Analytica Chimica Acta 1144 (2021) 14–25

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Comprehensive supramolecular solvent-based sample treatment platform for evaluation of combined exposure to mixtures of bisphenols and derivatives by liquid chromatography-tandem mass spectrometry

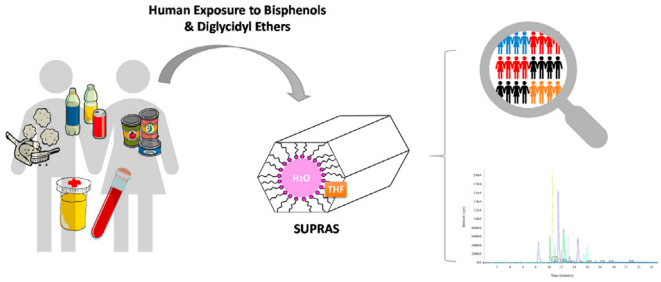
Noelia Caballero-Casero [a, 1, *], Soledad Rubio [a]

[a] Department of Analytical Chemisty, Institute of Fine Chemistry and Nanochemistry, Marie Curie Building (Annex), Campus of Rabanales, Universidad de Córdoba, 14071, Córdoba, Spain

HIGHLIGHTS

- A comprehensive sample treatment is developed for determination of 21 bisphenols.
- It is based on the use of a supramolecular solvent with restricted access properties.
- Matrix-independent quantitation of selected bisphenols by LC-MS/MS.
- Liquid and solid exposure samples and biological fluids are efficiently treated.
- The method meets the analytical and operational features for epidemiological surveys.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 August 2020
Received in revised form
3 November 2020
Accepted 30 November 2020
Available online 3 December 2020

Keywords:
Bisphenols
Comprehensive sample treatment
Supramolecular solvents
Biomonitoring
Matrix-independent methods

ABSTRACT

The growing demand for a better understanding of the effects of chemical mixtures on human health has fostered the need for extensive estimation of uptake rates from identified sources and/or biomonitoring, which has encouraged the development of analyte- and matrix-independent analytical methods. In this paper, we report a comprehensive sample treatment platform for the efficient extraction and interference removal in the determination of twenty-one bisphenols and derivatives (log K_{ow} from 1.254 to 6.564) in a variety of human exposure sources and biological fluids. Treatment of both liquid (canned beverages, urine and serum) and solid (canned food, dust) samples was based on the use of low volumes (190–200 μL) of a hexanol-based supramolecular solvent having properties of restricted access materials. The efficient extraction of bisphenol and derivatives (absolute recoveries 70–114%) was due to the mixed-mode mechanisms (hydrogen bonding, polar and dispersion interactions) and the huge number of binding sites offered by the supramolecular solvent with properties of restricted access materials for solute solubilization. Signal suppression or enhancement (SSE) values kept in the range 78–116% for samples encompassing a wide range of macromolecules content (e.g. protein, fat, carbohydrates, etc.). Quantification was carried out by liquid chromatography, electrospray tandem mass spectrometry using external calibration. Method quantitation limits for bisphenols in liquid and solid samples were in the interval 0.019–0.19 μg L^{-1} and 0.06–0.81 μg kg^{-1}. The method was applied to the determination of

* Corresponding author. Department of Analytical Chemisty, Institute of Fine Chemistry and Nanochemistry, Marie Curie Building (Annex), Campus of Rabanales, Universidad de Córdoba, 14071, Córdoba, Spain.
E-mail address: a42caasn@uco.es (N. Caballero-Casero).
[1] Current Address: Department of Analytical chemistry, Nutrition and Food Science, University of Salamanca, Salamanca, 37008 Spain.

https://doi.org/10.1016/j.aca.2020.11.057

bisphenols and derivatives in thirteen human exposure sources and biological fluids. Only four bisphenols out of twenty-one were not found in the analyzed samples. This supramolecular solvent-based bisphenol- and matrix-independent method constitutes a valuable strategy in terms of analytical and operational characteristics for the assessment of human exposure to mixtures of bisphenols and derivatives.

1. Introduction

It has become increasingly evident that human exposure to mixtures of chemicals, at individual concentrations considered safe, may cause harmful effects [1]. Guidance for conducting risk assessment for combined exposure to multiple chemicals has been published by US EPA [2], the WHO/IPCS [3], and the European Commission (EC) [4,5]. However, except for intentional mixtures such as formulated products and mixtures included in specific legislative frameworks (e.g. pesticides), most of the chemical risk assessment is based on substance-driven and sector-specific [6–9].

Human exposure to chemicals can be assessed by estimating uptake rates from identified sources and/or biomonitoring and, in this respect, both the quality and quantity of available data, along with the low cost, easy use and speediness of the procedures involved, are essential for reliable exposure assessment [7]. Thus, the ideal analytical method for supporting chemical risk assessment would have to cope with the sensitive and selective determination of a large number of chemicals in a variety of exposure sources and biological matrices, using simple, short and cost-effective analytical runtimes.

Bisphenol mixtures meet several of the criteria set by the EC to be considered of potential concern [4]. Thus, exposure of the human population to bisphenols is widespread; they are pseudo-persistent; there is potential for adverse effects to the likely exposure levels; there is a scientific base to predict that they will probably act similarly; and threshold limits for the effects of most of bisphenols and derivatives have been not established. So, the knowledge about the extent and magnitude of human exposure to these mixtures is essential for the development of the respective legislative provisions.

Analytical methods reported so far do not meet the analytical and/or operational requirements for simultaneously evaluating the extent and magnitude of human exposure to a mixture of bisphenols [10]. Major drawbacks arise from both sample treatment and LC-MS/MS quantification, which results in matrix- and analyte-dependent procedures. Thus, solvent extraction, mandatory for the treatment of solid samples and widely used for the processing of the liquid ones, is not well suited for the extraction of analytes covering a wide polarity range [11,12].

On the other hand, multimode solid-phase extraction (SPE), able to retain analytes based on different interaction mechanisms, is only applicable to liquid samples and also involves multiple steps (conditioning, loading, washing, elution, and evaporation), which increases both time and costs compared to solvent extraction [13]. Molecular imprinted polymers (MIPs) are usually more effective than conventional SPE sorbents for matrix components removal, although their application is only limited to liquid samples (i.e. canned energy drinks) [14]. Albeit QuEChERS has shown promising results as applied to dairy products, major drawbacks include the consumption of high volume of organic solvent (15 mL/sample), the need for sample clean-up and evaporation of 6 mL of extract, and the requirement of using matrix-matched standard calibration for bisphenols quantitation [15]. Thus, matrix effects continue being the major concern for quantification of bisphenols by LC-MS/MS, particularly using electrospray ionization (ESI), which, in most cases, makes it mandatory the use of matrix-matched calibration or extensive sample preparation procedures for interference removal [11–13]. On the other hand, the chromatographic conditions required for achieving adequate sensitivity for bisphenol diglycidyl ethers by LC-ESI-MS/MS (e.g. formation of $[M + NH_4]^+$ adducts) cause ion suppression in the determination of bisphenols, that hindering their sequential determination [16].

This work was intended to develop a comprehensive sample treatment platform able to simultaneously extract bisphenols in a wide polarity range and remove matrix interferences from a large variety of samples of interest for human exposure evaluation. For this purpose, several advanced functional features of environment-responsive supramolecular solvents (SUPRAS) [17] were brought together, namely the high number of binding sites and polarity microenvironments they offer, their capability to act as restricted access materials (SUPRAS-RAM) through physical and chemical mechanisms [18,19] and their ability to significantly reduce phospholipid-based matrix effects [20]. These combined properties, in addition to the capability of SUPRAS to offer multiple extraction mechanisms (e.g. dispersion, hydrogen bonding, polar, etc) [21], should be able to extract efficiently analytes in a wide polarity range and remove major matrix components (i.e. proteins, carbohydrates, lipids, humic acids, etc). As a result, we hypothesize that matrix-independent sample treatments could be developed prior to LC-MS/MS quantification of mixtures of chemicals of interest for evaluation of human exposure.

The approach was applied to the determination of a mixture of 21 bisphenols and derivatives (see Tables S–1 in Supporting Information, SI), encompassing a wide polarity range (log K_{ow} from 1.254 to 6.564), in a variety of human exposure sources (canned food, beverages, dust) and biological fluids (urine and serum). These samples involve matrices of very different composition (e.g. see the composition of some canned foodstuffs in Tables S–2) where bisphenols are at concentrations in the low ng g^{-1} or ng mL^{-1}.

