Original Article

Determination of Bisphenol in Indoor Dust Samples by Liquid Chromatography in Combination with Quadrupole Mass Spectrometry (LC-MS/MS)

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Abstract: This study has focused on the development of the method LC-MS/MS to determine 2,2-bis(4-hydroxyphenyl)propane (bisphenol A: BPA) and 4,4′-dihydroxydiphenylmethane (bisphenol F: BPF) in indoor samples by using ultrasonication extraction and solid-phase extraction for preparation. The chromatographic procedure was performed on Kinetex C18 2.1 x 150 mm, 2.6 µm column with mobile phases: 2 mM HCOONH₄ in MeOH and H₂O:MeOH (90:10,…). The method quantification limits (MQL) of BPA and BPF were 15.0 and 12.0 ng/g, respectively. The mean recoveries were 80.4% for BPA and 93.8% for BPF (RSD<15%). The calibration curve for both BPA and BPF was linear over a concentration range of 1.0 to 500 ng/mL (R² ≥ 0.999). Twenty-one samples were collected from private houses, laboratories, drug stores, and plastic recycling factories in Hanoi. BPA and BPF were found in dust samples with frequencies of 85.7 and 100%, respectively. Concentrations of BPA and BPF in dust samples were in the ranges of <MQL–2230 ng/g (mean: 626) and <MQL–149,000 ng/g (mean: 14,700), respectively. Based on the measured concentration, the human exposure to bisphenol via indoor dust ingestion was estimated for various age groups. The estimated exposure dose for people working at plastic recycling factories was the highest level (23.6 ng/kg-bw/day) but lower than the oral reference dose by the EPA and the European Food Safety Authority (50 µg/kg-bw/day).

Keywords: Bisphenol; Ultrasonication extraction; SPE extraction; LC-MS/MS; Indoor dust; Human exposure.

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1. Introduction

Bisphenols are organic compounds including two rings of phenol with hydrocarbon-bridge in their structures [1]. Common compounds are 2,2-bis (4-hydroxyphenyl)propane (bisphenol A: BPA), 4,4’-dihydroxydiphenylmethane (bisphenol F: BPF). They are important components in various producing plastic materials such as polycarbonate (PC) plastics, epoxy resins, tetrabromobisphenol A [2, 3]. PC is used in a variety of consumer products such as water bottles, sports equipment, medical devices, dental fillings, sealants, and household electronics. Epoxy resin is widely used as a protective lining for canned foods and beverages and as a coating on metal lids for glass jars and bottles [2, 4]. Annually worldwide, about 8 billion pounds of BPA were synthesized and used in polycarbonate plastic materials [4, 5]. Due to their wide use in consumer products, they can easily leach out into the environment over time. Previous studies reported the distribution of bisphenols in indoor dust, sediment, food, and even human urine, blood and plasma [4-9]. Among them, dust is one of the most important ways for BPA and BPA analogs to transfer between a variety of microenvironments and humans. The level of BPA in indoor dust samples collected from Shanghai, China (residences, dormitories, offices) and Spain (public environments) reached 23,033 ng/g and 4400 ng/g, respectively [10, 11]. Even so, BPA was observed at a mean concentration of 48,647 ng/g in high schools in Barcelona [12]. A previous study has documented that BPA and BPF were also detected from 12 indoor house dust samples in Vietnam with a concentration range of 27–1400 ng/g (mean 330 ng/g) and <1–1500 ng/g (mean 200 ng/g) [13]. In general, little is known about the occurrence of BPA and BPF in environments including dust samples in Vietnam.

Bisphenols were classified as emerging endocrine disruptors due to their impact directly on antioxidant enzymes and testosterone secretion [2, 5, 14, 15]. Human exposure risk to them via various pathways such as inhalation, dermal absorption, dust ingestion, and food/beverage consumption is unavoidable [16, 17]. Healy et al., (2015) reported that about higher than 90% of children in the United States, Europe, Asia, and Australia are exposed to BPA [2]. Especially, children, who are exposed to BPA at a low level can lead at risk of adverse health outcomes such as obesity, asthma, preterm birth, and type II diabetes [2, 18]. Based on the guideline of the EPA Integrated Risk Information system (IRIS), the maximum permissible dose for causing reproductive toxicity to humans is 3 µg/day [16]. In the face of evidence of the adverse effects on human health, worldwide authorities have enacted laws to limit or ban the use of bisphenols in some consumer products. Baby bottles and infant-feeding cups which contain BPA were stopped producing in markets in the USA [19]. In Vietnam, the allowable limit of BPA in PC plastics was required lower than 0.05 mg/kg [20].

