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RESEARCH PAPER

High-performance thin-layer chromatography coupled with HPLC-DAD/HPLC-MS/MS for simultaneous determination of bisphenol A and nine brominated analogs in biological samples

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Abstract

Bisphenol A (BPA) and its brominated analogs exhibiting bioaccumulation potential, endocrine disruption, and reproductive toxicity have been worldwide detected in water, air, soil, and sediments. But few methods have been proposed for simultaneously determining a variety of these compounds in biological matrices, hindering the further study on their biological transformation/degradation and health risks. In this study, a simple, solvent-saving and sensitive method based on high-performance thin-layer chromatography (HPTLC) for sample pretreatment coupled with high-performance liquid chromatography-diode array detector (HPLC-DAD) (UV = 214 nm)/triple quadrupole mass spectrometry (MS/MS) was developed for determining BPA and its nine brominated analogs in biological samples. The method detection limits (MDLs) and method quantification limits (MQLs) for ten BPA analogs ranged from 0.8 to 685.7 ng g^{-1} dw (S/N = 3) and 2.7 to 2285.7 ng g^{-1} dw (S/N = 10), respectively. The recoveries were 64–124% with SD less than 10%. The RSD of intermediate precision was less than 11%, and matrix effects were lower than 19%. Compared with traditional purification procedures, HPTLC largely reduced the workload and procedures for complex biological sample cleanup without inducing decomposition of the analytes. The proposed method exhibited good performance when detecting these ten chemicals in chicken samples from a nearby yard of brominated flame retardant plants, indicating its great potential for investigating their environment level, behavior, and fate in organisms.

Keywords Bisphenol A · Brominated analogs · Biological matrices · HPTLC · Samples cleanup

Introduction

Bisphenol A (BPA) and its brominated analogs (e.g., tetrabromobisphenol A (TBBPA) and other derivatives) are commonly applied as important raw materials in epoxy resin and brominated flame retardants (BFRs), which are further used for producing plastics, packing materials, electronics, textiles, etc. [1–3]. BPA, TBBPA, and their associated analogs released from the process of their production and application have been widely detected at high levels in water, soil, sediment, indoor dust, air, biologicals, etc. [4–6]. Their accumulation and biomagnification via food chain have been proven to increase the associated potential risks to wild animals and human beings, such as endocrine disruption, reproductive toxicity, and neurotoxicity [7–10]. The frequent detections and the potential risks lead to subsequent urgent demand on the study of their environmental behaviors, fate, and health risks [1, 2, 11], especially for their associated novel derivatives and unknown analogs. To date, developing rapid and sensitive methods for effectively monitoring BPA and its associated analogs in the environment is still a great challenge, especially when studying their environmental behaviors and ecological risks.

To date, methods based on mass spectrometry are still the most commonly used strategy for quantitative and qualitative analysis of these chemicals [1, 2, 6, 12–14]. Gas

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chromatography mass spectrometry (GC-MS) and high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) present higher sensitivities for BPA and TBBPA in marine organism and food samples [9, 15–17]. Due to the weak ionization by common electrospray ionization (ESI)-MS/atmospheric pressure chemical ionization (APCI)-MS and the thermolability of some TBBPA derivatives, some novel MS ion source or derivatization of chemicals have been proven to be useful strategies for detecting TBBPA and its associated derivatives. By enabling atmospheric pressure photoionization (APPI)-MS/MS coupled with HPLC, method detection limits (MDLs) of TBBPA bis(allyl ether) (TBBPA-BAE), TBBPA bis(2,3-dibromobispropyl ether) (TBBPA-BDBPE), and TBBPS bis(2,3-dibromobispropyl ether) (TBBPS-BDBPE) in herring gull eggs can be decreased to 0.03–1.28 ng g^{-1} ww [12, 18]. Beyond that, isotopic-labeled techniques have also been successfully used to track and to quantify the metabolites of TBBPA when studying its metabolism processes [19, 20]. But current references show that identifying unknown products of TBBPA, BPA, and their associated analogs still depend on high-resolution mass spectrometry (HRMS) [21–23]. For example, BPA analogs in model experiments, e.g., tribromobisphenol A (TriBBPA), dibromobisphenol A (DBBPA), monobromobisphenol A (MBBPA), and TBBPA momo(methyl ether) (TBBPA-MME), have been identified as transformation products of TBBPA in biological models [23–25].

