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Glucuronide and Sulfate Conjugates of Bisphenol A: Chemical Synthesis and Correlation Between Their Urinary Levels and Plasma Bisphenol A Content in Voluntary Human Donors

Ka-Lok Ho[1] · Ka-Ki Yuen[1] · Man-Shan Yau[1] · Margaret B. Murphy[1] · Yi Wan[2,9] · Bonnie M.-W. Fong[3,4] · Sidney Tam[3] · John P. Giesy[1,2,5,6,7,8] · Kelvin S.-Y. Leung[4] · Michael H.-W. Lam[1]

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Abstract Bisphenol A (BPA) glucuronide and sulfate conjugates are major products of Phase II metabolism of BPA in humans. In the past, their determination in body fluids usually involves tedious enzymatic hydrolysis and multiresidual analysis. The recent availability of authentic standards of these conjugates enables our better understand of the human metabolism of BPA and the distribution of their metabolites in body fluids. In this work, we report the chemical synthesis and purification of BPA mono- and di-glucuronide and BPA mono- and di-sulfate. Their levels, as well as that of BPA, in 140 paired human plasma and urine samples collected randomly from voluntary donors in Hong Kong SAR, China, were determined by solid-phase extraction and liquid chromatography–tandem mass spectrometry (LC–MS/MS). BPA was found in more than 135 human plasma and urine samples. Its Phase II metabolites, ranging from N.D. to 36.7 μg g^{-1}-creatinine, also were detected in 139 of the 140 urine samples. Good correlation (r = 0.911) between molar concentration of BPA in the plasma and that of "total urinary BPA" (i.e., ln [(BPA + $\sum$ BPA phase II conjugate)$_{\text{molar concentration}}$]) was observed. Direct quantification of Phase II metabolites of BPA in human urine can be a useful assessment tool for population exposure to this potent endocrine disrupting chemical.

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✉ Michael H.-W. Lam
bhmhwlam@cityu.edu.hk

[1] State Key Laboratory for Marine Pollution, Department of Chemistry, City University of Hong Kong, Tat Chee Avenue, Kowloon, Hong Kong SAR, People's Republic of China

[2] Department of Biomedical Veterinary Sciences and Toxicology Centre, University of Saskatchewan, Saskatoon, Canada

[3] Division of Clinical Biochemistry, Queen Mary Hospital, Kowloon, Hong Kong SAR, People's Republic of China

[4] Department of Chemistry, Hong Kong Baptist University, Kowloon, Hong Kong SAR, People's Republic of China

[5] Department of Zoology and Center for Integrative Toxicology, Michigan State University, Lansing, MI, USA

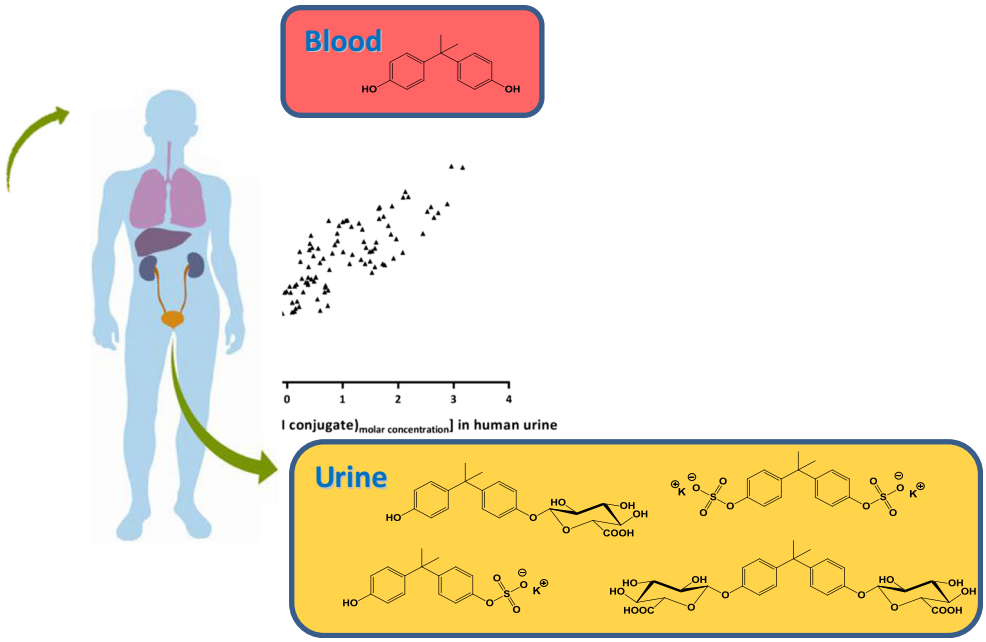
[6] School of Biological Sciences, The University of Hong Kong, Kowloon, Hong Kong SAR, People's Republic of China

[7] Department of Zoology, and Center for Integrative Toxicology, Michigan State University, East Lansing, MI, USA

[8] State Key Laboratory of Pollution Control and Resource Reuse, School of the Environment, Nanjing University, Nanjing, People's Republic of China

[9] Present Address: Laboratory for Earth Surface Processes, College of Urban and Environmental Sciences, Peking University, Beijing, People's Republic of China

