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Simultaneous Measurement of Urinary Bisphenol A and Alkylphenols by Automated Solid-Phase Extractive Derivatization Gas Chromatography/ Mass Spectrometry

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Bisphenol A (BPA) and alkylphenols (APs) are widely used industrial chemicals. BPA is used to manufacture polycarbonate plastic and epoxy resins; APs are used to make alkylphenol ethoxylates, common nonionic surfactants. BPA and APs can leach into the environment during industrial production and after degradation of the polycarbonate plastics and nonionic surfactants. Environmental exposure to these phenolic compounds has been associated with adverse reproductive and developmental effects in wildlife. We developed a sensitive and robust method for measuring BPA and six APs; 3-*tert*-butylphenol, 4-*tert*-butylphenol, 4-*n*-octylphenol, 4-*tert*-octylphenol, 4-*n*-nonylphenol, and technical-grade nonylphenol in urine. The method is based on the use of automated solid-phase extraction (SPE) coupled to isotope dilution-gas chromatography/mass spectrometry (GC/MS). During the automated SPE process, the phenols are both extracted from the urine matrix and derivatized, using pentafluorobenzyl bromide, on commercially available styrene−divinylbenzene copolymer-based SPE cartridges. After elution from the SPE column, the derivatized phenols in the SPE eluate are analyzed by GC/MS. The method, validated on spiked pooled urine samples and on urine samples from exposed persons, has limits of detection of ∼0.1 ng in 1 mL of urine.

Bisphenol A [2,2-bis(4-hydroxyphenyl)propane; BPA] and alkylphenols (APs) are industrial chemicals produced worldwide in the millions of tons each year.[1] BPA is used to manufacture polycarbonate plastic and epoxy resins, which are used in baby bottles, as protective coatings on food containers,[2,3] and for composites and sealants in dentistry.[4] APs are used to make alkylphenol ethoxylates (APEs), widely used nonionic surfactants. APEs are applied as emulsifying, wetting, dispersing, or stabilizing agents in numerous industrial, agricultural, and domestic consumer products including detergents and pesticide formulations.[5] BPA and APs can be released into the environment during the manufacturing process and by leaching from the final products.[6−8]

Toxicologic studies on laboratory animals suggest that exposure to BPA and APs is associated with morphologic, functional, and behavioral anomalies related to reproduction. Several APs are estrogenic in vitro and in vivo[9] and interfere with the estrous cycle and pubertal onset in rats.[10,11] APs also show aquatic toxicity at low micrograms per liter concentrations.[5,12] In turn, exposure of rodent fetuses to low BPA doses of 20−400 μg kg^{-1} d^{-1} produces postnatal estrogenic effects, including reduced daily sperm production and increased prostate gland weight in males and alteration in the development and tissue organization of the mammary gland, disruption of sexual differentiation in the brain, long-term deleterious effects in the vagina, and accelerated growth and puberty in females.[13−20] Very low doses (2−20 μg kg^{-1} d^{-1}) of BPA administered perinatally modify sexual behavior in rats.[13]

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Interestingly, in mice treated with BPA at oral doses comparable to environmental exposure levels (20−100 μg kg^{-1} d^{-1}), BPA appears to be a potent disruptor of meiosis, the cell division process that creates sperm or eggs, leading to aneuploidy.[21] This finding may be relevant to the genesis of meiotic aneuploidy in humans. Aneuploidy, an error in cell division, during meiosis is thought to be the most common mechanism causing birth defects in people. In the Unites States, 10−25% of fertilized human eggs are aneuploid. Although most aneuploid embryos undergo spontaneous miscarriage, less severe cases result in birth defects, including Down syndrome.

