Talanta 204 (2019) 145–152

Contents lists available at ScienceDirect

Talanta

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

Development and validation of an LC–MS/MS method for the simultaneous determination of bisphenol A and its chlorinated derivatives in adipose tissue☆

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ARTICLE INFO

Keywords:
Adipose tissue
Bisphenol A and its chlorinated derivatives
Endocrine disruptors
Breast
Prostate
Hormone-dependent cancer

ABSTRACT

Bisphenol A (BPA) and its chlorinated derivatives (Clx-BPA) are environmental pollutants exhibiting endocrine-disrupting (ED) properties suspected to be involved in the pathogenesis of hormone-dependent cancers, such as breast and prostate cancers. Due to their lipophilic properties, they may accumulate in adipose tissue which could therefore be a suitable matrix to assess long-term exposure to these compounds and relationships with the tumorigenesis of these cancers. An LC-MS/MS assay for the determination of BPA and Clx-BPA in adipose tissue samples was developed and fully validated according to current bioanalytical validation guidelines. Ionization was achieved using an electrospray source operating in the negative mode and quantification of target analytes was obtained in the multiple reaction monitoring mode. Both standard and quality control (QC) samples were prepared in blank adipose tissue samples. Linearity was demonstrated over the ranges 0.125 to 8.000 and 0.0125–0.8000 ng/mL for BPA and Clx-BPA, respectively. Accuracy and precision were demonstrated over the whole concentration range: intra and inter-day bias values were in the 85–114% range and imprecision of the method did not exceed 14%. Lower limits of quantification were validated using QCs at 0.1250 and 0.0125 ng/mL for BPA and Clx-BPA, respectively. Internal standard-corrected matrix effects were comparable in breast and prostate adipose tissues, demonstrating that this method could be used to reliably assay BPA and Clx-BPA in both tissues. The method was sensitive enough to determine BPA and Clx-BPA in breast adipose tissue obtained from women undergoing breast surgery, enabling identification of different patterns of exposure to these ED chemicals. The method enables the reliable quantification of BPA and Clx-BPA in adipose tissue and could be used to assess long-term exposure to these compounds and potential associations with hormone-dependent cancers.

1. Introduction

Bisphenol A (BPA) is a well-known ubiquitous environmental pollutant exhibiting endocrine-disrupting properties [1]. Chlorinated derivatives of bisphenol A (Clx-BPA) are formed by chlorination of bisphenol A present in water during water-disinfection process. As a result Clx-BPA have essentially been observed in various aqueous media such as tap water [2,3], sewage sludge [4] and wastewater of paper recycling plant [5]. Clx-BPA have also been found in human biological matrices: urine [6–8], placental tissue [9], breast milk [10] and colostrum [11]. Clx-BPA exhibit endocrine-disrupting properties: *in vitro* they show higher (up to 100 times) estrogenic activities than BPA [12]. Riu et al. have demonstrated that Clx-BPA are human PPARγ ligands and that activation of PPARγ depends on the degree of halogenation [13]. In humans, Clx-BPA exposure has been associated with the occurrence of type 2 diabetes [14] and obesity [15].

Due to direct activation of the estrogen receptor, BPA may have an impact on carcinogenesis in hormone-dependent cancers such as breast [16] and prostate cancers [17]. This is also the case for Clx-BPA [18]. The agonist effect of BPA and Clx-BPA on PPARγ receptors may also

☆ Declarations of interest: none.

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https://doi.org/10.1016/j.talanta.2019.05.103
Received 18 January 2019; Received in revised form 22 May 2019; Accepted 27 May 2019
Available online 28 May 2019
0039-9140/

have an impact on tumoregenesis in hormone-dependent cancer [19,20] and they may exert indirect tumoregenesis activity through obesity and inflammation [21,22].

Assessing human exposure to environmental pollutants is a major component of health risk assessment [23]. For that purpose, several approaches have been proposed and determination of exposure can be either indirect or direct. However, direct measurement of environmental pollutants in human body fluids, commonly urine or blood, is now considered as the method of reference. This is the so-called Human Biomonitoring (HBM) that reflects the total body burden resulting from all routes of exposure [24].

As a means of relating BPA and Clx-BPA exposure to cancer pathogenesis, blood or urine concentrations are probably poor biomarkers of exposure due to short biological half-life, at least for BPA [25]. It has been demonstrated that persistent [26] as well as non-persistent pollutants [27,28] may accumulate in adipose tissue due to their lipophilic properties. These results suggest that concentration in adipose tissue may be a suitable biomarker in assessment of long-term exposure [28].

Not many methods have been reported for the determination of BPA in adipose tissue. One is based on gas chromatography-mass spectrometry (GC-MS) [29] while others are based on LC-MS/MS [27,28,30]. To our knowledge, no LC-MS/MS method has been reported for the simultaneous determination of BPA and Clx-BPA. Fernandez et al. have briefly reported on a GC-MS method [31].

Therefore the objectives of this study were to develop and validate an LC-MS/MS assay for simultaneous determination of BPA and Clx-BPA in adipose tissue and to apply the validated method to a set of real samples in view of demonstrating its suitability and relevance.

