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Short Communication

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

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ABSTRACT

Human exposure to bisphenol A (BPA) is widespread. However, in recent years, bisphenol analogs such as bisphenol S (BPS) and bisphenol F (BPF) are replacing BPA in the production of some consumer products. Because human exposure to these alternative bisphenols may occur, biomonitoring of these bisphenol analogs is warranted. In the present study, we developed and validated a sensitive and selective method that uses on-line solid phase extraction coupled to high performance liquid chromatography-isotope dilution tandem mass spectrometry with peak focusing to measure BPA, BPF, BPS, and 11 other environmental phenols in urine. The method required a small amount of sample (100 μL) and minimal sample pretreatment. The limits of detection were 0.03 ng/mL (BPS), 0.06 ng/mL (BPF), 0.10 ng/mL (BPA), and ranged from 0.1 ng/mL to 1.0 ng/mL for the other 11 phenols. In 100 urine samples collected in 2009–2012 from a convenience group of anonymous adults in the United States, of the three bisphenols, we detected BPA at the highest frequency and median concentrations (95%, 0.72 ng/mL), followed by BPS (78%, 0.13 ng/mL) and BPF (55%, 0.08 ng/mL). This sensitive, rugged, and labor and cost-effective method could be used for the analysis of large number of samples for epidemiologic studies.

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1. Introduction

Human exposure to bisphenol A (BPA) is widespread due to the extensive use of this chemical in consumer products [1]. Scientific debate continues about the toxicity of BPA in animal studies and its potential implications for human health [2], and government organizations in several countries have banned the use of BPA in certain products [3–5]. In response to these restrictions and public pressure, the use of bisphenol analogs, including bisphenol S (BPS, 4,4′-sulfonyldiphenol) and bisphenol F (BPF, 4,4′-dihydroxydiphenylmethane), may be increasing.

Limited data suggest that BPS and BPF, similar to BPA, possess slight to moderate acute toxicity and weak estrogenicity, although the activity of BPF and BPS seems to be lower than that of BPA [6,7]. Nonetheless, the potential environmental and health impacts of BPF and BPS are largely unknown, and human exposure data to these compounds are warranted.

Several analytical techniques involving extraction, purification and/or derivatization followed by detection with gas chromatography–mass spectrometry, high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) or ultra performance liquid chromatography have been used to measure BPF and BPS in sediments, paper products, and food [8–12]. Analytical methods to measure BPF and BPS in biological fluids, on the other hand, are rather limited. BPS was recently quantified by HPLC–MS/MS in urine after automated off-line solid-phase extraction (SPE) [13].

In the present study, we developed and validated a sensitive and selective method that uses on-line SPE–HPLC–MS/MS with peak focusing to measure the urinary concentrations of BPA, BPF, and BPS. The same method can be also used to simultaneously measure 11 other phenols: 2,4- and 2,5-dichlorophenols; 2,4,5- and 2,4,6-trichlorophenols; o-phenylphenol, benzophenone-3, triclosan, and methyl-, ethyl-, propyl- and butyl parabens. These environmental phenols are used in consumer and personal care products and human exposure to these chemicals is of public health interest.

Abbreviations: CDC, Centers for Disease Control and Prevention; BPA, bisphenol A; BPF, bisphenol F; BPS, bisphenol S; HPLC, high performance liquid chromatography; LOD, limit of detection; On line-SPE, on line-solid phase extraction; QC, quality control; QCH, quality control high; QCL, quality control low; QCM, quality control medium; RAM, restricted access materials; RSD, relative standard deviation.

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2. Experimental

2.1. Analytical standards and reagents

BPA, BPF, and BPS were purchased from Sigma-Aldrich Laboratories, Inc. (St. Louis, MO, USA). $^{13}C_{12}$-BPA was obtained from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA) and $^{13}C_{12}$-BPS from CanSyn Chem Corp. (Toronto, Canada). The sources of the other standards and reagents are given in section 1 of the Supplemental Material.

2.2. Collection of human urine for method validation

We collected 100 urine samples in Atlanta, GA in 2009–2012 from 100 adult volunteers with no documented occupational exposure to the target phenols. The Centers for Disease Control and Prevention's (CDC) Human Subjects Institutional Review Board reviewed and approved the study protocol. A waiver of informed consent was requested under 45 CFR 46.116(d). No personal or demographic data were available.

2.3. Preparation of standard stock solutions and quality control materials

We prepared the stock solutions of individual analytical standard and stable isotope-labeled internal standard in MeOH. Ten mixed stock solutions containing all 14 analytes were generated by serial dilution of the individual stock with MeOH. The final concentrations of the mixed stock standards ranged from 0.01 to 100 ng/mL for BPF and BPS, and from 0.1 to 100 (or 1000) ng/mL for BPA and the other 11 analytes. The mixed internal standard solution, containing the stable isotope-labeled analogs for all target analytes except BPF, was prepared so that a 50 μL spike would result in a concentration of 25 ng/mL; we used $^{13}C_{12}$-BPA as the internal standard for BPF. We prepared a second set of individual stock and mixed stock standards and used them to fortify quality control (QC) materials and to check the method accuracy. The mixed stock solutions and mixed internal standard solution, dispensed into 1.5 mL glass vials and 10 mL glass vials respectively, were stored at −70 °C until used.

