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Letter

Simultaneous Determination of Multiple Classes of Phenolic Compounds in Human Urine: Insight into Metabolic Biomarkers of Occupational Exposure to E-Waste

Meiqing Lin, Shengtao Ma, Yingxin Yu, Guiying Li, Bixian Mai, and Taicheng An*

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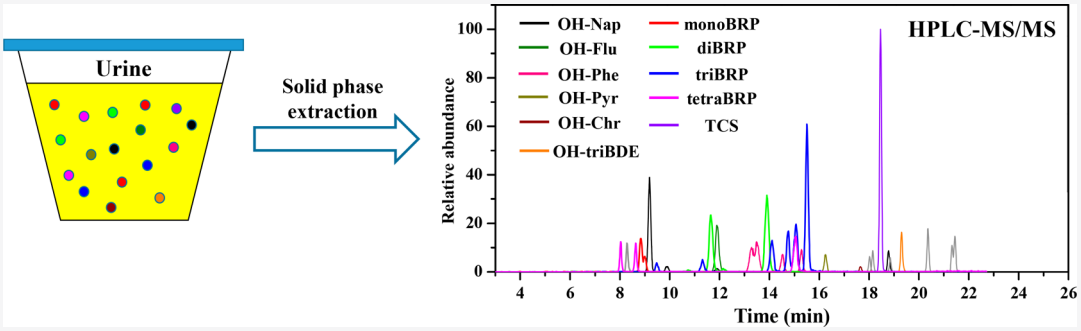

ABSTRACT: A new method using solid-phase extraction (SPE) and high-performance liquid chromatography coupled with triple quadrupole mass spectrometry (HPLC-MS/MS) was established for simultaneously analyzing 35 types of phenolic compounds in human urine samples, including 12 hydroxyl polycyclic aromatic hydrocarbons (OH-PAHs), 16 brominated phenols (BRPs), 5 hydroxyl polybrominated diphenyl ethers (OH-PBDEs), triclosan, and tetrabromobisphenol A (TBBPA). The presence of NH_4Ac in mobile phase played a crucial role in achieving good separation of isomers in HPLC-MS/MS analysis. The efficient SPE condition was obtained at pH 2 with a mixture eluent of methanol and dichloromethane. The recoveries of all analytes were 64.5%−143%, 56.2%−135%, and 52.5%−123% for low-, medium-, and high-spiked levels in a urine matrix, respectively, with less than 20% of RSDs of four of repetitive measurements. Moreover, the method was successfully applied to screen the potential urinary biomarker for population exposure to multipollutants from e-waste recycling areas, and 3,4-diBRP, 2,4,5-triBRP, 2,3,4,6-tetraBRP, and 2′-OH-BDE-28 were first tentatively detected in human urine. Specifically, 3,4-diBRP, 2,3,4,6-tetraBRP and, 2′-OH-BDE-28 were only detected in urine samples from e-waste dismantling workers, which should be considered as the urinary biomarkers for occupational exposure. Finally, this developed method will support a more comprehensive health risk assessment for human exposure to multipollutants.

INTRODUCTION

As numerous studies reported, humans are exposed to various pollutants through respiration, diet, and dermal contact.[1] Among these, phenolic compounds have drawn extensive attention due to their negative effects on human health. Many kinds of phenols are synthetic chemicals, including triclosan (TCS) and tetrabromobisphenol A (TBBPA). TCS has been widely used in human personal care due to its antibacterial properties,[2] and TBBPA is an important brominated flame retardant (BFR) used after the Stockholm Convention phasing out penta- and octa-BDE-based flame retardants in consumer products.[3,4] Another kind of phenolic compound is endogenous metabolites within the living body, such as hydroxyl polycyclic aromatic hydrocarbons (OH-PAHs), hydroxyl polybrominated diphenyl ethers (OH-PBDEs), and brominated phenols (BRPs). OH-PAHs and OH-PBDEs are metabolites of PAHs and PBDEs from cytochrome P450 enzyme-catalyzed hydroxylation, respectively.[5,6] BRPs were cleavage products of BFRs or OH-PBDEs through biological metabolisms.[6,7] Specifically, 2,4-dibromophenol and 2,4,6-tribromophenol are also commercial flame retardants used like TBBPA.[8,9]

There are resemblances between the molecular structures of some phenols (such as TCS, TBBPA, and OH-PBDEs) and thyroid hormones (THs). Therefore, TCS, TBBPA, and OH-PBDEs might disrupt or mimic THs activity to cause endocrine disruption.[2,10] As single-ring organohalogen com-

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pounds, the chemical structures of BRPs differ from THs but still have the capability to bind transthyretin.[10,11] Besides endocrine disruption characteristics, phenols are also potential neurotoxicity, carcinogenicity, and mutagenicity compounds.[12−18] It is therefore important to develop a time- and labor-saving, as well as reliable, analysis method for biological samples to support the health assessment of multipollutant exposure.

