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Determination of bisphenol-type endocrine disrupting compounds in food-contact recycled-paper materials by focused ultrasonic solid–liquid extraction and ultra performance liquid chromatography-high resolution mass spectrometry

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ABSTRACT

Focused ultrasonic solid–liquid extraction (FUSLE) and reverse-phase ultra performance liquid chromatography (UPLC) coupled to a quadrupole-time of flight mass spectrometer (Q-TOF-MS) was applied to the determination of bisphenol-type endocrine disrupting compounds (EDCs) in food-contact recycled-paper materials. Recycled paper is a potential source of EDCs. Bisphenol A (BPA), bisphenol F (BPF) and their derivatives bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE) are used for the production of epoxy resins employed in the formulation of printing inks. The FUSLE of bisphenol-type EDCs from packaging is reported for the first time. First, different extraction solvents were studied and methanol was selected. Then, the main FUSLE factors affecting the extraction efficiency (solvent volume, extraction time and ultrasonic irradiation power) were studied by means of a central composite design. The FUSLE conditions selected for further experiments were 20 ml of methanol at ultrasonic amplitude of 100% for 5 s. Finally, the number of extraction cycles necessary for complete extraction was established in two. The analysis of the FUSLE extracts was carried out by UPLC-Q-TOF-MS with electrospray ionization and the determination of the four analytes took place in only 4 min. The FUSLE and UPLC-ESI-QTOF-MS method was validated and applied to the analysis of different food-contact recycled-paper-based materials and packaging. The proposed method provided recoveries from 72% to 97%, repeatability and intermediate precision under 9% and 14%, respectively, and detection limits of 0.33, 0.16, 0.65 and 0.40 μg/g for BPA, BPF, BADGE and BFDGE, respectively. The analysis of paper and cardboard samples confirmed the presence of EDCs in these packaging.

1. Introduction

There are a number of chemical substances that disturb regular performance of the hormonal system. They are referred to as endocrine disrupters (EDCs) [1,2] and their undesirable effects are felt by both men and women. These substances, including organochlorine pesticides, alkylphenols, phthalates, polychlorinated biphenyls and dioxins, organic tin compounds and bisphenols among others, disturb the hormonal equilibrium of organisms, which is particularly dangerous at developmental age, when changes are in most cases irreversible.

Bisphenol A (BPA) is considered to have a oestrogenic activity and it has been recently related to thyroid hormone action disruption [3]. The toxicity of bisphenol F (BPF), which has also been proven, is mainly related to its oestrogenic and antiandrogenic effects [4]. Regarding bisphenol A diglycidyl ether (BADGE) and bisphenol F diglycidyl ether (BFDGE), they are related to their cytotoxic effects, which make them tumorigen and mutagen [5]. The chemical structures of these four bisphenol-type compounds are shown in Fig. 1.

BPA and BPF have been used as a raw substance for mass production of epoxy resin, polycarbonate, polyester and polyacrylate plastics. Epoxy resins are used in a great number of applications: as tank coatings, structural steel coatings, aircraft finishes, can and drum linings, furniture finishes, in printing inks, in dental surgical and prosthetic applications, etc., [6]. The most popular coating varnishes and lacquers used in drink and food cans are those based on vinyl organosols (novolacs), which include in their composition epoxy resins obtained from BADGE or from BFDGE [7]. BPA and BPF can be released from packaging material and migrate into beverages and foods, being the rate of migration enhanced by treatments such as heat processing.

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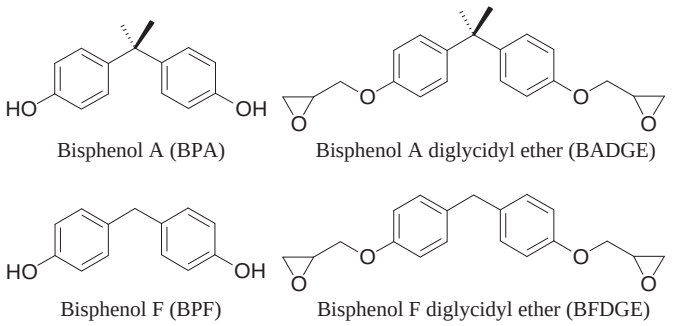

Fig. 1. Chemical structures of the four p–p bisphenol-type compounds.

Paper and cardboard are widely used as food packaging materials, directly in contact with food or more frequently protected by a barrier layer from direct contact with foodstuffs. It has, however, been demonstrated that these materials could contain pollutants of different origin, and these chemicals could be transferred to food in contact with the material [8–12]. BPA has been found in recycled paper and paperboard used for food packaging (pizza cardboard, paper bags) and in kitchen towels made from recycled paper, probably due to its use in printing inks [13]. For this reason, it is very important to establish the criteria to ensure that paper containing recycled pulp is safe enough to be used as food contact materials. Further data would be needed to quantify the impact of these sources in terms of BPA exposure in the population.

