Highlights

- Tunable solvents (solvency properties) are obtained from THF:water mixs
- THF:water:NaCl (salting-out) mixs are proposed for liquids and THF:water for solids
- Solvency, extraction efficiency and exclusion of interferents are discussed.
- Methods were applied to the analysis of BPA in urine and OTA in baby-foods

Tunable solvency mixtures of tetrahydrofuran:water for efficient and fast extraction/clean-up of trace contaminants

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Abstract

In this study, we investigated the potential of mixtures of tetrahydrofuran (THF) and water as tunable solvents for the microextraction of contaminants in solid and in liquid matrices. These two miscible solvents have very different dielectric constant and Hildebrand solubility parameters, so that tunable mixtures spanning a wide range of dispersion and hydrogen bonding forces could be easily prepared by simply changing their composition. In this way, rapid and more efficient extraction methods can be developed. A liquid-liquid and a solid-liquid microextraction method for the determination of bisphenol A (BPA) in urine and ochratoxin A (OTA) in cereal baby food were developed as a proof of concept. Both, the chemical composition and the relative solvency of the THF-water mixtures, expressed as Teas solubility parameters, were studied in order to gain some insights into the chemical interactions governing analyte extraction. For urine, the salting-out extraction with THF:water and NaCl was evaluated, a process which is still scarcely investigated for analytical purposes. These methods featured good recoveries (above 95%), satisfactory standard deviation (5-6 %) and good sensitivity (detection limits of 0.l µg L^{-1} for BPA and of 0.l ng g^{-1} for OTA) with the advantages of simplicity, rapidity and low consumption of reagents. Recoveries for other compounds and matrices (bisphenols ad phosphorus flame retardants in dust and in tap water, dyes in tap water and OTA in powder milk) were also assessed to prove the wide potential of these tunable solvent mixtures.

KEYWORDS: tunable solvents; organic aqueous mixtures; microextraction; ochratoxin A; bisphenol A.

1. Introduction

Current trends in the analysis of complex food and biological samples move towards the simplification of sample preparation, and in this respect, major efforts have focused on the development of efficient, green, cost-effective and high-throughput extraction methods [1]. Miniaturization of solvent extraction, a strategy highly encouraged for isolation of trace contaminants from food and biological fluids [2], requires the use of solvents with high extraction efficiency, and this need has boosted the search for solvents with tunable properties able to establish mixed mode mechanisms for contaminant solubilization [3]. Among solvents with tunable properties, supercritical fluids [4], ionic liquids [5], supramolecular solvents [6] or eutectic solvents [7], have been intensively investigated to increase extraction efficiency for structurally unrelated compounds.

Tunability of the solvency properties of conventional solvents, still the most widely used extractants, has been traditionally tried by the mixing of solvents [8]. As contaminants cover a wide polarity range, their extraction from both solid and liquid samples is usually carried out by mixtures of water with miscible polar solvents (e.g. methanol, acetonitrile, acetone, etc.), the last application (viz. liquid-liquid extraction, LLE) requiring the production of a biphasic system under the action of salting out agents (both organic and inorganic salts) [9]. However, tunability of the solvency properties of these mixtures is quite limited owing to the high polarity of the solvents involved. So, solvents having the highest possible difference in solvency power should be more appropriate for producing tunable solvency extractants.

In this work, we evaluate the use of mixtures of THF and water as tunable solvency extractants of contaminants, in both liquid and solid samples, based on the substantial difference in dielectric constants (ε_{water} = 80.1, ε_{THF} = 7.5) and Hildebrand solubility parameters (δ_{water} = 23.3 $cal^{1/2}$ $cm^{-3/2}$; δ_{THF} = 9.5 $cal^{1/2}$ $cm^{-3/2}$) [10]. Thus, the THF values for ε and δ are quite similar to those of very nonpolar water-immiscible solvents such as ethyl acetate (ε_{EAc} = 6.0, δ_{EAc} = 9.1 $cal^{1/2}$ $cm^{-3/2}$) or chloroform (ε_{CHCL13} = 4.8 δ_{CHCL13} = 9.3 $cal^{1/2}$ $cm^{-3/2}$), and considerable lower than those for methanol (ε_{MeOH} = 32.7, δ_{MeOH} = 14.5 $cal^{1/2}$ $cm^{-3/2}$) or acetonitrile (ε_{ACN} = 37.5, δ_{ACN} = 11.9 $cal^{1/2}$ $cm^{-3/2}$) [10]. The polarity of solvent mixtures could be modulated in a wider range for THF:water mixtures compared to that for ACN:water or MeOH:water, which should favor the

extraction of analytes in a broader polarity interval. Particularly interesting is the fact that two of the three types of intermolecular interactions that contribute to the Hildebrand solubility parameter, namely dispersion (f_d), polar (f_p) and hydrogen bonding (f_h) forces (Teas or fractional solubility parameters), are very different for water and THF. Thus, Teas parameters (%) for water and THF are f_d =21, f_p =22, f_h =57 and f_d =55, f_p =19, f_h =26, respectively [10]. This means that both dispersive interactions and hydrogen bonding can be tuned by simply changing the THF:water mixture composition, while polar (dipole-dipole) interactions will remain almost constant (see Fig. 1).

The tunability of the solvency properties of the mixture THF-water was here explored by the development of a liquid-liquid and a solid-liquid microextraction method for the analysis of bisphenol A (BPA) in urine and ochratoxin A (OTA) in cereal baby food as a proof of concept. For the application to urine, a biphasic system was obtained from THF:water mixtures under the addition of sodium chloride. This separation process has been recently described in the literature [27- 30].

BPA (Log K_{ow} 3.32; hydrogen bonds donor/acceptor groups 4) is a known endocrine disruptor with a widespread occurrence in humans [11]. OTA (Log K_{ow} 4.74 hydrogen bonds donor/acceptor groups 9) is a carcinogenic contaminant with a restrictive maximal residue limit of 0.5 ng g^{-1} in baby food [12]. Methods for the extraction of OTA in cereal-based food use mostly conventional solvent extraction (acetonitrile:water mixtures [13-15]), although some alternatives such as pressurized liquid-strategies [16,17] and matrix solid phase dispersion [18,19] have been proposed. The volume of organic solvent consumed per sample is usually relatively high (50-250 mL) and further clean-up is needed, usually with immunoaffinity columns [14,20]. Regarding the analysis of BPA, conventional SPE [21,22] and on-line SPE coupled to LC-MS/MS have been the most used approaches for its analysis in urine [23]. Some methods using selective sorbents, i.e immunoaffinity columns [24] and molecularly imprinted polymers (MIPs) [25,26] have been proposed. The background contamination coming from the use of water and solvents (containing always trace amounts of this ubiquitous contaminant) in the SPE conditioning steps and the high cost of MIPs are common inconveniences in these strategies.

