Analytical and Bioanalytical Chemistry
https://doi.org/10.1007/s00216-018-1062-2

RESEARCH PAPER

Rapid and sensitive determination of nine bisphenol analogues, three amphenicol antibiotics, and six phthalate metabolites in human urine samples using UHPLC-MS/MS

Yuan Yao[1,2] · Yijun Shao[1,2] · Ming Zhan[3] · Xiaoli Zou[4] · Weidong Qu[1,5] · Ying Zhou[1,2,3]

Received: 28 December 2017 / Revised: 28 February 2018 / Accepted: 3 April 2018

Abstract

Bisphenol analogues, amphenicol antibiotics, and phthalate have widely aroused public concerns due to their adverse effects on human health. In this study, a rapid and sensitive method for determination of nine bisphenol analogues, three amphenicol antibiotics, and six phthalate metabolites in the urine based on ultra-high-performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry was developed and validated. The sample pretreatment condition on the base of mixed-mode anion-exchange (Oasis MAX) SPE was optimized to separate bisphenol analogues and amphenicol antibiotics from phthalate metabolites: the former were detected with a mobile phase of 0.1% ammonium water solution/methanol containing 0.1% ammonium water solution in negative mode, whereas the latter were determined with a mobile phase of 0.1% acetic acid solution/acetonitrile containing 0.1% acetic acid in negative mode. The limits of detection were less than 0.26 ng/mL for bisphenol analogues, 0.12 ng/mL for amphenicol antibiotics, and 0.14 ng/mL for phathalate metabolites. The recoveries of all target analytes in three fortification levels ranged from 72.02 to 117.64% with the relative standard deviations of no larger than 14.51%. The matrix effect was adjusted by isotopically labeled internal standards. This proposed method was successfully applied to analyze 40 actual urines and 13 out of 18 studied compounds were detected.

Keywords Bisphenol analogues · Amphenicol antibiotics · Phthalate metabolites · Mixed-mode solid-phase extraction · Ultra performance liquid chromatography-tandem mass spectrometry · Urine sample

Introduction

Bisphenol A (BPA) and phthalates are widely used in the manufacture of common consumer products such as food containers, paper, water pipes, toys, or personal care and medical products [1, 2]. Numerous researches have documented effects of BPA and DEHP as endocrine disruptor and have been potentially linked to adverse health effects as diabetes, obesity, or cancer [3–6]. As a result of the public concern and governmental regulations on BPA, some compounds are being used as alternative substances to replace BPA. These chemicals are structurally similar to BPA and collectively referred to as bisphenol analogues. Although a total of 16 bisphenol analogues have been documented for industrial applications, BPF (4,4′-methylenediphenol), BPS (4-hydroxyphenyl sulfone), and BPAF (4,4′-hexafluoroisopropylidene diphenol) are among the main substitutes of BPA in the manufacturing of polycarbonate plastics and epoxy resins. Elevated concentrations of BPF, BPS, and BPAF have been reported in the biotic

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00216-018-1062-2) contains supplementary material, which is available to authorized users.

✉ Ying Zhou
yingchou@fudan.edu.cn

[1] Key Laboratory of Public Health Security, School of Public Health, Ministry of Education, Fudan University, Shanghai 200032, China

[2] Department of Chemistry in Public Health, School of Public Health, Fudan University, 130 Dong-an Road, Xuhui District, Shanghai 200032, China

[3] Pudong New Area for Disease Control and Prevention, Fudan University Pudong Institute of Preventive Medicine, Shanghai 200136, China

[4] Department of Sanitary Technology, West China School of Public Health, University of Sichuan, Chengdu 610041, Sichuan, China

[5] Department of Environmental Health, School of Public Health, Fudan University, Shanghai 200032, China

Published online: 19 April 2018

environment and human urine from some regions [7, 8]. Moreover, laboratory studies have found that many bisphenol analogues exhibited endocrine disrupting effects, cytotoxicity, genotoxicity, reproductive toxicity, dioxin-like effects, and neurotoxicity [7]. However, the population epidemiological studies on the associations between bisphenol analogues exposure and adverse health effects are still limited.

Amphenicol antibiotics including chloramphenicol (CAP), thiamphenicol (TAP), and florfenicol (FF) were used as veterinary drugs to treat infections in animals. The association of CAP with many health side-effects such as bone marrow suppression and carcinogenic properties was reported in human [9]. However, there is up to date lack of epidemiological data to assess CAP carcinogenicity and effects on reproduction which makes the establishment of an ADI (acceptable daily intake) for CAP not feasible [10]. Although thiamphenicol (TAP) and florfenicol (FF) are widely used as the replacement of CAP in animals, the information on human exposure to TAP and FF through intaking animal food is scarce.

The determinations of bisphenol analogues, phthalates metabolites, and CAP in urinary species have been done by liquid chromatography-tandem mass spectrometry (LC-MS/MS) [11–17] or gas chromatography-mass spectrometry (GC-MS) [18, 19]. However, to our best knowledge, there is no study performed to simultaneously assess the human exposure to bisphenol analogues, amphenicol antibiotics, and phthalates as well as their health risks in epidemiological study. The aim of this study is to develop and validate a reliable and sensitive analytical method for the simultaneous determination of selected bisphenol analogues, amphenicol antibiotics, and phthalate metabolites in human urine species by ultra-high performance liquid chromatography coupled with triple quadrupole tandem mass spectrometry (UHPLC-MS/MS). The application of the proposed method is investigated through analysis of authentic school children urine samples.

Experimental

Chemicals and reagents

Analyte standards included nine bisphenol analogues (bisphenol A, bisphenol B, bisphenol AF, bisphenol AP, bisphenol E, bisphenol F, bisphenol P, bisphenol S, and bisphenol Z), three amphenicol antibiotics (chloramphenicol, thiamphenicol, and florfenicol), and six phthalate metabolites (monoethyl phthalate, mono(2-ethylhexyl)phthalate, mono(2-ethyl-5-carboxypentyl)phthalate, mono[(2-carboxymethyl)hexyl]phthalate, mono(2-ethyl-5-oxohexyl)phthalate, and mono(2-ethyl-5-hydroxyhexyl) phthalate. Other standards included 4-methylumbelliferone glucuronide and 4-methylumbelliferone (4-MU). All of these standards were purchased from Sigma-Aldrich (St Louis, MO, USA) or Dr. Ehrenstorfer (Augsburg, Germany). Thirteen isotopically labeled internal standards (IS) of MEP-$^{13}C_2$, MEHP-$^{13}C_2$, MECPP-$^{13}C_4$, MEOHP-$^{13}C_4$, MCMHP-$^{13}C_4$, MEHHP-$^{13}C_4$, BPA-d_4, BPF-d_{10}, BPS-$^{13}C_{12}$, BPAF-$^{13}C_{12}$, CAP-D_3, FF- D_3, and TAP-D_3 were supplied by Cambridge Isotope Laboratories (Andover, MA, USA). Compound names, chemical structures, and physical-chemical properties of the target analytes are listed in Table 1. HPLC grade of ethyl acetate, LC-MS grade of methanol (MeOH), acetonitrile (ACN), ammonium water, acetic acid, and ultra-pure water were from Sigma-Aldrich (St Louis, MO, USA) or Fisher Scientific (Fair Lawn, NJ, USA).

