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# Quantification of the urinary concentrations of parabens in humans by on-line solid phase extraction-high performance liquid chromatography–isotope dilution tandem mass spectrometry

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**Abstract**

Parabens (alkyl esters of *p*-hydroxybenzoic acid) are widely used as antimicrobial preservatives in cosmetic products, pharmaceuticals, and food processing. However, weak estrogenicity of some parabens has been revealed from several studies. Human exposure to parabens may be assessed by measuring the conjugated or free species of these compounds or their metabolites in urine. We have developed a method using on-line solid phase extraction-high performance liquid chromatography–isotope dilution tandem mass spectrometry with peak focusing to measure the urinary concentrations of methyl, ethyl, propyl, *n*- and *iso*- butyl, and benzyl parabens. This method has good reproducibility and accuracy with detection limits for all analytes below 0.2 ng/mL in 100 μL of urine, and permits quick and accurate analysis of a large number of samples in epidemiologic studies for assessing the prevalence of human exposure to parabens. Using this method, we detected methyl, ethyl, and propyl parabens, mostly as conjugated species, in 22 urine samples collected from anonymous adults.
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*Keywords:* Paraben; Exposure; Human; Biomonitoring; Urine; Biomarker

## 1. Introduction

Parabens are a group of alkyl (e.g., methyl, ethyl, propyl, butyl) esters of *p*-hydroxybenzoic acid widely used as antimicrobial preservatives, especially against molds and yeast, in cosmetic products and pharmaceuticals, and in food and beverage processing. Parabens have been used in cosmetics for over half century [1]. Individually or in combination, parabens are used in over 13,200 formulations [2] in nearly all types of cosmetics. Used in pharmaceuticals since the mid-1920s, parabens are also present in a wide variety of drug formulations, usually at concentrations no greater than 1% [3]. The antimicrobial activity of parabens increases, but water solubility decreases with the length of the alkyl chain [2]. For this reason, methyl and propyl parabens are the most extensively used in cosmetics and food processing [4].

The widespread use of parabens arises from their low toxicity, broad inertness, worldwide regulatory acceptance, and low cost [5]. However, parabens may have estrogenic activity, the extent of which is dependent upon their structure [6]; however, this is many orders of magnitude lower than that of estradiol [6,7]. In vitro studies suggest that parabens exhibit weakly estrogen activities in yeast-based assays [7–10], induce the growth of MCF-7 human breast cancer cells, and influence the expression of estrogen-dependent genes [11–14]. Similarly, in vivo studies suggest increased uterine weight in the immature mice after exposure to butyl, isobutyl, and benzyl parabens [12]. Male rodents also exhibited decreased excretion of testosterone and some reproductive tract alterations after exposure to butyl and propyl parabens [15–17], but not to methyl and ethyl parabens [18]. Ethyl, propyl, and butyl parabens evoked estrogenic responses in vivo in sexually immature rainbow trout. However, the estrogenic potency of ethyl paraben was weaker than those of propyl and butyl paraben [19].

Parabens have been found in human breast tumors [20], but the toxic effects of parabens in humans are mostly unknown.

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In particular, butyl paraben was nominated by the National Institute of Environmental Health Sciences for toxicological characterization, including reproductive toxicity studies [21]. The estrogenic activity of parabens in animals and the presence of these compounds in human breast tissue have raised some concerns about their safety. Moreover, the use of parabens in our daily life is extensive. Human exposure to parabens is estimated at 1.3 mg/kg day or 76 mg/day (cosmetics and personal products, 50 mg/day; drugs, 25 mg/day; food, 1 mg/day) [22]. The widespread use of parabens, and their potential risk to human health have prompted interest in assessing human exposure to these compounds.

