Determination of priority carcinogenic polycyclic aromatic hydrocarbons in wastewater and surface water by coacervative extraction and liquid chromatography-fluorimetry

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Abstract

The US Environmental Protection Agency (EPA) and the European Union (EU) have set restrictive limits for priority carcinogenic polycyclic aromatic hydrocarbons (CPAHs) in surface waters (EPA 3.8 ng $L^{-1}$and EU 2-100 ng L^{-1}) in order to protect aquatic life and human health. Currently, methods meeting these sensitivity criteria are not suitable for routine analysis of CPAHs. Here, we present a simple, rapid and low-cost method for the routine monitorization of these pollutants in aquatic environments based on their extraction with coacervates of decanoic acid reverse micelles in the nano and microscale, and determination by liquid chromatography-fluorimetry (LC-FL). The method involves the stirring of filtered aqueous samples (36 mL) with 4 mL of THF containing 70 mg of decanoic acid for 5 min, its centrifugation for 10 min and the analysis of 20 μL of the resulting coacervate containing the CPAHs by LC/FL. The method is robust, the extractions being independent on salt concentration (up to 1M), temperature (up to 60 ºC) and pH (below 4).Besides, the coacervate prevents the CPAHs from adsorption onto the surface of containers during sample storage. No clean-up steps are necessary and the method is matrix-independent. The quantification and detection limits of the method ranged between 0.4 and 3.5 ng L^{-1} and 0.1 and 1 ng L^{-1} respectively, for the seven priority CPAHs. The method has been successfully applied to the determination of these pollutants in raw and treated sewage from three mechanical-biological treatment plants, two rivers and a reservoir with frequent motorized recreational craft activities, all of them located in the South of Spain. Recoveries for spiked samples in the range 2-30 ng L^{-1} were between 88% and 95% with relative standard deviations from 1 to 7%. CPAHs were present in wastewater influents at concentrations in the range 3.9-37 ng $L^{-1,}$ while the treatment at the WWTPs studied reduced their concentration in their respective effluents in a percentage near 100%. Three CPAHs were present at quantifiable levels in Guadajoz river (1.8-6.6 ngL^{-1}) and six in La Breña reservoir (1.39-4.8 ng L^{-1}).

Keywords: Coacervative extraction; Liquid-liquid extraction; Environmental analysis; Priority carcinogenic polycyclic aromatic hydrocarbons; Liquid chromatography; Fluorescence dectection; Reverse micelles

1. Introduction

Among the large number of polycyclic aromatic hydrocarbons (PAHs) polluting the aquatic environments, the group of carcinogenic PAHs (CPAHs) stands out because of its great environmental and health concern. The US Environmental Protection Agency (EPA) has included seven CPAHs in the priority pollutant list and it has recommended a limit of 3.8 ng L^{-1} in surface waters as a criterion for water quality [1]. Table 1 gives the structures, octanol-water constants (log K_{ow}) and estimated carcinogenic potency of CPAHs according to the classification of the International Agency for Research on Cancer (IARC) [2]. The European Union (EU) has fixed environmental quality standards in surface waters for some of the CPAHs [3,4]. These standards are expressed as a maximum allowable concentration (benzo(a)pyrene, BaP, 100 ng L^{-1}) or as an annual average concentration (BaP, 50 ng L^{-1}; the sum of benzo(b)fluoranthene, BbF, and benzo(k)fluoranthene, BkF, 30 ng L^{-1}; and the sum of indeno(1,2,3-cd)pyrene, IP, and benzo(g,h,i)perylene, BP, 2 ng L^{-1}, the latter being not considered carcinogenic by IARC).

The routine quantitation of CPAHs in surface waters at the low levels fixed by the EPA and EU requires the development of analytical methods that both provide very efficient extraction and concentration steps and permit their simple, rapid and low cost determination. To our knowledge, no specific methods have been reported for the determination of CPAHs, although they have been routinely determined along with other PAHs by liquid chromatography and fluorescence (LC-FL) [5-7] or ultraviolet (LC-UV) [7] detection. Gas chromatography combined with mass spectrometry (GC-MS) has also been employed [8-10]. LC-MS is used to a lesser extent because of the difficulty in ionizing and fragmenting PAHs employing atmospheric pressure ionisation (API)-MS techniques [11,12], which results in detections limit higher than those required for quantification of CPAHs in aquatic environments, even after applying sample treatments that achieve concentration factors of 1000 [13]. LC-FL/UV is the technique used by EPA methods for the analysis of priority PAHs, including CPAHs, in drinking waters (methods 550 and 500.1), wastewaters (method 610) and ground waters (methods 8100 and 8310), but the quantitation limits of these methods (0.1-1.2 μg L^{-1}) are well above the quality standards set for CPAHs.

Most of the problems associated with the determination of CPAHs in surface waters are derived from their low concentration. As a result, sample treatment constitutes the bottleneck of the reported analytical methods. The most common extraction methods for CPAHs are solid phase extraction (SPE) [14] and liquid-liquid extraction (LLE) [10], both of them giving recoveries above 70%. However, these methods present major drawbacks, such as the large sample volume necessary (1- 50 L), the high consumption of hazardous organic solvents in LLE, the irreversible adsorption in SPE and the laborious evaporation and clean-up steps required. Many of these problems have been overcome with the use of solid-phase microextraction (SPME) [15] and stir bar sorptive extraction (SBSE) [16] which have achieved quantification limits for CPAHs in the ranges 0.20-0.98 ng L^{-1} [15] and 0.7-4.9 ng L^{-1}[16] when combined with GC-MS/MS and LC-FL, respectively. These techniques allow a considerable reduction in the volume of sample treated (10-20 mL) and do not require the use of clean-up steps, however some important drawbacks still remain. Thus, extraction times are too long (60-120 min) and recoveries are affected by CPAH concentration (e.g. they were 60.5 and 91.2% for 5 and 50 ng L^{-1} of IP, respectively) [16] and sample matrix (e.g. recoveries for IP ranged between 59 and 95% for superficial and tap water, respectively) [15].