Phenols, xenobiotic or endobiotic compounds, are generally considered to form glucuronide and sulfate conjugates by phase II metabolic enzymes. The major elimination route of phenols and their conjugated metabolites is through urine excretion.[5,19,20] The presence of TCS at detectable levels in the majority of human urine suggests wide use of and human exposure to TCS.[21] Many studies have used OH-PAHs as urinary biomarkers to evaluate human exposure to PAHs.[22,23] As potential metabolites of BFRs, urinary BRPs and OH-PBDEs could be considered as potential biomarkers of occupational exposure, including electronic waste (e-waste) dismantling activities. However, few data are available on urinary biomarkers for occupational exposure to BFRs. So far, only two studies have reported urinary BRPs from the general population, and only 4 out of 19 BRP congeners (namely, 2-BRP, 4-BRP, 2,4-diBRP, and 2,4,6-triBRP) were detectable.[20,24] However, these four compounds are natural compounds or commercial materials,[25,26] which are not likely to be biomarkers of occupational exposure.

Hitherto, it is still challengeable to simultaneously determine multiple classes of phenolic compounds in human urine to support the health assessment of multipollutant exposure. Recently, because it is rapid, has superior sensitivity and selectivity, and avoids derivatization commonly used in GC-MS analysis, high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) has been introduced to qualitatively and quantitatively analyze various phenolic compounds in biological samples.[27,28] However, overestimating the quantification results or overlooking some important compounds would occur due to many co-occurrence phenolic compounds with unseparated peaks. Thus, developing a reliable new method to separate the isomeric compounds in urine is still very changeable.

The aim of this study was to develop an effective and reliable method to simultaneously quantify and qualify multiple classes of phenols (namely, TCS, TBBPA, 19 BRPs, 4 OH-PBDEs, and 12 OH-PAHs) in human urine with HPLC-MS/MS. The developed method was validated to analyze human urine samples from e-waste recycling areas to screen the potential urinary biomarkers for occupational exposure to multipollutants.

EXPERIMENTAL SECTION

Chemicals and Reagents. All chemicals and reagents used in this work are provided in Text S1 in the Supporting Information.

Urine Samples Collection. Pooled urine samples for method development were collected from 15 anonymous healthy nonsmoking donors from a university in Guangzhou Higher Education Mega Center, South China. Another 20 individual urine samples for method application were obtained from 10 occupationally exposed e-waste dismantling workers and 10 nonoccupationally exposed residents living in an e-waste recycling area in South China. Consents were obtained from all participants after being clearly informed of the purposes of this study. All samples were frozen and stored at −80 °C until analysis.

Sample Analysis. Urine samples were thawed at room temperature and vortex mixed. Then, urine (2 mL) was transferred into a Teflon tube and spiked with a mixture of internal standards (10 μL) according to the detailed procedures in Text S2. After gentle mixing, samples were added to a β-glucuronidase/arylsulfatase enzyme (10 μL) and acetic acid−sodium acetate buffers (1 mol L^{-1}, 1 mL) to adjust to pH 5.5 and then incubated at 37 °C overnight. After cooling to room temperature, the samples were added to hydrochloric acid (0.5 mol L^{-1}, 1.7 mL) to adjust to pH 2. The target analytes were extracted through Oasis HLB SPE cartridges. The procedure is as follows: SPE cartridges were conditioned with 6 mL of methanol (MeOH):dichloromethane (DCM) (1:1, v/v), 6 mL of MeOH, 6 mL of water, and 8 mL of a 25 mmol L^{-1} KH_2PO_4 buffer. Then, samples were loaded onto cartridges and washed with a KH_2PO_4 buffer (25 mmol L^{-1}, 3 mL) and purified water (3 mL). Finally, the cartridges were dried using a vacuum pump, and the target analytes were eluted with MeOH or the mixture solvent of MeOH and DCM (1:1, v/v) to optimize the eluent and elution volumes. During these procedures, the flow rate was held below 1 mL min^{-1}. Eluates were evaporated to nearly dry, and the residues were reconstituted with MeOH (200 μL) for HPLC-MS/MS analysis. For HPLC separation, different mobile phases were tested. Purified water, aqueous acetic acid (HAc) solution, or aqueous ammonium acetate (NH_4Ac) solution was used as solvent A, and MeOH or acetonitrile (ACN) was used as solvent B. Gas chromatography-triple quadrupole mass spectrometry (GC-MS/MS) with diazomethane derivatization was used to confirm the detection of OH-PBDEs in human urine. The detailed analytical parameters of the HPLC-MS/MS and GC-MS/MS are presented in Text S3, Text S4, Tables S1, and S2.

