QUANTITATION OF BISPHENOL A AND BISPHENOL A GLUCURONIDE IN BIOLOGICAL SAMPLES BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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ABSTRACT:
Bisphenol A (BPA) is a weak estrogen. Pharmacokinetic studies of BPA have demonstrated a rapid and extensive metabolism of BPA to the nonestrogenic BPA-monoglucuronide (BPA-gluc). Some investigators have reported that BPA was found at parts per billion concentrations in the tissues or urine of humans without known exposure to BPA. This work developed a rapid and sensitive method for the determination of BPA and BPA-gluc in plasma and urine based on liquid chromatography-tandem mass spectrometry. The liquid chromatography-electrospray ionization-tandem mass spectrometry method for quantitation of BPA and BPA-gluc uses stable isotope-labeled internal standards. A linear ion trap mass spectrometer permits identification and quantitation of BPA-gluc and BPA without sample workup. Development of separation conditions reduced the BPA-background in solvent samples to below 2.5 pmol/ml for BPA. Limit of quantitation (LOQ) for BPA in control urine was 15 pmol/ml; LOQ for BPA-gluc was 65 pmol/ml.

Application of the method to urine samples from human subjects (n = 6) after administration of 25 μg of BPA/person (estimated maximum human daily intake) permitted the determination of excretion kinetics for BPA-gluc; BPA was below the LOD in all except two of the samples. In urine or blood samples of human subjects (n = 19) without intentional exposure to BPA, BPA concentrations were always below the limit of detection (~2.5 pmol/ml) with or without prior glucuronidase treatment. The results show that care is required for analysis of BPA and its major metabolite BPA-gluc. The LOD obtained and the absence of detectable levels of BPA in samples from individuals suggests that general exposure of humans to BPA is much lower than the worst-case exposure scenario developed.
$d_{16}$-BPA to assess BPA toxicokinetics in humans after low doses (Völkel et al., 2002).

Therefore, sensitive and selective methods to determine BPA in a biological matrix are needed with reduced sample workup to reduce possible contamination of samples with BPA from unknown sources and to quantitate both BPA and BPA-gluc based on stable isotope-labeled standards. The method development also considered the issue of background contamination with BPA present in solvents or released from material used for sample preparation by organic solvents. The combination with a linear ion trap mass spectrometer permits multiple reaction monitoring for quantitation and simultaneous recording of a product ion spectrum for conclusive identification.

Materials and Methods

Chemicals. $d_{16}$-BPA (98 atom % D, fully labeled) and BPA were obtained from Sigma-Aldrich (Deisenhofen, Germany). Water and acetonitrile used as HPLC solvents were purchased in HPLC gradient-grade quality from Carl Roth GmbH & Co. (Karlsruhe, Germany). The glucuronides of $d_{16}$-BPA and BPA were generated and characterized as described previously (Völkel et al., 2002). All other reagents and solvents were reagent grade or better and obtained from several commercial suppliers. Glucuronidase/sulfatase from Helix pomatia (EC 3.2.1.31) was obtained from Sigma-Aldrich.

Exposure of Human Subjects to BPA. BPA (25 μg/person) was orally administered in 50 ml of water to three healthy female and three healthy male human subjects (Table 1). All subjects enlisted in the study had to refrain from alcoholic beverages and medicinal drugs 2 days before and throughout the experiment. Subjects did not abuse alcohol and were nonsmokers. Subjects were healthy as judged by medical examination and clinical blood chemistry. The study was carried out according to the Declaration of Helsinki, after approval by the Regional Ethical Committee of the University of Würzburg, Germany, and after written informed consent by the subjects. Urine samples from the subjects were collected in glass vessels at 0, 1, 3, 5, and 7 h after giving BPA. After urine volume was determined, two aliquots (1 ml and 50 ml) were stored at $-20^\circ$C. Urine and plasma samples from human subjects without known exposure to BPA (age 26–50 years) were also collected in glass vessels (urine) or heparinized syringes at random times in the morning or in the afternoon. After urine volume was determined, two aliquots (1 ml and 50 ml) were stored at $-20^\circ$C. For analysis, part of the plasma samples (100 μl) were diluted with an equal volume of methanol and subjected to centrifugation, and 100 μl of acetonitrile was added, followed by a second centrifugation. From the obtained supernatant, 20 μl were analyzed by LC-MS/MS. Quantitation of creatinine was performed in the 1-ml urine samples separately frozen for creatinine determination as described elsewhere (Amberg et al., 1999). Glucuronidase treatment of urine and plasma samples was performed as previously described (Völkel et al., 2002).