To the best of our knowledge, this is the first proposal of a supramolecular-based comprehensive sample treatment platform prior to LC-MS/MS for the evaluation of human exposure to a mixture of bisphenols and derivatives through the estimation of their content in both exposure sources and biological fluids. Below, the most relevant results are presented and discussed.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade and used as supplied by manufacturers. Twenty-one bisphenols and derivatives (chemical names in Table S1) were obtained as follows: bisphenol A (BPA), bisphenol F (BPF), bisphenol A diglycidyl ether (BADGE), monochloro-BPA (MCBPA), dichloro-BPA (DCBPA) and trichloro-BPA (TCBPA) from Aldrich (St. Louis, USA); BADGE monohydrate

(BADGE·H_2O) (≥95%), BADGE dihydrate (BADGE·$2H_2O$) (≥97%), BADGE hydrochloride (BADGE·HCl), (≥90%), bisphenol F diglycidyl ether (BFDGE) (≥95%), BADGE chlorohydroxy (BADGE·H_2O·HCL) (≥95%) and BADGE dihydrochloride (BADGE·2HCL) (≥97%) from Fluka Chemika (Bush, Switzerland); bisphenol S (BPS) (98%); bisphenol AF (BPAF) (97%), bisphenol AP (BPAP) (99%), bisphenol P (BPP) (99%); bisphenol Z (BPZ) (98%) and BFDGE dihydrate (BFDGE·2H2O) (≥95%) from Sigma Aldrich (Steinheim, Germany); bisphenol B (BPB) (98%) and bisphenol E (BPE) (98%) from TCI Europe (Zwijndrecht, Belgium) and tetrachloro-BPA (TeCBPA) from Tokyo Chemical Industries (Tokyo, Japan). Isotopically labeled BPA ($12C^{13}$-BPA) and BADGE (d6-BADGE) were supplied by Cambridge Isotope Laboratories (Andover, USA) and Toronto Research Chemicals (Toronto, Canada), respectively. Methanol, 1-hexanol, and tetrahydrofuran (THF, 98%) were obtained from VWR-Prolabo (Bois, France), ammonium formate (≥99%) from Sigma Aldrich (St. Louis, USA), formic acid (98%) from Panreac Química, (Barcelona, Spain), and ultra-pure quality water (18.2 MΩ cm) from a Milli-Q purification system (Merck Millipore, Madrid, Spain). Lichrosolv® water was supplied by Merck KGaA (Darmstadt, Germany). Stock solutions for individual bisphenols and isotopically labeled internal standards (1–2.5 mg mL^{-1}) were prepared in methanol and stored at −20 °C. Intermediate solutions containing mixtures of bisphenols plus chlorinated derivatives or bisphenol diglycidyl ethers (10 μg L^{-1} each) were prepared in methanol. Working solutions were prepared daily by appropriate dilution of the intermediate solutions with methanol:water (50:50, v/v) for bisphenols plus chlorinated derivatives and methanol:ammonium formate/formic acid buffer (12.5 mM, pH 3.75) (50:50 v/v) for bisphenols diglycidyl ethers.

2.2. Determination of bisphenols and derivatives in canned food and beverages, dust and biological fluids

2.2.1. Sample collection and pretreatment

A variety of samples, known to be representative sources of human exposure to bisphenols and derivatives, were selected for the study. They included canned food (sweet corn, lentils, chickpeas, sausages and meatballs), canned beverages (tonic water, tea, cola and beer), bottled water, and indoor dust. Likewise, representative fluids for bisphenol biomonitoring (urine and serum) were included in the study. Canned food and beverages were purchased in local supermarkets in Córdoba (South Spain) in September 2019, and they were stored at 4 °C, under dark conditions, until analysis. The whole solid content of canned foodstuffs was taken for analysis and homogenized using a high-speed Ultra-Turrax (T-25 Basic from Ika. Werke, Germany) for around 5min. Carbonated beverages were degasified with nitrogen. Dust was collected from domestic carpets and furniture with a vacuum cleaner equipped with non-reusable paper vacuum cleaner bags, from five private residences located in Córdoba (Spain) during the spring of 2019. The urine and serum sampling were conducted between May and July of 2019 on six healthy volunteers (3 men and 3 women from 25 to 32 years) from the University of Córdoba (Córdoba, Spain) in compliance with the Ethics and Data Protection regulations of the University of Córdoba. Serum samples were taken with serum separator vacuum tubes (Vacu-test®) to avoid possible contamination during collection and frozen at −20 °C until analysis. Spot urine samples were centrifuged for 20 min at 3000 rpm to remove possible sediments and kept frozen at −20 °C until analysis.

2.2.2. Control of background contamination

Several measurements were taken to control the background contamination of bisphenols and derivatives that arises from the widespread use of polycarbonate plastics and epoxy resins in lab materials and equipment and usually occurs at ng L^{-1} - μg L^{-1} levels with a random pattern. Thus, sample preparation was performed in a separate room designated exclusively for bisphenol handling. Working benches were cleaned with methanol on a daily basis and covered with aluminum foil, over which clean materials were left. Nitrile gloves were always worn. Glassware, pipette tips, microtubes and other materials were sequentially washed (twice each step) with soap and tap water, ultra-pure quality water and methanol. Ultra-high-quality water with BPA content below the LOD was obtained by filtration of Lichrosolv® water through Empore® styrene-divinylbenzene disks (Análisis Vínicos S.L. Tomelloso, Spain). Labware, solvents and reagents were periodically checked for the presence of bisphenols and derivatives using a quick chromatographic elution program (mobile phase water (A) and methanol (B)), linear gradient from 90 to 98% of B in 5 min and 98 to 90 %B for column equilibration at the initial mobile phase composition in 5 min with a flow rate of 300 μL min^{-1}). Labware giving signals higher than noise was re-subjected to the cleaning protocol and retested again. Procedural blanks were conducted for each sample batch to account for background contamination, and when bisphenols concentrations were above the method limit of quantification the analysis of the whole sample batch was repeated.

2.2.3. SUPRAS-based extraction/cleanup of bisphenols in liquid samples

A volume (1500 μL) of sample (beverage, urine or serum) was added to a 2 mL-microtube Safe-Lock from Eppendorf Iberia (Madrid, Spain) containing hexanol (100 μL) dissolved in THF (400 μL). The water content present in the liquid samples promoted the self-assembly of hexanol and caused the instantaneous *in-situ* formation of an environment-responsive SUPRAS [17,20]. The mixture was vortex-shaken at 2500 rpm for 5 min (Reax Heidolph from Schwabach, Germany) to favor analyte extraction, and then centrifuged at 14,160 g for 30 min (MPW-350R from MPW Medical Instruments, Warschaw, Poland). Fig. 1 (bottom) shows a schematic of the sample treatment platform for liquid samples. Two aliquots of the SUPRAS extract (75 μL each) were transferred to 10 mL-glass centrifuge tubes and evaporated to dryness under a gentle nitrogen stream and temperature (60 °C). Then, the residues were reconstituted in 300 μL of methanol:water (50:50 v/v) for determination of bisphenols/chlorinated derivatives and 300 μL of methanol: ammonium formate/formic acid (12.5 mM, pH 3.75) buffer (50:50 v/v) for quantitation of diglycidyl ethers (Fig. 1, right).

2.2.4. SUPRAS-based extraction/cleanup of bisphenols in solid samples

A SUPRAS volume (~6.2 mL) able to treat 31 solid samples was obtained by mixing hexanol (3 mL), THF (6 mL) and water (21 mL) in a 50 mL glass centrifuge tube. The SUPRAS formed instantaneously under the addition of water and the mixture was centrifuged at 2400 g for 30 min to speed up liquid-liquid phase separation. Then, the SUPRAS was separated from the equilibrium solution with a syringe and both were stored in closed glass vial until use (see Fig. 1, top). Under these conditions, the SUPRAS and equilibrium solution were stable at room temperature for at least one month. A SUPRAS aliquot (200 μL) and the homogenized solid subsample (200 mg) were mixed in safe-lock 2 mL microtubes. In the case of dust samples, they were previously wetted with 300 μL of the equilibrium solution obtained in the synthesis of the SUPRAS, otherwise the SUPRAS was almost completely adsorbed into the dust matrix. So, three phases were obtained for dust during extraction; the wetted dust, the equilibrium solution and the SUPRAS. Four glass balls (3 mm diameter) were introduced in the microtube in all the cases to improve sample dispersion during

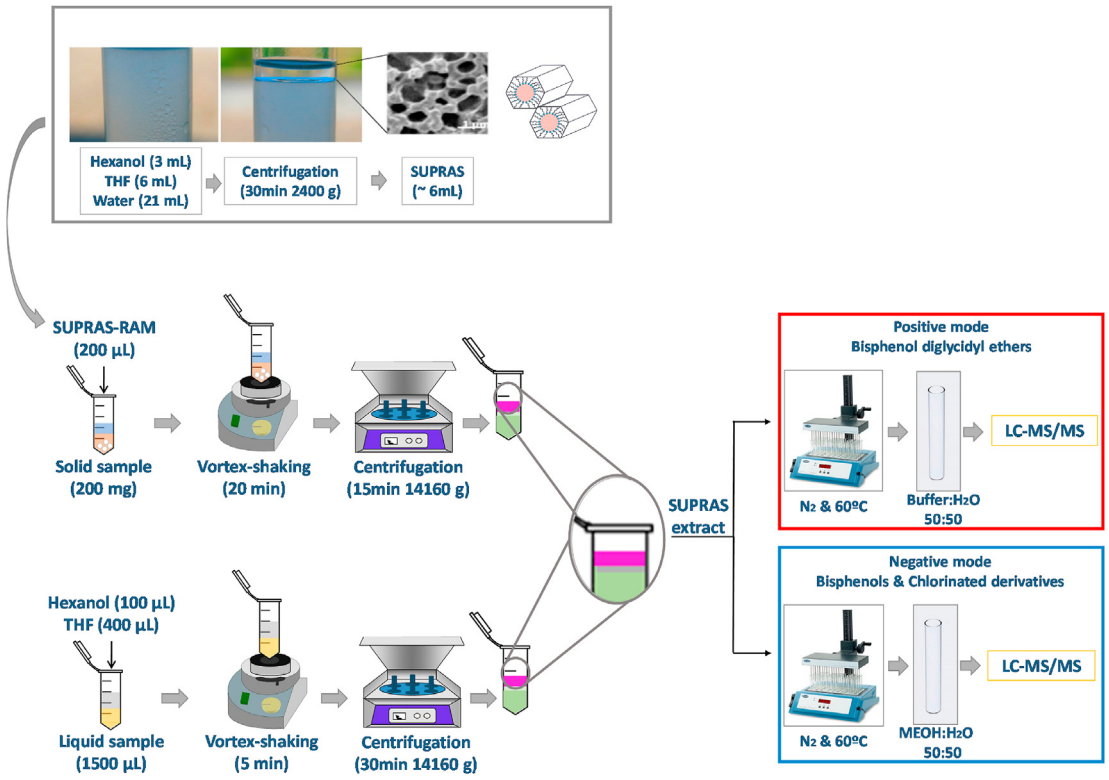


Fig. 1. Schematic illustrations for the production and structure of the SUPRAS used (top) for the extraction/cleanup of solid samples (middle), the formation of the SUPRAS into the liquid sample and simultaneous extraction/cleanup (bottom) and LC-ESI-MS/MS sequential analysis of bisphenol diglycidyl ethers and bisphenols/chlorinated bisphenols (right).

extraction, which was performed by means of vortex-shaking at 2500 rpm for 20 min. The mixture was then centrifuged at 14,160 g for 15 min to separate the solvent from the solid residue (see Fig. 1, middle). Two aliquots (75 µL) of the SUPRAS extract were evaporated to dryness and reconstituted according to the procedure described above for liquid samples (Fig. 1, right).