Graphical Abstract



Bisphenol A (BPA) is a synthetic chemical and a potent environmental estrogen, commonly used in the production of polycarbonate plastic and epoxy resins that possess many desirable properties, such as high-impact strength, high transparency, good adhesive properties, and malleability (Ben-Jonathan and Steinmetz 1998). BPA-based materials are being extensively used in food packaging. There is mounting evidence that humans are ubiquitously exposed to this endocrine-disrupting chemical, because BPA can leach from its synthetic materials under high temperature and acidic/basic conditions and transfer onto food and beverage products (Brotons et al. 1995; Calafat et al. 2008; Cao et al. 2010; Cooper et al. 2011; Greens et al. 2012; Liao and Kannan 2013; Sajiki et al. 2007; Schecter et al. 2010). Upon dietary exposure, the first-pass metabolism of BPA occurs in the wall of the gut and liver, with the formation of BPA-glucuronide and sulfate conjugates as the major Phase II metabolites (Inoue et al. 2001, 2003; Kuester and Sipes 2007; Pritchett et al. 2002; Ye et al. 2005; Zalko et al. 2003). These highly water-soluble BPA conjugates are mainly excreted in urine (LaKind and Naiman 2015; Thayer et al. 2015). In many previous works, quantification of these BPA metabolites in biota tissues and body fluids was hampered by the unavailability of their authentic standards at the time. The mostly adopted analytical strategy was to hydrolyze them enzymatically with the corresponding β-glucuronidases and arylsulfatases followed by chromatographic determination of the free BPA (Ballestos-Gómez et al. 2009; Jiménez-Díaz et al. 2015; Schauer et al. 2006; Suzuki et al. 2004). This has greatly hindered studies on the biotransformation of this endocrine-disrupting chemical and the use of its metabolites as exposure markers for public health risk assessment.

The first direct determination of BPA metabolites in human serum and urine was reported by Liao and Kannan (Liao and Kannan 2012) using authentic standards of BPA monoglucuronide and BPA disulfate conjugates. BPA monoglucuronide was found to be the dominant BPA metabolite in serum, whereas levels of BPA monoglucuronide and disulfate were comparable in urine. On the other hand, Gerona et al. (2013) analyzed levels of BPA, BPA monoglucuronide, and BPA monosulfate in midgestation umbilical cord serum samples from 85 human fetuses in the Northern and Central California population and reported that BPA monosulfate was the dominant BPA-related compound. A number of recent studies of population exposure to BPA have directly quantified BPA monoglucuronide and BPA monosulfate in human urine using the newly accessible authentic calibration standards (Battal et al. 2014; Kubwabo et al. 2014). In this work, we report the chemical synthesis and purification of all the four most common BPA Phase II metabolites in mammalian model organisms, including human. They are BPA mono- and diglucuronide and BPA mono- and disulfate conjugates (Fig. 1). Using them as authentic standards for quantification, levels of BPA Phase II metabolites in the urine of 140 voluntary donors randomly recruited in Hong Kong Special

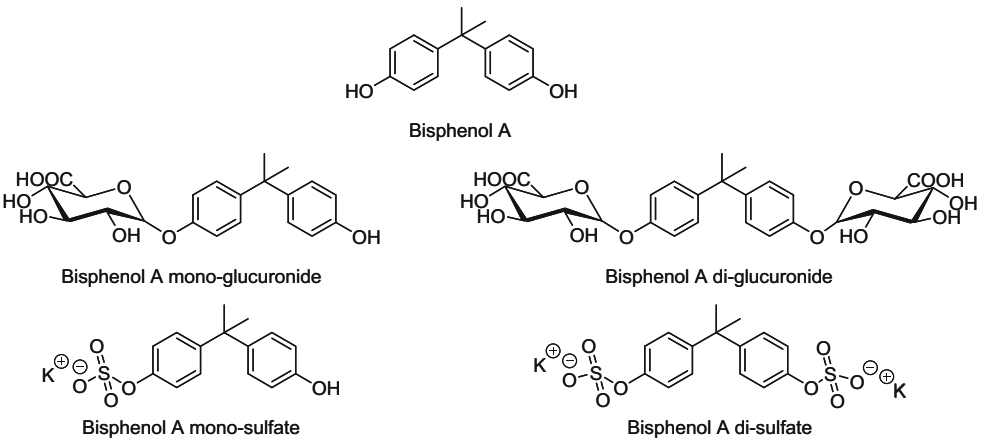


Fig. 1 Chemical structures of bisphenol A (BPA) and its four Phase II metabolites: BPA mono-/diglucuronide and BPA mono-/disulfate

Administrative Region (HK SAR), China, were determined and compared to the corresponding BPA contents in their blood plasma. Our results reveal good relationship between BPA content in plasma and the total amount of BPA-related compounds in urine. This suggests that BPA compounds in human urine may be useful in assessing population exposure to the endocrine-disrupting chemical.

Experimental

Safety Precautions

Extra precaution was practiced in the handling of human body fluid samples. Double-latex gloves, facemasks, and eye-protection goggles were worn always during the handling, spiking, and transferring of those samples. All of the spent samples after analysis were collected in separated, close-lipped containers with proper clinical waste labels. The spent samples and used personal protection items were treated as clinical wastes and were collected and disposed of in accordance with the "Code of Practice for the Management of Clinical Waste" issued by the Environmental Protection Department of the Hong Kong SAR Government.