An important factor to consider in the development of an extraction method for CPAHs is their strong adsorption onto the surface of containers [5,17,18], which is a consequence of their high hydrophobicity (see log K_{ow} in Table 1) and results in considerable losses of these pollutants during storage of samples (e.g. around 15% in 24 h and 30% in 96 h for 2 μg $L^{-1}$of BaP in glass containers) [5]. The adsorption can be prevented by the addition to the sample of an organic solvent (e.g. acetonitrile, isopropanol or methanol) in percentages between 20 and 40% v/v [17,19,20], but its effect on the extraction recovery of techniques such as SPE, SPME and SBSE should be always checked. Surfactant aggregates have also been proposed for the preservation of CPAHs in aqueous samples on account of their high stabilizing capacity for the solubilized analytes [5,17,21,22].

In this work, coacervates made up of decanoic acid reverse micelles are proposed for both the microextraction and preservation of CPAHs prior to LC/FL determination at the levels set by the EPA and EU as quality standards for surface waters. Coacervates are water immiscible liquids that separate from colloidal solutions by the action of a dehydrating agent (e.g. changes in temperature or pH of the solution, or addition of an electrolyte or a non-solvent for the macromolecule) [23,24]. They have long been used in analytical extractions where the approach has been named *cloud point technique* [25-27]. The aqueous sample solution is made colloidal by the addition of surfactants at concentrations above their critical aggregation concentration. So the coacervate, that is the extractant, is produced in situ in the bulk sample solution. A major feature of coacervates is the high concentration of amphiphiles, and therefore of binding sites, they contain (typically 0.1-1 mg μL^{-1}). Consequently, high extraction efficiencies can be achieved using low coacervate volumes which results in high concentration factors (typically 100-500).

Coacervates made up of Triton X-114 [28] and sodium dodecane sulphonic acid (SDSA) [5] micelles have previously been proposed for the extraction of PAHs from environmental samples, however the quantitation limits for CPAHs (0.5-20 ng L^{-1}) are not low enough to meet EPA criteria. In addition, some clean-up steps were required to avoid the coelution of Triton X-114 with CPAHs during chromatographic separation [28],while strong acidic conditions were required for the coacervation of SDSA (4M of HCl) [5].

Coacervates consisting of reverse micelles of alkyl carboxylic acids, recently proposed by our research group [29], have the potential to effectively extract and concentrate CPAHs on account of the suitability of reverse micelles to solubilize hydrophobic compounds and the low volume of coacervate obtained (~1.67 μL mg $^{-1}$). Below, parameters affecting extraction efficiency and concentration factors are optimized and the method is applied to the determination of CPAHs in wastewater and surface waters.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent-grade and were used as supplied. Decanoic acid (capric) was obtained from Fluka (Madrid, Spain). Tetrahydrofuran (THF) and LC-grade acetronitrile were supplied by Panreac (Sevilla, Spain) and ultra-high-quality water was prepared with a Milli-Q water purification system (Millipore, Madrid, Spain). The target compounds chrysene (Chry), benzo(b)fluoranthene (BfF), benzo(a)pyrene (BaP), dibenzo(a,h)anthracene (DahAn) and indeno (1,2,3-cd)pyrene (IP) were obtained from Sigma-Aldrich (Steinheim, Germany), while benzo(a)

anthracene (BaA) and benzo(k) fluoranthene (BkF) were obtained from Fluka (Steinheim, Germany). Stock standard solutions containing individual CPAHS at a concentration of 100 mg L^{-1} were prepared in acetonitrile and stored under dark conditions at 4ºC. Working solutions containing a mixture of CPAHs were made by appropriate dilutions of the stock solutions with acetonitrile.

2.2. Apparatus

The liquid chromatographic system used (Spectra System SCM1000, ThermoQuest, San Jose, CA, USA) consisted of a P2000 binary pump, a UV1000 detector and a FL3000 fluorescence detector. In all experiments a PEEK Rheodyne 7125NS injection valve with a 20 μL sample loop was used (ThermoQuest, San Jose, CA, USA). The stationary-phase column was a Supelcosil TM LC-PAH (5μm, 25 cm). A Mixtasel Selecta centrifuge (Barcelona, Spain) was used for sample preparation.

2.3. Determination of CPAHs in surface and wastewater samples

2.3.1 Sample collection and preservation

Wastewater samples were collected in dark glass containers in February 2008 from different municipal wastewater treatment plants (WWTPs) (Lucena, Mengíbar and Arahal) in the South of Spain. Lucena WWTP receives an estimated industrial percentage of the total wastewater input of 40-50% (from the furniture and bronze factories) while those from Mengíbar and Arahal receive mainly domestic influents. Surface waters were collected from the rivers Guadalquivir and Guadajoz and the reservoir La Breña, all of them located in the South of Spain. Samples were transported to the lab and immediately filtered through 0.45 μm filters (Watman GF/F Osmonics, France) in order to remove suspended solids. Then, they were adjusted to pH 2 by the addition of concentrated nitric acid. Aliquots of 36mL were transferred to specially designed centrifuge tubes with a narrow neck (Figure 1), which contained 70 mg of decanoic acid dissolved in 4 mL of THF. The tubes were wrapped with aluminium foil to prevent the photodegradation of CPAHs and sealed with parafilm to avoid THF evaporation. The resulting solution, in which the coacervate formed instantaneously, was either subject to coacervative extraction as specified below or mechanically homogenized and stored at room temperature (20-25 ºC) when immediate extraction was not possible. Under these conditions, samples were stable for at least 1 month.

2.3.2. Coacervative Extraction

The mixture of water sample, THF and decanoic acid, was stirred (700 rpm, 5 min) and then centrifuged (1850 g, 10 min) to speed up the complete separation of the coacervate. The volume of the coacervate (~115 μL), which was standing at the top of the solution in the narrow neck of the tube, was calculated by measuring its height with a digital calliper. Aliquots of 20 μL were withdrawn using a microsyringe and directly injected into the LC-FL system for CPAHs analysis. At this point, as immediate CPAHs analysis was not possible, a volume of coacervate (~100 μL) was transferred to a sealed amber glass vial with insert (~150μL capacity) at 4ºC and analysed within 7 days.

