



# Reliable quantification of bisphenol A and its chlorinated derivatives in human breast milk using UPLC–MS/MS method

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## ABSTRACT

Bisphenol A is a widespread industrial chemical which over the past decade has demonstrated its toxicity as an endocrine disruptor. Chlorine present in drinking water may react with bisphenol A to form chlorinated derivatives, which have demonstrated a heightened level of estrogenic activity. In this work, we have comprehensively validated a method using on-line SPE–UPLC–MS/MS and isotope dilution quantification to measure bisphenol A and its chlorinated derivatives in human breast milk according to accepted guidelines. Deuterated bisphenol A was used as internal standard. The matrix calibration curve ranged from 0.40 to 6.40 ng/mL for each of the target compounds and provided good linearity ( $r^2 > 0.99$ ). This method was precise (the intra and inter-day coefficient of variation was  $< 20\%$  at two different concentrations (0.40 and 3.20 ng/mL) and accurate (recovery ranged from 81% to 119%). The limits of detection obtained for BPA and its chlorinated derivatives ranged from 0.01 to 0.09 ng/mL. The limit of quantification for all the compounds validated at 0.40 ng/mL when using 500  $\mu$ L of milk was found to be suitable for the concentration existing in real samples. The analytical method developed in this study is in accordance with the requirements applicable to biomonitoring of BPA and its chlorinated derivatives in human breast milk.

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## 1. Introduction

More than one hundred man-made chemicals have been found to disrupt the endocrine systems of animals, and human beings are likewise affected. Among these endocrine-disrupting chemicals (EDCs), bisphenol A (BPA) is one of the high-volume compounds produced, and it is widely used in the production of polycarbonate plastics and epoxy resins [1]. As a result, it may be found in many common consumer products and is abundantly retrieved in the environment leading to widespread exposure to BPA among the general population.

BPA toxicity has been intensively investigated over the past decade and although it shows only weak estrogenic activity, recent studies have demonstrated the effects attributable to even minute doses of BPA [2]. Due to its toxicity, human exposure to BPA needs to be closely assessed in order to evaluate the potential health risk arising from its different sources. With this consideration in mind,

wide-ranging works have developed an analytical method of variable complexity meant to quantify BPA in numerous environmental settings. In these studies, BPA has been found throughout the environment: in natural water, air, dust, sewage, consumer products, food, drinking water, tickets, etc. Moreover, as has been demonstrated in wastepaper recycling treatment plants [3], when released into the environment, BPA may produce chlorinated derivatives in the presence of free chlorine [4]. In addition, the estrogenic activity of chlorinated derivatives may be higher than in parent compounds [5].

In environmental health impact assessment, human exposure can be evaluated using through ultratrace level determination of micro-pollutants in biological fluids and tissues (i.e. biological monitoring or biomonitoring) [6]. Biomonitoring has been proven to be at least as valuable as environmental measures in the estimation of human exposure to environmental contaminants [7]. In this field, a number of authors have proposed analytical methods measuring trace levels of BPA in different biological matrices (urine, blood, etc.) and thereby facilitating biomonitoring studies [8]. These different studies have employed analytical methods for BPA determination as widely ranged as enzyme-linked immunosorbent assay (ELISA), liquid chromatography coupled with fluorescence or electrochemical detection, gas

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chromatography with mass spectrometric detection and liquid chromatography–tandem mass spectrometry (LC–MS/MS). Among the existing alternatives, tandem mass spectrometry is considered the most specific, accurate and precise detection method used to measure trace levels of environmental chemicals, particularly in complex biological matrices [9].

Human breast milk has been proposed in the assessment of human exposure to environmental chemicals [10], particularly to EDCs [11]. Human breast milk is conducive to biomonitoring studies due to both the possibility of non-invasive sampling and to the relatively large volumes available in the framework of large-scale biomonitoring programs [12]. Moreover, human breast milk may serve as a biomarker of both maternal and prenatal exposure to many different environmental chemicals. Human breast milk could at once be a major route of exposure for breastfed infants and a valuable biological fluid in assessment of exposure to BPA and its chlorinated derivatives. As a consequence, several papers have reported on methods of BPA quantification in human breast milk [13–17]. There does not exist a large amount of published data on quantification of BPA chlorinated derivatives and as far as we know, only a limited number of methods have been dedicated to their determination in biological media (plasma, adipose tissue, placental tissue and recently urine) [18–21]. Unlike BPA, determination of BPA chlorinated derivatives in human breast milk has not to our knowledge been the object of any published study.