Urine sample

The synthetic urine devoid of all target compounds was prepared as described in the report from Bruno Alves Rocha et al. [13] and Uzqueda et al. [20]. Briefly, 3.8 g of potassium chloride, 8.5 g of sodium chloride, 24.5 g of urea, 1.03 g of citric acid, 0.34 g of ascorbic acid, 1.18 g of potassium phosphate, 1.4 g of creatinine, 0.64 g of sodium hydroxide, 0.47 g of sodium bicarbonate, and 0.28 mL of sulfuric acid were added into 500 mL of ultra-pure water and stirred for 60 min. All reagents were at least analytical grade and obtained from Sigma-Aldrich (St Louis, MO, USA).

Authentic urine samples were acquired from our previous studies [21] in which first morning urinary specimen were collected from 40 school children aged 8–11 years in the Chinese primary schools. All samples of the subjects were stored at − 80°C after collection for analysis.

Preparation of buffer solutions, stock solutions, calibration solutions, and quality control samples

The sodium dihydrogen phosphate buffer (50.0 mM, pH 6.0) was prepared by dissolving 1.560 g of sodium dihydrogen phosphate dihydrate in 200.0 mL of ultra-pure water. The pH value was adjusted to 6.0 with an aqueous 14.0 M solution of sodium hydroxide. The ammonium acetate buffer (1.0 M, pH 6.0) was prepared by dissolving 7.710 g of ammonium acetate in 100.0 mL of ultra-pure water. The pH value was adjusted to 6.0 with the concentrated acetic acid solution.

Stock solutions of standards of bisphenol analogues, amphenicol antibiotics, and phthalate metabolites and isotopically labeled IS were prepared gravimetrically in methanol at a concentration of 100 μg/mL. Working stock solutions were made at a concentration of 1.0 μg/mL for each analyte by diluting the stock solutions with methanol. Calibration standards were prepared by dilution of working standard mixtures to provide concentrations. All stock solutions were stored at − 20 °C, while all the calibration solutions were stored at 4 °C and allowed to equilibrate to laboratory temperature for at least 15 min before use.

Table 1 Physicochemical properties and structures of all targeted analytes

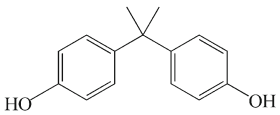
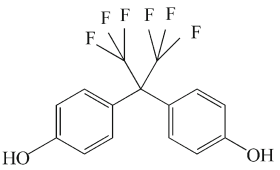
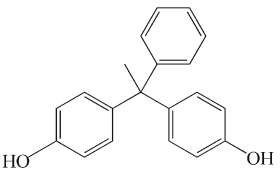
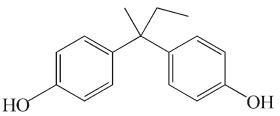
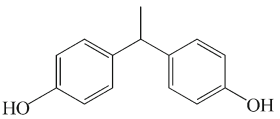
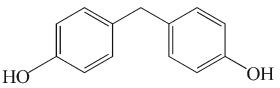
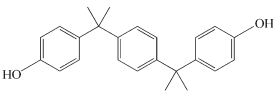
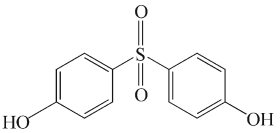
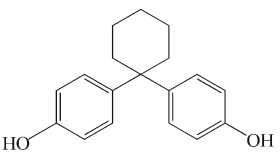
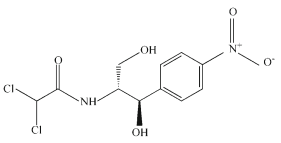
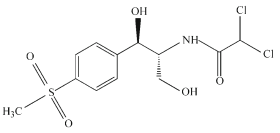
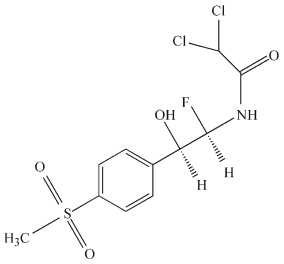
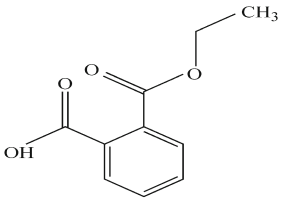
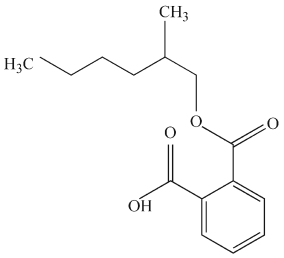
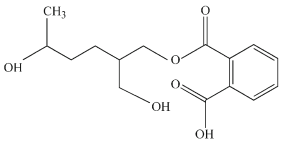
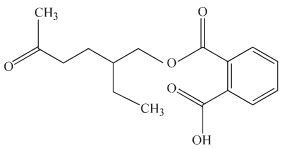
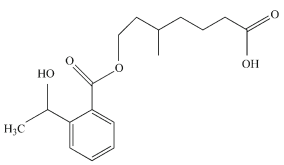
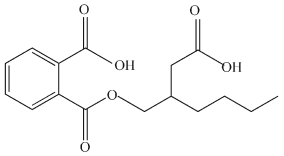
Compound	Acronym	CAS number	MW	Log K_{ow}[a]	pKa[b]	Structure
Bisphenol A	BPA	80-05-7	228.115	4.04	9.78-10.39	
Bisphenol AF	BPAF	1478-61-1	336.0585	4.77	9.13-9.74	
Bisphenol AP	BPAP	1571-75-1	290.1307	5.18	9.66-10.27	
Bisphenol B	BPB	77-40-7	242.1307	4.49	9.77-10.38	
Bisphenol E	BPE	2081-08-5	214.0994	3.74	9.81-10.39	
Bisphenol F	BPF	620-92-8	200.0837	3.46	9.84-10.45	
Bisphenol P	BPP	2167-51-3	346.1933	6.72	9.78-10.38	
Bisphenol S	BPS	80-09-1	250.0300	2.32	7.42-8.03	
Bisphenol Z	BPZ	843-55-0	268.1463	4.91	9.76-10.37	
Chloramphenicol	CAP	56-75-7	323.129	0.88~1.22	7.49~9.61	
Thiamphenicol	TAP	15318-45-3	356.222	-0.33	9.03	

Table 1 (Continued)

Compound	Acronym	CAS number	MW	Log K_{ow}[a]	pKa[b]	Structure
Florfenicol	FF	73231-34-2	358.213	-0.04	9.76	
Monoethyl phthalate	MEP	2306-33-4	194.184	1.99	3.08	
Mono(2-ethylhexyl) phthalate	MEHP	4376-20-9	278.343	4.66	3.08	
Mono(2-ethyl-5-hydroxyhexyl) phthalate	MEHHP	40321-99-1	294.347	3.19	3.08	
Mono(2-ethyl-5-oxohexyl) phthalate	MEOHP	40321-98-0	292.337	3.22	3.08	
Mono[2-(ethyl-5-Carboxypentyl)hexyl] phthalate	MECPP	40809-41-4	308.326	3.34	3.03	
Mono[2-(carboxymethyl)hexyl] phthalate	MCMHP	82975-93-7	294.347	3.49	3.08	

[a] Data were obtained from Human Metabolome Database, Chemspider, or [22]

[b] Data were from Human Metabolome Database

Quality control (QC) samples consisting of bisphenol analogues, amphenicol antibiotics, and phthalate metabolites were prepared by adding appropriate volume working stock solutions into a 10-mL volumetric flask and diluting with synthetic urine to volume. QC samples of 10 ng/mL were used for solid-phase extraction (SPE) optimization. QC samples of 1, 5, 25, 50, and 150 ng/mL were applied to method validation and quality control for analysis of real urine sample.

