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Development and validation of a specific and sensitive gas chromatography tandem mass spectrometry method for the determination of bisphenol A residues in a large set of food items

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ABSTRACT

BPA-containing products are widely used in foodstuffs packaging as authorized within the European Union (UE no. 10/2011). Therefore, foods and beverages are in contact with BPA which can migrate from food contact material to foodstuffs. An accurate assessment of the exposure of the consumers to BPA is crucial for a non-ambiguous risk characterization. In this context, an efficient analytical method using gas chromatography coupled to tandem mass spectrometry (GC–MS/MS), in the selected reaction monitoring (SRM) mode, was developed for the quantification of BPA in foodstuffs at very low levels (<0.5 μg kg^{-1}). A standard operating procedure, based on the combination of two successive solid phase extractions (SPE), was developed for various liquid and solid foodstuffs. The use of $^{13}C_{12}$-BPA as internal standard allowed accurate quantification of BPA by isotopic dilution. Control charts based on both blank and certified materials have been implemented to ensure analytical data quality. The developed analytical method has been validated according to in-house validation requirements. R^2 was better than 0.9990 within the range [0-100 μg kg^{-1}], the trueness was 4.2%. Repeatability and within-laboratory reproducibility ranged from 7.5% to 19.0% and 2.5% to 12.2%, respectively, at 0.5 and 5.0 μg kg^{-1} depending on the matrices tested for. The detection and quantification limits were 0.03 and 0.10 μg kg^{-1}, respectively. The reporting limit was 0.35 μg kg^{-1}, taking into account the mean of the laboratory background contamination. The global uncertainty was 22.2% at 95% confidence interval.

1. Introduction

Bisphenol A (BPA), 2,2-bis(4-hydroxyphenyl) propane, CAS Registry number 80-05-7, is an industrial chemical used in the production of polycarbonate and epoxy resins. BPA-containing products are widely used in foodstuffs packaging such as beverage and food containers, as well as in the manufacture of food cans inner coatings, as currently authorized within the European Union [1].

The tolerable daily intake (TDI) has been recently set at 0.005 mg kg^{-1} body weight/day by the European Food Safety Authority [2]. To characterize the risk of the French consumers regarding their exposure to BPA in food, an exhaustive study has been initiated at the national scale on a large range of food items. The food contamination pattern has been possible thanks to the development of a sensitive and specific analytical method applicable to a large set of foodstuffs. Within this scope, various options are offered to laboratories as reviewed by Ballesteros-Gómez et al. [3].

Even if microwave-assisted extraction [4,5] and pressurized liquid strategies [6,7] are reported for BPA extraction, liquid–liquid or liquid–solid extraction approaches are by far the most popular [3]. They frequently used acetonitrile, methanol, ethanol, ethyl acetate, dichloromethane or acetone as extraction solvents, depending on the considered matrices (solid or liquid). Purification is classically

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based on reversed SPE columns filled with Hydrophilic-Lipophilic Balance (HLB), divinylbenzene-N-vinylpyrrolidone copolymer or C18 stationary phases, while the use of molecularly imprinted polymers (MIP) is also reported for the determination of BPA in biological matrices [8]. Additionally, other extraction techniques are available and may be use for BPA extraction purpose, such as solid-phase microextraction (SPME) [9], matrix solid-phase dispersion (MSPD) or stir bar sorptive extraction (SBSE) [10].

Liquid chromatography–mass spectrometry (LC–MS) is often preferred to gas chromatography–mass spectrometry (GC–MS) as it does not need any derivatization step prior chromatographic separation. However, the major drawback of LC-MS methodology remains charge competition observed in electrospray ionization leading to questionable results especially when complex biological matrices – such as foodstuffs – are concerned [11–13].

The use of GC–MS has been chosen by several authors for the analysis of BPA in certain categories of food items [14–18]. Nevertheless, a drastic standard operating procedure has to be applied to allow an efficient derivatization of the targeted molecule. Indeed, the presence of residual lipids competes for the reagent and impact significantly the derivatization efficiency and thus the global analytical performances of the method [19–21]. Silylation using MSTFA, BSTFA or MTBSTFA have been very often reported for BPA analysis [5,16,22–35] while BSTFA is the most frequently used. Such strategy has successfully been applied for the determination of BPA in several complex matrices, such as meat, fish, fruits, vegetables and numerous solid food items [16,25,27,29,36–38]. Moreover, while tandem mass spectrometry (MS/MS) [17,18] or high resolution mass spectrometry (HRMS) may be available as detection technique, GC–MS is usually implemented in combination with either no use of any internal standard or the use of deuterated BPA. Therefore, analytical performances as published in the literature are difficult to compare in terms of performances. Reported detection and quantification limits are significantly different insofar as reported detection limits ranged from 0.4 ng L^{-1} in food stimulant [39] without any internal standard used to 1 μg kg^{-1} in beverages [37] using BPA-d_{14} as internal standard. Regarding the complex matrices such as fish [36], a LOD of 0.41 μg kg^{-1} was reported using BPA-d_{16} as internal standard. Nevertheless, few of the published analytical methods are fully validated in particular regarding trueness and accuracy.

The ubiquitous character of BPA is an additional challenge of BPA analysis [40,41]. From the sampling of the food items until the spectrometric signal production, BPA may enter unintentionally in the analytical chain. Sample collection material, analytical glassware, SPE cartridges, solvents, ultrapure water, various parts of the chromatograph are potentially BPA sources; they have to be tested before use and regularly checked batch-to-batch [3]. It is highly recommended to establish "blank" control chart to accept/reject analytical batches as well as to determine a concentration level below which it is not reasonable to report values for the samples.

Method validations follow different standards such as 2002/657/EC decision [42] and/or LAB GTA 26 [40] and SANCO/10684/2009 guideline [43,44].