Identification of BPA-gluc by Information-Dependent Acquisition (IDA). To identify BPA-gluc in urine samples from humans by LC-MS/MS, an IDA method consisting of multiple reaction monitoring (MRM), enhanced resolution (ER), and enhanced product ion (EIPI) scan mode was developed. Urine samples (100 μl) were diluted with 100 μl of acetonitrile containing internal standard ($d_{16}$-BPA-gluc, 42 nM). Samples (10 μl) were separated by a Reprosil-Pur ODS-3 column (5 μm, 150 × 4.6 mm; Maisch, Ammerbuch, Germany) using an Agilent 1100 autosampler and an Agilent 1100 HPLC pump (Agilent Technologies, Waldbronn, Germany). Gradient elution with water (solvent A) and acetonitrile (solvent B) with the following conditions was applied: 0% B for 2 min, followed by a linear gradient linear to 80% B within 20 min. Solvent composition was then kept at 80% B for 2 min. Flow rate was 300 μl/min. IDA experiments were performed on a linear ion trap mass spectrometer (QTrap; Applied Biosystems, Darmstadt, Germany) equipped with a TurboIonSpray source connected to the HPLC system. To record spectral data, a vaporizer temperature of 450°C and a TurboIonSpray voltage of $-4.5$ kV in the negative ionization mode were applied. Declustering potential (DP) was set to $-40$ V and $N_2$ was used as collision gas. The MRM mode used transitions from m/z 403 to 227 and from m/z 417 to 241 with a collision energy of $-30$ V and a collision gas setting of collision-activated dissociation = 4. The ER was performed with a scan rate of 250 amu/s and a fill time of 50 ms. For EPI scans, a scan rate of 4000 amu/s, a collision energy of $-30$ V, and a fill time of linear ion trap of 400 ms were used. In all experiments DP was set to $-40$ V. All IDA experiments were performed in the range of m/z 50 to m/z 420 with an entrance potential of 8 V and enabled Q3-trapping.

Quantitation of BPA-gluc. All quantitations were performed using a triple quadrupole instrument (API 3000; Applied Biosystems) equipped with a TurboIonSpray source. The HPLC parameters used were identical to those described above for identification. The HPLC system was directly coupled to the mass spectrometer. Analytes were detected in the negative-ion mode at a vaporizer temperature of 400°C and a TurboIonSpray voltage of $-4.0$ kV. Spectral data were recorded with $N_2$ as collision gas (collision-activated dissociation = 4) in the MRM mode with a dwell time of 500 ms for each transition monitoring the MS/MS ion transitions shown in Table 1. Quantitation of BPA-gluc was based on calibration curves obtained after addition of known amounts of BPA-gluc (0 nM, 20 nM, 39 nM, 78 nM, 157 nM, and 314 nM) to urine samples from the subjects collected before the exposure. Calibration curves were constructed from six data points using Analyst 1.3.1 (Applied Biosystems) with $R^2 > 0.99$. Intra-assay variability was below 15%. Acquisition conditions for mass spectra are detailed in Table 2. Under these conditions, calibration curves for BPA-gluc were linear up to concentrations of 1900 nM with $R^2 = 0.998$ and an accuracy between 88% and 115% (Table 3).

Quantitation of BPA. Samples both before and after glucuronidase treatment were analyzed by LC-MS/MS using the API 3000. Separations were performed with water (A) and acetonitrile (B) as solvents: 60% B for 2 min, followed by a linear gradient to 80% B within 20 min. The solvent composition was then kept at 80% B for 2 min; flow rate was 300 μl/min. All other parameters were identical to those described for the analysis of BPA-gluc. Quantitation of BPA was also performed relative to the stable isotope-labeled

