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Title: Determination of bisphenols A and F and their diglycidyl ethers in wastewater and river water by coacervative extraction and liquid chromatography-fluorimetry

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Abstract

A simple, rapid and sensitive analytical method was developed for the simultaneous determination of bisphenol A (BPA), bisphenol F (BPF) and their corresponding diglycidyl ethers (BADGE and BFDGE) in wastewater and river water, in order to have a useful tool for evaluating their fate and distribution in aquatic environments. It was based on their extraction with coacervates made up of decanoic acid reverse micelles and subsequent determination by liquid chromatography/fluorimetry. The procedure involved the extraction of 10.8 mL of water sample for 5 min, its centrifugation for 10 min to accelerate phase separation and then the chromatographic analysis of the target compounds. Clean-up or solvent evaporation steps were not necessary to get the required sensitivity and selectivity. Extraction efficiencies and concentration factors mainly depended on the amount of decanoic acid and tetrahydrofuran making up the coacervate. A general equation for the prediction of the volume of the coacervate as a function of its components has been proposed and fitted by nonlinear regression. This equation permits to know a priori the maximum concentration factors that can be achieved under given experimental conditions. Extractions were independent of salt addition (up to 1M), the temperature (up to 60 ºC) and the pH (below 4) rendering the method robust. Recoveries in samples ranged between 80 and 96% and the actual concentrations factors were between 87 and 102, which resulted in practical detection limits around 30-32 ng L^{-1}. The method was successfully applied to the determination of the target pollutants in raw and treated sewage from four mechanical-biological treatment plants and three rivers. Bisphenols and their diglycidyl ethers were present in wastewater influents at concentrations in the range 0.96 to 1.6 $\mu g\ L^{-1}$. The biological treatment at the WWTPs studied reduced the concentration of BPA and BPF in a percentage above 75%, while diglycidyl ethers were not detected in most of the effluents investigated. Only BPA was detected in surface waters and its concentration was above the general limit recommended by the EU for organic pollutants in waters.

1. Introduction

Bisphenols A (BPA) and F (BPF) are extensively used as leading chemicals in plastics [1] with a variety of industrial applications, including digital media products (e.g. CDs, DVDs), electrical and electronic equipment, lacquer coatings in cans, and dental composites and sealants [2,3]. Bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) are epoxy resins obtained by reaction of their respective monomers, BPA and BPF, with epichlorohydrin [4].

Occurrence of BPA in the aquatic environment has been widely reported [5-10], its presence largely arising from factories where it is produced or used as a starting material. Levels found for BPA in surface water and wastewater vary over a wide range (e.g. 5-500 ng L^{-1} in several German rivers, lakes and channels and 20-700 ng L^{-1} in different German sewage effluents [6]), nevertheless they do not usually exceed 1 µg L^{-1} [11]. Data for BPF in the aquatic environment are scarce [6]. On the whole, BPF is present at significantly lower levels than BPA (e.g. 0.1-180 and 22-123 ng L^{-1} in German surface waters and wastewaters, respectively [6]), which has been explained in terms of the much lower quantities in present use. To our knowledge, no data have been reported on the concentrations of BADGE and BFDGE in the aquatic environment.

Concern has been raised in recent years about the toxicity of BPA to aquatic organisms, which has been mainly related to its estrogenic activity [12]. A predicted no-effect concentration ($PNEC_{water}$) for BAP of 1 µg L^{-1}[11] has been proposed from the results obtained in different ecotoxicity studies using fish, invertebrates and algae as test organisms and considering a safety factor of 1000 [13]. The lowest reported PNEC for BPA has been 0.1 µg L^{-1} [14] on the basis of BPA effects on some fish [15]. Although no information is available about the ecotoxicity of BPF, BADGE or BFDGE [16], as with BPA, release of these chemicals into the environment is possible during manufacturing and by leaching from final products. According to their water solubility (e.g. 360, 89, 14 and 5 mg L^{-1} for BPF, BPA, BFDGE and BADGE, respectively), their distribution in aquatic ecosystems should be comparable, to a certain extent, to that of BPA. The toxicity of BPF has been proven and is mainly related to its estrogenic [17] and strong antiandrogenic [18] effects, while that of BADGE and BFDGE is related to their cytotoxic effects [19], which make them tumorigen and mutagen [20]. So, in order to have a more real assessment of the environmental impact caused by the chemicals released from plastic processes and products, the environmental concentrations and ecotoxicity of BPF, BADGE and BFDGE should be also taken into consideration [6] and this issue demands research.