RESULTS AND DISCUSSION

Development of Reliable Analytical Method. Most analytes could be quantified and qualified by MRM transitions using ESI-MS/MS (Table S1). However, the separation of isomers should be paid more attention due to their undistinguishable MRM transitions, such as triBRPs and tetraBRPs. For the development and modification of the HPLC-MS/MS analytical method for phenolic compound determination, the aqueous−organic mobile phase additives play important roles in chromatographic separation.

Here, two organic phases (MeOH and ACN) and two water phase additives (HAc and NH_4Ac) were optimized. Figures S1−S6, showing peak shapes and chromatographic separation efficiency in six different mobile phase systems. The chromatographic separations were achieved and comparable in all mobile phase conditions for OH-PAHs isomers and low brominated phenol isomers. However, the mobile phase additive has a notable effect on the separation of tri- and tetra-brominated phenol isomers. Poor separations and peak shapes were obtained for 2,3,5-triBRP, 2,4,5-triBRP, and 3,4,5-triBRP and tetra-brominated phenols using the mobile phase of purified water and MeOH or ACN (Figures S1 and S2). While, poor separations but better peak shapes were achieved for 2,3,5-triBRP, 2,4,5-triBRP, and 3,4,5-triBRP and tetra-brominated phenols with HAc solution and MeOH or ACN (Figures S3 and S4). However, all tri- and tetra-brominated phenol isomers obtained better separations and good peak

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Table 1. Mean Recovery Percentages and Relative Standard Deviations (n = 4) for Analytes in Pooled Urine Spiked at Various Concentrations, MDL[a], and Matrix Effect[b]