In order to obtain more information on trace levels of chemicals and possible unknown products/metabolites from environmental samples, complicated sample pretreatments and preconcentration processes are often inevitable. For example, lipid, protein, and pigments in biological samples could be co-extracted with the targets in accelerated solvent extraction (ASE) [26] or Soxhlet extraction [27], which set a higher request on the purification procedures. In order to remove these interferences, gel-permeation chromatography (GPC) [28] and acid silica gel [29] are often applied for first-step cleanup, followed by solid phase extraction (SPE) [12], column chromatography [15, 30], etc. Treating one sample by GPC often need solvent equal to or higher than 500 mL and cost more than 2 h [15]. Concentrated sulfuric acid used in acid silica gel can significantly induce the oxidation decomposition of TBBPA [31], and then decrease the recovery and analysis accuracy. Therefore, developing a rapid, simple, and cost-effective method for analyzing BPA and its associated analogs is still necessary, especially for biological samples containing higher contents of lipid, protein, and pigments.

High-performance thin-layer chromatography (HPTLC) is an efficient separation strategy in chemical synthesis, pharmaceutical, and biomedical fields [32–39]. HPTLC with the advantages of large sample loading volumes, visible separation process, effective cost, and time-saving has been proven to be one of the most useful strategies for chemical concentration [40]. Here, an effective analytical method based on HPTLC coupled with HPLC-DAD/HPLC-MS/MS has been developed for analyzing BPA and its nine brominated analogs, including BPA, TBBPA, TBBPA-BAE, TBPA-BDBPE, TBBPA mono(allyl ether) (TBBPA-MAE), TBBPA mono(2,3-dibromobispropyl ether) (TBBPA-MDBPE), TBBPA-MME, MBBPA, 2,2′-DBBPA, and TriBBPA in biological samples. The method performances for treating biological samples have been well optimized and evaluated by analyzing these chemicals in chicken samples. To our knowledge, there is still no strategy for simultaneously determining BPA and these nine important target-brominated analogs in environmental matrix to date.

Materials and methods

Chemicals and materials

Ethyl acetate (Fisher Scientific, Trinidad, UK), acetone (Fisher Scientific, Trinidad, UK), *n*-hexane (Fisher Scientific, Trinidad, UK), dichloromethane (DCM) (Merck, Darmstadt, Germany), acetonitrile (Merck, Darmstadt, Germany), and methanol (Merck, Darmstadt, Germany) are all HPLC grade. Glacial acetic acid (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) of analytical reagent grade was used. Ultra-pure water (18 MΩ cm^{-1}) was prepared from Purelab Ultra system (Veolia Water Systems, UK). BPA (Sigma-Aldrich, Steinheim, Germany), TBBPA (Sigma-Aldrich, Steinheim, Germany), TBBPA-BAE (Sigma-Aldrich, Steinheim, Germany), TBBPA-BDBPE (Sigma-Aldrich, Steinheim, Germany) were obtained from the market. TBBPA-MAE, TBBPA-MDBPE, TBBPA-MME, MBBPA, 2,2′-DBBPA, and TriBBPA were synthesized according to literatures [41–43] and characterized by ^{1}H-NMR and HPLC-UV (214 nm) with purities higher than 97%. HPTLC plates (20 cm × 10 cm) of $HSGF_{254}$ (Yantai Jiangyou Silica Gel Development Co., Ltd., Yantai, China) were purchased with the precoated silica gel GF_{254} of 0.40–0.50 mm. Chicken breast samples were cleaned, freeze-dried, grinded, and stored in a refrigerator at − 20 °C before analysis.

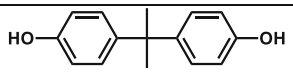
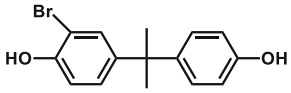
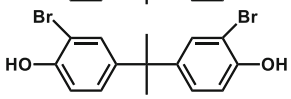
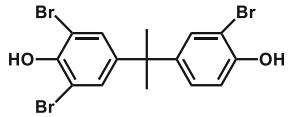
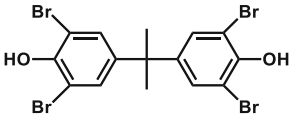
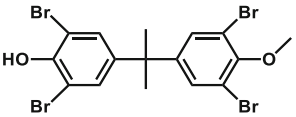
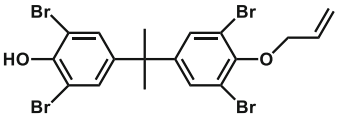
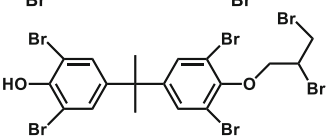
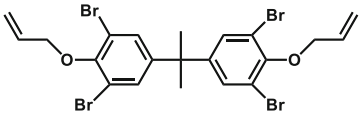
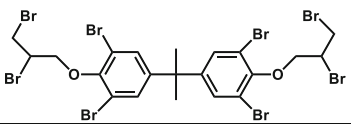
Instrumentation