Most analytical methods proposed in recent years to determine endocrine disruptors in rivers and municipal/industrial wastewaters include the determination of BPA. In general, because of the complexity of the mixtures analysed, these methods are based on GC/MS or LC/MS and require a laborious sample treatment step, largely involving the solid phase extraction (SPE) or liquid-liquid extraction (LLE) of sample volumes between 0.5 and 10 litres [7,21-23]. Measurement of BPA in the aquatic environment can be made simpler using solid-phase microextraction (SPME) [24] or stir bar sorptive extraction (SBSE) [25] combined with GC/MS. In this way, quantification limits around 10 ng L^{-1} can be obtained. Few methods have been proposed for the simultaneous determination of BPA and BPF in wastewater and rivers [6,7]. They are based on LLE or SPE of high sample volumes (1-2 litres) and subsequent solvent evaporation. To our knowledge, only one analytical method has been reported for the simultaneous determination of BPA, BPF, BADGE and BFDGE in aquatic environments [26]. It consist in a LLE of 0.5-L water sample, evaporation to dryness and subsequent determination by GC-MS. Quantification limits for BFDGE and BADGE were 450 and 300 ng L^{-1}, respectively, which was well above the concentrations expected for these toxics in environmental waters.

This work deals with the development of a simple, rapid and sensitive method for the simultaneous determination of BPA, BPF, BADGE and BFDGE in aquatic environments. It is based on the coacervative extraction of the analytes and their subsequent determination by LC-fluorescence detection. Coacervates are water immiscible liquids that separate from colloidal solutions by the action of a dehydrating agent, namely temperature, pH, electrolyte or a non-solvent for the macromolecule [27,28]. After separation, the coacervate contains most of the colloid and is in a dynamic equilibrium with the initial solution. In analytical extractions, 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. The most used surfactant aggregates in analytical extractions have been aqueous non-ionic [29-31], amphoteric [32], anionic [33] and cationic [34] micelles. Recently, coacervates made up of vesicles [35] and reversed micelles [36] of alkyl carboxylic acids have been reported by our research group, which permit the extraction of analytes in a wide polarity range. The main advantages of coacervative extraction, usually named in the analytical literature as cloud point extraction (CPE), are high efficiency and concentration factor, low cost, safety and environmental friendliness.

In this study the suitability of coacervates made up of reverse micelles of alkyl carboxylic acids to extract bisphenols from wastewater and rivers was assessed. The selection of this coacervate was based on its capacity to establish both hydrogen bond and hydrophobic interactions with analytes and its low volume. Below, the parameters affecting the extraction efficiency and concentration factor are optimised and the method is applied to the determination of the target compounds in river and wastewater.

2. Experimental

2.1. Chemicals

All chemicals were of analytical reagent-grade and were used as supplied. The alkyl carboxylic acids, decanoic (capric), dodecanoic (lauric) and tetradecanoic (miristic) were obtained from Fluka (Madrid, Spain). Octanoic acid (caprilic) was acquired from Riedel-de Haën (Seelzen, Germany). Tetrahydrofuran and LC-grade acetronitrile were supplied by Panreac (Sevilla, Spain) and ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). Tetrabutylammonium bromide was obtained from Sigma-Aldrich (Milwaukee, WI). Bisphenol A [BPA; 2,2'-Bis(4-hydroxyphenyl)propane], bisphenol F [BPF; Bis(4-hydroxyphenyl)methane], bisphenol A diglycidyl ether [BADGE; Bis(4-hydroxyphenyl)dimethylmethane diglycidyl ether] and bisphenol F diglycidyl ether [BFDGE; Bis(4-hydroxyphenyl)methane diglycidyl ether] were obtained from Fluka (Madrid, Spain). BFDGE was supplied as a mixture of three position isomers (ortho-ortho, ortho-para, para-para) whose relative proportions were unknown. A stock standard solution containing a mixture of bisphenols, 1 g L^{-1} each, was prepared in acetonitrile and stored under dark conditions at 4ºC. Working solutions were made by appropriate dilutions of the stock solution with acetonitrile.

2.2. Apparatus

The liquid chromatrographic system used (Spectra System SCM1000, ThermoQuest, San Jose, CA, USA) consisted of a P4000 quaternary pump, a UV6000LP diode-array detector and a FL3000 fluorescence detector. In all experiments, a Rheodyne 7125 NS injection valve, with a 20 μL sample loop, was used (Thermo Quest). The stationary-phase column was a Hipersil ODS C_{18} column (5μm, 4.6 x 150 mm) from Análisis Vinicos (Tomelloso, Spain). A Mixtasel Selecta centrifuge (Barcelona, Spain) was used for sample preparation.