Whatever be their purpose, the development and application of chemical measurement methods require implementation of rigorous quality assurance/quality control procedures in accordance with acknowledged guidelines. In the field of biomonitoring, several papers have made recommendations pertaining to the characteristics of a scientifically robust analytical method [7,22,23], with some of them focused on human breast milk biomonitoring [24,25]. It may be concluded that the ideal analytical method for assessment of human exposure to environmental chemicals, in particular BPA and its chlorinated derivatives, should be ultrasensitive, with LOD and LOQ suited to a low level of exposure, highly specific, reproducible, conducive to comparison of results, and simple enough to be successfully applied to a large number of samples. Moreover, such a method will scrupulously respect several reliability criteria such as accuracy (trueness and precision), cross-contamination and correct validation of LOD and LOQ. Several international institutions have proposed an appropriate framework along with the attendant recommendations (NORMAN, FDA, ISO). However, most of the methods reported in the literature fail to provide sufficiently detailed data on these critical points. Concerning BPA, special attention has to be paid in order to avoid cross-contamination during the different steps of the assessment procedures [26]. And once again, it bears mentioning that most of the published data do not contain enough detailed information to ensure that no BPA contamination has occurred.

Given both the poor sensitivity of analytical techniques and BPA contamination arising from collection procedures, urinary concentrations of total BPA (free plus conjugated) have been proposed as a means of monitoring BPA exposure [27]. However, since conjugated BPA does not display any estrogenic activity, an issue has been raised as regards exposure assessment using total BPA, and that is yet another reason why every effort should be made to develop a method efficiently facilitating determination of unconjugated BPA in the media under consideration.

In light of these factors, we have fully developed a highly sensitive and accurate method to determine not only BPA, but also and for the first time the chlorinated derivatives of BPA, in human breast milk, using liquid–liquid extraction (LLE) followed by an online solid phase extraction–ultrahigh performance liquid

chromatography–isotope dilution tandem mass spectrometry method (SPE–UPLC–MS/MS).

## 2. Materials and Methods

### 2.1. Chemicals and reagents

BPA (CAS 80-05-7) and internal standard (IS) bisphenol A- $d_{16}$  (CAS 96210-87-6) were obtained from Sigma-Aldrich Inc. (St. Louis, USA). Chlorinated BPA were custom synthesized by @rtMolecule (Poitiers, France). The chlorinated BPA (CBPA, 2,6-DCBPA, 2,2'-DCBPA and TCBPA) were obtained from bisphenol A or its suitably protected precursor with regard to phenol function by direct chlorination using sulfuryl chloride [28]. The purity obtained for these compounds was >98%. The chemical structures of these compounds are shown in Table 1.

Of very high analytical grade quality, the methanol (Pestipur<sup>®</sup>) and water (Optima<sup>®</sup>) used during sample preparation were purchased from Carlo Erba Reagents (Val-de-Reuil, France) and Fisher (Illkirsch, France), respectively. The methanol used during LC–MS/MS analysis was LC–MS grade and supplied by Fisher (Illkirsch, France). Similarly, the water used during LC–MS/MS analysis was pre-treated and then purified by a Synergy<sup>TM</sup> system (Millipore, Bedford, USA). Nitrogen alphagaz-1 was purchased from Air Liquide (Paris, France). All the solvents and reagents were tested to ensure that they were free of contamination from compounds.

### 2.2. Milk samples

The breast milk used in standard solutions and quality controls was collected, under a well-designed protocol (briefly described below), from multiple anonymous donors who had been breast-feeding for over 1 month in order to minimize the possible presence of target compounds. On the other hand and in view of assessing the validity of the analytical method, three breast milk samples were collected from donors under the same conditions but within a few days after delivery. All of the samples were obtained under strictly controlled collection. In order to avoid contamination of target compounds, the milk was drawn manually and directly in pre-treated glass tubes, without using any device, materials, wipes or gloves. All samples were kept frozen at  $-20^{\circ}\text{C}$  until analysis.

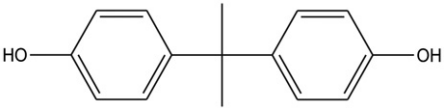
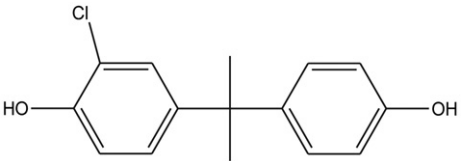
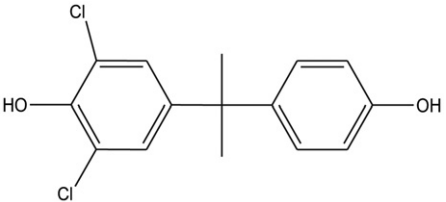
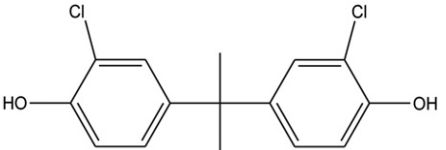
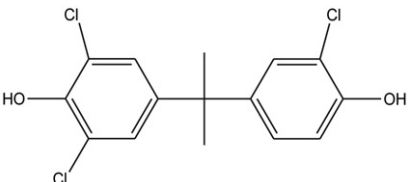
### 2.3. Preparation of standard solutions

A 200 mg/L methanol stock solution of each compound (BPA, CBPA, 2,6-DCBPA, 2,2'-DCBPA, TCBPA) was stored at  $+4^{\circ}\text{C}$ . Extemporaneously, the initial stock solutions were diluted in methanol/water 50/50 (v/v) to obtain working standard solutions at 4, 8, 16, 32 and 64 ng/mL used for spiked milk. Internal standard solution (BPA- $d_{16}$ ) was prepared in methanol/water 50/50 (v/v) at 32 ng/mL from initial stock solution (200 mg/L).