Instrumentation

NMR spectra were recorded by a Bruker AV400 (400 MHz) FT-NMR spectrometer. Electrospray (ESI) mass spectra were measured by a PE SCIEX API 365 LC/MS/MS system and Applied Biosystems SCIEX QSTAR Elite hybrid quadrupole/time-of-flight (Q-TOF) tandem high-resolution mass spectrometer. Elemental analyses were performed on a Vario EL III CHN elemental analyzer. HPLC purification of the synthesized BPA-conjugates was performed using a Waters 515 HPLC isocratic pump, a Xbridge Prep C18 100 mm × 19 mm i.d. × 5 μm preparative column at a flow rate of 5 mL/min and water:methanol (1:1 v/v) and water: acetonitrile (1:1, v/v) as the mobile phases, and a Waters 2487 dual λ absorbance detector (Milford, MA). Quantification of glucuronide and sulfate conjugates of BPA were performed on an Agilent 1290 Series HPLC (Agilent Technologies, Waldbronn, Germany) coupled to a MDS Sciex API 3200 QTrap triple quadrupole/linear ion trap mass spectrometer with a Turbo V ion spray source (Applied Biosystems, Foster City, CA). To improve sensitivity and selectivity, analytes were detected in the Multiple Reaction Monitoring (MRM) mode with the dwell time of 70 ms. The ionization source parameters were as follow: ion spray voltage: −4500 kV; curtain gas (N_2): 15 psig; collision gas (N_2), high; temperature of ionization source, 550 °C; ion source gas 1 (nebulizer gas), 60 psig; ion source gas 2 (heater gas), 50 pisg. Declustering potential (DP), entrance potential (EP), collision energy (CE), and collision cell exit potential (CXP) of all analytes were optimized to obtain maximum sensitivity. Details are tabulated in Table 1.

The chromatographic separation was performed using a Waters XBridge C18 2.5-μm × 3.0-mm × 50-mm column. A guard column XBridge C18 2.5-μm × 3.0-mm × 20-mm was placed in front of the analytical column. Separation was obtained using gradient elution at a flow rate of 300 μL min^{-1}, with solvent A (Milli-Q water contains 0.1% NH_4OH) and solvent B (acetonitrile contains 0.1% NH_4OH) at the composition of A:B (99:1, v/v) at $t = 0$ to $t = 5$ min, changed linearly to 15:85 (v/v) in a period of 5 min then held at such composition for a further 5 min. After the separation, the eluent composition was switched back to 99:1 (v/v) and held for 20 min before the next injection. The injection volume was 10 μL.

Identification and quantifications of the parent BPA, d_{16}-BPA (recovery standard), and d_{10}-pyrene (internal standard) were performed on a gas chromatography (Bruker 450-GC gas chromatograph) equipped with a tandem mass-selective

Table 1 Performance of the SPE-LC–MS/MS analytical protocol for the determination of the BPA glucuronide and sulfate conjugates (upper) and the SPE GC–MS/MS analytical protocol for the determination of the BPA (lower) in an artificial urine matrix

BPA/BPA-conjugates	Relative repeatability (%)	Inter-day reproducibility (%)	Recovery (%)	MDL (ng g^{-1}-creatinine)	MQL (ng g^{-1}-creatinine)
SPE-LC–MS/MS					
BPA monoglucuronide	4.9	7.9	94.4	3.3	17.0
BPA monosulfate	4.3	6.9	85.9	1.1	5.7
BPA diglucuronide	4.7	7.6	71.1	2.7	13.9
BPA disulfate	3.5	5.7	80.9	1.1	5.6
SPE-GC–MS/MS					
BPA	1.4	2.3	87.4	1.8	9.3
d_{16}-BPA	1.7	2.7	83.6	1.7	8.7

detector (Bruker 320-MS triple quadrupole mass spectrometer), using electron ionization (EI). The temperature of the GC injector was set to 280 °C with injecting volume of 2 μL under splitless mode. BPA and related analytes were analyzed by a 30-m × 0.25-mm × 0.25-μm VF-5MS column. The temperature program was as follows: 110 °C for 1 min; 20 °C min^{-1} to 300 °C held for 10 min. Helium was used as carrier gas and its flow rate was adjusted to 1.5 mL min^{-1}. The total running time for each analysis was 20.5 min. Mass spectrometric parameters were set as following: the ion source temperature was set at 200 °C, whereas temperature of manifold was set at 40 °C. Electron impact voltage was set to 70 eV. Argon at 1.5 mTorr was used as the collision gas in the collision cell. Solvent delay was set to 4.0 min. The detector was an electron multiplier with extended dynamic range (EDR). Mass transitions for each of the targeted compounds are shown in Table 1. Quantification of each analyte was based on the peak area using one target and one quantifier ion (Scheme 1).

Synthesis of BPA Glucuronide and Sulfate Conjugates

The general synthetic routes for the BPA glucuronide and sulfate are outlined in Scheme S1 in the Supporting Information. Intermediate A and B were synthesized according to literature methods (Bollenback et al. 1955; Pilgri and Murphy 2010). Detail synthetic, purification procedure, and characterization data are given in the Supporting Information.