2.3. Optimisation studies

The optimisation of the extraction process was carried out by studying the influence of different parameters on both the extraction efficiencies and concentration factors obtained for bisphenols. Experiments were made by dissolving alkyl carboxylic acids (C_8-C_{14}, 0.1-4%) in tetrahydrofuran (2-40%) into specially designed centrifuge tubes that had narrow necks (~7 mm i.d.). Then, an appropriate volume of aqueous solution containing a mixture of BPA, BPF, BADGE, BFDGE, 5 μg L^{-1} each, at pH between 1

and 4 was added (final volume of the solution = 40 mL). Immediately, an immiscible alkyl carboxylic acid-rich phase, named coacervate, separated from the bulk solution. The mixture was stirred (700 rpm, 1-60 min, 20-60 ºC) to extract the analytes and then centrifuged (3500 rpm, 10 min) to speed up the separation of the two phases. The volume of the coacervate was calculated by measuring its height in the cylindrical neck of the tube with a digital calliper. Finally, the coacervate was transferred to a 2-mL flask using a microsyringe, made up to the mark with acetonitrile and 20μL of the resulting solution was injected into the LC-FL system. Given that the presence of the surfactant in the extract did not alter the chromatographic signals or the retention times of bisphenols, calibration curves were performed using standards dissolved in acetonitrile. The coacervate volume estimates were analysed by nonlinear regression (statistical program SPSS V.11.5) in order to define a prediction equation for the volume of the coacervate under given experimental conditions. All the optimisation experiments were performed in triplicate.

2.4. Determination of bisphenols in river and wastewater samples.

2.4.1. Sample collection and preservation.

Wastewater samples were collected in March 2007 from different municipal wastewater treatment plants (WWTPs) (Linares, Bailén, Mengíbar and Lucena) and rivers (Guadalquivir, Rabanales and Dos Torres) all of them located in the south of Spain. Although the composition of influents in these WWTPs, which are a mixture of industrial and domestic wastewater, is very variable, estimated data of the industrial percentage were supplied by the personnel of the WWTPs: 30-50% for Linares WWTP (mainly from the car and engineering industries); 20-40% for Bailén WWTP (principally from brickworks, ceramic and olive oil industries) and 40-50% for Lucena (from the furniture and bronze factories). Mengíbar WWTP receives mainly domestic influents. After collecting the samples in dark glass containers, they were filtered through 0.45 μm filters (Watman GF/F Osmonics, France) in order to remove suspended solids and adjusted to pH 2 by the addition of concentrated nitric acid. They were finally stored in amber bottles at 4 ºC until analysis.

2.4.2. Coacervate-based extraction.

Decanoic acid (60mg) was dissolved in THF (1.2 mL) into centrifuge tubes which had a narrow neck (~7 mm i.d.). Afterwards, a river or wastewater sample (10.8 mL) at pH 2 was added, which induced the formation of a water immiscible decanoic acid-rich coacervate. The mixture was stirred (700 rpm, 5 min) to increase the extraction rate of analytes, and then centrifuged (3500 rpm, 10 min) to accelerate the separation of the coacervate from the bulk solution. After the measurement of the volume of the coacervate, which was standing at the top of the solution in the narrow neck of the centrifuge tube, an aliquot (20μL) was withdrawn using a microsyringe and directly injected into the LC-FL system.

2.4.3. Liquid chromatography/ Fluorimetry

Separation and quantification of BAP, BPF, BADGE and BFDGE was carried out by liquid chromatography-fluorimetry. Acetonitrile (solvent A) and water (solvent B) were used as eluents at a flow rate of 1mL/min. The gradient elution program was as follows: from 45 to 55% A in 7 min, from 55 to 70% A in next 23 min and finally from 70 to 100% A for 5 min. The column effluent was monitored at 280 nm of exciting wavelength and 306 nm of emission wavelength. Quantification was performed by measuring peak areas. The selected chromatographic conditions permitted the separation of the three BFDGE isomers. Calibration for this pollutant was based on the sum of the peak areas corresponding to the three isomers. Calibration curves obtained for bisphenols A, F and their diglycidyl ethers were linear from 10 to 25000 $\mu g\ L^{-1}$.

3. Results and discussion

3.1. Coacervative extraction of bisphenols.

3.1.1. Description and bonding capabilities of reverse micelle-based coacervates

Protonated alkyl carboxylic acids (pK_a= 4.8±0.2) are sparingly soluble in water. They dissolve in THF, where they self-assemble as reverse micelles according to a sequential-type self-association model. The addition of water to these solutions causes partial desolvation of the reverse micelles, which makes easier micelle-micelle interaction and leads to the formation of bigger aggregates. As a result, these aggregates become insoluble in the water:THF solution and separate as a immiscible liquid. At a microscopic level, the coacervate consists of spherical droplets, made up of a variable number of reverse micelles, dispersed in a water:THF continuous phase. The excellent dissolution properties of reverse micelles and the low volume of the coacervates obtained make them very attractive for analytical extractions. Since the coacervation phenomenon occurs from protonated alkyl carboxylic acids, extractions must be carried out below pH 4.

