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Synthesis and Characterization of Bromophenol Glucuronide and Sulfate Conjugates for Their Direct LC-MS/MS Quantification in Human Urine as Potential Exposure Markers for Polybrominated Diphenyl Ethers

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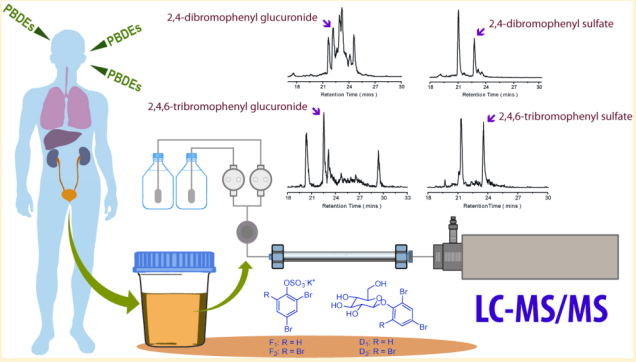
Supporting Information

ABSTRACT: Bromophenol glucuronide and sulfate conjugates have been reported to be products of mammalian metabolism of polybrominated diphenyl ethers (PBDEs), a group of additive flame-retardants found ubiquitously in the environment. In order to explore their occurrence in human urine, four water-soluble bromophenol conjugates, namely, 2,4-dibromophenyl glucuronide, 2,4,6-tribromophenyl glucuronide, 2,4-dibromophenyl sulfate, and 2,4,6-tribromophenyl sulfate, were synthesized, purified, and characterized. An analytical protocol using solid-phase extraction and ion-paired liquid chromatography−electrospray tandem mass spectrometry (LC-ESI-MS/MS) quantification has been developed for the direct and simultaneous determination of these glucuronide and sulfate conjugates in human urine samples. The limit of detections for all analytes were below 13 pg mL^{-1}, with 73−101% analyte recovery and 7.2−8.6% repeatability. The method was applied to analyze 20 human urine samples collected randomly from voluntary donors in Hong Kong SAR, China. All the samples were found to contain one or more of the bromophenol conjugates, with concentration ranging from 0.13−2.45 $\mu g\ g^{-1}$ creatinine. To the best of our knowledge, this is the first analytical protocol for the direct and simultaneous monitoring of these potential phase II metabolites of PBDEs in human urine. Our results have also suggested the potential of these bromophenol conjugates in human urine to be convenient molecular markers for the quantification of population exposure to PBDEs.



Polybrominated diphenyl ethers (PBDEs) are a class of additive brominated flame retardant used commonly in polymers, electric and electronic goods, and textiles. As they are not chemically bonded to materials, PBDEs can be released into the environment during product usage.[1] Their hydrophobicity and resistance to degradation and metabolism have led to their ubiquity in the natural environment and in humans. PBDEs with four to seven bromine substituents have been formally recognized as persistent organic pollutants (POPs) by the Stockholm Convention in 2009 as a result of the strong evidence showing that these compounds have been entering the global ecosystem at an alarmingly rapid rate.[2,3] Even after the ban of their production and use, their levels in human may further increase because of their persistence in the ecosystem and our continued utilization of manufactured goods containing PBDEs. Therefore, monitoring the accumulation of PBDEs in humans is important to assess the risk of PBDE exposure. This calls for the reliable estimation of the population exposure to PBDEs. However, the reliability of many of the current practices for the measurement of population exposure to PBDEs is seriously limited by difficulties in the acquisition of adequate quantities of human tissue samples.

Direct quantification of selected BDE congeners and their hydroxylated and methoxylated metabolites in human blood or serum[4] and human breast milk[5] is the most commonly adopted approaches for the assessment of human exposure to PBDEs.