Sample Collection

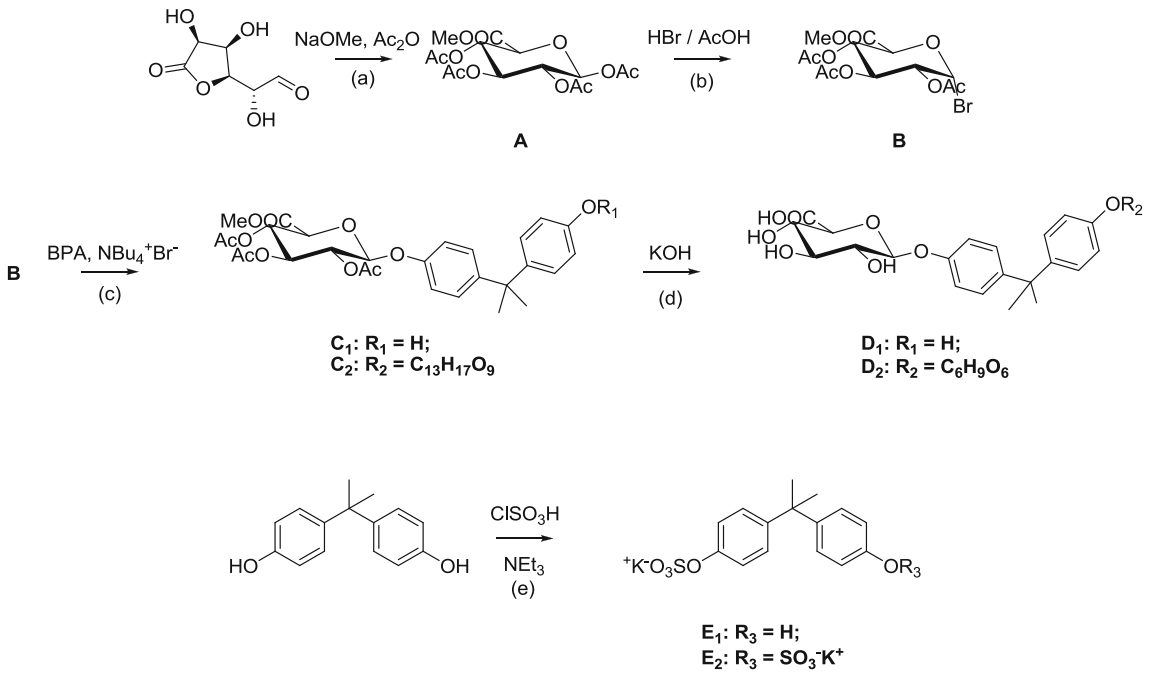
Human studies were performed in accordance with the guidelines and approval of the research ethics committee of City University of Hong Kong. Parallel human blood plasma and urine samples ($n = 140$) were collected from voluntary donors from April to August 2012, by registered doctors and nurses, at the Queens Mary Hospital, Hong Kong SAR, China. Of the 140 donors, there were 66 males and 74 females. The age range of these volunteers was from 18 to 96 years (mean age = 47.1 ± 18.2 years). Whole blood samples and urine samples were collected as describe in our previous study (Ho et al. 2015, 2017). Details are given in Supporting Information.

Sample Extraction and Cleanup

BPA in human blood: literature method (Dirtu et al. 2008; Hovander et al. 2004; Wan et al. 2010) with slight modifications was adopted for the extraction of BPA in human plasma samples. Details are given in the Supporting Information.

BPA in human urine: in a typical determination, d_{16}-BPA (1 ng) was spiked into 5 mL of human urine. The sample was allowed to stand for 10 min for equilibrium. Then, 50 μL of formic acid was added, followed by extraction with ethyl acetate (3 × 5 mL). The organic fractions were combined and evaporated to dryness under a gentle steam of nitrogen. This was followed by reconstitution in 5% acetone in hexane (1 mL), and SPE clean-up with a Sep-Pak Florisil cartridge previously conditioned by DCM/MeOH (4:1 v/v, 6 mL) and 5% acetone in hexane (6 mL) at a rate of 1 drop per second. The cartridge was then eluted by 5% acetone in hexane (6 mL). The cartridge was the dried under reduced pressure and the BPA and d_{16}-BPA were eluted by DCM / MeOH (4:1 v/v, 10 mL). The eluate was evaporated to dryness under a gentle stream of nitrogen. Analytes was reconstituted in 50 μL iso-octane containing 5 ng pyrene-d10 as an internal standard and the phenolic analytes were derivatized by 50 μL of BSTFA with 1% TMCS at 70 °C for an hour.

BPA glucuronide and sulfate conjugates in human urine: human urine (5 mL) was mixed with 2 M of sodium acetate buffer (5 mL, pH 5) and then loaded at the flow rate 1 drop per second onto an Oasis® WAX cartridge previously conditioned with 5 mL of methanol, 5 mL of Milli-Q



Scheme 1 General synthetic procedure of glucuronide and sulfate conjugates of bisphenol A: **a** NaOMe, Ac_2O, perchloric acid; **b** HBr in HOAc (33%), DCM; **c** bisphenol A, NBu_4Br, $CHCl_3$, K_2CO_3, H_2O; **d** KOH, MeOH; **e** *N*,*N*-diethylaniline, $ClSO_3H$, $CHCl_3$, NEt_3

water, and 5 mL of 2 M sodium acetate buffer. The cartridge was then washed by a 2 M of sodium acetate buffer (5 mL, pH 5), Milli-Q water (5 mL), and 2% acetic acid in methanol (5 mL). The sorbent was then dried under reduced pressure and the glucuronide and sulfate conjugates were eluted by 5% NH_4OH in methanol (10 mL). The elute was evaporated to dryness under a gentle stream of nitrogen at 50 °C and was then reconstituted in a mixture of acetonitrile and water (1:1, 100 μL).

Quality Control, Quality Assurance, and Statistical Analysis

Procedural blanks and matrix spikes were analyzed for every batch of 18 samples in analysis of blood BPA and urinary BPA and BPA conjugates. d_{16}-BPA was used as a surrogate to estimate the BPA recovery throughout whole extraction process. All equipment was rinsed with acetone and hexane before use to avoid sample contamination. During analysis of human plasma samples, laboratory blanks and spiked matrices were analyzed per every batch of 18 samples to check for instrumental drift, matrix interference and contamination of solvent and glassware. Estimation of the method detection limit (MDL = $t \times \sigma$, where σ is the standard deviation of the data and t is the compensation factor from the Student's t test with $n - 1$ degrees of freedom at a confidence interval of 95%) and method quantification limit (MQL = $10 \times \sigma$, where σ is the standard deviation of the data) was carried out using a lower spike concentration. Details of quality assurance/quality control are given in Supplementary Information.