	Low level			Medium level			High level				
	Spiked (ng mL^{-1})	Mean recovery (%)	RSD (%)	Spiked (ng mL^{-1})	Mean recovery (%)	RSD (%)	Spiked (ng mL^{-1})	Mean recovery (%)	RSD (%)	MDL (ng mL^{-1})	Matrix effect (%)
1-OH-Nap	1.0	68.0	17.1	2.5	56.2	1.8	5.0	52.5	8.5	0.040	−7
2-OH-Nap	1.5	102.1	10.8	3.75	104.2	8.3	7.5	96.1	7.6	0.015	1
2-OH-Flu	1.0	107.0	3.3	2.5	95.9	0.1	5.0	109.4	2.6	0.019	1
3-OH-Flu	1.0	110.7	14.0	2.5	99.9	14.3	5.0	122.5	1.4	0.125	−13
2-OH-Phe	0.5	114.1	4.2	1.25	110.7	3.1	2.5	114.3	0.8	0.012	14
3-OH-Phe	0.5	91.4	4.3	1.25	113.3	2.2	2.5	105.6	0.7	0.018	19
4-OH-Phe	0.5	65.0	0.7	1.25	95.9	3.8	2.5	91.8	7.8	0.029	8
1/9-OH-Phe[c]	0.5	64.5	8.8	1.25	90.1	4.8	2.5	85.3	2.6	0.039	−4
1-OH-Pyr	0.5	98.0	14.2	1.25	82.4	16.5	2.5	87.2	5.8	0.064	10
6-OH-Chr	0.5	100.3	0.8	1.25	89.1	2.4	2.5	67.9	8.9	0.161	28
3-OH-BaP	0.5	65.4	11.9	1.25	94.6	18.5	2.5	60.2	14.7	0.041	−3
TCS	0.6	109.8	16.3	1.5	111.5	2.8	3.0	99.5	4.1	0.021	5
TBBPA	0.5	120.3	10.6	1.25	107.1	3.8	2.5	74.7	7.4	0.029	7
4-BRP	0.5	90.0	19.6	1.25	88.3	9.8	2.5	87.6	19.3	0.027	−10
3-BRP	0.5	108.1	19.3	1.25	94.1	6.3	2.5	104.5	10.9	0.080	29
2,3-diBRP	0.5	120.0	12.5	1.25	114.3	6.2	2.5	74.7	7.5	0.093	22
3,4-diBRP	0.5	122.7	2.0	1.25	102.3	1.2	2.5	97.4	1.7	0.025	−2
2,4-diBRP	0.5	128.1	6.8	1.25	104.7	5.4	2.5	75.8	4.3	0.086	−18
3,5-diBRP	0.5	107.3	12.7	1.25	111.7	5.8	2.5	107.1	9.6	0.015	4
2,3,6-triBRP	0.5	65.9	10.8	1.25	67.0	2.1	2.5	70.4	0.2	0.068	31
2,4,6-triBRP	0.5	79.7	12.1	1.25	96.0	8.9	2.5	76.1	13.2	0.051	6
2,3,4-triBRP	0.5	85.2	0.3	1.25	89.0	7.4	2.5	74.0	4.0	0.033	7
2,3,5-triBRP	0.5	87.7	1.6	1.25	97.4	6.0	2.5	73.8	5.7	0.021	0
2,4,5-triBRP	0.5	91.3	14.5	1.25	105.7	12.2	2.5	83.4	4.4	0.013	14
3,4,5-triBRP	0.5	104.8	1.3	1.25	128.1	18.9	2.5	96.4	11.5	0.008	4
2,3,5,6-tetraBRP	0.5	69.9	3.1	1.25	64.1	14.3	2.5	103.0	7.4	0.021	58
2,3,4,6-tetraBRP	0.5	80.8	10.2	1.25	82.7	10.8	2.5	101.9	19.5	0.023	63
2,3,4,5-tetraBRP	0.5	77.0	5.7	1.25	74.6	15.6	2.5	79.8	11.5	0.015	11
PBP	0.5	96.3	15.1	1.25	86.1	6.0	2.5	93.6	2.0	0.024	18
2′-OH-BDE-28	0.2	136.7	18.8	0.5	128.6	2.8	1.0	81.6	2.5	0.012	9
6-OH-BDE-47	0.2	142.6	5.5	0.5	135.2	9.9	1.0	81.1	1.4	0.011	5
6′-OH-BDE-99	0.2	137.5	12.4	0.5	114.2	2.3	1.0	68.7	1.4	0.015	9
3′-OH-BDE-154	0.2	110.2	5.3	0.5	115.5	1.6	1.0	84.5	19.5	0.017	11
4′-OH-BDE-201	0.2	133.0	3.4	0.5	125.0	12.6	1.0	84.4	19.2	0.023	−7

[a]The method detection limit (MDL) was calculated using the US EPA recommended method[36] as follows: MDL = $t^{(n-1,1-\alpha=0.99)} \times S$, where $t^{(n-1,1-\alpha=0.99)}$ is 2.998, namely, the student's t-value appropriate for the single-tailed 99th percentile t statistic and a standard deviation estimate with 7 degrees of freedom based on eight replicates in this study; S is sample standard deviation of the replicate low level spiked sample analyses (n = 8). [b]The matrix effect was calculated by the following formula: Matrix effect = $\frac{B-A}{A} \times 100\%$, where A is the peak area of an analyte in a standard solution (in MeOH); B is the peak area of postextract spiked with the analyte at the same concentration as the standard solution. If matrix effect = 0%, there is no matrix effect. If matrix effect > 0%, an ion-enhancement occurs. If matrix effect <0%, ion-suppression occurs. [c]1- and 9-OH-Phe could not be separated chromatographically and were consequently quantified together.

shapes with the mobile phase of NH_4Ac solution and MeOH or ACN (Figures S5 and S6). Furthermore, NH_4Ac possessed the highest chromatographic separation efficiency with an increased concentration of NH_4Ac to 5 mM under the ACN phase for BRP isomers. Thus, complete separation of six triBRP isomers and three tetraBRP isomers was obtained with HPLC-MS/MS with the mobile phase of NH_4Ac solution and ACN (Figure S6). However, it is worth noting that the intensities of 2-BRP, 2,6-diBRP, and 2,5-diBRP signals were too low to be discerned even at high injection levels no matter what mobile phase additive was added. This was attributed to the difficult ionization of phenolic compounds with Br atoms on the ortho position related to the OH group.[29,30] Above all, 5 mM NH_4Ac with ACN was selected as the optimal mobile phase to achieve a good peak shape, separation, and sensitivity for HPLC-MS/MS analysis for all target phenolic compounds except 2-BRP, 2,6-diBRP, and 2,5-diBRP.