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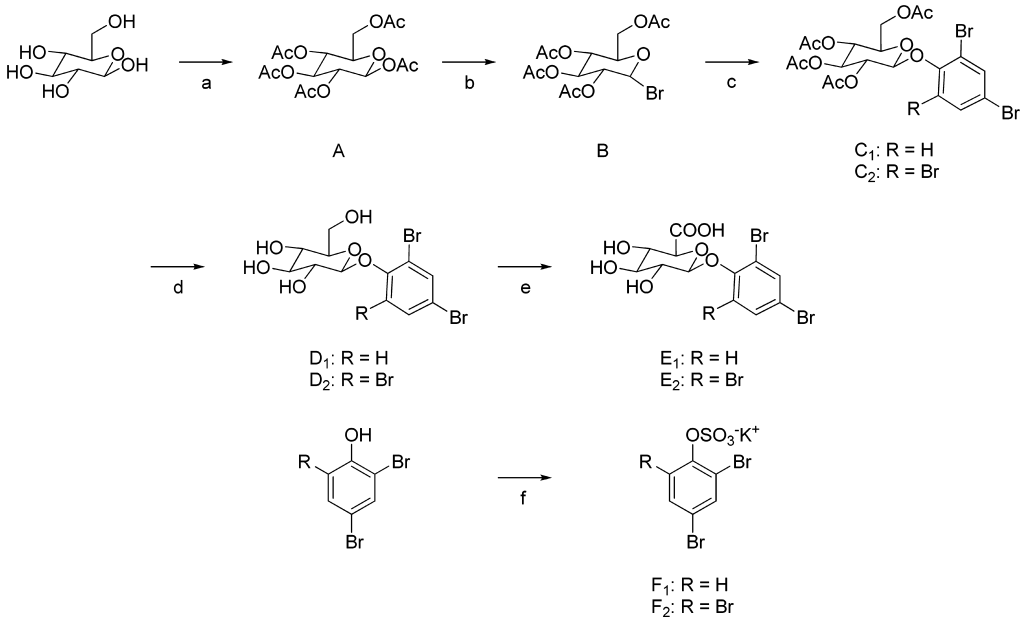
Scheme 1. General Synthetic Procedure of Glucuronide and Sulfate Conjugates of Bromophenols[a]



[a](a) Acetic anhydride, NaOAc; (b) HBr in CH_3COOH; (c) 2,4-dibromophenol/2,4,6-tribormophenol, tetrabutylammonium bromide, NaOH(aq); (d) NEt_3, MeOH, THF, H_2O; (e) TEMPO, NaOCl(aq), NaOH(aq); (f) $ClSO_3H$, $CHCl_3$.

Other less commonly used tissues for exposure estimation include hair, lung, liver, and adipose tissues.[6] However, sampling human tissues for contaminant analysis is intrusive and difficult to achieve on a large scale, especially in the case of healthy subjects who are not hospitalized or required to go through clinical diagnostic tests. Sampling of breast milk can be considered a nonintrusive method, but that restricts the sampling to lactating women within a relatively narrow age distribution, and therefore, it is not clear whether these samples can truly reflect population-level exposure.[7] Sampling of human urine is a truly nonintrusive approach, making it much easier to obtain urine samples from much more voluntary donors for large-scale population surveys.[8] Detection of PBDE metabolites in human urine as exposure markers for PBDEs enables the collection of more representative data on population exposure to the contaminants in order to make comparisons and public health risk assessment on both nationwide and international scales.

The occurrence of phase II metabolites of selected BDE congeners in the urine of exposed mammalian animal models has already been well established by numerous pharmacokinetic and toxicokinetic studies (Figure S1 of the Supporting Information).[9] These metabolites are mainly glucuronide and sulfate conjugates of dibromophenols (DBPs) and tribromophenols (TBPs). To the best of our knowledge, there is no literature report on the occurrence of these potential phase II PBDE metabolites in human urine. Authentic standards of these phase II metabolites are generally not available. Thus, the aims of this study are to synthesize, purify, and characterize selected dibromophenyl- and tribromophenyl-glucuronide and sulfate conjugates as authentic standards for the development of a liquid chromatography−tandem mass spectrometry (LC-MS/MS) analytical protocol for their quantification in human urine samples.

EXPERIMENTAL SECTION

Safety Precautions. Extra precaution was practiced in the handling of human urine samples. Double latex gloves, facemasks, and eye-protection goggles were worn all the time during the handling, spiking, and transferring of human urine samples. All the spent urine samples after analysis were collected in a separated close-lipped container with proper clinical waste labels. The spent urine 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. ^1H NMR spectra were recorded by a Bruker AV400 (400 MHz) FT-NMR spectrometer. Electrospray ionization (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 carried out on a Vario EL III CHN elemental analyzer. Purification of the authentic standards was performed using a Waters 515 high-performance liquid chromatograph (HPLC) isocratic pump and a Waters 2487 dual λ absorbance detector (Milford, MA) using a Zorbax Eclipse XDB-C18 5 μm 150 mm × 4.6 mm i.d. analytical column at a flow rate of 1 mL min^{-1} with water/methanol (1:1 v/v) as the mobile phase. Quantification of the bromophenol glucuronide and sulfate conjugates was performed using an Agilent 1200 Series HPLC (Agilent Technologies, Waldbronn, Germany) coupled to a MDS Sciex API 3200 QTrap triple quadrupole/linear ion trap MS with a Turbo V ion spray source (Applied Biosystems, Foster City, CA, USA). For improved detection sensitivity and selectivity, analytes were detected in the multiple reaction monitoring (MRM) mode with a dwell time of 150 ms. The ionization source parameters were as follows: ion spray voltage, −4500 kV; curtain gas (N_2), 15 psig; collision gas (N_2), high;

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temperature of heater gas, 600 °C; ion source gas 1 (nebulizer gas), 60 psig; ion source gas 2 (heater gas), 50 pisg. Declustering potential (DP), entrance potential (EP), and collision energy (CE) for all analytes were optimized to obtain maximum sensitivity. The collision cell exit potential (CXP) was held constant at −1 V.

