$

Where C_0, C_{aq}, V_0 and V_{aq} correspond to the concentration of

analytes in the organic extract, the spiked concentration of analytes in the aqueous solution, the volume of the organic extract, and the volume of aqueous solution, respectively.

Quantification was accomplished by using relative peak area (RPA) corresponding to the ratio of PAHs peak area and the internal standard peak area. All experiments were made using four milliliters of distilled water spiked with PAHs at 50µg/L and 10 µg/L of internal standard.

3.2. Effect of the type and volume of extraction solvent

In the selection of extraction solvent, several factors should be considered: (1) higher density than water; (2) low solubility in water, (3) good gas chromatographic behavior, and (4) high extraction capability of compounds of interest. Halogenated hydrocarbons are usually selected because of their high density. Thereby, the following solvents, methylene chloride, trichloroethylene and chloroform were tested. The effect of these solvents was investigated by mixing aqueous solution of PAHs with 100 µL of each extraction solvent and 900 µL of acetonitrile in order to achieve the appropriate amount of sedimented phase at the bottom of the EC.

Comparison of the ER obtained with different extraction solvents (Figure S-2 supplementary material) revealed that trichloroethylene has the highest extraction efficiency (75 - 104 %) in comparison with methylene chloride (40 -73 %) and chloroform (42 - 81 %). Therefore, trichloroethylene was selected as the extraction solvent.

To optimize the volume of the extraction solvent, different volumes of trichloroethylene were evaluated, while keeping the other experimental parameters constant. Lower volumes were avoided due to the very small volume of sedimented phase formed with subsequent harmful effects on reproducibility. The response of all analytes reached a maximum when the volume of trichloroethylene was 50 µL, and decreased with the increase of the volume from 100 to 250 µL due to the dilution effect (Figure S-3 of supplementary material). However, for this volume the sedimented phase was very small, making difficult to collect always the same volume in the injection valve, for this reason 100 µL of trichloroethylene were employed in further experiments.

3.3. Effect of the type and volume of disperser solvent

In DLLME, most important factors for the selection of a suitable dispersive solvent are: its miscibility in both phases and its dispersive capability into the aqueous solution, in order to form the cloudy state. Methanol, acetone and acetonitrile were selected for this purpose, since these have low surface tension and high surface activity. Results illustrated in Figure S-4 of supplementary material, show that the highest extraction recovery was achieved by using acetonitrile as the dispersive solvent.

To study the effect of the volume of disperser solvent, different volumes of acetonitrile (500, 900, 1200, 1500, and 2000 µL) were investigated. Results showed that a higher volume of acetonitrile increased the PAHs solubility in water, reducing the extraction efficiency due to the decrease of the distribution coefficient. Nevertheless, a low volume of acetonitrile implies that the cloudy state is not well formed and centrifugation is needed. For this reason 900 µL were chosen as a compromise solution. Graphical results are provided in Figure S-5 of supplementary material.

3.4. Effect of aspiration sequence and mixing flow rate

One of the main factors influencing the successful dispersion of the organic phase is the aspiration sequence of the reagents and sample in the holding coil before their delivery into the extraction chamber and the mixing flow rate. Various aspiration sequences were tested and differences depending on the loading sequence were found. Best results were obtained with the sample being the last one when dispensing into the EC, making possible that the mixture solvents pass through it achieving an effective extraction. Thus, the following aspiration sequence: sample solution, acetonitrile, and trichloroethylene was used in further studies. Furthermore, the syringe pump allows working in a wide range of flow rates. Ranges from 5 to 15 mL/min were tested. A propelling flow rate of the reagents mixture of at least 10 mL/min was required to achieve satisfactory dispersion and efficient contact between the phases and consequently maximal extraction efficiency. When the reaction plug was dispensed at the maximum flow rate of the syringe pump (15 mL/min) to the EC, the extraction efficiency was maintained. Therefore, in order to achieve the shorter time of analysis, this flow rate was chosen for further investigations.

