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Rapid, automated online SPE-LC-QTRAP-MS/MS method for the simultaneous analysis of 14 phthalate metabolites and 5 bisphenol analogues in human urine

A.L. Heffernan [a], K. Thompson [a], G. Eaglesham [a], S. Vijayasarathy [a], J.F. Mueller [a], P.D. Sly [b], M.J. Gomez [a,*]

[a] National Research Centre for Environmental Toxicology, The University of Queensland, 39 Kessels Rd, Coopers Plains, Brisbane, QLD 4108, Australia
[b] Queensland Children's Medical Research Institute, and Children's Health and Environment Program, The University of Queensland, Centre for Children's Health Research, Level 7, 62 Graham St South, Brisbane, QLD, 4101, Australia

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ABSTRACT

Phthalates and bisphenol A (BPA) have received special attention in recent years due to their frequent use in consumer products and potential for adverse effects on human health. BPA is being replaced with a number of alternatives, including bisphenol S, bisphenol B, bisphenol F and bisphenol AF. These bisphenol analogues have similar potential for adverse health effects, but studies on human exposure are limited. Accurate measurement of multiple contaminants is important for estimating exposure. This paper describes a sensitive and automated method for the simultaneous determination of 14 phthalate metabolites, BPA and four bisphenol analogues in urine using online solid phase extraction coupled with high-performance liquid chromatography/tandem mass spectrometry using a hybrid triple-quadrupole linear ion trap mass spectrometer (LC-QTRAP-MS/MS), requiring very little sample volume (50 μL). Quantification was performed under selected reaction monitoring (SRM) mode with negative electrospray ionization. The use of SRM combined with an enhanced product ion scan within the same analysis was examined. Unequivocal identification was provided by the acquisition of three SRM transitions per compound and isotope dilution. The analytical performance of the method was evaluated in synthetic and human urine. Linearity of response over three orders of magnitude was demonstrated for all of the compounds ($R^2 > 0.99$), with method detection limits of 0.01–0.5 ng/mL and limits of reporting of 0.07–3.1 ng/mL. Accuracy ranged from 93% to 113% and inter- and intra-day precision were $< 22\%$. Finally, the validated method has been successfully applied to a cohort of pregnant women to measure biomarker concentrations of phthalates and bisphenols, with median concentrations ranging from 0.3 ng/mL (bisphenol S) to 18.5 ng/mL (monoethyl phthalate).

1. Introduction

Phthalates, dialkyl or alkyl aryl esters of 1,2-benzemedicarboxylic acid, are used widely in industrial applications. High molecular weight phthalates are predominantly used as plasticisers (e.g. in polyvinyl chloride), and some lower-molecular weight phthalates are used in personal care products, thus there are many potential sources of human exposure [1]. Phthalates are rapidly metabolized

Abbreviations: ACN, acetonitrile; APCI, atmospheric pressure chemical ionisation; BPA, bisphenol A; BPAF, bisphenol AF; BPB, bisphenol B; BPF, bisphenol F; BPS, bisphenol S; CE, collision energy; CXP, exit potential; DEHP, di(2-ethylhexyl) phthalate; DP, declustering potential; DIDP, diisodecyl phthalate; DINP, diisononyl phthalate; DnOP, di-n-octyl phthalate; ESI, electrospray ionisation; EP, entrance potential; EPI, enhanced product ion; GM, geometric mean; IPA, isopropanol; IS, internal standard; (LC–MS/MS), liquid-chromatography tandem mass spectrometry; LOR, limit of reporting; MBP, mono-nbutyl phthalate; MBzP, monobenzyl phthalate; MCHP, monocyclohexyl phthalate; MCNP, monocarboxy-isononyl phthalate; MCOP, monocarboxyoctyl phthalate; MCPP, mono-3-carboxypropyl phthalate; MDL, method detection limit; MDP, mono-isodecyl phthalate; MECPP, mono-2-ethyl-5-carboxypentyl phthalate; MEHP, mono-(2-ethylhexyl) phthalate; MEHHP, mono-(2-ethyl-5- hydroxyhexyl) phthalate; MeOH, methanol; MEOHP, mono-(2-ethyl-5-oxohexyl) phthalate; MEP, monoethyl phthalate; MiBP, mono-isobutyl phthalate; MMP, monomethyl phthalate; MNP, mono-isononyl phthalate; MOP, mono-n-octyl phthalate; MRM, multiple reaction monitoring; QC, quality control; QCH, high-concentration quality control; QCL, low-concentration quality control; RSD, relative standard deviation; RT, retention time; S/N, signal-to-noise; SPE, solid phase extraction; SRM, selected reaction monitoring; TIC, total ion chromatogram; XIC, extracted ion chromatogram

* Corresponding author.
E-mail addresses: amy.heffernan@uqconnect.edu.au (A.L. Heffernan), k.leechue@uq.edu.au (K. Thompson), g.eaglesham1@uq.edu.au (G. Eaglesham), s.vijayasarathy@uq.edu.au (S. Vijayasarathy), j.mueller@uq.edu.au (J.F. Mueller), p.sly@uq.edu.au (P.D. Sly), m.gomezramos@uq.edu.au (M.J. Gomez).

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Table 1
List of target compounds and abbreviations, including phthalates and their primary hydrolytic monoesters; and bisphenol analogues.

Parent compound	Abbreviation	Monitored biomarker	Abbreviation
Phthalates (short chain)			
Dimethyl phthalate	DMP	Monomethtyl phthalate	MMP
Diethyl phthalate	DEP	Monoethyl phthalate	MEP
Diisobutyl phthalate	DiBP	Mono-isobutyl phthalate	MiBP
Dicyclohexyl phthalate	DCHP	Monocyclohexyl phthalate	MCHP
Di-n-butyl phthalate	DBP	Mono-butyl phthalate	MBP
Butyl benzyl phthalate	BBzP	Monobenzyl phthalate	MBzP
Phthalates (long chain)			
Di(2-ethylhexyl) phthalate	DEHP	Mono(2-ethylhexyl) phthalate	MEHP
		Mono(2-ethyl-5-hydroxyhexyl) phthalate[a]	MEHHP[a]
		Mono(2-ethyl-5-oxohexyl) phthalate[a]	MEOHP[a]
		Mono(2-ethyl-5-caboxypentyl) phthalate[a]	MECPP[a]
Di-n-octyl phthalate	DnOP	Mono-n-octyl phthalate	MOP
		Mono(3-carboxypropyl) phthalate[a]	MCPP[a,b]
Diisononyl phthalate	DINP	Mono-iso-nonyl phthalate	MNP
Diisodecyl phthalate	DIDP	Mono-iso-decyl phthalate	MDP
Bisphenols			
Bisphenol A	BPA	Bisphenol A	BPA
Bisphenol S	BPS	Bisphenol S	BPS
Bisphenol AF	BPAF	Bisphenol AF	BPAF
Bisphenol B	BPB	Bisphenol B	BPB
Bisphenol F	BPF	Bisphenol F	BPF

[a] Secondary (oxidized monoester) phthalate metabolites.
[b] MCPP is also a minor metabolite of DBP and other HMW phthalates like DINP and DIDP.

to their respective hydrolytic monoesters, which can be further metabolized to oxidative products. Monoester and oxidative metabolites may be glucuronidated before excretion through urine and faeces [2], and have been used as biomarkers of exposure to phthalates [3,4] (Table 1). Evidence from animal studies suggests that phthalates are reproductive and developmental toxicants (reviewed in Martino-Andrade and Chahoud [5]), with some evidence for adverse effects on the reproductive system, especially in males [6], and impaired development in humans [7–10].