The analytical method presented in this paper has been developed in the frame of the 2nd French Total Diet Study (TDS). It covers a large panel of solid and liquid foodstuffs and both detection and quantification limits performances were better than 0.03 and 0.09 μg kg^{-1}, respectively. Gas chromatography coupled to a tandem mass spectrometry (triple quadrupole device) has been preferred. Electron ionization and Selected Reaction Monitoring were found optimal to assure the robust quantification of BPA in foodstuffs. Quantification relied on the isotopic dilution principle using $^{13}C_{12}$-BPA as internal standard. The standard operating procedure is based onto a first liquid–liquid or liquid–solid extraction, depending on the matrix of interest and two consecutive solid phase extractions (non-polar stationary phase and MIP) followed by a derivatization step.

A particular attention was paid to environmental contamination by using a control chart with specified alert limits. Different possible analytical pitfalls have been pointed out during the study and propositions to manage them have been proposed. A validation protocol was eventually performed; performances are discussed in the light of exposure data generation.

2. Materials and methods

2.1. Standards and reagents

Bisphenol A [2,2-bis(4-hydroxyphenyl)propane, BPA] and $^{13}C_{12}$-BPA were obtained from Cambridge Isotope Laboratories (Andover, MA, USA). Acetonitrile and methanol (HPLC gradient-grade quality), cyclohexane and formic acid were purchased from Carlo-Erba Reagents (Rodano, Italy). Ultrapure water was purified using a Milli-Q-osmosis system from Millipore (Milford, MA, USA). Both solid phase extraction columns, i.e. HR-X and Affinimip BPA were obtained, respectively, from Macherey-Nagel (Hoerdt, France) and Polyintell (Val de Reuil, France). The derivatization reagent (N-methyl-N(trimethylsilyl)-trifluoroacetamide—MSTFA) was purchased from Sigma-Aldrich (Saint Quentin Fallavier, France).

Stock standard solutions of both BPA and $^{13}C_{12}$-BPA were prepared in acetonitrile at a concentration of 5 ng μL^{-1}. Working solutions were obtained by an appropriate dilution in acetonitrile. All standard solutions were stored at 4 °C, in the dark.

2.2. Scope of the analytical method

Samples analyzed in the French Total Diet Study (TDS2) were included in the scope of the developed method. Therefore, a list of more than 1200 food items or individual foods was concerned and was divided into around forty food groups including bread and dried bread products, breakfast cereals, pasta, rice and wheat products, croissant-like pastries, sweet and savoury biscuits and bars, pastries and cakes, milk, ultra-fresh dairy products, cheese, eggs and egg products, butter, oils, margarine, meat, poultry and game, offal, delicatessen meats, fish, crustaceans and molluscs, vegetables (excluding potatoes), potatoes and potato products, pulses, fruit, dried fruits, nuts and seeds, ice creams, sorbets and frozen desserts, chocolate, sugars and sugar derivatives, water, non-alcoholic beverages, alcoholic beverages, coffee, other hot beverages, pizzas, quiches and savoury pastries, sandwiches and snacks, soups and broths, mixed dishes, dairy-based desserts, compotes and cooked fruit, seasonings and sauces, specific foods.

2.3. Sample pretreatment

The analytical strategy was developed for the determination of BPA in a wide number of different categories of foodstuffs as expected in a total diet study. The matrices covered liquid food items such as water, milk, oil, wine, soft drink, coffee, tea, cream, as well as solid state matrices prepared as consumed such as vegetables, fruits, fish, crustacean, meat, ready-cooked dishes, biscuits, chocolate, cheese, desserts. The equivalent of 5 g of sample were homogenized after a milling step, and accurately weighted in a polypropylene tube. Then, 50 μL of an internal standard solution ($^{13}C_{12}$-BPA, 0.5 ng μL^{-1}) were directly added to the samples and mixed.

Table 1
MS/MS parameters for each molecule of interest.

Molecule	Signal	Transition	Retention time	Collision energy (eV)
Bisphenol A	1	357.2 > 191.2	8.21	10
	2	372.2 > 357.2		7
	3	357.2 > 207.2		7
	4	357.2 > 341.1		7
$^{13}C_{12}$-Bisphenol A	1	369.2 > 197.2	8.21	10
	2	384.2 > 369.2		7

2.4. Sample extraction and lipid content removal

Fifteen milliliters of acetonitrile were added to the fresh sample which was shaken 1 min in the vortex. The sample and acetonitrile were kept around 12 h at 20 °C. And then centrifuge at 4500 rpm, 30 min, 4 °C for protein precipitation. The supernatant was transferred to another polypropylene tube for further sample delipidation by 15 mL cyclohexane. After centrifugation, the acetonitrile phase was transferred to another polypropylene tube. Acetonitrile was then evaporated to dryness under a gentle stream of nitrogen at 45 °C. Final extracts were reconstituted by 2 mL water/methanol (90:10, v/v).

2.5. SPE clean up

For clean-up purposes two successive solid phase extractions (SPE) were performed. The first SPE was carried out using a polystyrene-divinylbenzene polymer with a moderate/high specific adsorption surface (>1000 $m^2\ g^{-1}$). The SPE cartridge was conditioned successively with 20 mL methanol and 10 mL water. After loading the 2 mL sample extract, the stationary phase was successively washed by 6 mL water, 8 mL water/methanol (90:10, v/v) and 4 mL water/methanol (40:60, v/v). Analytes elution was done by 10 mL methanol. After evaporation (N_2, 45 °C), 200 μL acetonitrile and 5 mL water were added. A specific Molecularly Imprinted Polymer (MIP) stationary phase was successively conditioned by 10 mL methanol/formic acid (98:2, v/v), 4 mL acetonitrile and 4 mL water. After loading the extract, the SPE was rinsed with 5 mL water, 3 mL water/acetonitrile (60:40, v/v) and 3 mL acetonitrile. The elution was carried out with 6 mL of methanol. The extracts were then evaporated to dryness under a gentle nitrogen stream (45 °C); the dry residue was reconstituted in 200 μL acetonitrile and finally transferred to an injection vial.