Alkyl carboxylic acid reverse micelles provide a 2-fold mechanism for substrate solubilization, namely hydrophobic interactions in the surfactant tails at the micellar surface and hydrogen bonds in the polar headgroups at the micellar core. Bisphenols are relatively polar compounds (their octanol-water partition coefficients, log K_{ow}, are 2.91, 3.25, 3.32, and 3.95 for BPF, BFGDE, BPA, and BADGE, respectively). They are neutral (pK_a for BPA and BPF are 9.73 and 9.67, respectively) in the pH range in which coacervates are produced (below 4). So, the expected driving forces for extraction of bisphenols were

both hydrogen bonds between the carboxylic groups of reverse micelles and the alcohol/ether groups of analytes, and Van der Waals interactions between the hydrophobic regions of analytes and coacervates.

3.1.2. Optimisation of coacervative extraction process

Optimisation studies were carried out following the procedure described in section 2.3. Selection of the optimal conditions was based on the recoveries (R_s) and actual concentration factors (ACF_s) obtained for bisphenols. Phase volume ratios (PVR_s) were calculated as the ratio of sample volume over coacervate volume, so they represented the maximum concentration factors that could be obtained under given experimental conditions.

Coacervates made up of octanoic, decanoic, dodecanoic and tetradecanoic acids were assessed as extraction solvents. The largest recoveries were obtained by using decanoic and dodecanoic acids (Table 1). The volume of coacervate obtained slightly increased as the alkyl chain of the carboxylic acid did (e.g. around 2% per carbon atom); accordingly, the PVR_s provided by the corresponding coacervates decreased. Maximal ACF_s for bisphenols were obtained for coacervates made up of decanoic acid, so they were selected as extractants. Figure 1 depicts the region encompassed by the coacervate as a function of THF and decanoic acid concentrations. THF percentages below and above the boundaries of this region caused precipitation and solubilization of decanoic acid, respectively.

A. Phase volume ratios

The coacervates used for extraction consisted of decanoic acid reverse micelles dispersed in a THF:water continuous phase and they were in equilibrium with the THF:water sample solution. It was checked that phase volume ratios (PVR_s) depended on both the decanoic acid amount and THF concentration.

A series of experiments were carried out to develop an equation for the prediction of the coacervate volume as a function of the decanoic acid amount and tetrahydrofuran percentage. The aim was to be able to predict the maximum concentration factor that could be obtained under given experimental conditions. For this purpose, a set of coacervates was prepared using a variety of decanoic acid amounts (10-500 mg) and THF concentrations (2.5-50%). The volumes obtained were measured with a digital calliper. The water percentage of the solution was not considered a predictor variable of the coacervate volume. Although the water content is implicit in the THF variable since they are expressed by volume percent, i.e. THF = 100-water (%, v/v) it was not expected to be incorporated into the extractant phase in a significant proportion, probably due to of its non-solvent character for the reverse micelles.

Table 2 shows the figures of merits of the linear relationship found between the amount of decanoic acid (10-500 mg) and the coacervate volume, at different THF percentages. This type of dependence is typical for surfactant-based extractions and it indicates that the composition of the surfactant rich-phase keeps constant as the other variables remain unchanged. The slope of the linear relationship gives the microliters of coacervate obtained per mg of surfactant, so maximum concentration factors will be found under conditions where the lower slope values are obtained (e.g. at low percentages of THF, table 2).

The relationship between the coacervate volume and the THF percentage was exponential (see table 3) and independent of the amount of decanoic acid considered. The parameter (b_1), which describes how rapidly the coacervate volumes increases as the THF (%) does, was found to be similar in all the experiments (mean value around ~0,04), thus indicating that it only depended on the THF percentage. On the contrary, the parameter b_0 was related to the decanoic acid amount, in fact there was a linear relationship between b_0 and the amount of surfactant (see Table 3).

Nonlinear regression was used to fit a model to the data obtained (n=60). This procedure uses an iterative approach to minimize the sum-of-squares of the vertical distances of the experimental points to a proposed curve based on preliminary estimates [37]. Taking into account that the dependence of the coacervate volume with THF percentage and decanoic acid amount was exponential and linear, respectively, the model proposed was $y = \theta_1 \, a \, e^{\theta_2 b}$. The dependent variable, *y* was the coacervate volume in μL, while the independent variables *a* and *b* were the amount of decanoic in mg and the THF in percentage (v/v), respectively. The units of the parameter θ_1 were μL mg^{-1}, while the parameter θ_2 was dimensionless. On the basis of the experimental data obtained, the initial values proposed for θ_1 and θ_2 were 1.00 and 0.04, respectively. The resultant equation, after 10 iterations using the Levenberg-Marquardt method, was $y = 1.035 \, a \, e^{0.0473b}$. Nonlinear regression summary statistics are presented in Table 4. The correlation coefficient was 0.995, thus indicating the high capability of prediction of this equation. So, the maximum concentration factors that can be achieved with decanoic acid reverse micelle-based coacervates under given conditions, can be known a priori and this makes easier method selection and optimisation.