### 2.4. Sample preparation

Human milk was thawed and vortex mixed before use. Then, 50  $\mu\text{L}$  of working standard solutions and 50  $\mu\text{L}$  of IS were added to 500  $\mu\text{L}$  of milk samples and homogenized by shaking. After that, 4 mL of methanol were added and the samples were vortexed for 1 min, sonicated for 10 min and centrifuged at 3500g for 10 min. Supernatants were evaporated at  $60^{\circ}\text{C}$  to dryness under a gentle nitrogen stream. Residues were dissolved in 1000  $\mu\text{L}$  of water/methanol (70/30) solution. Finally, 50  $\mu\text{L}$  of extract were injected onto the SPE–LC–MS/MS apparatus.

**Table 1**  
Chemical structure of BPA and its chlorinated derivatives.

Compound	Structure
BPA	
2-Chloro-4-[1-(4-hydroxy-phenyl)-1-methyl-ethyl]-phenol CBPA	
2,6-Dichloro-4-[1-(4-hydroxy-phenyl)-1-methyl-ethyl]-phenol 2,6-DCBPA	
2-Chloro-4-[1-(3-chloro-4-hydroxy-phenyl)-1-methyl-ethyl]-phenol 2,2'-DCBPA	
2,6-Dichloro-4-[1-(3-chloro-4-hydroxy-phenyl)-1-methyl-ethyl]-phenol TCBPA	

### 2.5. On-line SPE–UPLC–MS/MS analysis

The concentrations of BPA and chlorinated derivatives were determined using a LC/MS/MS system consisting of an UPLC system Acquity<sup>®</sup> (Waters, Milford, USA), coupled to a Xevo<sup>®</sup> TQ triple quadrupole mass spectrometer (Waters, Milford, USA). An external six-port switching high pressure valve (Rheodyne<sup>®</sup> MXT 715-000 CIL, Ste Foy la Grande, France), controlled by the work station, was inserted between the autosampler and the chromatographic column in order to switch on or off the SPE column in the chromatographic system. The SPE column was an Xbridge<sup>®</sup> C8 10 µm 2.1 mm × 30 mm (Waters, Milford, USA) and the UPLC column was an ACQUITY CSH<sup>™</sup> C18 (1.7 µm particle size, 2.1 mm × 100 mm, Waters, Milford, USA).

Fifty microliters of the sample were loaded onto the SPE column using a 515 HPLC pump (Waters, Milford, USA), with MeOH/water (20/80) at a flow rate of 2 mL/min. For 2 min, the SPE column was washed with MeOH/water (20/80). After that, the valve switched on the SPE column so that the analyte retained on the SPE column was back-eluted by the UPLC pump at a flow rate of 0.40 mL/min. The gradient was programmed as follows: 2.0–2.5 min MeOH/water (50/50), then the amount of methanol increased linearly up to 90% (2.5–4.0 min) then up to 99% (4.0–4.5 min), then the methanol was kept constant at 99% during column clean up step (4.5–10 min), then the methanol linearly decreased to MeOH/water (50/50) (9.9–12 min), and the column

equilibrated with MeOH/water 50/50 (10–13 min). Concomitantly, the valve switched in the initial position at 11 min and SPE column equilibrated with MeOH/water 20/80 at 2 mL/min for 2 min. The temperature of the chromatography column was maintained at 40 °C in a column oven.

The MS–MS detector was equipped with an electrospray ionization (ESI) interface, operating in negative ionization mode. Quantitative analysis was carried out in the multiple reaction monitoring mode (MRM), using two specific combinations of a precursor–product ion transition for each compound. Precursor product transitions along with their corresponding collision energies are shown in Table 2. MS/MS detector conditions were set as follows: source temperature 150 °C; desolvation temperature 550 °C, cone gas flow 50 L/h, desolvation gas (nitrogen) 1000 L/h; collision gas (argon) 0.28 mL/min, capillary potential 3.5 V, cone potential –66 V, extractor potential –29 V.

### 2.6. Method validation

BPA contaminations may arise from laboratory accessories, reagent, SPE procedure, or the apparatus. In order to avoid contamination, only pre-treated glassware (500 °C, 5 h), teflon seals and high-quality solvent were used throughout the study.

Linearity of the chromatographic response was assessed on five different days using standard curves including 5 calibration points ranging from 0.40 to 6.40 ng/mL.