The use of plastic materials for food contact is regulated in many countries, but recycled paper and board is not regulated by law. However, the guidelines on paper and board materials and articles, made from recycled fibres, intended to come into contact with foodstuffs are established in the Proposal approved by the Council of Europe (Resolution RESAP (2002) 1, at www.coe.int/soc-sp).

Analytical methods for the determination of BPA in food have been recently reviewed [14]. The extraction of bisphenol-type compounds from liquid samples has been accomplished by different techniques including liquid–liquid extraction (LLE) [15–19], microliquid–liquid extraction (MLLE) [20], microliquid–liquid dispersive extraction (MLDE) [21], solid-phase extraction (SPE) [7,22–28], solid-phase microextraction (SPME) [29,30], and stir bar sorptive extraction (SBSE) [31]; while bisphenols have been extracted from solid samples by conventional lixiviation with solvents [32,33] for migration studies, matrix solid-phase dispersion (MSPD) [34], microwave-assisted extraction (MAE) [35–37], ultrasound-assisted extraction [38–41] with ultrasonic bath and pressurized liquid extraction [42–44]. However, to the best of our knowledge, only once BPA has been extracted by FUSLE (focused ultrasound solid–liquid extraction) [45] but from a different solid matrix (sewage sludge). Few methods have been proposed for the simultaneous determination of BPA, BPF and their corresponding diglycidyl ethers (BADGE and BFDGE) [7,14,16,24,46]. Besides, these have been developed for aqueous matrices using other extraction methods and this is the first time that FUSLE has been used for the extraction of bisphenol-type endocrine disrupters from packaging.

FUSLE is a relative new technique that has been successfully applied chiefly in the environmental field, for instance, for the determination of BPA, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phthalate esters, and nonylphenols in environmental samples [47–50], and it has also been applied for the determination of UV-filters in packaging [51].

FUSLE is a consequence of the cavitation phenomena. When a cavitating bubble collapses near the surface of a solid sample particle, micro-jets of solvent propagate towards the surface at high velocities, causing pitting and mechanical erosion of the solid surface, leading to particle rupture, and consequently, to smaller particle size [52]. Likewise, the cavitational bubbles implosion cause microscopically very high temperatures (up to 5000 K) and pressures (up to 2000 atm), which also favour an exhaustive extraction, without appreciable changing the extraction macroscopic conditions [53] because of the very small size of the bubbles. For this reason, it is an interesting technique for labile compounds. It is also worth mentioning that the microtip of the focused ultrasound, which emitted a high ultrasound power, is directly immersed in the slurry. This makes the power of FUSLE is 100 times higher than the traditional ultrasonic bath [47].

The determination of bisphenol-type EDCs has been usually developed through chromatographic techniques because of the complexity of the mixtures analysed, mainly high performance liquid chromatography (HPLC) coupled to mass detector (MS) [34,35–42] or fluorescence detection [54–57], and gas chromatography–mass spectrometry [46,56–58]. However, the gas chromatography method seems to be limited, for BADGE and BFDGE, due to their low volatility [7].

In this work, reverse-phase ultra performance liquid chromatography (UPLC) coupled to a quadrupole-time of flight mass spectrometer (Q-TOF-MS) was applied to the determination of BPA, BPF, BADGE and BFDGE. This UPLC–HRMS method is advantageous over conventional HPLC-MS methods in terms of shorter analysis time and improved selectivity. The FUSLE and UPLC–Q-TOF-MS detection of bisphenol-type EDCs from packaging is reported for the first time and has proved to be fast and efficient.

2. Experimental

2.1. Standards and material

Bisphenol A (99%), bisphenol F (98%), bisphenol A diglycidyl ether (97%) and bisphenol F diglycidyl ether (97%, a mixture of 3 isomers: ortho–ortho, ortho–para, para–para) were obtained from Fluka (Switzerland). The isotopically labelled BPA standard (BPA-d_{16}) used as internal standard for the GC–MS determination was purchased from Cambridge Isotope Laboratories (USA), and the derivatisation agent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTEFA) was obtained from Alfa Aesar GMbH (Karlsruhe, Germany).

Deionised water was obtained from a MilliQ water purification system (Millipore, USA). Acetonitrile, diclorometane, tetrahydrofuran, ethyl acetate and acetic acid, all LC-MS quality, were purchased from Scharlab (Barcelona, Spain). LC–MS-grade methanol and anhydrous sodium acetate (99%) were obtained from Panreac (Barcelona, Spain).

2.2. Samples and sample preparation

Standard solutions containing 1000 mg/l of the each compound were prepared in acetonitrile and subsequently diluted in methanol as necessary.

Different food-contact paper-based materials and packaging, including kitchen paper, tablecloth, food boxes and bags, were obtained from local supermarkets and fast-food outlets.

The samples were ground for 6 min using a cryogenic mill 6750 freezer/mill (SPEX CertiPrep, UK) before analysis.

Spiked cardboard was used for the study of variables and the method validation. It was spiked with 20 μg/g of each analyte by adding a standard solution to the milled cardboard. Solvent was let evaporate and then the spiked cardboard was triturated again to ensure proper homogenization of the sample. Before analysis,

all spiked samples were stored in glass containers at room temperature, protected from light for at least one month. For the recovery study, a paper sample free from bisphenols was used.