For sample preparation, an Oasis HLB (500 mg, 6 cc) SPE cartridge was chosen for urine sample extraction due to its high retention capacity to polar and nonpolar compounds at a full pH range (1−14),[31] as well as its better performance than C18 and C8 cartridges.[29] However, the charged molecule will not stay in stationary phase of HLB column. According to the "pK_a-rule", sample pH should be two units lower than pK_a to ensure a protonated and neutral molecule for the best extracting acidic compounds.[32] Among all the target analytes, PBP is the analyte with the lowest pK_a of 4.40 (Table S3). Therefore, the pH of urine samples was adjusted to 2 before all

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the extraction processes. MeOH and a mixture solvent of MeOH and DCM (1:1, v/v) were used to test the elution efficiency of target analytes. For OH-PAHs, the recoveries of 1-, 2-, 3-, 4-, and 9-OH-Phe, 1-OH-Pyr, 6-OH-Chr, and 3-OH-BaP in the first fraction (F1) of the MeOH elution solution were 0% (Figure S7a). The result was the same as OH-PBDEs. Recoveries of other compounds in F1 ranged from 2% (2,3,5,6-tetraBRP) to 88% (2-OH-Nap). Furthermore, the elution flow rates tended to decrease with an increasing log K_{ow} of the target compounds (Table S3 and Figure S7a), suggesting that the MeOH solution was not strong enough to elute those phenols with high log K_{ow} values. When 50% of DCM was mixed in MeOH, recoveries of the target compounds significantly increased in F1 (Figure S7b). Specifically, all OH-PAHs were completely eluted in F1 with recoveries of 58%−111%. Other compounds also achieved considerable recoveries in F1: 70%−115% for BRPs, 108%−125% for OH-PBDEs, 101% for TCS, and 100% for TBBPA. The rest of the small amounts of phenolic organohalogen compounds were washed out in F2. Consequently, 8 mL of a mixture solvent of MeOH and DCM (1:1, v/v) was selected as the most efficient solvent to elute all target phenols from HLB cartridges.

Method Validation. On the basis of the above developed method, the instrument detection limits (IDLs) and method detection limits (MDLs) of all target analytes ranged from 0.2 to 15.0 pg and 0.008 to 0.161 ng mL^{-1}, respectively (Table 1 and Table S1). The matrix effects were calculated as shown in Table 1, which ranged from −18% to 63%, suggesting the suppression or enhancement of ESI-MS/MS signals of the target compounds. To compensate for the matrix effects, structural analogs and stable isotopically labeled compounds were used as internal standards (Text S1).

Good linearity was achieved with linear regression coefficients ≥0.990 (Table S1). Three concentrations (C_{Low}, C_{Medium}, and C_{High}) were prepared to spike into the diluted pooled urine samples with four replicates for each level to evaluate recoveries. The mean recoveries of all analytes were 64.5%−143%, 56.2%−135%, and 52.5%−123% for low-, medium-, and high-spiked levels, respectively (Table 1). Relative standard deviations (RSDs) of repetitive measurements were as low as 20% with three spiked concentrations. All of the calibration standard solutions and blank samples were processed with the real samples in the same batch run. The recoveries of internal standards in the real urine samples were 50.3%−131%. No target analytes were detected in the procedural blank.