Animal studies show that after being absorbed, parabens are mainly hydrolyzed to *p*-hydroxybenzoic acid, which can then excreted in the urine as glycine, glucuronide and sulfate conjugates [5,23,24]. The unchanged parabens can also be excreted in various forms [5,23], and differences in metabolic profiles have been found depending upon the exposure route [5]. Following oral administration of propyl paraben in humans (2 g daily for 5 days), only 17.4% of the administered dose was recovered as *p*-hydroxybenzoic acid and its glycine conjugate [25]. Furthermore, measuring *p*-hydroxybenzoic acid and its conjugates in urine may not be the best approach for assessing human exposure to parabens because *p*-hydroxybenzoic acid measurements are not specific, and different parabens can possess quite different estrogenic bioactivities. Therefore, measuring the unchanged precursor parabens may be a viable alternative.

Several analytical techniques have been reported for the quantification of parabens in different matrices. Specifically, parabens have been measured by solid-phase microextraction-ion mobility spectrometry in pharmaceutical formulations [26], by flow injection-chemiluminescence in foods [27], by microemulsion electrokinetic chromatography in a pharmaceutical formulation [28], by gas chromatography–mass spectrometry (GC–MS) in virgin and recycled paper [10], by high performance liquid chromatography (HPLC) in a pharmaceutical preparation [29] and in cosmetic products and foods [30], and by HPLC–tandem mass spectrometry (HPLC–MS/MS) in human breast tumors [20]. We developed a novel and highly sensitive method to measure free and conjugated species of methyl, ethyl, propyl, *n*- and *iso*-butyl, and benzyl parabens in human urine using a unique on-line solid phase extraction (SPE)-HPLC–isotope dilution MS/MS system with peak focusing. Compared to most of the previous methods, ours provides higher sensitivity, and similar accuracy and precision, without complicated and time-consuming sample preparation. Furthermore, our method provides high throughput because it permits concurrent SPE and HPLC–MS/MS. We applied the method for measuring these five parabens in 22 urine samples collected from a convenience group of demographically diverse anonymous adult volunteers. To our knowledge, this is the first study to report the concentrations of individual parabens in human urine. Our method could be used for quick, accurate, and cost-effective analyses of large numbers of samples in epidemiologic studies to assess the prevalence of human exposure to parabens.

## 2. Experimental

### 2.1. Analytic standards and reagents

Analytical or HPLC-grade methanol and water were obtained from Tedia (Fairfield, OH). Formic acid (98%) was purchased from EM Science (Gibbstown, NJ). Methyl, ethyl, propyl, *n*- and *iso*-butyl, and benzyl paraben, 4-methylumbelliferyl glucuronide, 4-methylumbelliferyl sulfate, and β-glucuronidase/sulfatase (*Helix pomatia*, H1) were purchased from Sigma–Aldrich Laboratories, Inc. (St. Louis, MO). We obtained $^{13}C_4$-4-methylumbelliferone from Cambridge Isotope Laboratories, Inc. (Andover, MA), $D_4$-methyl paraben from CDN Isotopes (Quebec, Canada), and $D_4$-ethyl, $D_4$-propyl, and $D_4$-*n*-butyl parabens from CanSyn Chem Corp. (Toronto, Canada).

### 2.2. Preparation of standards and quality control materials

We prepared initial stock solutions by dissolving measured amounts of the analytes of interest in methanol. Nine working standard spiking solutions containing all five compounds were generated by serial dilution of the initial stock solutions with methanol. These standards covered concentration ranges of 0.1–100 ng/mL. The isotope-labeled standard stock solutions were also prepared in methanol. The internal standard working solution, containing $D_4$-methyl, $D_4$-ethyl, $D_4$-propyl, and $D_4$-*n*-butyl parabens, was prepared by diluting the stock solutions in MeOH, so that a 50-μL aliquot in 100 μL urine resulted in a concentration of 50 ng/mL. Therefore, the response factors (peak area ratios of native to labeled compounds) for all analytes ranged from 0.02 to 2.0. All standard stock solutions and spiking solutions were dispensed into vials and stored at −20 °C until use. Quality control (QC) materials were prepared with pooled urine from multiple anonymous donors. The urine pool was divided into two subpools that were enriched with native target compounds to create QC low (QCL) and QC high (QCH) concentration pools. These pools were mixed thoroughly after preparation, dispensed in aliquots of 1.5 mL in glass autosampler vials, and stored at −20 °C until use. The QC pools were characterized to define the mean and the 95% and 99% control limits of parabens concentrations using a minimum of 40 repeated measurements during a 2-week period.