2.3. FUSLE procedure

A 70-W power 20-kHz frequency SONOPULS HD 2070 sonicator/homogeniser (Bandelin electronic GMBH & Co. KG, Berlin, Germany) provided with a 3-mm cylindrical titanium alloy probe was used for focused ultrasound assisted extraction. Extractions were carried out in 34 × 100-mm round-bottom centrifuge glass vessels.

The effect of different factors on extraction yield was investigated by experimental design, using Statgraphics Centurion XV software (Statpoint Technologies, USA) to generate the matrix of experiments and to estimate the effect of each factor on the efficiency of the extraction.

Under final conditions, samples (0.5 g) were accurately weighed in the extraction vessel and 20 ml of methanol were added. The vessel was cooled with an ice–water bath (0 °C) during extraction. The probe titanium tip was immersed 2 cm from the upper surface of the mixture. The sample was exposed to 2 cycles of ultrasonic irradiation at 100% power irradiation for 5 s. After each FUSLE cycle, solid and liquid phases were separated by centrifugation (Orto Alresa, Spain) at 2000 rpm for 2 min. Next, liquid extracts were evaporated to about 1 ml under nitrogen stream using a TurboVap II evaporator (Zymark, Hopkinton, MA, USA). Then, it was transferred to a 5-ml volumetric flask and filled up to 5 ml with methanol. This dilution step can be avoided in order to increase sensitivity. Finally, extracts were filtered through a 0.22-μm-teflon filter using a glass syringe (Hammilton, Las Vegas, NV). It should be pointed out that plastic syringes showed a significant interference with m/z=282. For this reason, plastic materials were restricted in this work.

2.4. UPLC–Q-TOF

Analytes were determined with a Waters Acquity UPLC TM chromatograph (Milford, MA, USA) equipped with a Waters Acquity BEH C18 column (1.7 μm × 50 × 2.1 mm) and a Waters VanGuard precolumn of the same material, and coupled to a Microtof-Q (Q-TOF) mass spectrometer from Bruker Daltonik (GMBH, Germany) with a electrospray interface. The chromatographic and mass data were acquired with the software Data Analysis Version 4.0 from Bruker Daltonik (GMBH, Germany).

A 1:1 acetonitrile:methanol mixture (solvent A) and a 0.5 mM sodium acetate 8.5 mM acetic acid aqueous solution (solvent B) were used as mobile phases.

The chromatographic separation took place in only 4 min. The mobile phase composition was varied according to a linear gradient that increased from 5% to 50% A within 1.0 min, was held at 50% A for an additional 1.0 min, increased again from 50% to 95% A within 2.0 min, and maintained at 95% A for 1.0 min and then returned to the initial conditions. Total run time was 7 min. The flow rate was set at 0.4 ml/min and the injection volume was 5 μL (half loop, 50% of the total loop volume). A chromatogram of the mixture of the four analytes is shown in Fig. 2. It is worth mentioning that although BADGE and BFDGE overlapped at the base, the quantification can be performed without interference because of chromatograms were obtained at their corresponding m/z.

The mass spectrometer was calibrated across the mass range of 50–2000 Da using internal references. Quantification was performed in full scan MS conditions by ion extraction with a ± 20 mDa m/z window. The full-scan data were acquired using a capillary voltage of 3.5 kV in negative mode whereas it was 4.5 kV in positive mode. A coaxial nebulizer N_2 gas flow (9.0 l/min) at 200 °C and 3.0 bar of pressure around the ESI emitter was used to assist the generation of ions. The signals at m/z 227.106, 199.074, 363.159 and 335.126 Da were selected for the determination of BPA, BPF, BADGE and BFDGE, respectively.

2.5. Selective PLE and GC–MS analysis

In order to compare the BPA concentrations found in pizza box and paper tablecloth by FUSLE and UPLC–Q-TOF, these samples were extracted by selective pressurised liquid extraction (SPLE) followed by GC–MS analysis as described by Martinez-Moral et al. [45].

SPLE was carried out using an ASE200 accelerated solvent extractor from Dionex, furnished with 11-mL stainless-steel extraction cells. Extraction cells were prepared as follows. Two cellulose filters were placed at the cell bottom, followed by a layer of 1 g of anhydrous sodium sulphate. Then, a mixture of 0.5 g of sample, 1 g of anhydrous sodium sulphate and 4 g of Florisil was added. Finally, the cell was completely filled with anhydrous