2. Materials and methods

2.1. Chemicals and reagents

Bisphenol A (BPA) and its deuterated analogue (BPA-d_{16}) were obtained from Sigma-Aldrich® (St Louis, USA). Chlorinated derivatives of BPA (Clx-BPA) and the deuterated analogue of dichlorobisphenol A (2,2'-DCBPA-d_{12}) were purchased from @rtMolecule® (Poitiers, France). Acetonitrile, methanol and water were acquired from Carlo Erba® (Val de Reuil, France). They were of Ultra-High Performance Liquid Chromatography (UHPLC) grade and we demonstrated that they were free of BPA and Clx-BPA. Materials in glass or polypropylene were used to avoid any BPA contamination. MIX I ($MgSO_4$, NaCl, Na_2H citrate - 1.5 H_2O, Na_3 Citrate - 2 H_2O) and MIX VI ($MgSO_4$, Diamino PSA (Primary Secondary Amine), C_{18} ec (Octadecyl modified silica phase, endcapped)) were purchased from Machery-Nagel® (Hœrdt, France).

2.2. Preparation of stock solutions, working solutions and spiking solutions

Analyte stock solutions were prepared in methanol at 10 µg/mL for BPA and 1 µg/mL for Clx-BPA. Deuterated internal standard (IS) stock solutions were at 10 µg/mL for BPA-d_{16} and 1 µg/mL for 2,2'-DCBPA-d_{12}. Stock solutions were stored at −20 °C before use. Each day of analysis, two working solutions were freshly prepared in methanol: one with analytes at 80 ng/mL and 8 ng/mL for BPA and Clx-BPA, respectively, and one at 40 ng/mL and 4 ng/mL for BPA-d_{16} and 2,2'-DCBPA-d_{12}, respectively. Working solutions were diluted with methanol to obtain spiking solutions ranging from 1.25 ng/mL to 80.00 ng/mL and from 0.125 to 8.000 ng/mL ng/mL for BPA and Clx-BPA, respectively. These solutions were used to prepare calibration standards in adipose tissue.

2.3. Sample collection

Samples of adipose tissue were obtained from patients during breast or prostate surgery at the university hospital of Poitiers, France. Patients who had given their informed consent after having been fully informed could be enrolled in this study. Samples were collected in glass tubes to avoid contamination with BPA. All samples were stored at −20 °C before use.

2.4. Sample extraction

Each sample of adipose tissue (100 mg) was homogenized in a clean glass tube with 500 µL of water using an Ultra-Turrax® (IKA® T18 Basic), and spiked with 50 µL of IS working standard solution. Calibrant samples were prepared in adipose tissue by spiking tissue homogenate with 50 µL of spiking solutions to obtain final concentrations ranging from 8.000 ng/mL to 0.125 ng/mL and 0.8000 to 0.0125 ng/mL, for BPA and Clx-BPA, respectively. For blank and unknown samples, spiking solution was replaced by 50 µL of pure methanol. For double-blank samples, spiking and IS solutions were replaced by 100 µL of pure methanol. Then 500 µL of acetonitrile were added to homogenates. Samples were vortex-mixed for 1 min and 100 mg of MIX I were added. pH was adjusted to 5.5 with NaOH 1 M, vortex-mixed again for 1 min and centrifuged at 2330 × *g* for 5 min. The upper organic layer was transferred into a clean glass tube, 100 mg of MIX VI were added, vortexed again for 30 s and centrifuged at 2330 × *g* for 5 min. The upper organic layer was transferred into a clean glass-tube and evaporated to dryness at 37 °C under a gentle nitrogen stream. Dry residues were dissolved in 200 µL of a water/methanol (70/30, v/v) solution, vortex-mixed and transferred into glass vials for injection. Finally, 20 µL of extract were injected into the LC–MS/MS system.

2.5. Liquid chromatography

A UHPLC Shimadzu® Nexera X2 (Kyoto, Japan) pump was equipped with a Waters® Acquity CSH C18 (3.5 µm particle size, 2.1 × 100 mm, Milford, USA) column heated at 40 °C. Chromatographic separation was achieved using a binary mobile phase (water (A)/methanol (B)) delivered in the gradient mode at a flow rate of 350 µL.min^{-1}. Gradient elution started at 30% of B (0–0.5 min); then linearly increased to 90% of B (0.5–7 min); then to 99% of B (7–7.5 min); maintained at 99% of B for 5 min (7.5–12.5 min); then decreased to 30% of B (12.5–13 min) and finally maintained at 30% of B for 2.5 min (13–15.5 min) for re-equilibration of the column.

2.6. Mass spectrometry

Analyte quantification was performed with an API6500 + mass spectrometer from Sciex® (Concord, Canada) using an electrospray ionization (ESI) interface, operating in negative ionization mode. Ion source and MS/MS conditions were optimized with infusion of a 1 µg/mL solution of the individual analytes at 10 µL/min. For each compound multiple reaction monitoring mode (MRM) with two specific daughter fragment transitions for one parent precursor ion was used. The first was used for quantification while the second was used for confirmation. Dwell and cycle times were optimized using the Scheduled MRM® algorithm. Data were acquired with Analyst® software and quantification was obtained with MultiQuant® software.