All statistical analyses were performed with the SPSS 16 (SPSS Inc., Chicago, IL), Prism 2.01 (GraphPad Software, Inc.) and Sigmastat 3.5 (Sigmastat, Jandel Scientific, CA). Normality of the distributions was assessed by the Kolmogorov–Smirnov test. Logarithmic or natural-logarithmic transformation was used to obtain normally distributed data sets for parametric statistical tests. Student's t test was used to statistically analyze the concentrations of plasma BPA and urinary BPA conjugates between male and female donors for normally distributed data sets. In case where data were not normally distributed, nonparametric Mann-Whitney rank-sum test was used for the comparison. Natural-log transformation on urinary BPA conjugates was applied to reduce their skewness in all statistically analyses. Pearson product moment was used to examine relationship between ln[BPA] in human plasma and ln Σ[BPA-conjugates] / ln[BPA] in human urine. $P < 0.05$ was considered statistically significant.

Results and Discussion

Synthesis and Purification of the Glucuronide and Sulfate Conjugates of BPA

Mono- and disulfate conjugates of BPA were prepared according to our previous published method with some

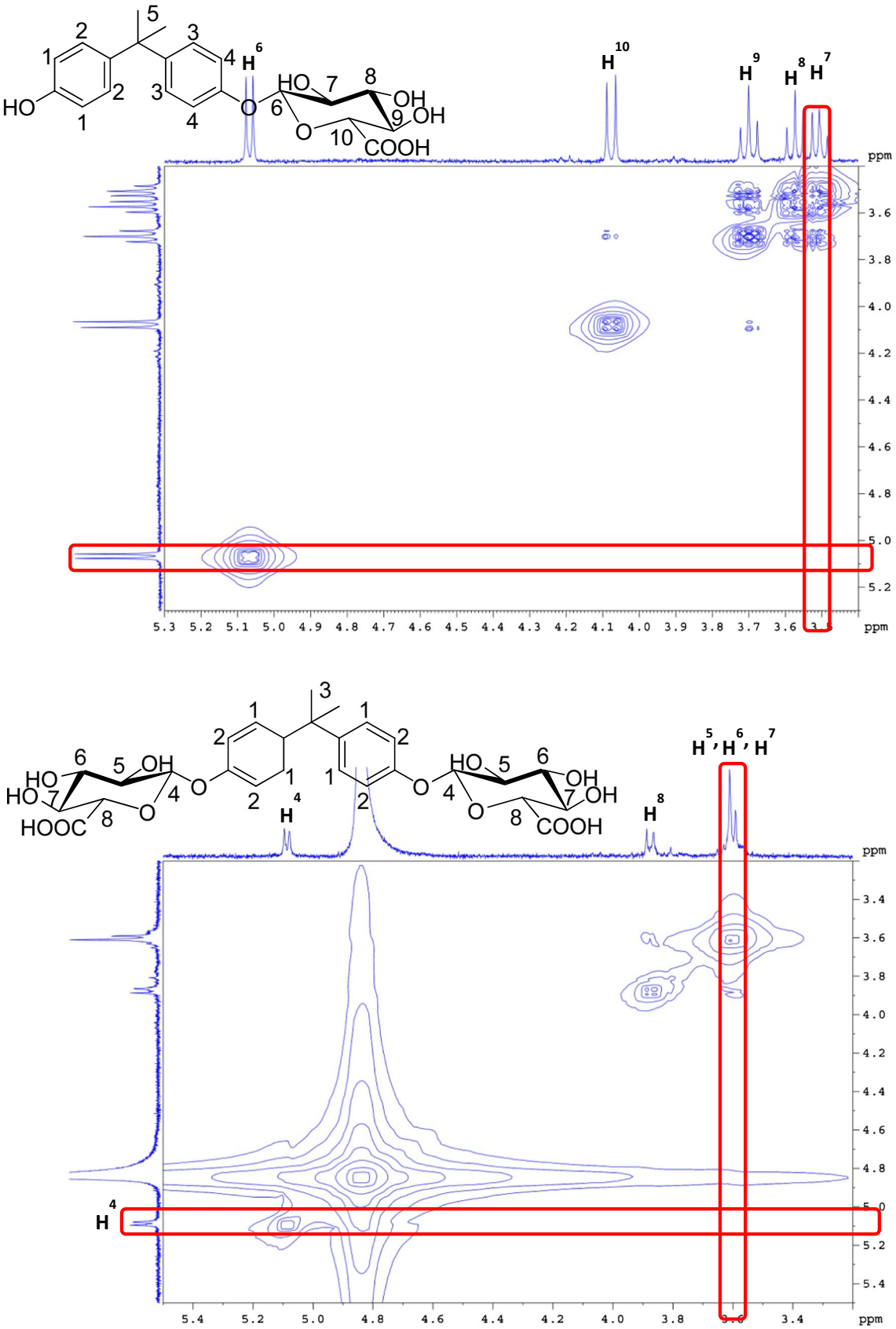


Fig. 2 ^{1}H–^{1}H ROESY 2D-NMR spectra of BPA mono-glucuronide (*upper*) and BPA di-glucuronide (*lower*)