High-performance liquid chromatography (Agilent 1290, CA, USA) coupled with a triple quadrupole mass spectrometer (Agilent 6430 Technologies, CA, USA) was employed for analyzing BPA, MBBPA, 2,2′-DBBPA, TriBBPA, TBBPA, TBBPA-MAE, TBBPA-MDBPE, and TBBPA-MME. Chromatographic separation was performed on an Agilent Zorbax ODS column (Agilent, CA, USA, 150 mm × 3 mm, 5 μm) at 30 °C with a 5-μL injection volume. The mobile

phase consisted of ultra-pure water (A) and methanol (B) at a flow rate of 0.3 mL min^{-1}. The gradient parameters were 0–5 min at 50% B, 5–18 min at 50 to 90% B, 18–34 min at 90% B, 34–36 min at 90 to 100% B, and 36–40 min at 100 to 50% B. The mass spectrometer was operated in negative ESI mode (ESI^-), using multiple reaction monitoring (MRM) mode for quantitation. Nitrogen was used as the sheath gas and auxiliary gas. The gas temperature was 350 °C, the nebulizer was 40 psi, the gas flow was 10 L min^{-1}, and the capillary was 4500 V. According the European Union concept of identification points for the confirmation of the identity of a compound, two transitions for each analyte were monitored in MRM mode (Table 1). Data were analyzed using Qualitative Analysis B.06.00 software (Agilent, CA, USA).

UHPLC of Ultimate 3000 series (Thermo Technologies, Trinidad, UK) equipped with a diode array detector (DAD) was used for determining TBBPA-BAE and TBBPA-BDBPE. ChemStation software of Chromeleon 7 (Thermo Technologies, Trinidad, UK) was used for data processing. Pretreated samples (20 μL) were injected by automatic sampler, separated on an Agilent Zorbax ODS column (150 mm × 3 mm, 5 μm) at 30 °C and detected by DAD (UV 214 nm). The flow rate was set at 0.6 mL min^{-1} with methanol and water as the mobile phase. The linear gradient started isocratic at 50% methanol for 3 min, then increased to 90% in 7 min and to 100% in another 8 min and kept for another 2 min, then the gradient returned to 50% methanol in 2 min and kept for 5 min.

Table 1 Analytical parameters for BPA and its nine brominated analogs

Compounds	Structures	Retention time (t_R)	Quantitation/confirmation transitions (m/z)	Frag (V)	CE (V)	Detection wavelength (nm)
BPA/ Bisphenol A		13.0	227.1-212.1/227.1-133.1	126	14/25	—
MBBPA/ Monobromobisphenol A		15.2	305.0-79/305.0-81	114	30/30	—
2,2'-DBBPA/ 2,2'-dibromobisphenol A		17.0	385.0-79/385.0-81	126	30/34	—
TriBBPA/ Tribromobisphenol A		18.4	462.8-79/462.8-340	150	40/38	—
TBBPA/ Tetrabromobisphenol A		19.6	542.7-417.8/542.7-445.8	170	40/30	—
TBBPA-MME/ TBBPA mono(methyl ether)		23.1	556.7-526.7/556.7-541.7	130	34/26	—
TBBPA-MAE/ TBBPA mono(allyl ether)		24.8	582.7-526.7/582.7-541.7	126	30/14	—
TBBPA-MDBPE/ TBBPA mono(2,3-dibromopropyl ether)		19.8	742.7-526.6/742.7-528.6	150	40/40	—
TBBPA-BAE/ TBBPA bis(allyl ether)		17.6	—	—	—	214
TBBPA-BDBPE/ TBBPA bis(2,3-dibromopropyl ether)		20.3	—	—	—	214