2.7. Validation

Bioanalytical method validation was performed according to EMA guidelines [32]. Selectivity, calibration curve linearity, accuracy, precision, lower limits of the method and matrix effect were determined.

To determine selectivity, for each analyte and deuterated standard we examined chromatograms at their corresponding retention time in blank adipose tissue samples. Acceptable selectivity was defined by the absence of any detectable peak.

Calibrant samples were prepared using a pool of adipose tissue obtained from donors. Calibration curves, ranging from 0.125 to 8 (0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0) ng/mL, and from 0.0125 to 0.8

(0.0125, 0.025, 0.05, 0.1, 0.2, 0.4, 0.8) ng/mL for BPA and Clx-BPA, respectively, were constructed with 1/x weighting. For each analyte five sets of calibration curves were examined. R squared, slope and y-intercept were determined. Linearity was demonstrated when correlation coefficient was over 0.99.

Inter- and intra-day accuracy and precision were determined using QCs prepared with blank breast adipose tissue samples. QC concentrations were 0.125, 1.0, 4.0 ng/mL and 0.0125, 0.10, 0.40 ng/mL for BPA and Clx-BPA, respectively. Trueness values within the 85–115% range were accepted for validation of accuracy; a maximal 80–120% range was accepted for low QC. Precision was evaluated and expressed as coefficient of variations (CV%). Values had to be lower or equal than 20% for low QC and 15% for medium and high QCs.

In each batch of analysis (for method validation and routine analysis), five blank samples were analyzed. The corresponding mean and standard deviation of the peak area were calculated for each target analyte. The limit of detection (LOD) was set to three times the standard deviation for each target analyte [8].

The limit of quantification (LOQ), defined as the lowest level of the calibration standards (0.125 ng/mL for BPA and 0.0125 ng/mL for Clx-BPA) was fully validated with low-level QCs prepared with breast adipose tissue spiked at this concentration [32].

Matrix effect (ME) and recovery (RE) were also determined over the whole concentration range according to the method described by Matuszewski et al. [33]. Briefly, the signals from the analysis of three sets of samples were compared: (A) extracted QCs prepared in breast adipose tissue, (B) blank breast adipose tissue samples spiked at the same concentrations after extraction and (C) corresponding neat standards prepared in mobile phase, all at three levels of concentration (low, medium and high). IS corrected matrix effect was also calculated [33].

A complementary set of experiments was conducted in order to demonstrate that this method could be applied to other types of adipose tissues for example prostate adipose tissue. Calibrant and QC samples were prepared using blank prostate adipose tissue. Matrix effect and recovery were assessed and compared to results obtained with breast adipose tissue. Linearity and accuracy were also assessed in prostate adipose tissue samples.

3. Results

3.1. LC-MS/MS conditions

Optimal conditions for the electrospray ionization source operating in the negative mode were determined as follows: ion spray voltage at −4500V, source temperature at 650 °C, curtain gas pressure at 30.0 psi, collision gas set as high, ion source gas 1 pressure at 40.0 psi and ion source gas pressure 2 at 60.0 psi. Specific mass spectrometer parameters (declustering potential, entrance potential, collision energy and collision cell exit potential) for each quantification and ion transition pairs are reported in Table 1.

Chromatographic conditions allowed peak separation for each target analyte and corresponding retention times are reported in Table 1.

3.2. Method validation

No significant chromatographic peaks were observed at the retention times of Clx-BPA in blank breast adipose tissue samples (Fig. 1), thereby demonstrating the specificity of the method. A peak of BPA was present due to baseline contamination of human matrices with this ubiquitous pollutant. Linearity of the method was demonstrated over the whole concentration range with correlation coefficients > 0.99 (Table 2). The high value of y-intercept found for bisphenol A was due to basal occurrence of this compound in adipose tissue (Fig. 1). The accuracy and precision of the method were demonstrated over the whole range of concentration (Table 3). Intra and inter-day bias values were in the range 85–114% in accordance with bioanalytical validation guidelines. Imprecision of the method (both intra- and inter-day) did not exceed 14%. The limits of quantification, corresponding to the lowest standards of the calibration curves, were validated at 0.1250 and 0.0125 ng/mL for BPA and Clx-BPA, respectively. At the LOQ, good accuracies and precisions were demonstrated (Table 3) and high signal-to-noise peaks were obtained (Fig. 2). Matrix effects in breast tissue were low to moderate since ion suppression and enhancement did not exceed 31 and 2%, respectively (Table 4). They were satisfactorily corrected by internal standards (Table 4). Recoveries were moderate but, importantly, were consistent over the whole range of concentration (Table 4).

Slightly higher absolute matrix effects were measured in prostate adipose tissue but they were also satisfactorily corrected by IS (Table 5). Recoveries of target analytes from prostate adipose tissues were in the same range as those from breast adipose tissues (Table 5). Linearity was also demonstrated using this matrix (Table 6).