3.5. Effect of extraction time

In DLLME, the extraction time is defined as the interval of time between the injection of the solvent mixture (extraction-dispersive) into the sample and centrifugation [11,24]. In our approach the use centrifugation is not necessary. Since the mixture of acetonitrile and trichloroethylene has a density significantly higher than water, the fine droplets containing the extracted analytes are sedimented in short time in the EC. In addition, the mass transfer of analytes from sample solution to extraction solvent is so fast that the extraction equilibrium can be achieved in a short time, diminishing the analysis time of the extraction procedure considerably. Consequently, 30 s of extraction time was chosen for the following experiments as the minimum time needed for self phase separation.

Summing up, most suitable analysis conditions for the LOV-DLLME-MSFIA-GC-MS were as follows: 100 µL of trichloroethylene as extraction solvent, 900 µL of acetonitrile as dispersive solvent, extraction time 30 s and sample volume 4 mL. All following experiments were carried under these conditions. Figure 2 shows the chromatograms corresponding to (a) spiked (50 µg/L) and (b) non spiked rain water sample (RW), after DLLME by the developed method under the described optimum conditions.

3.6. Evaluation of the method performance

Analytical characteristics of the optimized DLLME method in terms of: limit of detection (LOD) and quantification (LOQ), precision, enrichment factor (EF) and linear working range; were calculated to gain an insight into the efficiency and the feasibility of application of the developed method (Table 1). Under optimum experimental conditions, the proposed methodology was evaluated using distilled water samples spiked with the

selected PAHs at nine different concentration levels: 0.25, 0.5, 1, 5, 10, 25, 50, 150, 250 µg/L, in order to obtain the respective calibration curves.

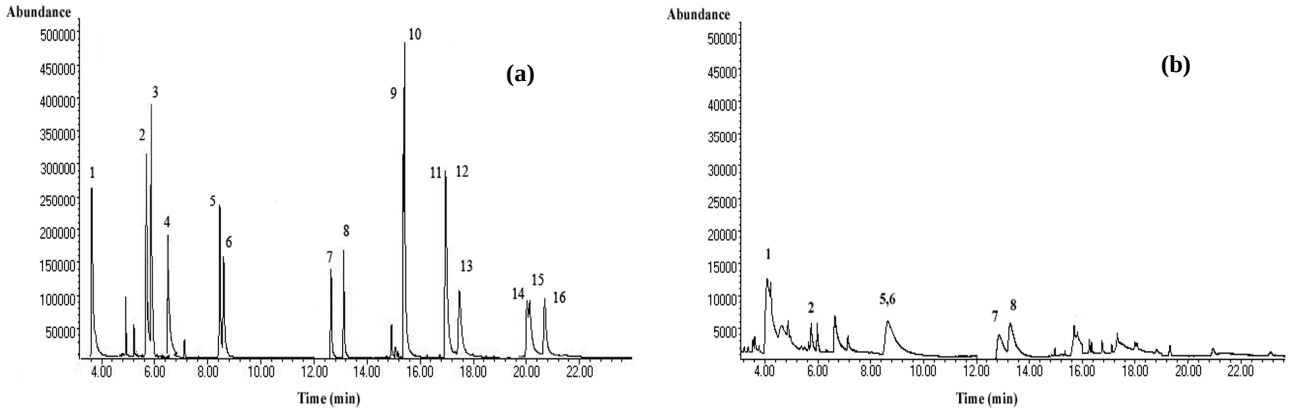

Fig 2. Chromatograms obtained by the LOV-DLLME-MSFIA-GC-MS corresponding to (a) spiked rain water at PAHs concentration 50 µg/L (b) non spiked rain water extracts: 1: Nap, 2: Acpy, 3: Acp, 4: Flu, 5: PA, 6: Ant, 7: FL, 8: Pyr, 9: BaA, 10: Chr, 11: BbFl, 12: BkFl, 13: BaP, 14: IP, 15: DBA and 16: BghiP.

Table 1. Linear range, correlation coefficient, enrichment factor, relative standard deviation, limit of quantitation (LOQ), limit of detection (LOD) and recoveries of PAHs obtained with the developed LOV-DLLME-MSFIA-GC-MS system.