4-Methylumbelliferyl sulfate, 4-methylumbelliferyl glucuronide, and $^{13}C_4$-4-methylumbelliferone were added to all samples and used as deconjugation standards to quantify the extent of the enzymatic reaction. After incubation, 4-methylumbelliferyl sulfate and 4-methylumbelliferyl glucuronide were deconjugated to free 4-methylumbelliferone, and the 4-methylumbelliferone/$^{13}C_4$-4-methylumbelliferone peak area ratio was monitored to check the extent of the deconjugation. The deconjugation standard solution was prepared by dissolving 240 μg of 4-methylumbelliferyl glucuronide, 200 μg of 4-methylumbelliferyl sulfate, and 200 μg of $^{13}C_4$ 4-methylumbelliferyl in 100 mL of methanol. The enzyme solution was prepared by dissolving 0.02 g of β-glucuronidase/sulfatase (*H. pomatia*, 463,000 U/g solid) in 10 mL of 1 M ammonium acetate buffer solution (pH 5.0).

### 2.3. Sample preparation

Urine samples were thawed and vortexed before aliquoting. A 100 μL aliquot of urine was mixed with 20 μL of internal standard solution in a 1.5-mL conical bottom autosampler vial. The rest of the sample preparation steps were performed automatically by a ThermoFinnigan Surveyor Plus liquid chromatograph autosampler (Thermo Electron Corp., San Jose, CA) [31]. The Surveyor autosampler, operated with the Xcalibur software (ThermoFinnigan) was programmed to spike the urine samples with solutions of 4-methylumbelliferyl glucuronide/4-methylumbelliferyl sulfate standard (10 μL) and β-glucuronidase/sulfatase (*H. Pomatia*) solution (50 μL), and mix the sample with the spiked solutions using the syringe autosampler. The sample tray temperature was set at 37 °C for the duration of the spike and mixing steps. The spiked urine samples were incubated at 37 °C for 4 h. Then the deconjugation reaction was stopped by adding 820 μL of 0.1 M formic acid and the contents of each vial were mixed using the syringe autosampler. Next, the sample tray temperature was lowered to 0 °C and the samples were kept at this temperature until analysis. For analysis, the samples were taken out of the autosampler used for sample preparation, vortex mixed, and centrifuged at 2500 rpm for 10 min before being placed on the HPLC autosampler for on-line SPE-HPLC–MS/MS analysis. To determine the concentrations of the free species, we followed the procedures described above, but used 50 μL of 1 M ammonium acetate buffer instead of the enzyme solution, and skipped the incubation step.

We prepared analytical standards, QCs, and blanks using a procedure analogous to the one used for the unknown samples, but replaced the urine by the same volume of standard stock solution, QC urine or HPLC-grade water (for blanks).

### 2.4. On-line SPE-HPLC–MS/MS

The on-line SPE-HPLC–MS/MS system consisted of several Agilent 1100 modules (Agilent Technologies, Wilmington, DE), namely two binary pumps with degassers, an autosampler with a 900-μL injection loop, a high-pressure mixing tee, and one column compartment with a 10-port switching valve. The API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) was equipped with an atmospheric pressure chemical ionization (APCI) interface. The system, previously described [32], was designed to allow for concurrent SPE-HPLC operation with peak focusing (i.e., diluting the SPE eluate before HPLC). The SPE column was a LiChrosphere RP-18 ADS (25 mm × 4 mm, 25-μm particle size, 60-Å pore size, Merck KGaA, Germany). Two Chromolith™ Performance RP-18 (100 mm × 4.6 mm, Merck KGaA, Germany) HPLC columns in tandem and a slow HPLC gradient program had to be used in order to separate two pairs of isomers (2,4- and 2,5-dichlorophenol, and 2,4,5- and 2,4,6-trichlorophenol), which were also included in the same method. The mass spectrometer and Agilent modules were programmed and controlled using the Analyst 1.4 software (Applied Biosystems).