3.3. Application of the method

This method enables the determination of BPA and Clx-BPA in adipose tissue samples from women receiving breast surgery for breast lesions (Figs. 3 and 4). BPA was systematically quantified in these samples with concentrations ranging from 0.238 to 1.745 ng/mL (1.19–8.73 ng/g) (Table 7). Exposure to Clx-BPA was variable among participants: overall detection frequency was 45%. Table 7 shows that some patients are exposed to several of these compounds (patient BE) whereas others are not (patient RS).

4. Discussion

For the first time, an LC-MS/MS method enabling the simultaneous determination of BPA and Clx-BPA in adipose tissue has been developed and validated according to well-established bioanalytical validation guidelines. Methods for assaying Bisphenol A in adipose tissue had previously been developed using LC-MS/MS [27,28,30] or GC-MS [29]. A GC-MS method for analysis of BPA and Clx-BPA initially developed for water and plasma samples has been succinctly described as a means of analysis of adipose tissue [31].

Several attempts were made to optimize extraction procedure. We started with a method similar to the one used for the analysis of Clx-

Table 1
Descriptive table of retention times, precursor ions, fragment ions and MS/MS optimization conditions (declustering potential, DP; entrance potential, EP; collision energy, CE; collision cell exit potential, CXP) for all analytes. For CE and CXP, first and second values are for quantification and confirmation ions, respectively.

Analyte	Retention time (min)	Precursor ion (m/z)	Quantification ion (m/z)	Confirmation ion (m/z)	DP (V)	EP (V)	CE (V)	CXP (V)
BPA	5.62	227.0	211.8	132.8	−65	−10	−24/-34	−19/-11
BPA-d16	5.55	241.1	222.9	141.9	−90	−10	−28/-34	−25/-15
MCBPA	6.20	260.9	181.7	209.7	−120	−10	−38/-30	−19/-21
DCBPA	6.72	294.9	215.8	279.8	−75	−10	−40/-30	−24/-13
TCBPA	7.20	329.0	249.7	277.7	−85	−10	−42/-34	−27/-31
TTCBPA	7.64	364.8	313.7	285.6	−110	−10	−36/-44	−33/-29
DCBPA-d12	6.65	307.0	224.9	252.8	−120	−10	−42/-34	−23/-25

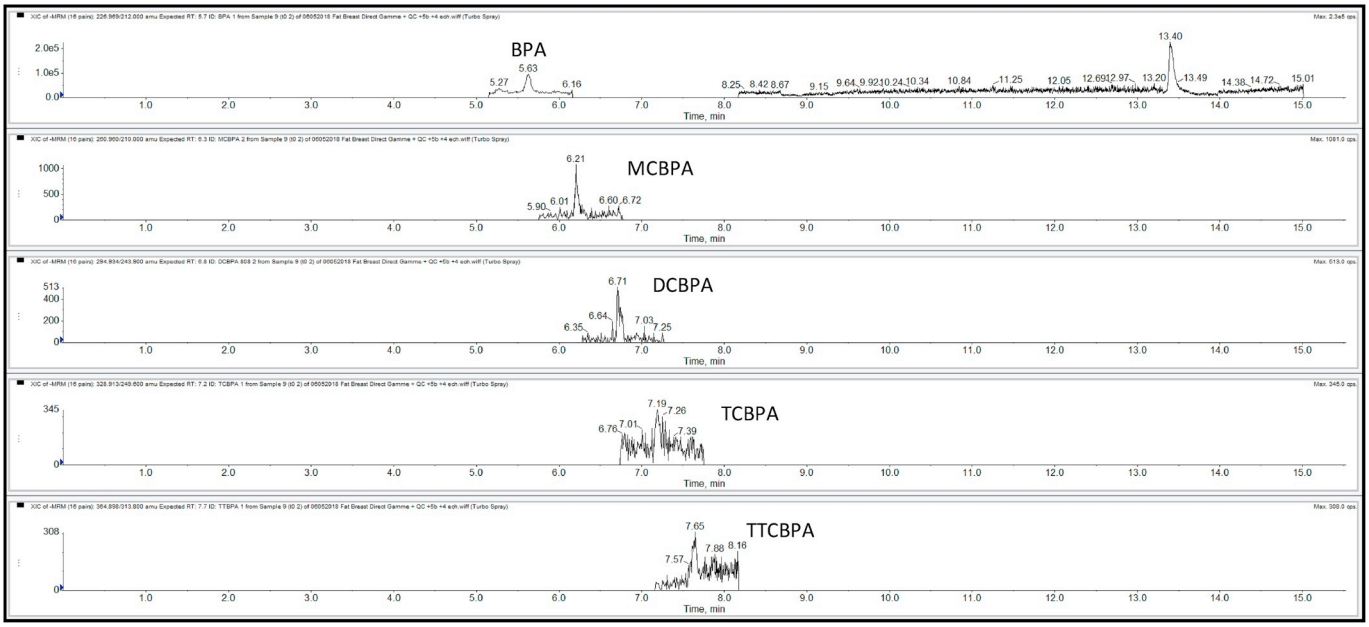

Fig. 1. Chromatogram of a blank adipose tissue sample. BPA: bisphenol A, MCBPA: monochlorobisphenol A; DCBPA: dichlorobisphenol A; TCBPA: trichlorobisphenol A; TTCBPA: tetrachlorobisphenol A.