2.3.3. Liquid chromatography/ Fluorescence detection

Separation and quantification of CPAHs was carried out by liquid chromatography-fluorimetry. Water (solvent A) and acetonitrile (solvent B) were used as eluent solvents at a flow rate of 1.5

mL min^{-1}. The gradient elution program selected was 75% B during the first 5 min, from 75% to 100% B in the next 30 minutes and from 100 to 75% for 5 min for reconditioning of the column. The fluorescence detection was performed at the following excitation and emission wavelengths: Chry and BaA (λ_{ex} 266 nm, λ_{em} 384 nm), BbF, BkF and BaP (λ_{ex} 284_n nm, λ_{em} 404 nm), DahAn (λ_{ex} 260 nm, λ_{em} 420 nm) and IP (λ_{ex} 284 nm, λ_{em} 496 nm). Quantification was carried out by measuring the chromatographic peak areas of CPAH standard solutions in acetonitrile in the following concentration ranges: BkF and BaP (0.1-500 μg L^{-1}), BaA, Chry and BbF, (0.2-500 μg L^{-1}), DahA (0.4-500 μg L^{-1}) and IP (1-500 μg L^{-1}).

3. Results and discussion

3.1. Coacervative extraction of CPAHs.

3.1.1. Formation and structure of decanoic acid reverse micelle-based coacervates

Decanoic acid (pK_a= 4.8±0.2) is sparingly soluble in water (e.g. ~0.2 g L^{-1}), while it dissolves well in THF and self-assembles as reverse micelles having 4-8 nm diameter according to a sequential-type self-association model [29,30]. When water (pH 1-4) is added to these solutions, reverse micelles result partially desolvated and micelle-micelle interactions becomes easier, which leads to the formation of larger reverse micelles (sizes in the range nm-μm) that separate as an immiscible liquid from the bulk solution and produce the coacervate. At a microscopic level, the coacervate consists of spherical droplets of different sizes dispersed in a water:THF continuous phase. The water content is only about ~1-2%, and it is expected to be either in the micellar core or mixed with THF in the continuous phase.The excellent solvation properties of reverse micelles and the low volume of the coacervates obtained (e.g. 1.67 μL mg^{-1} when using 10% THF) make them very attractive for analytical extractions. Since the coacervation phenomenon occurs from protonated decanoic acid, extractions must be carried out below pH 4.

Figure 2 depicts the region at which coacervation is produced as a function of the relative concentration of the three coacervate components (THF, water and decanoic acid). Below and above the boundaries of this region, the decanoic acid precipitates or solubilizes in the THF:water mixture, respectively. This type of phase diagram has been previously observed for the coacervation of phospholipids [31,32] and gelatine [33] in miscible water/alcohol binary mixtures and it is very different to those obtained from surfactants in immiscible solvents binary mixtures [34,35].Figure 3 shows a picture of the surfactant aggregates present in the different steps of the extraction process. The driving forces for the extraction of CPAHs in the decanoic acid reverse micelles are mainly hydrophobic and consequently the more probable solubilization site is the surfactant tails at the micellar surface.

3.1.2. Optimisation

Optimal conditions for the extraction of CPAHs were investigated by changing each variable in turn while keeping the others constant. Actual concentration factors (ACFs), calculated from recoveries (Rs) and phase volume ratios (sample volume/coacervate volume; PVRs), were used as a criterion for the selection of the experimental conditions. In order to meet the restrictive water quality criteria set by EPA for CPAHs (3.8ng L^{-1}) and taking into account the instrumental quatitation limits of fluorescence detection for these compounds (0.1-1 μg L^{-1}), ACFs around 280

should be achieved. So, conditions giving these ACF value were selected provided that recoveries were above 70% and relative standard deviations were below 10%, as it has been recommended for the determination of pollutants in environmental samples [36-38].

Phase volume ratios (PVRs) depended on both the decanoic acid amount and THF concentration, linearly and exponentially, respectively. Water hardly incorporated to the extractant phase due to its non-solvent character for the reverse micelles. For the optimisation process, PVRs were estimated from a general equation previously reported for the prediction of the volume of the coacervate made up of decanoic acid as a function of its major components (THF and decanoic acid) [39]. The equation was $y = 1.035\ \mu L\ mg^{-1}\ a\ e^{0.0473b}$, where y was the volume of coacervate in μL, a the amount of decanoic in mg and b the THF in percentage (v/v). The correlation coefficient for this equation, fitted by nonlinear regression, was 0.995, thus indicating its high capability of prediction.

Recoveries were investigated as a function of the amount of decanoic acid (20-400 mg), percentage of THF (2.5-30%, v/v), pH (1-4), salt concentration (10^{-3}-1M NaCl), temperature (25-60 ºC) and extraction time (0-30 min, at 700 rpm with a magnetic bar). Experiments were made in triplicate. Distilled water was spiked at two CPAHs concentration levels depending on the PVRs estimated, namely 10 and 50 ng L^{-1} for PVRs above and below 250, respectively. The final volume of the solution (distilled water + THF) was 40 mL.

Table 2 shows the results obtained for recoveries and ACFs as a function of the amount of decanoic acid. Because of their high hydrophobicity (see log K_{ow} in Table 1), CPAHs behaved similarly with regard to extraction. Recoveries around 90% were obtained for all the target compounds at decanoic acid amounts as low as 60 mg. The recovery was quantitative as the amount of decanoic acid was about 400 mg but the volume of coacervate, which is linearly dependent on this component, increased too much and the corresponding ACFs did not meet the EPA water quality criteria for CPAHs. Standard deviations were always low enough and they were not considered for the selection of this variable. An amount of decanoic acid of 70 mg was selected for further experiments on account of the scarce dependence of recoveries on decanoic acid in the range 60-80 mg and the ACFs obtained.