The procedure for extracting the parabens from the urine involved concurrent SPE and HPLC–MS/MS cycles as previously described [32]. While the autosampler and pump 1 were used for the SPE cleanup of one sample, the 10-port switching valve, pump 2, and mass spectrometer were used to collect data from the previous sample (Table 1). The HPLC–MS/MS acquisition method was built in "no sync" mode (i.e., all devices

Table 2
Analyte retention time, and precursor ion → product ion transitions monitored for quantification (and confirmation) of native compounds and corresponding isotope-labeled internal standards

| Analyte | RT (min) | Precursor ion → product ion (*m/z*) | |
|---|---|---|---|
| | | Native analyte | Internal standard |
| Methyl paraben | 9.02 | 151 → 92 (136) | 155 → 96 |
| Ethyl paraben | 10.99 | 165 → 92 (137) | 169 → 96 |
| Propyl paraben | 13.64 | 179 → 92 (136) | 183 → 96 |
| *n*-Butyl paraben | 15.99 | 193 → 92 (136) | 197 → 96 |
| Benzyl paraben | 15.96 | 227 → 92 (136) | 197 → 96[a] |

RT: retention time.
[a] $D_4$-*n*-Butyl paraben was used as the internal standard for benzyl paraben.

Table 1
Concurrent on-line SPE and HPLC solvent gradient programs, and time schedules of the autosampler and 10-port switching valves

| Time (min) | Pump 1 | | | Autosampler valve position | Time (min) | Pump 2 | | | 10-port valve position |
|---|---|---|---|---|---|---|---|---|---|
| | A (%) | B (%) | Flow (μL/min) | | | A (%) | B (%) | Flow (μL/min) | |
| 0 | 80 | 20 | 250 | HPLC–MS | 0 | 50 | 50 | 500 | SPE-HPLC–MS |
| | | | | | 2 | 50 | 50 | 500 | SPE-HPLC–MS |
| | | | | | 2.1 | 50 | 50 | 750 | HPLC–MS |
| 2.5 | 80 | 20 | 250 | HPLC–MS | | | | | |
| 2.6 | 0 | 100 | 1000 | SPE/waste | | | | | |
| 5.0 | 0 | 100 | 1000 | SPE/waste | | | | | |
| 5.1 | 80 | 20 | 1000 | SPE/waste | | | | | |
| | | | | | 10 | 35 | 65 | 750 | HPLC–MS |
| | | | | | 17 | 0 | 100 | 750 | HPLC–MS |
| 19 | 80 | 20 | 1000 | SPE/waste | 19 | 0 | 100 | 750 | HPLC–MS |
| 19.1 | 80 | 20 | 0 | Waste | 19.1 | 50 | 50 | 750 | HPLC–MS |
| 21 | 80 | 20 | 0 | Waste | 21 | 50 | 50 | 750 | HPLC–MS |

Mobile phase A is HPLC grade water, and mobile phase B is methanol.