To accurately quantify the concentration of bisphenols on various samples, several analytical methods are continuously being improved. Bisphenols and their derivatives can be qualified and quantified by methods of capillary electrophoresis (CE) and gas/liquid chromatography in combination with mass spectrometry such as GC-MS [11, 21], LC-MS, LC-MS/MS [3, 5, 7, 22], UPLC-MS/MS [8, 23]. In general, the LC method is more commonly used to measure bisphenols because it avoids the demand to derivatize prior analysis as the GC method. Various techniques such as traditional Soxhlet extraction, ultrasonic-assisted liquid extraction, accelerated solvent extraction (ASE), QuEChErs have been developed for solid sample preparation. The ultrasonication technique is considered green extraction because of low toxic solvent consumption, high extraction efficiency, and less extraction time [24]. This work has
introduced a method for analysis of BPA and BPF in dust samples by LC-MS/MS, using a fast and green extraction (ultrasonication) combined with solid-phase extraction clean-up for preparation.

2. Materials and Methods

2.1. Chemicals

Target compounds: 2,2’-bis(4-hydroxyphenyl)propane (BPA) and 4,4’-dihydroxydiphenylmethane (BPF) with their purities of 99.0% were purchased from Tokyo Chemical Industry. 13C-labeled Bisphenol A (13C12–BPA, P/N B519498) was received from Toronto Chemical (Canada) and used as an internal standard. Deionized water was obtained from Milli-Q Integral 3 (Merck Millipore, France). All chemicals including methanol (MeOH), dichloromethane (DCM), acetone, n-hexane, ammoniac solution (NH4OH), ammonium formate (HCOONH4) with analytical purities were acquired from Merck. All standard compounds were prepared in MeOH. The 1000 µg/mL of individual target standard and internal standard solutions were prepared by weighing 10.10 mg solid standard compounds by an analytical balance (EX125D, Ohaus) in a volumetric flask (10 mL), followed by adding an appropriate amount of MeOH. The mixture of target standard (50 µg/mL, 1 µg/mL, 25 ng/mL) and internal standard (5 µg/mL, 2 µg/mL) solutions were prepared by mixing and diluting 1000 µg/mL single standard and internal standard solutions. Working standard solutions at seven levels of 1, 5, 10, 50, 100, 200, and 500 ng/mL with the same internal standard concentration of 200 ng/mL were prepared by diluting the mixed standard solutions.

2.2. Instrumental Analysis

In this study, BPA and BPF were analyzed by a liquid chromatography-tandem quadrupole mass spectrometry system (LC-MS/MS-8040, Shimadzu). The separation was performed on a Kinetex C18- column (150 mm x 2.1 mm, 2.6 µm, P/N 00F-4462-AN). The column oven temperature was set at 35 °C. The flow rate was kept constant at 0.3 mL/min. Two µL of each sample was injected by an autosampler into the LC-MS/MS system. The gradient of mobile phases with 2mM HCOONH4 in MeOH (A channel) and H2O:MeOH (90:10;...) (B channel) was started at 20% B in 2 min, increased linearly to 75% B in 1 min, raised continuously to 95% B in 2 min (held for 3 min). The gradient was returned to the initial condition at 10 min and held for 2 min to equilibrium for the next injection. The quadrupole mass spectrometry was operated at the negative electrospray ionization (ESI(-)) mode. Parameters for this part of the system were set up as follows: drying gas flow: 15 L/min, CID gas: 230 kPa, DL temperature: 250 °C, and heat block temperature: 400 °C. The multiple reaction monitoring (MRM) mode was used for the detection and quantification of BPA and BPF (Table 1). Their identification was based on precursor, retention time (RT), and two transitions. The internal standard was applied for their quantification in real samples.