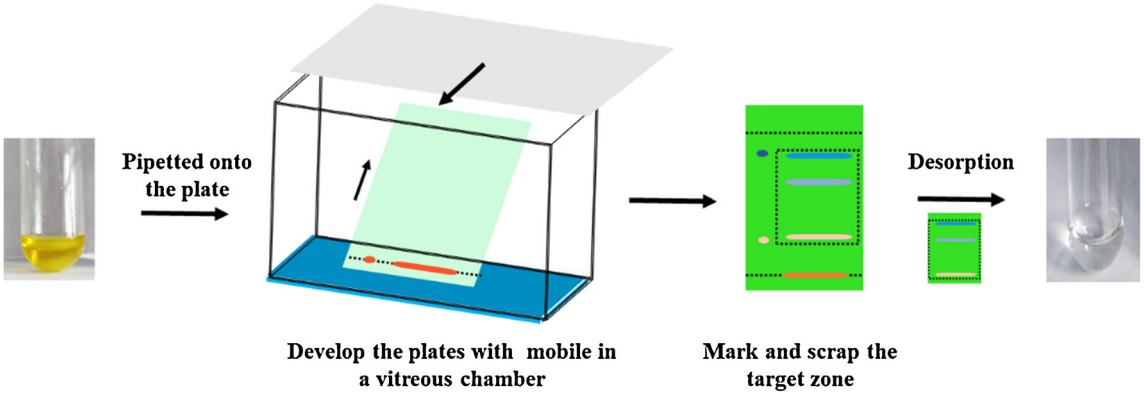


Fig. 1 Illustration of HPTLC separation and desorption of target compounds

Sample extraction

Chicken breast samples (0.5 g) were mixed with 5 g of anhydrous Na_2SO_4, soaked with 500 μL of methanol sufficiently and ultrasonically extracted with 30 mL solvent mixture of ethyl acetate:acetone (1:1, *v*/*v*) for three times (20 min per time) with centrifugation speed of 3000 r min^{-1}. The extracts were merged and concentrated using a rotary evaporator and solvent exchanged to 0.2 mL acetone before HPTLC sample pretreatment. For the recovery experiments, each sample was spiked with the standards, mixed sufficiently, and kept overnight before extraction. Natural samples were treated following the procedure described above.

HPTLC separation and desorption

The proposed method consisted of two steps: (1) analytes separation from the interferences on HPTLC plate and (2) desorption of target compounds from HPTLC plate (Fig. 1). Standard solution, consisting of BPA and TBBPA-BAE used as marker and concentrated sample extracts (100 μL) were pipetted onto HPTLC plate at the starting line, 10 mm from the bottom edge. Then, HPTLC plate was developed with *n*-hexane:ethyl acetate:DCM:acetic acid (25:5:5:1, *v*/*v*) as the mobile phase (migration distance, 15 cm) in a capped vitreous chamber at room temperature (20 °C). After development and drying, the plate can be observed visually at 254 nm in ZF-20C camera obscura ultraviolet analyzer. The developed zones containing target compounds (4.5–13.65 cm off the baseline) were then marked according to the marker standard and scraped from the surface of the plate layer. Subsequently, target compounds were desorbed from the scraped layer by 20 mL of acetonitrile (containing 2% acetic acid), solvent changed to methanol (500 μL), and further filtered through a 0.22-μm nylon syringe filters for UHPLC-DAD/HPLC-MS/MS analysis.

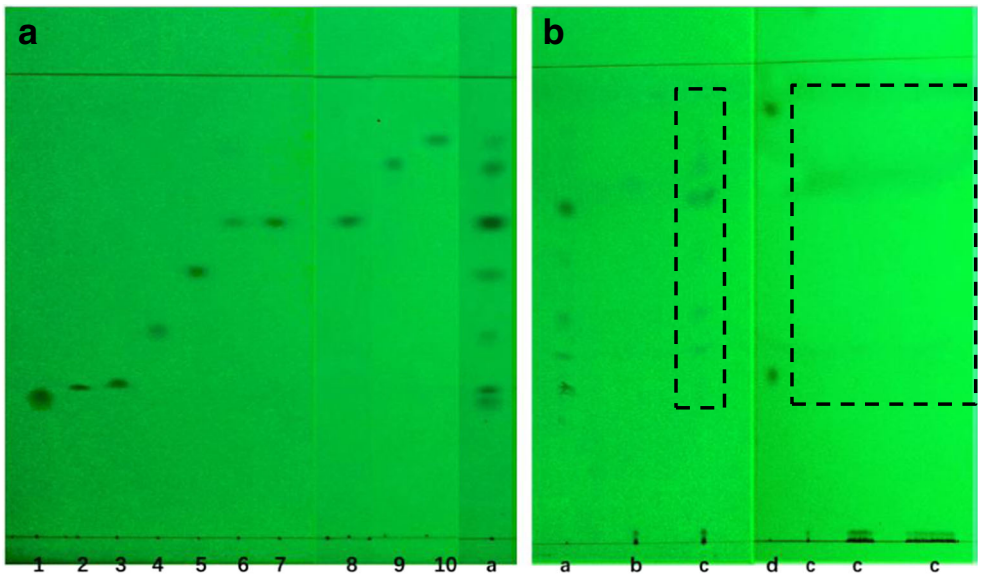


Fig. 2 HPTLC fingerprint profile of standards and chicken samples at 254 nm. **A** Profile of standards; **B** profile of standards and samples: 1, BPA; 2, MBBPA; 3, 2,2′-DBBPA; 4, TriBBPA; 5, TBBPA; 6, TBBPA-MME; 7, TBBPA-MAE; 8, TBBPA-MDBPE; 9, TBBPA-BDBPE; 10, TBBPA-BAE; a, standard mixture; b, blank sample; c, spiked samples; and d, marker (BPA, TBBPA-BAE)