2.6. Derivatization process

The derivatization step for GC–MS/MS analysis consisted in a silylation reaction of BPA using N-methyl-N(trimethylsilyl)-trifluoroacetamide (MSTFA). Therefore, the 200 μL acetronitrile were evaporated to dryness and 20 μL of MSTFA was added to the dry residue and the vial was heated for 30 min at 45 °C.

2.7. GC–MS/MS instrument

A HP-6890 gas chromatograph coupled to a QuattroMicro GC triple-quadrupole (Waters®, Milford, MA, USA) was used for analytes identification and quantification. The GC column was a 30 m × 0.25 mm i.d., 0.25 μm film thickness, ZB-5MS (Phenomenex, Le Pecq France). The temperature program was set as follows: 120 °C (1 min), 40 °C min^{-1} until 250 °C (0 min), 5 °C min^{-1} until 280 °C (0 min), 10 °C min^{-1} until 300 °C (3 min). Injector and transfer line temperatures were set at 300 and 320 °C, respectively. Source temperature was set to 250 °C. Electron energy was set to 70 eV. Helium (Alpha II purity grade) was used as carrier gas at 1 mL min^{-1}. Argon was used as collision gas at 2.8×10^{-3} mbar in the collision cell. The splitless mode was set as follows: initial temperature: 300 °C, initial pressure: 1 kPa, purge pressure: 40 kPa, purge time: 1.5 min. The injection volume was 2 μL.

Four SRM transitions: 357.2 > 191.2, 372.2 > 357.2, 357.2 > 207.2 and 357.2 > 341.1 were monitored for BPA identification and two other transitions: 369.2 > 197.2 and 384.2 > 369.2 for $^{13}C_{12}$-BPA (see Table 1). BPA quantification was performed on the most intense BPA transitions (357.2 > 191.2) and the corresponding transition of ^{13}C-BPA (369.2 > 197.2). The other selected transitions of BPA (372.2 > 357.2, 357.2 > 207.2 and 357.2 > 341.1) were used for identification purpose. The dwell time was set at 0.1 s allowing about 15–20 points per peak. Then the quadrupole resolution was set at 12.5 and 13.5 on Q1 and Q3, respectively.

2.8. Quantification procedure

BPA quantification was achieved according to isotopic dilution method with the use of ^{13}C-BPA as internal standard (25 ng directly added to each homogenized sample). Area signals of BPA (357.2 > 191.2) and ^{13}C-BPA (369.2 > 197.2) were determined and the ratio between both molecules (BPA area/^{13}C-BPA area) was calculated. In parallel, a calibration curve was carried out in the range [0–100 μg kg^{-1}] and allowed the calculation of the Relative Response Factor (RRF) between BPA and its corresponding internal standard. Therefore, the quantity of BPA was determined according to the calculated ratio, taking into account RRF. Finally, BPA concentration was determined regarding the calculated quantity of BPA and its corresponding test sample weight.

2.9. Validation procedure

An in-house validation strategy based on the 2002/657/EC decision (guideline for validating method dedicated to chemical residues in food) was conducted. Specific elements of LAB GTA 26 (French accreditation Body, COFRAC) [40] and SANCO/10684/2009 [41] documents were used during the validation process. Specificity, trueness, stability, repeatability, within-laboratory reproducibility, linearity, recovery determination and detection/quantification limits were investigated. Specificity was determined by checking the absence of interfering compounds in the range of the expected retention time of the targeted analyte for different categories of foodstuffs (vegetable oil, dairy products, meat, fish, various solid matrices, butter and fruits). The trueness of the method was calculated by repeated analyses of a certified material (FAPAS, York, UK) and interlaboratory results (FAPAS proficiency test, ref. 1244) [45]. More than 60 repetitions of the certified material were performed under reproducibility conditions, i.e. operators, instrument, time range, batches of both solvents and standards, procedure materials. The corresponding intra-laboratory relative standard deviation (RSD) for the repeated certified material analyses was calculated. The within-laboratory reproducibility was also evaluated by performing minor and major changes such as matrices, levels of supplementation, operators, different GC–MS/MS instruments, and different batches of standards, solvents and materials. The global impact of these variations was