2.3. Sample Collection

Twenty-one indoor dust samples were collected from private houses (7), laboratories (5), drug stores (4), and plastic recycling factories (5) in 2021. They were collected from different points at each location by a broom and then homogenized into one sample. Before being wrapped in aluminum foil and transported to the laboratory. Samples were sieved through a sieve with a pore size of 100 µm then immediately analyzed or stored in a dark glass jar at 4 °C.

2.4. Factors Affecting Sample Preparation

In this work, the procedure of sample preparation was optimized by examining individual independent factors including the types of SPE columns, the volume of eluent solvent, and the ratio of extractive solvents. All experiments were carried out three times. Recoveries were taken as the objective parameters for optimization. The recovery value
was determined by comparing the signal measured on the LC/MS/MS instrument of target and internal standard in a known solution (X) and final solutions (Y) after each investigation. The known solution (X) was prepared by diluting from 100 µL of the target and internal standard solution (1000 ng/mL) in MeOH to exactly 1 mL.

Three types of SPE columns including Poly-Sery HLB 60 mg, 3 mL (P/N 2.CA3179.0001, ANPEL); Copure HLB 60 mg, 3 mL (P/N COHLB360, Biocomma); Oasis HLB 60 mg, 3 mL (P/N WTWAT094226, Waters) were investigated. One hundred ng (100 µL of a 1000 ng/mL standard solution) of individual target and internal standards were spiked directly onto each column which was previously conditioned with 5 mL MeOH. After the equilibration time (15 min), the analytes were eluted with 15 mL MeOH. The eluted solution was concentrated exactly to 1 mL (final solution: Y) under a gentle stream of nitrogen and transferred to an LC-vial (1.5 mL).

The best recoveries (comparing the signal of compounds in solutions Y and X) correspond to which column was selected for the next investigation (section 3.1).

The volume of eluent solvent: Experiments were conducted on the column which was selected from the prior investigation. The experimental steps are similar to those of the column type selection survey, except that the changes of eluent MeOH volume. The volume of MeOH was investigated at three values $V_1 = 5$ mL, $V_2 = 10$ mL and $V_3 = 15$ mL (section 3.1).

The ratio of extractive solvents: To literature, the mixture of MeOH and DCM was commonly used for the extraction of organic contaminants and its hydrophilic/lipophilic character makes it suitable for interaction with analytes of a wide polarity range. In this work, three available HLB cartridges including Poly-Sery HLB 60 mg, 3 mL (P/N 2.CA3179.0001, ANPEL); Copure HLB 60 mg, 3 mL (P/N COHLB360, Biocomma); Oasis HLB 60 mg, 3 mL (P/N WTWAT094226, Waters) were investigated. To our results from Figure 1, the Oasis HLB column has shown the highest recovery (>80%) with the smallest standard deviation (RSD). Therefore, Oasis HLB 60 mg, 3 mL (Waters) was the best choice to investigate elution volume.

2.5. QA/QC

All glassware was rinsed thoroughly with acetone and hexane to remove organic contaminants and baked at 400 °C for 3 hours. For each sample batch, solvent blank and QC samples (at least 20% total number of injections) were simultaneously analyzed to confirm the contamination and stability of the method. The relative standard deviation (RSD, %) was controlled to less than 15% for both BPF and BPA. Besides, the detection limit (LOD) and quantitation limit (LOQ) was calculated as 3- and 10-times signal-to-noise ratio obtained from sample spiked at the level of 1 ng/mL [25-28].

3. Results and Discussion

3.1. Optimization of Sample Preparation

For sample preparation, the reason for the selection of SPE with HLB stuffing material is its universal sorbent that has been extensively used for extraction of organic contaminants and its hydrophilic/lipophilic character makes it suitable for interaction with analytes of a wide polarity range. In this work, three available HLB cartridges including Poly-Sery HLB 60 mg, 3 mL (P/N 2.CA3179.0001, ANPEL); Copure HLB 60 mg, 3 mL (P/N COHLB360, Biocomma); Oasis HLB 60 mg, 3 mL (P/N WTWAT094226, Waters) were investigated. To our results from Figure 1, the Oasis HLB column has shown the highest recovery (>80%) with the smallest standard deviation (RSD). Therefore, Oasis HLB 60 mg, 3 mL (Waters) was the best choice to investigate elution volume.
The volume of MeOH used to elute BPF and BPA from the solid-phase extraction column was tested at three levels: $V_1 = 5$ mL, $V_2 = 10$ mL, and $V_3 = 15$ mL. Figure 2 has illustrated that recovery of target analytes at both values of $V_2 = 10$ mL and $V_3 = 15$ mL (>93%) was in the allowable range (80-120%). However, to help save solvents as well as shorten the time during sample concentration, the elution volume of 10 mL was chosen as the optimal value.