Sample preparation

An aliquot (1.0 mL) of urine sample was spiked in succession with 10.0 μL of 13 isotope labeled IS mixture at a concentration of 1000 ng/mL, 10.0 μL of 4-MU-$^{13}C_4$ at a concentration of 10.0 μg/mL and 10.0 μL of 4-methylumbelliferone glucuronide at a concentration of 10.0 μg/mL. And then the sample was buffered with 200.0 μL of 1.0 M ammonium acetate (pH 6.0) and 20.0 μL of β-glucuronidase aqueous solution (197,114 units/mL) from *Helix pomatia* (type H-2, Sigma-Aldrich). The mixture was thoroughly vortexed for 1 min followed by incubation in a water bath at 37.0°C overnight to hydrolyze the analyte conjugates. Deconjugation efficiency was approximately 100% under such conditions.

After hydrolysis, the urine sample was loaded onto an Oasis MAX cartridge (6 cm^3/150mg, Waters, Milford, MA, USA) which was preconditioned with 4.0 mL of methanol and 4.0 mL of water. The cartridge was then sequentially washed with 4.0 mL water and 4.0 mL of 50.0 mM sodium dihydrogen phosphate buffer (pH 6.0). The analytes were eluted into two separate fractions. Firstly, the bisphenol analogues and amphenicol antibiotics were eluted with 5.0 mL of ethyl acetate after neutral or basic urine impurities were eliminated with 4.0 mL of 30% methanol solution (*v*/*v*). Secondly, the phthalate metabolites were eluted with 5.0 mL of methanol containing 1% formic acid (*v*/*v*) after the cartridge was sequentially washed by 4.0 mL of water to wash off the buffer and 4.0 mL of 30% methanol solution containing 1% formic acid (*v*/*v*) to remove acidic urine impurities. Two fractions of eluate were separately concentrated to dryness at 40 °C under a gentle stream of nitrogen. The residue containing bisphenol analogues and amphenicol antibiotics was redissolved in 200.0 μL of 50% methanol solution (*v*/*v*), whereas another residue containing phthalate metabolites was redissolved in 200.0 μL of 50% acetonitrile solution (*v*/*v*). All the reconstituted solutions were centrifuged at 16000 rpm for 15 min and transferred to glass vials for UHPLC-MS/MS analysis.

Instrumentation

UHPLC-MS/MS analysis was performed using an ultra-high performance liquid chromatography Nexera X2 coupled to an API 8050 triple stage quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). MS/MS detection was via electrospray ionization (ESI) in negative ion mode using multiple-reaction monitoring (MRM) transitions for quantification of each compound (see Table 2). Nitrogen was used as the desolvation gas, and argon was as the collision gas. The source conditions were set as follows: the flow rates of nebulizing gas, drying gas, and heating gas were set to 3.0, 10.0, and 10.0 L/min, respectively. The interface voltage was set to 4.0 kV. The DL temperature and heat block temperature were set to 250 and 400 °C, respectively. Transitions with optimized conditions for MS/MS are listed in Table 2.

Chromatographic separation of bisphenol analogues and amphenicol antibiotics were achieved using a C_{18} column (100 mm × 2.1 mm × 1.7 μm, Acquity UPLC BEH). Mobile phases was composed of 0.1% ammonium water solution (*v*/*v*) (A) and methanol containing 0.1% ammonium water solution (*v*/*v*) (B) with a flow rate of 0.2 mL/min. The gradient program of mobile phase B was as follows: staring at 10% for 0.20 min, then increasing to 100% over 3.8 min and held for 1.5 min, finally returning to 10% over 0.2 min and kept for 5.6 min to re-equilibrate the mobile phase. The run time was 11.30 min. The column temperature was maintained at 40 °C. The phthalate metabolites were analyzed with a Phenyl-Hexyl column (50 mm × 2.1 mm × 1.7 μm, Acquity UPLC CSH). The mobile phases included 0.1% acetic acid solution (*v*/*v*) (A) and 0.1% acetic acid in acetonitrile (*v*/*v*) (B) with a flow rate of 0.2 mL/min. The gradient program of mobile phase B was as follows: starting with 10% for 0.20 min, then increasing to 85% over 2.65 min and held for 0.20 min, and finally returning to 10% over 2.95 min and kept for 2.80 min. The run time was 8.80 min. The column temperature was set at 40 °C. Retention time of each analyte is given in Table 2.

Before analysis, injections of a double procedural blank were performed to ensure system stability and cleanliness followed by a system suitability test, performed by injecting the low-level QC sample containing all targeted analytes. After the injection of a further double blank and a reagent blank, analysis was started with injections of six-point calibration curve followed by a blank injection. The QC samples were interspersed evenly throughout the study samples. Following analysis of 15–20 study samples, four QC samples (two low-level and two high level) and a reagent blank were injected. The background concentrations obtained from the procedural blanks were deducted from the sample analysis. And glassware used for experiments was baked for 4 h at 400 °C in a muffle furnace to remove phthalate metabolites and bisphenol analogues contamination.

Quantification of the samples

All analytes were quantified by means of isotopic dilution internal standard calibration. BPA-d_4 was used for BPA, BPZ, BPB, BPE, and BPB, while BPF-d_{10} was for BPF, BPS-$^{13}C_{12}$ was for BPS, BPAF-$^{13}C_{12}$ was for BPAF and BPAP, CAP-d_3

Table 2 Chromatographic and mass spectrometric characteristics of the analytes in human urine

