Determination of Bisphenol A in canned fatty foods by coacervative microextraction, liquid chromatography and fluorimetry

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Determination of Bisphenol A in canned fatty foods by coacervative microextraction, liquid chromatography and fluorimetry
Abstract

Decanoic acid reverse micelle-based coacervates were used to provide simple, rapid and almost solventless extraction of bisphenol A (BPA) from canned fatty foods. The procedure involved the extraction of 200-400 mg of homogenized food sample with an aqueous solution containing 20% THF and 200 mg of decanoic acid, conditions under which the coacervate (around 550 µl) formed in situ and instantaneously. The overall sample treatment took about 30 min and several samples could be simultaneously treated using conventional lab equipment. No clean-up or solvent evaporation were required before determination of BPA by liquid chromatography and fluorescence detection. Recoveries in samples were between 90 and 99%, with relative standard deviations in the range 2-7%. The limit of quantification ranged from 29-15 ng g⁻¹ for 200-400 mg of sample being far below the current specific migration limit (SML) set by the European Commission, EC (600 ng g⁻¹). The method was successfully applied to the determination of BPA in the solid content of canned fish (from 20 to 129 ng g⁻¹) and meat (from undetected to 37 ng g⁻¹).

Keywords: bisphenol A; canned fatty food; microextraction; coacervate; food analysis; liquid chromatography-florescence; reverse micelles

Introduction

Bisphenol A (4-4'-isopropylidenediphenol, CAS: 80-05-7; BPA) is a recognized environmental estrogen (Brotons et al. 1995; Chen et al. 2002; Vom Saal & Hughes 2005) widely used in the manufacturing of the most important internal polymeric coatings of food cans (Goodson et al. 2004). Human exposure to BPA occurs from the migration of this contaminant into food (FSA 2001; Kang et al. 2006). The tolerable daily intake (TDI) level established by the U.S. Environmental Protection Agency, EPA (IRIS 1988), as well as that recently recommended by the European Food Safety Authority, EFSA (IRIS 1988; Vom Saal and Hughes 2005), is 50 ng g⁻¹ body weight.
The amount of BPA legally permitted to migrate from packaging into food, known as the specific migration limit (SML), is based on the TDI and it was set at 600 ng g\(^{-1}\) by the EC in 2004 (EC 2004).

Occurrence of BPA in canned foods has widely been reported (FSA 2001; Munguía-López et al. 2002; Braunrath et al. 2005; Munguía-López et al. 2005; Thomson and Grounds 2005; Sun et al. 2006; Podlipna and Cichna-Markl 2007). In 1995, Brotons and colleagues detected BPA in the liquid portion of several types of canned vegetables in the range 22-76 ng g\(^{-1}\) (Brotons et al. 1995). Later, Goodson et al. conducted a wide study in the UK that involved the analysis of 62 samples of canned foods and drinks (Goodson et al. 2002). BPA was quantified in 37 samples of canned food at levels from 7 up to 70 ng g\(^{-1}\), with one sample of meat containing a mean level of 380 ng g\(^{-1}\). Recently, a number of studies have confirmed the presence of BPA in canned meat (7.6-140 ng g\(^{-1}\)), fish (0.3-110 ng g\(^{-1}\)), beverages (0.1-3.4 ng mL\(^{-1}\)), vegetables (1-212 ng g\(^{-1}\)), fruits (1.7-24 ng g\(^{-1}\)), pet food (12-206 ng g\(^{-1}\)) and so on (Brotons et al. 1995; FSA 2001; Imanaka et al. 2001; Yoshida et al. 2001; Kang and Kondo 2002; Braunrath et al. 2005; Munguía-López et al. 2005; Thomson and Grounds 2005; Sun et al. 2006; Podlipna and Cichna-Markl 2007, García-Prieto et al. 2008). The widespread human exposure to BPA from food and beverage containers has opened a strong debate in the scientific community about the low-dose reproductive and developmental effects of this contaminant, the conclusions going from no risk to human health (FSA 2001) to the need for a new risk assessment (Chen et al. 2002; Vom Saal and Hughes 2005).