From the results of three previous investigations, the optimal sample treatment procedure was described in detail as follows:

Two hundred-fifty milligrams of dust sample which was spiked 200 ng/mL internal standard was exacted with 5 mL MeOH:DCM (1:1,...) twice, using ultrasonication technique before centrifuged to collect the supernatant. The combined supernatant was concentrated to about 3 mL and then cleaned up by a solid-phase extraction cartridge (OASIS HLB 3cc, 60 mg/30 µm), which was previously conditioned by 5 mL MeOH. Target compounds were eluted by 10 mL MeOH. The eluted solution was concentrated under a gentle stream of nitrogen to exactly 1 mL. All sample solutions were filtered through 0.45 µm NY-membrane (Sartorius), transferred to an LC-vial, and then injected into LC/MS/MS system.

3.2. Validation of the Method

Based on optimal sample preparation and instrumental analysis mentioned in section 2.2, the validation of this method was performed triplicate in spiked indoor dust samples at levels of 1 ng/mL and 100 ng/mL.

For analysis, internal calibration curves for both BPA and BPF were built at seven points of concentration 1, 5, 10, 50, 100, 200, and 500 ng/mL (cal1–cal7) with 200 ng/mL of internal standards in MeOH. Linearity was obtained with a regression coefficient higher than 0.999. Moreover, the method detection limit (MDL) and method of quantitation limit
(MQL) of this method were defined based on the signal-to-noise ratio (S/N) of 3 and 10, respectively. MDL was calculated by the formula (1): \[ \text{MDL} = \text{IDL} \times \frac{V_1}{m_0} \] (1); where IDL, \( V_1 \), and \( m_0 \) are the instrumental detection limit (ng/mL), the final volume of sample (mL), and the mass of extracted sample (g), respectively. In this work, MDL and MQL values were 4.0 ng/g and 12.0 ng/g; 5.0 ng/g and 15.0 ng/g for BPF and BPA, respectively, more sensitive than the study taken by Fan et al., (2019) (MDL for BPA 80 ng/g) [18]. On the other hand, the overall recovery of sample preparation including ultrasonication and clean-up was 80.4% and 93.8% for BPA and BPF, respectively, and fell in the allowable range according to AOAC guidelines (80–110%). In addition, the relative standard deviations (RSD) lower than 15% have indicated the good repeatability of this method.

Table 1. Method validation

<table>
<thead>
<tr>
<th>Analytes</th>
<th>Quantifier (CE)</th>
<th>Qualifier (CE)</th>
<th>MDL (ng/g)</th>
<th>MQL (ng/g)</th>
<th>Re (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPF</td>
<td>199.1\textsuperscript{&gt;93.1} (22)</td>
<td>199.1 &gt; 104.8 (21)</td>
<td>4.0</td>
<td>12.0</td>
<td>93.8</td>
<td>12.8</td>
</tr>
<tr>
<td>BPA</td>
<td>226.9 &gt; 211.9 (17)</td>
<td>226.9 &gt; 132.9 (25)</td>
<td>5.0</td>
<td>15.0</td>
<td>80.4</td>
<td>10.5</td>
</tr>
<tr>
<td>\textsuperscript{13}C-BPA</td>
<td>239.05 &gt; 223.2 (23)</td>
<td>240.7 &gt; 142.3 (24)</td>
<td>3.3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3.3. Concentration of BPA and BPF in Indoor Dust Samples

The validated method was applied for the analysis of BPA and BPF in twenty-one dust samples which were divided into four groups of places: private houses (n=7), drug stores (n=4), laboratories (n=5), and plastic recycling factories (n=5). The concentration of analytes in extracted samples was calculated by the formula (2): \[ C_0 = C_1 \times \frac{V_1}{m_0} \] (2); where \( C_0 \), \( C_1 \), \( V_1 \), and \( m_0 \) are the concentration of analytes in the extracted sample, the concentration of analytes calculated from the calibration curve, the final volume (mL), respectively.