Chromatographic separations were performed using a Waters XBridge C18 2.5 μm 3.0 mm i.d. × 50 mm column. A guard column (XBridge C18 2.5 μm 3.0 mm i.d. × 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 (5 mM DHAA in Milli-Q water) and solvent B (5 mM DHAA in methanol) at the composition of 90:10 (v/v) at t = 0 to t = 2 min, changed linearly to 30:70 (v/v) over a period of 18 min and then held at such composition for a further 10 min. After the separation, the eluent composition was switched back to 90:10 (v/v) and held for 20 min before the next injection. The injection volume was 10 μL.

Synthesis, Purification, and Characterization of Bromophenol Glucuronide and Sulfate Conjugates. The general synthetic routes for the bromophenol glucuronide and sulfate conjugates are outlined in Scheme 1. Detail synthetic and purification procedures and characterization data are given in the Supporting Information.

Sample Extraction and Cleanup. A human urine sample (5 mL) was partitioned with 3 × 5 mL ethyl acetate. The combined organic solution was evaporated to dryness under a gentle stream of nitrogen. Residues were dissolved in 15 mL of 0.67 M sodium acetate buffer at pH 5.2. The resultant solution was applied, at a rate of 1 drop s^{-1}, to an Oasis WAX solid-phase extraction (SPE) cartridge that had been preconditioned sequentially by 5 mL of methanol, 5 mL of Milli-Q water, and 5 mL of 2 M sodium acetate buffer at pH 5.2. The loaded WAX SPE cartridge was then washed in turn by 5 mL of 2 M sodium acetate buffer at pH 5.2, followed by 5 mL of methanol. The glucuronide fraction was then eluted with 4 mL of a formic acid/methanol (1:9, v/v) mixture, and the sulfate fraction was eluted with 4 mL of an aqueous ammonia/methanol (1:9, v/v) mixture. The eluates were evaporated to around 100 μL under a gentle stream of nitrogen. $^{13}C_6$-2,4-dibromphenol (200 μL, 500 ng mL^{-1}) was added as an internal standard for LC-MS/MS quantitation.

Stability of Glucuronide and Sulfate Conjugates of Bromophenols in Human Urine. The stability of the bromophenol glucuronide and sulfate conjugates was examined by spiking 300 ng mL^{-1} of the authentic standards into 2 L of human urine samples to assess the persistence of these conjugates 14 times over a period of 30 days. Three parameters related to the storage conditions of the urine samples were assessed: (i) addition of formaldehyde; (ii) addition of sodium azide (NaN_3) as preservatives; and (iii) storage at −20 °C.

Collection of Human Urine Samples. Human urine samples were obtained from 20 healthy volunteers randomly recruited in Hong Kong SAR, China. Urine samples were collected in 100 mL sterilized glass bottles and stored at −80 °C, within 15 min after sampling, until being analyzed. The urine sample from each donor was subdivided into three replicate samples before low temperature storage. All samples were carefully labeled and documented. Upon analysis, samples were thawed, and 10 mL of each sample was taken for creatinine content determination.

RESULTS AND DISCUSSION

Synthesis and Purification of the Glucuronide and Sulfate Conjugates. Synthesis of the sulfate esters of the bromophenols was relatively straightforward. The literature method reported by Burkhardt and Lapworth[10] was generally adopted, with slight modifications. Chloroform was used instead of carbon disulfide because the bromophenols were not too soluble in the latter solvent, leading to low synthetic yields. The synthesis of the glucuronide conjugates involved the nucleophilic substitution reaction between 2,3,4,6-tetra-*O*-acetylglucopyranosyl bromide and the bromophenols using tetrabutylammonium bromide as the phase-transfer catalyst. Deprotection of the hydroxyl groups on the glucuronide moiety was carried out using Et_3N. The final products were obtained by the selective oxidation of the primary alcohol by TEMPO and NaOCl. The glucuronide six-membered rings of the bromophenol conjugates can have two stereisomeric configurations, the α-anomer and β-anomer, depending on the position of the hydrogen atom on its C1 carbon. In the α-anomer, the C−H on C1 is *cis* to the C−H on C2. In the β-anomer, those two C−H groups are *trans* to each other. In human, phenolic compounds are conjugated to form their β-anomers with glucuronic acid as the conjugation reactions follow an S_n2 mechanism.[11] $^1H-^1H$ ROESY two-dimensional (2D) NMR was used to measure the coupling between the C1 and C2 protons of the glucuronide moieties through space so as to determine their configuration. A crossed signal can be observed in α-anomers but not in β-anomers. Figure 1 shows the typical $^1H-^1H$ ROESY 2D NMR spectra of the synthesized 2,4-dibromophenyl glucuronide and 2,4,6-tribromophenyl glucuronide. The absence of any crossed signal between H1 and H2 confirmed the β-anomeric configuration of the glucuronide moiety on the two synthesized bromophenol conjugates.