Application in Human Urine Samples Analysis. To further validate our developed method and screen out potential urinary biomarkers for occupational exposure population, 10 urine samples from e-waste dismantling workers and 10 from residents living in an e-waste recycling area were analyzed. As shown in Figure 1 and Table S1, all target OH-PAHs except 3-OH-BaP were detected in urine samples from the two populations with comparable detection frequencies. 2-OH-Nap, 2-OH-Flu, 2-OH-Phe, and 3-OH-Phe were detected in all samples. Concentrations of total OH-PAHs in the samples tested ranged from 2.37−104 and 2.77−117 ng mL^{-1}, respectively. This is consistent with our previous study that no significant difference existed in urinary OH-PAHs concentrations between occupational and nonoccupational populations from e-waste recycling areas.[33] OH-PAHs are the metabolites of PAHs which widely exist in the environment. Therefore, they could be easily detected in any individual.

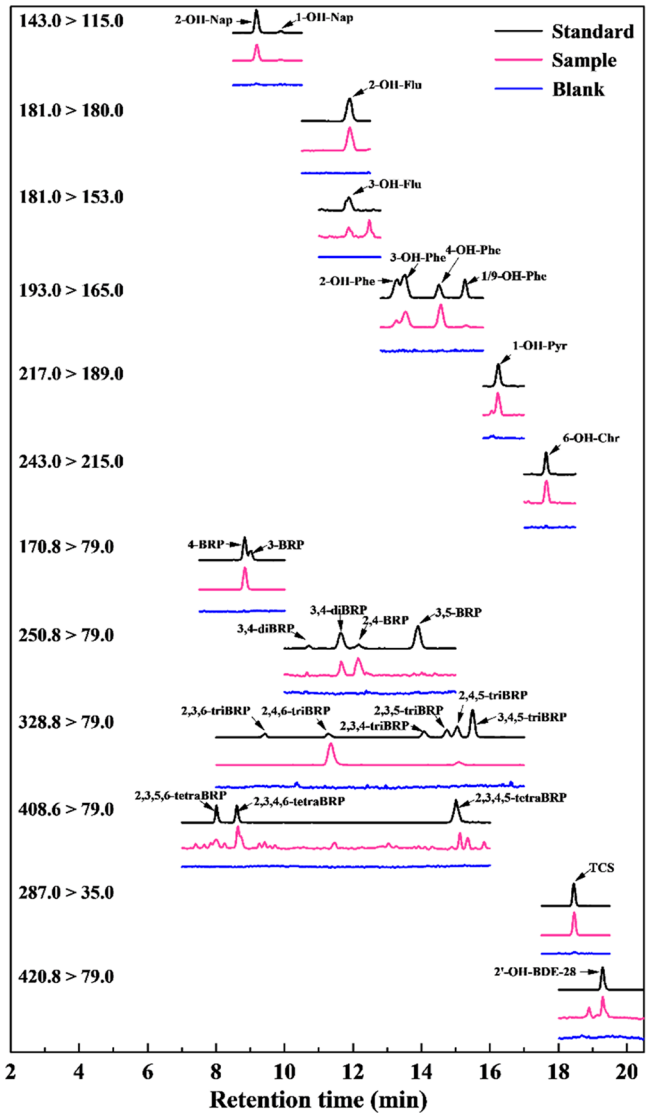

Figure 1. HPLC-MS/MS typical MRM ion chromatograms of target analytes resulting from a standard solution of 5−37.5 ng mL^{-1} (black line), a representative urine sample (red line), and a blank (blue line).

Similarly, TCS was also detected in urine samples from two populations with comparable detection frequencies with the concentration ranging from less than MDL to 10.2 ng mL^{-1} for e-waste workers and from less than MDL to 25.7 ng mL^{-1} for residents. The level of urinary TCS was responsible for the usage of personal care products. The above results suggest that OH-PAHs and TCS are the ubiquitous metabolites or compounds in the human body.

For BRPs, 6 out of 19 BRPs were detectable in human urine samples. 4-BRP and 2,4,6-triBRP had the highest and comparable detection frequencies (90% and 100%, respectively) in theses urine samples (Table S4). However, the concentrations of 4-BRP and 2,4,6-triBRP in urine from e-waste dismantling workers (ranging from less than MDL to 12.3 and 0.93 to 52.7 ng mL^{-1}, respectively) were higher than those from the residents (ranging from less than MDL to 0.48 and 0.24 to 7.39 ng mL^{-1}, respectively), while the detection frequencies of 2,4-diBRP and 2,4,5-triBRP in urine from workers (60% and 80%, respectively) were significantly higher than those of the residents (20% and 10%, respectively).

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Furthermore, 3,4-diBRP and 2,3,4,6-tetraBRP were only detected in urine samples from workers with 40% detection frequencies.