**Table 2**  
MS/MS parameters of BPA and its chlorinated derivatives.

Compound	Retention time (min)	MRM ( <i>m/z</i> )	Cone voltage (V)	Collision energy (V)	Dwell time (s)
BPA	4.55	227.0–211.9	38	18	0.150
		227.0–133.0	38	27	0.150
BPA- <i>d</i> <sub>16</sub>	4.55	241.1–223.1	38	20	0.120
		241.1–142.0	38	26	0.120
CBPA	4.71	260.1–181.9	41	27	0.007
		260.1–209.9	41	24	0.007
DCBPA	4.86	294.9–215.9	37	30	0.015
		294.9–243.9	37	24	0.015
2,2'-DCBPA	4.86	294.9–166.9	37	24	0.015
TCBPA	5.01	328.8–249.9	44	30	0.020
		328.8–277.8	44	25	0.020

Sample concentrations were determined for each compound using the corresponding spiked milk standard curve calibration. Calibration curves were constructed using compounds/IS peak area ratio versus compound concentration. BPA-*d*<sub>16</sub> was used as an internal standard for all target compounds since the corresponding labeled standards were not readily available.

Accuracy was determined by analysis of quality controls performed using human breast milk (exempt from target compounds) spiked at two different concentrations (0.40 ng/mL and 3.20 ng/mL).

Intra-day coefficient of variation was obtained with replicates of quality control sample at 0.40 ng/mL (*n*=5) and 3.20 ng/mL (*n*=3). Inter-day coefficient of variation was obtained from experiments performed on five separate days of quality control sample at 0.40 ng/mL and 3.20 ng/mL.

The limit of detection (LOD) was defined according to NORMAN guidelines based on ISO/DIS 13530 as three times the standard deviation of a blank sample of human breast milk [29]. According to several guidelines, the limit of quantification (LOQ), set at the level of the lowest calibration standard, was fully validated using quality controls performed using milk samples spiked at this low concentration.

### 3. Results and discussion

#### 3.1. Method optimization

Several studies have likewise reported BPA contamination from reagents or solvents or leaching from the materials during sampling, storage, processing, and analysis [8,26,30]. Therefore, special attention should be paid when proceeding. In the present study, use of high-purity solvents along with glass instead of plastics, and the comprehensive implantation of specific purification procedures allowed us to eliminate BPA contamination. Chromatograms of a blank solvent are presented in Fig. 1. While BPA was detected in some of the pooled breast milk used for standards and quality controls, it was always at a level markedly lower than the limit of quantification (between 0 and 0.12 ng/mL) and has been taken into account in the calculation method (Fig. 2). To summarize, the BPA/IS peak area ratio of blank milk was subtracted from the BPA/IS ratio of each standard used to construct the calibration curve. On the other hand, no BPA chlorinated derivatives were detected in any of the pooled breast milk.

Only a limited number of studies have reported analytical methods of BPA quantification in human breast milk. One of them

used ELISA [15] but due to the non-specificity of the anti-BPA antibody, cross-reactivity may occur. Moreover, the mean concentration of BPA determined in this study was higher than in others; as previously shown, this may have been due to its having been overestimated [31]. Consequently, ELISA cannot be considered suitable for BPA exposure monitoring [8]. Among the other papers, some used fluorescence detection [13,16] or GC–MS [17], whereas only one study employed the isotope dilution tandem mass spectrometry method [14] so as determine BPA. On the other hand, to our knowledge, no study has been published concerning the determination of BPA chlorinated derivatives in human breast milk. Moreover, there does not exist a substantial amount of published data on quantification of BPA chlorinated derivatives. As far as we know, few methods have been suggested as means of determining chlorinated BPA in biological fluids (plasma, adipose tissue, placental tissue and, recently, urine) and not all of them have used the isotope dilution tandem mass spectrometry method. This work is consequently the first to put forward an analytical method adapted to large-scale biomonitoring studies aimed at assessing exposure to BPA and its chlorinated derivatives through use of human breast milk.

BPA chlorination may provide two dichlorobisphenol A isomers (2,2'-DCBPA+2,6-DCBPA, Table 1). Since the response in mass spectrometry is structurally dependent, accurate determination of these two isomers requires the use of pure synthetic reference compounds for calibration [32]. Contrarily, use of a mixture or only one of the isomers as a calibration standard of dichlorobisphenol A may provide biased results [33]. To our knowledge, no independent determination of both dichlorobisphenol A isomers existed prior to the present study. Under our chromatographic conditions, the two isomers could not be separated.

Moreover, if a specific transition was available for quantitation of 2,2'-DCBPA, no specific transition providing sufficient intensity was found to be available for 2,6-DCBPA. On the other hand, two transitions were shared by the two isomers, which produced the same signal intensity and enabled quantification of the total amount of DCBPA (2,2'-DCBPA+2,6-DCBPA) (Table 2). Finally, quantification of 2,6-DCBPA was obtained by subtracting the amount of 2,2'-DCBPA from the total DCBPA obtained. In this way, we were able to independently quantify both dichlorobisphenol A isomers.