Both, the chemical composition and the relative solvency of the THF:water and THF:water (NaCl) mixtures were studied in order to gain some insights into the chemical interactions governing analyte extraction. The solvency tunability of the mixtures was exploited to enhance recoveries and to prevent as much as possible the co-of matrix interferents (e.g. proteins, polysaccharides, etc.). In this way we obtained certain degree of selectivity that was enough to avoid further clean-up steps. Methods were simple, rapid and efficient with low consumption of reagents. The extraction efficiency of other compounds (bisphenols, flame retardants and dyes) and samples (tap water, dust, powder milk) was further evaluated to prove the potential of the THF:water mixtures for the extraction of a wide polarity range of compounds.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical reagent-grade and were used as supplied. THF, HPLC-grade acetonitrile, methanol and acetic acid were supplied by Panreac (Sevilla, Spain). Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). Sodium chloride, ammonium acetate, ochratoxin A (OTA) and bisphenol A (BPA) and β-glucuronidase/sulfatase H1 enzyme from Helix pomatia were obtained from Sigma (St. Louis, MO, USA). The internal standard of BPA ($^{13}C_{12}$-BPA) was obtained from Cambridge Isotope Laboratories, Inc. and was used as method internal standard to control potential losses of BPA during sample preparation and fluctuations in the MS performance (e.g. ion suppression and enhancement). Stock standard solutions of BPA and OTA at concentrations of 10 mg L^{-1} were prepared in methanol and stored in closed glass tubes at 4ºC. The deconjugation solution was made from β-glucuronidase/sulfatase H1 enzyme from Helix pomatia (2563300 U g^{-1}) at a concentration of 926 U mL^{-1} in a buffer of ammonium acetate 1M (adjusted at pH 5).

Further recovery experiments were done with a) bisphenols: 4,4'Methylenediphenol (bisphenol F, BPF) and 4,4'-Sulfonyldiphenol (bisphenol S, BPS) that were obtained from Sigma-Aldrich (St. Louis, MO, USA), 4-(4-phenylmethoxyphenyl)sulfonylphenol (BPS-MAE), 4-(4-propan-2-yloxyphenyl)sulfonylphenol (D-8) and 4-(4-hydroxy-3-prop-2-enylphenyl)sulfonyl-2-prop-2-enylphenol (TGSA) and the internal standards

(IS) Bisphenol A-d_6 diglycidyl Ether (BPA-d_6) and bis(4-hydroxyphenyl) Sulfone-d8 (BPS-d_8) which were acquired from Toronto Research Chemicals (Toronto, Canada); b) phosphorus flame retardants: TPHP (triphenyl phosphate) and TPHP-d_{15} which were obtained from Sigma Aldrich (Zwijndrecht, the Netherlands) and bisphenol A bis(diphenyl phosphate) (BDP) which was obtained from AccuStandard (New Haven, CT) and c) dyes: Trypan blue (CAS 72-57-1) and Malachite Green (CAS 2437-29-8) that were obtained from Fluka (Steinheim, Germany).

2.2. Apparatus

For the analysis of BPA, the LC-MS system used was an AB Sciex 4000 Qtrap® mass spectrometer (Foster city, California, USA), with a negative-ion TurboSpray interface coupled to an Agilent 1200 Series LC system (Palo Alto, CA, USA). The stationary phase was a SymmetryShieldTM RP 18 column (particle size 3.5 µm, i.d. 2.1mm, length 50 mm) from Waters (Milford, MA, USA). An Ascentis C18 guard column (particle size 3 µm, i.d. 4 mm, length 20 mm) was inserted before the analytical column. Styrene divinylbenzene (DVB) (SDB-XC, disks 47 mm) were obtained from Empore (3M, St Paul, Minnesota, USA). For the analysis of OTA, a liquid chromatographic system (Breeze HPLC, Waters, Milford MA) consisting of a 1525 binary pump, a 717 plus automatic injector, a 1500 series column heater and a 2475 multiwavelength fluorescence detector was used. The stationary phase was a Kromasil C_8 (particle size 5 µm, i.d. 4 mm, length 25 cm) from Análisis Vínicos (Tomelloso, Spain). A Karl Fischer coulometric tritator from Metrohm (Herisau, Suize) was employed to measure the water content in the organic solvent-rich phase after the salting-out process. For sample treatment, an ultracentrifuge MPW-350R (Warsaw, Poland), was used for the precipitation of solids in the extracts.

2.3. Phase behavior and chemical composition of THF-water-NaCl mixtures

Mixtures (10 mL) of THF and water (0.05-6 M NaCL) were prepared in centrifuge tubes, mixed (30 sec, vortex) and centrifuged (2,000 rpm, 5 min). THF:water ratios varied between 5:95 and 95:5% v/v. Phase behavior was visually determined and, in certain ratios, the volume and composition of the resulting phases were determined. The L-L-S and L-S regions were determined by the NaCl saturation limit. Phase volumes were calculated by measuring their cylindrical volume in the centrifuge tubes (the phase

volume height and the internal diameter of the tube were measured with a digital caliper). Water content (%, w/w) was determined by Karl Fischer coulometric tritation after proper dilution with methanol. NaCl (%, w/w) was determined gravimetrically after removal of THF and water by evaporation. THF (%, w/w) was determined by difference in the ternary mixture THF:water:NaCl.

2.4. Determination of OTA in baby food

Cereal-based baby food samples (300 mg) were weighed in a 2 mL Eppendorf microtube. Water (240 µL) and tetrahydrofuran (960 µL) were added to the sample and extraction was carried out by vortex shaking for 10 min. Samples were then ultracentrifuged (15,000 rpm, 15ºC, 10 min) for precipitation of solids and aliquots of 20 µL injected in the LC-FL system. The mobile phase consisted in water (A) and acetonitrile (B) containing both 1% v/v of acetic acid at a flow of 1 mL min^{-1}. The LC program was as follows: isocratic conditions at 30% B for 5 min, linear gradient from 30% to 40% B for 3 min, linear gradient from 40% to 48% B in 15 min, isocratic at 48% for 4.5 min and finally a linear gradient to 100% B in 0.5 min and isocratic at 100% B for 2 min for elution of hydrophobic matrix components. After that, the ratio was returned to initial conditions for 5 min to re-equilibrate the column for the next injection. OTA was monitored at λ_{ex} 334 nm and λ_{em} 460 nm. Quantification was made by external calibration with standards prepared in THF:water 80:20 v/v.

2.5. Determination of BPA in urine

The urine samples (n=8) belonged to pregnant women and children and were previously analyzed by a conventional solid-phase extraction (SPE) method and results published elsewhere [31]. Conjugated BPA was analyzed after an enzymatic treatment by adding 625 µL of the deconjugation solution to 1.25 mL of urine sample containing 5 µg L^{-1} of internal standard and stored in closed 2 mL glass vials at 37 °C overnight. Samples were then homogenized (vortex) and transferred to Eppendorfs microtubes for ultracentrifugation (15,000 rpm, 10 min, 15 ºC) and precipitation of solids. Two aliquots of around 1 mL were transferred to 2 mL Eppendorfs tubes. To each aliquot, an amount of 145 mg of NaCl was added and dissolved followed by addition of 650 µL of THF. The samples were vortex-mixed during 5 min and then ultracentrifuged (15,000 rpm, 10

212 min, 15 ºC) for phase separation. The upper organic phase of both tubes was evaporated
213 to dryness (N_2, 40ºC) and reconstituted with 0.5 mL of methanol:water 50:50 v/v.
214 Aliquots of 30 µL were then injected in the LC-MS system. The mobile phase consisted
215 of water and methanol at a flow rate of 0.4 mL min^{-1}. The gradient elution was
216 programmed as follows: linear gradient from 90 to 70% of water for 10 min, linear
217 gradient from 70 to 47% of water for 20 min, then isocratic conditions at 47% of water
218 for 5 min and then reverting to initial conditions allowing 10 min for stabilization. The
219 column and pre-column were operated at 35 ºC. Quantitative analyses were performed
220 on the Scheduled MRM mode recording the quantitation and confirmation transitions
221 for BPA (227→ 132.9; 227→ 211.9) and internal standard (239→ 144.9; 239→ 223.9).
222 The Turbo spray settings were as follows: curtain gas (N_2) 27 psi; ion spray voltage -
223 4500 V; temperature 600ºC; ion source gas (1) 70 psi and ion source gas (2) 50 psi. Unit
224 resolution was used for both Q1 and Q3 quadrupoles. Quantification was made by
225 internal standard solvent calibration and calibration standards prepared in
226 methanol:water 50:50 v/v. BPA concentrations were calculated from the calibration
227 curve obtained by plotting the ratio of analyte peak area to method IS peak area against
228 the analyte concentration. Linear regression with a weighing 1/x was selected for
229 quantitation.