Both of them were found in almost all collected samples with their high frequencies of 100% and 85.7% for BPF and BPA, respectively. Total concentrations of BPF and BPA in dust samples ranged from not detection to 150,000 ng/g (mean/median: 15,300/853 ng/g). In general, BPF was reported as abundant in all collected samples, ranging from <MQL to 149,000 ng/g (mean 14,700 ng/g), accounting for 96% of the total bisphenol concentrations. The measured concentration of BPF in dust was a hundred times higher than that in the USA (22 ng/g), China (21 ng/g), Japan (45 ng/g), and Korea (500 ng/g) [22]. This result is also significantly higher than the previous report in Vietnam in 2021, the mean level of BPF found in indoor dust was 200 ng/g [22]. In contrast, the distribution of BPA in indoor dust collected from Vietnam tends to decrease. Data reported in 2015 with the mean BPA concentration in dust samples was 330 ng/g [22] compared to 2021 (in this study) was 626 ng/g (range: <MQL–2230 ng/g). Although the distribution of BPF and BPA in the environment depends on various environmental, meteorological, and biodegradability factors, however, BPA has received a lot of attention in terms of toxicity and has been regulated by the allowable limits in commercial products and environments [19, 22]. To our knowledge, Vietnam also has the National technical regulation on the safety and hygiene of BPA in plastic packaging and tools in direct contact with food [20]. Meanwhile, the understanding of the distribution of BPF in the environment is still limited and regulations on controlling this chemical need more attention in the future.

Considering individual microenvironments, their highest total concentrations of BPF and BPA were observed in samples collected from plastic recycling factories (49,600 ng/g), followed by samples from private houses (1,430 ng/g). One reason can be the concentration of huge numbers of plastic materials in plastic recycling factories. Whereas, these values in investigated laboratories and drug stores were at the lower level (about 600-700 ng/g). Besides, BPF was found in all collected samples with a higher level than BPA, except for samples from private houses. Contamination of BPF in
samples from factories was at the top leading with a mean concentration of 49,000 ng/g, a hundred times higher than that in others. Facts have shown that BPF is becoming an alternative chemical for BPA in various plastic consumer products in our lives because of the negative impact of BPA on humans [14]. However, bisphenol analysis in various indoor dust microenvironments where people can be easy to contact them is still a high concern. BPA contamination was detected at very high level in kindergarten in Guang Zhou, China (980-9730 ng/g, mean 2860 ng/g) [5], preschools, Sweden (<LOD-15,000 ng/g, mean 1200 ng/g) [30], public microenvironments in Spain (192-4440 ng/g, mean 1880 ng/g) [11], high schools, museums, libraries in Barcelona, Spain (14,300–245,000 ng/g, mean 48,700 ng/g; 8020–188,000 ng/g, mean 43,200 ng/g; 2910–445,000 ng/g, mean 32,600 ng/g, respectively) [12].

For assessment of the correlation between BPA and BPF concentrations in collected samples, Spearman correlation was used. Results have indicated that there was no significant relationship in their concentration (p-value >0.05). Their concentrations in indoor dust can be affected by several factors, containing floor type, room furnishings, ventilation, and time of sampling [22]. BPA originated mainly from epoxy-based floorings, adhesives, paints, electronic devices, and printed circuit boards [18]. Nowadays, bisphenol analogs such as BPF, BPS, and BADGE have been used as the replacement of additives in plastic consumer products.

Especially, the occurrence of these target compounds in private house dust was published in most studies because all people contact them every day and they were represented in Figure 4. They were found in spread house dust samples collected from other countries in the range of BPA and BPF mean concentration 360 ng/g (India) - 4730 ng/g (France) and <MQL-5500 ng/g (Greece), respectively [13, 18]. As clearly seen that BPA and BPF observation in this study in Vietnam (<MQL–2230 ng/g, mean 1340 ng/g and <MQL–819 ng/g, mean 365 ng/g, respectively) belonged to group countries with low contamination. There was a tendency in these countries that BPA concentration was about three times higher than BPF. To our results, BPA contamination in Vietnam in this study was much lower than that in Singapore (<MQL–22,500 ng/g, mean 3420) and higher than in the previous study carried out in Vietnam (27–1400 ng/g, mean 330 ng/g) [13, 16]. Overall, the polluted levels of BPA and BPF in house dust were significantly different from various studies. These results suggest the different habits and usage levels of products containing BPA and BPF by region. Environmental factors also affect their distribution in the dust sample [3].