#### 3.2. Matrix effects and extraction recovery

Even though liquid chromatography with MS/MS detection is considered the method of choice in quantitative determination of environmental chemicals in biological fluids, matrix effects may compromise accuracy [34]. Nevertheless, most of the published papers dealing with BPA or BPA and its chlorinated derivatives do not mention matrix effects, which were observed on a significant scale during our experiments and led to correspondingly significant signal intensity inhibition. Several approaches involving sample preparation (LLE, SPE, SPE online), LC separation (UPLC), or use of isotopically labeled standards may help to overcome these effects [33]. In this study, we coupled UPLC to tandem mass spectrometry, but even after LLE plus online SPE, significant matrix effects were still observed. While BPA-*d*<sub>16</sub> was used as internal standard, corresponding labeled compounds were not readily available for BPA chlorinated derivatives, so we decided to attempt another approach in order to compensate for matrix effects and to guarantee accuracy. Since sample dilution (the so-called dilute and shoot procedure) has been proposed as a strategy to reduce matrix effects [35], we evaluated the impact on matrix effects of the dilution of samples prior to injection, thereby increasing the volume used to dissolve the residue obtained after LLE. Following LLE extraction, blank samples of

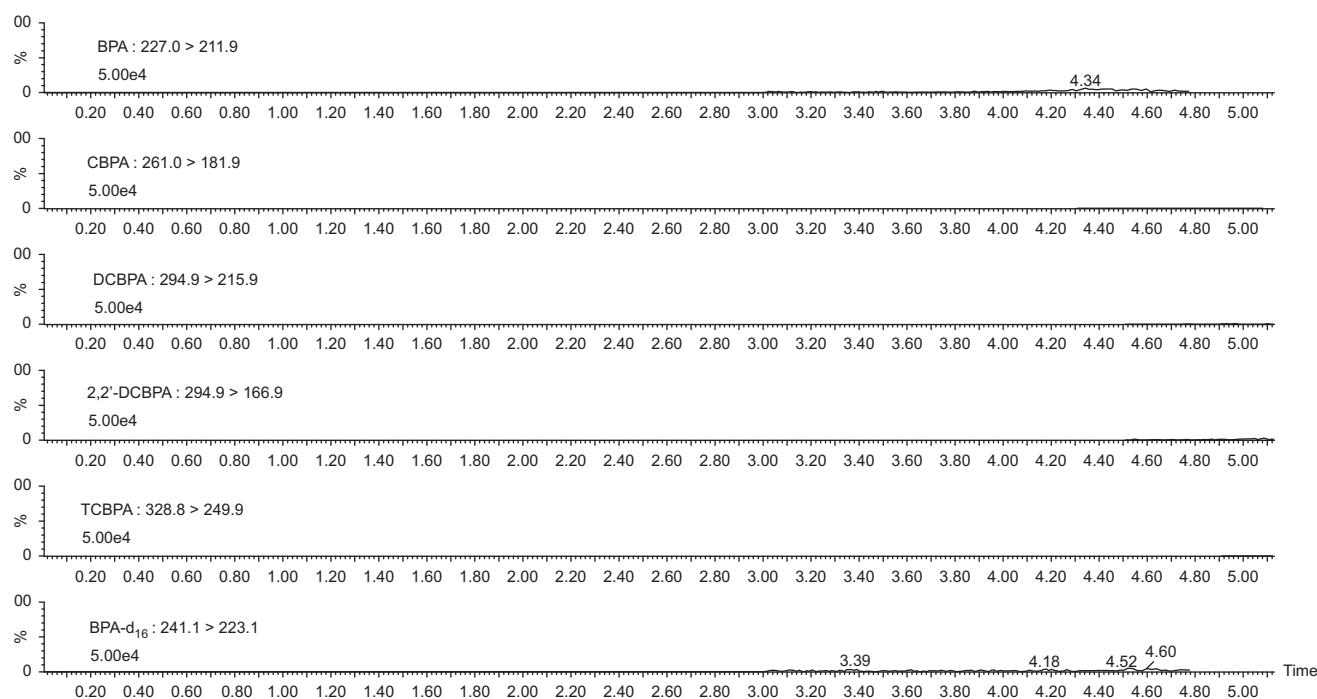


Fig. 1. Chromatograms of a blank solvent.