Bisphenol A (BPA) is a chemical intermediate in the production of polycarbonate and epoxy resins used widely in consumer products [11,12], and exposure occurs predominantly through contaminated food [13,14]. BPA is rapidly metabolized via glucuronidation and excreted in urine [15,16]. The endocrine-disrupting potential and relevance for human health is under debate [17–19]. In response to restrictions on the use of BPA in some countries worldwide, the use of bisphenol A analogues, such as bisphenol S (BPS; 4,4′-sulfonyldiphenol), bisphenol AF (BPAF; 4,4′-(hexafluoroisopropylidene)diphenol), bisphenol B (BPB; 2,2′-bis(4-hydroxyphenyl)butane) and bisphenol F (BPF; 4,4′-dihydroxydiphenylmethane) may be increasing. Limited data suggests estrogenic activity similar to that of BPA [20,21]. These chemicals are used extensively in consumer and personal care products, and exposure assessment is of public health interest.

Phthalates have been extensively studied in recent years. Liquid-chromatography tandem mass spectrometry (LC–MS/MS) with electrospray ionisation (ESI) is generally the method of choice. Various LC–MS/MS methods with offline [22] or online-solid phase extraction (SPE) have been described for between 7 and 22 phthalate metabolites in urine [23–26]. Some gas chromatography mass spectrometry methods have been reported [27–29], but these rely on laborious liquid–liquid extraction and derivatisation techniques.

Similarly to phthalates, BPA, measured together with other phenols and/or parabens, are typically analysed using SPE-LC-MS/MS with either ESI [30–32] or atmospheric pressure chemical ionisation (APCI) [33]. Two methods was identified for the analysis of five [34] and seven [35] monoester phthalate metabolites plus BPA in urine [34]. A recent study describes the analysis of BPA, BPF, BPS and triclosan in urine [36]; and a second study reports on the analysis of seven bisphenol analogues in urine [37]. To our knowledge no published analytical method exists for the simultaneous analysis of phthalate metabolites and bisphenol analogues.

Here we present a rapid, automated online SPE-LC-MS/MS method for the analysis of 14 phthalate metabolites (monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), monocyclohexyl phthalate (MCHP), mono-*n*butyl phthalate (MBP), monobenzyl phthalate (MBzP), mono-(2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP), mono-2-ethyl-5-carboxypentyl phthalate (MECPP), mono-*n*-octyl phthalate (MOP), mono-3-carboxypropyl phthalate (MCPP), mono-isononyl phthalate (MNP), mono-isodecyl phthalate (MDP)) and five bisphenol analogues (BPA, BPS, BPAF, BPB, and BPF) in human urine, using only 50 μL of sample.

2. Materials and methods

2.1. Standards and reagents

Phthalate metabolites (> 99.9%) and their $^{13}C_4$-labelled internal standards (> 99.9%) and bisphenols and ring-labelled $^{13}C_{12}$-BPA (> 99%) were all purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Acetonitrile (ACN), methanol (MeOH) and isopropanol (IPA) (HPLC-grade) were purchased from Merck (Darmstadt, Germany). Ultra-pure water was obtained from a Milli-Q filtration unit (Merck Millipore, MA, USA), or purchased from VWR International (Radnor, PA, USA) (HiPerSolv-grade for use in mobile phases). Ammonium acetate (≥ 99%) and acetic acid (≥ 99.8%) were purchased from Sigma Aldrich Laboratories, Inc. (St Louis, MO, USA). Formic acid (≥ 99.5%) was purchased from

Thermo Fisher Scientific (Waltham, MA, USA). β-glucuronidase (*Escherichia coli*-K12) was purchased from Roche Biomedical (Mannheim, Germany).

2.2. Preparation of standards and quality control material

Stock solutions of phthalate metabolites, bisphenols and isotopically-labelled phthalate metabolites were prepared in acetonitrile and stored at −20 °C in amber glass vials. Working solutions were prepared in MeOH from serial dilutions of the stock solutions containing phthalate metabolites and bisphenols. The internal standard solution was prepared from isotopically-labelled phthalate metabolites and $^{13}C_{12}$-BPA (0.1 and 0.2 μg/mL, respectively). Working solutions were stored at 4 °C in amber glass vials until use. Calibration standards were prepared from working solution using serial dilution via an automated liquid handler (Tecan Genesis, Chiron Healthcare). The enzyme solution was prepared daily by adding β-glucuronidase (60 μL, 200 units/mL) to 3 mL ammonium acetate buffer (1 M, pH 6.5).

Quality control (QC) materials were prepared from stock pooled urine obtained from multiple anonymous adult donors. The pool was divided into two sub-samples and fortified with native phthalate metabolites to create low-concentration (QCL) and high-concentration (QCH) QC materials which were mixed, aliquoted and frozen prior to use. Synthetic urine was prepared according to Calafat and Sampson [38].

2.3. Sample preparation

Human urine samples were thawed and mixed prior to extraction. Sample pretreatment steps were performed using a liquid handling workstation. Urine samples (50 μL) were added to 2 mL glass vials (Agilent Technologies, Santa Clara, CA, USA); fortified with internal standard solution (80 μL), enzyme solution (25 μL) and milli-Q water (440 μL), and incubated at 37 °C for 90 min for the enzymatic hydrolysis of phthalate metabolites and bisphenol conjugates. The reaction was quenched by addition of formic acid (400 μL, 0.5% solution), and the sample filtered in series using 0.45 μm and 0.2 μm regenerated cellulose membrane syringe filters (Phenomenex, Lane Cove, NSW, Australia) prior to injection on the SPE-LC-MS/MS system.

2.4. Instrumental method

2.4.1. System configuration

Samples were injected directly using a Gilson GX-271 liquid handler (John Morris Scientific, Murrarie, QLD, Australia) to facilitate large-volume injection, and analysed using online SPE-LC-MS/MS. The LC–MS/MS system consisted of Prominence UFLC (Shimadzu Scientific, Columbia, MD, USA) with two LC30AD pump modules (Pump A, B) and 1 LC20AB module (pump CB) coupled to a linear ion trap quadrupole 5500 QTRAP (Sciex, Framingham, MA, USA) equipped with a turbospray interface in the multiple reaction monitoring (MRM) mode. The online SPE system consisted of a 10-port switching valve and mixing T-piece, as described by Ye et al. [33]. The system was programmed using Trilution Liquid Handling software version 2.0 (John Morris Scientific) and Analyst software version 1.5.2 (Sciex).

2.4.2. Online solid phase extraction

The online-SPE-LC-MS/MS involves simultaneous processes of solid phase extraction (concentration, clean up and elution); chromatographic separation and MS analysis (Appendix B). Analytes were trapped on a Strata X 20 × 2.0 mm, 25 μm cartridge (Phenomenex). Chromatographic separation was achieved using a Synergi MAX-RP 150 × 3.00 mm, 4 μm column (Phenomenex) maintained at 50 °C. A pre-filter (2 μm, 2.00 mm) was included before the switching valve to retain potential contaminants from the mobile phases (A: H_2O+0.05% acetic acid (v/v); B: 95% ACN+0.05% acetic acid (v/v)). The timing and gradient protocols are described in Appendix A. Briefly, 0.5 mL sample is loaded onto the SPE cartridge (2 mL/min for 2.5 min). Retained analytes are eluted using 95% B using pump 2; diluted to 40% B for peak focusing, and transferred to the head of the analytical column. Chromatographic separation is achieved using non-linear gradient elution (4.1 min, 40% B; 8.80 min, 100% B; 13 min, 100% B). Simultaneously, the SPE cartridge was flushed with 98% B (2 mL/min for 4 min), then equilibrated at 0% B prior to the next injection. These processes occurred cyclically, where the next sample is held in the sample loop ready for injection once the SPE cartridge is re-equilibrated. The sample loop was flushed with IPA:MeOH (10:90) and the needle rinsed with 20% MeOH between each injection to avoid carryover. Peak focusing was achieved by a simple T-junction combined with a 10-port valve.