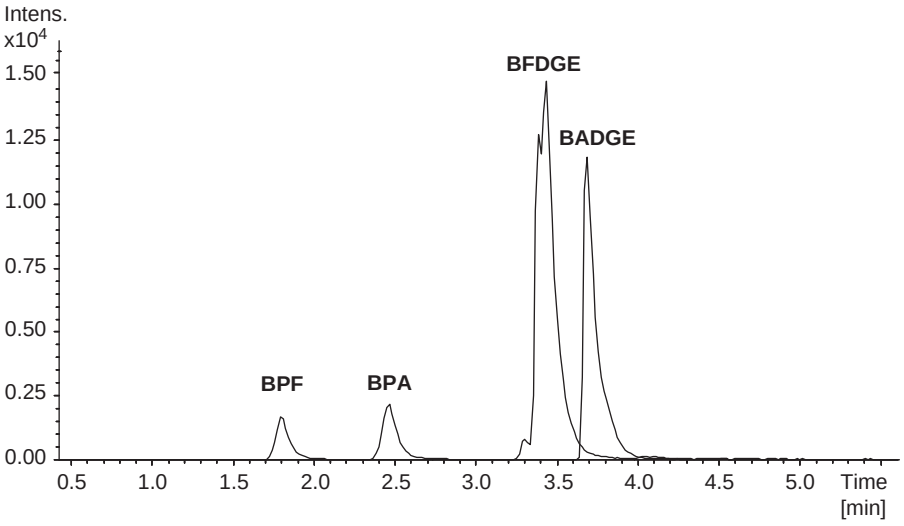

Fig. 2. UPLC–MS chromatogram of a bisphenol mixture.

sodium sulphate, and a cellulose filter was placed on top. Extractions were carried out at 130 °C with dichloromethane for 1 min in one cycle. PLE extracts (around 15 ml) were evaporated to dryness under a nitrogen stream using a Turbo Vap II concentrator (Zymark, Hopkinton, MA, USA). The residue was reconstituted in 2 mL of ethyl acetate, extracts were filtered through a 0.45-mm nylon filter and 500 μL were derivatised before GC analysis.

In order to perform the derivatization, 50 μL of BSTFA were added to 500 μL of extract. Then the mixture was thoroughly shaken and maintained at 50 °C for 15 min in a closed vial. Finally, derivatised extracts were quickly cooled and injected into the GC–MS system.

A Varian 3900 gas chromatograph with a Varian 2100T ion trap MS detector (Walnut Creek, CA, USA) equipped with a VF-5-MS fused-silica column (30 m × 0.25 mm id, 0.25 μm 5% polydiphenylsiloxane/95% polydimethylsiloxane phase) was used to carry out GC–MS analysis. BPA-d_{16} was used as internal standard. Injection volume was 1 μL and injector temperature was set at 280 °C. An initial 1:20 split ratio for 0.01 min was followed by splitless injection for 2 min and a split ratio of 1:50 until the end of the separation. The initial oven temperature was 50 °C for 2 min and was then increased to 100 °C at a rate of 30 °C/min; increased again at a rate of 10 °C/min to 200 °C and finally increased at a rate of 30 °C/min to 300 °C and held for 8 min. Helium (99.996%) at a flow rate of 2.0 mL/min was used as carrier gas.

Electron ionisation and selected ion storage MS detection mode were used; ions *m*/*z* 357 and 368 were used for BPA and BPA-d_{16} quantification, respectively.

3. Results and discussion

3.1. Study of UPLC–MS conditions

Water and acetonitrile were tested as mobile phases during the studies of the ESI conditions. In order to achieve better signals for all analytes, ESI in negative ion mode was selected for BPA and BPF while positive ion mode was chosen for BADGE and BFDGE. The negative ionization mode for BPA and BPF showed the deprotonated molecule $[M\text{-}H]^-$ to be the most abundant ion while positive ion mode was chosen for BADGE and BFDGE because of they showed poor signal or even no signal in negative ion mode.

However, when water was used as an aqueous phase in a carrier containing a 50% of methanol/acetonitrile, the ESI mass spectra of BADGE and BFDGE showed peaks at *m*/*z* ratios corresponding to the sodiated, potassiated and ammoniated molecular ions. A BADGE mass spectra under these conditions is shown in Fig. 3A. Different aqueous phase compositions were tested with the aim of promoting one single kind of positive ion and thus to improve sensitivity. There are some studies [33] regarding the promotion of the ammoniated ion formation that suggest the use of ammoniated salts. However, the best results were obtained by using a 0.5 mM sodium acetate and 8.5 mM acetic acid aqueous solution which promoted the formation of only sodiated ions. BADGE mass spectrum under these conditions is shown in Fig. 3B.

Regarding the BPA and BPF determination in negative ion mode, the addition of 0.1% acetic acid produced 14-fold increased signals for both BPA and BPF, probably because protons promoted the ion formation and desorption in electrospray ionization. However, the addition of sodium acetate caused a signal suppression of around 40% compared to the signals obtained in acetic acid. BPA and BPF signals in different solvents are shown in Fig. S1A and B in supplementary material available on-line. Moreover, the full-scan ESI(-)–MS mass spectra (*m*/*z* of 0 to 600 amu) of BPA showed than the $[M\text{-}H]^-$ ion at *m*/*z* 227 is the major ion formed in acetic acid solution, while in water the formation of ion clusters with higher *m*/*z* is significant. BPA mass spectra under these conditions is shown in Fig. S2 in supplementary material.