The influence of the concentration of THF on the recoveries and ACFs is given in Table 3. Recoveries progressively increased as the percentage of THF did and then they decreased at the highest concentration investigated. This behaviour was related to the composition of the coacervate as a function of the THF percentage. Thus, according to previous results [29], maximal transfer of decanoic acid from the bulk solution to the coacervate occurs at THF concentrations around 10%. Above this concentration, the amount of decanoic acid in the coacervate keeps constant while the amount of THF exponentially increases until a maximum at which the coacervate starts to dissolve. So, the increasing recovery for CPAHs between 2.5 and 20% could be explained by the higher amount of decanoic acid and THF in the coacervate. Above 20%, the decrease in recoveries was probably due to the dissolution of a portion of the coacervate in the THF:water bulk solution, since a decrease of about 30% in the measured volume of the coacervate (with regard to the theoretical volume predicted by the general equation cited above) was observed. No significant differences were known between the recoveries obtained for the different CPAHs investigated. A concentration of 10% THF was selected for

further studies. The volume of coacervate obtained under these conditions was around 115 μL which permitted to get 2-3 different chromatographic runs per sample in a reliable way (20 μL each injection).

CPAHs were not affected by the pH in the range 1 to 4, which is logical considering the type of interactions expected to be the driving forces for the extraction. The pH of the samples, previously adjusted to 2 during sample treatment (see section 2.3.1) was maintained for extraction. On the other hand the addition of NaCl to samples over the concentration range 10^{-3}–1 M or the increase of the temperature of the sample solution from 25 to 60ºC did not affected CPAHs extraction efficiencies or concentration factors. The time necessary to reach extraction equilibrium conditions using the procedure proposed (stirring rate 700 rpm) was about 1 min, although 5 min was proposed to assure complete homogenisation of samples.

3.2. Study of the adsorption of CPAHs onto the surface of glass containers under different preservation conditions

The extent of adsorption of CPAHs onto the surface of amber glass containers was assessed using spiked wastewater influent samples, previously filtered and adjusted to pH 2 with concentrated nitric acid, and subjected to different preservation conditions. Firstly, adsorption was investigated in samples stored at 4ºC, with no additives, for a period of 7 days. Aliquots of these samples were directly analysed by LC/FL, so the spiked concentrations (2 μg L^{-1} for each CPAH except for IP, 5 μg L^{-1}) were selected to be able to asses until at least a percentage of adsorption around 80%. Experiments were made in triplicate. Table 4 shows some representative results. CPAHs were rapidly adsorbed onto the glass surface at the start, the adsorption being higher as the hydrophobicity of the target compounds increased (see octanol-water constants in Table 1). The adsorption was progressively increasing and it was in the range 64-80% after 7 days of sample storage. The addition of THF at the concentration required to produce the coacervate, 10%, did not avoid adsorption but it was lower than without additives, especially for the less hydrophobic compounds (see Table 4).

At this point, we studied the capacity of decanoic acid reverse micelle-based coacervates for the desorption of CPAHs from the surface of glass containers for samples stored at pH 2 without additives. With this aim filtered wastewaster influent samples (36 mL, pH 2), were spiked with 30 ng L^{-1} of each CPAH and stored during 24 or 48h in specially designed centrifuge tubes (4ºC, in the dark). Then, they were subjected to coacervative extraction by adding 70 mg of decanoic acid dissolved in 4mL of THF. Extractions were carried out by stirring the samples (700 rpm) at 10-min intervals and then centrifuged at 1850 g for 10min. Aliquots of the extracts (20 μL) were analysed by LC-FL. According to the results, the coacervate was able to recover the previously adsorbed CPAHs, but the time required for their complete recovery increased as the time of sample storage did. Thus, 40 and 60 min were necessary for quantitative extraction of CPAHs from samples stored without additives (4ºC, in the dark) during 24 and 48 h, respectively. So, if this option is selected for sample preservation, the time for coacervative extraction after longer periods of storage should be optimised.

Finally, the suitability of coacervates for the preservation of CPAHs after their collection was assessed. With this purpose, aliquots of wastewater influent samples (36 mL) were filtered and adjusted at pH 2, spiked with 30 ng L^{-1} of each CPAH and transferred to specially designed

centrifuge glass tubes (Fig. 1), that contained 70 mg of decanoic acid dissolved in 4 mL of THF. The tubes were wrapped with aluminium foil to prevent CPAHs from photochemical degradation. As the equilibrium partition of CPAHs between the coacervate and the bulk aqueous solution was rapidly reached after just a mechanical homogenization, the solubilization of CPAHs into the reverse micelles should prevent them from adsorption onto the surface of the glass tubes. To confirm this hypothesis and in order to give working flexibility to labs, two storage conditions were investigated. First, the glass tubes containing the sample and the coacervate were sealed with parafilm to prevent THF evaporation and then they were kept at room temperature (20-25 ºC) until analysis. At this point, the sample was centrifuged at 1850 g for 10min and an aliquot of 20 μL was analysed for CPAHs by LC/FL. Secondly, samples were centrifuged and about 100 μL of the coacervate was transferred to sealed amber glass vials with inserts (~150μL capacity) and stored at 4ºC until analysis by LC/FL. In both cases, CPAH analysis was carried out at three-day intervals during one month. The results obtained showed that the coacervate stabilized all the CPAHs for at least 1 month, independently of the storage conditions, so it constitutes a good tool for preservation of the target compounds.