230 With the aim of preventing BPA background contamination, LiChrosolv water was
231 filtered through a Styrene DVB disks and glassware and Eppendorf microtubes were
232 rinsed with methanol several times before their use. As a precautionary measure, an
233 additional column (Water Symmetry® 3.5 µm, 4.6 mm × 75 mm) was inserted between
234 the pump and injector in order to trap BPA that could be released from the LC
235 equipment. Contamination blanks were routinely run with each batch of samples and
236 were always below the quantification limit.

237 ***2.6. Extraction recoveries of other organic contaminants with mixtures THF:water***

238 THF:water mixtures (20, 50 and 80% THF v/v, total volume 1.2 mL) were tested for
239 the extraction of bisphenols (BPs) and phosphorus flame retardants (PFRs) in dust (50
240 mg, blank sample from a previous study [32]) and OTA in powder milk sample (200
241 mg, blank sample, bought in a local supermarket in Córdoba, Spain). In the case of
242 milk, water was acidified (0.1M HCl) to favour the precipitation of proteins. BPs and

PFRs were fortified at 500 ng g^{-1} and OTA at 0.5 ng g^{-1}. Samples were vortex shaked for 10 min and then ultracentrifuged (15,000 rpm, 15ºC, 10 min). Milk extracts were analysed by LC-FL as described in section 2.4. Dust extracts were diluted 1:1 with methanol and spiked with internal standards (BPS-d_8, BPA-d_6, TPHP-d_{15}, level 200 ng g^{-1}) and aliquots of 5 µL injected in an LC-MS/MS instrument to estimate the recoveries. Details about the analysis and quantitation of BPS and PFRs can be found in references 32 and 33.

Mixtures of THF:tap water (2 M NaCl in water; 30 and 40 % v/v THF, total volume 10 mL) were tested for the extraction of dyes, BPs and PFRs. Tap water was obtained from Córdoba (Spain). For dyes, the sample was fortified either at 50 mg L^{-1} with either Trypan Blue or Cresyl Violet Acetate. The Mixtures were stirred for 10 min (800 rpm) and then centrifuged for 15 min (2,000 rpm) to accelerate phase separation. The absorbance of the aqueous solution (after further dilution 1:10 with water) was measured in a spectrophotometer (λ= 300-790) for estimating the residual concentration of dyes in the extracted water. Bisphenols and phosphorus flame retardants were fortified at 5 µg L^{-1}. The samples were vortex-mixed during 5 min and then ultracentrifuged (15,000 rpm, 10 min, 15 ºC) for phase separation. The upper organic phase was evaporated to dryness (N^2, 30ºC) and reconstituted with 0.5 mL of methanol (containing 5 µg L^{-1} internal standard). Aliquots of 5 µL were injected in an LC-MS/MS instrument to estimate the recoveries (analysis details in references 32 and 33).

3. Results and discussion

3.1. Mixtures THF:water for solid-liquid extraction of OTA in baby foods

For the optimization of the extraction of OTA in baby food, cereal samples (0.5 g) were extracted by vortex-mixing for 15 min under different THF:water ratios (total volume of 4 mL). Table 1 shows that the recoveries obtained for OTA were constant for mixtures containing 10-50% v/v water. These values corresponded to Hildebrand values in the range ~11-16 $cal^{1/2}$ $cm^{-3/2}$ and a ratio f_d/f_h in the range of ~1-2. So, both dispersion forces and hydrogen bonds binding interactions play major roles in the extraction of

OTA. This behavior is logical given the fact that OTA have both hydrophobic moieties and hydrogen bonds donor/acceptor groups.

The mixture composition was also investigated for exclusion of interferences at the lowest THF percentages in order to have the highest possible concentration factors. Clearer extracts and chromatograms with lower background noise were obtained for THF:water ratios of 80:20 compared to those obtained at ratios of 90:10. So, an optimal value of 80:20 v/v THF:water (Hildebrand 12 cal1/2 cm-3/2; f_h = 32, f_p = 20 and f_d = 48; f_d / f_h =1.5) was finally selected as optimal as a good balance of dispersion, dipole-dipole and hydrogen bond interactions for the extraction of OTA and to avoid the presence of high concentration of lipids, proteins and polysaccharides in the extracts.

The ratio of solvent (80:20 THF:water, µL) to sample (mg) was studied between values of 8:1 and 3:1 and the extraction efficiency kept constant along this range. A ratio 1:4 (300 mg sample, 960 µL THF, 240 µL water) was selected for a good dispersion of the sample in the extraction solvent during the vortex stirring and to obtain enough volume of extract (~ 0.4 mL). In this way we could operate at a low solvent consumption per sample.

Finally, the extraction time (vortex) was studied between 5 and 30 min, being 10 min enough for obtaining maximal recoveries.

3.2. Mixtures THF:water for liquid-liquid extraction of BPA in urine

The production of biphasic systems in aqueous-solvent mixtures under the action of salting-out agents has been widely exploited in bioanalysis [9]. Applications have been mainly based on acetonitrile [34-36] because of alcohols and acetone give organic phases with higher water and salt content due to their strong interaction with water through hydrogen bonding [37]. Thus, they usually produce dirty extracts owing to the co-extraction of hydrophilic matrix components. To the best of our knowledge, the salting-out of THF has not been explored in bioanalysis and it is still scarcely investigated for analytical purposes, e.g. recent applications have been reported for the determination of diuron in water [27] and sulfonamides in honey [28].

3.2.1. Biphasic systems in THF:water:NaCl mixtures: phase behavior, chemical composition and solubility parameters

Figure 2 shows the phase diagram obtained for aqueous THF solutions in the presence of increasing concentrations of NaCl, expressed as molar concentration in water. Four regions were obtained as a function of THF and salt concentration. Below the NaCl saturation limit (5.75 M, point A in the phase diagram), there were a monophasic region, corresponding to homogeneous solutions of THF and water, and a biphasic region, where binary mixtures of these solvents underwent separation and formed two coexisting liquid phases; a THF-rich organic phase at the top and a THF-poor aqueous phase at the bottom. At NaCl concentrations above the saturation limit (point A), further addition of salt resulted in a non-solubilized precipitate, which gave triphasic (L-L-S) and biphasic (L-S) regions, respectively. Table S1 shows a power equation to predict the minimal content of THF in the initial solution causing phase separation at different NaCl molar concentrations based on the experimental data.