4-BRP, 2,4-diBRP, and 2,4,6-triBRP have been determined in urine from the general population as potential biomarkers for PBDE exposure.[24] Moreover, 2,4,6-triBRP has been proven to be a metabolite of BDE-100 and BDE-154, as well as 1,2-bis(2,4,6-dibromophenoxy) ethane (BTBPE).[7,8] 2,4-diBRP has been proven to be a metabolite of BDE-47.[6] However, 2,4-diBRP and 2,4,6-triBRP are not only the metabolites of BFRs but also of the flame retardants.[8,9] Therefore, it is hard to differentiate their sources in human urine. Nevertheless, 3,4-diBRP, 2,4,5-triBRP, and 2,3,4,6-tetraBRP were neither natural compounds nor commercial materials. Further, 3,4-diBRP was hardly detected in any biological fluids, and 2,4,5-triBRP has been proven as a BDE-99 metabolite and detectable in human serum.[6] In addition, Eguchi et al. found 2,3,4,6- and 2,3,5,6-tetraBRP in human serum samples and suggested tetraBRP as the potential metabolite of higher-brominated PBDEs.[34,35] In this study, 3,4-diBRP, 2,4,5-triBRP, and 2,3,4,6-tetraBRP were first thought to be in human urine samples from e-waste dismantling workers. Further animal studies are needed to find out the precursors of 3,4-diBRP and tetraBRP. Therefore, we could conclude that higher concentrations of 4-BRP and 2,4,6-triBRP as well as higher detection frequencies of 3,4-diBRP, 2,4-diBRP, 2,4,5-triBRP, and 2,3,4,6-tetraBRP in urine samples from e-waste dismantling workers were attributed to BFRs exposure. Furthermore, the MRM transitions of OH-triBDE were only found in urine samples of workers with concentrations from less than MDL to 0.09 ng mL^{-1} and 70% detection frequency (Table S4). However, the specific compound could not be determined, even though the retention time of this OH-triBDE was well matched with the standard of 2′-OH-BDE-28 (Figure 1). Unlike BRPs, the separation of OH-triBDE isomers in HPLC-MS/MS was not confirmed without more standard OH-triBDE isomers. Therefore, the urine sample containing OH-triBDE was further analyzed using GC-MS/MS with diazomethane derivatization. The result confirmed that OH-triBDE was 2′-OH-BDE-28 in the urine sample through matching the MRM transitions and the retention time of the standard (Figure S8). It is worth noting that 2′-OH-BDE-28 was also first tentatively detected in the human urine sample.

To summarize, the developed method did achieve simultaneous quantification and qualification of multiple classes of phenols in human urine and achieved good separation for the isomers during HPLC-MS/MS, which was the key to fulfilling the global analysis of urine samples from a population exposed to multipollutants and identifying a series of BFR potential metabolites in human urine. 3,4-diBRP, 2,4,5-triBRP, 2,3,4,6-tetraBRP, and 2′-OH-BDE-28 were first tentatively detected in human urine from e-waste dismantling workers and considered as urinary biomarkers for occupational exposure to BFRs. Finally, this developed method will also support a more comprehensive health risk assessment for human exposure to multipollutants.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.estlett.0c00187.

Detailed description of chemicals and reagents preparation, instrumental analysis parameter, pK_a values and log K_{ow} of target analytes, HPLC-MS/MS MRM chromatographic profiles of target analytes in six different mobile phase systems, elution efficiency of target analytes in SPE with different elution solution, and GC-MS/MS typical MRM ion-chromatograms of OH-triBDE. (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Taicheng An − *Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China;* orcid.org/0000-0001-6918-8070; Email: antc99@gdut.edu.cn

Authors

Meiqing Lin − *Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China; State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, Guangdong, China; University of Chinese Academy of Sciences, Beijing 100049, China*

Shengtao Ma − *Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China; Synergy Innovation Institute of GDUT, Shantou 515100, China*

Yingxin Yu − *Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China*

Guiying Li − *Guangdong Key Laboratory of Environmental Catalysis and Health Risk Control, School of Environmental Science and Engineering, Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, China; Synergy Innovation Institute of GDUT, Shantou 515100, China*

Bixian Mai − *State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, Guangdong, China;* orcid.org/0000-0001-6358-8698

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.estlett.0c00187

Notes

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