The mechanism underlying the low-dose toxicologic effects of BPA and APs is not fully understood. In rats, a major percentage of orally administered ^{14}C-BPA is excreted as free ^{14}C-BPA in feces and as ^{14}C-BPA monoglucuronide in urine.[22] Because BPA monoglucuronide is biologically inactive,[23,24] the conversion to the BPA monoglucuronide after oral administration of BPA results in low bioavailability of free BPA,[22] the biologically active form. After injection of BPA in pregnant CD1 mice, both free and conjugated BPA were detected in the placenta, the amniotic fluid, and the fetus, indicating that BPA can be transferred from the mother to the fetus.[25]

Because of the widespread use of BPA and APs, the potential for human exposure is high. The implications of the laboratory toxicologic studies in wildlife and in people is a subject of intense scientific debate.[11,14,26] A major concern is that, although BPA and APs display only weak estrogenic properties, continuous exposure to low levels of these compounds could induce changes in growth, development, reproduction, or behavior.[13,20,27] Furthermore, parallel exposure to BPA and APs and to other hormonally active compounds such as phthalates, polychlorinated biphenyls, and phytoestrogens may induce combined adverse health effects.[13,20,27−29]

BPA and APs have been measured in biological matrixes by using gas chromatography (GC) or high-performance liquid chromatography (HPLC) coupled with different detection techniques.[30−32] To increase sensitivity and selectivity, the phenols have been derivatized to alkyl or acyl derivatives before GC/mass spectrometry (MS) analysis. GC/MS methods offer the highest sensitivity with detection limits of 0.01−0.1 μg/L.[17,33−40] We have developed a sensitive method for measuring BPA and six APs: 4-*n*-nonylphenol (nNP), technical-grade nonylphenol (techNP, a mixture of branched-alkyl chain isomers of 4-nonylphenol), 4-*tert*-octylphenol (tOP), 4-*n*-octylphenol (nOP), 3-*tert*-butylphenol (3tBP), and 4-*tert*-butylphenol (4tBP) in urine. The method uses automated solid-phase extractive derivatization coupled to isotope dilution GC/MS. During the automated solid-phase extraction (SPE) step, the phenols are both extracted from the urine matrix and derivatized using pentafluorobenzylbromide (PFBBr), one of the most selective derivatizing reagents for phenolic OH groups[36] on commercially available SPE cartridges. The derivatized phenols then are separated from other extracted components by GC and quantitatively determined by isotope dilution MS. With the use of isotopically labeled internal standards, the limits of detection (LODs) are ∼0.1 ng in 1 mL of urine.

EXPERIMENTAL SECTION

Preparation of Standards and Quality Control Materials. All organic solvents were HPLC grade. To minimize interferences, all aqueous solutions were filtered through a Bond Elute PPL SPE cartridge (500 mg/6 cm^3; Varian Inc., Walnut Creek, CA); one cartridge was used for every 500 mL. Initial stock solutions of native BPA and APs (∼100 mg/L) were prepared by dissolving measured amounts of BPA, tOP, nOP, 3tBP, and 4tBP (97−99%; Sigma-Aldrich, Milwaukee, WI), nNP (99%, 100 mg/L in nonane; Cambridge Isotope Laboratories Inc., Andover, MA), and techNP (90−93%; Sigma-Aldrich, Milwaukee, WI) in methanol. Serial dilutions of these stock solutions were made in methanol to create eight standard solutions, containing BPA and the APs (except techNP), such that a 100-μL aliquot of each solution in 1 mL of urine resulted in the desired BPA and APs concentrations (i.e., from 0.01 to 100 ng/mL). Because techNP typically contains 7−10% of other APs, techNP standards were prepared separately at concentrations such that 25-μL aliquots of 1 mL of urine provided a calibration range of 1−200 ng/mL. Stock solutions of $^{13}C_{12}$-BPA and $^{13}C_6$-nNP were prepared in methanol by dilution of a 100 mg/L solution (99% in nonane; Cambridge Isotope Laboratories Inc., Andover, MA). Stock solutions of 4tBP-d_{13} and of nOP-d_{17} were prepared in methanol from measured amounts of the solid compounds (CDN Isotopes, Quebec, Canada). The internal standard working solution was prepared by diluting the stock solutions in ethanol, so that a 50-μL aliquot resulted in an approximate amount of 10 ng of each labeled compound in 1 mL of urine. Both the native and the isotope-labeled standard solutions were divided into 1-mL aliquots and stored at −20 °C. $^{13}C_6$-nNP was used as the internal standard for nNP and techNP; 4tBP-d_{13}

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was used for 4tBP and 3tBP; and nOP-d_{17} was used for nOP and tOP. We used β-glucuronidase (*Escherichia coli*, 463 000 U/g solid; Sigma-Aldrich Co., Milwaukee, WI) to deconjugate the glucuronidated BPA and APs. The concentration of the β-glucuronidase enzyme solution was 20 mg/mL in 1 M ammonium acetate pH 5 buffer. A working spiking solution containing 1-naphthyl β-D-glucuronide sodium salt (Sigma-Aldrich, Milwaukee, WI) and naphthol-d_7 (97%; Sigma-Aldrich) was prepared in ethanol (2.5 μg/mL each). After enzymatic deglucuronidation, the naphthol/naphthol-d_7 peak area ratio was monitored to check the extent of the deconjugation reaction.