B. Recoveries and actual concentration factors

The influence of variables on recoveries was studied and the actual concentration factors, ACF_s (0.01*R(%)*PVR) were calculated from the coacervate volumes predicted by the general equation proposed above. Decanoic acid concentration was the most influential parameter on recoveries. Recoveries higher than 79% were obtained for all the target compounds at decanoic acid concentrations as low as 0.5% (Table 5). A concentration of 0.5% was selected as optimal on the basis that it provided

the best possible ACF_s for bisphenols at the threshold recovery recommended by the IUPAC for the determination of pollutants in environmental samples (75%).

Recoveries for bisphenols were independent of THF concentrations higher than 10% and decreased as the THF concentration did at lower percentages (Table 6). According to previous studies [36], decanoic acid incorporates progressively to the coacervate from the bulk solution at low THF concentrations and it reaches maximal incorporation at 10% THF and remains steady from here on. So, recoveries for bisphenols depended only and directly on the amount of decanoic acid in the coacervate and THF did not influence them. We selected 10% THF, which gave the maximal ACF_s for recoveries higher than 75%.

Table 7 shows the phase volume ratios inferred from the general equation and the range of ACF_s calculated for bisphenols, considering the recovery values shown in Table 2. A practical aspect to be considered was the volume of environmental sample to analyse, because although it does not influence recoveries or concentration factors, it determines the total mass of decanoic acid at a given surfactant concentration and consequently the volume of coacervate obtained. Our criterion was to get around 100 µL of coacervate per sample, which permitted 2-3 different chromatographic runs in a reliable way (20 µL each injection). Table 7 indicates the minimal sample volume that should be analysed to meet this requirement for each of the decanoic acid concentrations investigated. Detection limits below 100 ng L^{-1}, the general limit set by the EU for organic pollutants, were obtained using decanoic acid concentrations lower than 1% of decanoic acid. Under the selected experimental conditions (0.5% decanoic acid, 10% THF) detection limits around 30-35 ng L^{-1} were obtained for all the target compounds. Sensitivities were very similar for all the bisphenols.

Recoveries for bisphenols 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 for their preservation, was maintained during extraction.

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 affect bisphenol extraction efficiencies or concentration factors. The time necessary to reach extraction equilibrium conditions using the procedure proposed was about 5 min. An increase of about 7% in recoveries for bisphenols was found from 1 to 5 min and remained steady from here on.

3.2. Analytical Performance

Calibration curves for the target compounds were run using standard solutions prepared in acetonitrile. No differences in peak areas or retention times were observed for the analytes injected in organic solvent or coacervates. The retention times of the analytes, expressed in min, were 3.7 for BPF, 5.2 for BPA, 10.1, 10.5 and 10.7 for the BFDGE isomers I, II and III; respectively and 11.7 for BADGE. Correlation between peak areas and bisphenols concentrations (10-25000 $\mu g\ L^{-1}$) were determined by linear regression and were in the range 0,995-0,998, indicating good fits. The slope of the calibration curves were 721±12, 689±20, 1311±38 and 1648 ± 32 L μg^{-1} for BPA, BPF, BADGE and BFDGE, respectively (n= 7). The instrumental detection limits were calculated from blank determinations by using a signal-to-noise ratio of 3. They were estimated to be ~3μg/L. From this value and considering the actual concentration factors, the detection limits of the method were estimated to be around 30 ng L^{-1} for BPA and BPF and 28 ng L^{-1} for BADGE and BFDGE. Under the experimental conditions proposed for their determination, recoveries for BPA/BPF and BADGE/BFDGE were ~80% and ~92%, respectively, in the whole range of concentrations tested, with standard deviations in the interval 2-5%.

Lower detection limits can be achieved for bisphenols by decreasing the amount of decanoic acid used to extract the samples (e.g. 0.1 or 0.25%, see table 7). However, the sample volume have to be increased and recoveries for all the bisphenols or some of them decrease below 75% making advisable to run calibration using the whole procedure, that is with the same experimental conditions (i.e. including extraction of the standards in distilled water) as selected for the analysis of unknown water samples.

The possible interference of matrix components that could elute with bisphenols was assessed by comparison of the calibration curves obtained from standards and those obtained from wastewater and river water, fortified with known amount of bisphenols. The figures of merits of both types of calibration curves were similar, and therefore, matrix components were not expected to interfere in the determination of the target compounds.