2.4.3. Mass spectrometry parameters

The 5500 QTRAP was operated in negative ion mode using ESI with the following settings: curtain gas: 35 psi; collision gas: medium; source temperature 650 °C; ion spray voltage −4500 V; ion source gas 1 and 2: 60 psi. The declustering potential (DP), collision energy (CE), entrance potential (EP), exit potential (CXP) and mass transitions were optimized for each analyte (Table 2), with a dwell time of 10 ms per channel. The MS was operated in selected reaction monitoring (SRM) mode with a detection window of 60 s and target scan time of 0.8 s.

Analytes were identified based on retention time of the analyte compared to the standard (±2%) and it's labelled internal standard; and ratio of abundance between first and second transitions (within 20% of value observed in the standard) using MultiQuant software version 2.1 (Sciex). The third transition was used for confirmation when matrix interferences obscured the second transition. Analytes were quantified via isotope dilution.

3. Validation study

The optimized method was validated for linearity, matrix effect, method detection (MDL) and reporting limits (LOR), selectivity, recovery, accuracy and precision. The validation studies were performed by using human urine, synthetic urine and MilliQ water extracts. On-line SPE recoveries (n=3) were calculated as area ratios by comparing the signal obtained from injection of 5 μL of urine extract fortified at 100 ng/mL (500 pg injected) directly onto the analytical column with the signal obtained after loading 500 μL of the same sample spiked at 1 ng/mL (500 pg injected) onto the SPE cartridge as per a standard injection. Calibration curves, weighted by the reciprocal of the standard concentration ($1/x$) of the peak area of each analyte ion divided by the peak area of its isotope-labelled standard versus standard concentration, were constructed. Where isotopically-labelled standards were unavailable (MiBP, MDP and bisphenols S, AF, B and F) a surrogate internal standard was used for quantitation ($^{13}C_4$-MBP, $^{13}C_4$-MNP and $^{13}C_{12}$-BPA, respectively). Each analytical batch included three QCL, three QCH, four procedural blanks, and 20 unknown samples.

Special attention should be paid to the analysis of BPA and simple monoesters of phthalates at trace levels: these metabolites are prone to external contamination as they can relatively easily be generated from omnipresent phthalate diesters before or during the analytical procedure [4,16,22,34]. Thus, precautions were taken to prevent contamination from personnel, organic solvents, equipment and glassware. Procedural or method blanks (n=4), of MilliQ water and synthetic urine, were employed with every batch

Table 2
Mass transitions for target analytes.

Compound (IS)	Q1/Q3 (m/z)	Type	DP (V)	EP (V)	CE (V)	CXP (V)	RT (min)
Phthalates							
MMP ($^{13}C_4$-MMP)	179.0/107.0	Quantitation	−50	−10	−14	−14	5.40
	179.0/77.0	Confirmation	−50	−10	−25	−12	
	183.2/79.1	Internal std.	−45	−10	−25	−13	
MEP ($^{13}C_4$-MEP)	193.0/77.0	Quantitation	−40	−10	−25	−12	5.80
	193.0/121.0	Confirmation	−40	−10	−17	−13	
	197.1/78.9	Internal std.	−70	−10	−25	−12	
MiBP[a]	221.0/134.0	Quantitation	−60	−10	−20	−14	7.90
	221.0/77.0	Confirmation	−60	−10	−25	−12	
MCHP ($^{13}C_4$-MCHP)	247.0/147.0	Quantitation	−60	−10	−23	−15	8.60
	247.0/77.0	Confirmation	−60	−10	−30	−12	
	251.2/97.0	Internal std.	−90	−10	−21	−15	
MBP ($^{13}C_4$-MBP)	221.0/77.0	Quantitation	−55	−10	−25	−12	8.00
	221.0/149.0	Confirmation	−55	−10	−16	−17	
	225.2/79.0	Internal std.	−75	−10	−28	−10	
MBzP ($^{13}C_4$-MBzP)	255.0/107.0	Quantitation	−60	−10	−20	−15	8.20
	255.0/77.0	Confirmation	−60	−10	−32	-12	
	259.2/107.1	Internal std.	−70	−10	−20	−10	
MEHP ($^{13}C_4$-MEHP)	277.0/134.0	Quantitation	−65	−10	−21	−10	9.80
	277.0/77.0	Confirmation	−65	−10	−40	−12	
	281.3/136.9	Internal std.	−120	−10	−22	−17	
MEOHP ($^{13}C_4$-MEOHP)	293.1/121.0	Quantitation	−70	−10	−28	−15	7.50
	293.1/145.0	Confirmation	−70	−10	−19	−17	
	297.2/123.9	Internal std.	−100	−10	−26	−16	
MEHHP ($^{13}C_4$-MEHHP)	291.1/121.0	Quantitation	−60	−10	−25	−15	7.80
	291.1/143.0	Confirmation	−60	−10	−20	−15	
	295.0/123.9	Internal std.	−95	−10	−20	−16	
MECPP ($^{13}C_4$-MECPP)	307.0/159.0	Quantitation	−60	−10	−20	−21	7.00
	307.0/113.0	Confirmation	−60	−10	−41	−18	
	311.0/159.0	Internal std.	−60	−10	−20	−18	
MOP ($^{13}C_4$-MOP)	277.0/77.0	Quantitation	−80	−10	−35	−11	9.90
	277.0/127.0	Confirmation	−80	−10	−22	−19	
	281.2/126.9	Internal std.	−100	−10	−22	−17	
MCPP ($^{13}C_4$-MCPP)	251.0/103.0	Quantitation	−44	−10	−13	−12	5.20
	251.0/165.1	Confirmation	−44	−10	−17	−13	
	255.0/103.0	Internal std.	−110	−10	−15	−13	
MNP ($^{13}C_4$-MNP)	291.1/77.0	Quantitation	−50	−10	−38	−12	9.90
	291.1/139.0	Confirmation	−50	−10	−24	−15	
	295.2/141.1	Internal std.	−105	−10	−25	−15	
MDP[b]	305.2/155.0	Quantitation	−90	−10	−26	−23	10.2
	305.2/77.0	Confirmation	−90	−10	−44	−11	
Bisphenols							
BPA ($^{13}C_{12}$-BPA)	227.0/133.0	Quantitation	−100	−10	−32	−17	7.60
	227.0/212.0	Confirmation	−100	−10	−26	−23	
	239.0/139.0	Internal std.	−90	−10	−38	−21	
BPS[c]	249.1/92.0	Quantitation	−80	−10	−50	−16	4.80
	249.1/108.0	Confirmation	−80	−10	−35	−21	
	249.1/156.0	Confirmation	−80	−10	−30	−19	
BPAF[c]	335.0/265.0	Quantitation	−130	−10	−32	−32	8.90
	335.0/69.0	Confirmation	−130	−10	−71	−15	
BPB[c]	241.1/211.0	Quantitation	−125	−10	−38	−25	8.40
	241.1/147.2	Confirmation	−125	−10	−34	−15	
	241.1/117.0	Confirmation	−125	−10	−68	−15	
BPF[c]	199.0/105.0	Quantitation	−105	−10	−30	−13	6.10
	199.0/93.0	Confirmation	−105	−10	−35	−11	
	199.0/77.0	Confirmation	−105	−10	−33	−18	

IS: internal standard; DP: declustering potential; EP: entry potential; CE: collision energy; CXP: collision exit potential; RT: retention time.

[a] Internal standard used: $^{13}C_4$-MBP.
[b] Internal standard used: $^{13}C_4$-MNP.
[c] Internal standard used: $^{13}C_{12}$-BPA.

of samples to monitor background contamination of the studied compounds.

To demonstrate the applicability and suitability of the validated method, 30 human urine samples were analysed. Urine samples were collected over two days from pregnant women ≥ 18 years at 14–18 weeks gestation from Brisbane, Australia who were participating in a larger study examining exposure to plastic components during pregnancy. This study was approved by the University of Queensland ethics committee (approval number 2013001156).