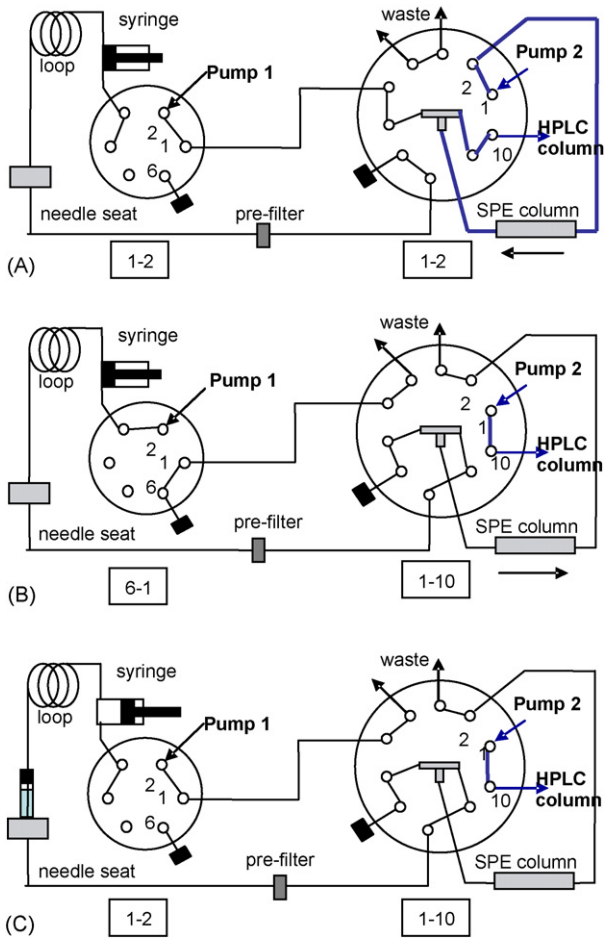


Fig. 1. Typical tubing configuration setup for the autosampler valve (6-port) and the 10-port valve for three selected periods: (A) analyte transfer and dilution, (B) SPE column regeneration and sample wash, and (C) sample loading.

were programmed to start at the same time). The solvent gradient programs of pumps 1 and 2, and the time schedules of the autosampler valve and 10-port switching valve are listed in Table 1. The analytes from the previously injected sample that had been retained by the SPE column were eluted by pump 2. The SPE eluate was diluted through a mixing tee with 20% methanol:80% water provided by pump 1, and the analytes were transferred to the HPLC column (Fig. 1A). At 2 min, the collection of the HPLC–MS/MS data began, while the SPE column was regenerated and equilibrated (Fig. 1B). At 8 min, the autosampler started to inject the next sample. The injection (800 μL of sample containing 100 μL urine) was programmed as two sequential "400 μL sample draw" and "400 μL eject into the needle seat" commands in Analyst 1.4. Tube connections inside the autosampler were modified in-house to connect the needle seat directly to the SPE column. In this way, the execution of the "eject into the needle seat" command resulted in loading the sample directly onto the SPE column by the autosampler syringe (Fig. 1C). After the sample loading was complete, the SPE column was washed while unbound urine components were carried to waste by pump 1 (Fig. 1B). The collection of HPLC–MS/MS data lasted 20 min, after which the HPLC pump was equilibrated for the next elution cycle while the flow through the SPE column was brought to a complete stop.

### 2.5. Mass spectrometry

The API 4000 mass spectrometer was used in negative ion APCI mode. The APCI settings were curtain gas ($N_2$) flow: 20 arbitrary units (au), collision gas flow: 9 au, nebulizer gas (air) flow: 50 au, nebulizing gas temperature: 500 °C, and corona needle voltage: −3 V. Unit resolution was used for both Q1 and Q3 quadrupoles. Ionization parameters and collision cell parameters were optimized separately for each analyte. The negative fragment ions used for quantification and the retention time for the analytes are listed in Table 2.