<table>
<thead>
<tr>
<th>Volunteer</th>
<th>Gender</th>
<th>Age</th>
<th>Height</th>
<th>Body Weight</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Male</td>
<td>37</td>
<td>175</td>
<td>90</td>
</tr>
<tr>
<td>B</td>
<td>Male</td>
<td>43</td>
<td>178</td>
<td>70</td>
</tr>
<tr>
<td>C</td>
<td>Male</td>
<td>49</td>
<td>188</td>
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<td>D</td>
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<td>F</td>
<td>Female</td>
<td>32</td>
<td>163</td>
<td>57</td>
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</table>
BPA and BPA-gluc are deprotonated to give \[[M-\text{H}]^-\]
under these conditions, both with negative ionization, and were optimized for both analytes for the quantitation of both BPA and BPA-gluc and LC-MS/MS analytical methods and may indicate higher BPA concentrations than samples with BPA from other sources may interfere with sensitive contamination of samples, reagents, and equipment, was addressed.

The problem of interference of BPA from other sources, e.g., other components of urine or plasma, which elute close to the solvent front. This method was initially introduced in the acquisition method, and quantitations of BPA and BPA-gluc were developed. In addition, the problem of interference of BPA from other sources, e.g., contamination of samples, reagents, and equipment, was addressed. Due to the presence of BPA in plastics, contamination of biological samples with BPA from other sources may interfere with sensitive analytical methods and may indicate higher BPA concentrations than are actually present.

The developed methods apply stable isotope-labeled standards as a basis for quantitation of both BPA and BPA-gluc and LC-MS/MS with negative ionization, and were optimized for both analytes for optimal sensitivity (Tables 2 and 3). Under these conditions, both BPA and BPA-gluc are deprotonated to give \([M-\text{H}]^-\) (227.0 amu) for BPA, respectively \([M-H]^+\) (403.2 amu) for BPA-gluc. Addition of buffer salts or acid to improve separation was not possible since the addition of buffer salts or acids to the HPLC solvents dramatically reduced sensitivity. The use of stable isotope-labeled internal standards permitted a clear identification of the signals for both BPA-gluc and BPA despite the small shifts in retention time observed in the application of this gradient did not permit the sensitive quantitation of BPA-gluc with a limit of detection of 0.9 pmol/ml when the LOD was determined in water 0.9 pmol/ml and 65 pmol/ml (signal to noise ratio (S/N) 8) in human urine samples.

**Method Development.** Optimized mass spectrometric conditions for the quantitation of BPA and BPA-gluc were developed. In addition, the problem of interference of BPA from other sources, e.g., contamination of samples, reagents, and equipment, was addressed. Due to the presence of BPA in plastics, contamination of biological samples with BPA from other sources may interfere with sensitive analytical methods and may indicate higher BPA concentrations than are actually present.

The developed methods apply stable isotope-labeled standards as a basis for quantitation of both BPA and BPA-gluc and LC-MS/MS with negative ionization, and were optimized for both analytes for optimal sensitivity (Tables 2 and 3). Under these conditions, both BPA and BPA-gluc are deprotonated to give \([M-\text{H}]^-\) (227.0 amu) for BPA, respectively \([M-H]^+\) (403.2 amu) for BPA-gluc. Addition of buffer salts or acid to improve separation was not possible since the addition of buffer salts or acids to the HPLC solvents dramatically reduced sensitivity. The use of stable isotope-labeled internal standards permitted a clear identification of the signals for both BPA-gluc and BPA despite the small shifts in retention time observed in the absence of buffers (Table 3). Moreover, collision-induced fragmentation results in characteristic product ions from the precursor ion at MS/MS transitions for BPA were present in urine samples. Unfortunately, the application of this gradient did not permit the sensitive quantitation of BPA-gluc in the same separation, since BPA-gluc eluted with the solvent front.