4. Results and discussion

4.1. Method development

Due to the different chemical properties and disparate typical endogenous concentrations of phthalates and bisphenols found in humans, most existing analytical methods measure BPA or phthalate metabolites using separate analytical methods. Recent studies have used a single method to analyse BPA and phthalate

metabolites [34], and BPA and bisphenol analogues [37] respectively, but to the best of our knowledge no methods have been reported for the simultaneous analysis of phthalate metabolites, BPA and bisphenol analogues. Our method has the added advantage of reduced urine volume required for analysis (50 μL) while maintaining adequate sensitivity, compared with the usual total volume of 0.1–2 mL used elsewhere [25,30,37]. With the increasing number of epidemiological samples for which only a limited volume of urine is available, sample volume has become an issue. The challenges in developing and optimizing a method to detect all these metabolites simultaneously in urine are described below.

4.1.1. Chromatographic separation and sensitivity enhancement

To achieve the highest level of sensitivity and maximize recoveries we selected optimal on-line SPE working conditions (SPE sorbent, sample load flow rate, loaded sample volume, elution gradient and washing steps). Special attention was also paid to optimizing chromatographic separation with the aim of adequately resolving all individual analytes, particularly isobaric compounds/fragments e.g. MiBP/MBP and MEHP/MOP that use the same mass spectrometric transitions for identification. To achieve this different chromatographic columns (Gemini C18 NX 50 × 2.00 mm, 3 μm; Kinetex phenyl-hexyl 100 × 2.10 mm, 2.6 μm; Kinetex Synergi Fusion-RP 100 × 2.00 mm, 4 μm; Kinetex Synergi Fusion-RP 50 × 2.00 mm, 2.5 μm; and Synergi MAX-RP 150 × 3.00 mm, 4 μm (Phenomenex)) and SPE cartridges (Alltima HP C18 7.3 × 3.0 mm, 5 μm (Grace Discovery Sciences, Bannockburn IL USA) and Strata X 20 × 2.0 mm, 25 μm) were tested.

Peak focusing, whereby an aqueous solvent is combined with the SPE eluent so the analyte bands enter the analytical column at low organic conditions (40% MeOH) and refocus into sharper bands on the head of the analytical column, has proven to be very efficient in both in this study, and previously [33,39]. The best sensitivity, peak shape and resolution were achieved using a Strata X cartridge and Synergi MAX-RP analytical column, and allowed separation of all analytes in less than 13 min. This is particularly noteworthy for pairs of isomers MiBP/MBP and MEHP/MOP that are difficult to separate chromatographically, and uses the same mass spectrometric transitions for detection (Fig. 1). While baseline resolution was not possible for these pairs of isomers with such a short run time, the optimized conditions allow sufficient separation for software-automated integration of the individual analytes in the majority of human urine samples. Only in a few cases was manual integration necessary. For correct operation of the method it is recommended to change the SPE cartridge and in-line filters approximately every 100 urine samples.

Both APCI [40–42] and ESI [25,30,34] have previously been used to measure phthalate metabolites and bisphenols in urine. APCI has the advantage of little matrix suppression compared with ESI, so the sensitivity of some analytes showing strong matrix suppression in urine with ESI such as BPA, MiBP, MEP, MMP or MDP, were improved with APCI. However, for other analytes (BPF, MCPP and MECPP) sensitivity with APCI was very low. In general ESI provided the best results for the greatest number of analytes and was selected as the ionisation technique of choice.

4.1.2. Enzymatic hydrolysis

β-glucuronidase/sulfatase (*Helix pomatia*, H-1) is the most commonly used enzyme for BPA deconjugation. However, the sulfatase activity is capable of converting parent phthalate diesters to their monoester metabolites during the enzymatic hydrolysis reaction [40]. Phthalates are ubiquitous environmental contaminants and can be incorporated into the sample during the collection, storage, and analysis. In order to distinguish between exogenous contamination and endogenous sample concentration, monoester metabolites are monitored rather than the diesters. Therefore to minimize the contribution from potential phthalate diester contaminants, we used an enzyme that is glucuronidase only (no sulfatase). The *E. coli* β-glucuronidase (K12, Roche Biomedical) had excellent β-glucuronidase activity and no measurable lipase activity on the phthalate diesters [40]. This enzyme lacks sulfatase activity, but no phthalate conjugates other than glucuronides have been detected in human urine [43] and only a small percentage of BPA (< 3%) is excreted as sulphate conjugates in humans [15,30,44]. In vitro and in vivo investigations of the biotransformation of some BPs (BPAF, BPF and tetrabromobisphenol A) suggest similar first-pass metabolism to glucuronidated metabolites as BPA [16,37,45–47]. Thus, the use of a glucuronidase-only enzyme is appropriate in this instance. It should be noted that for BFB, hydroxylated metabolites may be the most relevant for human biomonitoring studies [46], and that for BPS, the most important BPA replacement, no information on dominant metabolites could be found, and that

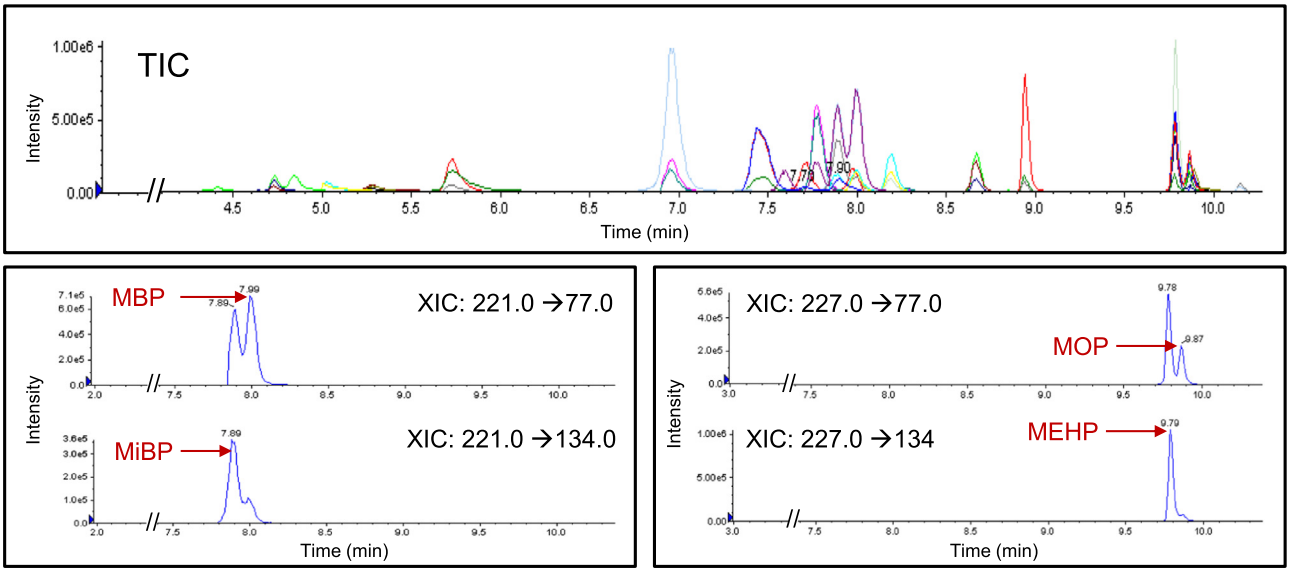

Fig. 1. Total ion chromatogram of fortified and extracted urine sample at 5 ng/mL, showing separation of 14 phthalate metabolites and 5 bisphenol analogues in 13 min; and extracted ion chromatograms of the pairs of isomers MiBP/MBP and MEHP/MOP. Note discontinuity in *x*-axes. TIC: total ion chromatogram; XIC: extracted ion chromatogram.

sulphate conjugates may prove important in future exposure assessments.