3.3. Analytical performance

Calibration curves for CPAHs were run using standard solutions prepared in acetonitrile since no differences in peak areas or retention times were observed for the analytes injected in organic solvent or the coacervate. The main analytical characteristic of the method are given in Table 5. The instrumental detection (LODs) and quantification (LOQs) limits were calculated from blank determinations (i.e. bidistilled water extracted similarly to the samples) using a signal-to-noise ratio of 3 and 10, respectively. The instrumental detection (LODs) and quantification limits (LOQs) were calculated from blank determinations by using a signal-to-noise ratio of 3 and 10, respectively. The quantification and detection limits of the method were estimated from these values and considering the ACFs obtained. The method LOQs were between 0.4 and 3.5 $ngL^{-1}$for all the CPAHs (see Table 5). Consequently, the method permitted their quantification below the ultra-trace level proposed by EPA as quality standards for CPAHs in surface water (3.8 ngL^{-1}). It also allowed to meet the quality standards established by the EU for CPAHs, except for IP, which could be detected but not quantified at the required level (2 ngL^{-1}). Under the experimental conditions proposed for their determination, recoveries for CPAHs varied between 88% and 95%, in the whole range of concentrations tested (~1-200 ngL^{-1}), with standard deviations in the interval 1-6%.

The possible interference of matrix components that could elute with CPAHs was assessed by comparison of the slopes of the calibration curves (n =7) obtained from standards in distilled water with those obtained from wastewaters and river water samples, fortified with known amounts of CPAHs, and run using the whole procedure. The slopes of the calibration curves performed in distilled water were 496±10, 737±35, 354±20, 1035±62, 860±50, 380±19, 37±2 mV L ng^{-1} for BaA, Chry, BbF, BkF, BaP, DahAn and IP, respectively. The difference between these slopes and those obtained from environmental samples were found to be not statistically significant by applying an appropriate Student's t test [40]. The calculated t-values were in the range 0.03–1.1 and were below the critical t-value (3.17), being significance established at 0.01 levels. Therefore, matrix components were not expected to interfere in the determination of the target compounds.

The precision of the method was evaluated by extracting 11 independent fortified samples using wastewaters (n = 6) and surface waters (n = 5). The values expressed as relative standard deviation (R.S.D.), were between 3% and 5% for the seven CPAHs.

3.4. Analysis of environmental water samples.

To prove the suitability of the method to work under real conditions, it was applied to the determination of CPAHs in two rivers, a reservoir and three different WWTPs. Table 6 shows the results obtained, expressed as the mean value of three independent determinations, besides their corresponding standard deviations. These samples were also spiked with different CPAHs concentrations according to the level of CPAHs found in the samples. The recoveries obtained ranged between 88% and 95% with relative standard deviations from 1 to 7%.

The target compounds, except IP, were present in all influents at concentrations in the range 3.9-37 ng L^{-1}, the highest concentrations being found in Lucena WWTP that receives a large percentage (40-50%) of industrial wastewater. The treatment at the WWTPs reduced the levels of CPAHs below their detection limits, being they only present in the Lucena effluent at very low concentrations (below ~2 ng L^{-1}).

Regarding surface waters, CPAHs were found in some samples at levels near or even higher than the water quality standards recommended by EPA. Six CPAHs were present in the river Guadajoz, five of them above the quantification limit and in the range 1.8-6.6 ng L^{-1}, while six CPAHs were found at levels between 1.39 and 4.8 ng L^{-1} in the reservoir La Breña. The fact that the sampling location in the river Guadajoz was near a divided highway while in river Guadalquivir was at countryside, may explain the difference in the concentrations of analytes in these two rivers. On the other hand, the presence of CPAHs in La Breña was probably due to the frequent aquatic motor activities at this reservoir, exhaust emissions resulting in large amounts of combustion products and unburned fuel being mixed into water [41]. In fact the increasing CPAHs pollution of surface waters by recreational water craft is an issue that demands research [41].

The chromatograms obtained from a standard solution in acetonitrile (A), La Breña reservoir (B) and Lucena influent (C) and effluent (D) samples are shown in Figure 4. No interference from matrix components were detected for any of the samples analysed.

4. Conclusions

Coacervates of reverse micelles of decanoic acid have been proven to be a valuable tool for the extraction of CPAHs from wastewater and surface water samples prior to their determination by LC/FL. The extraction procedure is robust (extractions are no dependent on the ionic strength, temperature or matrix components), simple (no clean-up of extracts or solvent evaporation are necessary), rapid (extractions require 5 min of stirring and 10 min of centrifugation and several samples can be simultaneously processed), and suitable for the preservation of the analytes during storage of samples (coacervates are able to prevent the adsorption of CPAHs onto the surface of glass containers for at least 1 month). Furthermore, it requires low volume sample (36 mL) and features low cost (no special equipment is required for extraction, so the method can be applied in labs without extra investment, and uses fluorimetry as detector that is cheaper than MS). The proposed method permits the routine monitorization of these compounds at the

thresholds set as quality standards in surface waters by the EPA and the EU. Only IP could not be quantified, although it may be detected, at the level fixed by the EU (2 ngL^{-1}), owing to its lower fluorimetric sensitivity.

Acknowledgment

The authors gratefully acknowledge financial support from Spanish MCyT (Project CTQ2005-00643). They also thank the personnel from the following municipal WWTPs for kindly collecting the sewage water samples: Arahal, Lucena and Mengíbar. A. Ballesteros-Gómez acknowledges to the Spanish MEC the doctoral fellowship awarded (AP2005-4275).

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Figure captions

Figure 1. Schematic picture of the glass centrifuge tube designed for coacervative extractions.

Figure 2. Phase diagram of decanoic acid in binary mixtures of THF:water.

Figure 3. Illustration of the different surfactant aggregates involved in the coacervative extraction.

Figure 4. LC/Fluorescence chromatograms obtained from (A) a standard solution in acetonitrile (10 μg L^{-1} of IP and 5μg $L^{-1}$of the rest of CPAHs); (B) a reservoir water sample (La Breña in Córdoba, Spain); and (C) an influent and (D) an effluent wastewater sample from Lucena´s WWTP in Córdoba, Spain.