2.2.5. Liquid chromatography-tandem mass spectrometry

The separation and quantification of the 21 bisphenols, chlorinated derivatives and diglycidyl ethers (Tables S–1) were carried out by reversed-phase LC (Agilent HP 1200 series, Palo Alto, CA, USA) coupled with a TurbeIonSpray (TIS)-tandem mass spectrometry (Applied Biosystems MSD Sciex 4000QTRAP, Foster City, CA, USA). The stationary phase was a C18 column (ACE 3 C18-PFP, UK, 150 mm × 3.0 mm, particle size 3.5 µm) thermostated at 35 °C. This column was preceded by a C18 Guard Cartridge (ACE 3 C18-PFP, UK, 30 mm × 4.6 mm, particle size 4 µm). Additionally, a Symmetry C18 column (Waters, 75 mm × 4.6 mm, particle size 3.5 µm) was placed between the LC pump and the injection valve to retain potential bisphenols coming from the solvents used as mobile phases or leached from the plastic components of the LC system. In this way, these bisphenols eluted after the respective ones coming from the sample. Chromatographic runs were sequentially obtained for bisphenols/chlorinated derivatives and bisphenol diglycidyl ethers, using the same mobile phase (water (A) and methanol (B)) and elution programs. The linear gradients were as follows: from 50% to 60% solvent B for 2 min, then from 60% to 80% B for 2 min, next from 80% to 90% B for 18 min and finally, from 90% to 100% B for 1 min. Column equilibration at the initial mobile phase composition took 5.5 min. The injection volume was 10 µL and the flow rate 0.3 mL min^{-1}. Mass analysis was carried out in both positive (ammonium diglycidyl ethers) and negative (bisphenols and their chlorinated derivatives) ESI modes. All data were acquired and processed using the Analyst 1.5.1 Software (Applied Biosystems). Quantitative analyses were carried out using two specific combinations of a precursor-product ion transition for each compound, with a dwell time set up at 100 m s. Compound specific MS/MS parameters for each compound are given in Tables S–1. Source settings were as follows: probe vertical y-axis position, 2 mm; probe horizontal y-axis position, 6 mm; curtain gas (N_2), 10 psig; nebulizer gas, 40 psig; turbo gas, 60 psig; temperature of the turbo gas, 650 °C; ion spray voltage: ± 4500 V. Collision gas 3.0×10^{-5} Torr. Quantification was carried out by external calibration with standards in methanol:water (50:50 v/v) for determination of bisphenols/chlorinated derivatives and methanol: ammonium formate/formic acid (12.5 mM, pH 3.75) buffer (50:50 v/v) for determination of diglycidyl ethers, and using the internal standards $12C^{13}$-BPA and d6-BADGE, respectively.

3. Results and discussion

3.1. Optimization of the sequential LC-MS/MS analysis of bisphenols and bisphenol diglycidyl ethers

Sensitive LC (ESI)-MS/MS analysis of bisphenols and derivatives requires two different chromatographic runs spaced by a thorough cleaning step or even conducted in two different instruments [16]. Usually, bisphenols and chlorinated derivatives are separated using mobile phases made up of methanol and water and quantified by measurement of fragment ions obtained from $[M-H]^-$ [22]. On the other hand, bisphenol diglycidyl ethers are prompted to form adducts, some of which (e.g. $[M+Na]^+$, $[M+K]^+$) are very stable and produce poor fragmentation in MS/MS, while others (e.g. $[M + NH_4]^+$) give efficient fragmentation [16]. In order to enable the formation of ammonium adducts and ensure signal reproducibility, ammonium acetate or formate buffers are generally used as

additives in the mobile phase [23]. However, these additives cause ion suppression in the MS/MS analysis of bisphenols and chlorinated derivatives and this fact hinders their simultaneous determination with diglycidyl ethers, at least at the low concentration levels required for evaluation of human exposure. So the sequential LC (ESI)MS/MS analysis of bisphenols and their respective diglycidyl ethers can be performed using methanol-water and methanol-aqueous ammonium buffer, respectively. However, this is only possible after thorough cleaning of the chromatographic system since the presence of minute traces of ammonium drastically reduces the response for bisphenols [16]. This cleaning is time-consuming, no cost-effective and, what is worse is that it hinders high sample throughput.

In an attempt to speed up this sequential analysis we tried to form $[M + NH_4]^+$ adducts for bisphenol diglycidyl ethers during sample treatment. Our working hypothesis was that, $[M + NH4]^+$ adducts would be stable enough to keep their integrity during chromatographic analysis. Thus, SUPRAS extracts (Fig. 1) were fortified with the bisphenol diglycidyl ethers included in Tables S–1 (1 μg L^{-1} each), evaporated to dryness and reconstituted with a mixture of 150 μL of methanol and 150 μL of ammonium formate/formic acid buffer at concentrations from 2.5 to 12.5 mM (pH 3.75). Then, they were subjected to LC-ESI-MS/MS analysis using methanol-water as the mobile phase.

Table 1 shows the responses obtained for the ammonium adducts formed during sample treatment, expressed as the percentage of the response obtained for adducts produced in the traditional way (i.e. into a mobile phase made up of 50:50 vol ratio of methanol and 25 mM formic acid:ammonium formate, pH 3.75, flow rate 0.3 mL min^{-1}). It can be checked that the same mass responses were obtained for ammonium adducts of diglycidyl ethers formed in SUPRAS-extracts containing buffer (12.5 mM) and mobile phases containing buffer as additive. So, our results prove that these adducts were stable enough under the chromatographic conditions set for bisphenol diglycidyl ether separation. Fig. 2 shows the total ion chromatograms obtained for (A) bisphenols and chlorinated derivatives and (B) bisphenol diglycidyl ethers when they were run sequentially using identical mobile phase composition and gradient elution without any intermediate cleaning step. Fragmentation patterns for the product ions obtained (Tables S–1) were in agreement with those previously reported [22,24].

3.2. SUPRAS-based sample treatment platform for bisphenols and derivatives: study of recoveries and matrix effects

A comprehensive sample treatment platform for LC-MS/MS determination of bisphenols and derivatives in exposure sources and biological fluids should be able to provide both analyte-independent recoveries and matrix-independent mass responses. For this purpose, we investigated the use of hexanol-based SUPRAS, which are nanostructured solvents made up of inverted hexagonal aggregates, obtained spontaneously in mixtures of tetrahydrofuran and water by self-assembly and coacervation [20]. In these SUPRAS, the alcohol groups of hexanol surround aqueous cavities while the hydrocarbon chains are dispersed in THF (see a schematic in Fig. 1, top) [17]. They have the potential to efficiently extract bisphenols and derivatives (log K_{ow} from 1.254 to 6.564, Tables S–1) and simultaneously remove major sample interferences based on:

1) The different polarity microenvironments (i.e. aqueous cavities, amphiphile headgroups and amphiphile hydrocarbon chains) offered for solute solubilization.
2) The mixed-mode mechanisms (acceptor-donor hydrogen bonds, polar interactions, dispersion interactions) and multiple sites (the concentration of hexanol in the SUPRAS is in the range 7.4–0.15 mg μL^{-1}) [20] provided for solute binding.
3) Their ability to provide extracts free of proteins, carbohydrates and lipids. Thus, proteins are removed as a white layer between the SUPRAS and the equilibrium solution in liquid samples and between the SUPRAS and sample residue in solid samples, owing to their denaturation and flocculation by the action of THF and hexanol respectively [17]. Regarding carbohydrates, they can be removed by size exclusion owing to the capability of alkanol-based SUPRAS to act as restricted access materials (RAM) [17], which arise from the fact that the size of the aqueous cavities of the inverted hexagonal arrangement of the hexanol, where polar macromolecules solubilize, can be tailored by controlling the THF:water ratio in the bulk solution [17]. Finally, lipids and phospholipids remain precipitated after hexanol volatilization and bisphenols reconstitution [20].

The ability of the hexanol-based SUPRAS-RAM to simultaneously extract bisphenols and derivatives and remove major sample matrix components was evaluated. For this purpose, three samples representative for human exposure (canned lentils and tonic) and biomonitoring (urine) were selected. Optimization of the sample treatment platform was based on the study of matrix effects, global recoveries (i.e. those corresponding to the whole process) and reconstitution recoveries (i.e. those corresponding to the solubilization of analytes from the residue remaining after evaporation of SUPRAS extracts, see Fig. 1).

Reconstitution recoveries were investigated from aliquots of SUPRAS (75 μL) fortified with 4 μg L^{-1} of either bisphenol and chlorinated derivative or bisphenol diglycidyl ethers. The fortified SUPRAS were evaporated to dryness under a nitrogen stream and

Table 1
MS/MS response[a] for ammonium adducts of bisphenol diglycidyl ethers formed during sample treatment.