Analyte	Linearity		Reproducibility (RSD%)[c]				Relative recovery (%)		EFs		LOD	LOQ
	DR (µg/L)	R^2	Low level[a]	Low level[b]	High level[a]	High level[b]	Low level[d]	High level[e]	Low level[d]	High level[e]	(µg/L)	(µg/L)
Nap[f]	0.25-250	0.9983	0.78	4.76	1.99	4.19	95.2 ± 1.7	92.6 ± 4.8	32.47	31.62	0.01	0.07
Acpy[f]	0.25-250	0.9982	1.32	1.35	1.97	5.51	100.0 ± 4.6	95.0 ± 3.7	36.15	33.04	0.03	0.13
Acp[f]	0.25-250	0.9991	3.03	4.04	4.35	5.04	95.6 ± 1.0	93.3 ± 5.8	35.08	31.38	0.05	0.26
Flu[f]	0.25-250	0.9997	3.12	4.06	1.08	2.82	83.7 ± 3.4	99.3 ± 5.3	28.78	31.38	0.05	0.21
PA[g]	0.25-250	0.9984	1.28	2.63	1.20	2.99	100.4 ± 2.7	101.0 ± 5.1	36.02	36.49	0.02	0.12
Ant[g]	0.25-250	0.9983	3.31	4.09	1.05	4.88	100.0 ± 4.4	101.6 ± 2.1	36.03	38.21	0.02	0.12
FL[g]	0.25-250	0.9996	0.97	1.61	4.31	1.39	100.0 ± 1.1	97.3 ± 1.3	36.06	35.53	0.02	0.10
Pyr[g]	0.25-50	0.9987	1.63	1.64	1.01	2.33	102.8 ± 1.2	86.2 ± 1.1	38.87	29.20	0.03	0.18
BaA[h]	0.25-50	0.9995	2.99	3.09	2.20	2.71	80.6 ± 1.2	86.7 ± 2.3	27.01	29.21	0.05	0.18
Chr[h]	0.25-50	0.9984	2.98	4.87	2.67	4.32	97.6 ± 3.0	100.7 ± 4.0	33.74	36.30	0.05	0.18
BbFl[h]	0.5-50	0.9993	3.77	4.61	2.60	2.96	101.3 ± 1.0	95.4 ± 2.2	37.05	35.11	0.07	0.36
BkFl[h]	0.5-50	0.9898	3.48	4.02	3.86	4.66	101.3 ± 2.9	100.1 ± 3.9	38.68	36.49	0.07	0.38
BaP[h]	0.5-50	0.9994	1.19	1.22	3.04	3.32	101.6 ± 4.1	102.7 ± 1.2	38.68	38.78	0.02	0.13
IP[i]	0.5-50	0.9984	3.38	5.38	4.03	4.34	100.4 ± 1.1	96.8 ± 3.0	37.09	35.21	0.07	0.37
DBA[i]	0.5-50	0.9998	3.33	5.91	2.50	2.54	99.2 ± 4.8	92.8 ± 4.4	35.81	31.28	0.06	0.31
BghiP[i]	0.5-50	0.9987	1.48	2.66	3.22	4.45	96.5 ± 1.3	99.8 ± 4.3	33.58	36.29	0.07	0.30

R2= correlation coefficient
DR= dynamic range
a Intra–day, b inter–day.
c n = 5 replicates
d Ultrapure water spiked with PAHs at concentration 1 µg/L.
e Ultrapure water spiked with PAHs at concentration 50 µg/L.
f Internal standard acenaphthene-d10
g Internal standard phenanthrene-d10

h Internal standard chrysene-d12
i Internal standard perylene-d12.

Determination coefficients (r2) ranging from 0.9984 to 0.9998 indicated an excellent linearity for all species. The precision of the method (n = 5) was evaluated by measuring five replicate samples in one day (intra-day precision) and in three consecutive days (inter-day precision), by treating distilled water samples at two addition levels of 1 and 50 µg/L. The method showed good intra-day and inter-day precision, i.e. 0.78 - 5.91 % and 1.01 - 5.51 %, respectively, expressed as relative standard deviation (%RSD).