In order to establish the best UPLC conditions to separate analytes, different usual organic mobile phases reported in literature were tested. Mobile phases studied consisted of methanol, acetonitrile or mixtures of both organic solvents. The best separation of the four analytes was achieved with a gradient of a 1:1 acetonitrile:methanol mixture.

3.2. Features of the UPLC–MS determination

The UPLC–MS method was characterised in terms of linearity, repeatability and limit of detection (LOD) using standard solutions of all analytes in methanol. Results are shown in Table 1. Linearity was studied in methanolic solutions and matrix extracts in order to determine if matrix effect was present. As can be seen in Table 1, slopes for BPA and BPF were statistically equal, whereas slopes were very different for BADGE and BFDGE. Ionisation efficiency in positive mode was markedly affected by the presence of sample matrix components. Therefore, standard addition method was mandatory for the quantification of BADGE and BFDGE in order to avoid matrix-effect errors.

Repeatability expressed as relative standard deviation of ten replicates ranged from 5% to 7%. The limit of detection of BPA was calculated from the mean and relative standard deviation of a filter paper sample used as blank. This blank does not show signals for the other three analytes, therefore their limits of detection were calculated as the analyte concentration corresponding to a signal-to-noise ratio of three. This concentration was calculated by plotting the signal-to-noise ratio as a function of concentration in a range from 20 to 80 ng/ml. As can be seen in Table 1, LODs were between 16 and 47 ng/ml.

3.3. Optimization of FUSLE conditions

In order to develop a FUSLE method the influence of several instrumental variables such as ultrasound power, pulse cycle, extraction time and probe depth in the solvent should be taken into account, as well as other variables such as solvent composition and volume, sample mass, temperature, extraction vessel shape [59] and number of FUSLE steps.

According to the technical specifications, the sample amount should be between 0.1 and 1 g. The influence of mass and solvent volume on recovery is correlated because the factor affecting the recovery is the volume ratio of the phases. Thus, in this study the sample mass was fixed at 0.5 g.

In the case of the pulse cycle and the ultrasound irradiation power, it should be taken into account that these two variables are related. Thus, we decided to set the pulse mode at 0.5 pulse cycle.

In order to get the maximum ultrasonic performance any experiment should be conducted at as low temperature as possible to avoid reducing the cavitation phenomena [47,48,59,60]. Thus, the solvent temperature was maintained at 0 °C during the extraction using an ice bath.

Once the above-mentioned parameters were fixed, several extraction solvents were tested under mild FUSLE conditions; then the influence of irradiation power, solvent volume and extraction time were investigated using an experimental design and finally, the number of FUSLE steps was studied.

Four different solvents: tetrahydrofuran, dicloromethane, acetonitrile, and methanol were tested in order to select the best solvent for the FUSLE of bisphenols from cardboard. All of them are polar solvents, however only methanol is a protic solvent.