Determination of BPA in food is required for both the control of the compliance of current legislation and the assessment of human exposure. Most analytical methods proposed in recent years for the determination of BPA in canned foods invariably involve the extraction of samples with organic solvents followed by liquid
chromatography-fluorescence (LC-FL) or gas chromatography-mass spectrometry (GC-MS). Sample treatment is by far the most laborious and critical step.

Concerning the extraction of fatty foods, Goodson et al. have proposed the simplest BPA isolation procedure so far (Goodson et al. 2002). It involves the blending of twenty grams of fatty-sample with 20 mL of n-heptane for fat removal, duplicate extraction with 20+20 mL of acetonitrile, filtration, addition of anhydrous sodium sulphate and evaporation under nitrogen up to 5 mL. Other authors have proposed some minor modifications to this procedure, namely the treatment of ten grams of fatty sample, which reduces by half the solvent required (Podlipna and Cichna-Markl 2007), and the replacement of n-heptane by trimethylpentane, which is more effective for fat removal (Thomson and Grounds 2005). More organic solvent-consuming procedures (e.g 100 mL of methanol and 100 mL of hexane) have been proposed recently (Munguia-López et al. 2005). After isolation of BPA, the extracts are usually subjected to GC-MS, previous derivatisation of BPA, or they are analyzed by LC-FL, after a clean-up step. For this purpose, high selectivity can be achieved using sol-gel immunoaffinity columns (Braunrath et al. 2005; Braunrath and Cichna 2005; Podlipna and Cichna-Markl 2007).

The strong demand for simple, rapid, low-cost and environmentally friendly methods for food analysis has forced researchers to introduce new approaches and highly innovative technologies in food laboratories. In this context, the use of solvent-free techniques and other isolation emerging technologies is essential to increase sample throughput. The present work proposes the coacervative microextraction of BPA from fatty-foods as an innovative isolation technique to reduce time, cost and toxicity of current extraction methodologies.

Coacervates are water immiscible liquids that separate from the bulk of colloidal solutions by the action of a dehydrating agent, namely temperature, pH, electrolyte or a
non-solvent for the macromolecule (IUPAC 1972; Gander et al. 2002). After separation, the coacervate, a low-volume phase, contains most of the colloid and it is in a dynamic equilibrium with the bulk solution. Coacervates are well-known in food industry where they are widely used for encapsulation of active ingredients (Gander et al. 2002). In extraction processes, applications have mainly focused on the use of surfactant aggregate-based coacervates for the extraction of pollutants from water (Saitoh and Hinze 1991; Jin et al. 1999; Casero et al. 1999; Carabias-Martinez et al. 2000; Rubio and Pérez-Bendito 2003; Merino et al. 2005; Ruiz et al. 2006; Ballesteros et al. 2007), soil, sediment (Merino et al. 2002) and sludge (Merino et al. 2003, Ruíz et al. 2003). The most used surfactant aggregates have been aqueous non-ionic (Ishii et al. 1977; Hinze and Pramauro 1993; Carabias-Martinez et al. 2000), amphoteric (Saitoh and Hinze 1991), anionic (Casero et al. 1999) and cationic (Jin et al. 1999) micelles. Recently, coacervates made up of vesicles (Ruiz et al. 2006; Ruiz et al. 2007a) and reversed micelles (Ruiz et al. 2007b; Ballesters-Gómez et al. 2007) of alkyl carboxylic acids have been reported, which permit the extraction of organic compounds in a wide polarity range. Because of their excellent solvation and stabilizing properties, which can be varied by changing the nature of the macromolecule or the coacervating agent, coacervates have the potential to simplify extraction procedures in food analysis.

This paper focuses on the use of decanoic acid reverse micelle-based coacervates for the extraction of BPA from canned fish and meat using minute amounts of food sample and coacervate. The research includes the study of the parameters affecting the extraction efficiency of BPA, the study of the quantitative performance of the method using LC-FL and its application to the determination of BPA in several samples of canned fish and meat purchased in local supermarkets.

**Materials and methods**

*Reagents*
Bisphenol A (BPA) and decanoic acid (capric) were obtained from Fluka (Buchs, Switzerland). HPLC-grade acetonitrile, hydrochloric acid 37% and tetrahydrofuran were purchased from Panreac (Barcelona, Spain). Stock solutions of BPA (0.5 g L$^{-1}$) were prepared in acetonitrile and stored under dark conditions at 4°C not more than three months. Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain).