LODs and LOQs were determined using five replicates of a solution containing 0.5 µg/L of each PAHs in distilled water following the Environmental Protection Agency Method Detection Limit (MDL) protocol [31]. The LOD was calculated as 3.747 times the standard deviation of the seven replicates. 3.747, is the value of t for 5−1 = 4 degrees of freedom and at the 99% level from the one-sided t distribution table [29] and LOQ was estimated as 10 times the corresponding standard deviation. LODs ranged from 0.01 - 0.07 µg/L. LOQs ranged for all target analytes from 0.07 to 0.38 µg/L. These values are lower than the maximum contaminant level (MCL) permitted in the EU in waters and leachates [32-36].

The evaluation of the DLLME efficiency was accomplished by means of the EF and the relative recovery (RR) [11]. The EF is defined as the ratio between the concentration of analyte in the sediment phase (Csed) and the initial concentration of analyte (C0) in the sample. RR was calculated with the following equation:

$$RR = ((C_{founded}-C_{real})/C_{added}) \times 100 \quad (2)$$

Where $C_{founded}$, C_{real}, C_{added} are the concentrations of analyte after addition of a known amount of standard in the real sample, the concentration of analyte in the real sample and the concentration of the known amount of standard which was spiked to the real sample, respectively.

In order to calculate the EF and RR, three replicates were performed spiking distilled water samples (1 and 50 µg/L), and resulting in the range of 27 - 39 and 80 -102 % for EF and RR, respectively.

The values reported in Table 1 are comparable with those obtained by DLLME-GC-FID [11], SPME-GC-MS [9], SPE-GC-MS [37], low toxic DLLME-GC-MS [13], USAEME-GC-MS [36], solidification of floating organic droplet method DLLME-SFO-CG-MS [39], low-density solvent-based solvent demulsification dispersive liquid-liquid microextraction LDS-SD-DLLME [40] and homogeneous liquid-liquid microextraction via flotation assistance HLLME-FA-GC-FID [41], (Table 2) making our approach competitive in terms of reproducibility, LOQs and LODs.

The possibility of performing the entire procedure, including the injection of the sample, in about 8 min is a compelling reason to use the present method with the advantages of being faster, simpler and environmentally friendly using smaller volumes of organic solvents than SPE and SPME counterparts. Moreover, the combination of the proposed sample preparation approach DLLME with a sensitive and selective determination technique, e.g. GC-MS provided very low detection and quantification limits and good selectivity. These results demonstrate that the LOV-DLLME-MSFIA-GC-MS procedure is a simple, rapid and low cost method for the simultaneous determination of PAHs traces in environmental water samples with suitable accuracy and precision.

In terms of cost and environmental concerns, the described method entails an important improvement compared with previously published extraction techniques and standard methods for the determination of PAHs in water samples.

Table 2. Comparison of the proposed method with other extraction methods for the determination of the PAHs in water samples

Technique	Sample treatment mode	EFs	Extraction time (min)[a]	Recovery (%)	Precision (RSD %)	LOQ (ng/L)	PAHs number[c]	Ref
SPME	Manual	---	45	---	<20	1 - 29	16	[9]
DLLME	Manual	603 - 1113	Few seconds	60 – 11	<11	23.3 – 100.0	16	[11]
HLLME-FA	Manual	---	5	100 - 108	<10	14000.0 – 41000.0	4	[41]
USAEME	Manual	1776–2714	Few seconds	ni	<7	20 – 50[b]	10	[38]
DLLME–SFO–CG–MS	Manual	88 - 118	1	88 – 106	<4.3	0.04 – 1.1[b]	5	[39]
LDS–SD–DLLME	Manual	----	1	65– 95	<10	10 - 150	16	[40]
LOV-DLLME-MSFIA-GC-MS	Automated		30 seconds	80 - 115	<5	10 - 70	16	Proposed method

[a] Time employed in extraction stage, any other operations were not included
[b]Limit of detection
[c] Number of PAHs measured in each procedure.
ni: not indicated

3.7. Application

To evaluate the reliability of the proposed method, six environmental water samples were examined, i.e. tap water from our laboratory (TW), rainwater from our campus university (RW), solid waste landfill leachate (SW), leachate from a landfill of bulky and construction equipment (SC), rain water (SP) collected from the streets around a waste treatment plant, and river water (SR).

To estimate the matrix effect of these samples, these were spiked

with 1 µg/L of the 16 standards of PAHs and the relative recovery and relative standard deviation of the target compounds were calculated.