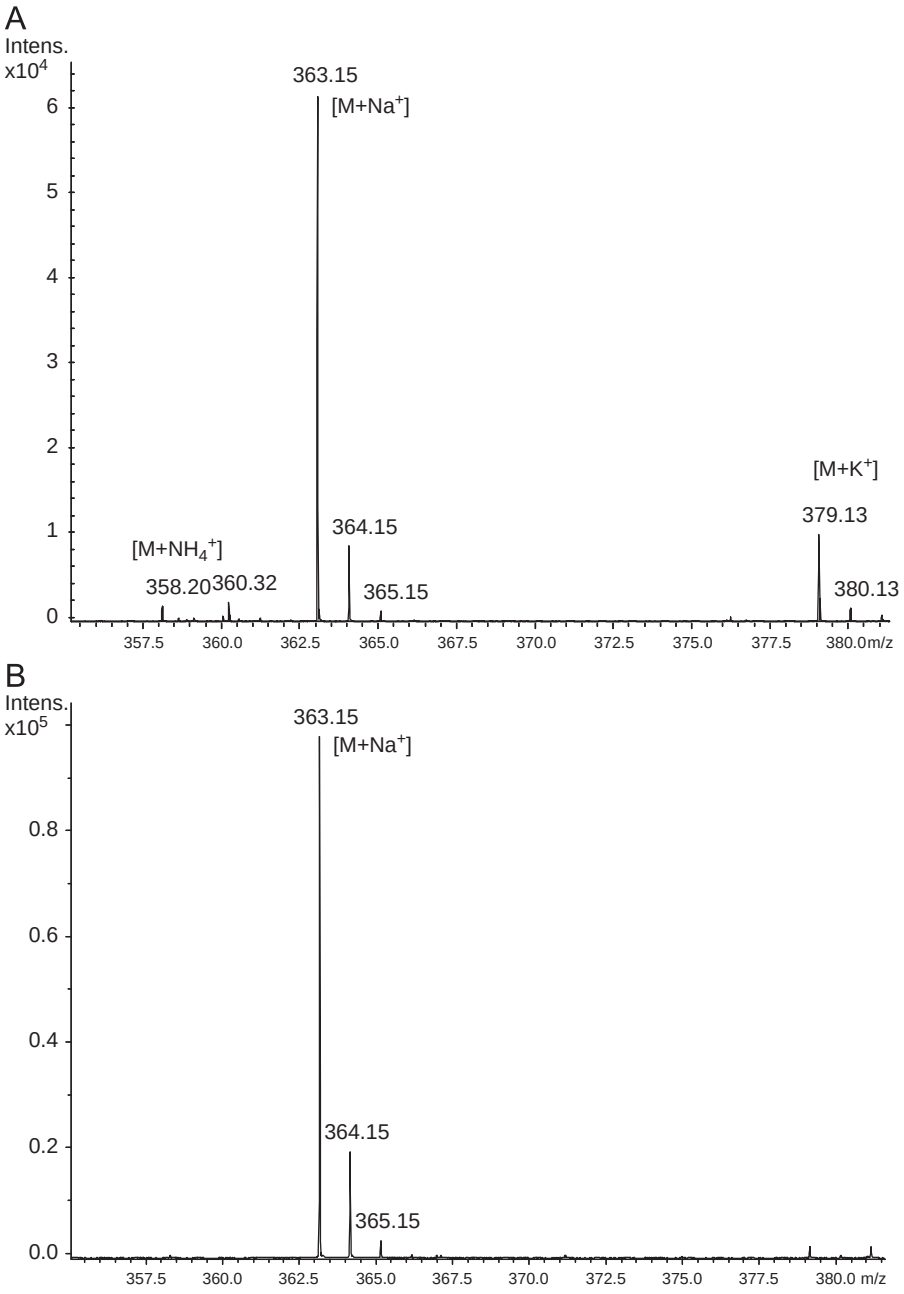

Fig. 3. Mass spectra of BADGE using (A) water and (B) an 8.5 mM acetic acid 0.5 mM sodium acetate aqueous solution in order to promote the formation of $[M+Na]^+$ ion.

Table 1
Figures of merit of the UPLC–MS method.

Analyte	Range (ng/ml)[a]	Repeatability[b] (RSD, %)	LOD (ng/mL)	Methanolic solution		Methanolic matrix extract	
				Slope ± SD	R^2	Slope ± SD	R^2
BPA	50–2000	5	34[c]	40.7 ± 1.2	0.999	40.3 ± 1.4	0.998
BPF	25–2000	7	16[d]	24.5 ± 1.2	0.997	24.3 ± 1.4	0.995
BADGE	25–2000	7	20[d]	251 ± 16	0.995	117 ± 21	0.993
BFDGE	50–2000	6	47[d]	515 ± 34	0.994	181 ± 14	0.993

[a] Calibration points: $n=9$.
[b] Relative standard deviation ($n=10$) at 500 ng/mL.
[c] Limit of detection calculated from the standard deviation of a filter paper sample used as blank.
[d] Limits of detection calculated for a signal-to-noise ratio of 3 (S/N=3).

Three consecutive extractions were carried out with three aliquot of 10 ml of solvent at mild conditions (50% irradiation power) for 2 min. The recovery values achieved for each solvent are shown in Fig. 4. In general, the lowest extraction efficiency corresponded to tetrahydrofuran. Methanol provided the highest extraction efficiency. Therefore, it was selected for further extractions.

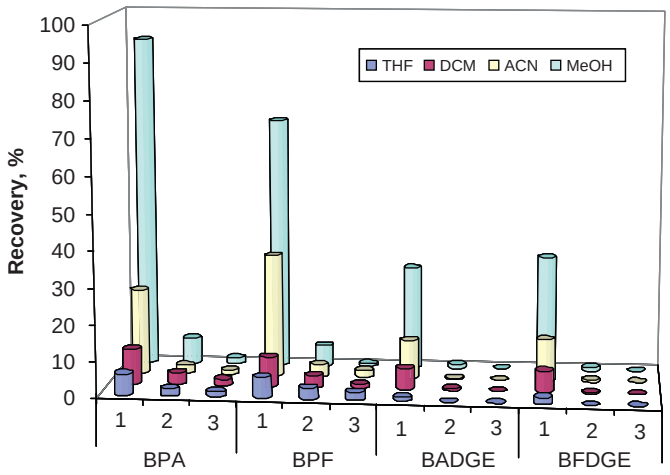

Fig. 4. Recovery of three consecutive extraction of a sample with different solvents. Extraction conditions: 10 ml of solvent, 50% of power irradiation, 0.5 cycles and 2 min.

Once methanol was selected as extraction solvent, the main FUSLE factors affecting the extraction efficiency including solvent volume, extraction time and ultrasonic irradiation power, were studied by means of a central composite design (CCD).

The experiments were carried out using 0.5 g of cardboard containing 20 μg/g of each analyte. This concentration was chosen according to bisphenol levels previously reported in literature [13,61,62].