Quality control (QC) materials were prepared from a base urine pool obtained from multiple anonymous donors. To two parts of the base QC pool, we added acetonitrile (0.3:1 acetonitrile:urine); this was necessary for keeping the highly hydrophobic long-chain APs in the polar urine matrix during freezing and storage. These two pools were enriched with native BPA and APs to create low-concentration (QCL, 2−5 ng/mL) and high-concentration (QCH, 12−20 ng/mL) QC materials. The third part, representing the native concentrations of BPA and APs, was used as the method blank and the standard matrix. The three pools were dispensed in 5-mL portions in prerinsed glass vials and stored at −20 °C. Each QC material was characterized by repeated measurements to define the mean concentrations and the 95th and 99th confidence intervals of BPA and APs.

Sample Preparation. One milliliter of urine, buffered with ammonium acetate (50 μL, 1 M, pH 5.5), was spiked with the labeled internal standards solution (10 ng) and with 10 μL of 1-naphthyl β-D-glucuronide sodium salt/naphthol-d_7 solution; 50 μL of the β-glucuronidase/ammonium acetate buffer was added to the urine, and the urine samples were gently mixed and incubated at 37 °C overnight. Standards and QCs were not incubated. After incubation, 0.3 mL of acetonitrile was added to each deconjugated urine sample. To prepare the calibration standards, 1-mL blank QC aliquots were spiked with native and labeled phenol standards, 0.3 mL acetonitrile, and 50 μL of 1 M ammonium acetate buffer (pH 5). The 1-mL aliquots of QCH and QCL were spiked with labeled internal standards, 10 μL of naphthyl glucuronide/naphthol-d_7 solution, and 50 μL of 1 M ammonium acetate buffer (pH 5).

Solid-Phase Extractive Derivatization. The automated SPE derivatization was performed on a RapidTrace extractor (Zymark Corporation, Hopkinton, MA) using 200 mg/3 cm^3 Bond Elute PPL SPE cartridges. The automated procedure was as follows: The SPE cartridges were conditioned with 1 mL of acetonitrile, 2 mL of ethyl acetate, and 1 mL of acetonitrile at a controlled flow of 1 mL/min. After drying with 12 mL of air, 0.1 mL of a 1:2 diluted solution of the derivatizing agent PFBBr (Sigma-Aldrich) in hexane (Caledon Laboratories Inc., Georgetown, ON, Canada) was applied to the SPE column at 0.5 mL/min; next, 12 mL of air was pushed through the cartridge at 2 mL/min. The buffered deconjugated urine mixture was diluted with 1 mL of 0.2 M NaOH/0.05 M tetrabutylammonium hydrogensulfate (97%, Sigma-Aldrich Co.) and loaded onto the cartridge at 0.38 mL/min. Next, the SPE cartridge was dried with 6 mL of air, washed with 1 mL of 20% acetonitrile/80% water, washed with 1 mL of 50% acetonitrile/50% water, and then dried under a N_2 flow. The derivatized analytes were eluted from the SPE cartridge with 1 mL of acetonitrile and 2 mL of ethyl acetate. The SPE extracts were evaporated to dryness under a stream of dry nitrogen in a Turbovap evaporator (Zymark Corp.) at 55 °C, and reconstituted in 200 μL of isooctane (HPLC grade; Burdick & Jackson, Muskegon, MI). The RapidTrace extractor modules were placed in a fumehood since PFBBr is a strong lachrymator.