**Apparatus**

The liquid chromatographic 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. The stationary-phase column was a Hypersil ODS C$_{18}$ column (5µm, 4.6 x 150 mm) from Análisis Vinicos (Tomelloso, Spain). A magnetic stirrer Basicmagmix from Ovan (Barcelona, Spain) and a digitally regulated centrifuge Mixtasel from JP-Selecta (Abrera, Spain) were used for sample preparation. Volumes of coacervate were measured using a digital calliper from Medid Precision, S.A. (Barcelona). Centrifuge tubes with narrow necks (Figure 1) were designed by authors in order to make easier the measurement and collection of the coacervate after extraction. Pobel S.A. (Madrid, Spain, web page: www.pobel.com) constructed them from commercial heavy-duty glass cylindrical centrifuge tubes with round-bottom (ref. 159050) by keeping their basic structure at the bottom (34 mm of outside diameter) but reducing the diameter from a specified height, which depended on the required tube capacity.

“[Insert Figure 1 about here]”

**Determination of BPA in canned fatty foods**

**Sample pretreatment.** Spanish manufactured cans of fish (tuna in olive oil, mackerel in vegetable oil, sardines in olive oil and mussels in pickled sauce) and meat (cooked and

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sterilized meatballs and lean pork cooked in its own juices) were bought in supermarkets in Córdoba (Spain). The unopened cans were stored at room temperature. For analysis, the overall content of each can was drained in a mesh strainer (holes size ~0.85 mm$^2$) and the liquid discarded. Then, the overall solid portion was blotted with a filter paper (base weight of 73 g/m$^2$, Anoia S.A., Barcelona, Spain) and homogenized using a kitchen food mixer (~9000 rpm, Thermomix TM 21, Vorwerk, Spain). Aliquots of 200-400 mg were taken for analysis and recovery experiments, which were made in triplicate. Spiking of samples for recovery rates was carried out by adding minute volumes of a BPA standard solution in acetonitrile to the solid portion of the food before its homogenization. Samples not immediately analyzed were stored at -20 ºC until analysis. The type of the internal coatings of the cans was not investigated.

Coacervative microextraction of BPA. Decanoic acid (200 mg) was dissolved in THF (2 mL) in a glass centrifuge tube (Figure 1). Then, 8 mL of distilled water and 140 µl of HCl 0.5 M were added. Immediately, the coacervate phase (around 550 µl) separated from the bulk solution. Next, the food (200-400 mg) was added using a small spatula (100 mm long and 4 mm width, supplied by Selecta, Barcelona, Spain). The mixture was magnetically stirred at 1200 rpm for 15 min to favour BPA extraction and then centrifuged at 4000 rpm for 15 minutes to accelerate phase separation. Next, the volume of the coacervate, which was standing at the narrow neck of the glass tube, was measured with a digital calliper. Finally, an aliquot of 20 µl of the coacervate was withdrawn with a microsyringe and injected into the chromatograph.

Quantitation by liquid chromatography-fluorescence. Quantification of BPA and separation from the matrix components was carried out by liquid chromatography-fluorescence. The mobile phase consisted of water (A) and acetonitrile (B). The flow-rate was 1 mL min$^{-1}$. The gradient elution program was: linear from 70:30 to 45:55
(A:B) in 13 minutes and then isocratic initial conditions (70:30) for 7 min to clean and stabilize the chromatographic system. The selected wavelengths were 276 nm (excitation) and 306 nm (emission). BPA eluted to a retention time of 8.8 min. Calibration was run by injecting 20 µl of standard solutions in acetonitrile containing between 0.2 and 60 ng of BPA. Quantification was performed by measuring peak areas. Correlation coefficients between peak area and BPA were in the range 0.9995-0.99990.

Results and discussion

Formation and structure of decanoic acid reverse micelle-based coacervates

Decanoic acid is highly soluble in THF where it self-assembles as reverse micelles (size around 4-8 nm), according to a sequential-type self-association model (Ruiz et al. 2007b). The addition of water, in which decanoic acid is scarcely soluble, causes the partial desolvation of the reverse micelles and makes easier micelle-micelle interaction. As a result, the reverse micelles self-assemble in larger aggregates with a wide size distribution in the nano and micro scale regimes and separate from the bulk solution as an immiscible liquid phase (the coacervate). At a microscopic level, the coacervate consists of spherical droplets made up of reverse micelles which are dispersed in a THF continuous phase containing a minimal amount of water that is essential to keep the coacervate structure.