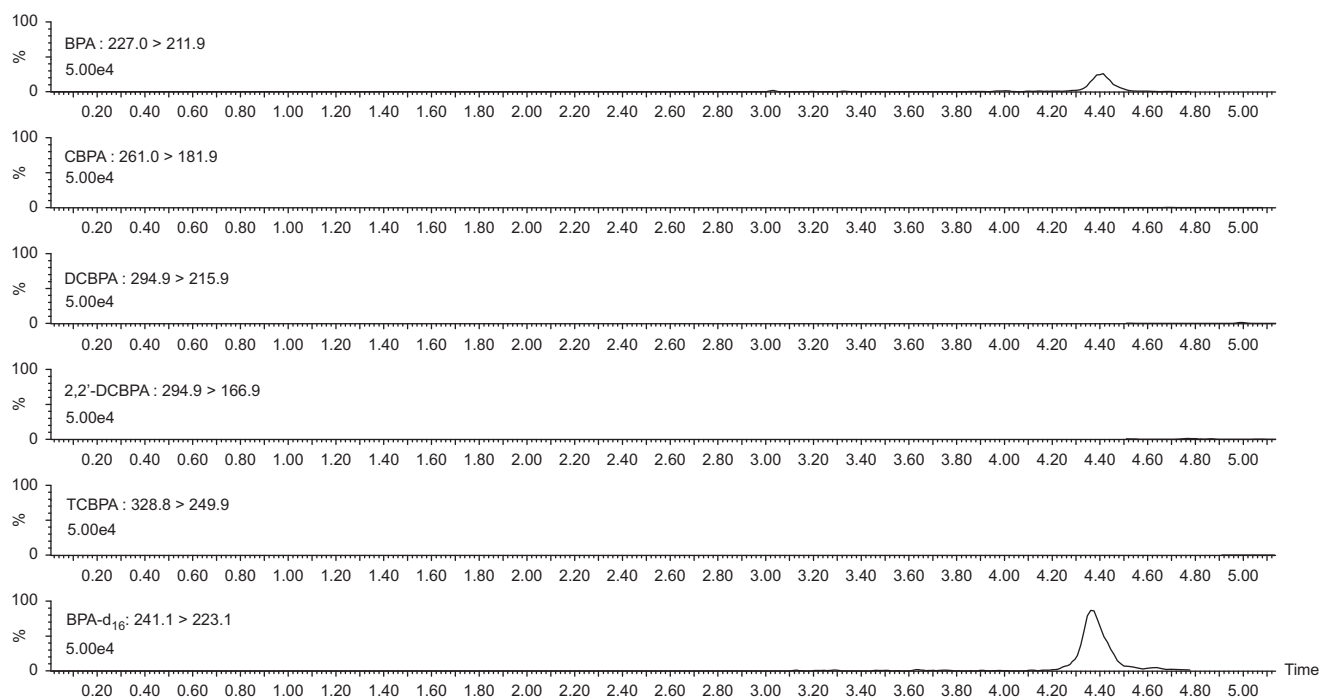


Fig. 2. Chromatograms of a blank milk containing BPA at a low level (0.10 ng/mL).

human breast milk ( $n=3$ ) were spiked, at 3.20 ng/mL and then diluted using different volumes of mobile phase (0.1, 0.2, 0.5, 1.0 and 2.0 mL) before injecting onto SPE-UPLC-MS/MS. Reconstitution with 1.0 mL of mobile phase yielded complete recovery of the signal for all the target compounds, compared to a standard injected under the same conditions. However, due to the variability of human breast milk composition, matrix effect may still exist. Therefore, the use of isotopically labeled standards should be maintained.

Finally, online SPE recovery was assessed by comparing the response obtained after direct injection ( $n=3$ ) of a standard

solution at 3.20 ng/mL to the response obtained after injection onto SPE-UPLC-MS/MS of the same standard. Levels of extraction recovery greater than 80% were obtained for the five target compounds compared to direct injection of the same samples onto UPLC-MS/MS.

### 3.3. Analytical performance

Calibration curves of BPA and chlorinated BPA provided adequate linearity as shown by the correlation coefficients, which are greater than 0.99. Chromatograms of a milk standard spiked at 0.8 ng/mL are