The CCD consisted of a 2^3 factorial design with six star points located at $\pm\alpha$ from the centre of the experimental domain and nine replicates of the central point. An axial distance α of 1.68 was selected in order to fulfil the rotatability condition. The design consisted of 23 randomly performed experiments. Ultrasonic irradiation power values ranged from 20% to 100%, including the following levels: 20%, 36%, 60% (central value), 84% and 100% of ultrasound power. Extraction time was studied between 5 and 300 s and the levels were 5, 65, 152 (central value), 240 and 300 s. Methanol volume used in extractions was between 5 and 20 ml with levels of 5.00, 8.00, 12.50 (central value), 17.00 and 20.00 ml. Conditions of the experiments are shown in Table S1 in supplementary material. The experimental domain was selected according to technical limitations. Ultrasound power was varied from a low but significant value (20%) to the maximum allowed by the sonicator (100%). Irradiation time was varied from a very short time (5 s) to a usual value found in literature (300 s) [47,51]. Times of 1 to 3 min are common for applications performed with ultrasound probes [63]. For the selection of the lower and upper solvent volume values, it is worth mentioning that the titanium microtip of the probe must be immersed into the vessel 1–2 cm from the upper surface of the slurry according to manufacturer's recommendations, and about 5 mm above the bottom of the vessel to minimize "dead zones". According to these recommendations and the vessel dimensions, the lower and the upper volumes selected were 5 and 20 ml, respectively.

Main effects, quadratic terms and two-factor interactions for variables involved in the design were calculated using analysis of variance (ANOVA). Standardized values for these terms are shown in Fig. 5. Dotted lines in this figure represent the upper and lower 95% confidence levels. As can be seen, time presented a negative and significant effect on the extraction of BADGE and BFDGE. The irradiation power also presented a significant negative effect in BADGE extraction. For three of the analytes (BPA, BPF and BADGE) the extraction efficiency increased significantly with volume. Related to quadratic terms and two-factor interactions, only the quadratic term of irradiation power and the volume-time interaction for BPA were statistically significant.

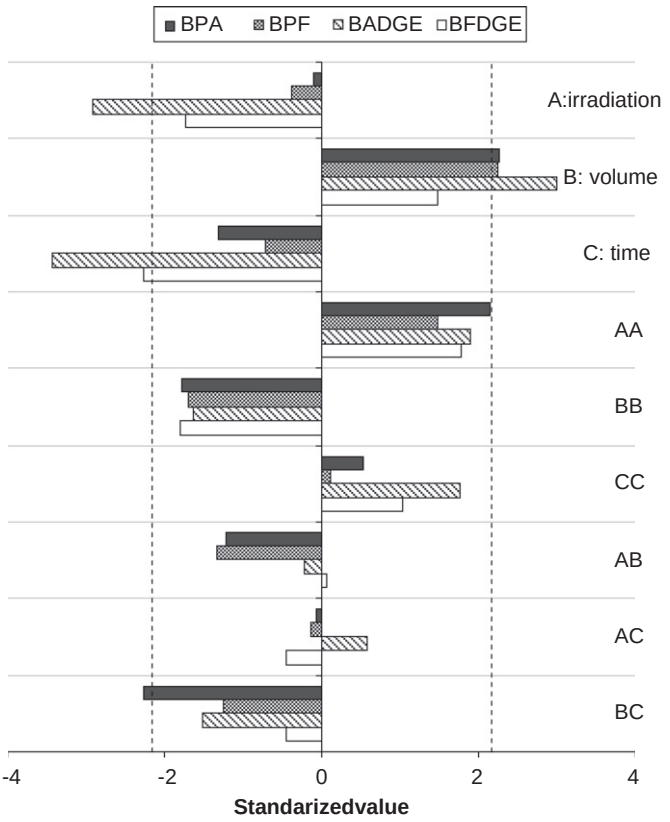

Fig. 5. Standardized coefficients for main effects, quadratic terms of factors and two-factor interaction considered in the CDD design. Codes: (A) irradiation power (%), (B) solvent volume (ml), (C) extraction time (s).

In order to determine the optimal values for time and volume, the response surface for BPA was drawn (it is shown in Fig. S3.A in supplementary material). As can be seen, the best extraction efficiencies were achieved at the highest volume and the lowest time studied, 20 ml and 5 s, respectively.

On one hand, irradiation power showed a negative effect in BADGE extraction. On the other hand, irradiation quadratic term presented a positive effect in BPA extraction. In order to find compromise conditions, the desirability function was obtained. First, BPA and BADGE response function obtained from the CCD were normalized, and then the desirability function was calculated as their geometric mean. A plot of this function vs. solvent volume and irradiation power, at an extraction time of 5 s, is shown in Fig. S3.B in supplementary material. The desirability function showed that the value of irradiation power was not significant in the overall response at the highest volume value previously selected.

Once the best FUSLE conditions were established, the amount extracted in three consecutive FUSLE steps of the same sample was determined at 20% and 100% of irradiation power (it is shown in Fig. S4 in supplementary material), in order to fix the number of FUSLE steps needed to exhaustive extraction. Results showed that the irradiation power has not got a significant effect in the amount extracted as expected, but a 100% of power provided better repeatability for BPA and BPF. In addition, two cycles were enough to extract more than 95% in all cases. Therefore, 2 cycles and 100% of irradiation power were selected.