QC materials were prepared from blank urine pre-screened to confirm that it did not contain detectable concentrations of the target analytes. The blank urine was divided into two aliquots to create QC low (QCL) and QC high (QCH) concentration pools. The QCL and the QCH pools were enriched with different levels of native target compounds that encompass the ranges described for the U.S. general population [13,14]. All QC materials were stored in 1.5 mL glass vials at −70 °C until used.

The enzyme solution was prepared daily as described in section 2 of the Supplemental Material. A mixture of $^{13}C_4$-4-methylumbelliferone, 4-methylumbelliferyl sulfate, and 4-methylumbelliferyl glucuronide was added to each sample and used as a deconjugation standard to confirm that the enzyme functioned properly. Additional details are given in the Supplemental Material.

2.4. Sample and standard preparation

To measure both the concentration of free and total (free plus conjugated) species of the target phenols, each unknown sample was prepared in two different ways: one sample was processed without enzyme treatment; the other was treated with β-glucuronidase/sulfatase. For estimating the concentrations of free species, we added 50 μL of internal standard solution and 100 μL of urine in a 1.5 mL conical silanized glass autosampler vial, and diluted the urine to 1 mL with 0.1 M formic acid. For measuring the total concentrations, we added 50 μL of internal standard solution, 50 μL of deconjugation standard, and 50 μL of β-glucuronidase/sulfatase to 100 μL of urine in a conical silanized glass autosampler vial. After being gently mixed, the urine was incubated at 37 °C for 4 h. Upon incubation, 750 μL of 0.1 M formic acid was added. All samples were vortex mixed and centrifuged at 812 g for 15 min before the on-line SPE–HPLC–MS/MS analysis.

We prepared standards, QCs, and reagent blanks daily for each batch using the same procedure as described above for study samples to be analyzed for total concentrations, but replaced the urine with the same volume of mixed standard stock solution, QC materials, and HPLC-grade H_2O (reagent blank). In addition to study samples, each batch includes analytical standards, QCH, QCL and reagent blanks. Calibration curves must have $r^2 > 0.99$. The concentrations of the QC materials are evaluated using standard statistical probability rules [15]. All laboratory operations are conducted under the requirements set forth in the Clinical Laboratory Improvement Act of 1988.

2.5. On-line SPE–HPLC–MS/MS with peak focusing

The on-line SPE–HPLC–MS/MS system was modified from previous methods [16,17], and consisted of several Agilent 1200 modules (Agilent Technologies, Wilmington, DE, USA) and an ABSciex 5500 QTRAP mass spectrometer (Applied Biosystems, Foster City, CA, USA) equipped with an atmospheric pressure chemical ionization (APCI) interface. The SPE column was LiChrospher™ RP-18 ADS (25 × 4 mm, 25 μm particle size, 60 Å pore size; Merck KGaA, Germany) and the HPLC column was Chromolith® High Resolution RP-18e (100 × 4.6 mm, Merck KGaA, Germany). The injection volume was 350 μL.

We designed the on-line SPE–HPLC–MS/MS system to allow for concurrent SPE and HPLC–MS/MS cycles with peak focusing (i.e., diluting the SPE elute before HPLC) as previously described [16]. The on-line SPE procedure details, the negative fragment ions used for quantification and confirmation and the retention times for all analytes are given in the supplemental material (Section 3, Tables S1–S2).

3. Results and discussion

3.1. On-line extraction of urine with restricted access material (RAM)

We used RAM, a type of extraction sorbent used for on-line sample clean up and extraction of biological samples [18]. As a result, the urine pre-treatment was minimal and simply involved the addition of internal standard, deconjugation with β-glucuronidase/sulfatase, and dilution with formic acid, followed by centrifugation. The SPE recoveries of the 14 phenols from urine were calculated as the ratio of response factors calculated from two experiments by adding internal standard before or after SPE (Section 4, supplemental material). The calculated SPE recoveries ranged from 77% to 106% (supplemental material, Table S3) suggesting adequate extraction of the target compounds from the urine matrix.

3.2. Method validation and quality control

Pre-screened blank urine spiked with standard and isotope-labeled internal standard solutions was analyzed repeatedly to determine the limits of detection (LODs). LODs were calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches zero [19]. S_0 was determined from measurements of three low-level standards prepared in urine. The calculated LODs of the three bisphenols were 0.1 ng/mL (BPA), 0.06 ng/mL