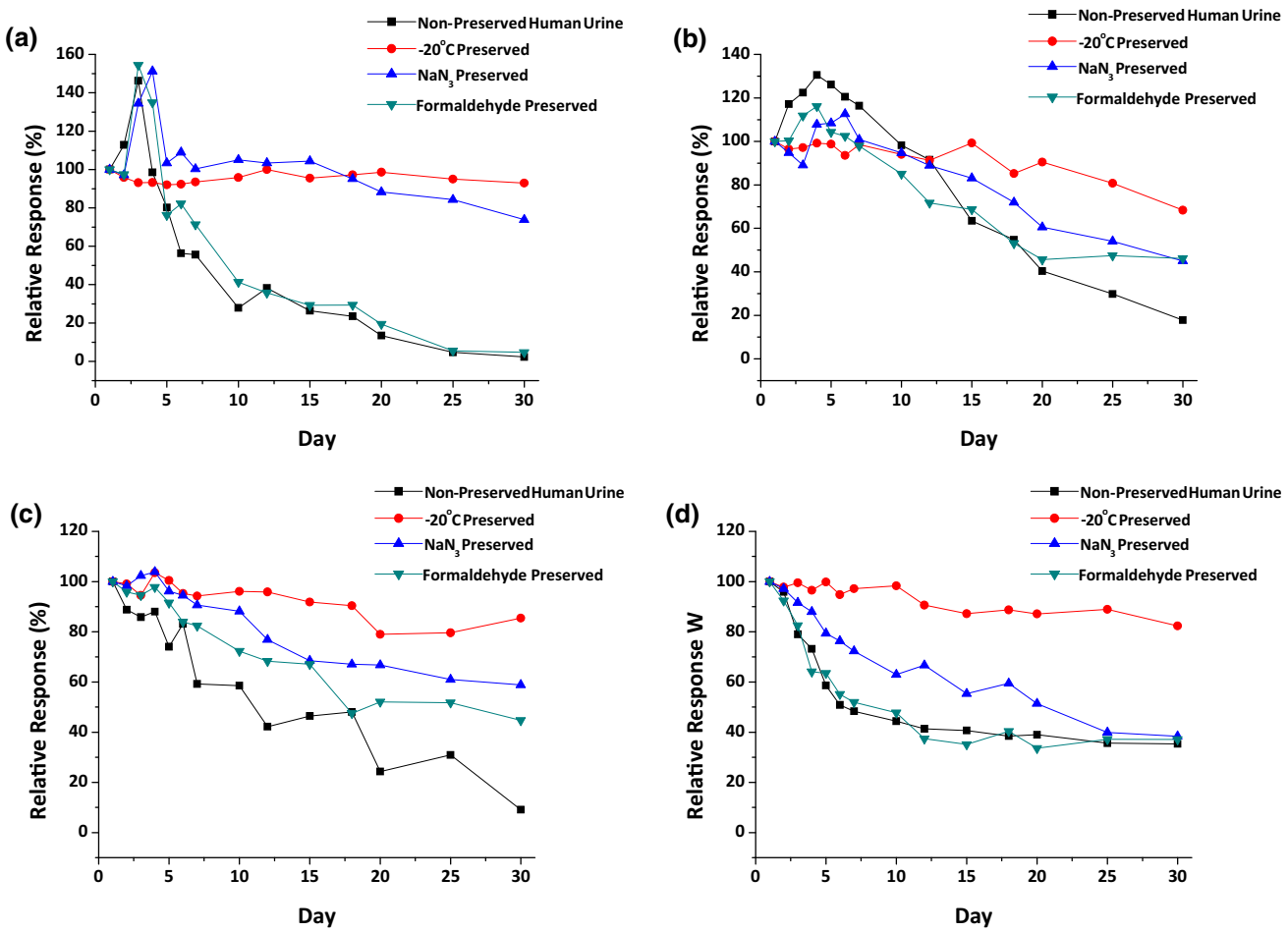


Fig. 3 Stability of BPA conjugates by chemical and physical measures: **a** BPA monoglucuronide; **b** BPA monosulfate; **c** BPA diglucuronide; **d** BPA disulfate

modifications (Ho et al. 2015, 2017). All of the conjugates were purified by semipreparative HPLC. Mass spectrums and ^{1}H NMR spectrum of bisphenol A Phase II conjugates are given in Supporting Information.

Figure 2 shows the typical the typical ^{1}H–^{1}H ROESY 2D-NMR spectra of the synthesized BPA monoglucuronide and BPA diglucuronide, showing no cross signal with respect to the protons on the C1 and C2 carbons of the glucuronide rings. This confirms the β-anomeric configuration of the glucuronide moiety on the two synthesized BPA glucuronide conjugates.[34]

Validation of Analytical Methods

All of the multiple reaction monitoring (MRM) transitions adopted for the identification and quantification of the BPA, BPA glucuronide, and sulfate conjugates, and interpretations of their mass spectrometric fragmentation patterns are given in Tables S1–S3 in the Supplementary Information. Good linear LC–MS/MS responses were obtained for different concentrations of mono- and diglucuronide and sulfate conjugates of BPA over the concentration range of 0.5–1000 ng mL^{-1}. The correlation coefficients of the best-fitted lines were in the range of 0.9962–0.9996 for the four different glucuronide and sulfate conjugates of BPA. Linearity of GC–MS/MS responses to the parent BPA and d_{16}-BPA was analyzed over the range from 0.2 to 1000 ng mL^{-1}. The correlation coefficients of the best-fitted lines are 0.9994 and 0.999 for BPA and d_{16}-BPA respectively. Repeatability and analyte recovery were evaluated by the consecutive analysis of seven independent artificial urine samples spiked with 0.5 ng mL^{-1} of each of the conjugates. Method detection limits (MDLs) and method quantification limit (MQL) of the SPE–LC–MS/MS analytical protocol for the conjugates were determined based on the lowest spiked levels of the BPA glucuronide and sulfate conjugates in the artificial urine matrix (5 mL) at their corresponding MRM chromatographic peaks over a series of seven consecutive analyses. Table 1 summaries the optimize performance

Table 2 Concentrations of BPA and BPA mono-/diglucuronide and sulfate conjugates in paired human plasma (ng mL^{-1}) and human urine samples (μg g^{-1}-creatinine) from Hong Kong SAR, China (n = 140)