Analyte	RT (min)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Q1 pre bias (V)	CE (V)	Q3 pre bias (V)
BPA	6.683	227.00	212.00[a]	11.0	18.0	13.0
			133.05[b]	11.0	24.0	13.0
BPA-d_4	6.680	231.20	216.20 [a]	11.0	19.0	14.0
			135.15 [b]	14.0	26.0	12.0
BPAF	6.517	335.95	266.00 [a]	17.0	22.0	12.0
			178.05 [b]	17.0	45.0	11.0
BPAF-$^{13}C_{12}$	6.512	346.05	276.05 [a]	16.0	25.0	22.0
			277.15 [b]	24.0	13.0	12.0
BPAP	6.986	289.10	274.05 [a]	11.0	22.0	12.0
			273.10 [b]	14.0	36.0	18.0
BPB	6.903	240.90	212.05 [a]	12.0	17.0	13.0
			211.05 [b]	12.0	28.0	21.0
BPE	6.492	212.95	198.00 [a]	11.0	18.0	12.0
			197.05 [b]	11.0	28.0	20.0
BPF	6.251	198.85	77.05 [a]	12.0	26.0	26.0
			105.05 [b]	11.0	22.0	10.0
BPF-d_{10}	6.233	209.15	97.10 [a]	15.0	23.0	13.0
			110.05 [b]	15.0	23.0	13.0
BPS	0.884	248.75	108.00 [a]	12.0	26.0	10.0
			91.95 [b]	12.0	34.0	13.0
BPP	7.587	345.05	330.15 [a]	17.0	28.0	15.0
			315.10 [b]	10.0	38.0	21.0
BPS-$^{13}C_{12}$	0.880	261.00	113.95 [a]	13.0	27.0	11.0
			98.00 [b]	13.0	36.0	13.0
BPZ	7.153	266.95	173.10 [a]	14.0	26.0	11.0
			223.00 [b]	13.0	32.0	14.0
CAP	5.996	321.05	152.15 [a]	11.0	17.0	15.0
			257.10 [b]	16.0	12.0	11.0
CAP-d_3	5.993	326.05	157.20 [a]	16.0	18.0	16.0
			262.10 [b]	16.0	13.0	12.0
TAP	5.094	354.10	185.15 [a]	13.0	20.0	12.0
			290.10 [b]	13.0	13.0	13.0
TAP-d_3	5.081	359.05	188.15 [a]	11.0	21.0	12.0
			295.10 [b]	11.0	13.0	13.0
FF	5.527	356.05	336.05 [a]	13.0	10.0	11.0
			185.15 [b]	10.0	19.0	12.0
FF-d_3	5.526	361.00	341.05 [a]	18.0	11.0	11.0
			121.15 [b]	18.0	13.0	11.0
MEP	4.978	193.10	77.10 [a]	11.0	17.0	16.0
			121.10 [b]	11.0	12.0	13.0
MEP-$^{13}C_2$	4.975	197.10	79.10 [a]	22.0	17.0	16.0
			124.15 [b]	22.0	14.	12.0
MEHP	6.279	277.15	134.10 [a]	14.0	15.0	26.0
			77.10 [b]	13.0	22.0	11.0
MEHP-$^{13}C_2$	6.278	281.10	137.10 [a]	13.0	15.0	27.0
			79.05 [b]	13.0	22.0	11.0
MEHHP	5.277	293.15	145.20 [a]	14.0	14.0	14.0
			121.05 [b]	14.0	18.0	11.0
MEHHP-$^{13}C_4$	5.275	297.20	145.25 [a]	14.0	15.0	14.0
			124.15 [b]	14.0	22.0	12.0
MEOHP	5.371	291.15	143.20 [a]	14.0	14.0	14.0
			121.10 [b]	15.0	18.0	12.0
MEOHP-$^{13}C_4$	5.366	295.15	143.20 [a]	14.0	14.0	14.0
			124.10 [b]	15.0	18.0	12.0

Table 2 (continued)

Analyte	RT (min)	Precursor ion (*m*/*z*)	Product ion (*m*/*z*)	Q1 pre bias (V)	CE (V)	Q3 pre bias (V)
MECPP	5.299	307.15	159.25 [a]	15.0	13.0	10.0
			121.10 [b]	15.0	23.0	12.0
MECPP-$^{13}C_4$	5.296	311.15	159.25 [a]	20.0	14.0	20.0
			113.20 [b]	15.0	29.0	11.0
MCMHP	5.471	307.15	159.25 [a]	15.0	11.0	10.0
			113.15 [b]	15.0	29.0	11.0
MCMHP-$^{13}C_4$	5.473	311.15	159.30 [a]	15.0	11.0	10.0
			141.25 [b]	15.0	24.0	13.0

[a] Quantification ion

[b] Confirmation ion

was for CAP, FF-d_3 was for FF, and TAP-d_3 was for TAP. The data were fitted to a linear least square regression curve with a weighting index of 1/*x*. All analytes and IS were identified by their chromatographic retention times and by two mass transitions (shown in Table 2) following the criteria in European Commission Decision 2002/657/EC [23].

Method validation

Method validation for the proposed method was performed in accordance with the guidelines on bioanalytical method validation from the European Medicine Agency (EMA) and US Food and Drug Administration (FDA) guidelines. Validation parameters include linearity, limit of detection (LOD), limit of quantitation (LOQ), precision, accuracy, matrix effects, and stability of analytes.

Linearity was assessed using an 8-point calibration curve with three replicates. The LOD and LOQ were determined by spiking decreasing concentrations of the mixed standard solution into blank synthetic urine. The LOD and LOQ were calculated as $3S_0$ and $10S_0$, where S_0 is the standard deviation as the concentration approaches zero [14]. Accuracy and precision were evaluated simultaneously by analyzing low, intermediate, and high concentration levels of QC samples injected with five replicates on five different days. The matrix effect was calculated as the ratio of the slope of the working curve obtained with synthetic urine matrix extracts to that of the calibration curve obtained from standard solutions [24, 25]. The stability of the injected analyte was investigated by storing the QC samples in the autosampler at 4 °C and by reanalysis of the QC samples maintained in the autosampler for 72 h. For the analyte to be considered stable, the difference had to be within ± 10% of the original value. The stability of working standard solutions was evaluated by determinations of freshly made QC samples spiked at low, intermediate, and high concentration levels of standard solutions at three replicates and reanalysis of the QC samples stored at 4 °C for 72 h. For the same level of QC samples, the difference of determinations at 0 and 72 h should be less than 10%.

Result and discussion

Optimization of LC-MS/MS conditions

Mass spectrometric conditions were investigated using the analyte standards on the base of peak intensity. To ensure optimal performance of mass spectrometer, compound-dependent parameters were individually optimized via direct infusion of each analyte standard. For each target compound and isotopically labeled analogous standard, two MRM transitions were respectively chosen as quantification and confirmation ions. The optimal mass spectrometric analysis characteristics are displayed in Table 2.