Table 2
Mean (+/− SD) slope, correlation coefficient (r^2) and y-intercept of calibration curves prepared with breast adipose tissue (n = 5).

Compound	BPA	MCBPA	DCBPA	TCBPA	TTCBPA
Slope (mean ± SD)	1.082 ± 0.148	1.514 ± 0.496	3.574 ± 1.148	3.321 ± 0.900	3.011 ± 1.203
Correlation coefficient r^2 (mean ± SD)	0.999 ± 0.000	0.997 ± 0.003	0.997 ± 0.003	0.997 ± 0.003	0.997 ± 0.002
Y-intercept (mean ± SD)	0.487 ± 0.058	0.007 ± 0.004	0.003 ± 0.005	0.009 ± 0.011	0.023 ± 0.025

Table 3
Intra and inter-day accuracy and precision for each analyte in breast adipose tissue at each level of QC (n = 5).

Compound	Concentration of QC (ng/mL)	Accuracy (%)		Precision (%)	
		Intra-day	Inter-day	Intra-day	Inter-day
BPA	0.125 (LOQ)	110	95	8	3
	1.0	106	101	6	4
	4.0	109	104	4	6
MCBPA	0.0125 (LOQ)	114	112	3	14
	0.10	97	102	10	8
	0.40	96	104	2	5
DCBPA	0.0125 (LOQ)	99	104	4	13
	0.10	96	102	5	6
	0.40	102	104	3	5
TCBPA	0.0125 (LOQ)	99	101	4	7
	0.10	100	102	8	4
	0.40	101	102	3	5
TTCBPA	0.0125 (LOQ)	85	109	8	7
	0.10	101	103	8	7
	0.40	106	108	6	13

BPA in milk samples [10] using either methanol or acetonitrile as a precipitation reagent. This method enabled to obtain high recovery rates of target analytes but also led to high matrix effects with adipose tissue. One key step to reduce matrix effect was removal of lipid content from adipose tissue samples. The method proposed by Cariou et al. [34] was not chosen due to a labor-intensive protocol. We also avoided the use of freezing for lipid removal, with freezing time ranging from 15 min to overnight [28,30], to shorten experimentation time. Our final method involves a very simple sample preparation step with QuEChERS reagents. While QuEChERS-based extraction has frequently been used in food analysis [35,36], to our knowledge this is the first time that it is considered as a means of extracting environmental pollutants from human adipose tissue. Our extraction method involves two simple and rapid steps: the first is a salting-out extraction step with acetonitrile and the second is a clean-up step using dispersive solid-phase extraction with a C18 sorbent to remove lipids. Using our method, recoveries were moderate but did not impact the overall performances. Indeed, aside from its simplicity and low cost, our method proves to be effective.

Separation of target analytes was achieved using a 3.5 µm particle size column. Although the HPLC system was suitable for UHPLC, this particle size was chosen for better column life time.

Our assay has been fully validated according to well-established bioanalytical validation guidelines: linearity, repeatability and reproducibility have been demonstrated using calibration standards and QCs prepared in adipose tissue, which is recommended by guidelines but not always performed. Special emphasis was put on the study of matrix effect and overall matrix effects were low for all target analytes in both tested matrices. In an ideal situation each target analyte would have its own corresponding stable isotope labelled internal standard (SIL-IS) to correct matrix effect. However, to our knowledge only 2,2'-dichlorobisphenol A is commercially available and other deuterated compounds should be custom synthetized which represents a significant cost. Moreover, our data demonstrates that deuterated DCBPA enables to correct matrix effect for other chlorine derivatives. Furthermore, IS-corrected matrix effects were comparable in breast and prostate adipose tissues, demonstrating that this method could reliably assay BPA and Clx-BPA in both tissues.

BPA is ubiquitous in the environment, therefore specific precautions were implemented to avoid contamination with target analytes that may cause misleading results: tissue samples were collected and stored in glass vials, ultrapure solvents were used throughout the study and all