Table 1. Retention Time and Negative Ion Fragment Masses (*m*/*z*) of Derivatized Analytes and Their Internal Standards[a]

analyte/labeled isotope internal standard	retention time (min)	fragment ion *m*/*z*
3-*tert*-butylphenol/4-*tert*-butylphenol-d_{13}	11.4/11.8	149/162
4-*tert*-butylphenol/4-*tert*-butylphenol-d_{13}	11.9/11.8	149/162
naphthol/naphthol-d_{17}	15.4/15.3	143/150
4-*tert*-octylphenol/4-*n*-octylphenol-d_{17}	14.4/14.8	205/222
4-*n*-octylphenol/4-*n*-octylphenol-d_{17}	14.9/14.8	205/222
tech-grade nonylphenol, peak 1[a]	17.05/18.3	219/225
4-*n*-nonylphenol/4-*n*-nonylphenol-$^{13}C_6$	18.3/18.3	219/225
bisphenol A/bisphenol A-$^{13}C_{12}$	23.4/23.4	407/419

[a] 4-*n*-nonylphenol-$^{13}C_6$ used as internal standard.

GC/MS Analysis. The GC 6890 (Agilent, Wilmington, DE), equipped with a 7683 autosampler (Agilent) and a programmable temperature vaporizer inlet (Gerstel Inc., Baltimore, MD), was interfaced to a single quadrupole mass selective detector (series 5973; Agilent). The chromatographic separation was achieved on a DB-5MS column (J&W Scientific, Folsom, CA; 30 m × 0.25 mm, 0.25 μm film thickness) using helium as the carrier gas (1.3 mL/min, constant pressure). Ten microliters of the extract was injected in solvent vent mode (inlet temperature gradient program: 60 °C for 0.3 min, then ramped at 500 °C/min to 300 °C; vent time: 0.25 min; vent flow: 50 mL/min). The following oven temperature program was used: initial temperature 50 °C, held for 1.0 min, ramped at 18 °C/min to 200 °C and held for 6 min, ramped at 60 °C/min to 300 °C and held until total run time of 24 min. The MS was operated in negative chemical ionization mode with methane as the reagent gas and with a 10-min solvent delay. The GC/MS transfer line was held at 280 °C, and the MS quadrupole and source heaters were maintained at 150 and 230 °C, respectively. Mass spectra were acquired in selected ion monitoring (SIM) mode with 50 ms dwell time. During chemical ionization, the derivatized phenols lost a pentafluorobenzyl group. The negative fragment ions used for quantification and the GC signals retention times are given in Table 1.

RESULTS AND DISCUSSION

Alkylation of phenols using PFBBr is traditionally a liquid−liquid extractive (LLE) derivatization reaction in which the phenolic anions are generated in a strongly basic aqueous medium and then extracted by an organic solvent, typically dichloromethane, where the reaction with PFBBr occurs. However, PFBBr is not selective enough in the presence of biological matrixes such as urine or serum. Therefore, phenols are normally extracted from urine or serum first and then derivatized with PFBBr.[33,41] Although this is a valid approach, the derivatization reaction is time-consuming and difficult to automate.

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Table 2. Solid-Phase Extractive (SPE) Derivatization Recoveries from Urine, Accuracy of Spiked Recoveries, Limits of Detection (LODs), and Daily Variation of Calibration Curve Slopes and Intercepts[a]

analyte	SPE/LLE[b] recovery	spiked recovery (%) at 1/5/50 ng/mL[c]			LOD	slope CV %	intercept CV %
3-*tert*-butylphenol	0.02	107	115	99	0.2	22	30
4-*tert*-butylphenol	0.11	102	113	91	0.4	11	21
4-*tert*-octylphenol	0.40	94	109	95	0.7	15	34
4-*n*-octylphenol	1.77	105	103	100	0.1	15	28
tech-grade nonylphenol		91	109	106	5[d]	10	28
4-*n*-nonylphenol	1.20	105	99	101	0.1	15	45
bisphenol A	1.18	104	116	95	0.1	6	10

[a] Expressed as the average coefficient of variation [CV %]). LOD reported in ng/mL. [b] LLE, liquid−liquid extractive derivatization from water. [c] Spiked recovery at 5, 50, and 100 ng/mL total techNP concentrations. [d] Reported for total tech-grade nonylphenol.