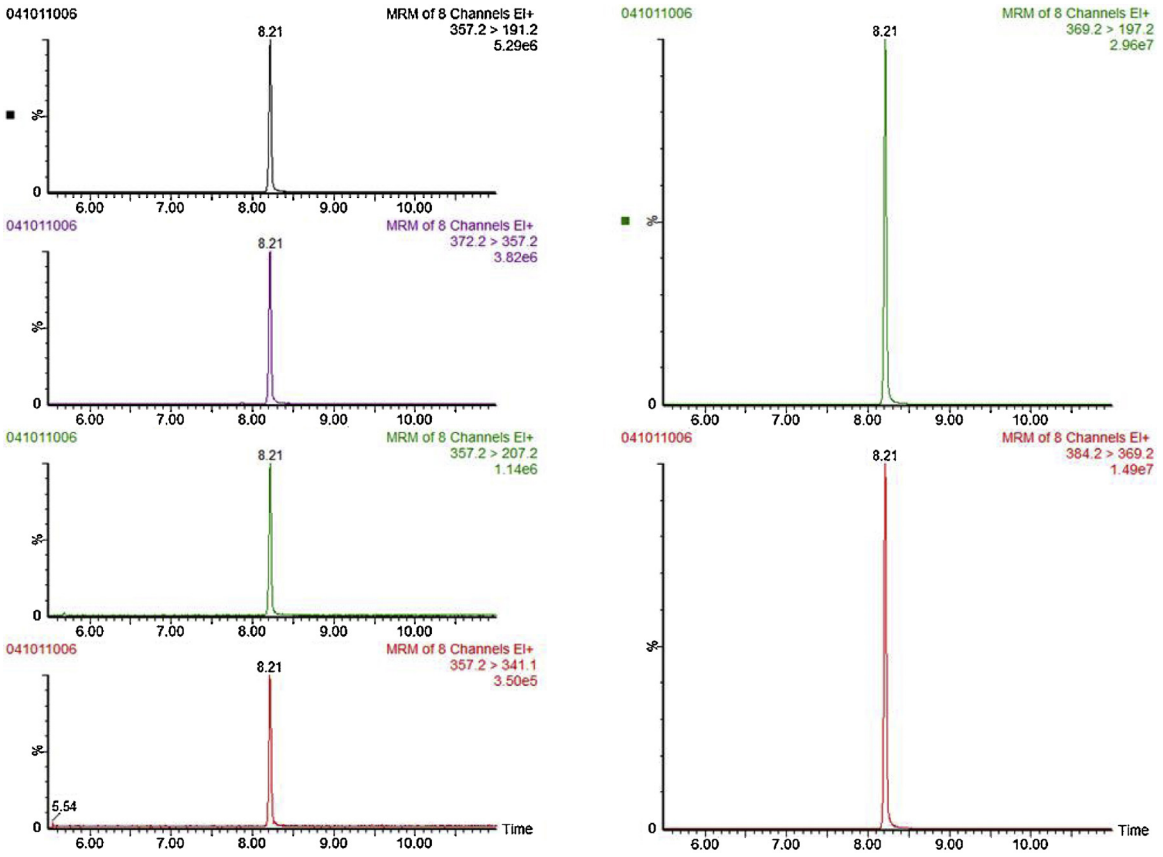

Fig. 1. GC–MS/MS chromatograms (SRM mode) of a standard solution corresponding to 1 ng injected on column. Four diagnostic transitions for BPA monitoring (left) and two diagnostic transitions for ^{13}C-BPA used as internal standard (right).

evaluated during the validation process. Stability of the analyte was checked throughout the development and validation process duration (more than 1 year). Linearity was determined by constructing a calibration curve using 12 levels of concentration in the range [0–100 μg kg^{-1}]. Moreover, repeatability was evaluated independently using 6 different representative food matrices (joint of pork, farmhouse bread, apple, butter, salmon and milk) spiked at two concentration levels in the range of interest, i.e. fortifications of 0.5 and 5.0 μg kg^{-1} which have been considered as realistic levels of food contamination by BPA. The recovery of the analyses was calculated for each sample; no correction factor was applied to derive BPA concentration (isotopic dilution). Both detection and quantification limits of the technique were calculated. Limit of detection (S/N = 3) and limit of quantification (S/N = 9) were calculated in all the samples included in the validation study.

3. Results and discussion

3.1. GC–MS/MS parameters

The MS/MS acquisition method was developed and optimized for providing both specificity [identification points (IP)] and sensitivity (as low as possible). The chromatograms shown in Fig. 1 illustrate the 4 monitored transitions for BPA and the 2 monitored transitions for $^{13}C_{12}$-BPA, which are identical to those used in other published results [10], at 1 ng BPA on column (equivalent to 1 μg kg^{-1}, 100% recovery). The Relative Response Factor (RRF) of BPA (357.2 > 191.2) *versus* $^{13}C_{12}$-BPA (369.2 > 197.2) was checked for each series of analysis on the basis of the results obtained with the calibration curve [0–100 μg kg^{-1}].

3.2. BPA background

The control of in-laboratory environmental contamination was considered as a major critical issue as already described in several articles [6,7,40,41,46]. Numerous contamination sources have been already highlighted, e.g. analytical measurement devices, solvents, SPE columns, glassware, plastic ware [10]. Therefore, since the various sources of contamination, the strategy applied in the present study consisted in a systematic measurement of the global BPA contamination due to the working environment in each series of samples. Three blank samples were included in each series; average and RSD of BPA was calculated for each series. A cut-off value was calculated on the basis of 60 series of analyses (±1200 samples) over a period 6 months. BPA values above this cut-off in a blank sample automatically generated the rejection of the corresponding series. This rejection limit was set at 0.20 μg kg^{-1}.

Finally, a corresponding mean was calculated to the equivalent of 0.150 μg kg^{-1} with an intra-series standard of variation of 0.065 (n > 60). The corresponding calculations of mean ± 2 and 3 × SD were, respectively, 0.28 and 0.35 μg kg^{-1}. Therefore, a Reporting Limit has been defined as the results of mean + 3 × SD. The developed strategy for the quantification of BPA in the analyzed samples finally allowed controlling the analytical contamination within each series of analysis throughout the analytical process and during the time.

3.3. Standard operating procedure

The standard operating procedure (Fig. 2) was developed and optimized for the determination of free BPA (active form) in foodstuffs, whatever the nature of the matrix of interest: i.e.