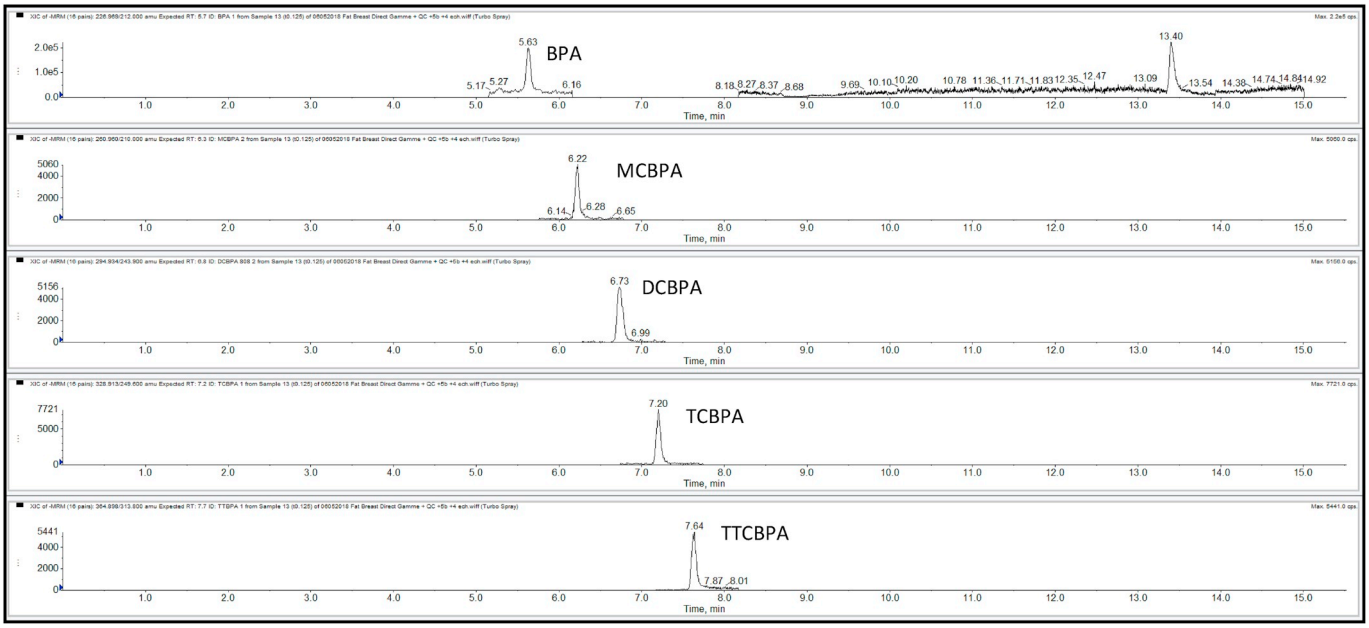

Fig. 2. Chromatogram corresponding to the lower limit of quantification (LOQ): lowest standard sample prepared in adipose tissue sample and spiked at 0.125 and 0.0125 ng/mL of BPA and Clx-BPA, respectively. BPA: bisphenol A, MCBPA: monochlorobisphenol A; DCBPA: dichlorobisphenol A; TCBPA: trichlorobisphenol A; TTCBPA: tetrachlorobisphenol A.

Table 4
Determination of matrix effect, IS-corrected matrix effect and recovery in breast adipose tissue at each level of QC (n = 3).

Compound	Concentration of QC (ng/mL)	Matrix effect (%)	IS-corrected matrix effect (%)	Recovery (%)
BPA	0.125	98	111	50
	1.0	77	83	22
	4.0	81	98	20
MCBPA	0.0125	80	100	19
	0.10	77	85	14
	0.40	69	83	15
DCBPA	0.0125	80	99	18
	0.10	94	98	15
	0.40	81	98	21
TCBPA	0.0125	92	114	17
	0.10	100	104	16
	0.40	97	116	20
TTCBPA	0.0125	100	126	17
	0.10	102	106	15
	0.40	94	112	17

Table 5
Determination of matrix effect, IS-corrected matrix effect and recovery in prostate adipose tissue at each level of QC (n = 3).

Compound	Concentration of QC (ng/mL)	Matrix effect (%)	IS-corrected matrix effect (%)	Recovery (%)
BPA	0.125	61	79	40
	1.0	66	82	18
	4.0	72	86	18
MCBPA	0.0125	67	87	15
	0.10	62	85	14
	0.40	67	90	16
DCBPA	0.0125	74	95	14
	0.10	67	92	15
	0.40	72	97	16
TCBPA	0.0125	68	88	19
	0.10	91	123	10
	0.40	66	87	15
TTCBPA	0.0125	94	121	14
	0.10	93	113	9
	0.40	63	87	15

reagents and material were tested for the absence of target analytes.

Calibration standards and QCs were prepared using a pool of blank matrix as recommended by bioanalytical validation guidelines. However, target analytes may be present in blank matrices due to basal contamination of individuals since these compounds may be ubiquitously found in the environment and consequently in an individual's organisms. This was particularly the case for BPA. As a result, the mean signal corresponding to 5 blank samples determined for each series was systematically subtracted from signal of standard and QC samples to improve the accuracy of BPA in the low range.

Our LOQ for BPA was validated using QCs and was in the same range as the one obtained by Reeves et al. [30] (0.38 ng/g). No LOQ was defined in the paper by Artacho-Cordon et al. but the lowest QC tested for BPA was 2 ng/g [28]. No LOQ was validated for Clx-BPA in the paper by Fernandez et al. [31] but values defined as LOD were high (0.5–3.0 ng/mL) in comparison to our LOQ. The sensitivity of our assay was adequate to detect and quantify BPA and Clx-BPA in real samples. Bisphenol A was systematically quantified with BPA concentration in the 0.238–1.745 ng/mL range (1.19–8.73 ng/g). These concentrations were of the same order of magnitude as those measured in adipose tissue originating in various sources [27,29,31] but higher than those found by Reeves et al. in breast adipose tissue [30]. Our method enables to identify different patterns of exposure to Clx-BPA, with some patients being exposed whereas others are not. Concentrations of Clx-BPA in our samples were lower than those found by Fernandez et al. [31] who measured median concentrations equal to 3.14, 7.77 and 0.74 ng/g for CBPA, DCBPA and TCBPA, respectively.