BPA and BPA Phase II metabolites	Total (n = 140)			Male (n = 66)			Female (n = 74)		
	GM (95% CI)	Min–max	% of detection	GM (95% CI)	Min–max	% of detection	GM (95% CI)	Min–max	% of detection
Human plasma samples									
BPA	0.53 (0.43–0.67)	N.D.–10.43	96.4	0.47 (0.31–0.69)	N.D.–10.43	95.5	0.59 (0.47–0.75)	N.D.–8.99	97.3
Human urine samples									
BPA	0.24 (0.18–0.31)	N.D.–74.23	99.3	0.20 (0.14–0.29)	0.026–74.23	100	0.28 (0.19–0.43)	N.D.–55.18	98.6
BPA mono-glucuronide	0.74 (0.58–0.54)	N.D.–15.28	86.4	0.56 (0.40–0.80)	N.D.–13.15	87.9	0.93 (0.67–1.30)	N.D.–15.28	85.1
BPA di-glucuronide	0.28 (0.21–0.39)	N.D.–26.34	82.1	0.17 (0.12–0.15)	N.D.–17.29	81.8	0.43 (0.29–0.65)	N.D.–26.34	82.4
BPA mono-sulfate	0.12 (0.09–0.15)	N.D.–2.22	99.3	0.09 (0.06–0.13)	N.D.–1.65	98.5	0.16 (0.11–0.22)	0.01–2.22	100
BPA di-sulfate	0.54 (0.42–0.70)	N.D.–30.45	82.9	0.41 (0.29–0.59)	N.D.–8.63	84.8	0.70 (0.48–1.00)	N.D.–30.45	81.1

GM geometric mean, *CI* confidence interval, *N.D.* not detected

parameters of the analytical protocol for the determination of the urinary BPA-conjugates. MDLs for all the conjugates were ≤3.3 ng g^{-1}-creatinine, while MQLs for all conjugates were ≤17.0 ng g^{-1}-creatinine. These levels of detection sensitivities and quantification sensitivities are considered adequate for environmental analysis purposes.

Stability of BPA Glucuronide and Sulfate Conjugate in Human Urine

Similar to the case of bromophenols and tetrabromobisphenol A, glucuronide and sulfate conjugates of BPA are stable at low temperature (Ho et al. 2015, 2017). These conjugates remained unchanged within 2 weeks of storage and have only decreased by approximately 25% over 30 days (Fig. 3). Other preservation methods, such as the use of sodium azide and formaldehyde as chemical preservatives, were not able to achieve similar storage stability. Also, for monosubstituted conjugates, e.g., BPA monoglucuronide, increased LC–MS/MS responses were observed when urine samples were stored at room temperature, even in the presence of chemical preservatives. This may be due to the stepwise degradation of the disubstituted conjugates.

Levels of BPA in Human Plasma and Human Urine

Paired blood plasma and urine samples were obtained from 140 voluntary donors in Hong Kong SAR, China, in 2012. BPA was detected in the blood plasma of 135 donors. The geometric mean level was 0.53 ng mL^{-1} (95% confidence interval 0.43–0.67 ng mL^{-1}), and the range was N.D. to 19.69 ng mL^{-1} (Table 2). Similar to previous studies reported in the literature, there is no statistical difference (natural-log transformed, P = 0.728) in the plasma concentration of BPA between male and female donors (Ballestos-Gómez et al. 2009; Jiménez-Díaz et al. 2015; Schauer et al. 2006; Suzuki et al. 2004). Our results are comparable to those reports of the plasma BPA levels in the general population as well as in women during pregnancy in Belgium, Germany, Italy, Japan, Korea, and the United States (Cobellis et al. 2009; Ikezuki et al. 2002; Lee et al. 2008; Mulder 1992; Padmanabhan et al. 2008; Schecter et al. 2010; Schönfelder et al. 2002). Excretion and metabolic biotransformation are the two major routes for the reduction of body burden of BPA. In animal feeding studies, excretion of parent BPA and BPA metabolites mainly occurred in feces and urine (Cunha and Fernandes 2010). Of the 140 voluntary donors, BPA was detected in the urine of 139 of them (Table 2). The geometric mean concentration was 0.24 μg g^{-1}-creatinine (95% confidence interval at 0.18–0.31 μg g^{-1}-creatinine). No statistical significant difference was found in the urinary BPA levels

Table 3 Correlation coefficients between parent BPA in human plasma and urinary BPA related compounds

	Total (n = 140)		Male (n = 66)		Female (n = 74)	
	r	P	r	P	r	P
ln (urinary BPA)	0.510	<0.05	0.419	<0.05	0.585	<0.05
ln (Σ urinary BPA-conjugates)	0.896	<0.05	0.906	<0.05	0.889	<0.05
ln (urinary BPA) + ln Σ (urinary BPA-conjugates)	0.911	<0.005	0.908	<0.05	0.914	<0.05

Analysis of urinary BPA relation compounds were conducted using natural-log transformed concentration

Concentration of BPA in human plasma was expressed in mol mL^{-1}; concentration of BPA conjugates in humn urine was expressed in mol g^{-1}-creatinine

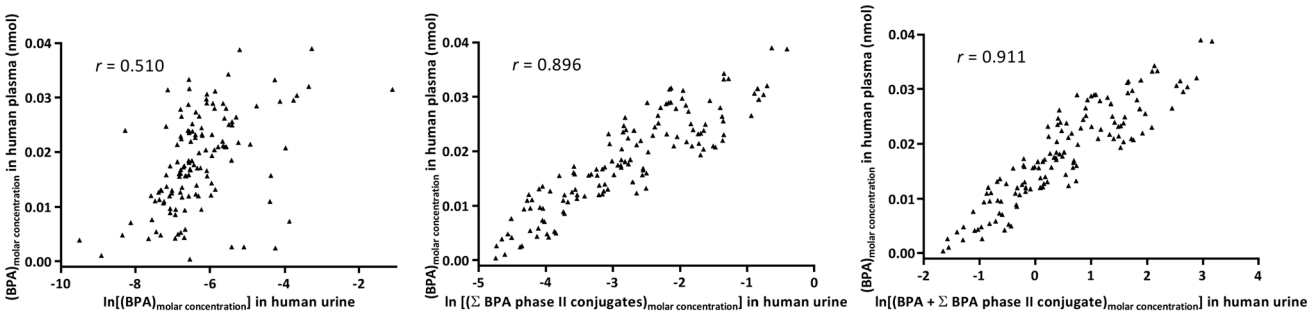


Fig. 4 Scatter plots of BPA content in human plasma versus that of different sets of urinary BPA-related compounds: molar concentration BPA in human plasma versus natural-log transformed molar concentration of BPA in human urine (*left*); molar concentraion of BPA in human plasma versus natural-log transformed total molar concentration of BPA conjugates in human urine (*middle*); molar concentration of BPA in human plasma versus natural-log transformed molar concentration of "total urinary BPA," i.e., BPA + BPA conjugates, in human urine (*right*)