Analyte	Buffer concentration[b] (mM)				
	2.5	**5**	**7.5**	**10**	**12.5**
BADGE	42 ± 9	45 ± 3	53 ± 3	89 ± 2	98 ± 3
BADGE·H_2O	48 ± 9	49 ± 7	54 ± 8	94 ± 1	98 ± 2
BADGE·$2H_2O$	37 ± 6	37 ± 6	65 ± 4	87.3 ± 0.7	95 ± 4
BADGE·HCl	67 ± 4	69 ± 3	68 ± 3	92 ± 7	101 ± 3
BADGE·2HCl	50 ± 6	59 ± 6	64 ± 3	74 ± 4	108 ± 5
BADGE·HCl·$2H_2O$	40 ± 2	59 ± 5	64 ± 5	89 ± 3	99 ± 3
BFDGE	40 ± 2	45 ± 3	53 ± 3	89 ± 6	98 ± 4
BFDGE·$2H_2O$	40 ± 5	46 ± 6	50 ± 1	85 ± 2	101 ± 4

[a] Expressed as percentage of the signal obtained, along with the respective standard deviation (n = 3), for ammonium adducts formed in a mobile phase made up of 25mMformic acid–ammonium formate buffer at pH 3.75 (solvent A) and methanol (solvent B).
[b] Ammonium formate/formic acid, pH 3.75.

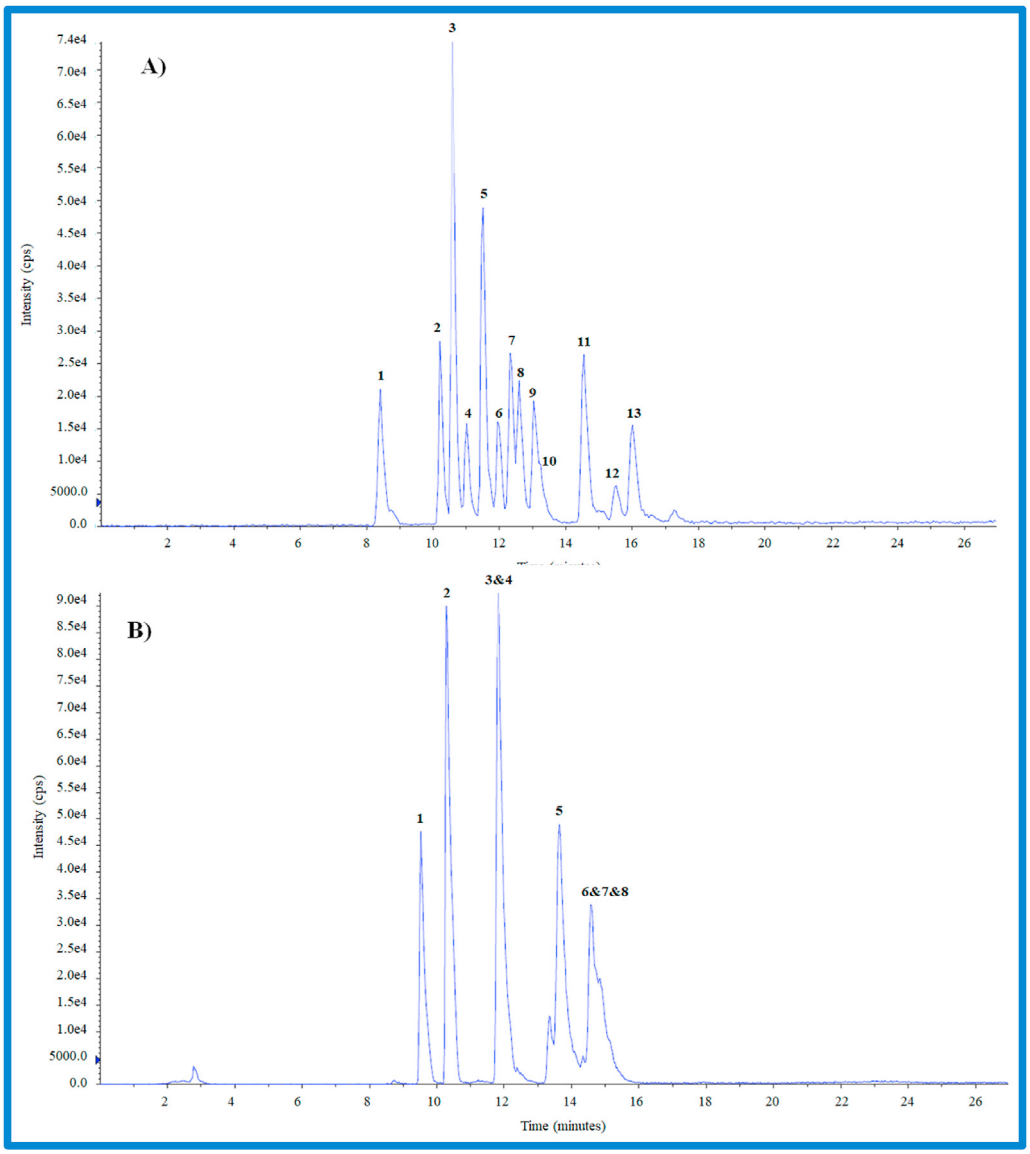


Fig. 2. LC-ESI-MS/MS total ion chromatograms obtained from a mixture of standards (20 μgL^{-1} each). A) bisphenols and chlorinated bisphenols: (1) BPS, (2) BPF, (3) BPE, (4) BPA+12C^{13}BPA, (5) BPB, (6) MCBPA, (7) BPAP, (8) BPZ, (9) BPAF, (10) DCBPA, (11) TCBPA, (12) BPP, (13) TeCBPA. B) diglycidyl ethers: (1) BFDGE·2H2O, (2) BADGE·2H2O (3) BADGE·H2O, (4) BADGE·H2O·HCL, (5) BFDGE, (6) BADGE·2HCL, (7) BADGE·HCL, (8) BADGE + d6-BADGE.

60 °C, and the residue were treated with solvent mixtures of different compositions for the reconstitution of analytes. Absolute recoveries were in the range 97–115% for bisphenols and chlorinated derivatives when extracted with 300 μL of methanol:water (50/50, v/v), and in the range 95–110% for bisphenol diglycidyl ethers when extracted with 300 μL of methanol: ammonium formate/formic acid buffer, 12.5 mM, pH 3.75 (50:50, v/v). As specified in section *3.1*, the presence of buffer in the reconstitution solvent was also effective in the formation of ammonium adducts of bisphenol diglycidyl ethers.

Matrix effects were investigated by extracting the selected model samples (canned lentils and tonic, and urine) with SUPRAS synthesized in different THF:water media (i.e. percentages of THF in the range 10–40%). The concentration of hexanol in the SUPRAS decreases and the size of the inverse hexagonal aggregates increases as the percentage of THF in the synthesis medium raises [20]. On the other hand, SUPRAS composition and structure remain unchangeable as the percentage of hexanol varies. Liquid and solid samples were treated according to the procedures specified in sections *2.2.3* and *2.2.4*, respectively, and the SUPRAS extracts obtained were fortified with 1 μg L^{-1} of each bisphenol and 5 μg L^{-1} of the internal standards 12C^{13}-BPA and d6-BADGE, which were used for the analysis of bisphenols and chlorinated derivatives and the bisphenol diglycidyl ethers, respectively. Matrix effects were calculated by measuring signal suppression or enhancement (SSE) by comparing mass response for analytes in samples and standards dissolved in methanol:water (50:50 v/v) for determination of bisphenols/chlorinated derivatives and methanol: ammonium formate/formic acid (12.5 mM, pH 3.75) buffer (50:50 v/v) for quantitation of diglycidyl ethers. The SSE calculated in this manner may be referred to as an absolute matrix effect; percentages higher than 100 indicate ion enhancement, while percentages lower than 100 are indicative of ion suppression.

Table 2 shows the SSE values obtained for the samples analyzed as a function of the percentage of THF and therefore, of the vacuole size of the SUPRAS. The behavior was very similar in the samples

Table 2
Values of signal suppression and enhancement (SSE, %), along with their corresponding standard deviations, for bisphenols and derivatives in lentils, tonic and urine as a function of the percentage of THF used in the synthesis of the SUPRAS.