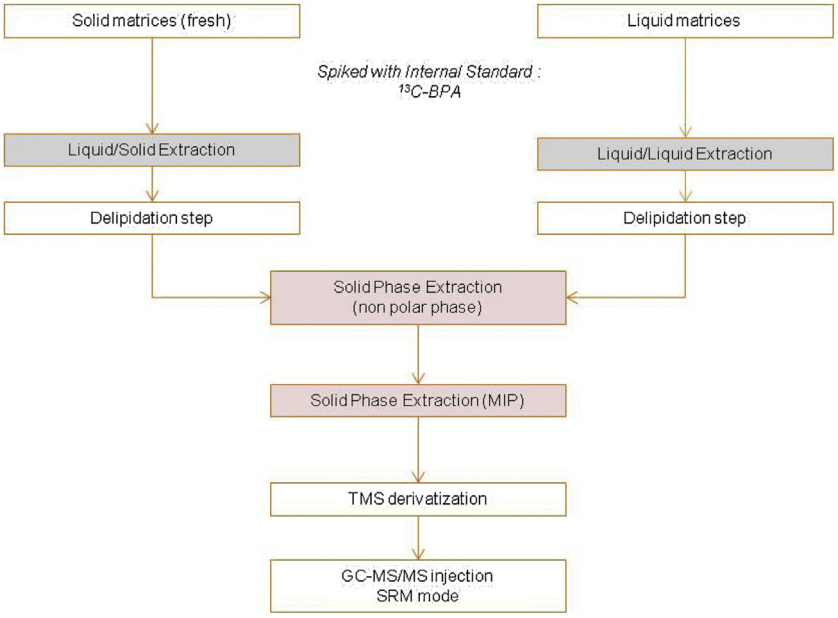

Fig. 2. Standard operating procedure for BPA determination in foodstuffs.

solids or liquids. Particular attention was given to the extraction step efficiency, especially for BPA determination in solid matrices. Recovery comparisons have been conducted on the solid–liquid extraction stages on freeze–dried. Acetonitrile was tested first. Representative food "incurred" samples including roast beef, sauerkraut and cassoulet were extracted with acetonitrile or water/acetonitrile (50:50, v/v). Measured concentrations were 0.59, 6.32 and 3.39 $\mu g\,kg^{-1}$ when acetonitrile was used, and 2.58, 12.6 and 24.2 $\mu g\,kg^{-1}$ when water/acetonitrile was tested. The efficiency was significantly concluded better when using water/acetonitrile (50:50, v/v). Additional trials showed that the results were not significantly different either the extraction was conducted using water/acetonitrile on freeze–dried samples or acetonitrile on fresh samples. These unexpected results clearly showed the different behaviors between incurred BPA and spiked ^{13}C-BPA added in freeze–dried samples. Indeed, acetonitrile extraction of freeze-dried sample lead to an underestimation of BPA concentrations in solid foodstuffs. To get the highest possible recovery, we investigated different SLE strategies. A vegetable sample (spinach), a ready-cooked dish (cassoulet) and a meat sample (calf) were used for that. Four successive and repetitive liquid/solid extractions have been carried out for each of the samples: a first extraction was done with acetonitrile at ambient temperature for 12 h, then the extracted samples undergone a second extraction with acetonitrile at 40 °C for 12 h, then a repetition of the second step was performed, and finally a pressurized liquid extraction (PLE) was conducted on the extract (acetonitrile, 60 °C, 100 bars, 2 cycles of 5 min). The first step permitted to extract 83–94% of the original BPA (83% for calf sample, 85% for spinach and 94% for cassoulet) [Table 2]. The sum of the three other accumulated extractions ranged from 6% to 17% of the total BPA extracted: the recovery observed ranged from 3% to 13%, 1% to 6% and 0% to 2% for the second, third and last extractions, respectively. Therefore, a single step extraction of BPA with acetonitrile (ambient temperature, 12 h) from fresh solid matrices was concluded to be adapted especially when isotope dilution approaches are used.

Table 2
Successive liquid/solid extractions efficiency.

Matrix of interest	First extraction: ACN, 15 h, ambient temperature	Second extraction: ACN, 15 h, 40 °C	Third extraction: ACN, 15 h, 40 °C	Fourth extraction: ASE (ACN, 60 °C, 100 bars, 2 cycles of 5 min)
Spinach	84.5	11.3	4.1	<0.1
Cassoulet	93.9	4.4	1.6	<0.1
Calf	81.5	13.7	2.7	2.1

Two consecutive orthogonal purification stages, i.e. a non-polar stationary phase combined to a selective molecular imprinted polymer were carried out. The selectivity was judged very good whatever the nature and the complexity of the food matrix. Fig. 3 illustrates the results obtained on various complex matrices. GC–MS/MS chromatograms representative of custard, crisps, salmon and corn can extracts are shown in Fig. 3a–d, respectively. Despite the complexity of the matrices, the detection and the identification were easily done even at trace concentration (0.06 $\mu g\,kg^{-1}$ for custard, 0.38 $\mu g\,kg^{-1}$ for crisps, 3.68 $\mu g\,kg^{-1}$ for salmon and 43.1 $\mu g\,kg^{-1}$ for corn can sample).

3.4. Validation results

3.4.1. Selectivity

The specificity of the method was assessed by verifying the absence of chemical and electronic interferences close to the chromatographic elution of BPA (±5 peak width). Various food matrices milk (skimmed, semi skimmed and whole milk), meat (pork roast), bread, butter, apple, fish (salmon) were fortified ($n = 6$) at two concentration levels (0.5 and 5.0 $\mu g\,kg^{-1}$). The relative BPA transition intensities (357.2 > 191.2/369.2 > 197.2) were calculated. The mean value of the transition's ratio and RSD did not exceed 20% (*cf.* 2002/657/EC decision), thus demonstrating the specificity of the method whatever the matrices of interest. The selectivity of the method was assessed by spiking blank samples with compounds characterized with close chemical structures, e.g. equol, bisphenol S (4,4′-sulfonyldiphenol), bisphenol E (4,4′-ethylidene-bisphenol) and bisphenol F (bis(4-hydroxyphenyl)methane). The absence of interferences was checked and eventually demonstrated in the chromatographic region of interest on the same MS/MS transition events. The capability of the method to provide even at low concentration levels unambiguous identification proofs of the identity