In order to gain some insights about the phase behavior of THF-water-NaCl mixtures, the chemical composition of the two coexisting liquid phases was analyzed in ten different points within the biphasic L-L and triphasic L-L-S regions of the phase diagram (points M1-M10 in Figure 2). Table S2 shows the chemical composition of the solution before phase separation (M1-M10) as well as those found for the top organic phase (M1T-M10T) and the bottom aqueous phase (M1B-M10B) after phase separation. In all cases, the composition of the THF-rich top phase was quite similar (M1T-M10T). Regarding the composition of the aqueous bottom phase, the initial amount of salting-out agent determined the content of THF not undergoing phase separation. Thus, the higher the salt content, the lower the percentage of residual THF in the aqueous phase (M1B-M10B). Equations (second order polynomial regression) for the estimation of the THF and water content in top and bottom phases were formulated based on the NaCl molar concentration of the initial solution (see Table S1).

The volume of organic phase obtained was dependent on both the initial content of THF and salt, this dependence being more critical as the THF decreased (see Table S2). Thus, the percentage of THF that underwent phase separation ranged from 7 to 60% for samples M1-M3, from 50 to 93% for samples M4-M6 and from 90 to 96% for samples

M7-M10. This means that the higher the content of THF in the initial solution, the more effective solvent separation under the action of the salting-out agent.

The Hildebrand and Teas parameters were calculated for both the bottom aqueous phase (M1B-M10B) and the top organic phase (M1T-M10T) by averaging their components [10]. The results obtained (Table S3) clearly reflect that the solvency behaviour of the top and aqueous phases was quite similar to that of THF and water, respectively (i.e. compare the respective parameter values with those corresponding to the pure solvents in the footnote of table S3). Thus, although the increase in salt concentration favoured the salting out of THF to the organic phase and consequently the Hildebrand and f_h parameters progressively decreased or increased in the organic and aqueous phase, respectively, all the solubility parameters calculated for both phases were within quite a narrow range. So, according to this behaviour, very similar partition coefficients are expected for analytes under application of different THF:water:NaCl ternary mixtures (e.g. M1-M10). On the other hand, preconcentration factors for analytes will be favoured by using the lowest salt and THF concentration since they give the lowest volume of organic phase (see as an example the data included in Table S2). In conclusion, the salting out of THF-water mixtures induced by NaCl follows a predictable behaviour. Thus, the composition of the separated phases fit equations that strongly depend on initial salt concentration (table S1), the volume of the organic phase depends on both the salt and THF content in the initial solution (table S2), and the solvency behaviour of both phases varies in a narrow range (table S3).

3.2.2. Optimization of the extraction of BPA in urine

The extraction efficiency for BPA by THF:water mixtures was first investigated in water samples. For this purpose, water spiked with 5 µg L^{-1} of BPA was mixed with NaCl (1-6 M) and THF (20-50% v/v, total solution volume 2 mL). Extractions were done in 2mL Eppendorf tubes by vortex-mixing for 5 min. Samples were then ultracentrifuged (15,000 rpm, 10 min) and the upper organic phase extracted with a glass Pasteur pipette, made up to 1mL with methanol and its BPA content measured by LC-MS/MS. The internal standard $^{13}C_{12}$-BPA was added (12.5 µg L^{-1}) at this dilution step as injection IS (for correction of possible MS signal fluctuations due to variations in MS performance) with the aim of accurately calculate the extraction recoveries.

As shown in Table 2, BPA recoveries were quantitative in the range of THF:water mixtures tested. Results were in agreement with the similar solvency behavior found for the organic and aqueous phases independently of the composition of the initial mixtures (see Table S3). Thus, the upper phase (Hildebrand parameters 10.1-11.7 and f_d/f_h 1.7-1.9) was more hydrophobic than the competing bottom one (Hildebrand parameters 20.2-22.6 and f_d/f_h ~0.5) but still offered a good balance between dispersion and hydrogen bond interactions for extraction of BPA.

Taking into account the high water content of urine (i.e. higher that 95%), phase separation was also observed by mixing urine with THF and salt. The same experiments as for water were carried out. Phase behavior of this ternary mixture was similar to that obtained in water, however lower volumes of organic phase were generally obtained and they only approached the volumes found for the THF:water:NaCl mixtures at the higher concentrations of THF (see Table 1). The reason was that THF caused the precipitation of urine proteins, which appeared as a white layer at the bottom of the organic phase after the centrifugation step. Due to this phenomenon, the volume of the top organic phase was lower in urine:THF this affecting also the recoveries (see Table 1). So, a value of 40% of THF was recommended for extraction of BPA in urine in order to ensure quantitative recoveries.

Regarding the ionic strength (1-6 M NaCl), the extraction efficiency slightly increased from 1 (83±1) to 2 M (94±1) and kept constant from here on. A value of 2.5 M NaCl was selected as optimal. The stirring time was optimized between 1 and 10 min. Maximal recovery was obtained after just 1 min, although 5 min was selected to ensure that the extraction equilibrium was reached.

3.3. Analytical performance for BPA and OTA determination

The standard solutions for calibration were prepared in THF:water 80:20 v/v for OTA and in methanol:water 50:50 v/v for BPA, the latter containing a concentration of 12.5 μg L^{-1} of $^{13}C_{12}$-BPA. The figures of merits of the methods are summarized in Table S4. The limits of detection and quantification (LODs and LOQs) of the methods were calculated experimentally from blank samples spiked at low levels (0.04-0.5 ng g^{-1} for OTA and 0.05-0.5 μg L^{-1} for BPA) that underwent the optimized extraction protocols.

393 The standard criteria of a signal to noise ratio of 3 and 10 were considered for the
394 calculation of the LODs and LOQs, respectively. LODs and LOQs were 0.1 and 0.2 ng
395 g^{-1} for OTA and 0.1 and 0.2 μg L^{-1} for BPA. These limits are low enough for the
396 quantification of OTA at the low level established for baby food by the UE (0.5 ng g^{-1})
397 and for analyzing the BPA levels commonly found in human urine (0.5-20 μg L^{-1}) [11].

398 The presence of matrix interferences, that could affect the external quantification of
399 OTA, was assessed by the comparison of the slopes of the calibration curves (n = 8)
400 obtained from standards prepared in THF:water with those obtained from extracts of
401 spiked blank from four samples of cereal-based baby food. The differences between the
402 slopes were not statistically significant by applying an appropriate Student's t-test. For
403 the quantification of BPA, the isotope labeled internal standard for BPA was added
404 before the extraction and total recoveries were in the range of 95-108%.

405 The analytical performance of our method was compared with those reported in the
406 literature (see Tables S6 and S7). Recoveries and precision were within the highest
407 reported values. SUPRAS treatment was advantageous in terms of lower cost and
408 simplicity without the need of further clean-up or multi-steps protocols which are
409 common in many of the reported methods. The organic solvent consumption per sample
410 was reduced to 0.96 mL in baby food (~11-61.6 mL in reported methods) and to 1.3 mL
411 in urine (~3-20 mL in literature).

412 ***3.4. Analysis of samples***

413 Three cereal baby food samples were analyzed before and after spiking at three different
414 concentration levels (0.5, 2 y 5 ng g^{-1}). OTA was only found in one of the samples of
415 cereal food at a concentration of 0.21±0.01 ng g^{-1}. Recoveries were independent on the
416 concentration and were between 96±7 and 109±10 %.