between male and female (natural-log transformed, P = 0.11) is observed. This level of urinary BPA content is relatively lower than those reported in the North America and Europe (Cunha and Fernandes 2010; Kim et al. 2003; Ye et al. 2009).

Levels of BPA Glucuronide and Sulfate Conjugates in Human Urine

Concentrations of mono- and diglucuronide and mono- and disulfate conjugates of BPA in the human urine samples are tabulated in Table 3. Of the 140 urine samples, at least one BPA-conjugate was detected in 139 of them, with the range from N.D. to 36.66 μg g^{-1}-creatinine. Among the four different conjugates, BPA monosulfate is the most frequently detected, whereas level of BPA mono-glucuronide is the highest (geometric mean 0.75 μg g^{-1}-creatinine). This is generally in line with the findings of Liao and Kannan (2012) via direct measurement using authentic standards of BPA conjugates, although they reported a higher occurrence rate of BPA monoglucuronide than BPA monosulfate. Women generally have higher level of urinary BPA conjugates. There are significantly differences in the urinary levels of all four BPA conjugates between male and female donors, BPA diglucuronide (natural-log transformed, P = 0.041), BPA diglucuronide (log transformed, P = 0.002); BPA monosulfate (log transformed, P = 0.027), and BPA disulfate (natural-log transformed, P = 0.02). This is consistent with a previous study performed in Korea where BPA and BPA conjugates in human urine were determined by enzymatic deconjugation (Becker et al. 2009).

The geometric mean of the overall BPA content in human urine, i.e., the summation of all the quantified BPA-related compounds in urine, among the 140 voluntary donors in this study is 2.79 μg g^{-1}-creatinine (95% confidence interval 2.26–3.34 μg g^{-1}-creatinine). This corresponds well with data obtained from other recent studies in Korea, China, Europe, Australia, and the United States (Covaci et al. 2015; Heffernan et al. 2014; Li et al. 2013; Völkel et al. 2002; Zhang et al. 2011).

Correlation Studies

Many previous studies on the administration, exposure, and metabolism of BPA have already revealed rapid metabolism of BPA in human after oral administration. The estimated half-life of BPA in human was ca. 6 h (Völkel et al. 2002), and its Phase II metabolites are excreted in urine. We make use of our present set of paired plasma and urinary BPA/BPA metabolites data to study their correlations. Pearsons product moment was employed to calculate the

correlation coefficients, and results are tabulated in Table 2.

To avoid the difference in molecular mass of BPA and its metabolites from interfering the correlation study, contents of BPA and its glucoronide and sulfate conjugates were expressed in terms of molar concentrations (mol mL^{-1} for plasma content and mol g^{-1}-creatinine for urinary content) (Gerona et al. 2016). Molecular mass of BPA (228.3 g mol^{-1}), BPA monoglucuronide (404.4 g mol^{-1}), BPA diglucuronide (580.5 g mol^{-1}), BPA monosulfate (potassium salt) (330.4 g mol^{-1}), and BPA disulfate (di-potassium salt) (404.6 g mol^{-1}) were used to convert the mass concentration of BPA and its metabolites into their corresponding molar concentration in the plasma and urine samples. Urinary contents of BPA and its metabolites were natural-log transformed.

Relative poor correlation, $r = 0.510$, was found between molar concentration of BPA in plasma and that in urine. This is expected as human are able to rapidly metabolize BPA. Urinary content of BPA is not a good indicator of the level of BPA in human plasma. To obtain a better correlation, products of BPA metabolism in the urine have to be taken into account. A much better correlation, $r = 0.896$, between BPA content in plasma and the total urinary content of BPA cojugates was revealed.

Next, we examined whether the combined content of BPA and all its conjugates in urine correlated better with BPA content in plasma. We define the "total urinary BPA" content as the sum of the natural-log normalized molar concentration of BPA content and that of BPA conjugate ($\ln([\text{BPA}] + \sum [\text{BPA equivalent of BPA-conjugates}])$ in urine. Indeed, an even stronger correlation, $r = 0.911$, was revealed. Figure 4 shows the scatter plots of BPA content in human plasma versus that of different sets of BPA-related compounds in human urine.

Conclusions

The chemical synthesis and purification of the four most commonly occurred BPA Phase II metabolites, i.e., mono- and disubstituted glucuronide and sulfate conjugates of BPA, have been performed successfully. These authentic standards facilitate direct quantification of these important BPA conjugates in our body fluids. This provides a clearer picture of the chemical speciation of the Phase II metabolic process on BPA, which is not achievable with previous analytical protocols based on enzymatic hydrolysis.

A survey of paired human blood plasma and urine samples from 140 voluntary donors recruited in Hong Kong SAR, China, revealed that the "total urinary BPA" content correlates well with plasma BPA content. This suggests that besides blood BPA content, which has been extensively used to reflect BPA exposure in human, the "total urinary BPA" content in humans can be a useful tool for the assessment of population exposure to BPA.

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