The bromophenol glucuronide and sulfate conjugates were purified by recrystallization and high-performance liquid chromatographic separation. Purity of all the resultant products was checked by HPLC-UV monitored at dual wavelengths (254 and 226 nm). No ghost peak was observed on their corresponding chromatograms. Q-ToF high-resolution mass spectrometry (HRMS) and LC-ESI-MS were used to confirm the identity of their parent ions. Results of elemental analyses and the exact molecular masses of the synthesized conjugates, revealed by HRMS, are shown in Table S1 of the Supporting Information. Differences between the actual m/z values of the parent ions of the synthesized conjugates to their corresponding theoretical m/z were less than 10 ppm. LC-ESI-MS spectra of the four bromophenol conjugates showing good matches between isotope distribution patterns of their parent ions and major fragments with the theoretical patterns are shown in Figure S2 of the Supporting Information. These conjugates were used as authentic standards for the development of the corresponding analytical protocol for their quantification in the human urine matrix by LC-MS/MS.

Optimization of Chromatographic Conditions. Ion-pairing liquid chromatography is a commonly adopted technique to improve peak shape, retention capacity, and detection sensitivity of highly polar, charged organic analytes.[12] The biggest drawback of ion-pairing chromatography is ion suppression during MS analysis.[13] The sensitivity of ESI-MS is strongly affected by the type, concentration, and pH of the ion-pairing agents in the mobile phase.[14] In this work, we have tried

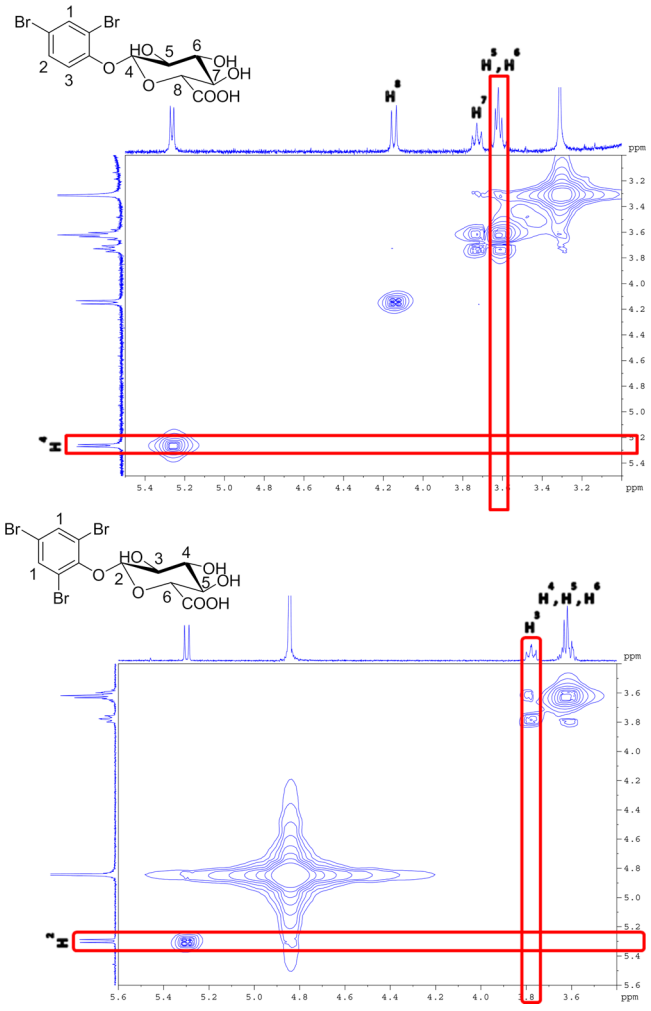

Figure 1. $^{1}H-^{1}H$ ROESY 2D NMR spectra of 2,4-dibromophenyl glucuronide (upper) and 2,4,6-tribromophenyl glucuronide (lower).