Figure 1

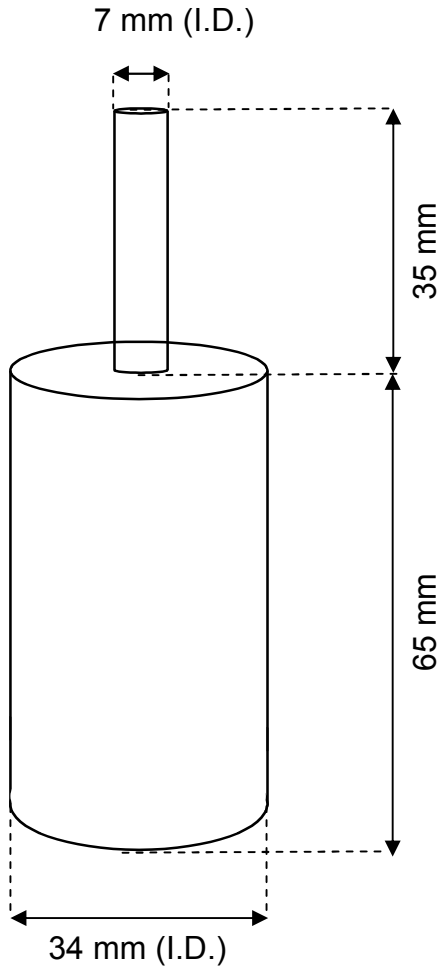


Figure 2

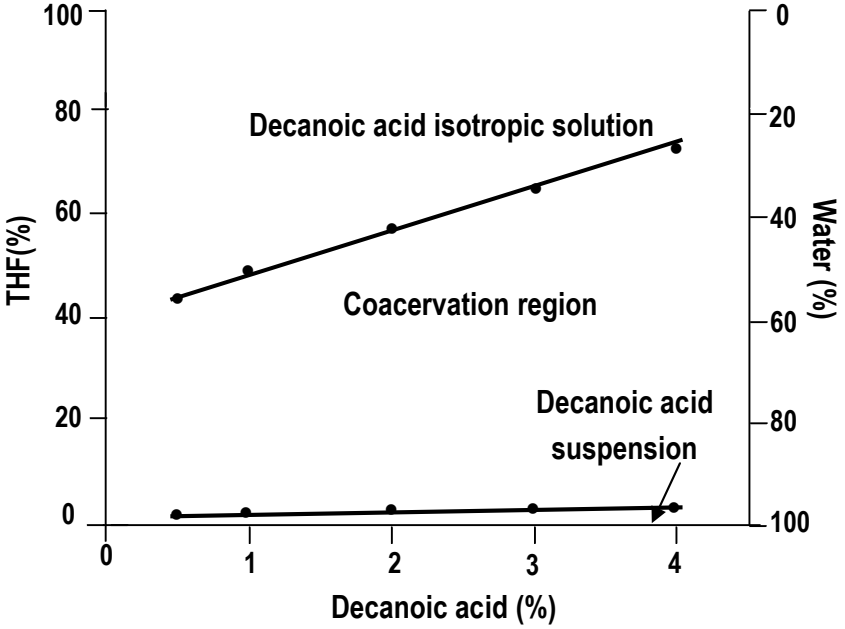

100
80
60
40
20
0
THF(%)
0
20
40
60
80
100
Water (%)
Decanoic acid isotropic solution
Coacervation region
Decanoic acid
suspension
0
1
2
3
4
Decanoic acid (%)

Figure 3

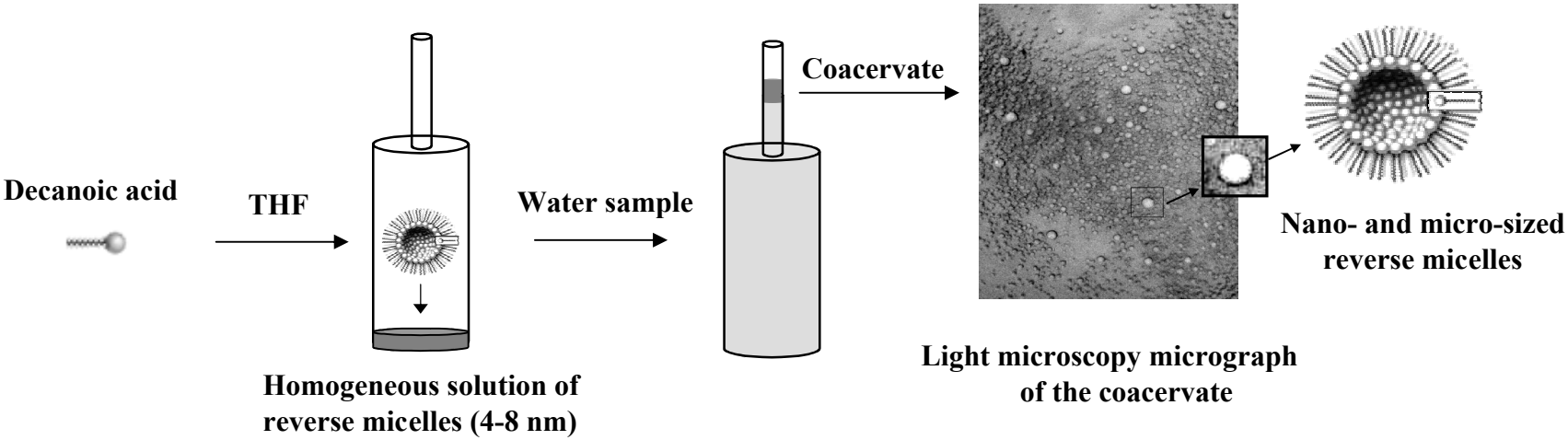

Decanoic acid
THF
Water sample
Coacervate
Nano- and micro-sized
reverse micelles
Homogeneous solution of
reverse micelles (4-8 nm)
Light microscopy micrograph
of the coacervate