3.4. Performance of the FUSLE-UPLC–MS method

Once the FUSLE conditions were established, the whole FUSLE-UPLC–MS method was characterised in terms of sensitivity (through detection limit determination), repeatability and intermediate

Table 2
Features of the FUSLE-UPLC–MS/MS method.

Analyte	Repeatability[a] (RSD, %)	Intermediate precision[b] (RSD,%)	LOD (μg/g)
BPA	6	7	0.33[c]
BPF	5	14	0.16[c]
BADGE	9	12	0.65[d]
BFDGE	7	4	0.40[d]

[a] Intra-day relative standard deviation ($n=3$ replicates).
[b] Inter-day relative standard deviation ($n=$ 3 replicates $\times$ 3 day).
[c] LOD calculated from the instrumental LODs reported in Table 1, the sample amount used and the extract volume obtained.
[d] LOD calculated from the calibration data in matrix extract, as three times the intercept standard error divided by the slope, in order to taken into account the sensitivity decrease caused by the matrix effect.

Table 3
FUSLE UPLC–MS/MS recovery values obtained at three spiking levels.

Analyte	Recovery[a] (%) at 2.5 μg/g	Recovery[a] (%) at 10 μg/g	Recovery[a] (%) at 20 μg/g
BPA	91 ± 7	84 ± 3	93 ± 12
BPF	82.6 ± 1.2	83 ± 10	92 ± 10
BADGE	77 ± 12	97 ± 12	84 ± 10
BFDGE	72 ± 17	86.7 ± 1.5	84 ± 7

[a] Confidence interval ($n=3$, $\alpha=0.05$).

Table 4
Bisphenol concentrations (μg/g) found in different recycled-paper materials.

Sample	Concentration[a] (μg/g)	
	BPA	BPF
Packaging box	9.12 ± 0.23	n.d.
Kitchen paper	< LOQ	n.d.
Hamburger pack	1.53 ± 0.08	n.d.
Milk brick	0.97 ± 0.05	n.d.
Supermarket bag	0.92 ± 0.05	n.d.
Pizza box	11.52 ± 0.07	< LOQ
Popcorn bag	< LOQ	< LOQ
Paper tablecloth	25.4 ± 2.5	n.d.

[a] Confidence interval ($n=3$, $\alpha=0.05$).
n.d.=not detected; concentration below the limit of detection.
< LOQ=concentration below the limit of quantification.

precision and recovery. Figures of merit of whole method are shown in Table 2.

Limits of detection of the whole method ranged from 0.16 to 0.65 μg/g. BPA and BPF LODs were calculated from the instrumental LODs reported in Table 2, according to the sample amount used and the extract volume obtained. However, the matrix-effect was taken into account to calculate BADGE and BFDGE LODs, and they were calculated from the calibration data obtained in matrix extract, as three times the intercept standard error divided by the slope.

Repeatability and intermediate precision, expressed as intra and inter-day relative standard deviation percentages were less than 9% and 14%, respectively.

A recovery study was carried out at three concentration levels: 2.5, 10.0 and 20.0 μg/g of each analyte (Table 3). Recovery values ranged from 72% to 97%.

3.5. Analysis of real samples

The FUSLE-UPLC–MS method was applied to determine BPA, BPF, BADGE and BFDGE in eight different food-contact recycled-paper materials including hamburger, pizza and popcorn packaging, kitchen paper and paper tablecloth, milk brick, supermarket bag and packaging box. Results are listed in Table 4. Diglycidyl ether derivatives were not detected in any sample. As can be seen, BPF was found only in two of the eight samples, although both concentrations were below the limit of quantification. It is important to highlight that BPA was found in all the samples. The highest BPA concentrations were found in paper tablecloth, pizza box and packaging box. The pizza box and the microwave pop-corn bag were the only packaging containing BPF and BPA.

The analysis of pizza box and paper tablecloth by SPLE and GC–MS showed BPA concentrations of 8.2 ± 2.0 and 31 ± 3 μg/g, respectively.

4. Conclusions

A new method based on FUSLE and UPLC–HRMS for the determination of BPA, BPF, BADGE and BFDGE in food-contact recycled-paper packaging and materials has been developed.

The separation of four analytes by UPLC is performed in only 4 min, whereas traditional liquid chromatography needs between 12 and 17 min [7,14,24]. Besides, FUSLE is a rapid, simple and cheap extraction technique. The complete extraction of the analytes is carried out in only two cycles of 5 s and the extraction method has a good efficiency for the bisphenol-type EDCs, with recoveries between 72% and 97%.

The whole method provided acceptable repeatability and intermediate precision, with relative standard deviations below 9% and 14%, respectively. The FUSLE-UPLC–MS method has been successfully applied to determine BPA in different food-contact paper-based materials and packaging.

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Appendix A. Supplimentary information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.05.035.

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