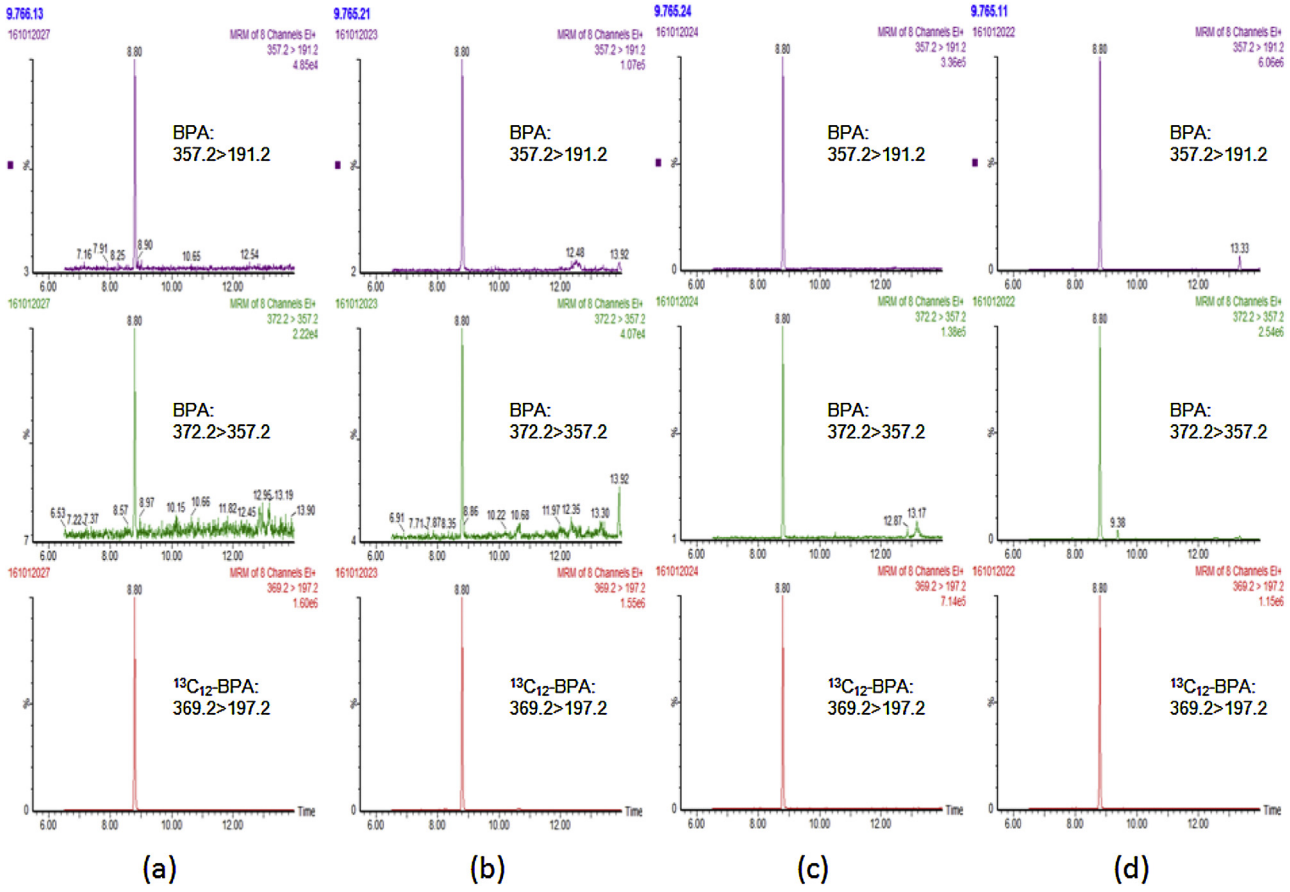

Fig. 3. GC–MS/MS chromatograms (SRM mode) of different foodstuffs: custard (a), crisps (b), salmon (c) and corn sample (d). Sample sizes were 5 g of fresh matrix. 357.2 > 191.2, 372.2 > 357.2 and 369.2 > 197.2 represent, respectively, the signals corresponding to the principal transition of BPA, the second one and the principal transition of $^{13}C_{12}$-BPA.

of BPA was investigated. The two criteria used were the relative chromatographic retention time (tolerance of 0.5% in unknown samples versus a reference sample) and the ratio between two specific MS/MS transitions tolerance of 20% between unknown and reference samples). Identification criteria have been demonstrated for BPA in all tested food items.

3.4.2. Linearity–calibration curve

The linearity of the developed method was determined by preparing calibration curves using standard solutions. The linearity was demonstrated in the range [0–100 $\mu g\,kg^{-1}$]. Thus, the calibration curve was constructed on the basis of 12 concentration levels with a majority of values ($n = 8$) in the [0.5–10 $\mu g\,kg^{-1}$] range. The linear regression was performed by plotting the relative peak height ratio (BPA: 357.2 > 191.2/^{13}C-BPA: 369.2 > 197.2) versus the analyte concentration. The intercept was not forced through the origin due to the contribution of BPA in-laboratory environmental contamination. The determination coefficient (R^2) was better than 0.9990 in most cases with residuals <20% on the relative response factors (RRF). A systematic dilution (×10) was carried out for all the standards which concentrations were above 15 $\mu g\,kg^{-1}$ to avoid any saturation of the corresponding signal. The applied strategy for the quantification of BPA took into account the relative factor response between BPA and its corresponding internal standard.

3.4.3. Trueness

The trueness of the method was also considered as a key issue in the validation process. Therefore, this parameter was evaluated on the basis of a repeated analysis ($n > 60$) of a certified material obtained from FAPAS with an assigned value (0.48 $mg\,kg^{-1}$). It has been completed by data generated during a participation in an international proficiency test. On the one hand, the mean of the certified material concentration in BPA was calculated upon 60 analyses to 0.50 $mg\,kg^{-1}$ which corresponds to a deviation of 4.2% in trueness. On the other hand, a Z-score of −0.2 was obtained to a 2011 international proficiency test organized by FAPAS (referenced 1244) [45]. The corresponding result was a deviation of −5.2% with respect to the assigned value. On the basis of both results, the trueness of the developed method was considered as fitting to the purpose.