The levels found were below the maximum allowed PAHs concentration (Table 3), with values under the LOD up to 2.3 µg/L. Similar concentration levels were reported in previous studies [9,12,32-36,39,40]. Total concentrations (sum of 16 PAHs) were between 2.2 and 8.5 µg/L in leachates, whereas in rain, river and tap water values encountered were between nd and 3.1 µg/L.

Highest values of the evaluated PAHs correspond to those with lower molecular weight (Nap, Acpy, Acp, Flu, PA, Ant).

This could be due to their relatively higher water solubility and lower vapor pressure in comparison with other PAHs, making these compounds easily trapped by rain droplets in the atmosphere [42].

Table 3. Recoveries of PAHs determined by the proposed LOV-DLLME-MSFIA –GC–MS in different water and leachate samples.

PAHs	TW			RW			SP			SR			SC			SW		
	Ca ($\bar{x}$)	Sa ($\bar{x}$)	RR (%)b	Ca ($\bar{x}$)	Sa ($\bar{x}$)	RR (%)b	Ca ($\bar{x}$)	Sa ($\bar{x}$)	RR (%)b	Ca ($\bar{x}$)	Sa ($\bar{x}$)	RR (%)b	Ca ($\bar{x}$)	Sa ($\bar{x}$)	RR (%)b	Ca ($\bar{x}$)	Sa ($\bar{x}$)	RR (%)b
Nap	nd	0.87	87.4 ± 1.4	0.46	0.90	90.0 ± 1.4	0.42	1.02	102.0 ± 1.4	nd	1.02	102.0 ± 1.5	0.33	1.10	110.0 ± 1.4	2.34	1.05	105.0 ± 1.4
Acpy	nd	1.02	102 ± 4.0	0.51	0.83	83.0 ± 4.0	0.39	1.05	105.0 ± 4.0	nd	1.07	107.0 ±5.0	nd	0.89	89.0± 4.0	1.41	1.09	109.0 ± 4.0
Acp	nd	0.95	95.0 ± 1.3	nd	0.95	95.0 ± 1.3	0.1	1.08	108.0 ± 1.3	nd	1.08	108.0 ±2.8	0.37	0.95	95.0 ±1.3	1.35	0.95	95.0 ± 1.3
Flu	nd	0.95	95.0 ± 2.7	nd	0.83	83.0 ± 4.9	nd	0.88	88.0± 4.9	0.4	0.98	98.0 ±1.0	0.35	1.00	100.0 ±4.9	0.46	1.03	103.0 ± 4.9
PA	nd	0.97	97.0 ± 1.2	0.47	0.95	95.0 ± 4.1	0.39	1.05	105.0 ± 1.1	0.18	0.98	98.0 ±5.2	0.22	1.05	105.0 ±3.1	0.57	1.05	105.0 ± 4.3
Ant	nd	0.93	93.0 ± 3.5	0.47	0.98	98.0 ± 1.2	0.5	0.97	97.0 ± 3.5	0.37	0.95	95.0 ±2.9	0.38	1.05	105.0 ±4.8	0.68	0.89	89.0 ± 3.5
FL	nd	0.91	91.0 ± 1.8	0.43	1.00	100.0 ± 1.7	0.28	0.98	98.0 ± 1.8	0.21	1.05	105.0 ±2.7	0.2	1.06	106.0 ±1.8	0.45	0.95	95.0 ± 1.7
Pyr	nd	0.94	94.0 ± 1.3	0.45	0.98	98.0 ± 1.3	0.29	1.02	102.0 ± 1.3	0.23	1.03	103.0 ± 2.1	0.24	0.99	99.0 ±1.3	0.46	0.96	96.0 ± 1.3
BaA	nd	1.01	101.0 ± 4.0	nd	0.95	95.0 ± 2.0	nd	1.03	103.0 ± 2.4	nd	1.10	110.0 ±5.6	0.56	1.00	100.0 ±4.5	0.20	0.88	88.0 ± 4.5
Chr	nd	0.94	94.0 ± 5.3	nd	0.95	95.0 ± 2.1	0.4	1.05	105.0 ± 3.9	0.36	1.03	103.0 ±1.2	0.37	0.97	97.0 ±3.	nd	1.00	100 ± 1.3
BbFl	nd	0.98	98.0 ± 3.2	nd	0.98	98.0 ± 1.3	nd	1.03	103.0 ± 1.8	nd	0.97	97.0 ± 4.9	nd	0.96	96.0± 4.7	0.21	0.98	98.0 ± 3.7
BkFl	nd	1.00	100.0 ± 2.1	nd	0.93	93.0 ± 3.6	nd	1.01	101.0 ± 2.8	nd	1.1	110.0 ±3.9	nd	1.01	101.0 ±2.9	nd	0.99	99.0 ± 3.9
BaP	nd	0.84	84 .0± 2.3	nd	0.94	94.0 ± 4.1	nd	0.86	86.0 ± 2.9	0.16	1.08	108.0 ±2.4	0.17	0.98	98.0 ±1.8	nd	1.04	104 ± 2.3
IP	nd	0.95	95.0 ± 4.6	nd	0.89	89.0 ± 1.7	0.29	0.97	97.0 ± 3.0	0.25	0.97	97.0 ±2.6	0.26	0.97	97.0 ±2.8	0.36	0.92	92.0 ± 2.8
DBA	nd	0.93	93.0 ± 3.1	nd	0.92	92.0 ± 0.9	nd	1.00	100 ± 1.2	nd	1.10	110.0± 1.4	nd	0.98	98.0 ± 2.3	nd	1.00	100 ± 2.3
BghiP	nd	0.90	90.0 ± 2.6	nd	0.88	88.0 ± 3.0	nd	0.97	97.0± 1.4	nd	0.98	98.0 ±1.5	nd	0.98	98.0 ±1.5	nd	1.00	100 ± 2.4
Total	nd			2.79			3.06			2.16			3.45			8.49		