	THF (%)	BPA	BPB	BPE	BPF	BPP	BPS	BPZ	BPAF	BPAP	MCBPA	DCBPA	TCBPA	TeCBPA	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE ·HCl	BADGE·2HCl	BADGE ·HCl·H_2O	BFDGE	BFDGE ·$2H_2O$
Lentils	10	85 ± 9	87 ± 6	79 ± 4	88 ± 6	83 ± 5	88 ± 1	85 ± 4	98 ± 1	89 ± 1	84 ± 1	88 ± 3	80 ± 7	82 ± 8	106 ± 9	100 ± 9	104 ± 8	101 ± 2	102 ± 9	100 ± 1	108 ± 5	105 ± 3
	20	88 ± 5	91 ± 1	85 ± 4	102 ± 3	90 ± 1	108 ± 5	93 ± 4	102 ± 6	86 ± 1	89 ± 1	88 ± 3	82 ± 2	85 ± 3	106 ± 7	102 ± 4	108 ± 7	103 ± 6	109 ± 3	98 ± 4	106 ± 5	108 ± 3
	30	93 ± 1	89 ± 2	89 ± 6	62 ± 6	70 ± 3	103 ± 6	70 ± 5	111 ± 1	71 ± 7	75 ± 9	90 ± 4	105 ± 1	85 ± 1	81 ± 1	98 ± 6	108 ± 9	107 ± 3	106 ± 1	103 ± 6	84.7 ± 0.3	100 ± 2
	40	115 ± 9	88 ± 7	85 ± 7	60 ± 2	65 ± 6	107 ± 8	66 ± 4	100 ± 3	90 ± 7	80 ± 3	89 ± 5	81 ± 1	84 ± 5	88 ± 1	94 ± 8	106 ± 6	102 ± 2	102 ± 2	105 ± 9	87 ± 2	105 ± 6
Tonic	10	101 ± 3	93 ± 6	93 ± 4	112 ± 10	98 ± 7	93 ± 3	101 ± 3	96 ± 7	106 ± 2	105 ± 2	93 ± 7	94 ± 9	88 ± 12	111 ± 2	107 ± 1	100 ± 4	101 ± 1	109 ± 4	102 ± 2	104 ± 4	103 ± 6
	20	97 ± 2	95 ± 5	89 ± 8	104 ± 3	100 ± 6	101 ± 5	106 ± 8	103 ± 7	103 ± 9	109 ± 1	101 ± 6	96 ± 4	94 ± 1	105 ± 2	108 ± 4	86 ± 1	111 ± 2	88 ± 3	107 ± 1	109 ± 5	109 ± 9
	30	109 ± 6	92 ± 4	95 ± 3	95 ± 7	88 ± 8	98 ± 4	104 ± 4	100 ± 6	107 ± 7	111 ± 3	98 ± 4	101 ± 6	92 ± 1	104 ± 1	109 ± 8	88 ± 4	104 ± 8	58 ± 5	95 ± 4	84 ± 8	92 ± 3
	40	108 ± 4	95 ± 6	104 ± 1	103 ± 2	93 ± 7	95 ± 5	107 ± 7	105 ± 1	106 ± 2	95 ± 1	98 ± 3	107 ± 4	83 ± 6	103 ± 6	107 ± 12	101 ± 2	80 ± 5	49 ± 2	98 ± 7	90 ± 10	97 ± 6
Urine	10	102 ± 7	101 ± 9	99 ± 6	114 ± 4	91 ± 6	113 ± 3	111 ± 3	98 ± 1	104 ± 4	98 ± 9	113 ± 5	96 ± 2	95 ± 4	108 ± 2	98 ± 17	83 ± 10	104 ± 8	95 ± 4	85 ± 1	102 ± 2	104 ± 1
	20	89 ± 2	109 ± 3	103 ± 2	94 ± 2	105 ± 9	97 ± 6	100 ± 4	107 ± 4	103 ± 8	110 ± 3	97 ± 3	96 ± 1	103 ± 6	103 ± 2	101 ± 3	88 ± 9	101 ± 5	106 ± 3	88 ± 8	107 ± 8	109 ± 3
	30	87 ± 9	110 ± 4	104 ± 2	92 ± 2	108 ± 4	103 ± 4	102 ± 6	102 ± 3	108 ± 1	103 ± 1	103 ± 3	109 ± 3	104 ± 4	101 ± 8	91 ± 5	95 ± 5	105 ± 7	98 ± 2	98 ± 2	109 ± 3	100 ± 5
	40	97 ± 10	94 ± 6	83 ± 4	99 ± 2	103 ± 8	105 ± 1	102 ± 5	108 ± 7	108 ± 9	103 ± 4	105 ± 5	101 ± 9	100 ± 2	97 ± 4	107 ± 7	96 ± 7	106 ± 9	105 ± 1	100 ± 2	105 ± 4	103 ± 2

Table 3
Absolute recoveries (%) and standard deviations (%) obtained in the extraction of bisphenols, chlorinated derivatives and bisphenols diglycidyl ethers from lentils, tonic and urine with SUPRAS synthesized in a hydro-organic medium containing 20% of THF.

Sample	BPA	BPB	BPE	BPF	BPP	BPS	BPZ	BPAF	BPAP	MCBPA	DCBPA	TCBPA	TeCBPA	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·HCl	BADGE·2HCl	BADGE ·HCl·H_2O	BFDGE	BFDGE·$2H_2O$
Lentils[a]	86 ± 4	75.5 ± 0.9	82 ± 4	87 ± 2	82 ± 1	106 ± 5	90 ± 5	107 ± 6	114 ± 4	87 ± 1	101 ± 6	97 ± 3	70 ± 2	76 ± 7	90 ± 3	102 ± 4	99 ± 6	100 ± 6	98 ± 8	77 ± 5	100 ± 4
Tonic[b]	103 ± 5	102 ± 3	104 ± 6	103 ± 2	111 ± 4	99 ± 8	99 ± 4	103 ± 8	97 ± 5	83 ± 5	90 ± 8	74 ± 8	110 ± 8	75 ± 3	97 ± 8	85 ± 1	73 ± 2	75 ± 8	107 ± 2	109 ± 5	109 ± 7
Urine[b]	100 ± 1	107 ± 3	101 ± 2	98 ± 9	83 ± 7	105 ± 7	92 ± 9	91 ± 7	108 ± 4	103 ± 7	99 ± 9	105 ± 4	97 ± 5	97 ± 9	108 ± 4	76 ± 4	78 ± 2	79 ± 9	88 ± 5	100 ± 3	88 ± 3

[a] Fortified sample with 0.64 μg kg^{-1} bisphenols, chlorinated derivatives and bisphenols diglycidyl ethers. The internal standards were added to the sample extract at 20 μg kg^{-1} $12C^{13}$-BPA or 3.5 μg kg^{-1} d6-BADGE; n = 3.
[b] Fortified sample with 1 μg kg^{-1} bisphenols, chlorinated derivatives and bisphenols diglycidyl ethers. The internal standards were added to the sample extract at 20 μg kg^{-1} $12C^{13}$-BPA or 3.5 μg kg^{-1} d6-BADGE.

Table 4
Linear range for the instrumental calibration of bisphenols and derivatives and quantification limits for solid and liquid samples.

Compound	t_R (min)	Linearity (μg L^{-1})	MQL[a] (μg kg^{-1})	MQL[b] (μg L^{-1})
BPA	11.03	0.04–250	0.16	0.035
BPB	11.50	0.02–250	0.12	0.019
BPE	10.50	0.04–250	0.06	0.029
BPF	10.20	0.09–250	0.39	0.091
BPP	15.58	0.04–250	0.18	0.031
BPS	8.79	0.03–250	0.12	0.023
BPZ	12.63	0.03–250	0.14	0.036
BPAF	13.05	0.04–250	0.23	0.035
BPAP	12.34	0.04–250	0.21	0.040
MCBPA	11.98	0.08–250	0.36	0.084
DCBPA	13.24	0.05–250	0.24	0.056
TCBPA	14.56	0.03–250	0.16	0.032
TeCBPA	16.01	0.04–250	0.24	0.041
BADGE	14.78	0.02–250	0.21	0.024
BADGE·H_2O	11.83	0.01–250	0.56	0.049
BADGE·$2H_2O$	10.29	0.09–250	0.48	0.19
BADGE·HCl	14.57	0.13–250	0.54	0.11
BADGE·2HCl	14.37	0.13–250	0.61	0.12
BADGE·HCl·H_2O	11.87	0.1–250	0.52	0.094
BFDGE	13.62	0.15–250	0.81	0.13
BFDGE·$2H_2O$	9.53	0.11–250	0.74	0.17

Method quantitation limits for [a] lentils and [b] urine samples.

investigated; the signal was within the interval recommended for analysis of contaminants (e.g. 70–120%) at the lowest percentages of THF tested (10–20%) while some suppression was observed for a few analytes at higher percentages of this solvent (e.g. BPF, BPP and BPZ in lentils or BADGE.2HCl in tonic). SSE values in the range 82–109% were obtained for 20% of THF for the three matrices tested and consequently, this value was selected as optimal in order to achieve a matrix-independent sample treatment platform.

Absolute recoveries for the target bisphenols were investigated by the analysis of fortified samples subjected to the whole analytical process. Solid and liquid samples were spiked with the analytes to give a final concentration of 4 μg kg^{-1} and 2.5 μg L^{-1} each, respectively. The concentrations added of ^{12}C13-BPA and d6-BADGE to the solid and liquid samples were 20 μg kg^{-1} and 3.5 μg L^{-1}, respectively. Table 3 shows the recoveries obtained, along with the respective standard deviations (n = 3), for the different analytes and samples investigated. Absolute recoveries were in the range 70–114% with relative standard deviations in the interval 0.9–9%. So, good extraction efficiency was obtained for bisphenols and derivatives, which encompass a wide polarity range (log K_{ow} from 1.254 to 6.564), and consequently, the method can be considered analyte-independent for the selected compounds.

3.3. Method performance

Method performance was investigated in terms of calibration range, method identification and quantification limits, matrix effects, precision and trueness. Quantification was based on the internal standard method using calibrations from standards in methanol:water (50/50) for bisphenols and chlorinated derivatives, and methanol: ammonium formate/formic acid buffer, 12.5 mM, pH 3.75 (50:50, v/v) for bisphenol diglycidyl ethers. A single internal standard (^{12}C13-BPA or d6-BADGE) was used in each chromatographic run, in order to correct for process variability. Calibration graphs for analytes were linear over the whole concentration range tested. Correlation between peak area ratios and analyte concentrations was determined by linear regression and 1/x weighted calibration. Correlation coefficients were in the range 0.997–0.9999. Method detection (MDLs) and quantification (MQLs) limits were calculated from samples of lentils and urine, which were considered representative of solid and liquid samples, respectively. The MDLs were calculated from six independent complete analysis of samples by using a signal-to-noise ratio of 3 (the ratio between the peak areas for each target analyte and peak area of noise). The MQLs were calculated in a similar way by using a signal-to-noise ratio of 10. For samples containing the analytes at detectable levels, the noise was calculated close to the analyte peak. Table 4 shows the elution times, calibration working range and the MDLs and MQLs for both liquid and solid samples. MDLs were low enough to determine common concentrations found in real-world samples.