3.3. Analysis of environmental water samples.

To prove the suitability of the proposed method to work under real conditions, it was applied to the determination of bisphenols in three rivers and four different WWTPs. Table 8 shows the recoveries and the concentrations obtained for BPA, BPF, BADGE and BFDGE, expressed as the mean value of three independent determinations, besides their corresponding standard deviations. Recoveries were ranged between 80 and 90 for BPA, 79 and 82 for BPF, 90 and 96 for BADGE and 88 and 91 for BFDGE with relative standard deviations ranged in 1-8%.

All the target compounds were present in most of the wastewater influents analysed indicating their ubiquity and the need of studying their fate and distribution in the aquatic environment. The biological

treatment at the WWTPs studied reduced the concentration of BPA and BPF in a percentage above 75%, while diglycidyl ethers were not detected in any of the effluents investigated, except for BADGE at Bailén WWTP. Only BPA was detected in surface waters although its concentration was above the limits recommended by the EU for organic pollutants in waters (0.1 μg L^{-1}). Special high levels were found in the river Dos Torres, which receives domestic wastewater from a mechanical treatment WWTP.

Chromatograms obtained from Mengíbar influent (A) and effluent (B) samples, as well as from river Guadalquivir sample (C) are shown in Figure 2. Identification of analytes in samples was based on retention times and UV spectra, obtained from the diode array in line with the fluorescence detector. No interference from matrix components were detected for any of the samples analysed.

Conclusions

Coacervates of reverse micelles of decanoic acid have proven to be a valuable tool for the simultaneous extraction of bisphenols and their diglycidyl ethers from wastewater and river water samples. The general equation proposed for the estimation of the coacervate volume as a function of its components permitted the accurate prediction of the maximum concentration factor that can be reached under given experimental conditions. The procedure is robust (extractions are no dependent on the ionic strength, temperature or matrix components), simple (treatment of samples only require the extraction of bisphenols for 5 min and no clean-up of extracts or solvent evaporation are necessary) and rapid (each complete extraction procedure takes about 15-20 min and several samples can be simultaneously extracted, so sample throughput will be dependent mainly on the chromatographic analysis of the target compounds). There are additional assets associated to the method here developed; it requires low volume sample (around 10 mL wastewater or river sample), features low cost (no special equipment is required for extraction and uses fluorimetry for detection, so the method can be applied in routine analysis in labs without extra investment), and achieves actual concentration factors in the range 87 to 102 for the target compounds, which results in practical detection limits around 30-35 ng L^{-1}. Thus, the method developed constitutes a good tool for the study of the fate and distribution of bisphenols and their diglycidyl ether in aquatic environments and it is valuable for their control at the levels permitted by the European Directives for organic pollutants in waters (100ng L^{-1}).

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Figure captions

Figure 1. Phase diagram of decanoic acid in binary mixtures of THF:water.

Figure 2. LC/Fluorescence chromatograms obtained from (A) a standard solution and (B) an influent wastewater sample (Mengíbar's WWTP in Jaén, Spain); (C) an effluent wastewater sample (Mengíbar´s WWTP in Córdoba, Spain) and (D) a river water sample (Guadalquivir, flowing by Córdoba, Spain).

Table 1.

Mean percent recoveries and standard deviations obtained for bisphenols using different alkylcarboxylic acids

Alkylcarboxylic acid (1%)	Recovery ± S[a] (%)			
	BPA	BPF	BADGE	BFDGE
Octanoic	87±2	91±2	88±4	86±2
Decanoic	94±2	96±3	93±2	93±5
Dodecanoic	94±3	95±4	91±3	90±4
Tetradecanoic	79±4	82±3	85±2	85±4

[a] standard deviation; *n* =3; THF= 20%

Table 2.

Figures of merits of the linear relationship (y = a+ bx) between the coacervate volume (y, μL) and the amount of decanoic acid (x, mg) at different THF percentages.

THF (%)	b ± S[a] (μL mg^{-1})	a ± S[a] (μL)	R^2 [b]
5	1.31±0.03	3.46±2.76	0.997
10	1.67±0.04	0.67±3.99	0.997
20	2.64±0.03	-3.19±3.36	0.998
30	4.00±0.04	20.10±10.65	0.998

[a] standard deviation ; [b] correlation coefficient ; n =10

Table 3.

Figures of merits of the exponential relationships ($y = b_0 e^{b_1 x}$) between the coacervate volume (y, μL) and the concentration of THF (x, %) at different amounts of decanoic acid.