Chromatographic conditions were evaluated by analyses of the QC samples and spiked real urinary samples on the base of peak intensity and chromatographic separation. It has been reported in the previous studies [12, 13, 15] that bisphenol analogues, amphenicol antibiotics, or phthalates metabolites were separated using different LC conditions. Similarly, in this study, bisphenol analogues, amphenicol antibiotics, and phthalates metabolites were divided into two fractions for analysis. In the preliminary experiments, the chromatographic separation condition adapted from one of our previous studies [15] was used for separation of phthalates metabolites. The results indicated that chromatographic separation of the phthalates metabolites especially for MECPP and MCMHP were not done well in the spiked real urinary samples when using 0.1% acetic acid/acetonitrile as mobile phase (data not shown). The minor change of mobile phase was performed and then the optimal chromatographic separation for all phthalates metabolites were achieved when using 0.1% acetic acid/0.1% acetic acid in acetonitrile as mobile phase (see Electronic Supplementary Material (ESM) Fig. S1). The chromatographic separations of bisphenol analogues and amphenicol antibiotics were also investigated with the following mobile phases, i.e., water/methanol, 0.1% ammonium water/methanol, 0.1% ammonium water/methanol containing 0.1% ammonium water, respectively. Their gradient elution programs of were adapted from the previous studies [13, 14,

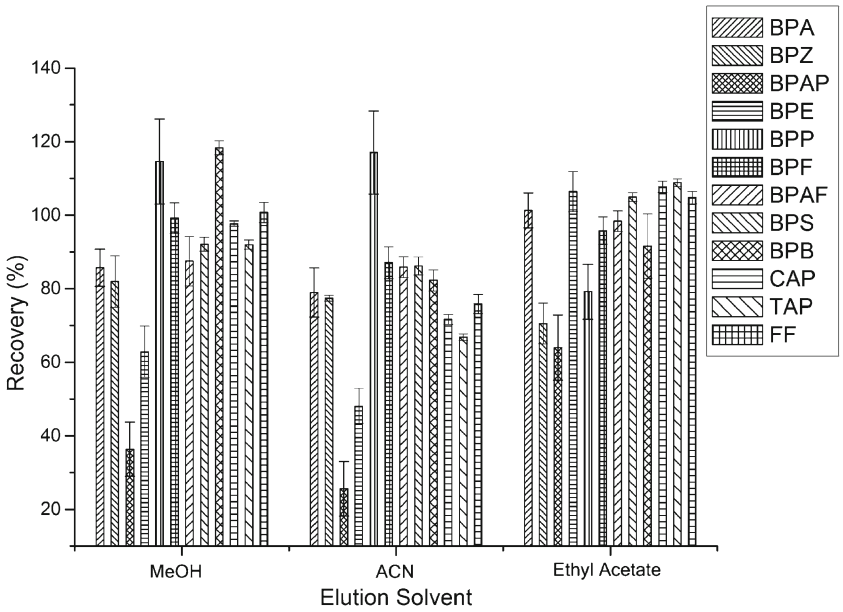


Fig. 1 Influence of elution solvent on the recovery of bisphenol analogues and amphenicol antibiotics in QC samples of 10 ng/ml

22, 26, 27]. As indicated in ESM Fig. S2, bisphenol analogues and amphenicol antibiotics were simultaneously separated well and better peak intensities were achieved for all of them using 0.1% ammonium water/methanol containing 0.1% ammonium as mobile phase. Therefore, 0.1% ammonium water/methanol containing 0.1% ammonium was chosen for chromatographic separation of bisphenol analogues and amphenicol antibiotics, and 0.1% acetic acid/0.1% acetic acid in acetonitrile for separation of phthalates metabolites.

Optimization of SPE procedure

Mixed-mode polymeric sorbents have a polymeric backbone capable of retaining compounds through both reversed-phase and ionic interactions. The presence of ionic interactions allows basic or acidic compounds to be selectively extracted from complex matrices. These sorbents have been applied to maximize the extraction of analytes in complex sample matrixes and minimize matrix effects by separating the basic, acidic, and neutral substances into different fractions, thus decreasing the extract complexity. [28–30]. According to our previous study [15], the mixed-mode SPE (reversed-phase and anion-exchange Oasis MAX sorbent) was selected to extract and clean-up all the analytes in this study. As inferred from for the pKa values in Table 1, bisphenol analogues and amphenicol antibiotics have lower acidic properties than phthalates metabolites. Theoretically, bisphenol analogues and amphenicol antibiotics might be protonated at low or intermediate pH values hereby decreasing ionic interactions with quaternary amine groups of Oasis MAX sorbent,

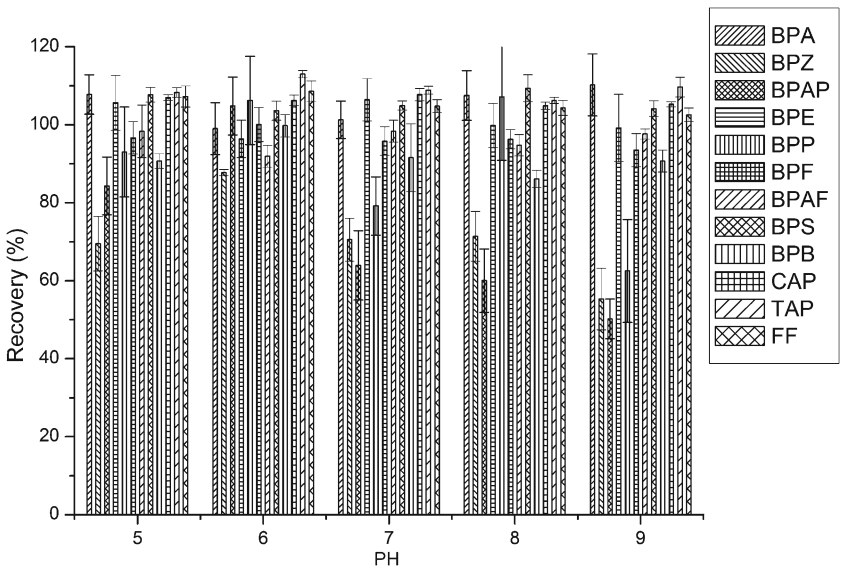


Fig. 2 Influence of pH value on recovery of bisphenol analogues and amphenicol antibiotics in QC samples of 10 ng/ml

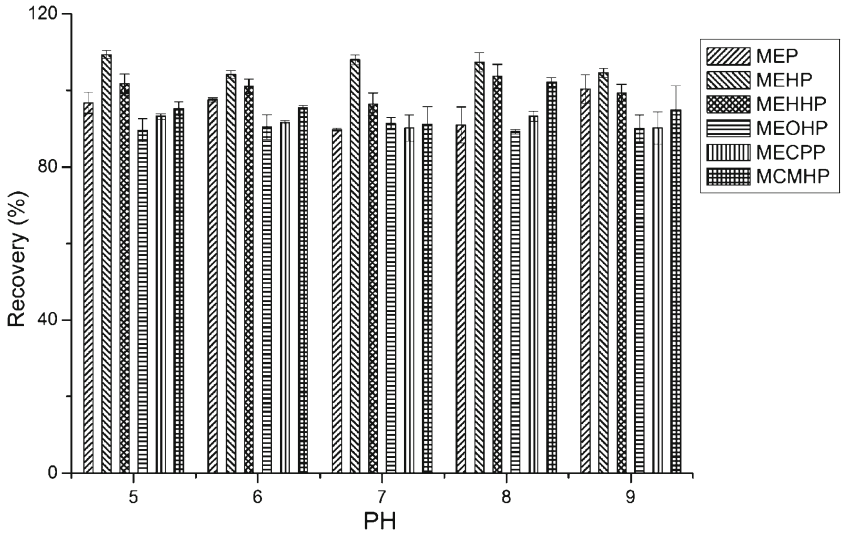


Fig. 3 Influence of pH value on recovery of phthalate metabolites in QC samples of 10 ng/ml

whereas phthalate metabolites could be deprotonated at high or intermediate pH values, thereby increasing retention by reversed-phase and ionic interactions with Oasis MAX sorbent. Therefore, all the analytes might be separated into two fractions resulting from optimization of elution solvent and pH value of the loaded buffer solution.