## 3. Results and discussion

### 3.1. Method performance and quality control

The limit of detection (LOD), limit of quantification (LOQ), and accuracy of the method were determined in synthetic urine since we could not obtain urine that did not contain the five parabens. The LOD and LOQ were calculated as $3S_0$ and $10S_0$, where $S_0$ is the standard deviation as the concentration approaches zero [33]. $S_0$ was determined from five repeated measurements of low-level standards prepared in synthetic urine [34]. The calculated LODs ranged from 0.1 ng/mL to 0.18 ng/mL (Table 3). Estimated LOQs were in the range of 0.3 ng/mL to 0.6 ng/mL. These values reflect the very good sensitivity of the method, especially considering the relatively low sample volume (100 μL) used. Typical chromatograms for a reagent blank and the lowest concentration standard are shown in Fig. 2. Method accuracy was assessed by five replicate analyses of synthetic urine spiked at four different concentrations and was expressed as the percentage of expected levels (Table 3). The intra-day

Table 3
Solid-phase extraction recoveries, spiked standard concentration recoveries, and limits of detection

| Analyte | SPE recovery (%) | (Standard concentration, ng/mL) spiked recovery (%) | | | | LOD (ng/mL) |
|---|---|---|---|---|---|---|
| Methyl paraben | 80 | (1) 98 | (5) 101 | (25) 98 | (50) 97 | 0.13 |
| Ethyl paraben | 84 | (1) 99 | (5) 106 | (25) 103 | (50) 98 | 0.10 |
| Propyl paraben | 88 | (1) 106 | (5) 109 | (25) 102 | (50) 100 | 0.18 |
| *n*-Butyl paraben | 96 | (1) 97 | (5) 105 | (25) 103 | (50) 99 | 0.10 |
| Benzyl paraben | 100 | (1) 105 | (5) 108 | (25) 103 | (50) 102 | 0.10 |

SPE: solid phase extraction; LOD: limit of detection.

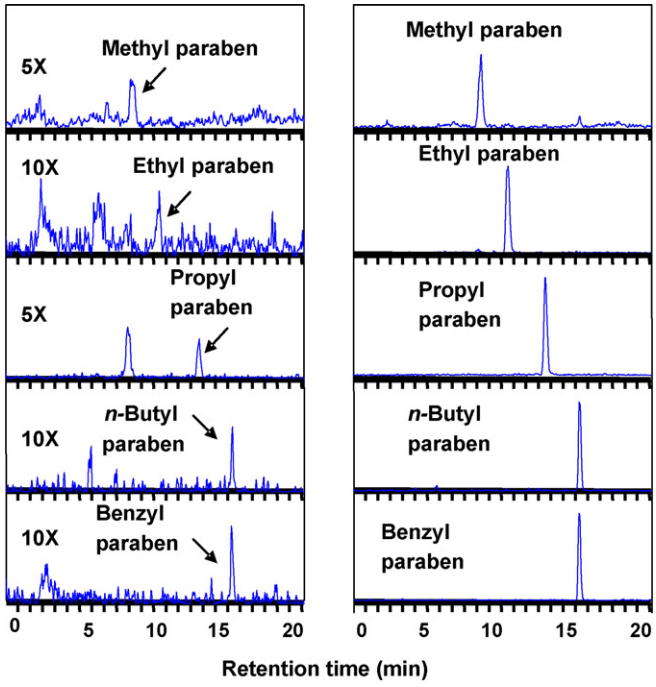


Fig. 2. Typical HPLC–MS/MS extracted ion chromatograms for a low concentration (1 ng/mL in urine) calibration standard (right) and a reagent blank (left, *y*-axis scale magnified 5× or 10×; calculated concentrations were <LOD for all parabens).

variability, reflected in method accuracy, ranged from 90% to 110% for all five analytes at the four spike levels (Table 3). We determined method precision in urine from 40 repeated measurements of QCL and QCH materials over a period of 2 weeks (Table 4). The RSDs, which reflect the intra- and inter-day variability of the method, ranged from 5.3% to 10.6% and demonstrate good precision for all of the analytes, including benzyl paraben for which a labeled internal standard was not available. We used $D_4$-butyl paraben as the internal standard for benzyl paraben.

Calibration curves were obtained from the standards spiked in water and synthetic urine. Because slopes of the calibration curves from water and from synthetic urine were very similar (data not shown), only the calibration curve obtained from water was used for quantification. Calibration curves in water showed adequate linearity (correlation coefficients greater than 0.99).