417 The method for the analysis of BPA was validated with the analysis of eight urine
418 samples by comparison with the results obtained by a standard SPE procedure [31]. As
419 we can see in Table 3 results were not significantly different. Figure S-1 shows the
420 chromatograms of two samples (baby food and urine) containing the target compounds
421 at low levels.

3.5. Potential of THF:water mixtures for the extraction of other contaminants

In order to further investigate the potential of THF:water mixtures as tunable solvents for a wide variety of compounds and matrices, we investigated extraction recoveries of other five bisphenols (BPS, BPF, BPS-MAE, D8 and TGSA) and two phosphorus flame retardants (TPPH and BDP) in dust and in tap water, two dyes (Trypan Blue and Malachite Green) in tap water and OTA in powder milk. The THF:water ratios tested are based on the previous optimal results for OTA and BPA in baby food and urine, respectively. Mixtures of THF:water (20-75% v/v water; extraction phase f_d/f_h= 0.6-1.5 see Table 1) were tested for the extraction of solid samples (dust and powder milk). For tap water, mixtures of THF:water (NaCl 2M) (60-70% v/v water; extraction organic phase f_d/f_h ~1.7-1.8; aqueous phase f_d/f_h ~0.5) were assessed. These compounds greatly varied in polarity (see Table S5) from highly hydrophobic compounds (BDP, log P 10.4) to medium polar compounds (BPS, log P 1.9) and an anionic (Trypan Blue, log P -1.35) and a cationic compound (Malachite Green, log P 0.62). Recovery results are shown in Tables S8 and S9. Recoveries for Trypan Blue and Malachite Green in water are not included and were below 15% in all the conditions tested.

OTA was extracted quantitatively from milk at 80% v/v THF (f_d/f_h= 1.5). Results were similar than those obtained for cereal-baby food (Table 1), although recoveries dropped faster with the decrease in THF. This is probably due to the need of a high organic solvent percentage for the efficient precipitation of matrix proteins. BPs and PFRs were extracted quantitatively from dust in the whole range of THF tested being the optimal at 80% v/v THF too (mean recoveries 91-110%). Regarding the salting-out extraction of tap water, recoveries of BPs and PFRs were maximum at 40:60 v/v THF:water (2M NaCl) with mean recoveries values in the range 70-90% (extraction organic phase f_d/f_h ~1.8-1.9).

In general, it can be observed that THF:water mixtures are suitable for medium polar to highly hydrophobic compounds (log P 1.9-10.4). For the most hydrophobic compounds of each class of contaminant recoveries were better at higher f_d/f_h ratios, e.g. for BPS-MAE in dust (log P 4.2) mean recoveries decreased from 91±4 at 80% v/v THF (f_d/f_h = 1.5) to 75±1% at 25% v/v THF (f_d/f_h = 0.6). The extraction efficiency of the most polar compounds of each class were less affected, e.g. recoveries for BPS in dust (log P 1.9)

were 95±3 at 80% v/v THF and 92±7 at 25% v/v THF, respectively. Ionic dyes were hardly extracted (recoveries below 15%) in the hydrophobic organic phase of the THF:water (2 M NaCl) mixture, so that ionic polar interferences are not expected to be co-extracted in these processes, which could be advantageous for clean-up purposes.

4. Conclusions

Tunable solvents can be easily obtained from aqueous organic mixtures featuring substantial differences in dielectric constants and Hildebrand solubility parameters. Values for fractional solubility parameters in the mixture can be modulated in terms of dispersion, polar and/or hydrogen bonding forces by proper choice of the organic solvent and the proportion of their components. In this paper, this strategy has been explored for tetrahydrofuran-water mixtures that have been proved to provide suitable extraction methods for ochratoxin A in cereal baby food and bisphenol A in urine.

Study of the chemical composition of the mixtures in the biphasic systems resulting under addition of salting-out agents, as well as the determination of Teas solubility parameters allow the understanding of the partition of contaminants between phases, thus avoiding the optimization of extraction parameters exclusively based on trial-and-error tests. Likewise, phase behavior of biphasic systems can be predicted through proper equations.

There are many other miscible aqueous organic mixtures, such as dioxane-water, where great differences in their Teas solubility parameters exist and that are also worthy of investigation.

Acknowledgments

Authors gratefully acknowledge financial support from Spanish MINECO (Project CTQ2017-83823-R). A. Ballesteros-Gómez acknowledges the funding from Spanish Ministry of Science, Innovation and Universities for a Ramón y Cajal contract (RYC-2015-18482).

Conflict of interest

Authors declare no conflict of interest

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Table 1. OTA extraction recoveries (R) in cereal baby food with different mixtures THF:water, and Hildebrand (δ, $cal^{1/2}\,cm^{-3/2}$) and Teas fractional (f_d f_p f_h) parameters

Water (%,v/v)	THF (%,v/v)	R±SD[a] (%)	δ	f_d	f_p	f_h	f_d/f_h
0	100	31±10	9.5	55	19	26	2.1
10	90	97±9	10.9	51.6	19.3	29.1	1.7
20	80	98±7	12.3	48.2	19.4	32.2	1.5
30	70	100±2	13.6	44.8	19.6	35.3	1.3
50	50	97±2	16.4	38.0	20.5	44.5	0.9
75	25	63±2	19.8	29.5	21.3	49.3	0.6
100	0	21±1	23.3	21	22	57	0.4

[a] *n*=3, sample size:0.5 g; THF:water total volume: 4 mL; extraction time (vortex): 15 min

Table 2. BPA extraction recoveries and organic phase volume after *salting-out* separation with different mixtures THF:water/urine (3M NaCl)

THF (%,v/v)	Water		Urine	
	Recovery (%) ±SD[a]	Volume (µL) ±SD[a]	Recovery (%) ±SD[a]	Volume (µL) ±SD[a]
20	88±7	75±7	22±15	30±14
30	85±6	475±30	78±7	260±14
40	95±3	810±25	95±3	625±35
50	100±4	900±35	101±1	860±56

THF:water or THF:urine total volume: 2 mL;[a] n=3; extraction time (vortex): 5 min

Table 3. BPA (µg L^{-1}) in urine samples (±SD, *n*=3) under the optimal THF:water (NaCl) microextraction method and the standard SPE protocol [31]