"[Insert Figure 2 about here]"

Figure 2 shows the relative concentration of decanoic acid:THF:water at which the coacervation occurs. Beyond the boundaries of this region, the decanoic acid precipitates or produces a homogeneous micellar solution where the size of the aggregates is considerable smaller than those in the coacervate. Figure 3 shows a picture of the surfactant aggregates present in the different steps of the extraction process. The reversed micelles in the coacervate provide a 2-fold mechanism for BPA...
extraction (BPA octanol-water partition coefficient, log $K_{\text{o/w}}$ 2.91 and $pK_a$ 9.73), namely van der Waals interactions in the decanoic acid hydrocarbon chains and hydrogen bonds in the micellar core, so it can be extracted efficiently from food samples. Since reverse micelles only occur from protonated decanoic acid ($pK_a = 4.8 \pm 0.2$), extractions must be carried out below pH 4.

“[Insert Figure 3 about here]”

**Optimisation of the coacervative microextraction of BPA**

Optimisation was carried out by extracting both fortified (n= 3; 50 ng BPA) and non fortified (n=3) tuna in olive oil samples (50-400 mg) under a variety of experimental conditions (100-600 mg decanoic acid; 5-40 % THF; pH 1-4; extraction time 10-60 min; temperature 20-60 °C, stirring rate 600-1500 rpm). Method limits of quantification (MQL) were used as a criterion for the selection of the optimal conditions for extraction. They were estimated from the volume of the coacervate (Vc), the recovery for BPA (R) and the instrumental quantification limit (IQL) for this contaminant, which was 0.2 ng (absolute amount injected in the chromatographic system). Conditions giving minimal method quantitation limits (MQL) were selected provided that the extraction for BPA was quantitative and the relative standard deviation of the method was below 10% (Green 1996).

**Influence of the coacervate amount and composition.** Coacervates are mainly made up of decanoic acid and THF, so the amount of these components in the samples determines the coacervate volume and greatly influences the BPA recovery. Figure 4 shows the dependence of Vc on both the amount of decanoic acid (a) and the percentage of THF (b). The volume of coacervate increased linearly as the amount of decanoic acid did independently of the percentage of THF investigated. This type of dependence indicated that the composition of the coacervate kept constant when the other variables remained unchanged. The corresponding equations for 5% and 20% of
THF were $y = (29\pm28) + (1.27\pm0.08) x$ and $y = (9\pm15) + (2.7\pm0.1) x$, respectively, where $y$ was the volume of coacervate in $\mu$l and $x$ the amount of decanoic acid in mg. The correlation coefficients were 0.996 and 0.998. The slopes of the linear relationship were similar to that obtained in the absence of food sample (Ballesteros et al. 2007), so although matrix components could be incorporated to the coacervate they did not influence its volume.

“[Insert Figure 4 about here]”

The relationship between the volume of coacervate and the percentage of THF was exponential (Fig. 4 (b)) and fit to the equation $y = b_0 e^{b_1z}$, where $y$ was the volume of coacervate in $\mu$l and $z$ the percentage of THF. The corresponding equations for 200 and 400 mg of decanoic acid were $y = (223\pm11) e^{(0.045\pm0.003)z}$ and $y = (433\pm17) e^{(0.046\pm0.002)z}$ respectively, with correlation coefficients of 0.997 and 0.998. This type of dependence indicated that progressively more THF was incorporated to the coacervate as the percentage of THF in the solution increased and consequently, the reverse micelles became more and more diluted. As these equations were also similar to those obtained in the absence of food sample, the general equation previously derived for the prediction of the volume of coacervate ($y$) obtained as a function of the amount of decanoic acid ($x$) and percentage of THF ($z$) was also applicable to tuna samples (Ballesteros et al. 2007). So, it is possible to estimate the volume of coacervate and therefore the maximum concentration factor that can be obtained under given experimental conditions through the equation $y = 1.06 x e^{0.04731z}$.