Table 4
Inter- and intra-day precision of concentration measurements ($N = 40$) in spiked quality control (QC) urine samples

| Analyte | QC low | | QC high | |
|---|---|---|---|---|
| | Mean (ng/mL) | RSD% | Mean (ng/mL) | RSD% |
| Methyl paraben | 8.9 | 8.3 | 60.8 | 6.8 |
| Ethyl paraben | 6.0 | 5.3 | 22.6 | 5.1 |
| Propyl paraben | 6.4 | 5.5 | 28.5 | 6.2 |
| *n*-Butyl paraben | 2.2 | 8.8 | 11.2 | 7.1 |
| Benzyl paraben | 1.8 | 9.1 | 9.0 | 10.6 |

Inter-day variation of calibration curve slopes, measured as the RSD, was less than 10%.

The SPE recoveries of compounds from urine were calculated on the basis of the following experiment: First, 100 μL of urine mixed with a known amount of native analyte standards and 0.1 M formic acid was injected on the SPE column. Right before the native compounds were backflushed from the SPE column and starting the HPLC separation, 50 μL of internal standard solution was injected into the HPLC gradient flow (using a second Agilent 1100 autosampler). Although native compounds and isotope-labeled standards were injected separately, they all eluted from the HPLC column and were detected by MS/MS at the same time. A response factor ($RF_a$) for each analyte was calculated from this experiment as the ratio of peak areas of native compound to its corresponding labeled analog. Second, 100 μL of urine spiked with the same amount of native and internal standards, was injected on the SPE column, and 50 μL of methanol was injected into the HPLC flow. Response factor ($RF_b$) was calculated as before. The two experiments differed in that for the first ($RF_a$), internal standards did not go through the SPE cleanup, but did for the second ($RF_b$). SPE recovery was calculated from $RF_a/RF_b$ because the internal standard amount used for both experiments was the same and matrix effects were equivalent.

Very good SPE recoveries (80–100%) (Table 3) were obtained for all of the analytes. Interestingly, the SPE recovery improved with the length of the alkyl chain (from 80% to 96% for methyl to butyl paraben). The relatively lower SPE recovery for the short compared to the longer alkyl chain parabens might be due to reduced retention of the short alkyl chain parabens on the SPE column. This would facilitate their breakthrough from the SPE column during the SPE loading and washing steps, while the long alkyl chain parabens would remain on the SPE column. However, the SPE recoveries of the short alkyl chain parabens are still acceptable for quantification purposes.

### 3.2. Urinary concentrations of five parabens and their conjugates in humans

We applied our method to determine free and total (free plus conjugated) concentrations of five parabens in 22 human urine samples collected in 2004 and 2005 from a convenience group of anonymous, demographically diverse adult volunteers who had no known occupational exposure to these compounds. Methyl, ethyl and propyl parabens were frequently detected in the urine samples (Table 5). The median concentrations of free methyl, ethyl, and propyl parabens were much lower than their corresponding median total concentrations suggesting that these parabens are mostly excreted in urine as glucuronide or sulfate conjugates (Fig. 3). The urinary concentrations of methyl and propyl parabens were the highest among the five parabens examined, with median total concentrations of 41.4 ng/mL and 10.2 ng/mL, respectively. This finding is in agreement with one report showing methyl paraben as the most abundant (mean value of 12.8 ng/g) among six parabens tested in human breast tumors [20]. Furthermore, the higher median urinary concentrations of methyl and propyl parabens