Therefore, separate methods were developed to quantify BPA-gluc and BPA. The method for BPA-gluc used the gradient starting with a high water content of the mobile phase and permitted the quantitation of BPA-gluc with a limit of detection of 0.9 pmol/ml when the standard was analyzed after dilution in purified water. However, despite the use of a specific analytical method including HPLC separation, stable isotope internal standard, and presumably selective MS/MS transition, several peaks were observed in the region where BPA-gluc eluted from the same column when analyzing urine samples from humans without intentional exposure to BPA (Fig. 3, A and B). Although different HPLC columns (Synergi Hydro 150 × 2 mm; 80 A, 4 μm; Nucleosil C18 150 × 2 mm, 100 A, 5 μm; ReproSil-Pur C18-AQ 150 × 4.6 mm, 120 A, 3 μm) were used, HPLC solvents were acidified, and gradient parameters were changed, the best resolution of these compounds and spiked BPA-gluc obtained is shown in Fig. 3B. The presence of these interfering compounds reduced the LOD for BPA-gluc to 65 pmol/ml [signal to noise ratio (S/N) 8] in

**TABLE 2**

<table>
<thead>
<tr>
<th>Transition</th>
<th>Compound</th>
<th>DP</th>
<th>CE</th>
<th>Loss of Fragment</th>
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<tr>
<td>227.0–212.0</td>
<td>BPA (量化)</td>
<td>–40</td>
<td>–28</td>
<td>–CH₃</td>
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<tr>
<td>227.0–133.0</td>
<td>BPA (量化)</td>
<td>–40</td>
<td>–38</td>
<td>–H, –C₆H₅O</td>
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<td>241.1–223.3</td>
<td>d₁₀-BPA (量化, 内标)</td>
<td>–40</td>
<td>–28</td>
<td>–CD₃</td>
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<tr>
<td>241.1–142.1</td>
<td>d₁₀-BPA (量化, 内标)</td>
<td>–40</td>
<td>–38</td>
<td>–H, –C₆D₅O</td>
</tr>
<tr>
<td>403.2–113.1</td>
<td>BPA-gluc (量化)</td>
<td>–36</td>
<td>–24</td>
<td>–H, –H₂O, –CO₂, –CH₅H₉O₂ (BPA moiety)</td>
</tr>
<tr>
<td>403.2–227.0</td>
<td>BPA-gluc (量化)</td>
<td>–36</td>
<td>–30</td>
<td>–C₆H₅O₂ (glucuronic acid moiety)</td>
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<tr>
<td>417.2–113.0</td>
<td>d₁₀-BPA-gluc (量化, 内标)</td>
<td>–36</td>
<td>–24</td>
<td>–H, –H₂O, –CO₂, –C₆H₉D₅O₂ (BPA moiety)</td>
</tr>
<tr>
<td>417.2–241.1</td>
<td>d₁₀-BPA-gluc (量化, 内标)</td>
<td>–36</td>
<td>–30</td>
<td>–C₆H₅O₂ (glucuronic acid moiety)</td>
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**TABLE 3**

<table>
<thead>
<tr>
<th>LOD/LOQ</th>
<th>Accuracy</th>
<th>Mean Retention Time ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>pmol/ml (μg/l)</td>
<td>pmol/ml (μg/l)</td>
<td>%</td>
</tr>
<tr>
<td>BPA</td>
<td>5 (1.14)</td>
<td>15 (3.42)</td>
</tr>
<tr>
<td>BPA-gluc</td>
<td>25° (10.1)</td>
<td>65 (26.26)</td>
</tr>
</tbody>
</table>

* LOD in water 0.9 pmol/ml.
urine, due to a 50-fold reduced S/N. Addition of BPA-gluc (final concentration 500 pmol BPA-gluc/ml) to a urine sample resulted in a defined peak with an S/N of 31 presented in Fig. 3C. The peaks at retention times 8.85, 9.09, and 9.18 min presumably represent isobaric glucuronides that show identical fragmentation.

In urine samples from humans administered BPA, a peak with the mass spectral information supposed to be characteristic for BPA-gluc eluted at the expected retention time (Fig. 3D). However, due to the closely eluting peaks of other compounds, confirmation of the identity of BPA-gluc was required, and the presence of presumably present isobaric glucuronides indicates a need for a more conclusive identification of BPA-gluc to avoid false-positive results. Therefore, urine samples from individuals given BPA were also analyzed by a LC-MS/MS QTrap instrument using the IDA scan method to confirm the
presence of BPA-gluc. The linear ion trap mass spectrometer combined with the software tool IDA allows mass scans including a MRM for both BPA-gluc and the internal standard \(d_{14}\)-BPA-gluc (no clear separation of BPA-gluc and \(d_{14}\)-BPA-gluc was achieved) to select the analytes, an enhanced resolution to record higher resolved \([M - H]^-\) spectra, and an enhanced product ion scan to record a mass spectrum of each analyte recognized by the MRM mode. These mass spectral data are obtained in a single separation. In Fig. 4A, the characteristic EPI mass spectrum of BPA-gluc is presented, and Fig. 4B shows the spectrum of BPA-gluc obtained by IDA analysis in a urine sample collected 3 h after application of \(25 \mu g\) of BPA to a human subject.