“[Insert Table I about here]”

Table I shows the volumes of coacervate estimated and the recoveries obtained for BPA as a function of the amount of decanoic acid and the percentage of THF. The
concentration of solvent became essential to get quantitative recoveries. A THF concentration of ca. 20% was found to be necessary for efficient coacervative extraction of BPA. The recovery decreased at THF percentages above 30%, which can be explained by the increase of BPA solubility in the THF:water solution in equilibrium with the coacervate, that resulting in decreased BPA partition coefficients. From these results, the corresponding MQL values were calculated and they are included in the Table I. Quantitative recoveries were obtained for BPA at the selected THF (20%) and decanoic acid (200 mg) values and the corresponding MQL (29 ng g⁻¹) was far below the specific migration limit (SML) set by the EC (600 ng g⁻¹, EC 2004).

Under these experimental conditions, it was found that the volume of coacervate measured with a digital calliper (550.0 ± 0.4µl) coincided with that calculated by the general equation (550 µl, Table I) thus confirming the high capability of prediction of the equation used.

**Influence of operational parameters.** The optimization of such parameters as extraction time and temperature and stirring rate was carried out in order to select those conditions under which the extraction became faster. None of these parameters modified the volume of coacervate yielded, so MQLs directly depended on the recoveries obtained for BPA.

The stirring rate influenced the kinetics of the extraction and consequently, more time was required to reach equilibrium conditions at the lowest stirring rates tested (600-1000 rpm). A value of 1200 rpm was selected as optimal since larger stirring rates did not reduce the extraction time. Equilibrium conditions and quantitative recoveries were obtained after 15 min of extraction and this time was selected as optimal for further studies. The temperature scarcely influenced the extraction kinetics for BPA in the range investigated (20-60 °C), so the whole procedure was carried out at room temperature.
Recoveries for BPA were not affected by the pH of the extraction solution in the range 1 to 4, which is logical considering the type of interactions expected to be the driving forces for the extraction. So, the pH of this solution was adjusted at 3-3.5 by the addition of hydrochloric acid.

_Influence of matrix components._ Table II shows the concentrations of the major components in the selected foods. The data were obtained from either the can label or significant databases. Preliminary experiments indicated that independently of the food analyzed, a whitish precipitate, which was standing as a very thin layer between the bottom of the coacervate and the THF:water solution, was extracted. This precipitate decreased as the amount of sample did, so it was matrix-dependent. It was found that the precipitate was caused by the proteins present in the samples, which were agglutinated by the reverse micelles and extracted by the coacervate, but they did not interfere in the recovery of BPA.

“[Insert Table II about here]”

As salt was a component of samples (see Table II), its influence on the extraction process was investigated by adding NaCl to 200 mg of tuna at the following concentrations: 1, 5, 10, 15 and 25 mg per gram of sample. Neither the recoveries for BPA nor the volume of coacervate were affected by NaCl in the range of concentrations usually present in these types of samples. The effect of larger amounts of salt: 500, 1170, 1750, 2300 and 2500 mg NaCl g⁻¹ tuna sample was also investigated in order to determine if they caused a salting out effect on the extraction of BPA. Salt did not influence on the efficiency of the extraction process, so its addition to samples is not recommended. It was clearly observed in this study that the volume of coacervate increased linearly with the NaCl concentration. The slope of this linear
relationship was $0.066 \pm 0.003 \, \mu l \, mg^{-1}$ NaCl g sample, so the volume of coacervate increased at a rate of $6.6 \, \mu l$ per $100 \, mg$ salt g$^{-1}$ sample. Such low rate was undetectable for the low concentrations of salt present in the samples but this increase of volume should be taken into account if samples with a high salt content are analyzed.