Table 2 Method performance results for determination of BPA and its nine analogs in biological sample

		Models										
		BPA A = 16.82C (− 163.52)	MoBBPA A = 22.68C (− 204.42)	DiBBPA A = 16.67C (+ 56.79)	TriBBPA A = 20.14C (+ 877.67)	TBBPA A = 15.78C (+ 660.38)	TBBPA-MME A = 271.13C (+ 8471.77)	TBBPA-MAE A = 151.49C (− 1632.77)	TBBPA-MDBPE A = 32.60C (− 861.50)	TBBPA-BAE A = 0.0039C (− 0.325)	TBBPA-BDBPE A = 0.0025C (− 0.23)
r^2	–	0.998	0.999	0.999	0.991	0.981	0.987	0.984	0.994	0.997	0.998
R_f	$n = 3$	0.30 ± 0.02	0.32 ± 0.00	0.33 ± 0.01	0.45 ± 0.02	0.58 ± 0.01	0.68 ± 0.01	0.69 ± 0.02	0.69 ± 0.02	0.91 ± 0.01	0.86 ± 0.01
HPTLC plate purification recovery (%)	400 ng (/plate) ($n = 3$)	82 ± 1	73 ± 5	81 ± 7	72 ± 9	72 ± 6	112 ± 2	100 ± 1	88 ± 6	94 ± 9	102 ± 10
Recovery (%)	4 μg g^{-1} ($n = 3$)	100 ± 2	72 ± 9	65 ± 7	70 ± 2	69 ± 8	66 ± 4	64 ± 2	89 ± 3	70 ± 2	86 ± 5
	8 μg g^{-1} ($n = 3$)	118 ± 8	113 ± 9	114±4	103 ± 8	79 ± 2	106 ± 5	66 ± 1	87 ± 9	106 ± 2	106 ± 1
	16 μg g^{-1} ($n = 3$)	88 ± 3	88 ± 6	95 ± 6	80 ± 2	71 ± 4	75 ± 5	65 ± 3	105 ± 5	124 ± 2	105 ± 9
Matrix effect (%)	200 ng mL^{-1} ($n = 3$)	3.4 ± 1.5	− 2.2 ± 4.3	− 3.6 ± 9.2	− 18.0 ± 11.6	− 4.6 ± 10.4	− 7.5 ± 6.8	− 18.6 ± 9.5	8.2 ± 0.2	4.6 ± 0.8	− 3.0 ± 3.1
	500 ng mL^{-1} ($n = 3$)	− 0.06 ± 7.00	− 7.6 ± 6.3	− 0.67 ± 0.41	− 9.7 ± 11.9	1.27 ± 0.6	0.6 ± 4.2	− 10.2 ± 3.2	7.7 ± 8.4	1.2 ± 2.0	− 5.9 ± 7.1
	1000 ng mL^{-1} ($n = 3$)	− 6.29 ± 3.74	− 8.1 ± 3.5	8.7 ± 4.6	2.2 ± 3.2	3.8 ± 4.9	6.1 ± 9.9	− 1.1 ± 2.5	0.4 ± 6.1	3.3 ± 3.2	1.2 ± 2.7
MDLs (ng g^{-1})		5.9	2.7	1.8	1.0	2.1	0.8	1.6	14.5	59.1	685.7
MQLs (ng g^{-1})		19.6	9.0	6.1	3.5	7.0	2.7	5.3	48.3	197.0	2285.7
Intermediate precision	Intraday	0.8	2.8	4.4	6.5	7.2	5.8	7.8	2.1	2.3	1.1
RSD (%)	Interday	5.5	8.6	9.7	1.8	10.7	9.8	9.7	6.7	5.4	3.1

Results and discussion

Extraction of BPA and its nine brominated analogs

Several solvents and their combination were examined for extracting target compounds from chicken sample by ultrasonic-assisted extraction. DCM was excluded due to its large density which often leads to separation difficulty via centrifugation. The addition of tiny amounts of methanol (500 μL) into the sample before extraction can improve the solid-liquid separation aggregation tendency via centrifugation. The extraction efficiencies using ethyl acetate:acetone (1:1, *v*/*v*), acetone, and methanol were compared. Ultimately, extraction by 30 mL ethyl acetate:acetone (1:1, *v*/*v*) was selected due to the higher extraction efficiency in ultrasonic extraction for 20 min three times with a centrifugation speed of 3000 r min^{-1}.