Matrix effects were investigated in complicated matrices of interest for the evaluation of human exposure to bisphenols, specifically canned chickpeas, sausages and meatballs, dust and serum. Table 5 shows the values of SSE obtained along with their respective relative standard deviations. SSE values for all analytes and matrices tested were in the range 78–116%, so the proposed sample treatment platform seems a good strategy to remove major interferences from a wide range of matrices.

Precision was studied in terms of repeatability and within-laboratory reproducibility. For this purpose, six aliquots of a urine sample spiked at 0.1, 0.2 and 1 μg L^{-1} for bisphenols and chlorinated derivatives and 0.3, 0.6 y 3 μg L^{-1} for bisphenol diglycidyl ethers, were analyzed in three days (six aliquots each) using freshly prepared mobile phases and standard solutions. The repeatability, expressed as standard deviation, was calculated as the square root of the average value of the intra-day variances obtained and, the within laboratory reproducibility as the square root of the mean intra-day variance plus the inter-day variance. The relative standard deviations under repeatability conditions were in the ranges 5–16%, 5–18% and 1–10% for bisphenols and chlorinated derivatives at concentrations of 0.1, 0.2 and 1 μg L^{-1}, and in the intervals 4–14%, 0.7–13% y 0.5–10% for bisphenol diglycidyl ethers at concentrations of 0.3, 0.6 y 3 μg L^{-1}. The reproducibility was in the ranges 6–20%, 6–13% and 7–12% for 0.1, 0.2 and 1 μg L^{-1} of bisphenol and chlorinated derivatives, while it varied in the ranges 4–15%, 5–15% and 3–12% for 0.3 and 0.6 y 3 μg $L^{-1}$of bisphenol diglycidyl ethers.

Due to the absence of certified reference materials for the target analytes, the trueness of the proposed method was assessed by measuring the recoveries of urine samples spiked at the same concentration level than that used for precision studies. Recoveries

Table 5
Matrix effects, expressed as signal suppression and enhancement (SSE, %) along with their corresponding standard deviation, obtained from different food, environmental and biological matrices.

Sample	BPA	BPB	BPE	BPF	BPP	BPS	BPZ	BPAF	BPAP	MCBPA	DCBPA	TCBPA	TeCBPA	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·HCl	BADGE·2HCl	BADGE ·HCl·H_2O	BFDGE	BFDGE·$2H_2O$
Chickpeas	110 ± 3	83 ± 1	82 ± 2	80 ± 7	93 ± 4	94 ± 2	88 ± 3	97 ± 5	85 ± 3	80 ± 2	95 ± 5	98 ± 6	81 ± 1	102 ± 7	97 ± 6	102 ± 4	90 ± 3	95 ± 1	109 ± 4	108 ± 6	109 ± 2
Sausages	100 ± 9	99 ± 4	104 ± 4	116 ± 2	88 ± 7	99 ± 8	100 ± 3	95 ± 7	107 ± 2	107 ± 1	93 ± 5	108 ± 5	89 ± 7	92 ± 3	105 ± 4	95 ± 3	100 ± 3	104 ± 1	100 ± 6	93 ± 2	109 ± 4
Meatballs	96 ± 1	95 ± 2	99 ± 1	116 ± 1	90 ± 1	107 ± 3	98 ± 7	78 ± 4	82 ± 9	91 ± 1	100 ± 3	89 ± 6	79 ± 1	108 ± 1	110 ± 2	101 ± 5	112 ± 3	109 ± 7	97 ± 2	106 ± 5	107 ± 3
Dust	110 ± 4	93 ± 1	105 ± 1	85 ± 4	98 ± 1	111 ± 9	100 ± 2	106 ± 3	100 ± 6	106 ± 2	88 ± 4	96 ± 2	90 ± 3	90 ± 2	86 ± 8	109 ± 8	94 ± 8	101 ± 2	117 ± 3	91 ± 5	91 ± 4
Serum	105 ± 3	86 ± 1	105 ± 3	90 ± 2	85 ± 5	111 ± 1	85 ± 1	96 ± 3	109 ± 1	103 ± 2	102 ± 2	90 ± 3	78 ± 3	96 ± 3	105 ± 3	98 ± 3	106 ± 3	104 ± 2	100 ± 1	109 ± 5	109 ± 6

SUPRAS extract fortified at 1 μg L^{-1} of each bisphenol and 5 μg L^{-1} of the internal standards $12C^{13}$-BPA and d6-BADGE; n = 3.

Table 6
Absolute recoveries (%) and standard deviations (%) obtained for bisphenols and chlorinated derivatives and bisphenols diglycidyl ethers from different food, environmental and biological matrices.

Sample	BPA	BPB	BPE	BPF	BPP	BPS	BPZ	BPAF	BPAP	MCBPA	DCBPA	TCBPA	TeCBPA	BADGE	BADGE·H_2O	BADGE·$2H_2O$	BADGE·HCl	BADGE·2HCl	BADGE ·HCl·H_2O	BFDGE	BFDGE·$2H_2O$
Chickpeas[a]	107 ± 3	74.7 ± 0.3	89 ± 2	77 ± 4	78 ± 4	95 ± 1	79 ± 3	92 ± 5	83 ± 10	78 ± 2	86 ± 4	78 ± 6	79 ± 3	84 ± 9	80 ± 5	102 ± 2	82 ± 4	92 ± 5	100 ± 5	99 ± 5	103 ± 1
Sausages[a]	87 ± 4	87 ± 3	87 ± 1	112 ± 2	77 ± 7	110 ± 5	84 ± 2	94 ± 6	82 ± 2	80 ± 1	93 ± 8	114 ± 6	79 ± 7	88 ± 2	83 ± 4	99 ± 2	96 ± 4	85 ± 1	85 ± 5	76 ± 2	84 ± 4
Meatballs[a]	97 ± 1	98 ± 2	93 ± 1	94 ± 7	84 ± 3	111 ± 2	76 ± 7	75 ± 8	80 ± 8	85 ± 2	79 ± 5	79 ± 5	88 ± 3	90 ± 6	82 ± 9	87 ± 6	88 ± 2	107 ± 3	105 ± 8	77 ± 6	101 ± 3
Dust[a]	82 ± 2	87 ± 5	105 ± 9	101 ± 2	84 ± 2	107 ± 9	90 ± 3	86 ± 3	82 ± 9	81 ± 2	77 ± 3	100 ± 10	89 ± 3	86 ± 7	83 ± 7	97 ± 6	74 ± 8	100 ± 2	86 ± 4	82 ± 4	84 ± 6
Serum[b]	107 ± 9	93 ± 5	100 ± 1	113 ± 10	87 ± 3	85 ± 4	72 ± 7	80 ± 9	77 ± 8	88 ± 6	76 ± 5	82 ± 8	92 ± 8	104 ± 4	104 ± 4	95 ± 7	107 ± 5	74 ± 1	97 ± 6	88 ± 8	91 ± 2

[a] Fortified sample with 0.64 μg kg^{-1} bisphenols, chlorinated derivatives and bisphenols diglycidyl ethers. The internal standards were added to the sample extract at 20 μg kg^{-1} $12C^{13}$-BPA or 3.5 μg kg^{-1} d6-BADGE; n = 3. [b] Fortified sample with 1 μg kg^{-1} bisphenols, chlorinated derivatives and bisphenols diglycidyl ethers. The internal standards were added to the sample extract at 20 μg kg^{-1} $12C^{13}$-BPA or 3.5 μg kg^{-1} d6-BADGE.

were in the ranges 90–106%, 97–107% and 95–110% for concentrations of bisphenols and chlorinated derivatives of 0.1, 0.2 and 1 μg L^{-1}, and in the intervals 90–111%, 93–107% and 91–105% for concentrations of bisphenol diglycidyl ethers of 0.3, 0.6 and 3 μg L^{-1}. Recoveries were also evaluated for spiked complicated matrices of interest for the evaluation of human exposure to bisphenols. Table 6 shows the values obtained along with their respective standard deviations. Absolute recoveries for all analytes in samples representing human exposure through food (i.e. canned chickpeas, sausages and meatballs) and inhalation (i.e. dust) as well as a biological sample (i.e. serum) were in the ranges 72–114%, which is illustrative of the wide scope of application of the sample treatment platform here proposed.

3.4. Quantification of bisphenols in human exposure sources and biological fluids

The method developed was applied to the determination of bisphenols and derivatives in human exposure sources and biological fluids. The content of protein (0–23%), carbohydrate (0.5–14%), fat (0.5–20%), and sugars (0–10%) in the selected foodstuffs, expressed as g per 100 g of food, encompassed a wide range. Table 7 shows the results obtained, expressed as the mean value of three determinations (μg L^{-1} or μg kg^{-1}), along with the corresponding standard deviations. Fig. 3 depicts, as an example, some chromatograms obtained for quantification of bisphenols (Fig. 3A) and bisphenol diglycidyl ethers (Fig. 3B) in liquid and solid samples, respectively.

Only four bisphenols out of twenty-one were not found in the analyzed samples (BPAF, MCBPA, DCBPA and TeCBPA). As was expected, BPA showed the highest occurrence and some BADGE derivatives the highest concentration, although the frequent occurrence of other bisphenols (e.g. BPP, FPE, BPF, BPP, etc.) in the samples analyzed provided pieces of evidence on the current replacement of BPA by alternative bisphenols. Found concentrations for bisphenols and derivatives were in agreement with those previously reported [10]. Thus, in canned beverages, Geen et al. reported BPA concentrations ranging from 0.02 to 8.1 μg L^{-1} [25]; Cunha et al. reported concentrations of BPA and BPS in the range 0.03–4.7 μg L^{-1} [26]; Gallo et al. quantified concentrations of 0.5–3.3 μg L^{-1} for BPA, BPS, BADGE and BFDGE [14]; and Gallart-Ayala et al. found BADGE and its derivatives at concentrations of 2.3–5.1 μg L^{-1} [27]. Likewise, the concentrations found in this study for bisphenols and derivatives were in agreement with those found in different canned foodstuffs marketed in Europe [28]. On the other hand, numerous biomonitoring studies have reported geometrical mean BPA concentrations of 0.85–4 μg L^{-1} in urine and 0.3–4.4 μg L^{-1} in serum [29,30].