Table 6
Slope, correlation coefficient (r^2) and y-intercept of calibration curves prepared with prostate adipose tissue.

Compound	BPA	MCBPA	DCBPA	TCBPA	TTCBPA
Slope	1.41	2.22	4.77	6.38	3.15
Correlation coefficient r^2	0.994	0.992	0.996	0.990	0.992
Y-intercept	0.480	0.002	0.004	−0.057	- 0.023

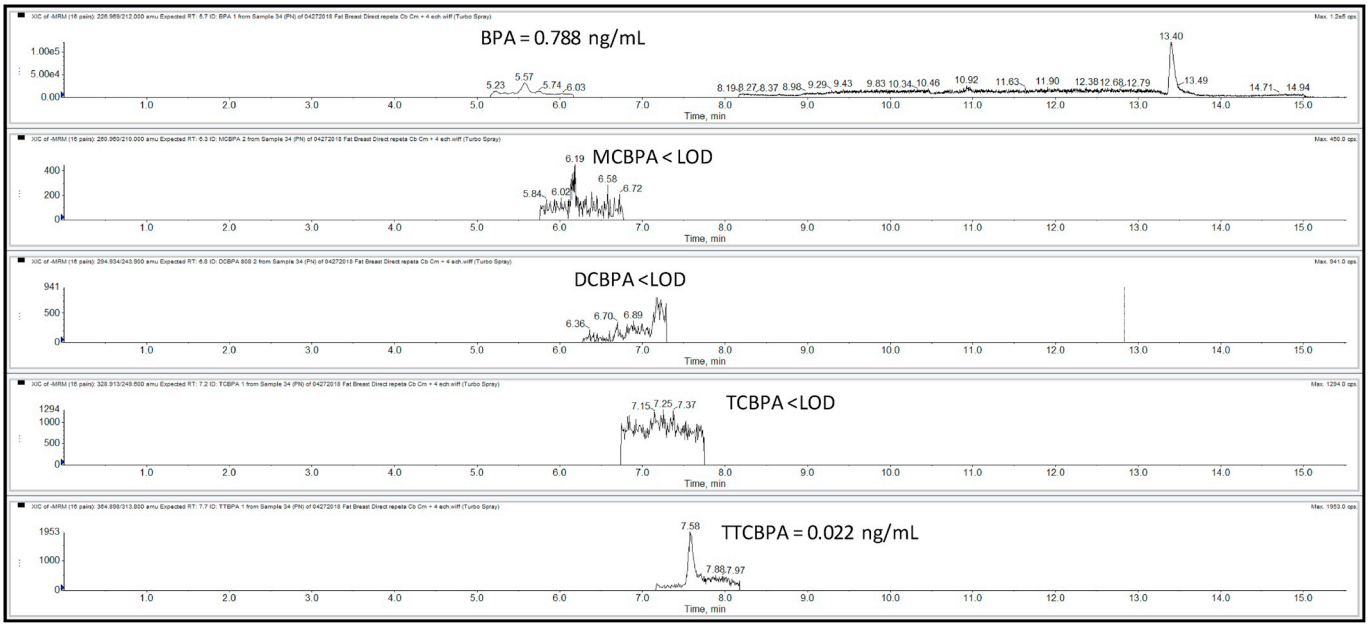

Fig. 3. Chromatogram of the adipose tissue sample from individual PN. BPA and TTCBPA were quantified while MCBPA, DCBPA and TCBPA were not detected (< LOD) (also see Table 7). BPA: bisphenol A, MCBPA: monochlorobisphenol A; DCBPA: dichlorobisphenol A; TCBPA: trichlorobisphenol A; TTCBPA: tetrachlorobisphenol A.

As suggested by others [28], adipose tissue may be a suitable biological matrix for assessment of mid to long-term exposure to environmental pollutants, especially non-persistent compounds presenting short half-lives. Collection of urine is undoubtedly more convenient and non-invasive but it may not reflect exposure over a prolonged period when half-life of target compound is short [37]. This is the case for bisphenol A of which the half-life is about 5 h whereas half-lives for Clx-BPA remain unknown. BPA and Clx-BPA are lipophilic compounds with logP values ranging from 3.8 to 6.4 for BPA and TTCBPA, respectively [2], thereby suggesting that they may accumulate in adipose tissue.

Table 7
BPA and Clx-BPA concentrations measured in 5 breast adipose tissue samples obtained from women undergoing breast surgery. < LOQ: compound detected but not quantified; < LOD: compound not detected.