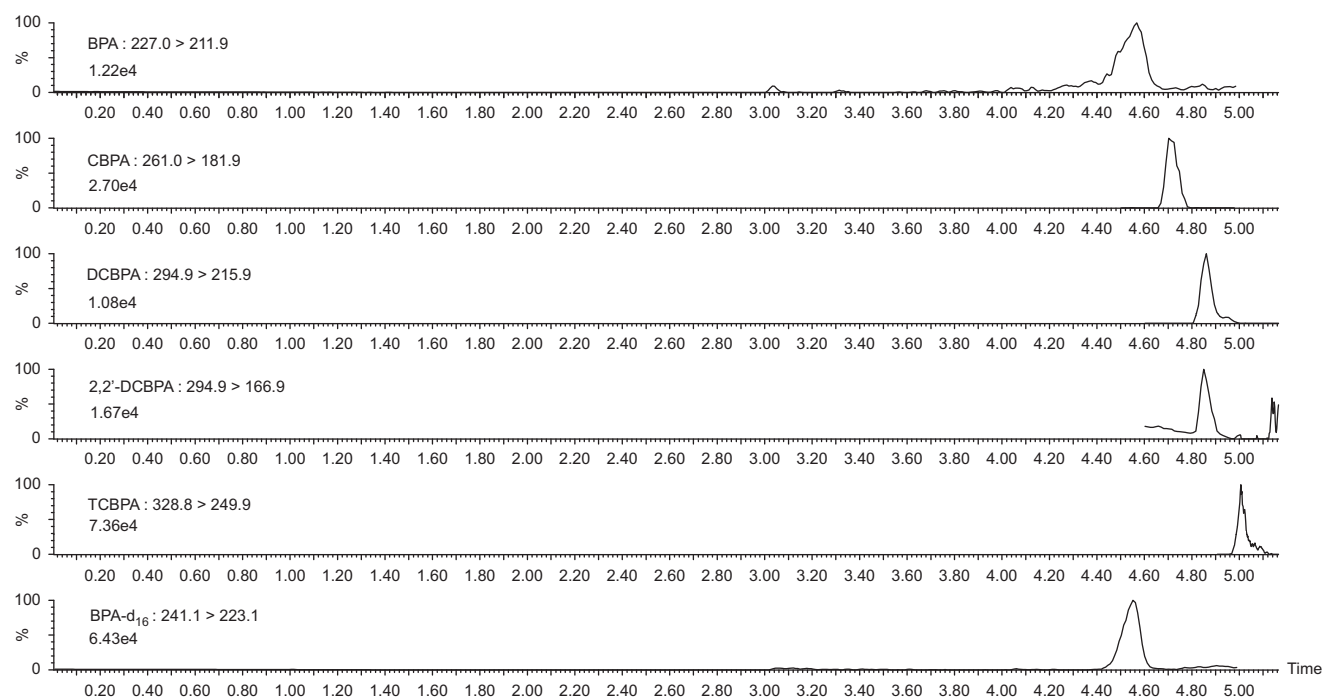


Fig. 3. Chromatograms of a milk standard spiked at 0.8 ng/mL.

**Table 3**  
Analytical performance parameters.

Compound	LOQ <sup>a</sup> (ng/mL)	LOD <sup>b</sup> (ng/mL)	Spiked concentration (ng/mL)	Intra-day mean (n=5)		Inter-day mean (n=13)	
				Trueness <sup>c</sup> (%)	Precision <sup>d</sup> (%)	Trueness <sup>c</sup> (%)	Precision <sup>d</sup> (%)
BPA	0.40	0.09	0.40	101	15	103	11
			3.20	93	1	98	14
CBPA	0.40	0.01	0.40	90	6	98	15
			3.20	81	15	99	17
2,6-DCBPA	0.40	0.05	0.40	81	18	98	19
			3.20	107	20	92	16
2,2'-DCBPA	0.40	0.05	0.40	91	6	103	15
			3.20	119	2	107	15
TCBCPA	0.40	0.04	0.40	103	18	109	14
			3.20	91	7	97	15

<sup>a</sup> Limit of quantification.

<sup>b</sup> Limit of detection.

<sup>c</sup> Expressed as [(mean observed concentration)/(nominal concentration)] × 100.

<sup>d</sup> Expressed as relative standard deviation.

presented in Fig. 3. The validation results for the method proposed in this study are presented in Table 3. Trueness of the five target compounds ranged from 81% to 119% and their precision was equally satisfactory, as evidenced by RSD values  $\leq 20\%$ . Moreover, these results are consistent with the current accepted guidelines, especially those applied in the field of bioanalytical or environmental chemistry [29,36].

The estimated LOD obtained for BPA and its chlorinated derivatives ranged from 0.01 ng/mL to 0.09 ng/mL (Table 3). While other studies have proposed lower LOD for BPA, particularly rigorous determination of LOD was performed in this work, as should be the case in any development of an analytical method suitable for biomonitoring studies in which concentration levels below the LOD have got to be statistically treated. Therefore, merely reporting measurements < LOD as non-detectable is not adequate, and it is clearly imperative that a precise number be assigned. Nevertheless,

in many biomonitoring papers, the method used to determine LOD is missing or poorly described. The S/N method is frequently proposed so as to estimate BPA LOD in human breast milk, and other researchers have used standard deviation of a low-level standard. As regards BPA quantification, these methods fail to take into account potential background contaminations. Therefore, LOD estimation would be improved through use of a blank sample (i.e. non-fortified media) in order to determine a value that statistically differs from zero, as proposed by the method applied in this study.

In this study, the LOQ determined for BPA was set at 0.40 ng/mL. Only one paper has reported a better LOQ (0.21 ng/mL) using GC–MS analysis, but this study proposed a highly time-consuming sample preparation step and required a relatively large volume of milk (25 g). The other authors did not report any LOQ or LOQ greater than the one validated in this study, in which chlorinated BPA presents the same LOQ, set at 0.40 ng/mL.