HPTLC separation and desorption of BPA and its nine brominated analogs

HPTLC was used for separating target compounds from the extraction residues of chicken sample. Various mobile phase systems have been examined, including *n*-hexane, dichloromethane, ethyl acetate, and mixed solvent (*n*-hexane:dichloromethane:ethyl acetate, 2:1:1, 4:1:1, 5:1:1, *v*/*v*). In our study, all the tested individual developing solvent showed poor separation efficiencies. For example, *n*-hexane induced much lower HPTLC mobility for all the analytes, while dichloromethane and ethyl acetate led to much higher HPTLC mobility near to the topline and then no separation between most of analytes (except BPA, MBBPA and 2,2′-DBBPA). Totally, mixed solvents presented improved separation performances from analytes themselves and interferences. Based on comparison results, the analytes could be successfully developed by a combination of *n*-hexane:ethyl acetate:DCM (5:1:1, *v*/*v*) in a narrower target zone. And the combined solvent was chosen as the mobile phase in our study. Further study showed that the addition of 2~3% acetic acid could obviously improve the polarity of the developing solvent and then reduce the tailed phenomenon of BPA, MBBPA, and 2,2′-DBBPA (see Electronic supplementary material (ESM) Fig. S1). Ultimately, *n*-hexane:ethyl acetate:DCM:acetic acid (25:5:5:1, *v*/*v*) was selected as mobile phase for HPTLC. The developed zone containing target compounds (4.5–13.65 cm off the baseline) could be marked using BPA as the rearmost end marker and TBBPA-BAE as the leading end marker of zone (Figs. 1 and 2). The purpose of using BPA and TBBPA-BAE as markers was to reduce the random error during HPTLC separation and to guarantee method stability. The retention factors (R_f) of BPA and nine analogs ranged from 0.30 to 0.91 with a SD of less than 0.02 (Table 2).

After separation and drying, the desorption of target analytes from HPTLC plate was optimized using acetone and acetonitrile (containing 2% acetic acid). As shown in ESM Fig. S2, acetonitrile (containing 2% acetic acid) presented higher desorption efficiency than that of acetone when eluting 0.4 μg of target compounds from plate surfaces. And all the analytes could be simultaneously eluted by 20 mL acetonitrile (containing 2% acetic acid). The recoveries of these target analytes loaded on HPTLC plate ranged from 72 to 112%, two to five times higher than that of acetone (Table 2). Besides, other related factors, such as, the applied volume, the stability of related compounds for storing temperature and on the plate, and so on, have been similarly optimized and evaluated by using one-way analyses of variance (ANOVA) in our previous study [44]. And these conditions have been simply validated and taken.

Commonly, biological samples can be pretreated by acid silica or GPC for preliminary elimination of the interferences, such as proteins, lipids, and pigments [45]. But using acid silica for TBBPA purification might induce decomposition and then result in lower recovery [31]. Here, it only needs less than 30 mL solvent and about 50 min when using HPTLC for cleanup, more convenient than using GPC which often consumes > 300 mL solvents and > 2 h per sample [15]. Beyond that, the advantages of visual operation and non-destruction physical absorption are also helpful for the operation and subsequent analysis.

Analytical performance

Under the optimum conditions, the proposed method was evaluated by spiking BPA and its nine brominated analogs in chicken breast samples. The linear ranges were 10–1000 ng/mL for all target compounds, and determination coefficients (r^2) were all higher than 0.99 (Table 2). MDLs were in the range of 0.8 to 685.7 ng g^{-1} dw (S/N = 3), and the method quantification limits (MQLs) were in the range of 2.7 to 2285.7 ng g^{-1} dw (S/N = 10), respectively (Table 2). TBBPA-BAE and TBBPA-BDBPE are more difficult to ionize under analysis with mass spectrometer, so the HPLC-DAD is more appropriate and widely used to detect them. As everyone knows, differences of instrument sensitivity cause the MDLs and MQLs value of TBBPA-BAE, and TBBPA-BDBPE is higher than other target compounds. The MDLs for BPA and TBBPA were 5.9 and 2.1 ng g−1 dw, which were comparable with that using GPC coupled with GC-MS (1.3–6.9 ng g^{-1} dw) [15] but a little higher than that using GPC/SPE-HPLC/GC-MS/MS (0.03–0.07 ng/g) [16, 17]. Although the methods for analyzing TriBBPA, DBBPA, MBBPA, BPA, and TBBPA in breast milk and in sediment samples have been