Decanoic acid (mg)	$b_0 \pm S^a$ (μL)	$b_1 \pm S^a$	$R^{2\,b}$
20	20.62±0.53	0.0471±0.0164	0.997
50	53.43±1.59	0.0479±0.0011	0.998
75	75.47±4.57	0.0489±0.0021	0.992
100	106.55±3.33	0.0454±0.0009	0.998
200	204.99±7.21	0.0454±0.0019	0.996

[a] standard deviation ; [b] correlation coefficient ; n =10

Table 4.

Nonlinear regression summary statistics for the equation $y = \theta_1 a e^{\theta_2 b}$

Parameters	Estimate	Asymptotic standard error	Asymptotic 95% confidence interval	
			Lower	Upper
θ_1 (µL mg^{-1})	1.0351	0.01874	0.9969	1.0734
θ_2	0.04731	0.0009	0.0454	0.4914

a= decanoic acid (mg); b = THF (%, v/v); *n*= 60.

Table 5.

Mean percent recoveries and standard deviations obtained for bisphenols using different decanoic acid concentrations.

Decanoic acid (%)	Recovery ± [a]S (%)			
	BPA	BPF	BADGE	BFDGE
1	52±6	45±5	66±4	65±5
0.25	76±4	67±3	87±5	87±4
0.5	83±3	79±2	94±3	90±4
1	92±3	90±1	92±4	92±2
2	95±4	93±2	99±3	100±1
3	100±3	96±2	100±4	100±2

[a] standard deviation; *n*= 3; THF =10 %

Table 6.

Mean percent recoveries and standard deviations obtained for bisphenols using different tetrahydrofuran concentrations.

%THF	Recovery ± [a]S (%)			
	BPA	BPF	BADGE	BFDGE
2	61±5	54±2	75±4	69±2
5	71±4	67±2	86±3	79±2
10	83±3	80 ±3	94±3	90±1
20	81±3	80±4	94±3	86±2
30	80±4	79 ±3	94±4	85±2
40	83±2	80±4	91±4	85±3

[a] standard deviation; *n*= 3; decanoic acid =0.5 %

Table 7.

Phase volume ratios, minimal sample volume required to get 100 μL of coacervate and method detection limits for different decanoic acid concentrations.

Decanoic acid (%)	Phase volumes ratios	Actual concentration factors	Minimal sample volume (mL)	LOD (ng L^{-1})
0.1	542	358-244	54	12-8
0.25	217	189-145	22	21-16
0.5	108	102-87	11	35-30
1	54	50-49	5	62-60
2	27	27-25	3	119-111

THF = 10%

Table 8.

Mean concentrations (µg L^{-1}) ± standard deviation (n=3) and recoveries (%) of target analytes found in wastewater influent and effluent samples and river water samples.

Sample Location	BPA	BPF	BADGE	BFDGE
WWTP Influent[a]				
Linares	1.13±0.05	0.90±0.08	0.77±0.03	n.d.
	82±3	78±6	94±3	89±3
Bailén	1.36±0.08	1.43±0.03	0.86±0.07	n.d.
	80±4	78±2	96±7	88±4
Lucena	0.96±0.03	n.d.	1.15±0.1	0.41±0.06
	83±2	81	94±7	87±2
Mengíbar	1.6±0.1	0.85±0.02	0.57±0.04	0.32±0.05
	81±5	80±2	92±6	89±6
WWTP Influent[b]				
Linares	0.26±0.02	0.15±0.002	n.d.	n.d.
	85±6	80±1	94±4	92±4
Bailén	0.35±0.03	0.15±0.002	0.25±0.02	n.d.
	88±7	81±1	93±7	89±1
Lucena	0.36±0.02	n.d.	n.d.	n.d.
	87±6	78±2	91±3	89±1
Mengíbar	0.26±0.03	0.1±0.002	n.d.	n.d.
	85±1	82±1	94±2	90±3
River Water[b]				
Guadalquivir	0.1±0.008	n.d.	n.d.	n.d.
	85±6	82±2	95±1	91±1
Rabanales	0.32±0.02	n.d.	n.d.	n.d.
	81±5	79±5	92±2	89±3
Dos Torres	0.25±0.01	n.d.	n.d.	n.d.
	81±3	81±3	90±4	88±2

[a]Spiked Sample (1 µg L^{-1}). [b]Spiked Sample (0.5 µg L^{-1}). nd: non detected