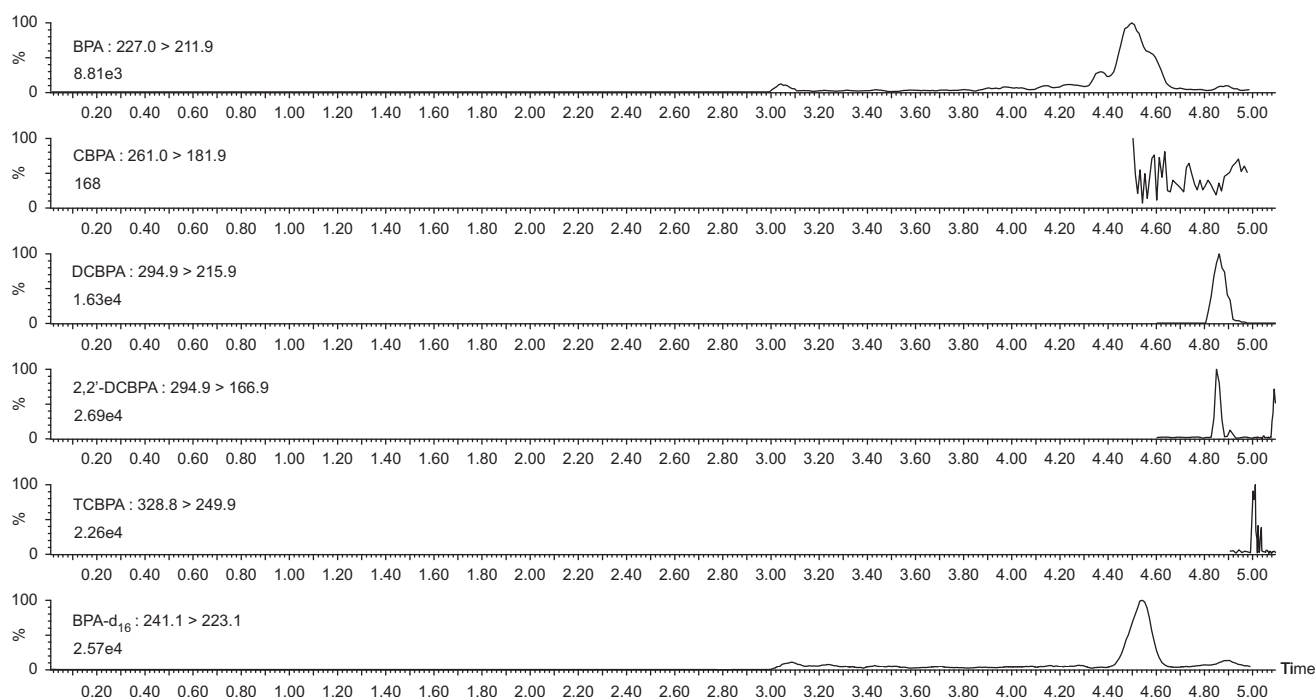


Fig. 4. Chromatograms of milk sample no. 3.

Table 4

Concentration (ng/mL) of BPA and its chlorinated derivatives in three human breast milk samples.

Samples	BPA	CBPA	2,2'-DCBPA	2,6-DCBPA	TCBPA
Milk 1	0.80	< LOD <sup>a</sup>	1.09	0.97	< LOD <sup>a</sup>
Milk 2	3.29	< LOD <sup>a</sup>	4.13	< 0.40	0.68
Milk 3	3.07	< LOD <sup>a</sup>	< 0.40	1.40	< LOD <sup>a</sup>

<sup>a</sup> Limit of detection.

Whereas no data are available concerning LOQ of BPA chlorinated derivatives in human breast milk, most of the studies carried out with other biological matrices reported equivalent LOQ. One of them reported better LOQ, but it had been determined in standard solution not using spiked matrix.

### 3.4. Method application

Fig. 4 shows a chromatogram obtained from a human breast milk sample collected within a few days after delivery in which BPA and some of its chlorinated derivatives were found. BPA, 2,2'-DCBPA, 2,6-DCBPA and TCBPA have been detected and/or quantified in the three samples analyzed, but none of them were found to contain CBPA (Table 4).

## 4. Conclusion

Comparability and reliability of monitoring data are essential to any meaningful environmental exposure assessment involving the management of environmental risks. With regard to emerging pollutants, there is cause for concern as to the comparability of data at the international level. Generally speaking, the methods used when monitoring emerging pollutants have yet to be validated to a sufficient extent.

It bears mentioning, in this respect, that the chemist has a particular responsibility to assume in ensuring the reliability of the results obtained in biomonitoring studies. Bearing this basic

exigency in mind, the method comprehensively developed and validated in this study provides ultrasensitive quantification allowing for reliable determination of BPA and its chlorinated derivatives, while the reported LOD and LOQ are altogether consistent with the concentrations observed in actual human breast milk. Full observance of the requirements mentioned above is essential to assessment of exposure to BPA and its chlorinated derivatives for individuals, along with the major decision-makers in public health.

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