4.1.3. Assessing exogenous contamination

A number of strategies have been adopted to monitor and avoid where possible exogenous contamination of ubiquitous BPA and phthalate contamination. As BPA and phthalates are known components of some plastics (e.g. polycarbonate), all laboratory plasticware was avoided during sample preparation and handling, and all glassware was washed twice with HPLC-grade solvents. Sample preparation and extraction steps were automated as much as practical using a liquid handling workstation and online SPE to minimize physical sample handling by the analyst, and limit contact with laboratory apparatus. Additionally, an ISO-5 clean laboratory was utilized for pooling, aliquoting, and storage of urine samples.

We observed notable contamination from acetic acid, ammonium acetate and some solvents. This was minimized by switching to ultrapure HPLC-grade solvents for extraction steps, and by purchasing ultra-high purity reagents packaged in glass rather than plastic, with each substitute tested for potential contamination before use. Similar contamination occurred with some phthalate metabolites and especially with BPA in ultra-pure water obtained from a Milli-Q filtration unit just after the filters were changed. To avoid future problems ultrapure HiPerSolv-grade water was purchased from VWR. In addition to the use of HPLC-grade solvents for the preparation of mobile phases, in-line filters were included on each of the analytical pumps, and a pre-column filter on the analytical column to avoid contamination of the sample with residual analytes from the mobile phases or analytical system itself. Further, four procedural blanks were included with each batch of samples to quantify average background contamination.

4.1.4. SRM and enhanced product ion mode

QTRAP systems combine a triple-quadrupole scanning functionality with sensitive linear ion trap scans. In addition to using two/three transitions per analyte and calculating ion ratios, the flexibility of the hybrid system offers the possibility of combining SRM and enhanced product ion (EPI) scans to simultaneously obtain accurate quantification and structural information. This system can be useful when the second confirmation transition is present at low intensity or not detected thus hampering compound identification at low concentrations. This approach has successfully been applied to confirm chemicals in waters with only one suitable SRM transition [48,49], and has been explored in this work. Reliable confirmation of phthalate metabolites and BPA was achieved using standard protocols (two or three transitions, ion ratio and retention time matching with labelled internal standard) (Fig. 2). However, difficulties were encountered with those compounds for which a commercial isotopically-labelled standard is currently unavailable. For example, Fig. 3 shows the analyte BPS in human urine fortified at 1 ng/mL and a human urine sample (not fortified), combining SRM and EPI operation modes. In SRM mode an interference is partially resolved in the fortified sample (SRM 1, at 4.84 min), but not in the patient sample. In an attempt to improve confirmation of BPS an EPI survey scan was included in the method. Unfortunately the EPI spectrum of BPS (4.73 min) and the interference (4.84 min) are almost identical, likely due to the complexity of the matrix and poor resolution, and cannot be used to confirm BPS in patient samples. The presence of 3 SRM transitions at the correct retention time and ratio provided adequate confirmation of BPS in patient samples, and the peak was recorded as a false positive (Fig. 2). Overall, EPI scan mode showed to be inadequate for confirmation of the target analyte in this complex matrix, and SRM mode in combination with isotope dilution is the best approach for accurate and reliable confirmation and quantification of phthalate metabolites and bisphenols in urine, including compounds for which a stable labelled internal standard is unavailable.

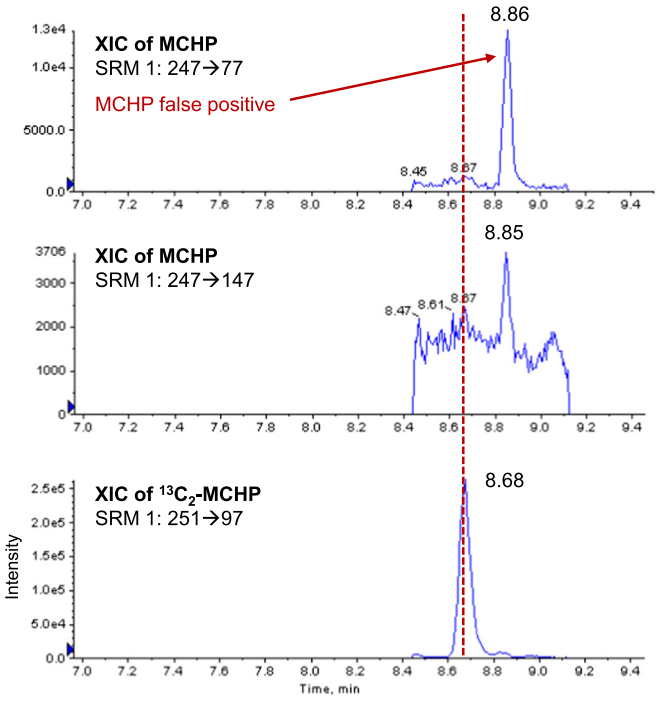

Fig. 2. Sample extracted ion chromatograms (XIC) of MCHP in human urine sample showing quantification (top), confirmation (middle) and isotopically-labelled internal standard (bottom) SRMs. There is an interference sharing the same transitions with MCHP and with a similar retention time (8.86 min), however a shift of 0.18 min in the retention time between MCHP and $^{13}C_2$-MCHP reveals a MCHP false positive.

4.2. Analytical validation

The analytical method was validated to demonstrate accuracy, precision, recovery, linearity, reproducibility, repeatability, sensitivity. Results of method validation are summarized in Table 3. The on-line SPE recoveries were very good for all analytes, ranging from 83% to 122%. Additionally, the use of isotope dilution mass spectrometry with dedicated isotopically-labelled internal standards for quantitation automatically corrects for any sample loss. Linearity was assessed from 0.1 to 200 ng/mL for all compounds except MEHHP, MEOHP, BPS and BPAF (0.1 to 100 ng/mL) and MEP, BPB and BPF (0.5 to 200 ng/mL). The method showed a linear response with determination coefficients (R^2) higher than 0.998 in all cases. Intra- and inter-day precision and accuracy were assessed by repeated analyses ($n=5$) of fortified samples of real urine, at 1–5 ng/mL and 20–25 ng/mL urine, for QCL and QCH, respectively, over a period of five days. Precision was calculated as relative standard deviation (RSD) for both intra- and inter-day variability; and accuracy calculated as the degree of closeness of the determined value to the nominal value. The intra-day precision was between 3.3% and 19.6%, and the inter-day precision was between 4.5% and 25.8% (Table 3). The accuracy was between 93.1% and 110%. These values show good accuracy and reproducibility of the method.

4.2.1. Limits of reporting

The MDL for individual compounds was calculated as three times the signal-to-noise ratio in low-level spiked synthetic urine. For compounds present in the synthetic urine blanks at