If all EPI spectra of \(m/z\) 403 (BPA-gluc) and \(m/z\) 417 (\(d_{14}\)-BPA-gluc) recorded within the peak width of BPA-gluc are summed up, the resulting EPI mass spectrum gives a typical fragment (\(m/z\) 241) of
$$d_{14-}\text{BPA-gluc} \text{ due to coelution and identical collision energy for both glucuronides (Fig. } 4B)\text{. In addition, the signal } m/z \ 113 \text{ formed from both precursors (} m/z \ 403 \text{ and } m/z \ 417) \text{ represents the sum of both precursors and is therefore higher compared with the spectrum of the precursor } m/z \ 403 \text{ alone (Fig. } 4A)\text{. In urine and calibration samples in water and acetonitrile (50:50 v/v) without the addition of the internal standard } d_{14-}\text{BPA-gluc, the fragment of } m/z \ 241 \text{ was not observed and the presumed isobaric glucuronides showed more or less changed mass spectra (data not shown). The combination of both LC-MS/MS methods permitted a conclusive identification of BPA-gluc even in low concentrations. These results are more reliable as compared with methods that only use single chromatographic traces and retention time.}$$

**Analysis of Urine Samples for BPA-gluc and BPA after Oral Administration of BPA to Human Subjects.** The two procedures were applied to analyze samples from an application of BPA to human subjects in a single oral dose in the range of the worst-case assumption for daily exposure. All samples were analyzed independently with both HPLC systems, and with and without glucuronidase treatment. This combination of analytical methods gives information on the presence of both BPA and BPA-gluc and on the total content of BPA in a sample. The excretion of BPA and BPA-gluc was quantitated over time in urine samples, but BPA-gluc was only quantified in samples in which its presence could be confirmed by EPI-mass spectrometry (time points 1, 3, and 5 h). In male subjects, 93 ± 19 nmol (85% of the applied BPA dose) and in female subjects, 83 ± 16 nmol (75% of the applied dose) were recovered as BPA-gluc (Fig. 5) within 5 h after application. An elimination half-life of approximately 4 h was calculated from the excretion rates. The half-life and the recovery is similar to that reported in a previous study (Völkel et al., 2002), which used higher doses of $$d_{14-}\text{BPA}$$ applied to human subjects. The highest concentrations of BPA-gluc from 221 to 611 pmol/mg creatinine and 117 to 345 pmol/mg creatinine were observed in urine samples collected 1 h and 3 h, respectively, after administration of 25 µg of BPA/subject (Fig. 5). In plasma samples from this exposure, BPA-gluc could also be detected due to the absence of interfering peaks (data not shown).

Application of the HPLC separation conditions to determine free BPA without background in the samples from the controlled exposure indicated BPA concentrations above the LOD in only two of the urine samples (volunteer A, 1 h; volunteer E, 5 h). The BPA concentrations in these two samples were approximately 10 pmol of BPA/mg of creatinine, corresponding to 1 to 2 nmol (<1 µg/l) of BPA in the total volume of urine released, or less than 2% of the BPA dose received.

BPA concentrations in all other samples were below the LOD (Fig. 6, A and B).

To determine the content of BPA representing both BPA-gluc and BPA, BPA-gluc was hydrolyzed by glucuronidase and the samples were then analyzed with the method for determination of BPA. BPA content determined in these samples was identical to the concentrations of BPA-gluc as determined by the method to quantitate BPA-gluc, further supporting the presence of very low levels of free BPA, even after a controlled exposure (Fig. 5). After glucuronidase treatment, 106 ± 16 nmol (97% of the applied dose) of BPA and 92 ± 16 nmol (84% of the applied dose) of BPA were recovered in male and female subjects, respectively (Fig. 5). The higher recovery of BPA as compared with BPA-gluc may be explained by the lower sensitivity of the QTrap system since only samples showing a characteristic EPI spectrum were used for quantitation of BPA-gluc.