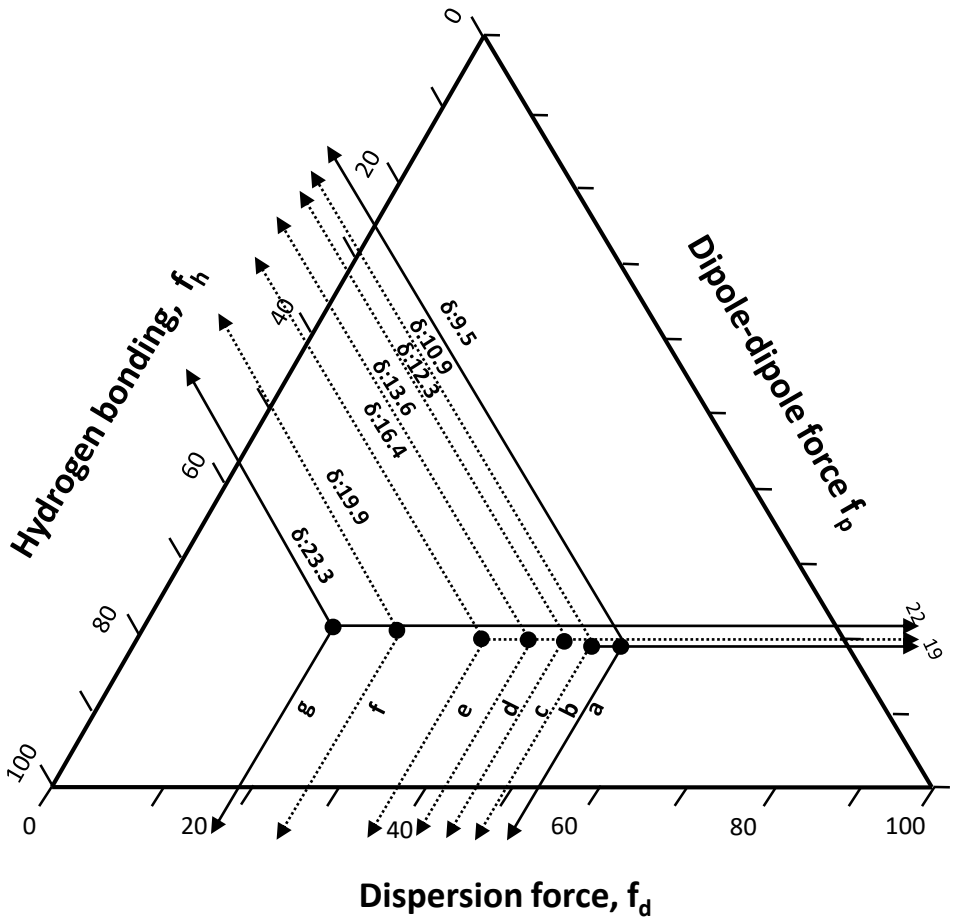
Sample code	***Microextraction***	***SPE***
1	2.3±0.2	2.3±0.1
2	1.0±0.1	0.7±0.1
3	3.0±0.2	3.1±0.2
4	0.60±0.05	0.6±0.1
5	4.7±0.4	4.6±0.2
6	1.5±0.1	1.51±0.04
7	2.4±0.1	2.31±0.08
8	2.1±0.3	2.2±0.1

n=3, non-significant variances between both methods based on appropriate t-test (homogeneous variance, t_{exp}:0.02-0.6; t_{cri}:2.78); extraction with 2 mL diluted urine (625 µL deconjugation solution + 1.25 mL of sample), 1.3 mL THF and 290 mg NaCl. Samples were vortex-mixed (5 min) and ultracentrifuged (15,000 rpm, 10 min)

Figure captions

Figure 1. Teas graph showing the Hildebrand solubility (δ) and fractional solubility (f_d, f_p and f_h) parameters for different THF:water mixtures: (a) 100:0, (b) 90:10, (c) 80:20, (d) 70:30, (e) 50:50, (f) 25:75 and (g) 0:100.

Figure 2. Phase diagram for THF:water (% v/v) solutions in the presence of NaCl (M) in water showing four regions (L, L-L, L-S and L-L-S), the NaCl saturation limit (point A) and the composition of the initial THF-water-NaCl mixtures subjected to further characterization (M1-M10).


Hydrogen bonding, f_h
Dipole-dipole force f_p
Dispersion force, f_d
0
20
40
60
80
100
δ:9.5
δ:10.9
δ:12.3
δ:13.6
δ:16.4
δ:19.9
δ:23.3
22
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a
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Figure 1

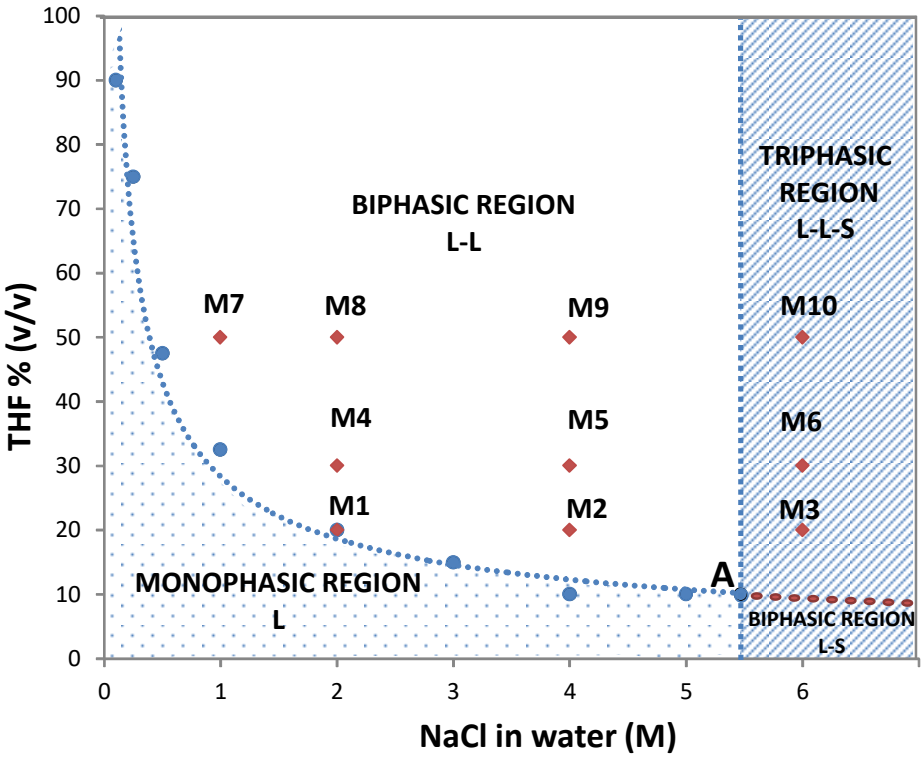
THF % (v/v)
NaCl in water (M)
BIPHASIC REGION
L-L
TRIPHASIC
REGION
L-L-S
MONOPHASIC REGION
L
BIPHASIC REGION
L-S
A
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10

Figure 2

SUPPLEMENTARY MATERIAL

Tunable solvency mixtures of tetrahydrofuran:water for efficient and fast extraction/clean-up of trace contaminants

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Table S1. Equations for prediction of the minimum THF content required for salting-out in THF:water mixtures, the composition (water and THF contents) of the resulting phases and for the relationship between THF and NaCl contents along the phase diagram

Dependent variable	Equation	R^2	Independent variables
Mimimum THF content required for salting-out ($THF_{minimum}$, % v/v in binary mix water:THF)	$THF_{minimum} = 28.321 \bullet NaCl_0^{-0.601}$	0.97	$NaCl_0$ (M in water, initial solution)
THF content (% w/w) of the bottom phase after salting-out (THF_B)	$THF_B = 0.426 \bullet NaCl_0^2 - 5.900 \bullet NaCl_0 + 25.326$	0.96	$NaCl_0$ (M in water, initial solution)
THF content (% w/w) of the top phase after salting-out (THF_T)	$THF_T = -0.497 \bullet NaCl_0^2 + 5.729 \bullet NaCl_0 + 76.975$	0.95	$NaCl_0$ (M in water, initial solution)
Water content (% w/w) of the bottom phase after salting-out ($water_B$)	$water_B = -0.289 \bullet NaCl_0^2 + 0.890 \bullet NaCl_0 + 75.386$	0.91	$NaCl_0$ (M in water, initial solution)
Water content (% w/w) of the top phase after salting-out ($water_T$)	$water_T = 0.576 \bullet NaCl_0^2 - 6.361 \bullet NaCl_0 + 22.269$	0.96	$NaCl_0$ (M in water, initial solution)