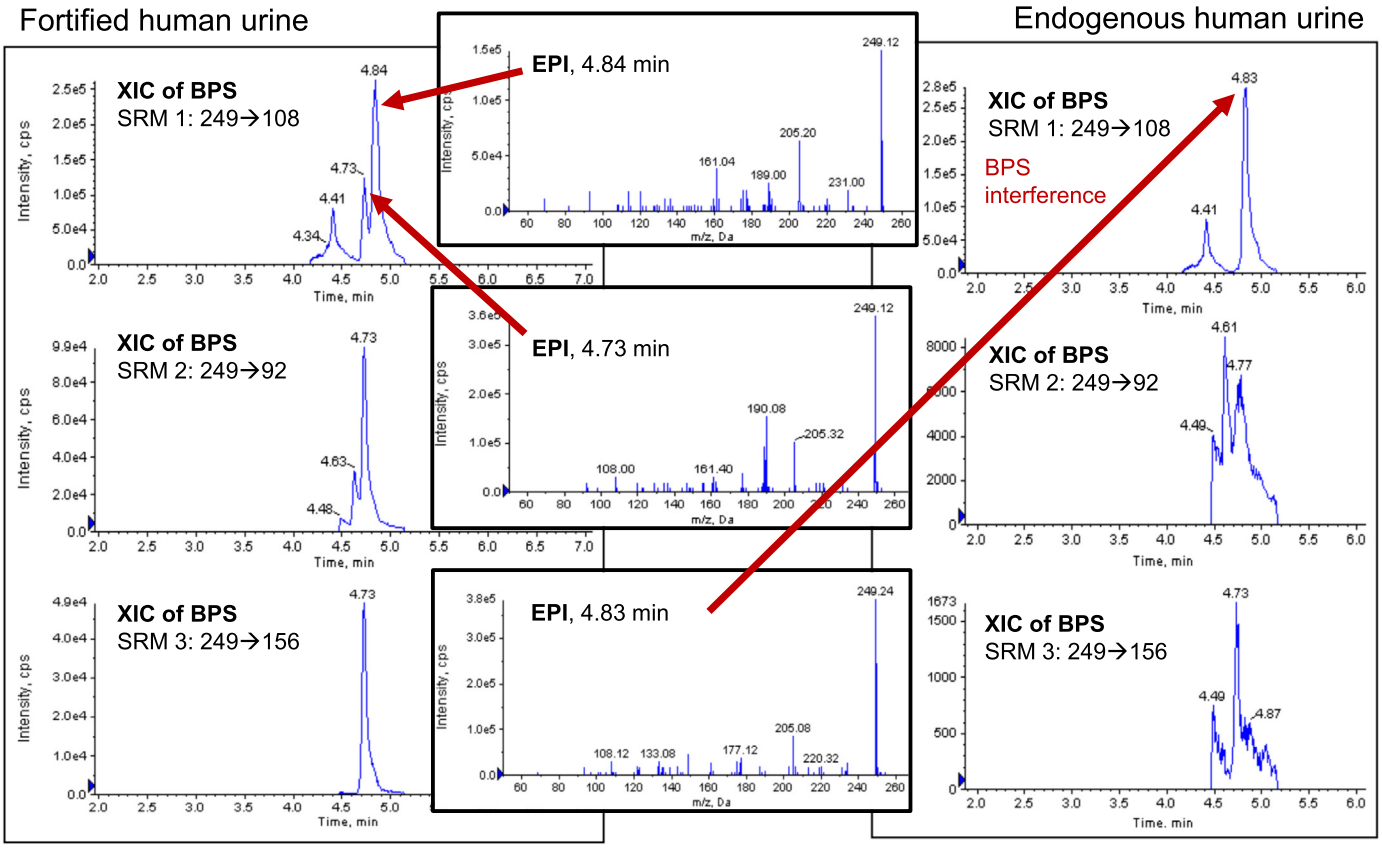

Fig. 3. Determination of BPS in synthetic urine fortified at 1 ng/mL (left) and a human urine sample (right), combining SRM (3 transitions) and EPI operation modes. Under SRM, an interference is observed in SRM 1. This is differentiated from BPS peak in the fortified sample (top left), but not in the patient sample (top right). EPI spectrum of BPS and interference are almost identical, and thus EPI cannot be used for identification. Unequivocal identification is provided by retention time matching to labelled standard and comparing ion ratios.

Table 3
Summary of method performance results.

Compound	**MDL** (ng/mL)	**LOR** (ng/mL)	**Intra-day** (n=5)				**Inter-day** (n=5)		**Matrix Effects** (%)	
			QCL		QCH		QCL	QCH		
			CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	CV (%)	Urine	Synthetic Urine
Phthalates										
MMP	0.031	0.10	10.8	103	5.44	96.5	20.5	18.9	32	0
MEP	0.19	0.62	9.85	93.8	6.85	96.9	18.0	18.7	35	−4
MiBP	0.039	0.12–2.9[a]	8.31	113	6.51	109	15.8	16.5	−8	13
MCHP	0.024	0.079	9.06	107	4.93	103	16.0	21.5	−4	0
MBP	0.051	0.64–2.1[a]	7.29	101	5.69	100	15.6	16.2	0	13
MBzP	0.038	0.13	4.79	101	6.91	103	12.8	19.1	1	−1
MEHP	0.020	1.0–3.1[a]	19.7	103	18.7	104	22.5	20.4	44	45
MEOHP	0.036	0.12	9.30	93.1	5.76	94.3	17.7	17.8	10	0
MEHHP	0.035	0.12	8.13	105	4.79	104	15.1	16.9	9	-5
MECPP	0.033	0.11	11.9	108	8.99	97.7	19.3	21.5	−5	−2
MOP	0.029	0.097–0.40[a]	5.8	102	14.0	98.5	16.2	12.6	5	15
MCPP	0.022	0.074	7.53	100	3.77	105	11.0	25.8	−6	9
MNP	0.044	0.15–0.52[a]	19.7	98.4	8.42	102	4.5	15.1	36	34
MDP	0.036	0.21–0.57[a]	18.1	99.4	17.5	105	15.6	19.7	70	68
Bisphenols										
BPA	0.10	0.21–0.41[a]	16.5	102	6.43	104	19.4	17.6	3	-2
BPS	0.067	0.22–0.47[a]	8.7	102	13.5	110	21.8	20.6	76	−1
BPAF	0.005	0.14	15.5	102	9.93	108	18.8	19.7	69	58
BPB	0.26	0.88	13.2	103	3.32	101	24.2	22.1	28	−3
BPF	0.39	1.3	18.2	102	7.25	105	22.5	19.8	46	−4

QCL: quality control-low concentration (1–5 μg/L); QCH: quality control-high concentration (20-25 μg/L); CV: coefficient of variation; MDL: method detection limit; LOR: limit of reporting.

[a] Range dependent on mean blank concentration per batch.

Table 4
Urinary concentrations of phthalate metabolites and bisphenols (ng/mL) detected in pregnant women ($n=30$) from Brisbane, Australia.

Compound	Av. Blank[a] (CV, %)	Mean	GM	Median	Range	Detection Frequency (%)
Phthalates						
MMP	< LOD	[b]	[b]	[b]	< LOR–22.3	10
MEP	< LOD	43.0	19.9	18.5	3.1–212	100
MiBP	0.40 (24)	14.5	10.4	7.3	3.9–40.3	100
MCHP	< LOD	[b]	[b]	[b]	< LOD	0
MBP	0.36 (26)	13.6	10.9	11.8	3.5–35.0	100
MBzP	< LOD	3.4	2.3	2.2	< LOR–9.96	93
MEHP	0.35 (16)	8.3	5.9	5.4	2.7–41.1	100
MEOHP	< LOD	14.7	6.7	7.3	0.86–105	100
MEHHP	< LOD	17.8	9.6	10.3	1.2–106	100
MECPP	< LOD	29.0	14.1	14.9	2.0–182	100
MOP	0.08 (11)	[b]	[b]	[b]	< LOD	0
MCPP	< LOD	3.7	1.9	2.2	< LOR–24.4	83
MNP	< LOD	[b]	[b]	[b]	< LOD	0
MDP	0.11 (8)	[b]	[b]	[b]	< LOR–1.0	33
Bisphenols						
BPA	0.11 (11)	8.7	5.0	3.8	1.7–44.8	100
BPS	0.09 (3)	[b]	[b]	[b]	< LOR–8.1	10
BPAF	< LOD	[b]	[b]	[b]	< LOD	0
BPB	< LOD	[b]	[b]	[b]	< LOD	0
BPF	< LOD	[b]	[b]	[b]	< LOR–74.0	10

The proportion of results beneath the limit of reporting was too high to produce a valid result; GM: geometric mean; LOD: limit of detection; LOR: limit of reporting.

[a] Procedural blanks ($n=4$).