Lipids in the samples are expected to be extracted by the coacervate by the formation of mixed micelles with decanoic acid. As incorporated at enough proportion, the lipids should produce an increase in the volume of coacervate obtained. The effect of lipids on the extraction process could not be directly investigated because of both the exact proportion of lipids in the solid portion of the food is unknown (Table II gives the amount of total fat) and it is very difficult to simulate the exact composition of the lipids (i.e. free, triglyceride, phospholipids, etc) and their exact interactions with the rest of matrix components. So, we decided to asses the overall effect of matrix components, which also include lipids, on the extraction of BPA through two experiments. In the first one, different amounts (150, 200 and 400 mg) of an unfortified tuna sample were analyzed by triplicate. The concentrations of BPA found (135±6, 129±6 and 130±5, respectively) were similar in the range investigated, so it was assumed that matrix components did not influence the extraction process. In the second one, different amounts (50-400 mg) of both tuna in olive oil and mackerel in vegetable oil were spiked with 50 ng of BPA and analyzed following the procedure recommended (see the section materials and methods). The results obtained are shown in Table III. Recoveries were quantitative and independent of the amount of sample thus confirming that matrix components, including lipids, did not affect the extraction process. On the other hand, the 95% confidence interval for the volumes of coacervate obtained in both experiments was $545 \pm 19 \, \mu L$, so the content of the lipids extracted from the minute amounts of sample analyzed was not enough to produce detectable changes in the volume of coacervate.
Analytical performance

Calibration curves for BPA were run using standard solutions prepared in acetonitrile. No differences in peak areas or retention times were observed for BPA injected in acetonitrile or coacervate. The retention time for BPA was 8.8 min. Correlation coefficient between peak areas and the amount of BPA injected every day (0.2, 1, 2, 10, 20, 30 and 60 ng) was in the range 0.9995-0.99990 indicating good fits. The slope of the calibration curve was $4.63 \pm 0.04 \times 10^3$ fluorescence intensity units ng$^{-1}$. The instrumental detection limit (IDL) was calculated from blank determinations by using a signal-to-noise ratio of 3 and it was calculated to be $\sim 0.06$ ng. From this value and the average percentage of recovery of BPA in food samples (95±8%, data calculated from Table IV), an average volume of coacervate of around 550 µl and an amount of sample handled of 200 mg, the minimum detection limit that could be reached by the method was calculated to be $\sim 9$ ng g$^{-1}$. Lower detection limits can be achieved if necessary by increasing the amount of sample treated (e.g. 400 mg) or decreasing the amount of decanoic acid used for extraction (e.g. see in Table I, data for 20% THF and 100-200 mg of decanoic) since recoveries above 70% are allowed (AOAC/FAO/IAEA/IUPAC 1999, Green 1996). The relative standard deviation, calculated from the analysis of a tuna sample during five days, two complete analyses per day, was ±6%.

The accuracy of the quantitation of BPA in fatty foods using external calibration was assessed by comparison of the slopes of the calibration curves obtained from standards in acetonitrile with those obtained from 200 mg of lean pork, tuna and mackerel samples fortified with known amounts of BPA (10-300 ng) and run using the whole procedure. Six different concentrations were used for construction of each calibration curve. The slopes and correlations coefficients found for lean pork, tuna and
mackerel samples were $(4.6 \pm 0.2) \times 10^3$, 0.998; $(4.4 \pm 0.1) \times 10^3$, 0.9990 and $(4.26 \pm 0.03) \times 10^3$, 0.9998; respectively. The differences found between these calibration slopes and that obtained in acetonitrile $(4.63 \pm 0.04) \times 10^3$ were exclusively due to the percentages of recovery obtained for BPA (e.g. mean recovery values of around 99, 95 and 92% for lean pork, tuna and mackerel, respectively), so calibration external is recommended for quantitation of BPA in fatty foods.

**Analysis of canned fatty foods**

The suitability of the proposed analytical method for the determination of BPA in canned fatty food was assessed by analyzing four samples of canned fish (tuna, mackerel, sardine and mussel) and two samples of canned meat (meatball and lean pork). Table IV shows some of the characteristics of the cans analyzed as well as the concentrations of BPA found and the recoveries obtained after spiking the samples with 50 ng of BPA. Values for concentrations and recoveries were expressed as the mean value of three independent determinations, besides their corresponding standard deviations. Recoveries of BPA were always above 90% and the standard deviations were between 2 and 7% thus indicating that the characteristic of the method were matrix-independent. BPA was present at quantifiable levels in all the canned foodstuffs analyzed except in the cooked meatballs. The quantification of BPA in the canned mackerel involved the analysis of 400 mg of sample owing to its low content. BPA in meatballs, if any, was below the detection limit of the method (i.e. 9 ng g$^{-1}$). Concentrations of BPA were always below the specific migration level of 600 ng g$^{-1}$ set by the European Commission (EC 2004).