4. Conclusions

The effect of bisphenols and derivatives on human health, especially on vulnerable populations such as infants, young children, pregnant and breastfeeding women, continues as a matter of debate [1]. The mixture effect of these compounds, an unexplored area so far, as well as their low dose-effects, demands for dedicated efforts to know how often and to what extent humans are exposed. There is a need to better understand human exposures by both monitoring and modeling.

Monitoring of chemical mixtures originating from multiple sources is a challenging task and there is a lack of matrix-independent methods able to deliver accurate results through simple and fast procedures. Analytical methods previously reported for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and

Table 7
Concentrations of bisphenols and derivatives, along with the respective standard deviations, found in canned food, beverages, dust and urine and serum samples.

	BPA	BPB	BPE	BPF	BPP	BPS	BPZ	BPAP	TCBPA	BADGE	BADGE ·H_2O	BADGE ·HCl	BADGE ·2HCl	BADGE ·HCl·H_2O	BFDGE	BFDGE ·$2H_2O$
Sweet Corn[a]	2.6 ± 0.4	nd	nd	nd	nd	Nd	nd	0.63 ± 0.05	nd	0.32 ± 0.01	nd	nd	nd	nd	nd	nd
Lentils[a]	2.88 ± 0.08	<MQL	nd	nd	0.198 ± 0.001	Nd	nd	nd	nd	0.23 ± 0.04	1.4 ± 0.2	84 ± 7	nd	2.8 ± 0.4	61 ± 6	0.81 ± 0.04	0.9 ± 0.2
Chickpeas[a]	5.1 ± 0.6	nd	nd	nd	nd	Nd	nd	nd	nd	0.216 ± 0.004	0.75 ± 0.03	36.4 ± 0.5		2.08 ± 0.04	9.4 ± 0.4	nd	nd
Sausages[a]	nd	nd	nd	0.42 ± 0.02	nd	nd	nd	nd	nd	nd	nd	1.12 ± 0.04	nd	nd	nd	nd	nd
Meatballs[a]	3.11 ± 0.01	nd	0.071 ± 0.003	0.60 ± 0.01	nd	nd	nd	nd	nd	nd	nd	45 ± 7	nd	0.77 ± 0.01	24 ± 3	nd	nd
Tonic[b]	1.2 ± 0.1	0.43 ± 0.09	0.81 ± 0.04	1.02 ± 0.03	0.46 ± 0.05	0.88 ± 0.05	1.0 ± 0.1	1.08 ± 0.05	nd	nd	nd	nd	nd	nd	nd	nd	nd
Bottled water[b]	8 ± 1	0.07 ± 0.01	0.052 ± 0.002	nd	0.82 ± 0.04	nd	nd	0.36 ± 0.05	nd	1.12 ± 0.02	nd	nd	nd	nd	nd	nd	nd
Tea[b]	7.2 ± 0.1	0.061 ± 0.003	nd	nd	0.56 ± 0.01	nd	nd	nd	nd	0.87 ± 0.02	nd	nd	3.1 ± 0.2	nd	nd	nd	nd
Cola[b]	6.4 ± 0.1	nd	nd	nd	0.285 ± 0.005	nd	nd	nd	nd	1.051 ± 0.009	nd	nd	nd	nd	nd	nd	nd
Beer[b]	9.1 ± 0.8	nd	nd	nd	0.95 ± 0.04	nd	nd	1.10 ± 0.02	nd	0.09 ± 0.02	nd	nd	0.24 ± 0.02	nd	nd	nd	nd
Dust[a]	5.3 ± 0.5	nd	nd	1.52 ± 0.08	0.18 ± 0.04	0.183 ± 0.002	nd	nd	0.28 ± 0.05	nd	nd	nd	nd	nd	nd	nd	nd
Urine[b]	1.0 ± 0.1	0.32 ± 0.02	1.12 ± 0.04	1.21 ± 0.05	nd	nd	0.82 ± 0.07	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Serum[b]	0.75 ± 0.05	nd	0.60 ± 0.04	1.2 ± 0.1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Concentration of bisphenols and derivatives expressed as [a] μg kg^{-1} and [b] μg L^{-1}. MQL: method quantitation limit; nd: non detected; n = 3.

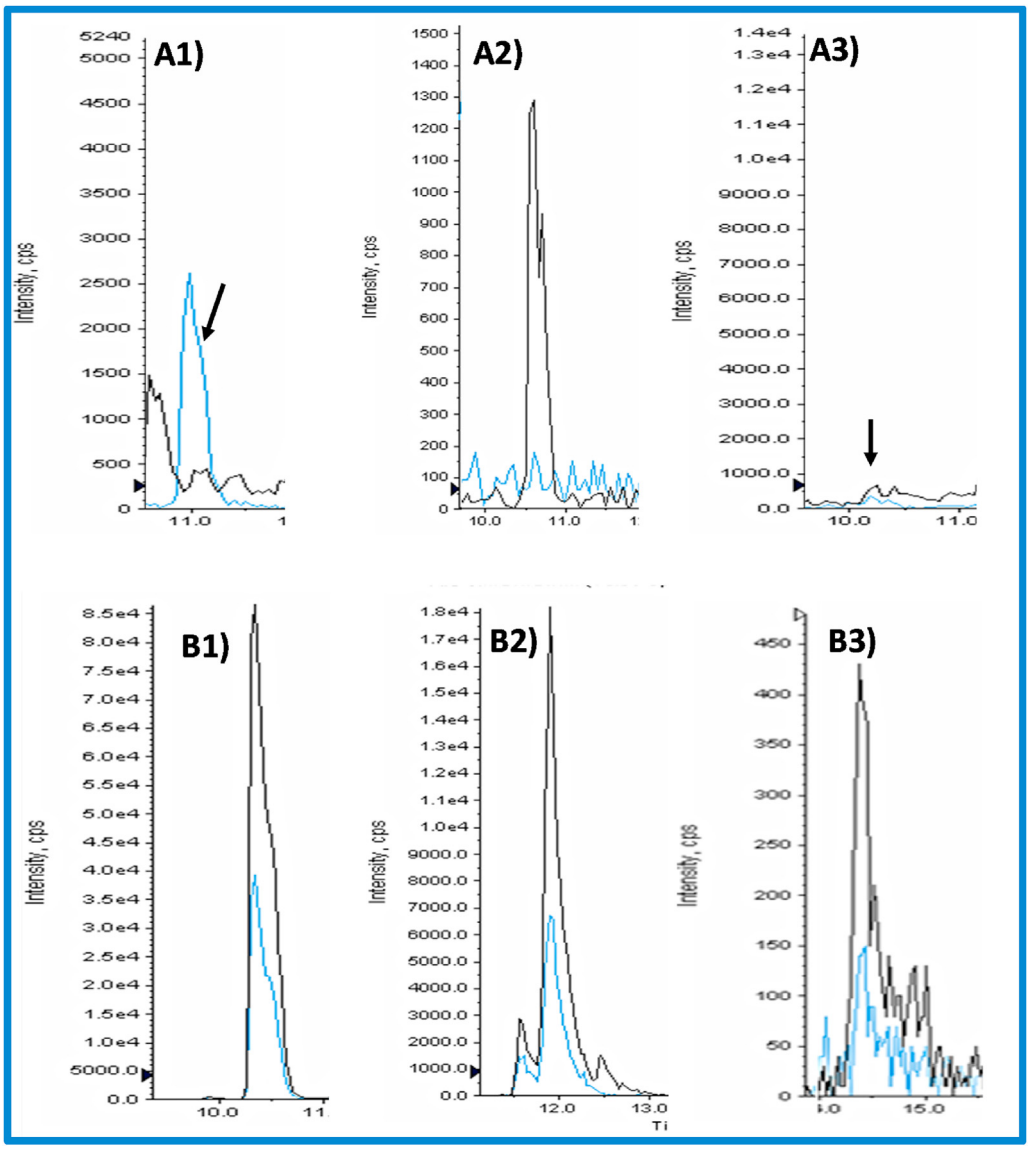


Fig. 3. Chromatograms obtained for unfortified samples of (A) bisphenols and chlorinated bisphenols in serum (A1: BPA; A2: BPE; A3: BPF) and (B) diglycidyl ethers in meatballs (B1: BADGE·2H2O; B2: BADGE·H2O·HCL; B3: BADGE·2HCL). Concentrations of analytes in Table 7. Line black: quantitative transition; line blue: qualitative transition, except for A1 that corresponds to the signal of the internal standard $12C^{13}$BPA.

biological fluids have been reviewed [10]. Most of these methods refer to the determination of a few bisphenols or bisphenols derivatives in specific samples (e.g. canned food, canned drinks, dust, urine, serum, etc.). Thus, Gallo et al. proposed a LC-FD method for the determination of three bisphenols and two diglycidyl ethers in canned drinks by a molecularly imprinted polymer developed for BPA [14]. Although the extraction efficiency was good for all the target compounds (94-78 %R) except for BPF (52 %R), their application to other bisphenols and derivatives and other matrices of interest for evaluation of human exposure has not been proved. Our research group has reported a method for determination of 12 bisphenols and derivatives using SUPRAS-LC-FD [31]. However, fluorescence detection does not provide unequivocal identification of bisphenols and problems might arise the co-elution BPA and the diol-epoxide formed by hydrolytic opening of BADGE under reversed-phase LC. Salafranca et al. developed a method based on direct immersion solid-phase microextraction (SPME) coupled with gas chromatography mass spectrometry (GC-MS) to the determination of BPA and BADGE in food simulants [32]. However, although it provides unequivocal bisphenol identification, the need for analyte derivatization hinders high sample throughput. Cheng et al. reported a method for the quantitation of twenty-one bisphenols, bisphenol diglycidyl ethers and their derivatives based on the use of QuEChERS and LC-MS [15]. Nevertheless, despite the use of two purification steps, matrix effect persisted and matrix-matched calibration was necessary for accurate quantification of bisphenols. On the other hand, although authors optimized the concentration of ammonium in the mobile phase trying to reach a balance between the responses obtained for bisphenols and diglycidyl bisphenols, high MQL were obtained for BADGE derivatives [15]. To the best of our knowledge, no method has been reported based on a generalized sample treatment for both bisphenols and their derivatives in major human exposure sources and samples of interest for their biomonitoring by LC-MS.