An alternative approach to LLE derivatization is SPE derivatization, used in the past for cannabinoids, in which PFBBr is immobilized on the surface of styrene−divinylbenzene beads.[40,42−47] The beads "coated" with the derivatizing reagent are added to the liquid medium (e.g., urine or serum), and the analytes are derivatized and adsorbed on the surface of the beads. Next, the liquid phase is removed by aspiration, and the derivatized analytes are extracted from the beads using an organic solvent. This method is simpler, is more selective, and has better potential for automation than the traditional LLE derivatization. However, one disadvantage is the relatively large amount (∼30 mL) of organic solvent (e.g., diethyl ether) needed to extract the derivatized analytes from the beads. We modified the SPE derivatization approach described above and developed a method with high throughput, reduced solvent waste, and suitable for automation. We used commercially available SPE cartridges instead of coated beads. The derivatized phenols were eluted from the SPE columns with a small volume (3 mL) of organic solvent. Furthermore, our SPE derivatization process was fully automated using a commercially available extractor system.

One critical aspect of our automated SPE derivatization method was flow control, especially while we were applying the derivatizing reagent onto the SPE column and loading the urine sample. We chose the Zymark RapidTrace extractor because of its unique capability of delivering precise programmable flows (from 0.38 to 30 mL/min). The urine sample was loaded at a very slow flow rate (0.38 mL/min) to allow enough time for completion of the derivatization. At this flow rate, the total reaction time per sample was 15 min. Automation of the method on the RapidTrace extractor was convenient for achieving high throughput. However, the SPE derivatization reaction also can be conducted on a manual SPE vacuum manifold as long as the sample is led through the cartridge by gravity (i.e., at a very slow flow rate).

The derivatization reaction required 30 μL of PFBBr per sample. However, the minimum volume that can be handled using the Zymark RapidTrace is 100 μL. Therefore, we diluted the PFBBr reagent with hexane in a 1:2 ratio. To effectively spread the 100 μL of PFBBr/hexane reagent on the sorbent, the PFBBr/hexane solution was applied at a slow flow rate (0.5 mL/min). To allow for evaporation of the hexane, thus leaving only PFBBr on the SPE sorbent, we applied 12 mL of air through the cartridge at a flow rate of 2 mL/min.

We tested two types of commercially available styrene−divinylbenzene copolymer SPE cartridges, PPL and ENV (Varian Inc., Walnut Creek, CA). We chose PPL, a functionalized polymer, because it was compatible with the ethyl acetate used for elution of the derivatized phenols. In contrast, the ethyl acetate seemed to degrade the ENV polymer.

Addition of acetonitrile to the urine samples (1 mL) after incubation was advantageous for reproducible recovery of the normal long alkyl chain APs, nOP and nNP, mainly because of their low solubility in water and hydrophobic nature. Without adding at least 0.3−0.5 mL of acetonitrile, the normal long alkyl chain APs seemed to partition out of the aqueous solution while waiting on the extractor. Similarly, the nonglucuronidated APs spiked into the urine QC pools tended to partition out of solution during storage at −20 °C. The addition of acetonitrile at the time the QC pools were prepared assisted in the solubilization of the APs during storage.

We assessed the SPE recoveries of the analytes by comparing the analyte peak areas from blank urine using our SPE derivatization method to the analyte peak areas from water using a LLE derivatization method.[33] The recoveries for BPA, nOP, and nNP using SPE derivatization were higher than with LLE (Table 2). In contrast, the recoveries for 3tBP, 4tBP, and tOP were lower with the SPE derivatization than with LLE. The recoveries for the short alkyl chain butyl phenols were lower probably because of the addition of acetonitrile to the urine. Although acetonitrile increased the aqueous solubility of the free long alkyl chain phenols, it suppressed adsorption of the *tert*-butylphenols and the branched alkyl chain tOP on the sorbent surface. The recoveries for all the analytes with the SPE derivatization method also may have been somewhat diminished by the presence of the ammonium acetate buffer; however, the buffer was necessary for pH control during the deconjugation of the phenol glucuronides.