Figure 4

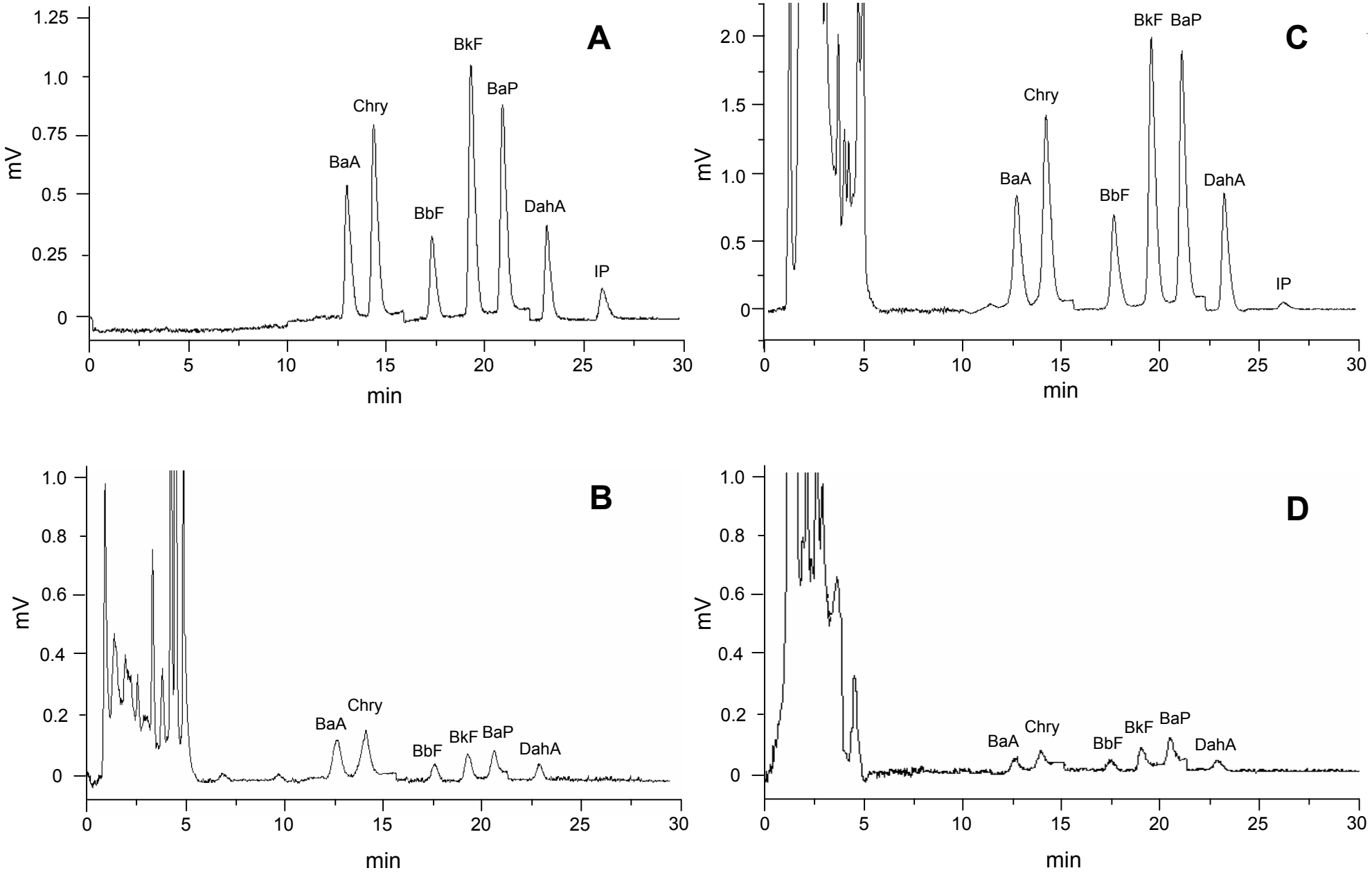
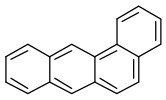
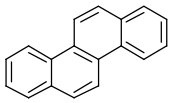
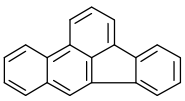
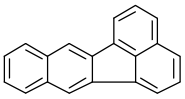
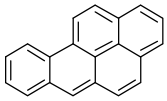
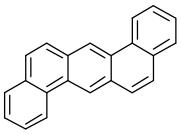
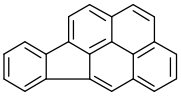


Table 1

Table 1.

Priority carcinogenic polycyclic aromatic hydrocarbons: Structures, octanol:water partition coefficients and estimated carcinogenic potency

PAH	Structure	Log K_{ow}[a]	Carcinogenic Potency[b]
Benzo(a)anthracene, BaA		5.91	2B
Chrysene, Chry		5.91	2B
Benzo(b)fluoranthene, BbF		6.40	2B
Benzo(k)fluoranthene, BkF		6.40	2B
Benzo(a)pyrene, BaP		6.40	1
Dibenzo(a,h) anthracene, DahAn		7.14	2A
Indeno(1,2,3-cd)pyrene, IP		6.89	2B

[a] Calculated using Advanced Chemistry Development (ACD/Labs) Software V9.04 for Solaris.

[b] IARC classification: carcinogenic to humans (1), probably carcinogenic to humans (2A), possibly carcinogenic to humans (2B).