[b] Not calculated.

concentration higher than the first point of the calibration curve (MBP, MEHP, MOP and BPA) MDLs were calculated from the corresponding isotopically-labelled standard. For compounds with very different matrix effects in synthetic urine compared with real urine (BPS and BPF, see discussion below) correction factors were applied. The limit of reporting (LOR) was calculated as 10 times the signal-to-noise in low-level spiked synthetic urine, or three times the average blank for compounds present in procedural blanks – whichever gave the higher value. The MDLs and LORs ranged from 0.01–0.5 ng/mL and 0.07–3.1 ng/mL, respectively (Table 3), and were similar to reported values for phthalate metabolites and BPA using on-line SPE-HPLC-MS/MS [25] and off-line SPE followed by HPLC–MS/MS [34]. For BPAF the detection limits is lower than previously reported, but LODs for BPS (0.067 ng/mL) and BPF (0.39 ng/mL) are higher than previously reported ($\leq$0.1 ng/mL) [31,36,37,50]. Human exposure data on bisphenol analogues is limited, with reported urinary concentrations up to a few ng/mL [31,36,37,50]. The detection limits reported here are sufficient to characterise mean concentrations typically measured in biomonitoring studies.

4.2.2. Matrix effects

One significant drawback of electrospray mass spectrometry is that the ionisation source is highly susceptible to co-extracted matrix components. The matrix effect typically results in suppression or, less frequently enhancement, of the analyte signal such that the response obtained from solvent/aqueous standards and matrix extracts may differ significantly. To determine the matrix effect, human urine and synthetic urine extracts fortified with the analytes at 10 ng/mL were compared to the same concentrations of analytes in neat standard solutions. Calculated values are shown in Table 3. A value of 0% indicates the absence of matrix effects, and negative and positive values are indicative of signal enhancement and suppression, respectively. Most compounds showed no or minimal (<15%) matrix effects in both synthetic and human urine. MMP, MEP, MNP and BPB had intermediate matrix suppression (28–46%), and only three compounds (MDP, BPS and BPAF) showed strong matrix suppression (58–76%). In general observed matrix effects were similar in human and synthetic urine, with the exception of BPS where values differed significantly (76% and −1%, respectively). MMP, MEP, BPB and BPF had intermediate suppression in human urine, but little suppression in synthetic urine. Taking into account the differences presented by MMP, MEP, BPS, BPB and BPF, synthetic urine is a good surrogate matrix for use in quality control materials, field and procedural blanks, and during the validation process.

Different method parameters were optimized with the aim of reducing matrix effects. Decreasing acetic acid mobile phase modifier from 0.1% to 0.05% increased ion intensity for many analytes by reducing matrix effects, while maintaining sufficient and reproducible retention. Similarly increasing the dilution factor by reducing urine volume from 100 μL to 50 μL decreased signal suppression for most of the compounds. Any remaining matrix effects are corrected by the use of stable isotope-labelled internal standards for each of the analytes.

4.3. Method utility

The usefulness of the method was assessed by analysing 30 samples from pregnant women with no known occupational phthalate exposure. Table 4 summarises the concentration and detection frequency of phthalate metabolites and bisphenols measured, and average blank concentrations ($n=4$). Trace levels of MBP, MiBP, MEHP, MOP, MDP, BPA and BPS were detected in the procedural blanks, but concentrations were low and consistent ($\leq$0.4 ng/mL; %CV < 25%), and at least one order of magnitude lower than concentrations typically reported at the population level [51]. MCHP, MOP and MNP were the only phthalate metabolites that were not detected in any of the urine samples. The remaining phthalate metabolites were measured in all samples with the exception of MMP, MBzP, MCPP and MDP, which were detected in 10, 93, 83 and 33% of samples, respectively. Oxidized metabolites of DnOP, DINP and DIDP (MCPP, monocarboxyoctyl phthalate (MCOP) and monocarboxy-isononyl phthalate (MCNP), respectively), are more valid biomarkers of exposure for these high molecular weight phthalates than their respective hydrolytic monoester phthalate metabolites (MOP, MNP and MDP, respectively) [25]. For example, for DnOP, MCPP (oxidized metabolite) and MOP (hydrolytic monoester metabolite) had detection frequencies of 83% and 0%, respectively. Similarly MNP and MDP had low detection frequencies (0% and 33%), but the oxidized metabolites MCOP and MCNP were not included in the current method and are not available for comparison. It should be noted that MCPP is a major metabolite of DnOP, but is also a minor metabolite of DBP and other high molecular weight phthalates like DINP and DIDP. Therefore, it is possible that the concentration of MCPP in humans reflect exposure not only to DnOP, but to other phthalates [52,53]. The highest concentrations were detected for MEP (213 ng/mL), MECPP (182 ng/mL), MEHHP (106 ng/mL) and MEOHP (105 ng/mL) with median concentrations of 18.5, 14.9, 10.3 and 7.3 ng/mL, respectively. For the bisphenol analogues, BPA was detected in all samples, and BPS and BPF in 10% of samples each. BPB and BPF were not detected in any of the analysed samples. In general, concentrations of phthalate metabolites and BPA were consistent with previously reported concentrations in pregnant women [54,55]. There are very few studies that report urinary concentrations of bisphenol analogues. In a recent study from China BPS and BPF were quantified in 22% and <30% of samples with GM of 0.029 and 0.228 ng/mL, respectively [37]. Liao et al. [31] report urinary BPS from different countries with median concentrations of 0.05–1.04 ng/mL. Based on this limited data, BPS

levels in this study were comparable to the reported levels and BPF levels in urine were higher than reported levels.

5. Concluding remarks

As a result of BPA restrictions in consumer goods bisphenol analogues are beginning to appear, with latest research indicating that these analogues have similar endocrine-disrupting effects as BPA in humans. However, in contrast with phthalates and BPA, few methods are available for the biomonitoring of other bisphenol analogues in humans. Analytical methods are required to measure the levels of exposure to these new emerging bisphenols in humans. This study presents a sensitive and automated on-line SPE-LC-QTRAP-MS/MS method for the fast and simultaneous determination of 14 phthalate metabolites and five bisphenol analogues in human urine for the first time. Compared with methods previously published for the analysis of environmental contaminants in urine, this method includes more analytes of interest in addition to requiring a smaller total urine volume. The developed method is robust, with wide linear ranges for all the compounds and MDL in the range of 0.01–0.5 ng/mL.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2016.01.037.