various alkylamines of different alkyl chain lengths as ion-pairing agents for the liquid chromatographic separation of the glucuronide and sulfate conjugates. Retention capacity, k', and suppression of the electrospray ionization efficiency of these conjugates by ammonium acetate (AA), triethylammonium acetate (TEAA), tributylammonium acetate (TBAA), and dihexylammonium acetate (DHAA) at a level of 10 mM in the mobile phase are shown in Figure S3 of the Supporting Information. All the ion-pairing agents were able to bring about sufficient retention of the analytes. Alkylamines with longer carbon chains, that is, TBAA and DHAA, gave higher retention capacity. Extents of ionization suppression on the analytes by the four alkylamine salts were comparable. DHAA was eventually chosen as the ion-pairing agent for subsequent method development of the LC-MS/MS determination. A series of concentrations (0.1 mM to 10 mM) of DHAA has been tried in order to evaluate the separation of the glucuronide and sulfate conjugates. Our results showed that the conjugates were effectively separated at a DHAA concentration of greater than 5 mM.

Mobile phase acidity is another important factor in LC-MS analysis as it has significant influence on analyte ionization in mass spectrometry.[15] In general, capacity factors for the conjugates decreased with increasing mobile phase pH, as higher pH reduced the ion-pairing ability of DHAA. The optimal mobile phase pH for analyte ionization at the electrospray ion source was found to be pH 5.4.

Selection of SPE Sorbents. Four different SPE sorbents, including Oasis HLB, Oasis WAX, Discovery DSX-SAX, and Supelclean LC-NH_2, were tested for the optimal extraction efficiency of the highly water-soluble glucuronide and sulfate conjugates from an artificial urine matrix. The spike level used was 10 ng mL^{-1}. A summary of the relative LC-MS/MS responses of the conjugates after extraction by the various SPE sorbents is shown in Figure S4 of the Supporting Information. Extraction efficiencies of polymeric sorbents HLB and WAX were higher than those of silica-based NH_2 and SAX sorbents. Of the two polymeric sorbents, WAX out-performed HLB, probably because of its weak anion exchange properties.[12a,16] Therefore, Oasis WAX SPE cartridges were used in all the subsequent sample extractions.

Validation of Analytical Protocol. Table 1 tabulates all the multiple reaction monitoring (MRM) transitions adopted for the identification and quantification of the bromophenyl glucuronide and sulfate conjugates. Good linearity of the LC-MS/MS determination for all the conjugates (with r^2 ranging from 0.9953 to 0.9999) was observed over the spiked concentration range of 1−500 ng mL^{-1}. 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) of the SPE LC-MS/MS analytical protocol for the conjugates were determined based on the lowest spiked levels of the glucuronide and sulfate bromophenol conjugates in the artificial urine matrix (5 mL) that were still able to produce a signal-to-noise ratio of >10 at their corresponding MRM chromatographic peaks over a series of seven consecutive analyses. Table 2 summaries the optimize performance of the analytical protocol for the determination of the bromophenol conjugates. The MDLs for all the conjugates were ≤13 pg mL^{-1}. This level of detection sensitivity is considered adequate for environmental analysis purposes. In fact, even lower MDLs are achievable by using a larger sample volume of urine. Finally, robustness of the analytical protocol was assessed by analyzing the same spiked sample once a week for seven weeks. No

Table 1. MS/MS Transitions and MS Parameters Adopted in the Analysis

bromophenol conjugate	MRM transitions (m/z)	declustering potential (V)	entrance potential (V)	collision energy (V)
2,4-dibromphenyl glucuronide	427.1 → 251.1	−27.5	−3.8	−32.7
	427.1 → 113.2	−24.0	−4.9	−22.1
2,4,6-tribromphenyl glucuronide	507.5 → 331.2	−15.9	−3.8	−27.0
	505.4 → 113.2	−12.0	−3.0	−23.9
2,4-dibromphenyl sulfate	330.9 → 250.9	−28.9	−4.5	−28.6
	330.9 → 81.3	−28.0	−4.9	−76.1
2,4,6-tribrophenyl sulfate	411.2 → 331.0	−17.0	−3.8	−28.5
	411.2 → 81.3	−20.0	−3.9	−99.0
$^{13}C_6$-2,4-dibromphenol I. S.	257.1 → 80.8	−37.5	−8.9	−39.6

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Table 2. Performance of the SPE LC-MS/MS Analytical Protocol for the Determination of the Bromophenyl Glucuronide and Sulfate Conjugates in Artificial Urine Matrix

bromophenol conjugate	relative repeatability (%)	recovery (%)	MDL (pg mL^{-1})
2,4-dibromophenyl glucuronide	8.5	93.4	12
2,4,6-tribromophenyl glucuronide	7.7	100.5	13
2,4-dibromophenyl sulfate	7.8	92.2	13
2,4,6-tribromophenyl sulfate	7.3	74.0	10

variation in the relative retention time (RRT) of the conjugates whatsoever was observed. Fluctuations in the corresponding chromatographic peak areas of the conjugates were within 5%.