3.4.4. Repeatability and within-laboratory reproducibility

The precision parameter was evaluated on the basis of results of both repeatability and within-laboratory reproducibility. Repeatability was evaluated by calculating the coefficient of variation (CV) for 6 matrices: meat, fish, bread, fruits, butter and milk. To this purpose, 6 replicates of the analyses of the various matrices fortified to 2 levels of concentration (+0.5 and +5.0 $\mu g\,kg^{-1}$) were carried out. The corresponding RSD ranged from 7.5% to 19.0% and from 2.5% to 12.2% for the fortification levels of 0.5 and 5.0 $\mu g\,kg^{-1}$, respectively. The different series were performed in repeatable conditions, i.e. same operator, same apparatus and same solvent batches. Repeatability – on the basis of the RSD – was below 20%. The results obtained for the repeatable analyses of the certified material obtained in reproducibility conditions (i.e.: different operators, periods of analysis, using different batches of solvents, materials and in some cases, another instrument) were used for

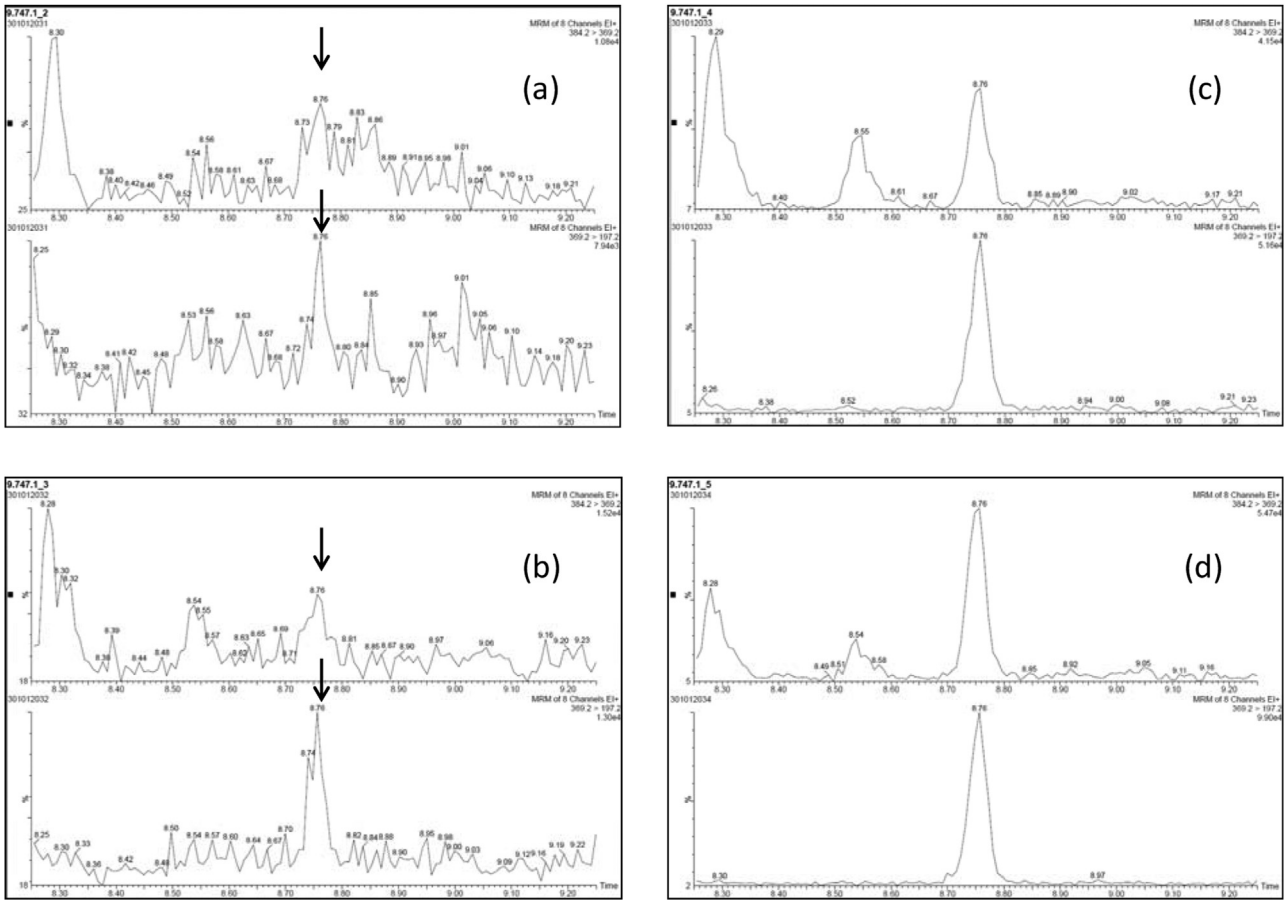

Fig. 4. LOD and LOQ determination. Cassoulet sample fortified with 0.01 μg kg^{-1} of ^{13}C-BPA (a), 0.03 μg kg^{-1} of ^{13}C-BPA (b), 0.10 μg kg^{-1} of ^{13}C-BPA (c) and 0.20 μg kg^{-1} of ^{13}C-BPA (d) for both diagnostic transitions of ^{13}C-BPA (384 > 369 and 369 > 197).

a global evaluation of the within-laboratory reproducibility. The corresponding coefficient of variation was 7.2%.

3.4.5. Detection and quantification limits

The limit of detection (LOD) is defined as the concentration for which the signal-to-noise ratio is better than 3 (S/N = 3). Because BPA is ubiquitous in the laboratory environment, the S/N has been calculated on the most intense transition of the internal standard ($^{13}C_{12}$-BPA). LODs were determined in 6 food matrices representative of the total diet study for which the developed analytical method will be used. The resulting LODs ranged from 0.01 to 0.03 μg kg^{-1} for milk and salmon, respectively.