The method here developed has valuable analytical and operational assets for the determination of major bisphenols and

derivatives in human exposure sources and biological fluids. Thus, it provides a generalized sample treatment for the intended analytes, in both liquid and solid samples based on the ability of the hexanol-based SUPRAS for efficient extraction and matrix macromolecule removal.

From a practical point of view, the sample treatment here proposed features low cost, it is fast, several samples can be simultaneously treated and it requires conventional lab equipment. So, it meets the requirements to be used in monitoring campaigns intended to know the occurrence in the different human exposure sources or epidemiological studies.

CRediT authorship contribution statement

Noelia Caballero-Casero: Conceptualization, Data curation, Funding acquisition, Formal analysis, Writing - original draft. **Soledad Rubio:** Conceptualization, Funding acquisition, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Spanish Ministry of Economy and Competitiveness [grant number CTQ2017-83823-R].

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aca.2020.11.057.

SUPRAS extract fortified at 1 μg L^{-1} of each bisphenol and 5 μg L^{-1} of the internal standards 12C^{13}-BPA and d6-BADGE; n = 3.

References

[1] A. Kortenkamp, M. Faust, Regulate to reduce chemical mixture risk, Science 361 (2018) 51–59.
[2] Usepa, Considerations for Developing Alternative Health Risk Assessment Approaches for Addressing Multiple Chemicals, Exposures and Effects- EPA/600/R-06/013A, Washington DC, 2006.
[3] I.P.C.S. Who, Assessment of Combined Exposures to Multiple Chemicals: Report of a WHO/IPCS International Workshop on Aggregate/cumulative Risk, in: World Health Organization, vol. 7, IPCS Harmonization Project document nº, 2009, p. 83.
[4] Brussels, 31.5 EC, Communication from the Commission to the Council, the combination effects of chemicals. Chemical mixtures, COM (2012) 252, https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A52012DC0252, 2012. last accessed 18/10/2019.
[5] Ec, Joint Research Centre, Science for policy brief. Something from nothing?. Ensuring the safety of chemical mixtures, European Commission, http://publications.jrc.ec.europa.eu/repository/bitstream/JRC111886/kjna29258enn.pdf, 2018. last accessed 18/10/2019.
[6] S.K. Bopp, R. Barouki, W. Brack, et al., Current EU research activities on combined exposure to multiple chemicals, Environ. Int. 120 (2018) 544–562.
[7] A. Kienzler, S.K. Bopp, S. van der Linden, E. Berggren, Regulatory assessment of chemical mixtures: requirements, current approaches and future perspectives, Regul. Toxicol. Pharmacol. 80 (2016) 321–334.
[8] T. Backhaus, M. Faust, A. Kortenkamp, Cumulative risk assessment: a European perspective on the state of the art and the necessary next steps forward, Integrated Environ. Assess. Manag. 9 (2013) 547–548.
[9] T. Svingen, A.M. Vinggaard, The risk of chemical cocktail effects and how to deal with the issue, J. Epidemiol. Community Health 70 (4) (2016) 322–323.
[10] N. Caballero Casero, L. Lunar, S. Rubio, Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids, A review. Anal. Chim. Acta 908 (2016) 22–53.
[11] J. Míguez, C. Herrero, I. Quintás, et al., A LC-MS/MS method for the determination of BADGE-related and BFDGE-related compounds in canned fish food samples based on the formation of [M + NH4]+ adducts, Food Chem. 135 (2012) 1310–1315.
[12] F. Vela-Soria, O. Ballesteros, A. Zafra-Gomez, et al., UHPLC-MS/MS method for the determination of bisphenol A and its chlorinated derivatives, bisphenol S, parabens, and benzophenones in human urine samples, Anal. Bioanal. Chem. 406 (2014) 3773–3785.
[13] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Analysis of bisphenols in soft-drinks by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry, Anal. Chim. Acta 683 (2011) 227–233.
[14] P. Gallo, I. Di Marco Pisciottano, F. Esposito, E. Fasano, G. Scognamiglio, G.D. Mita, T. Cirillo, Determination of BPA, BPB, BPF, BADGE and BFDGE in canned energy drinks by molecularly imprinted polymer cleaning up and UPLC with fluorescence detection, Food Chem. 220 (2017) 406–412.
[15] Y. Cheng, X. Nie, H. Wu, Y. Hong, B. Yang, T. Liu, D. Zhao, J. Wang, G. Yao, F. Zhang, A high-throughput screening method of bisphenols, bisphenols diglycidyl ethers and their derivatives in dairy products by ultra-high performance liquid chromatography-tandem mass spectrometry, Anal. Chim. Acta 950 (2017) 98–107.
[16] H. Gallart-Ayala, O. Núñez, P. Lucci, Recent advances in LC-MS analysis of food-packaging contaminants, Trends Anal. Chem. 42 (2013) 99–124.
[17] A. Ballesteros-Gómez, S. Rubio, Environment-responsive alkanol-based supramolecular solvents: characterization and potential as restricted access property and mixed-mode extractants, Anal. Chem. 84 (2012) 342–349.
[18] N. Caballero Casero, S. García-Fonseca, S. Rubio, Restricted access supramolecular solvents for the simultaneous extraction and cleanup of ochratoxin A in spices subjected to EU regulation, Food Contr. 88 (2018) 33–39.
[19] S. García-Fonseca, A. Ballesteros-Gómez, S. Rubio, Restricted access supramolecular solvents for sample treatment in enzyme-linked immuno-sorbent assay of mycotoxins in food, Anal. Chim. Acta 935 (2016) 129–135.
[20] J.A. Salatti Dorado, N. Caballero Casero, M.D. Sicilia, M.L. Loreto, S. Rubio, The use of a restricted access volatile supramolecular solvent for the LC/MS-MS assay of bisphenol A in urine with a significant reduction of phospholipid-based matrix effects, Anal. Chim. Acta 950 (2017) 71–79.
[21] A. Ballesteros-Gómez, M.D. Sicilia, S. Rubio, Supramolecular solvents in the extraction of organic compounds, A review, Anal. Chim. Acta 677 (2010) 108–130.
[22] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Liquid chromatography/multi-stage mass spectrometry of bisphenol A and its halogenated derivatives, Rapid Commun. Mass Spectrom. 21 (2007) 4039–4048.
[23] J. Yonekubo, K. Hayakawa, J. Sajiki, Concentrations of Bisphenol A, bisphenol A diglycidyl ether, and their derivatives in canned foods in Japanese markets, J. Agric. Food Chem. 56 (2008) 2041–2047.
[24] H. Gallart-Ayala, E. Moyano, M.T. Galceran, Multiple-stage mass spectrometry analysis of bisphenol A diglycidyl ether, bisphenol F diglycidyl ether and their derivatives, Rapid Commun, Mass Spectrom. 24 (2010) 3469–3477.
[25] T. Geens, T.Z. Apelbaum, L. Goeyens, H. Neels, A. Covaci, Intake of bisphenol A from canned beverages and foods on the Belgian market, Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 27 (2010) 1627–1637.
[26] S.C. Cunha, C. Almeida, E. Mendes, J.O. Fernandes, Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry, Food Addit. Contam. Part A Chem. Anal. Control Expo Risk Assess 28 (2011) 513–526.
[27] H. Gallart Ayala, E. Moyano, M.T. Galceran, Fast liquid chromatography-tandem mass spectrometry for the analysis of bisphenol A-diglycidyl ether, bisphenol F-diglycidyl ether and their derivatives in canned food and beverages, J. Chromatogr. A 1218 (2011) 1603–1610.
[28] G. Russo, F. Barbato, D.G. Mita, L. Grumetto, Occurrence of Bisphenol A and its analogues in some foodstuff marketed in Europe. Review, Food Chem. Toxicol. 131 (2019), 110575.
[29] R. Huang, Z. Liu, H. Yin, Z. Dang, P. Wu, N. Zhu, Z. Lin, Bisphenol A concentrations in human urine, human intakes across six continents, and annual trends of average intakes in adult and child populations worldwide: a thorough literature review, Sci. Total Environ. 626 (2018) 971–981.
[30] L.N. Vandenberg, R. Hauser, M. Marcus, N. Olea, W.V. Welshons, Human exposure to bisphenol A (BPA), Review. Reprod. Toxicol. 24 (2) (2007) 139–177.
[31] A. Alabi, N. Caballero Casero, S. Rubio, Quick and simple sample treatment for multiresiude analysis of bisphenols, bisphenols diglycidyl ethers and their derivaties in canned food prior to liquid chromatography and fluoresecence detection, J. Chromatogr., A 1336 (2014) 23–33.
[32] J. Salafranca, R. Batle, C. Nerín, Use of solid-phase microextraction for the analysis of bisphenol A and bisphenols A diglycidyl ether in food simulants, J. Chromatogr. A 864 (1999) 137–144.