Optimization of elution solvent

In the preliminary experiments, elution solvents were adapted from a previous study [15] in which methanol was applied to elute bisphenol analogues and amphenicol antibiotics from Oasis MAX sorbent and methanol containing 1% formic acid was used to elute phthalate metabolites from the same sorbent. The results indicated that the methanol containing 1% formic acid was suitable for elution of phthalate metabolites with good recoveries; however, BPS and BPAF were not completely eluted by methanol (data not shown). Given the bisphenol analogues and amphenicol antibiotics have low water solubility except BPS, three types of organic solvents including methanol, acetonitrile, and ethyl acetate were used to evaluate the elution capabilities of bisphenol analogues and

Table 3 The linearity, sensitivity, and matrix effect of all targeted analytes

Compound	Linearity		Sensitivity		Matrix effect (%)	
	Range (ng/mL)	*r*	LOD (ng/mL)	LOQ (ng/mL)	Before-adjusted	After-adjusted
BPA	0.5–50	0.997	0.13	0.38	58.81	− 9.36
BPF	0.5–50	0.999	0.13	0.42	35.63	− 13.29
BPS	0.25–50	0.999	0.06	0.22	81.49	− 15.42
BPAF	0.2–50	0.995	0.05	0.17	45.13	− 10.39
BPAP	0.2–50	0.998	0.03	0.12	47.17	19.63
BPZ	0.5–50	0.998	0.26	0.81	68.37	25.17
BPB	0.2–50	0.998	0.04	0.14	62.44	19.78
BPE	0.2–50	0.999	0.21	0..71	64.81	24.87
BPP	0.5–50	0.984	0.15	0.52	46.75	3.68
CAP	0.05–50	0.997	0.02	0.05	43.63	6.24
TAP	0.5–50	0.998	0.12	0.33	49.84	3.12
FF	0.06–50	0.993	0.02	0.07	34.36	13.30
MEP	0.5–200	0.999	0.14	0.48	68.12	− 10.20
MEHP	0.1–200	0.998	0.03	0.12	35.25	− 1.09
MEHHP	0.1–200	0.997	0.03	0.09	16.57	− 3.85
MEOHP	0.1–200	0.998	0.02	0.07	25.19	5.37
MECPP	0.1–200	0.998	0.01	0.04	12.80	3.19
MCMHP	0.1–200	0.998	0.02	0.07	32.50	− 9.26

Table 4 The accuracy and precision of the proposed method for all targeted analytes ($n = 5$)

Compound	Spiked (ng/mL)	Recovery (%)	Precision (%)	
			Intra-day	Inter-day
BPA	1	104.32	2.85	3.43
	5	102.47	2.32	1.53
	25	112.85	6.80	2.60
BPF	1	101.23	5.55	3.48
	5	94.97	7.69	9.84
	25	113.28	9.07	5.98
BPS	1	98.72	7.06	6.28
	5	104.89	5.22	1.34
	25	113.42	2.02	0.26
BPAF	1	89.08	4.86	8.32
	5	96.00	6.66	2.71
	25	115.80%	3.93	6.26
BPAP	1	83.32	5.98	4.82
	5	94.36	9.65	4.36
	25	83.34	9.02	3.15
BPZ	1	81.23	8.85	8.59
	5	72.02	12.31	9.99
	25	83.03	10.10	7.55
BPB	1	87.18	5.72	4.85
	5	81.18	5.55	3.27
	25	95.55	5.38	4.25
BPE	1	91.08	10.18	4.32
	5	94.31	1.82	11.56
	25	111.34	8.92	1.31
BPP	1	98.54	2.86	7.83
	5	87.67	3.63	12.23
	25	108.24	10.39	3.92
CAP	1	101.32	9.82	7.43
	5	104.35	4.93	5.28
	25	112.13	5.99	7.27
TAP	1	101.34	6.14	3.82
	5	117.64	1.45	0.85
	25	106.16	2.99	3.10
FF	1	93.29	5.17	6.24
	5	95.95	3.22	1.48
	25	100.77	4.46	1.04
MEP	1	108.11	7.35	2.96
	5	111.21	5.31	5.21
	150	109.37	2.30	3.79
MEHP	1	110.98	5.55	8.86
	5	103.90	9.62	4.45
	150	101.37	2.20	4.46
MEHHP	1	100.70	5.25	3.56
	5	104.59	9.32	3.91
	150	92.84	3.85	1.47
MEOHP	1	108.29	4.46	7.21
	5	112.59	2.55	1.33
	150	103.23	4.68	0.33
MECPP	1	88.26	10.38	3.13
	5	109.13	9.33	6.91
	150	95.58	8.49	4.58
MCMHP	1	85.68	7.82	9.76
	5	97.28	14.51	2.87
	150	104.91	3.79	6.46

amphenicol antibiotics. As shown in the Fig. 1, the recoveries of bisphenol analogues and amphenicol antibiotics were found optimal when using ethyl acetate rather than methanol or acetonitrile as elution solvent. Finally, ethyl acetate was selected for eluting bisphenol analogues and amphenicol antibiotics and then methanol containing 1% formic acid was chosen for eluting phthalate metabolites.

Optimization of pH values of the loaded buffer solution

The pH value was one important factor for changing the retention of compounds on mixed-mode SPE sorbents. In the present study, a series of buffer solutions across a pH range of 5.0–9.0 were investigated by the recoveries of each analyte in QC samples of 10 ng/mL. As shown in Figs. 2 and 3, the average recoveries of amphenicol antibiotics and phthalate metabolites varied in small scale at pH between 5.0 and 9.0 with deviations of less than 3.11 and 4.52%, respectively. However, the recoveries of nine bisphenol analogues altered inconsistently at pH from 5.0 through 9. For example, the recoveries of BPAP varied from 73.02 to 108.76% with a deviation of 23.70%. In the case of all bisphenol analogues, the maximum recoveries of the analytes were acquired while the pH value of buffer solution was adjusted to 6.0 (as shown in Fig. 2). Consequently, a 50-mM sodium dihydrogen phosphate buffer of pH 6.0 was chosen as optimal.

Method validation

The validation of the method was performed with synthetic urine due to the absence of true blank urine samples without all targeted analytes.