	Endocrine disruptor concentrations (ng/mL)				
Patient ID	BPA	MCBPA	DCBPA	TCBPA	TTCBPA
RS	1.433	< LOD	< LOD	< LOD	< LOD
PN	0.788	< LOD	< LOD	< LOD	0,022
PC	1.510	0.046	< LOD	< LOD	< LOD
LC	0.238	< LOQ	< LOQ	< LOQ	0.026
BE	1.745	0.013	< LOD	0.013	0.014

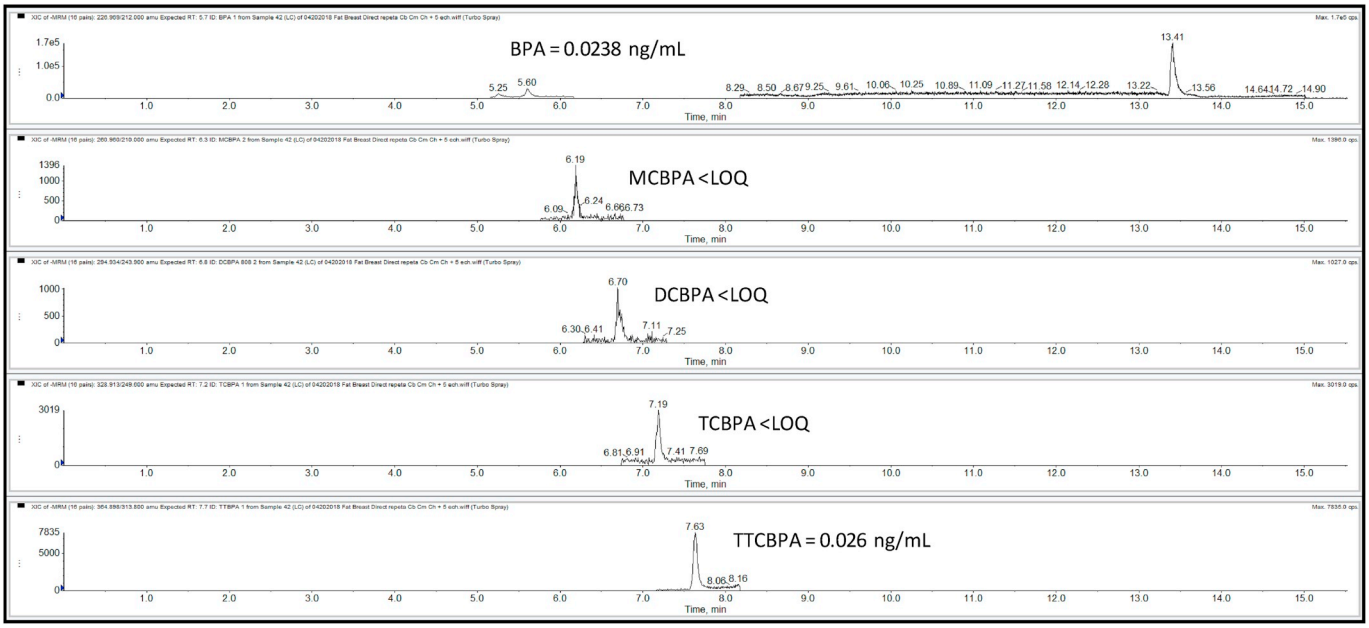

Fig. 4. Chromatogram of the adipose tissue sample from individual LC. BPA and TTCBPA were quantified while MCBPA, DCBPA and TCBPA were detected but not quantified (< LOQ) (also see Table 7). BPA: bisphenol A, MCBPA: monochlorobisphenol A; DCBPA: dichlorobisphenol A; TCBPA: trichlorobisphenol A; TTCBPA: tetrachlorobisphenol A.

This method could also be used to assess the relationships between exposure to BPA and Clx-BPA and local tumorigenesis effects of these endocrine-disrupting compounds. Indeed, adipose tissue is important in tumor microenvironments and is now recognized as a major factor in the development, growth and progression of cancer [38]. Adipose tissue inflammation may be a key process in promotion of cancer [39] and it has been shown in *ex vivo* experiments that low doses of BPA may promote secretion of inflammatory adipokines such as interleukin-6 and tumor necrosis factor α [40]. Obesity is also considered as a risk factor for breast adipose tissue inflammation suggesting an indirect effect of obesogenic pollutants [15,41]. Moreover, it has been shown *in vitro* that BPA and TTCBPA presented an affinity to the estrogen receptor and expressed a proliferative potential in the human breast cancer cell line MCF-7 [18]. Finally, as a biomarker of long-term exposure, this method could be applied in epidemiological studies.

5. Conclusion

In conclusion, this method enables the reliable determination of BPA and Clx-BPA in adipose tissue and could help to assess long-term exposure to these compounds as well as their possible associations with hormone-dependent cancers. For instance, this method will be applied to a study aiming to compare BPA and Clx-BPA exposure in women undergoing breast surgery for different breast lesion classes (benign, epithelial atypia and confirmed breast cancer).

Contributors

Conception or design of the work: NV, SR, CN, SR, VM, AD.
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Funding

Part of this research was funded by Roche Pharmaceuticals.

Acknowledgments

We wish to thank Jeffrey Arsham for his highly helpful reading of our original text.

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