Stability of the Bromophenyl Glucuronide and Sulfate Conjugates in Human Urine. One of the criteria for molecular markers to be useful for population exposure assessment is their stability in the sample matrices. If a marker can be further chemically or biochemically transformed or degraded after sampling, it has to be effectively preserved. Figure 2 shows the stability of spiked bromophenyl glucuronide and sulfate conjugates in human urine. Without any sample preservation, levels of the conjugates drop significantly (for >50%) within the initial week, and then, the rate of degradation gradually plateaus off. Nevertheless, within the first month after sampling, concentrations of the bromophenyl glucuronide and sulfate conjugates in human urine samples could have dropped to <30% of their original levels. The 2,4,6-tribromophenyl glucuronide content fell below the detection limit after storage at room temperature for five days. Previous studies have suggested that chemical additives such as NaN_3 and formaldehyde could prevent the microbial biodegradation of glucuronide conjugates of testosterone and epitestosterone.[17] Controlling storage temperature has also been used extensively to ensure long-term stability of samples and analytes. In this study, we compared the effectiveness of the above three approaches to preserve the bromophenyl glucuronide and sulfate conjugates in human urine samples. As shown in Figure 2, low-temperature storage (at <−20 °C) is the most effective way to preserve these conjugates, while the use of chemical preservatives does not slow down their degradation at all. In all subsequent experiments, urine samples were stored at −80 °C, within 15 min after sampling, until being analyzed.

Determination of Urinary Bromophenol Conjugates in Humans. To assess the applicability of the analytical protocol and to verify the occurrence of bromophenyl glucuronide and sulfate conjugates in human, we have collected and analyzed urines from 20 healthy voluntary donors (10 males and 10 females), selected randomly in Hong Kong SAR, China. Hong Kong is a Special Administrative Region, with an area of 1104 km^2 and a population of seven million, located in the southern coast of the Pearl River Delta, one of the most