The correlation coefficients (r) of all target compounds were greater than 0.993, except for BPP (0.984). The LOD and LOQ for each analyte are shown in Table 3. The LOD of bisphenol analogues ranged from 0.03 to 0.26 ng/mL, those of amphenicol antibiotics ranged from 0.02 to 0.12 ng/mL, and those of

Table 5 Analysis of urine samples of school age children by the proposed method

Compound	All (ng/mL)	Boy (ng/mL)	Girl (ng/mL)	Detection frequency (%)	Range (ng/mL)
BPA	2.03[a] (1.61)[b]	2.21 (1.79)	1.85 (1.53)	97.5	< LOD–7.4362
BPS	1.58 (4.24×10^{-2})	2.32 (4.24×10^{-2})	8.55×10^{-1} (4.24×10^{-2})	25.0	< LOD–37.562
BPF	1.91×10^{-1} (9.19×10^{-2})	2.76×10^{-1} (9.19×10^{-2})	1.06×10^{-1} (9.19×10^{-2})	17.5	< LOD–1.9857
BPAF	4.05×10^{-2} (3.53×10^{-2})	4.23×10^{-2} (3.53×10^{-2})	3.86×10^{-2} (3.53×10^{-2})	7.5	< LOD–0.1076
BPE	n.d	n.d	n.d	0	n.d
BPB	n.d	n.d	n.d	0	n.d
BPAP	n.d	n.d	n.d	0	n.d
BPZ	n.d	n.d	n.d	0	n.d
BPP	n.d	n.d	n.d	0	n.d
CAP	7.22×10^{-2} (3.76×10^{-2})	7.51×10^{-2} (3.42×10^{-2})	6.92×10^{-2} (4.19×10^{-2})	70.0	< LOD–0.3904
TAP	1.26×10^{-1}(8.48×10^{-2})	1.68×10^{-1}(8.48×10^{-2})	8.48×10^{-2}(8.48×10^{-2})	7.5	< LOD–0.7833
FF	2.47×10^{-2}(1.41×10^{-2})	1.71×10^{-2}(1.41×10^{-2})	3.15×10^{-2}(1.41×10^{-2})	17.5	< LOD–0.2419
MEP	78.18(21.90)	25.00(17.99)	131.35(28.50)	100	1.111–1838
MEHP	12.45(5.93)	15.33(6.01)	9.58(5.93)	100	0.3864–140.4
MEHHP	62.41(35.42)	78.12(41.42)	46.71(33.62)	100	3.644–542.1
MEOHP	39.21(25.53)	48.10(26.81)	30.32(24.68)	100	1.839–322.3
MECCP	68.77(34.71)	85.59(44.06)	51.96(33.05)	100	3.099–810.7
MCMHP	35.59(21.43)	43.26(26.60)	27.92(18.90)	100	2.254–285.4

n.d not detected

[a] Mean value

[b] Median value

phathalate metabolites ranged from 0.01 to 0.14 ng/mL. This method provided better sensitivity than previous studies [13, 15].

The accuracy and precision of the method were assessed by the recovery and relative standard deviation (RSD), respectively. Table 4 showed the information on accuracy and precision of bisphenol analogues, amphenicol antibiotics, and phthalate metabolites at three different concentrations levels. For bisphenol analogues, the recoveries ranged between 72.02 and 115.80% with intra-day RSDs from 1.82 to 12.31% and inter-day RSDs from 0.26 to 12.23%; for amphenicol antibiotics, the recoveries ranged between 93.29 and 117.64% with intra-day RSDs from 1.45 to 9.82% and inter-day RSDs from 0.85 to 7.43%; for phthalate metabolites, the recoveries ranged between 85.68 and 112.59% with intra-day RSDs from 2.20 to 14.51% and inter-day RSDs from 0.33 to 9.76%.

Matrix effects during ESI were caused by non-target analyte from the sample, which could suppress or enhance the ionization of target compound. Table 3 indicates the after-adjusted isotopically labeled internal standard curve method favoring the decrease of matrix effects compared to before-adjusted standards curve method. The working standard solutions in the QC samples used for the validation of the method were stable at 4 °C for 72 h (see ESM Table S1). The analytes in the injection sample were also stable at 4 °C for 72 h (data not shown). The comparison of performance characteristics of the proposed method with that of the previously published methods is shown in ESM Table S2.

Application to human samples

Following validation, the proposed method was applied to analyze the urine samples collected from 40 Chinese school age children. The results are summarized in Table 5. All the studied analytes were found in urine samples except for BPAP, BPB, BPE, BPP, and BPZ. For bisphenol analogues, the BPA was most widely detected followed by BPS, BPF, and BPAF, which was similar with other reported studies [13, 14, 31]. The median concentrations of bisphenol analogues found were 1.61 ng/mL for BPA, 4.24×10^{-2} ng/mL for BPS, 9.19×10^{-2} ng/mL for BPF, and 3.53×10^{-2} ng/mL for BPAF, respectively. The result tended to be lower than other studies [14, 31].

The medium concentrations of amphenicol antibiotics were 1.61 ng/mL for CAP, 8.48×10^{-2} ng/mL for TAP, and 1.41×10^{-2} ng/mL for FF and the detection frequency ranged from 7.5 to 70.0%. All the phthalate metabolites were detected over the study participants and the concentrations were comparable with the previous studies [15, 21, 32, 33].

Conclusions

In this study, a rapid and sensitive method was developed and validated for simultaneous determination of 18 bisphenol analogues, amphenicol antibiotics, and phthalate metabolites in

human urine. Mixed-mode SPE was used for selective extraction and clean-up of all the studied analytes which resulted in superior chromatographic separation and mass spectrometric analysis of them. The results showed the method had satisfactory accuracy and precision. The proposed method was applied to human samples and occurrence of the target compounds was found in the samples of the subjects of school age children. The analytical method should be of great benefit in supporting exposure assessment and epidemiological study of targeted environmental pollutants.

Acknowledgements This study was supported by the National Natural Science Foundation of China (No. 81373089), Scientific Research Foundation of Shanghai Municipal Commission of Health and Family Planning (No. 201540053), the National Science Fund for Distinguished Young Scholars of China (No. 81325017), and the Key Program of the National Natural Science Foundation of China (No. 81630088).

Compliance with ethical standards

The study was approved by the Institutional Review Board (IRB) of the School of Public Health, Fudan University (ref: IRB#2013-03-0437). Written informed consent was obtained from all participants and the parents/LAR of the participants.