**BPA and BPA-gluc in Urine and Plasma Samples from Human Subjects without Intentional Exposure to BPA.** Urine and plasma samples from human subjects (7 males and 12 females) were randomly collected between 10 AM and 7 PM on different days and analyzed for BPA-gluc and BPA. In most of the urine samples, small peaks with the characteristic MS/MS transitions for BPA-gluc were detected at retention times very close to those of authentic BPA-gluc, but the concentrations were lower than 65 pmol/ml (LOQ), corresponding to <65 nmol of BPA-gluc in an average urine volume of 1 liter. Due to the low concentrations and the presence of other analytes with identical mass spectral characteristics, an unequivocal identification of BPA-gluc with IDA-MRM-ER-EPI was not possible. After glucuronidase cleavage, the BPA concentrations determined in urine were always below the LOD of 1.14 µg/l (5 pmol/ml), suggesting the presence of very low concentrations of BPA, at best. However, addition of even small concentrations of BPA or BPA-gluc to the samples gave definite responses (Figs. 6C and 7). None of the plasma samples analyzed (both with and without glucuronidase treatment) contained detectable concentrations of BPA or BPA-gluc, also indicating plasma concentrations of BPA below 0.5 µg/l.
The aim of the work presented here was to develop an optimized method for exposure analysis of BPA in humans using sensitive and selective methods such as LC-MS/MS. The original intention was to quantify both free BPA and BPA-gluc in one separation without detailed sample workup to avoid degradation of BPA-gluc and/or sample contamination. Due to the sensitivity and selectivity of LC-MS/MS, this was considered feasible with sufficient sensitivity. How-
ever, since glucuronides are urinary metabolites of many endogenous and exogenous compounds (Tephy and Burchell, 1990), signals with the presumed specific transitions for BPA-gluc were present at similar HPLC retention times in urine samples from human subjects not intentionally exposed to BPA. For example, some isoflavones widely present in human diet, such as resveratrol, have a molecular weight identical to that of BPA and are also excreted as glucuronides (Bayer et al., 2001; Wang et al., 2004). Therefore, a more conclusive identification of BPA-gluc was necessary to avoid false-positive results.

BPA-gluc was analyzed after fragmentation of the \([\text{M} - \text{H}]^-\) anion formed in the negative ionization mode of the electrospray source (Inoue et al., 2002; Völkel et al., 2002). With this technique, BPA-gluc may be identified by monitoring specific mass transitions. However, since the method included a loss specific for glucuronides, presumed isobaric glucuronides of unknown structures eluted closely to BPA-gluc. The fragmentation of these compounds gave identical spectral information (fragment with \(m/z\) 113 and \([\text{M} - \text{H}]^-\) of the glucuronide moiety).

A linear ion trap mass spectrometer was, therefore, additionally used to confirm the presence of BPA-gluc in low concentrations. With this instrument, an EPI spectrum can be recorded simultaneously to the MS/MS transitions specific for BPA-gluc (Fig. 4). The detection of the M-glucuronide fragment of \(d_{14}\)-BPA-gluc in these spectra, not seen in samples without \(d_{14}\)-BPA-gluc, serves as additional evidence for the presence of BPA-gluc. Application of this combination of mass spectrometry procedures clearly confirmed that, after controlled exposure to low doses of BPA, BPA-gluc is excreted as a metabolite of BPA.