The limit of quantification (LOQ) was deduced from the LOD by a factor of 3. The method ability, to provide identification criteria at this level of concentration, was checked, i.e. detection (S/N > 3) of two independent transitions and acceptable repeatability of the transition intensity ratio. LOQs ranged from 0.03 to 0.08 μg kg^{-1} Calculated LODs and LOQs were checked by spiking three different samples (spinach, cassoulet and veal escalope). The results obtained for the cassoulet sample are illustrated in Fig. 4. Therefore, the three samples have been fortified with ^{13}C-BPA at 0.01, 0.03, 0.10 and 0.20 μg kg^{-1}, concentrations which are equivalent to 1/3LOD (Fig. 4a), LOD (Fig. 4b), LOQ (Fig. 4c) and 2 × LOQ (Fig. 4d). ^{13}C-BPA has been fortified instead of native BPA for the same reason that explained before. Two specific transitions of ^{13}C-BPA were monitored (369.2 > 197.2 and 384.2 > 369.2); the observations at the different tested levels confirmed our previous calculated values, i.e. LOD < 0.03 μg kg^{-1} and LOQ < 0.10 μg kg^{-1}.

3.4.6. Other parameters

The quantification of the low BPA concentration levels was checked on the basis of the standard addition method. Several trials were performed for different types of foodstuffs (fish, biscuit and dessert). For example, the concentration of BPA in a chocolate spread was determined as <LOD. Different standard levels were added to 0, 0.1, 0.2 and 0.4 μg kg^{-1}. A calibration curve with a coefficient of determination (R^2) of 0.9906 was obtained [Table 3]. The concentration of BPA was calculated according to the intercept of the regression line, and was completely in accordance with the previous result (<LOD). In the same way, a first determination of BPA in a breaded fish and in pepper biscuits samples indicated a concentration of 0.18 and 0.17 μg kg^{-1}, respectively. The BPA standard

Table 3
Comparison of both quantification methods: single isotopic dilution and standard addition method.

Matrix of interest	[BPA] (μg kg^{-1}) "classical" method	Levels of fortifications (μg kg^{-1})	R^2	Intercept	[BPA] (μg kg^{-1}) determined according to the standard addition method
Chocolate spread	<LOD	0/0.05/0.1/0.2/0.4	0.9906	0.20	<LOD
Breaded fish	0.18	0/0.05/0.1/0.2/0.4	0.9753	0.39	0.21
Pepper biscuits	0.17	0/0.05/0.1/0.2/0.4	0.9774	0.37	0.19

was added to the same level of 0, 0.1, 0.2 and 0.4 μg kg^{-1}. The concentration of BPA was calculated to 0.21 μg kg^{-1} for the breaded fish sample and 0.19 μg kg^{-1}. Corresponding coefficients of determination of 0.9753 and 0.9774 were obtained; these results were also in accordance with the previous determination.

Moreover, the evaluation of the different validation parameters allowed the calculation of a global uncertainty taking into account the uncertainties of precision, trueness, homogeneity and standard purity [47–49]. The relative combined uncertainty was calculated to 11.1%. The resulting widened uncertainty, expressed with a 95% confidence range was determined to 22.2%.

3.5. Quality controls

Several quality controls were implemented to accept/reject each series of analyses. First criteria consisted in three repetitions of blank samples; the mean of the residual contamination was systematically calculated. The contamination was monitored batch-to-batch using a control chart. A certified material was introduced in each batch of samples and monitored by a second control chart. Both criteria have been used for the acceptation (or not) of the sample batch.

3.6. Application of the method to a large range of samples

The developed and validated method was successfully applied to a large variety of foodstuffs studied in the frame of the French Total Diet Study (TDS) for which more than 1000 samples have been analyzed. Samples such as liquid (water, soda, milk, wine, oil, etc . . .) and solid (fruits, vegetables, meat, fish, cheese, cakes, pre-cooked meal, etc., . . .) were quantified for free BPA level. In the course of this work, both maximum BPA concentrations were highlighted at 395 and 224 μg kg^{-1} for a pooled liver and a cooked meal, respectively. Generally, canned food products were quantified above 5 μg kg^{-1} (vegetables, meat and fish) with a maximum value of 83 μg kg^{-1} in a green beans sample. Additionally, "significant" BPA concentrations were found in several food items such as salmon (98 μg kg^{-1}) and canned tuna (57, 52 and 30 μg kg^{-1}). The corresponding full results of BPA contamination together with consumer's exposure will be published in the near future [50].

4. Conclusions

The accurate and sensitive determination of BPA in food sample is a pre-requisite for assessing human exposure. Sample preparation remained the most sensible stage for the determination of BPA in food and was therefore shown as the main drawbacks of available analytical methods [10]. The liquid–solid extraction was performed using acetonitrile and fresh solid samples. Moreover, we included a dedicated MIP sorbent in the SOP. GC–MS/MS was preferred to LC–MS/MS for its significant better chromatographic resolution capabilities as well as to decrease the probability of competitions for the charges during the ionization process. BPA trimethylsilylation was judged necessary prior GC–MS/MS analysis. Specificity and *in fine* sensitivity were enhanced by SRM acquisition of BPA residues. The trueness of the analytical approach were significantly improved thanks to isotopic dilution approach. Accurate quantification was made possible down to concentration level sensibly below than 0.50 μg kg^{-1}; detection and quantification limits were as low as 0.03 and 0.10 μg kg^{-1}, respectively. A full validation protocol was carried out for the determination of the parameters of interest: LOD and LOQ, specificity, linearity, trueness, precision and the uncertainty parameter. Global extended uncertainty (2U) was evaluated to 22.2%. This analytical approach has been already successfully implemented for the determination of more than one thousand foodstuff samples analyzed in the frame of the second French Total Diet Study [51].

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