Because urine samples vary in both their composition and ion strength, we expected significant variability in the recovery during the SPE procedure and in the extent of ionization of the analytes. To minimize this variability, we used isotopically labeled internal standards. As expected, the accuracy and precision of the method

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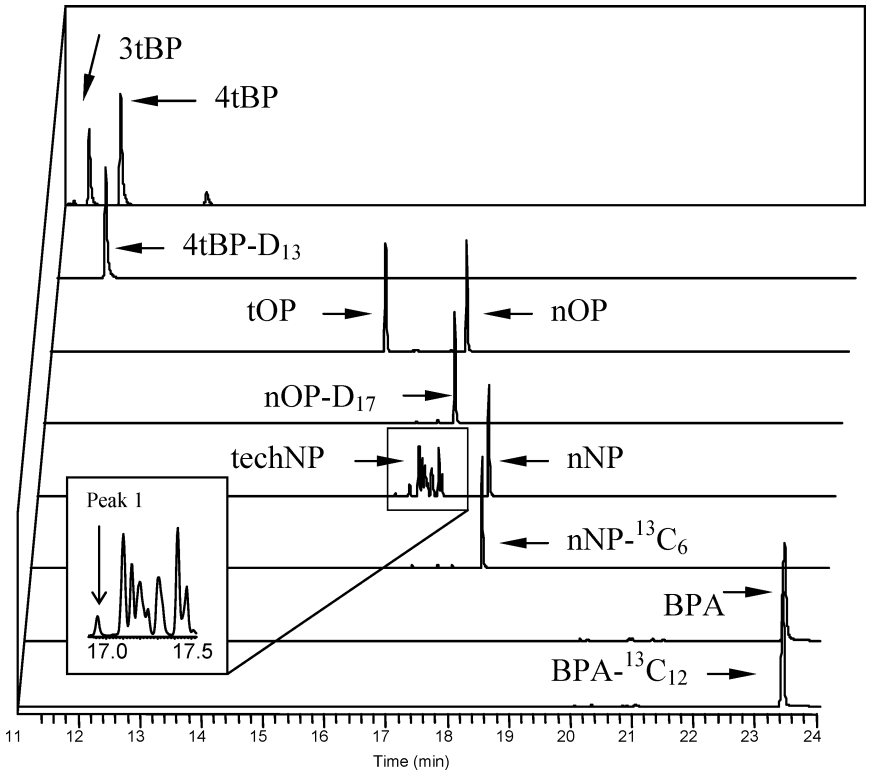

Figure 1. Typical chromatogram of a urine sample spiked with 10 ng/mL bisphenol A (BPA)/alkylphenols and 50 ng/mL techNP standards.

was best for the analytes for which there was a chemically identical isotopically labeled internal standard (i.e., BPA, 4tBP, nOP, nNP). For these analytes, the calibration curves (with $1/x$ weighting) showed excellent linearity with linear correlation coefficients ~0.99. For 3tBP, tOP, and techNP, the correlation coefficients also were good but slightly lower (0.93−0.98). The interday variability of the calibration curve slopes was excellent for all analytes (Table 2).

Spiked urine was analyzed repeatedly to determine the method recovery, reproducibility, and LOD. The LOD was calculated as $3S_0$, where S_0 is the standard deviation as the concentration approaches 0. S_0 was determined from the replicate analysis of low-level standards.[48] The calculated method LODs were ~0.1 ng/mL for BPA, nNP, and nOP. For tOP, 4tBP, and 3tBP, the LODs were higher (0.2−0.7 ng/mL (Table 2). We established the accuracy by determining the recovery of spiked urine samples. To examine the consistency of the recovery over a range of analyte levels, the measurements were taken by quintuplicate at three different concentrations. The mean recoveries in urine, expressed as a percentage of the expected value, were very good with values ranging from 91% to 116% (Table 2).

The precision of the SPE derivatization method was determined by calculating the average coefficient of variation (CV) of 19 repeated measurements of the QC materials over 1 month (Table 3). The average CVs for BPA, nOP, and nNP were 4−13%. For 3-tBP, 4-tBP, and tOP, CVs were higher (17−33%), probably because of the lack of chemically identical isotopically labeled internal standards (tOP) or the comparatively lower recovery and signal-to-noise ratio (3tBP and 4tBP). The precision of the overall method was assessed from the CV of 30 measurements over 2 weeks of the enzymatic deconjugation and derivatization of the 1-naphthyl β-D-glucuronide, measured by the naphthol/naphthol-d_7 peak area ratio. The CV was 7%, which indicates the excellent reproducibility of the method.