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

References

1. Muhamad MS, Salim MR, Lau WJ, Yusop Z. A review on bisphenol A occurrences, health effects and treatment process via membrane technology for drinking water. Environ Sci Pollut Res Int. 2016;23(12):11549–67.
2. Guo Y, Alomirah H, Cho HS, Minh TB, Mohd MA, Nakata H, et al. Occurrence of phthalate metabolites in human urine from several Asian countries. Environ Sci Technol. 2011;45(7):3138–44.
3. Rubin BS. Bisphenol A: an endocrine disruptor with widespread exposure and multiple effects. J Steroid Biochem Mol Biol. 2011;127(1–2):27–34.
4. Swan SH. Environmental phthalate exposure in relation to reproductive outcomes and other health endpoints in humans. Environ Res. 2008;108(2):177–84.
5. Diamanti-Kandarakis E, Bourguignon JP, Giudice LC, Hauser R, Prins GS, Soto AM, et al. Endocrine-disrupting chemicals: an Endocrine Society scientific statement. Endocr Rev. 2009;30(4):293–342.
6. Casals-Casas C, Desvergne B. Endocrine disruptors: from endocrine to metabolic disruption. Annu Rev Physiol. 2011;73:135–62.
7. Chen D, Kannan K, Tan H, Zheng Z, Feng YL, Wu Y, et al. Bisphenol analogues other than BPA: environmental occurrence, human exposure, and toxicity-a review. Environ Sci Technol. 2016;50(11):5438–53.
8. Caballero-Casero N, Lunar L, Rubio S. Analytical methods for the determination of mixtures of bisphenols and derivatives in human and environmental exposure sources and biological fluids. A review. Anal Chim Acta. 2016;908:22–53.
9. Liu HY, Lin SL, Fuh MR. Determination of chloramphenicol, thiamphenicol and florfenicol in milk and honey using modified QuEChERS extraction coupled with polymeric monolith-based capillary liquid chromatography tandem mass spectrometry. Talanta. 2016;150:233–9.
10. Hanekamp JC, Bast A. Antibiotics exposure and health risks: chloramphenicol. Environ Toxicol Pharmacol. 2015;39(1):213–20.
11. Chen M, Tao L, Collins EM, Austin C, Lu C. Simultaneous determination of multiple phthalate metabolites and bisphenol-A in human urine by liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2012;904:73–80.
12. Zhang S, Liu Z, Guo X, Cheng L, Wang Z, Shen J. Simultaneous determination and confirmation of chloramphenicol, thiamphenicol, florfenicol and florfenicol amine in chicken muscle by liquid chromatography-tandem mass spectrometry. J Chromatogr B Anal Technol Biomed Life Sci. 2008;875(2):399–404.
13. Rocha BA, da Costa BR, de Albuquerque NC, de Oliveira AR, Souza JM, Al-Tameemi M, et al. A fast method for bisphenol A and six analogues (S, F, Z, P, AF, AP) determination in urine samples based on dispersive liquid-liquid microextraction and liquid chromatography-tandem mass spectrometry. Talanta. 2016;154:511–9.
14. Zhou X, Kramer JP, Calafat AM, Ye X. Automated on-line column-switching high performance liquid chromatography isotope dilution tandem mass spectrometry method for the quantification of bisphenol A, bisphenol F, bisphenol S, and 11 other phenols in urine. J Chromatogr B Anal Technol Biomed Life Sci. 2014;944:152–6.
15. Wang HX, Wang B, Zhou Y, Jiang QW. Rapid and sensitive analysis of phthalate metabolites, bisphenol A, and endogenous steroid hormones in human urine by mixed-mode solid-phase extraction, dansylation, and ultra-performance liquid chromatography coupled with triple quadrupole mass spectrometry. Anal Bioanal Chem. 2013;405(12):4313–9.
16. Heffernan AL, Thompson K, Eaglesham G, Vijayasarathy S, Mueller JF, Sly PD, et al. Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine. Talanta. 2016;151:224–33.
17. Wang HX, Wang B, Zhou Y, Jiang QW. Rapid and sensitive screening and selective quantification of antibiotics in human urine by two-dimensional ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry. Anal Bioanal Chem. 2014;406(30):8049–58.
18. Kim M, Song NR, Choi JH, Lee J, Pyo H. Simultaneous analysis of urinary phthalate metabolites of residents in Korea using isotope dilution gas chromatography-mass spectrometry. Sci Total Environ. 2014;470-471:1408–13.
19. Cunha SC, Fernandes JO. Quantification of free and total bisphenol A and bisphenol B in human urine by dispersive liquid-liquid microextraction (DLLME) and heart-cutting multidimensional gas chromatography-mass spectrometry (MD-GC/MS). Talanta. 2010;83(1):117–25.
20. Gustafsson JBC, Uzqueda BHR. The influence of citrate and phosphate on the mancini single radial immunodiffusion technique and suggested improvements for the determination of urinary albumin. Clin Chim Acta. 1978;90(3):249–57.
21. Wang B, Wang H, Zhou W, Chen Y, Zhou Y, Jiang Q. Urinary excretion of phthalate metabolites in school children of China: implication for cumulative risk assessment of phthalate exposure. Environ Sci Technol. 2015;49(2):1120–9.
22. Regueiro J, Breidbach A, Wenzl T. Derivatization of bisphenol A and its analogues with pyridine-3-sulfonyl chloride: multivariate optimization and fragmentation patterns by liquid chromatography/Orbitrap mass spectrometry. Rapid Commun Mass Spectrom. 2015;29(16):1473–84.
23. Commission Decision (2002/657/EC) of 12 August 2002 implementing Council Directive 96/23/EC concerning the

performance of analytical methods and the interpretation of results. Off J Eur Commun L221:8–36.
24. Benijts T, Dams R, Lambert W, De Leenheer A. Countering matrix effects in environmental liquid chromatography–electrospray ionization tandem mass spectrometry water analysis for endocrine disrupting chemicals. J Chromatogr A. 2004;1029(1–2):153–9.
25. Li M, Wang Q, Zhu J, Li N, Zou X. A simple analytical method of determining 1-hydroxypyrene glucuronide in human urine by isotope dilution with ultra performance liquid chromatography-tandem mass spectrometry. Anal Bioanal Chem. 2017;409(6):1513–8.
26. Yang Y, Lu L, Zhang J, Yang Y, Wu Y, Shao B. Simultaneous determination of seven bisphenols in environmental water and solid samples by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr A. 2014;1328:26–34. https://doi.org/10.1016/j.chroma.2013.12.074.
27. Vela-Soria F, Ballesteros O, Zafra-Gomez A, Ballesteros L, Navalon A. UHPLC-MS/MS method for the determination of bisphenol A and its chlorinated derivatives, bisphenol S, parabens, and benzophenones in human urine samples. Anal Bioanal Chem. 2014;406(15):3773–85.
28. Gonzalez-Marino I, Quintana JB, Rodriguez I, Gonzalez-Diez M, Cela R. Screening and selective quantification of illicit drugs in wastewater by mixed-mode solid-phase extraction and quadrupole-time-of-flight liquid chromatography-mass spectrometry. Anal Chem. 2012;84(3):1708–17.
29. Salas D, Borrull F, Marce RM, Fontanals N. Study of the retention of benzotriazoles, benzothiazoles and benzenesulfonamides in mixed-mode solid-phase extraction in environmental samples. J Chromatogr A. 2016;1444:21–31.
30. Laven M, Alsberg T, Yu Y, Adolfsson-Erici M, Sun H. Serial mixed-mode cation- and anion-exchange solid-phase extraction for separation of basic, neutral and acidic pharmaceuticals in wastewater and analysis by high-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. J Chromatogr A. 2009;1216(1):49–62.
31. Yang Y, Guan J, Yin J, Shao B, Li H. Urinary levels of bisphenol analogues in residents living near a manufacturing plant in South China. Chemosphere. 2014;112:481–6.
32. Lewis RC, Meeker JD, Peterson KE, Lee JM, Pace GG, Cantoral A, et al. Predictors of urinary bisphenol A and phthalate metabolite concentrations in Mexican children. Chemosphere. 2013;93(10):2390–8.
33. Myridakis A, Balaska E, Gkaitatzi C, Kouvarakis A, Stephanou EG. Determination and separation of bisphenol A, phthalate metabolites and structural isomers of parabens in human urine with conventional high-pressure liquid chromatography combined with electrospray ionisation tandem mass spectrometry. Anal Bioanal Chem. 2015;407(9):2509–18.