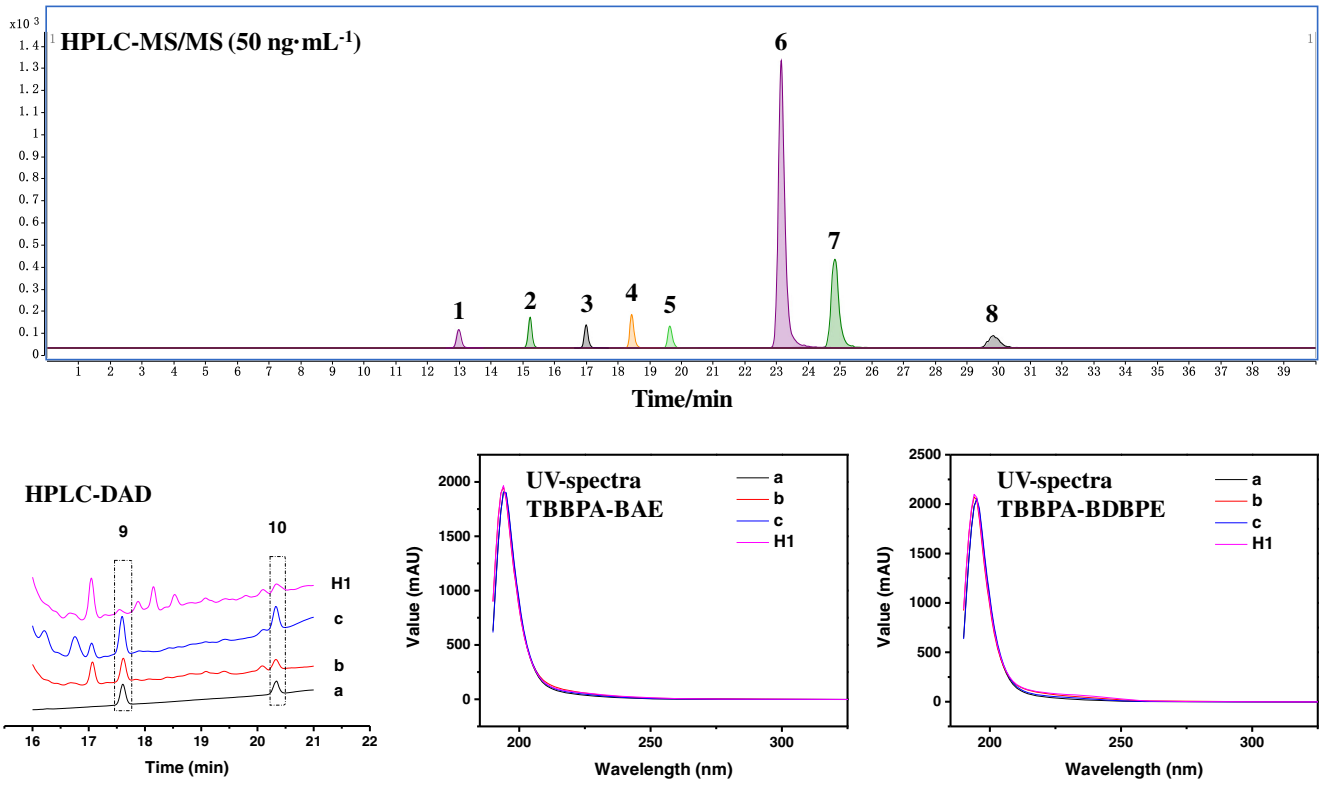


Fig. 3 Chromatograms and UV spectra of BPA and its nine brominated analogs: 1, BPA; 2, MBBPA; 3, 2,2′-DBBPA; 4, TriBBPA; 5, TBBPA; 6, TBBPA-MME; 7, TBBPA-MAE; 8, TBBPA-MDBPE; 9, TBBPA-BAE; 10, TBBPA-BDBPE; a, standard solution (600 ng mL^{-1}); b, the spiked solution (200 ng mL^{-1}); c, the spiked chicken sample (1000 ng mL^{-1}); and H1, natural samples

developed based on GC-MS [46] and HPLC-MS/MS [47], their validity in other biological samples have not been evaluated. Compared with these two methods, our analytes have been extended to BPA and its nine associated analogs and the application fields have been expanded to more complex biological samples.

The specificity of the method was ascertained by comparing R_f and t_R, where R_f represents the fingerprints from the HPTLC plate images and t_R represents UHPLC chromatogram of TBBPA-BAE and TBBPA-BDBPE and HPLC-MS/MS chromatogram of BPA and other seven brominated analogs (Fig. 3; Table 1). The R_f of the analytes was in the range of 0.30~0.91 (Table 2), corresponding to the t_R of 8.24~22.76 (Fig. 3) in chromatogram. Meanwhile, further confirmation for each analyte were performed by comparing their UV spectra (purity examination) and MS qualitative ions with their authentic standards.

The accuracy of the proposed method was evaluated by spiking the mixed standards in blank chicken breast samples at three different levels, 4, 8, and 16 μg g^{-1} dw ($n = 3$). The recoveries were in the range of 64–124% with SD lower than 10% (Table 2). The intermediate precision including intra- and interday precision results was determined by injection of freshly prepared standard solutions (500 ng mL^{-1}) in triplicate in 1 day (intraday) and in consecutive 3 days (interday). The RSD of intermediate precision ranged from 0.8 to 10.7% (n = 3, Table 2).