“[Insert Table IV and Figure 5 about here]“
Figure 5 shows the LC-fluorescence chromatograms obtained for the analysis of unfortified (a1-d1) and fortified with 50 ng de BPA (a2-d2) canned foods. Chromatograms were clear enough for the detection and quantitation of BPA in all the samples analyzed. Identification of the target analyte was based on the retention time and the UV spectrum, which was obtained from the diode array in line with the fluorescence detector. Analysis of the UV spectrum included both peak purity testing and spectrum matching. To calculate the purity a scan threshold of 1 mAU and peak coverage of 95% were considered. With regard to spectrum matching, the similarity threshold was set at 0.98.

Conclusions
Coacervates of decanoic acid reverse micelles constitute a valuable alternative to the current methodologies available for the extraction of BPA from canned fatty foods, which are based on the use of 30-200 mL of toxic organic solvents followed by clean-up and/or solvent evaporation. Comparatively, coacervative extraction is simpler and faster since sample treatment just requires a single extraction with an aqueous solution containing 2 mL of THF and 200 mg of decanoic acid, and no clean-up or solvent evaporation is necessary. As a result, the whole treatment procedure takes about 30 min and several samples can be simultaneously extracted, so sample throughput is considerable increased. There are additional assets associated with the proposed method; such as requires only minute amounts of sample (200-400 mg), features low cost (the consumption of organic solvent is greatly reduced and the use of SPE columns is avoided), no special equipment is required for sample treatment and it uses liquid chromatography-fluorescence for separation-detection, so the method can be applied in routine analysis in labs without extra investment. The quantitation limit of the method is about 29 ng g⁻¹, so it can be used for the routine control of BPA in canned fish and meat below the current specific migration limit (SML) of 600 ng g⁻¹. The experimental conditions established for the coacervative extraction of BPA from fatty-
food and those previously obtained by the authors for the extraction of BPA from canned vegetable and fruits (García-Prieto et al. 2008) are not essentially different, which proves the suitability of decanoic-based coacervates for the efficient and simple extraction of this contaminant from a variety of canned foods. Because of the absence of clean-up steps, chromatographic conditions are simpler for canned fruits than those for canned vegetable and fatty-foods in order to assure the exact quantitation of BPA.

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References


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IUPAC Compendium Chemical Terminology. 1972. 31:611.


Figure captions

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

Figure 2. Phase diagram of decanoic acid in binary mixtures of THF:water. Final volume of the solution (THF+ Water) = 40 mL. Experiments made at room temperature.

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

Figure 4. Volume of coacervate as a function of: a) the amount of decanoic acid at two percentages of THF, and b) the concentration of tetrahydrofuran at two amounts of decanoic acid. Extraction of 200 mg of tuna with a water:THF solution (10 mL)

Figure 5. LC-fluorescence chromatograms obtained from 200 mg of canned sardines (a), meatballs (b), mussels (c) and lean pork (d) without spiking (1) and after spiking with 50 ng of BPA (2). Experimental conditions described in the section Materials and Methods.
Figure 2

254x190mm (96 x 96 DPI)
Figure 3
Figure 4

254x190mm (96 x 96 DPI)
Figure 6

190x254mm (96 x 96 DPI)
Figure 6

190x254mm (96 x 96 DPI)
Table I. Coacervate volumes, mean recoveries and method quantification limits obtained for BPA as a function of THF and decanoic acid concentrations

<table>
<thead>
<tr>
<th>THF (%)</th>
<th>Decanoic acid (mg)</th>
<th>Coacervate volume* (µl)</th>
<th>Recovery†± S‡ (%)</th>
<th>Method quantification limit (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>200</td>
<td>269</td>
<td>58 ± 2</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>404</td>
<td>48 ± 1</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>538</td>
<td>59 ± 5</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>600</td>
<td>807</td>
<td>48 ± 6</td>
<td>84</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>342</td>
<td>53 ± 3</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>684</td>
<td>45 ± 5</td>
<td>76</td>
</tr>
<tr>
<td>20</td>
<td>100</td>
<td>275</td>
<td>76 ± 1</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>413</td>
<td>89 ± 1</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>550</td>
<td>95 ± 2</td>
<td>29</td>
</tr>
<tr>
<td>30</td>
<td>100</td>
<td>444</td>
<td>75 ± 5</td>
<td>30</td>
</tr>
<tr>
<td>40</td>
<td>100</td>
<td>714</td>
<td>54 ± 6</td>
<td>66</td>
</tr>
</tbody>
</table>