References

[1] J.D. Meeker, et al., Phthalates and other additives in plastics: human exposure and associated health outcomes, Philos. Trans. R. Soc. B: Biol. Sci. 364 (2009) 2097–2113.
[2] M. Wittassek, J. Angerer, Phthalates: metabolism and exposure, Int. J. Androl. 31 (2008) 131–138.
[3] H.M. Koch, A.M. Calafat, Human body burdens of chemicals used in plastic manufacture, Philos. Trans. R. Soc. B: Biol. Sci. 364 (2009) 2063–2078.
[4] M. Wittassek, et al., Assessing exposure to phthalates-the human biomonitoring approach, Mol. Nutr. Food Res. 55 (2011) 7–31.
[5] A.J. Martino-Andrade, I. Chahoud, Reproductive toxicity of phthalate esters, Mol. Nutr. Food Res. 54 (2010) 148–157.
[6] A. Miodovnik, et al., Developmental neurotoxicity of ortho-phthalate diesters: review of human and experimental evidence, Neurotoxicology 41 (2014) 112–122.
[7] J.D. Meeker, K.K. Ferguson, Relationship between urinary phthalate and bisphenol A concentrations and serum thyroid measures in U.S. adults and adolescents from the National Health and Nutrition Examination Survey (NHANES) 2007–2008, Environ. Health Perspect. 119 (2011) 1396–1402.
[8] J.M. Braun, et al., Phthalate exposure and children's health, Curr. Opin. Pediatr. 25 (2013) 247–254.
[9] J.D. Meeker, K.K. Ferguson, Urinary phthalate metabolites are associated with decreased serum testosterone in men, women, and children from NHANES 2011–2012, J. Clin. Endocrinol. Metab. 99 (2014) 4346–4352.
[10] R.U. Halden, Plastics and health risks, Annu. Rev. Public Health 31 (2010) 179–194.
[11] C. Brede, et al., Increased migration levels of bisphenol A from polycarbonate baby bottles after dishwashing, boiling and brushing, Food Addit. Contam. 20 (2003) 684–689.
[12] J.H. Kang, et al., Factors influencing the migration of bisphenol A from cans, J. Food Prot. 66 (2003) 1444–1447.
[13] N. von Goetz, et al., Bisphenol a: how the most relevant exposure sources contribute to total consumer exposure, Risk Anal. 30 (2010) 473–487.
[14] T. Geens, et al., A review of dietary and non-dietary exposure to bisphenol-A, Food Chem. Toxicol. 50 (2012) 3725–3740.
[15] W. Volkel, et al., Metabolism and kinetics of bisphenol a in humans at low doses following oral administration, Chem. Res. Toxicol. 15 (2002) 1281–1287.
[16] W. Dekant, W. Volkel, Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures, Toxicol. Appl. Pharmacol. 228 (2008) 114–134.
[17] L.N. Vandenberg, et al., Bisphenol-A and the great divide: a review of controversies in the field of endocrine disruption, Endocr Rev. 30 (2009) 75–95.
[18] L.N. Vandenberg, et al., Human exposures to bisphenol A: mismatches between data and assumptions, Rev. Environ. Health 28 (2013) 37–58.
[19] J.G. Hengstler, et al., Critical evaluation of key evidence on the human health hazards of exposure to bisphenol A, Crit. Rev. Toxicol. 41 (2011) 263–291.
[20] R. Kuruto-Niwa, et al., Estrogenic activity of alkylphenols, bisphenol S, and their chlorinated derivatives using a GFP expression system, Environ. Toxicol. Pharmacol. 19 (2005) 121–130.
[21] E. Grignard, et al., Weak estrogenic transcriptional activities of Bisphenol A and Bisphenol S, Toxicol. In Vitro 26 (2012) 727–731.
[22] L. Dewalque, et al., Simultaneous determination of some phthalate metabolites, parabens and benzophenone-3 in urine by ultra high pressure liquid chromatography tandem mass spectrometry, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 949–950 (2014) 37–47.
[23] H.M. Koch, et al., On-line clean-up by multidimensional liquid chromatography–electrospray ionization tandem mass spectrometry for high throughput quantification of primary and secondary phthalate metabolites in human urine, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 784 (2003) 169–182.
[24] M.J. Silva, et al., Analysis of human urine for fifteen phthalate metabolites using automated solid-phase extraction, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 805 (2004) 161–167.
[25] M.J. Silva, et al., Quantification of 22 phthalate metabolites in human urine, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 860 (2007) 106–112.
[26] K. Kato, et al., Determination of 16 phthalate metabolites in urine using automated sample preparation and on-line preconcentration/high-performance liquid chromatography/tandem mass spectrometry, Anal Chem. 77 (2005) 2985–2991.
[27] W. Gries, et al., Analytical method for the sensitive determination of major di-(2-propylheptyl)-phthalate metabolites in human urine, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 908 (2012) 128–136.
[28] M. Kim, et al., Simultaneous analysis of urinary phthalate metabolites of residents in Korea using isotope dilution gas chromatography-mass spectrometry, Sci. Total Environ. 470–471 (2014) 1408–1413.
[29] L. Herrero, et al., Feasibility of ultra-high performance liquid and gas chromatography coupled to mass spectrometry for accurate determination of primary and secondary phthalate metabolites in urine samples, Anal. Chim. Acta 853 (2015) 625–636.
[30] G. Provencher, et al., Determination of bisphenol A, triclosan and their metabolites in human urine using isotope-dilution liquid chromatography-tandem mass spectrometry, J. Chromatogr. A 1348 (2014) 97–104.
[31] C. Liao, et al., Bisphenol S in urine from the United States and seven Asian countries: occurrence and human exposures, Environ. Sci. Technol. 46 (2012) 6860–6866.
[32] M. Chen, et al., Determination of nine environmental phenols in urine by ultra-high-performance liquid chromatography–tandem mass spectrometry, J. Anal. Toxicol. 36 (2012) 608–615.
[33] X. Ye, et al., Automated on-line column-switching HPLC–MS/MS method with peak focusing for the determination of nine environmental phenols in urine, Anal. Chem. 77 (2005) 5407–5413.
[34] M. Chen, et al., 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. 904 (2012) 73–80.
[35] A. Myridakis, et al., 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. 407 (2015) 2509–2518.
[36] X. Zhou, et al., 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. 944 (2014) 152–156.
[37] Y. Yang, et al., Urinary levels of bisphenol analogues in residents living near a manufacturing plant in south China, Chemosphere 112 (2014) 481–486.
[38] C. AM and S. EJ, Laboratory Procedure Manual: Bisphenol A and Other Environmental Phenols and Parabens in Urine, ⟨www.cdc.gov/nchs/data/nhanes⟩, 2009 (Method number 6301.01).
[39] Z. Kuklenyik, et al., Automated solid-phase extraction approaches for large scale biomonitoring studies, J. Chromatogr. Sci. 47 (2009) 12–18.
[40] B.C. Blount, et al., Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS, Anal Chem. 72 (2000) 4127–4134.
[41] A.L. Heffernan, et al., Age-related trends in urinary excretion of bisphenol A in Australian children and adults: evidence from a pooled sample study

using samples of convenience, J. Toxicol. Environ. Health A 76 (2013) 1039–1055.
[42] Q.W. Gavin, et al., Development of HPLC-MS/MS method for the simultaneous determination of environmental phenols in human urine, Int. J. Environ. Anal. Chem. 94 (2014) 168–182.
[43] P.W. Albro, et al., Pharmacokinetics, interactions with macromolecules and species differences in metabolism of DEHP, Environ. Health Perspect. 45 (1982) 19–25.
[44] K.A. Thayer, et al., Pharmacokinetics of bisphenol A in humans following a single oral administration, Environ. Int. 83 (2015) 107–115.
[45] U.M. Schauer, et al., Toxicokinetics of tetrabromobisphenol a in humans and rats after oral administration, Toxicol. Sci. 91 (2006) 49–58.
[46] N. Cabaton, et al., Biotransformation of bisphenol F by human and rat liver subcellular fractions, Toxicol. In Vitro 22 (2008) 1697–1704.
[47] M. Li, et al., Biotransformation of bisphenol AF to its major glucuronide metabolite reduces estrogenic activity, PLoS One 8 (2013) e83170.
[48] M.J. Bueno, et al., Application of liquid chromatography/quadrupole-linear Ion trap mass spectrometry and time-of-flight mass spectrometry to the determination of pharmaceuticals and related contaminants in wastewater, Anal. Chem. 79 (2007) 9372–9384.
[49] M.J. Bueno, et al., Evaluation of various liquid chromatography-quadrupole-linear ion trap-mass spectrometry operation modes applied to the analysis of organic pollutants in wastewaters, J. Chromatogr. A 1216 (2009) 5995–6002.
[50] X. Ye, et al., Urinary concentrations of bisphenol A and three other bisphenols in convenience samples of U.S. adults during 2000–2014, Environ. Sci. Technol. 49 (2015) 11834–11839.
[51] CDC, Fourth national report on human exposure to environmental chemicals, February 2015, Updated Tables; U.S Department of Health and Human Services: Atlanta, GA, USA, ⟨www.cdc.gov/exposurereport⟩, 2015.
[52] H.M. Koch, et al., Phthalate exposure during cold plastisol application – a human biomonitoring study, Toxicol. Lett. 213 (2012) 100–106.
[53] A.M. Calafat, et al., Mono-(3-carboxypropyl) phthalate, a metabolite of di-n-octyl phthalate, J. Toxicol. Environ. Health A 69 (2006) 215–227.
[54] D.E. Cantonwine, et al., Urinary phthalate metabolite concentrations among pregnant women in Northern Puerto Rico: distribution, temporal variability, and predictors, Environ. Int. 62 (2014) 1–11.
[55] L. Casas, et al., Urinary concentrations of phthalates and phenols in a population of Spanish pregnant women and children, Environ. Int. 37 (2011) 858–866.