Assessment of matrix effect

The matrix effects of chicken breast samples were evaluated at three spiked levels of 200, 500, and 1000 ng·mL^{-1} for BPA and its nine brominated analogs. The matrix effects were calculated according to Eq. (1).

$$\text{Matrix effect} = \frac{C_s - C_b}{C_s} \times 100\% \quad (1)$$

where C_s is the concentration of standard solution and C_b is the measured concentration of the standards (concentration equals to C_s) spiked in the HPTLC-cleaned extracts of blank chicken sample.

The matrix effects for BPA and its nine brominated analogs by HPTLC purification strategy ranged from 0.06 to 18.6%, with eight analytes lower than 8.7% (Table 2). The lower interferences indicated that HPTLC can effectively remove matrix effects of interferences when analyzing these chemicals in complex biological samples.

Performance for analyzing chicken samples

Chicks were raised in a closed yard around large BFR plants located in Weifang, Shandong Province, China, and all the

Table 3 Concentration of BFRs in natural samples ($n = 3$)

Samples (ng g^{-1})	BPA	MBBPA	DBBPA	TriBBPA	TBBPA	TBBPA-MME	TBBPA-MAE	TBBPA-MDBPE	TBBPA-BAE	TBBPA-BDBPE
H1	357,034.0 ± 1229.5	134.9 ± 10.0	–	3.8 ± 0.4	55.9 ± 7.6	4.5 ± 0.2	8.2 ± 0.3	19.1 ± 2.1	434.0 ± 41.1	1148.8 ± 7.5
H2	6060.0 ± 886.6	–	–	–	194.6 ± 20.0	5.7 ± 0.4	8.0 ± 0.7	–	688.0 ± 22.0	1587.8 ± 16.3
H3	5396.4 ± 50.8	702.0 ± 30.6	135.7 ± 34.6	1545.6	599.3 ± 5.4	6.0 ± 0.3	137.1 ± 4.6	503.8 ± 12.5	1143.2 ± 30.8	–
H4	165.1 ± 10.1	95.8 ± 4.3	62.3 ± 8.7	180.9 ± 7.9	82.3 ± 2.3	7.8 ± 0.7	35.8 ± 1.8	65.9 ± 2.8	844.6 ± 20.9	–
C1	3625.5 ± 60.6	–	–	6.0 ± 1.6	66.8 ± 3.9	4.9 ± 0.06	14.8 ± 3.7	12.8 ± 2.9	329.3 ± 2.0	189.9 ± 7.1
C2	162.5 ± 5.6	46.0 ± 2.3	8.8 ± 4.3	84.4 ± 3.2	24.1 ± 1.2	–	14.9 ± 1.6	–	472.4 ± 15.8	–

tested chicks were 400 days old. Chicken samples, including four hens and two cocks, were treated by the proposed method and determined by HPLC-DAD/HPLC-MS/MS. The samples were named as H1, H2, H3, H4, C1, and C2. The contents of BPA and its nine brominated analogs ranged from 3.8 to 357,034.0 ng g^{-1} (Table 3). Higher levels of BPA, TBBPA, TBBPA-BAE, and TBBPA-BDBPE indicated their possible direct pollution source from the BFR factory. Occurrence of MBBPA, DBBPA, TriBBPA, TBBPA-MME, TBBPA-MAE, and TBBPA-MDBPE are likely to be the byproducts or the degradation products of TBBPA [30].

Conclusion

HPTLC technique coupled with HPLC-DAD/HPLC-MS/MS for simultaneous analysis of BPA and its nine brominated analogs in biological samples has been developed and evaluated. It presents the advantages of time and solvent saving, good cleanup efficiency, satisfactory recoveries, specificity, good precision, and low matrix effects. Compared with GPC, SPE, and acid silica, HPTLC can dramatically reduce manipulation steps and associated workload for biological sample cleanup without inducing high interference and targets decomposition. Also, TLC used for biological sample cleanup is simple and avoided the possible degradation of BPA analogs in pretreatment. Using the developed method, high level of BPA, TBBPA, TBBPA-BAE, and TBBPA-BDBPE together with their possible degradation/byproducts MBBPA, TriBBPA, TBBPA-MME, TBBPA-MAE, and TBBPA-MDBPE were detectable in the chicken samples, indicating the application potential of this method for further research on distribution, migration, and transformation of TBBPA series BFRs in organisms.

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Compliance with ethical standards

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the care and use of animals in china. The studies have been approved by the Academic Committee (Ethics Committee) of Qingdao Institute of Bioenergy and Bioprocess Technology, China Academy of Sciences.

Conflict of interest The authors declare that they have no conflict of interest.

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