Table 5
Frequency of detection, mean and median urinary concentrations of free and total (free plus conjugated) species, and range of urinary concentrations of five parabens in humans

| Compound | Frequency of detection (%) | Mean (ng/mL) | Median (ng/mL) | Range (ng/mL) |
|---|---|---|---|---|
| Methyl paraben-free | 64 | 1.8 | 0.6 | <LOD–10.9 |
| Methyl paraben-total | 100 | 142.7 | 41.4 | 4.5–726.0 |
| Ethyl paraben-free | 27 | 0.2 | <LOD | <LOD–2.4 |
| Ethyl paraben-total | 100 | 23.0 | 2.5 | 0.8–89.3 |
| Propyl paraben-free | 45 | 0.5 | <LOD | <LOD–2.2 |
| Propyl paraben-total | 100 | 48.1 | 10.2 | 0.3–461.0 |
| *n*-Butyl paraben-free | 5 | <LOD | <LOD | <LOD–0.2 |
| *n*-Butyl paraben-total | 41 | 2.3 | <LOD | <LOD–29.1 |
| Benzyl paraben-free | 0 | <LOD | <LOD | <LOD |
| Benzyl paraben-total | 5 | <LOD | <LOD | <LOD–0.5 |

$N = 22$. The limits of detection (LODs) were 0.13 ng/mL (methyl paraben), 0.18 ng/mL (propyl paraben), and 0.10 ng/mL (ethyl, *n*-butyl, and benzyl parabens). Concentrations <LOD were imputed a value of LOD divided by the square root of 2 for the statistical calculations.

could be due to the fact that these two parabens are the most widely used [4]. Previous reports suggested that parabens are quickly metabolized and may not be found in urine [23]. Our preliminary data on these 22 human urine samples suggest that parabens can be present in urine mostly in their conjugated form, and parabens may therefore be valid biomarkers of exposure. Additional information, including a better understanding of the metabolism of parabens in humans, is needed to link these biomarker measurements to exposure and internal dose.

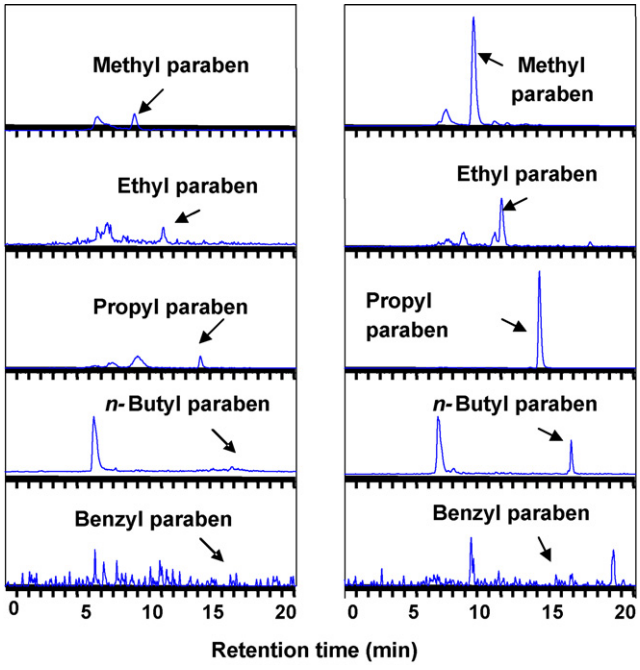


Fig. 3. HPLC–MS/MS extracted ion chromatograms of a urine sample with enzyme treatment (right) and without enzyme treatment (left, with the same *y*-axis scale). The calculated total (free) concentrations were 248 ng/mL (13.5 ng/mL) for methyl paraben; 1.34 ng/mL (0.12 ng/mL) for ethyl paraben; 41.7 ng/mL (1.08 ng/mL) for propyl paraben; 1.8 ng/mL (<0.1 ng/mL) for *n*-butyl paraben; and <0.1 ng/mL (<0.1 ng/mL) for benzyl paraben.

## 4. Summary

We developed a sensitive, selective, and precise automated on-line SPE-HPLC–isotope dilution MS/MS method to measure simultaneously several parabens in urine. To our knowledge, this is the first report on the urinary concentrations of individual parabens and their conjugates in humans. Our research suggests that human exposure to parabens may be assessed by measuring the free and conjugated forms of parabens in urine.

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