Figure 1

Figure 1

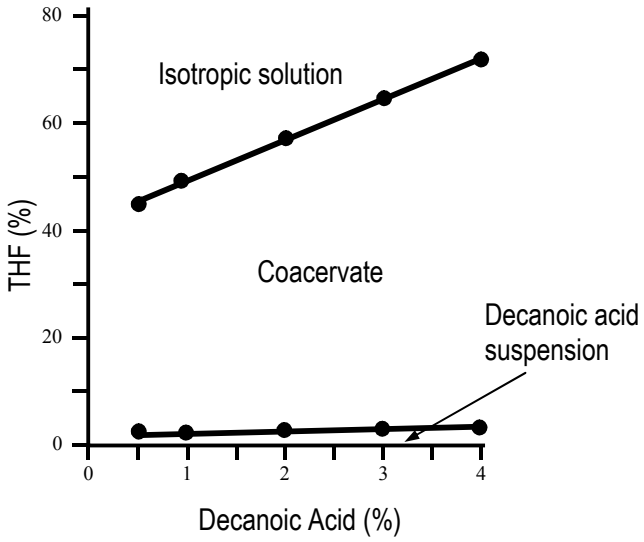

Figure 2

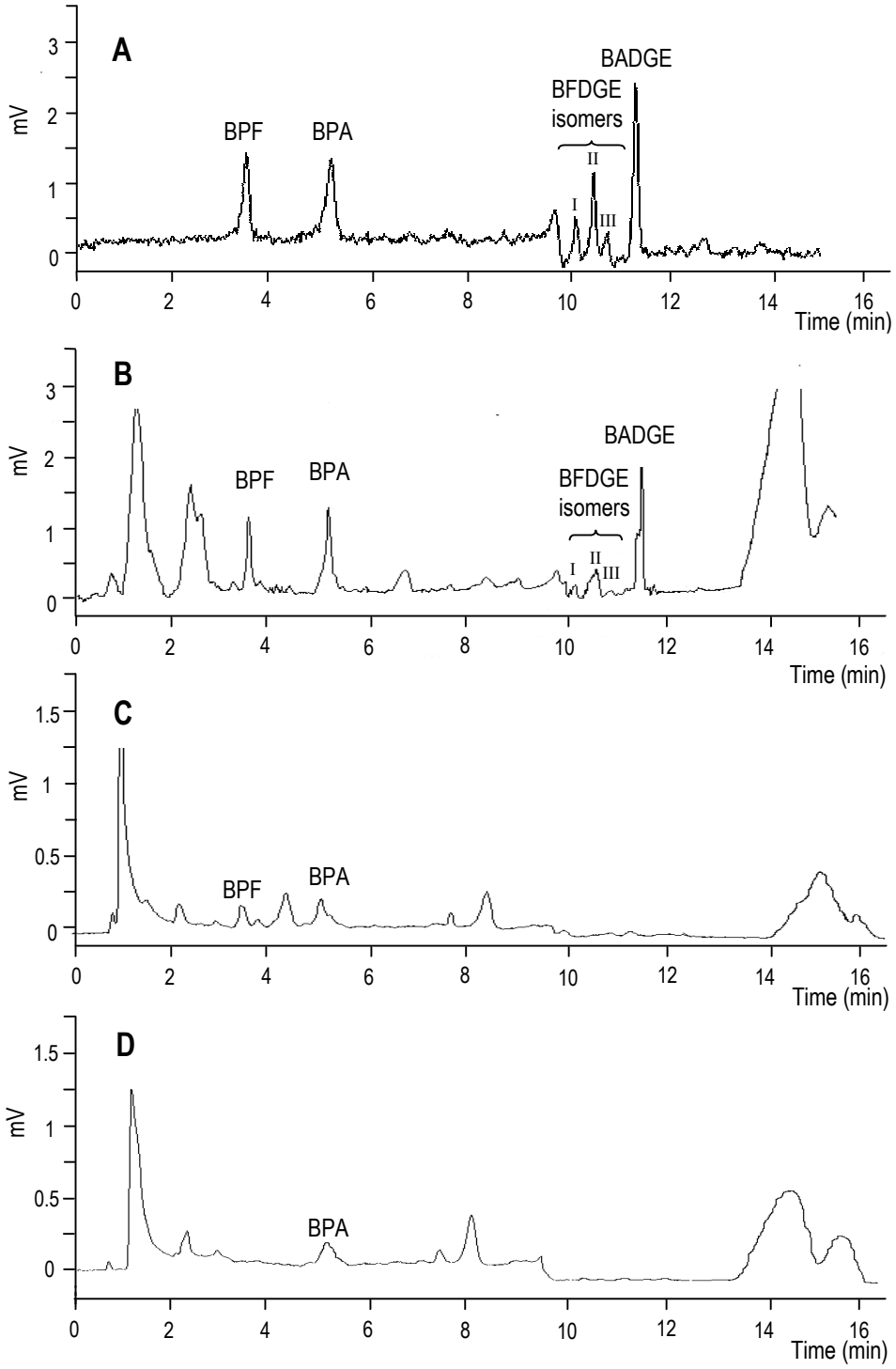

Figure 2