Table 3. Precision of Concentration Measurements in Spiked Quality Control (QC) Pools[a]

	QC high		QC low	
analyte	mean	CV %	mean	CV %
3-*tert*-butylphenol	13.1	17	2.2	21
4-*tert*-butylphenol	13.4	19	2.3	30
4-*tert*-octylphenol	42.3	33	14.1	16
4-*n*-octylphenol	22.8	9	4.7	13
4-*n*-nonylphenol	15.1	4	3.5	5
bisphenol A	13.8	6	3.7	7

[a] Mean concentrations in ng/mL. CV % is the average coefficient of variation.

We believe that human exposure to nonylphenol would be best addressed by measuring both nNP and techNP. TechNP, a mixture of branched alkyl chain isomers of *p*-nonylphenol, can be resolved into 18 isomers on a high-resolution GC column.[49] The signal with the shortest retention time, peak 1, was postulated to be a 1-methyl-1-isopropyl branched isomer based on its electron ionization MS and FT-IR spectra.[49] The area for peak 1 accounts for ~4% of the total area of all techNP GC signals.[49] We used peak 1 to estimate the amount of techNP in standards, QCs, and unknown samples (Figure 1). The techNP calibration curves using peak 1 area divided by $^{13}C_6$-nNP area versus the total techNP concentration showed good linearity (correlation coefficient 0.94)

(48) Taylor, J. K. *Quality Assurance of Chemical Measurements*; Lewis Publishers: Chelsea, MI, 1987.
(49) Wheeler, T. F.; Heim, J. R.; LaTorre, M. R.; Janes, A. B. *J. Chromatogr. Sci.* **1997**, *35*, 19−30.

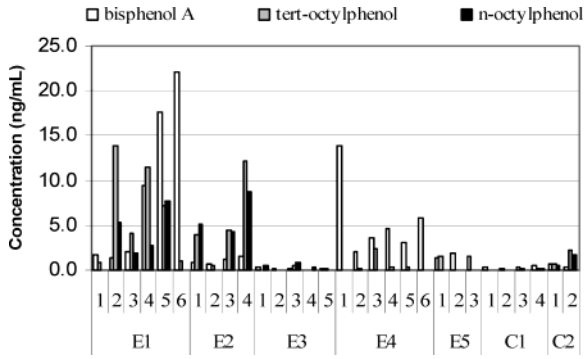

Figure 2. Concentrations (in ng/mL) of bisphenol A and alkylphenols in 30 urine samples collected from people painting their houses. Specimens from exposed persons (E1−E5) and controls (C1 and C2) are numbered in order of collection.

and precision (Table 2). However, the interday precision of the concentration measurements in the QC and unknown samples was poor probably because of the structural chemical differences between the highly branched alkyl chain techNP peak 1 isomer and the normal alkyl chain $^{13}C_6$-nNP used as the internal standard. These data suggest that application of our SPE derivatization method for measuring the concentrations of the *p*-nonylphenol isomers present in the techNP mixtures would require using an isotopically labeled 1-methyl-1-isopropyl branched *p*-nonylphenol isomer, which is not available. Until then, the current method can be used to provide qualitative concentration data for techNP.

To test the usefulness of our method, we analyzed 30 urine samples collected from a study of people who used paint products while painting their homes (Figure 2). BPA, tOP, and nOP were commonly detected. The frequency of detection and (range of concentrations) were 96% (0.4−22.1 ng/mL), 35% (0.4−13.9 ng/mL), and 43% (0.13 and 8.7 ng/mL) for BPA, tOP, and nOP, respectively. Only 13% of the samples had 4tBP, with the highest value being 6.7 ng/mL. 3tBP and nNP were not detected in any of the samples analyzed. These findings confirm the high sensitivity of our analytical method and its value for future studies assessing environmental exposure to BPA and APs in the general human population.

In summary, we have developed an analytical method based on an automated SPE derivatization coupled with GC/MS for the quantitative determination of urinary BPA and APs. The method is sensitive and accurate, uses a small amount of urine (1 mL), is not labor intensive, involves minimal manual sample preparation, and uses an automated SPE system commercially available. This method is suitable for large epidemiologic studies to assess the relevance of human exposure to BPA and APs.

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