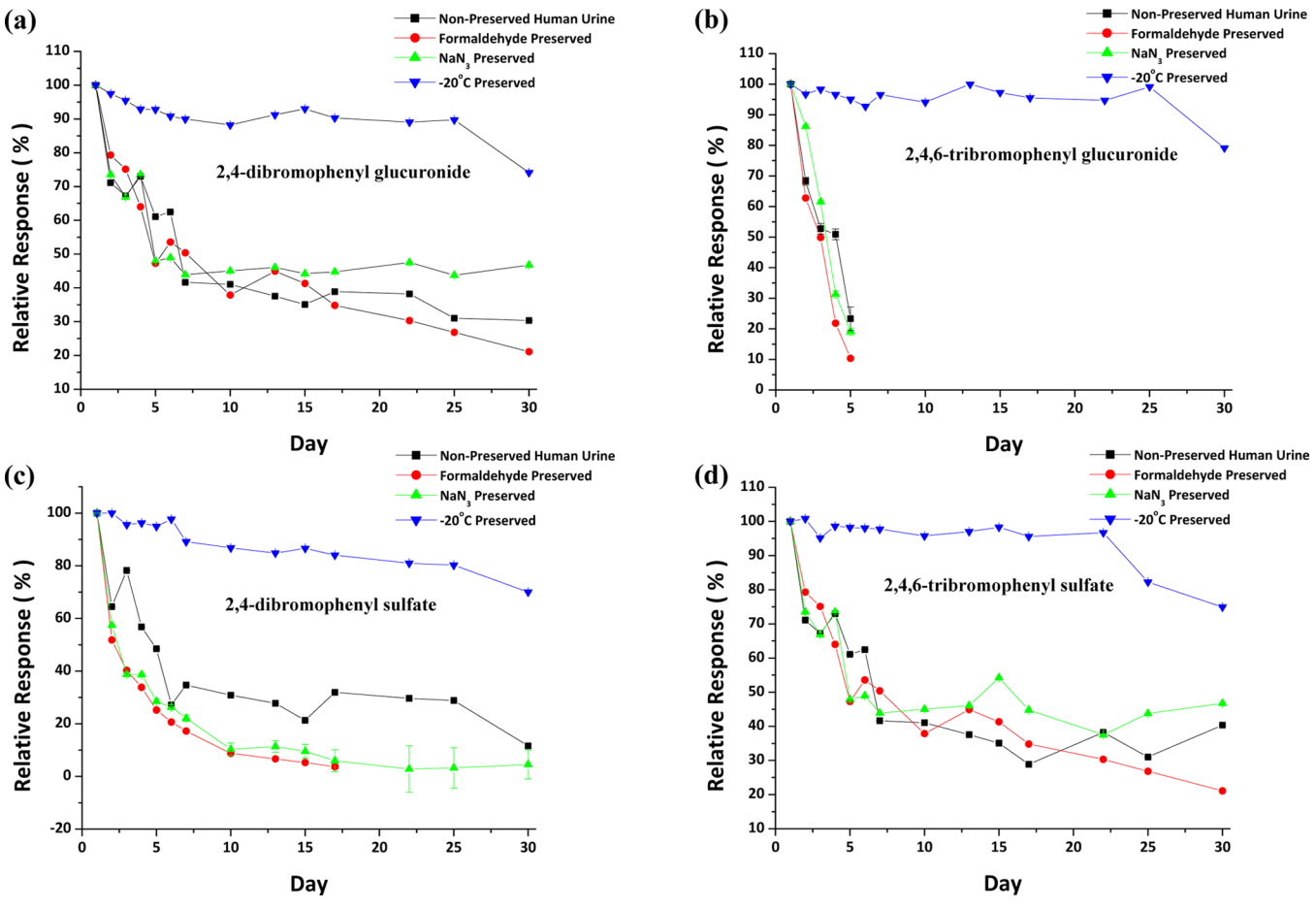

Figure 2. Effects of various chemical preservatives and storage temperature on the stability of the bromophenol conjugates in human urine. Spike level of the conjugates adopted was 300 ng mL^{-1}.

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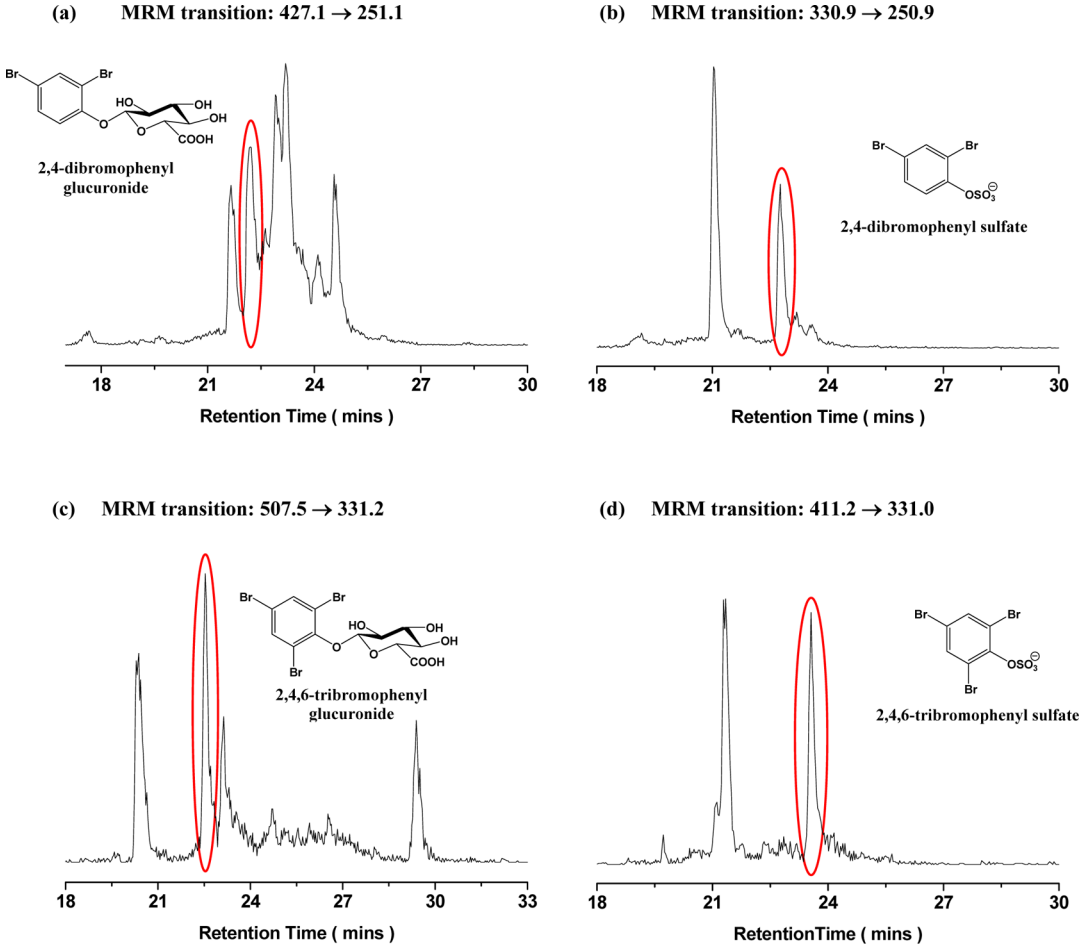

Figure 3. Typical HPLC-MS/MS chromatograms of human urine sample monitored at the corresponding MRM transitions of selected bromophenol conjugates: (a) 2,4-dibromophenyl glucuronide; (b) 2,4-dibromophenyl sulfate; (c) 2,4,6-tribromophenyl glucuronide; and (d) 2,4,6-tribromophenyl sulfate.