Table S2. THF, water and NaCl (%. w/w) in the aqueous and upper organic phase under different initial mixture composition

[a] Composition before phase separation						Composition after phase separation								Organic phase (mL)	Phased-out THF (%)
						Aqueous phase (bottom)				Organic phase (top)					
Point	**NaCl (M)**	**THF :water**	**NaCl (w/w)**	**THF (w/w)**	**Water (w/w)**	**Point**	**NaCl (w/w)**	**THF (w/w)**	**Water (w/w)**	**Point**	**NaCl (w/w)**	**THF (w/w)**	**Water (w/w)**		
M1	2	20:80	8.7	16.6	74.7	M1B	9±1	15.80±0.04	75±6	M1T	1.9±0.2	85.9±0.2	12±1	0.14±0.02	7
M2	4	20:80	16.1	15.3	68.7	M2B	17±2	8.78±0.03	74±5	M2T	1.6±0.3	91.9±0.3	6.5±0.7	1.01±0.03	50
M3	6	20:80	22.3	14.1	63.6	M3B	24.3±0.8	6.54±0.01	69±5	M3T	1.23±0.04	94.20±0.04	4.6±0.5	1.2±0.1	60
M4	2	30:70	7.8	25.5	66.7	M4B	9±1	16.59±0.04	74±6	M4T	2.01±0.09	85.5±0.7	12.5±0.7	1.51±0.06	50
M5	4	30:70	14.5	23.6	61.9	M5B	17±3	7.54±0.02	75±8	M5T	1.71±0.05	91.7±0.4	6.6±0.2	2.40±0.07	80
M6	6	30:70	20.2	22.0	57.7	M6B	25.3±0.4	3.34±0.01	71±5	M6T	1.5±0.1	93.20±0.03	5.3±0.5	2.80±0.05	93
M7	1	50:50	3.0	45.7	51.3	M7B	4.25±0.03	19.50±0.01	76±4	M7T	1.09±0.03	81.8±0.1	17.1±0.8	4.5±0.2	90
M8	2	50:50	5.8	44.3	49.8	M8B	9±1	13.96±0.05	77±7	M8T	1.4±0.3	88.7±0.3	9.9±0.1	4.51±0.04	90
M9	4	50:50	11.0	41.9	47.1	M9B	17±3	8.87±0.05	74±8	M9T	2.5±0.3	91.7±0.7	5.8±0.7	4.7±0.1	94
M10	6	50:50	15.6	39.7	44.6	M10B	23±1	6.05±0.01	71±7	M10T	2.5±0.1	93.1±0.1	4.4±0.7	4.80±0.04	96

[a] Solution volume = 10 mL

Table S3. Teas fractional (f_d. f_p. f_h) and Hildebrand ($cal^{1/2}\,cm^{-3/2}$) parameters in the aqueous and upper organic phase under different initial mixture composition

Initial mixture composition			Aqueous phase (bottom)					Organic phase (top)				
Point	**NaCl (M)**	**THF: water**	**Point**	**f_d**	**f_p**	**f_h**	**Hildebrand**	**Point**	**f_d**	**f_p**	**f_h**	**Hildebrand**
M1	2	20:80	M1B	27.5	21.4	51.1	20.7	M1T	51.2	19.3	29.5	11.0
M2	4	20:80	M2B	25.0	21.6	53.3	21.7	M2T	53.0	19.2	27.8	10.3
M3	6	20:80	M3B	24.3	21.7	54.0	22.0	M3T	53.6	19.1	27.3	10.1
M4	2	30:70	M4B	27.8	21.4	50.8	20.5	M4T	51.1	19.3	29.6	11.1
M5	4	30:70	M5B	24.5	21.7	53.8	21.9	M5T	53.0	19.2	27.9	10.3
M6	6	30:70	M6B	22.7	21.8	55.4	22.6	M6T	53.4	19.1	27.5	10.2
M7	1	50:50	M7B	28.6	21.3	50.1	20.2	M7T	49.7	19.5	30.9	11.7
M8	2	50:50	M8B	26.7	21.5	51.8	21.0	M8T	51.9	19.3	28.8	10.8
M9	4	50:50	M9B	25.0	21.6	53.4	21.7	M9T	53.2	19.2	27.6	10.2
M10	6	50:50	M10B	24.0	21.7	54.3	22.1	M10T	53.6	19.1	27.3	10.1

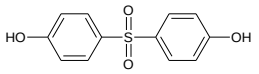
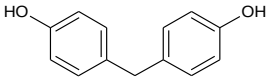
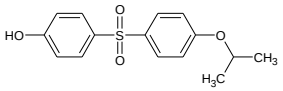
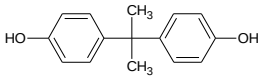
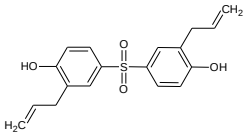
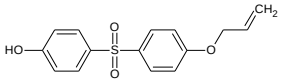
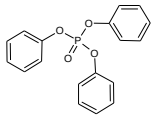
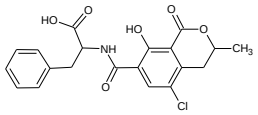
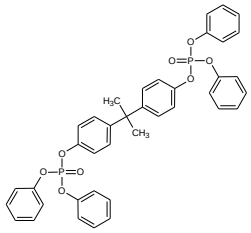
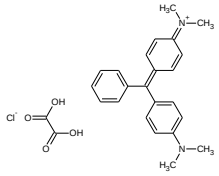
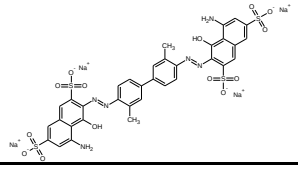
Teas parameters: water f_d =21, f_p =22, f_h =57; THF: f_d =55, f_p =19, f_h =26; Hildebrand parameter: water: 23.3 $cal^{1/2}\,cm^{-3/2}$; THF= 9.5 $cal^{1/2}\,cm^{-3/2}$

Table S4. Analytical performance of the methods for the analysis of BPA in urine and OTA in cereal-based baby food

	Instrumental linear range ($\mu g\ L^{-1}$)	Instrumental calibration R^2 (*n*=7)	Method LOD	Method LOQ	Precision (RSD, %)[a]
BPA	0.25-50	0.998	0.1 $\mu g\ L^{-1}$	0.2 $\mu g\ L^{-1}$	6%
OTA	0.02-50	0.998	0.1 $ng\ g^{-1}$	0.2 $ng\ g^{-1}$	5%

[a]The precision of the methods was evaluated by extracting eleven independent blank samples that were spiked with 0.5 $ng\ g^{-1}$ of OTA or 2.5 $\mu g\ L^{-1}$ of BPA

Table S5. Other organic contaminants investigated in this study (name and CAS, structure, and logP)