Table 2

Table 2

Mean percent recoveries along with their standard deviations (R ± SD, %) and actual concentration factors (ACF) obtained for CPAHs as a function of the amount of decanoic acid

CPAH	Decanoic acid (mg)													
	20		40		60		70		80		200		400	
	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[b]R±[c]SD	ACF	[b]R±[c]SD	ACF
BaA	56±2	607	65±3	353	88±1	318	91±3	282	92±2	249	94±3	102	98±1	53
Chry	56±3	607	65±1	353	87±4	314	91±1	282	93±2	252	95±2	103	97±1	53
BbF	54±1	585	66±5	358	87±2	314	92±3	285	92±2	249	96±4	104	99±4	54
BkF	54±3	585	64±4	348	89±4	322	93±2	288	92±3	249	96±2	104	98±5	53
BaPy	54±4	585	64±2	348	88±1	318	93±3	288	91±3	247	95±6	103	98±2	53
DahAn	53±2	574	63±1	342	89±2	322	91±4	282	92±2	249	95±3	103	97±2	53
IP	51±1	553	62±1	342	87±1	314	92±2	285	93±4	252	97±2	105	99±5	54

Spiking levels: [a] 10 ng L^{-1}, [b] 50 ng L^{-1}; [c] *n* = 3; THF =10 %

Table 3

Table 3

Mean percent recoveries and standard deviations (R ± SD, %), and actual concentration factors (ACFs) obtained for CPAHs as a function of tetrahydrofuran concentration

	THF (%, v/v)									
	2.5		**5**		**10**		**20**		**30**	
	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[a]R±[c]SD	ACF	[b]R±[c]SD	ACF	[b]R±[c]SD	ACF
BaA	82±1	392	84±3	348	91±3	282	99±6	170	74±5	69
Chry	85±2	406	84±2	348	93±6	288	97±5	166	72±4	67
BbF	83±1	397	84±4	348	93±4	288	100±6	172	76±4	71
BkF	85±5	406	86±4	356	93±4	288	102±5	175	80±4	75
BaPy	85±3	406	89±1	368	92±4	285	100±3	172	76±5	71
DahAn	83±2	397	87±1	360	92±5	285	100±2	172	80±6	75
IP	85±4	406	89±1	368	92±3	285	99±4	170	79±6	74

Spiking levels: [a] 10 ng L^{-1}, [b] 50 ng L^{-1}; [c] *n*= 3; decanoic acid= 70 mg

Table 4

Sorption of CPAHs onto glass containers as a function of time.

CPAH	Percentage of adsorption (%) ± [a]standard deviation							
	Without additives				Addition of 10% THF			
	4 h	24 h	48 h	168 h	4 h	24 h	48h	168 h[b]
BaA	25±1	33±2	52±3	69±2	27±1	28±1	27±1	27±1
Chry	26±1	35±2	51±2	64±3	26±1	27±1	27±2	29±2
BbF	30±1	44±2	60±3	65±5	32±1	31±1	50±4	51±3
BkF	27±2	44±2	61±4	64±2	28±2	40±2	49±3	49±2
BaPy	30±3	50±1	63±5	69±4	29±2	50±5	55±2	56±4
DahAn	35±3	55±4	60±3	80±4	34±2	55±4	65±2	65±4
IP	38±2	58±1	60±3	80±4	37±2	58±3	65±2	64±4

[a]*n*= 3; influent sample spiked with 2 μg L^{-1}(except for IP, 5 μg L^{-1}); storage conditions: 4ºC, dark.

Table 5

Table 5

Analytical performance of the method

Target compound	Retention time (min)	External calibration			Method [b]LOQ (ng L^{-1})	Method [c]LOD (ng L^{-1})
		Linear range (µg L^{-1})	Slope ±SD [(x10^3) mV L µg^{-1}]	[a]r		
BaA	13.2	0.2-500	1.99±0.03	0.998	0.7	0.3
Chry	14.4	0.2-500	2.96±0.05	0.998	0.7	0.3
BbF	17.7	0.2-500	1.41±0.02	0.998	0.7	0.3
BkF	19.6	0.1-500	4.2±0.06	0.998	0.4	0.1
BaP	21.1	0.1-500	3.46±0.05	0.998	0.4	0.1
DahA	23.4	0.4-500	1.53±0.03	0.998	1.4	0.4
IP	26.3	1-500	0.148±0.007	0.996	3.5	1

[a] correlation coefficient; *n*=7; [b] estimated quantification limits of the method; [c] estimated detection limits of the method.

Table 6

Table 6

Mean concentration (ngL^{-1}) ±standard deviation (n = 3) of the CPAHs found in wastewater and surface water samples, and recoveries (%) ±standard deviation (n = 3) obtained after spiking the samples with the target analytes.

Sample Location	BaA	Chry	BbF	BkF	BaPy	DahA	IP
WWTP Influent							
Lucena[a]	35±1	34±1	38±1	34±1	35±2	37±2	23±1
	90±4	91±4	90±3	90±4	94±5	93±5	90±4
Mengíbar[b]	8.0±0.3	12.4±0.5	9.3±0.6	6.6±0.4	7.1±0.6	8.6±0.5	n.d.
	93±4	96±4	92±6	90±5	92±6	93±6	93±5
Arahal[b]	3.9±0.2	5.2±0.4	16.5±0.9	11.4±0.4	4.1±0.1	6.9±0.2	n.d.
	91±5	92±5	92±5	90±3	94±2	94±5	95±4
WWTP Effluent							
Lucena[c]	2.11±0.05	1.16±0.03	1.08±0.01	0.77±0.03	1.09±0.01	1.7±0.1	n.d.
	92±2	95±2	90±1	94±4	92±1	90±5	93±5
Mengíbar[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	90±3	96±2	94±3	92±3	90±4	94±4	93±2
Arahal[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	91±2	93±1	92±1	91±6	92±6	92±7	93±6
Surface water							
Guadalquivir river[c]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	91±4	89±3	90±2	90±3	95±6	90±6	95±4
Guadajoz river[c]	2.5±0.1	3.4±0.2	3.4±0.1	<LOQ	6.6±0.2	1.8±0.1	n.d.
	88±4	89±4	90±2	92±3	95±1	91±3	92±5
La Breña	4.8±0.3	3.2±0.1	2.2±0.1	1.39±0.02	1.46±0.02	2.2±0.1	n.d.
reservoir[c]	92±5	92±2	94±6	94±2	92±3	92±5	93±6

[a]: sample spiked with 30 ng L^{-1} ; [b]: sample spiked with 10 ng L^{-1}; [c]: sample spiked with 2 ng L^{-1}; except for IP (5 ngL^{-1}); n.d. non detected; <LOQ: detected but below the quantification limit.