C: concentration found
S: Concentration spiked (1µg/L)
RR: relative recovery
nd: not detected or below the limits of detection
[a] Average (µg/L)
[b] Average ± relative standard deviation (RSD %) n = 3
Total: sum of the 16 PAHs considered in this study
TW: tap water collected in our chemistry laboratory
RW: rain water collected in our university campus
SP: rain water collected in the streets around a treatment waste plant
SR: river water
SC: leachate collected in a landfill of bulky and construction equipment
SW: solid waste landfill leachate
Recoveries of PAHs determined by the proposed LOV-DLLME-MSFIA –GC–MS in different water and leachate samples

On the other hand, higher molecular weight PAHs, i.e. those with five and six benzene rings, are present in rainwater and leachates in relatively larger amounts, due to their persistence in nature. In addition, these PAHs have relatively higher Henry's law constants (or low octanol-water coefficients) than the lighter ones, and tend to be efficiently scavenged by cloud or rain droplets. Also, PAHs emitted in local sources involving incomplete combustion of fossil fuels such as urban vehicular traffic, chemical industries, and power plants are highly absorbed by organic compounds in the urban solid waste [43].

Relative recoveries varied from 87% to 110% for all samples, and relative standard deviations were found to range from 0.9 - 5.3. A t test of comparison of means revealed the inexistence of significant differences between the expected and found concentrations for all the analytes at the 0.05 significance level.

4. Conclusions

In the present study, a LOV-DLLME-MSFIA-GC-MS system was developed, allowing rapid extraction and quantification of PAHs at very low levels in water and leachate samples. The factors affecting DLLME efficiency were studied in detail and optimal conditions were established. The simplicity, facility, low solvent consumption, low cost, high sensitivity, good precision and short analysis time are clear advantages of the proposed methodology. Furthermore full automation of the sample processing and coupling to GC-MS was successfully accomplished for the first time exploiting DLLME. Sample matrix cleanup, extraction, and injection of PAHs is performed within a total time of 8 min, increasing the overall sample throughput, with the overlapping of LOV-DLLME-MSFIA sample processing procedure with GC-MS.

Finally, the sensitivity and accuracy achieved with the proposed method, together with the high frequency of sample treatment, permit this approach to be used as a routine method for quantitative analysis of PAHs in environmental water control, reaching established levels in legislation.

Notes and references

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