Compound, CAS	Molecular structure	logP
4,4'-Sulfonyldiphenol (BPS) ***80-09-1***		1.9[b]
4,4'-Methylenediphenol (BPF) ***620-92-8***		2.91[a] 2.9[b]
4-(4-propan-2-yloxyphenyl) sulfonylphenol (D-8) ***95235-30-6***		3[b]
4,4'-(propane-2,2-diyl) diphenol (BPA) ***80-05-7***		3.32[a] 3.3[b]
4-(4-hydroxy-3-prop-2-enylphenyl)sulfonyl-2-prop-2-enylphenol (TGSA) ***41481-66-7***		4.1[b]
4-(4-phenylmethoxyphenyl) sulfonylphenol (BPS-MAE) ***97042-18-7***		4.2[b]
Triphenyl phosphate (TPHP) ***115-86-6***		4.6[b]
Ochratoxin A (OTA) ***303-47-9***		4.74[a] 4.7[b]
Bisphenol A bis(diphenyl phosphate) (BDP) ***5945-33-5***		10.8[b]
Malachite green ***2437-29-8***		0.62[a]
Trypan Blue ***72-57-1***		-1.35[b]

LogP values obtained from PubChem, [a]experimental, [b]calculated

Table S6. Analytical features of reported methods for the determination of OTA in cereal food in comparison with the present study

Sample size	Extraction conditions	Clean-up and/or further preparation steps	Total solvent consumption per sample	Mean recovery	Precision	Ref.
50 g	100 mL of acetonitrile water (6:4 v/v); 1 min at high speed (waring blender)	Filtration (filter paper) and dilution of 10mL extract with PBS buffer; filtration (glass microfiber filter) and immunoaffinity column clean-up; evaporation and reconstitution to 1 mL of mobile phase	~61.6 mL	91-92%	<12%	[13]
10 g	40 mL of acetonitrile:water:acetic acid (79:20:1 v/v/v); 60 min orbital shaker	Centrifugation (10 min, 3,000 rpm); dilution 1:1 v/v with acetonitrile:water:acetic acid (20:79:1 v/v/v) and filtration (0.22 µm)	~31.6 mL	87-88%	<13%	[14]
5 g	20 mL of acetonitrile:water (80:20 v/v); vortexing (2800 rpm); soaking (20 min); 30 min with overhead shaker	Centrifugation (3 min, 3,000 rpm); dilution 1:1 v/v with acetonitrile and filtration (0.2 µm)	16 mL	70-120%	<20%	[15]
10 g	Pressurized liquid extraction (PLE) with acetonitrile: water (80:20 v/v) at 40 °C 500 psi and 5 min cycle.	-	Not specified	82%	<11%	[16]
2.5 g	PLE with methanol at 80 °C, 2000 psi and a 5-min cycle.	12 mL extract was evaporated to dryness (rotavapor) and re-dissolved in 2 mL of methanol; this extract was evaporated at 55 °C (N_2) and taken to a final volume of 0.5mL	Not specified	92%	5%	[17]
2.5 g	Matrix solid-phase dispersion (MSPD); 1.5 g C_8 mixed with sample for 5 min using a pestle. Extract packed on a glass column and OTA was eluted with 20 mL methanol:formic acid (99:1 v/v).	Evaporation to 3mL, filtration (0.45 µm) and centrifugation (5,000 rpm, 10 min); further filtration and concentration to 0.5 mL (N2 at 45 °C)	~ 20mL	78-89%	4%	[18]
1 g	MSPD; 1 g C_{18} mixed with sample for 5 min using a pestle. Extract packed on a glass column and OTA was eluted with 10 mL acetonitrile	Evaporation to dryness at 35 °C (N_2) and reconstitution to 1 mL with acetonitrile; filtration 0.45 µm.	~11 mL	64-91%	<19%	[19]
0.3 g	THF:water (20:80 v/v)	Vortex shaking (10 min) and centrifugation (15,000 rpm, 15ºC, 10 min)	0.96 mL	96-109%	5%	This study

Table S7. Analytical features of reported methods for the determination of BPA in urine in comparison with the present study

Sample size	Extraction conditions	Clean-up and/or further preparation steps[a]	Total solvent consumption per sample	Mean recovery	Precision	Ref.
0.2 mL	Solid-phase extraction (C_{18}); conditioning with methanol and water, washing with water, elution with 1 mL acetonitrile.	Evaporation to dryness (40°C, N_2), reconstitution with 0.15 mL 0.1% acetic acid in acetonitrile:water (1:9 v/v)	≥3 mL	95-100%	4-6%	[21]
5 mL	Addition of 0.1 mL isopropanol and SPE (polymeric sorbent Strata-X); conditioning with dichloromethane, methanol and water, washing with water, elution with methanol:dichloromethane (70:30 v/vv)	Evaporation to dryness and reconstitution with 0.1 mL methanol:water (80:20 v/v)	20 mL	87-88%	<13%	[22]
1 mL	Online SPE (C_{18} fused core column); conditioning with methanol:water (5:95 v/v), chromatographic separation with acetonitrile:methanol:water (in gradient mode)	-	~ 4 mL	85-100%	3-14%	[23]
10 mL	Immunoaffinity column (for free BPA); washing with of acetonitrile-water (5:95 v/v), elution with acetonitrile:water (40:60 v/v)	Previous dilution of sample with 1.5 mL PBS and pH adjustment to 7.2-7.4; final evaporation to 1 mL (N_2).	~10 mL	78%	3.4%	[24]
3mL	Molecularly imprinted polymer (MIP-SPE); conditioning with methanol (2% acetic acid v/v) and water, washing with water:acetonitrile(40:60 *v/v*), and elution with methanol.	Concentration up to a final volume of 0.5 mL at 45 °C	~15.4 mL	>94%	≤ 8.1 %	[26]
1.25 mL	THF:water (20:80 v/v)	Vortex shaking (5 min) and centrifugation (15,000 rpm, 15ºC, 10 min)	1.3 mL	95-108%	6%	This study

[a]An enzymatic treatment step for determination of for free BPA is included in most methods

Table S8. Extraction recoveries ±SD[a] of OTA in powder milk (200 mg) and bisphenols and aryl-phosphate flame retardants in dust (50mg) with mixtures of THF:water (total volume 1 mL).

Water (%,v/v)	THF (%,v/v)	f_d/f_h[b]	OTA[c]	BPS	BPF	D8	BPA	TGSA	BPS-MAE	TPPH	BDP
20	80	1.5	101±4	95±3	104±5	92±4	98±11	99±5	91±4	110±2	109±1
50	50	0.9	65±3	92±3	107±4	84±2	100±3	88±5	82±2	110±1	93±5
75	25	0.6	35±3	92±7	108±4	85±3	106±4	75±8	75±1	92±10	86±10

[a]*n*=3, [b]water in extraction solvent acidified with 0.1M HCl; extraction time (vortex): 15 min,

Table S9. Extraction recoveries ±SD[a] of and bisphenols and aryl-phosphate flame retardants with mixtures of THF:tap water (NaCl 2 M) (total solution volume 10 mL)

Water (%,v/v)	THF (%,v/v)	f_d/f_h[b]	BPS	BPF	D8	BPA	TGSA	BPS-MAE	TPPH	BDP
70	30	~1.7	72±5	57±2	79±5	69±4	81±5	82±5	72±10	60±10
60	40	~1.7-1.8	74±5	70±4	82±5	83±5	80±4	84±5	90±3	80±7

[a]*n*=3, extraction time (vortex): 5 min

Figure S1. (A) LC-ESI(-)-MS/MS extracted ion chromatogram of BPA in a urine sample extract and (B) LC-FL chromatogram of a cereal baby food sample extract showing OTA (fortified sample).