However, the method using the triple quadrupole was not sufficiently selective to be applied to samples from human subjects without intentional exposure to BPA. Therefore, the strategy was changed to determine free BPA in urine or blood samples both with and without glucuronidase cleavage. BPA may be quantified with high sensitivity by LC-MS/MS; however, issues with background contamination needed to be solved before the method could be applied. Likely, water and/or acetonitrile used as HPLC solvents contain traces of BPA (Kuch and Ballschmiter, 2001; Völkel et al., 2002). Another source for contamination may be the release of BPA from plastics by organic solvents (Krishnan et al., 1993; Hutson, 1998; Fung et al., 2000). Indeed, sample workup with glassware gave a trend to lower BPA background levels. However, only a major change in HPLC separation conditions gave BPA concentrations below the limit of
When applying this procedure to a number of randomly collected blood and urine samples from human subjects without intentional exposure to BPA, the content of BPA in all samples was below the LOD of 5 pmol/ml (~1.14 μg/l), suggesting that the human exposure to BPA from diffusse sources in the environment is low. The levels observed in European subjects are well below those reported previously for populations in Korea and Japan, where mean values of 2.8 μg/l and 9.5 μg/l, respectively, were found in urine (Ouchi and Watanabe, 2002; Kim et al., 2003; Yang et al., 2003). In recent studies in the United States, mean urinary concentrations of BPA were 1.33 μg/l determined by GC-MS after glauconidase treatment (Calafat et al., 2005). In Japan, daily excretion of BPA with urine was reported as 1.2 μg/day (mean) using GC-MS/MS (Arakawa et al., 2004). The data presented here suggest that BPA concentrations in human urine in Europe are, at best, in a similar range.

Due to the rapid and complete elimination of BPA in humans as BPA-gluc in urine (Völkel et al., 2002), the determined concentration of BPA in urine suggests that the human exposure to BPA is less than 2.3 μg/pers/day (based on a volume of 2 liters of urine excreted). This value corresponds to a BPA dose of 38 ng/kg b.w. per day for a 60-kg adult and, thus, is well below the daily doses of BPA that have sometimes been reported to cause responses of unknown toxicological relevance in highly sensitive animal systems (20 μg/kg/day) (Timms et al., 2005).

The lack of detection of BPA in plasma with a LOD of 0.5 ng/ml also suggests that the plasma levels of BPA and BPA-gluc, respectively, in Europeans are below the BPA plasma concentrations reported in nonobese Japanese women (0.71 ± 0.09 ng/ml as analyzed by enzyme-linked immunosorbent assay) (Takeuchi et al., 2004) and lower than the previously reported median of 3 ng of BPA/ml of plasma in pregnant females in Europe (Schönfelder et al., 2002).

The capacity of the procedure to detect low levels of BPA and BPA-gluc in samples from human subjects administered a low dose of BPA, which represents the estimated maximum human exposure (EU Report, 2003), showed the capability of the procedure for exposure monitoring and also confirms previous observations on the rapid and almost complete excretion of BPA as BPA-gluc within a few hours after oral exposure. The rapid elimination of BPA as seen in this and a previous study of human excretion kinetics of BPA also indicated a need for a very frequent monitoring of BPA in blood and urine, since BPA taken up from food will be rapidly eliminated and will only be present in detectable concentrations for a relatively short period.

In summary, the results show that exposure monitoring to potentially ubiquitous chemicals requires a very detailed approach to account for possible interference by other chemicals present in the media and also requires specific analytical methods such as MS/MS. However, even when using these specific methods, structure confirmation is required. Therefore, methods to quantify BPA or BPA-gluc by HPLC-UV, HPLC-FLD, or HPLC with electrochemical detection or after fluorophore derivatization in urine of nonintentionally exposed humans may have only limited applicability (Ouchi and Watanabe, 2002; Takeuchi and Tsutsumi, 2002; Matsumoto et al., 2003; Mao et al., 2004; Kawaguchi et al., 2005), since the use of internal standards with these methods is difficult and many possibly interfering compounds with fluorophores or electrochemical potential are present in biological samples. Quantitation of parent BPA with GC-MS has a sufficient sensitivity and a higher power of separation as compared with HPLC (Schönfelder et al., 2002), but the procedure requires solvent extraction and/or further sample preparation as a possible source of contamination with BPA and needs to be well evaluated to avoid production of false-positive results. GC-MS analysis for BPA in human blood or urine samples also needs to account for the fact that, due to the rapid conjugation of BPA to BPA-gluc, most of the BPA is present as BPA-gluc. Therefore, a separation of BPA-gluc and free BPA must be achieved before extraction and derivatization, since the derivatization at higher temperature may hydrolyze BPA-gluc and overestimate the level of free BPA. Therefore, a LC-MS/MS method is more reliable and permits the quantitation of both free and conjugated BPA, which is relevant for assessment of postulated human health risks due to low-dose BPA exposures.

References


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