Table 3. Mean Concentrations (± 1 SD) of Bromphenol Conjugates Detected in Human Urine Samples, μg g^{-1} Creatinine[a]

sample	2,4-dibromophenyl glucuronide	2,4,6-tribromophenyl glucuronide	2,4-dibromophenyl sulfate	2,4,6-tribromophenyl sulfate
sample 1 (male 1)	N.D.	0.27	0.07	0.06
sample 2 (female 1)	0.22	N.D.	N.D.	N.D.
sample 3 (female 2)	0.29	4.80	0.02	0.05
sample 4 (male 2)	0.12	0.08	0.01	0.03
sample 5 (female 3)	0.91	2.96	0.13	0.18
sample 6 (female 4)	0.63	0.41	0.13	0.15
sample 7 (female 5)	0.08	0.09	N.D.	0.01
sample 8 (male 3)	0.16	0.18	N.D.	0.01
sample 9 (male 4)	N.D.	6.85	0.10	0.27
sample 10 (female 6)	0.09	0.26	N.D.	N.D.
sample 11 (male 5)	N.D.	3.52	0.29	0.61
sample 12 (female 7)	2.93	9.14	0.52	0.50
sample 13 (male 6)	N.D.	0.24	0.05	0.05
sample 14 (male 7)	N.D.	3.17	0.15	0.15
sample 15 (male 8)	0.08	0.86	0.08	N.D.
sample 16 (female 8)	0.87	1.29	0.22	0.25
sample 17 (male 9)	0.10	0.41	0.04	0.03
sample 18 (male 10)	0.49	4.32	0.31	0.87
sample 19 (female 9)	2.73	10.14	0.19	0.17
sample 20 (female 10)	0.23	0.06	N.D.	0.06
mean ± SD	0.50 ± 0.85	2.45 ± 3.15	0.13 ± 0.14	0.17 ± 0.23

[a]N.D. = not detected.

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industrialized regions, of China. Previous surveys have already demonstrated a relatively high level of PBDE contamination of the coastal marine environment of Hong Kong.[18] The local population is also suspected to have a high exposed to PBDEs. In this study, samples obtained were immediately frozen at −80 °C and were analyzed within one week after sampling. Figure 3 shows typical LC-MS chromatograms of urine samples monitored at the MRM transitions specific for the four bromophenol conjugates. All the peaks observed on the same chromatogram possessed similar MRM transitions to the selected bromophenol conjugates. They were originated most probably from isomers of the selected conjugate with bromine and glucuronide/sulfate substitutions at different positions on the aromatic ring. The method of standard addition was adopted to identify and directly quantify levels of the bromophenyl glucuronide and sulfate conjugates in the samples. These levels of conjugates were then normalized by the corresponding levels of creatinine in the urine samples. Our results are tabulated in Table 3. In all the tested samples, one or more of the bromophenol conjugates were detected, with the concentration ranging 0.13−2.45 μg g^{-1} creatinine. Among these four conjugates, the level of 2,4,6-tribromophenyl glucuronide was the highest in the human urine samples. In general, levels of the glucuronide conjugates were higher than those of the sulfate conjugates. These results confirmed the occurrence of the potential phase II metabolites of PBDEs in human urine, which in turn, suggested considerable population exposure to the fire retardants in Hong Kong and probably the southern China region.

CONCLUSION

The glucuronide and sulfate conjugates of 2,4-dibromophenol and 2,4,6-tribromophenol have been successfully synthesized, purified, and characterized. These conjugates were used as authentic standards for the development of a SPE LC-MS/MS analytical protocol for their quantification in human urine samples. Subsequent analyses of samples from voluntary donors revealed the presence of these potential phase II metabolites of PBDEs in their urine, which further hinted the considerable population exposure to the persistent organic pollutants in the study region. Further correlation between levels of these metabolites in human urine and the extent of PBDE exposure is warranted so as to explore the feasibility of using these metabolites in human urine as molecular markers for the quantification of population exposure to the POPs.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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