* Estimated from the equation \( y = 1.06 \times e^{0.0473x} \), where x is the amount of decanoic acid (mg) and z the percentage of THF.
† 200 mg of tuna spiked with 50 ng of BPA
‡ Standard deviation, n = 3
Table II. Major components of the food samples analysed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Fat (g 100 g⁻¹ food)</th>
<th>Protein (g 100 g⁻¹ food)</th>
<th>Carbohydrate (g 100 g⁻¹ food)</th>
<th>Salt (g 100 g⁻¹ food)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna in olive oil</td>
<td>12</td>
<td>24</td>
<td>0</td>
<td>0.77</td>
</tr>
<tr>
<td>Mackerel in vegetable oil</td>
<td>12</td>
<td>24</td>
<td>0</td>
<td>0.81</td>
</tr>
<tr>
<td>Mussels in pickled sauce</td>
<td>9</td>
<td>16</td>
<td>4</td>
<td>1.11</td>
</tr>
<tr>
<td>Sardines in olive oil</td>
<td>11†</td>
<td>25†</td>
<td>0†</td>
<td>0.67</td>
</tr>
<tr>
<td>Meatballs</td>
<td>16</td>
<td>8</td>
<td>7</td>
<td>1.20</td>
</tr>
<tr>
<td>Lean pork</td>
<td>12</td>
<td>16</td>
<td>0.3</td>
<td>0.99</td>
</tr>
</tbody>
</table>

Sources:  
† USDA (United States Department of Agriculture) National Nutrient Database for Standard Reference.  
Table III. Mean recoveries and method quantification limit as a function of the amount of sample analysed

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample amount (mg)</th>
<th>Recovery* ± S† (%)</th>
<th>MQL‡ (ng g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuna in olive oil</td>
<td>50</td>
<td>97 ±4</td>
<td>113</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>104 ± 5</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>95 ± 2</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>98 ± 7</td>
<td>14</td>
</tr>
<tr>
<td>Mackerel in vegetable oil</td>
<td>50</td>
<td>104 ± 6</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>111 ± 3</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>108 ± 3</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>98 ± 4</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>400</td>
<td>97 ± 2</td>
<td>15</td>
</tr>
</tbody>
</table>

* Spiking level: 50 ng of BPA  
† Standard deviation, n = 3  
‡ Method quantification limit estimated from an average volume of coacervate of 550 µl
Table IV. Characteristics of the cans analysed, mean concentrations found for BPA and recoveries obtained after spiking of the samples

<table>
<thead>
<tr>
<th>Product</th>
<th>Lacquer area (cm²)</th>
<th>Best before date</th>
<th>Net weight (g)</th>
<th>Drained weight (g)</th>
<th>BPA ± S (ng g⁻¹)</th>
<th>Recovery ± S (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuna in olive oil</td>
<td>110</td>
<td>12/2011</td>
<td>80</td>
<td>52</td>
<td>129 ± 6</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>Mackerel in vegetable oil</td>
<td>120</td>
<td>12/2012</td>
<td>115</td>
<td>81</td>
<td>120 ± 5</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>Sardines in olive oil</td>
<td>120</td>
<td>12/2011</td>
<td>125</td>
<td>87</td>
<td>119 ± 5</td>
<td>90 ± 5</td>
</tr>
<tr>
<td>Mussels in pickled sauce</td>
<td>110</td>
<td>12/2010</td>
<td>80</td>
<td>43</td>
<td>121 ± 2</td>
<td>95 ± 6</td>
</tr>
<tr>
<td><strong>Meat</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meatballs</td>
<td>300</td>
<td>12/1012</td>
<td>420</td>
<td>390</td>
<td>&lt;MDL</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>Lean pork</td>
<td>243</td>
<td>08/2009</td>
<td>220</td>
<td>210</td>
<td>37 ± 5</td>
<td>99 ± 7</td>
</tr>
</tbody>
</table>

Analysis of ¤ 200 and † 400 mg of fatty food. ‡ Standard deviation, n = 3. ¶ Food samples spiked with 50 ng of BPA. § below the detection limit of the method.