![Figure 4. Mean of BPA and BPF concentrations in house dust compared with previous studies.](image)

3.4. Human Exposure to Bisphenol Via Indoor Dust Ingestion

An earlier study has indicated that food ingestion accounted for higher than 90% of total BPA exposure for all age groups [16]. Dust is considered a minor exposure source but dust absorption via ingestion has been a dramatic exposure route for some environmental chemicals in humans, including bisphenols [3, 22]. Based on previous studies [22, 31–32], human exposure to bisphenol in this way was estimated as the equation (3) follows: \( DI = \frac{C \cdot f \cdot BM}{R_M} \) (3); where DI, C, f, and BM are daily intakes (ng/kg-bw/day), the concentration of bisphenol in collected samples (ng/g), the average rate of indoor dust ingestion (g/day) and body weight (kg-bw), respectively.
Literature, values of BW for Asian people can be divided into as follows: infants (<1 year, 6 kg), toddlers (1–5 years, 15 kg), children (6–10 years, 25 kg), teenagers (11–17 years, 48 kg) and adults (>18 years, 63 kg). Moreover, the f-value was 0.03 g/day for infants and 0.06 g/day for others. Results in private houses and drug stores have indicated about the range of exposed dose to bisphenol from 0.29-7.15 ng/kg-bw/day, approximately a half lower than reports in Korea (18.6 ng/kg-bw/day), Japan (15.8 ng/kg-bw/day), the USA (12.6 ng/kg-bw/day) [29]. Compared between different groups of age, the higher risk of being impacted by these chemicals in infants and children than the older. The reason can be explained by the frequent hand-to-mouth contact of children compared to adults [5, 18]. In contrast, laboratories and plastic recycling factories have focused on working with adults. There was a significant difference in daily intake for people there. The estimated value in plastic recycling factories (23.6 ng/kg-bw/day) was about 70 times higher than in laboratories (0.33 ng/kg-bw/day). So, working for a long time in this environment without enough safety can lead to negative effects on human health. However, DI values calculated in this work were much lower than the oral reference dose of 50 µg/kg-bw/day established by the EPA and the European Food Safety Authority [3, 22, 29], suggesting that bisphenol intake via dust ingestion in this study should be in the safety of threshold for Vietnamese. It is necessary to perform further studies with large sample sizes in various microenvironments, even bio-metabolites to assess human exposure to these contaminants. Moreover, the transformation of bisphenols in human bodies was assessed through analysis of them in blood and urine samples. According to a study taken for Chinese adults in 2012, the mean concentration of BPA was 0.19 ng/mL and 1.01 ng/mL in blood and urine samples, respectively [15]. Exposure to these compounds at enough high level can lead to a change in metabolism in genders and ages. Even that, low-dose and long-term exposure to BPA could influence the brain and neuron development of the embryo, fetus, and infant.

4. Conclusions

This study has illustrated that LC-MS/MS with good selectivity and sensitivity has been a modern and effective method for BPA and BPF analysis in dust samples. The linearity ranged from 1–500 ng/mL. Other parameters were mean recoveries: 93.8% and 80.4%; MDL: 4.0 ng/g and 5.0 ng/g for BPF and BPA, respectively. The optimum method was applied for the analysis of 21 dust samples from private houses, laboratories, drug stores, and plastic recycling factories. It was noticed that occurrence of target compounds in this study (15,300 ng/g) was at a high level in comparison with several previous studies in other countries. In addition, people working at plastic recycling factories have a high risk of negative impacts on their health if not protect themselves. However, the daily exposure to bisphenol via dust ingestion was much lower than the oral reference dose recommended by the EPA and the EFSA (50 µg/kg-bw/day). Considerably, people can be exposed to bisphenol in various pathways instead of only dust ingestion. So, the authors have planned for expanding sampling and analyzing bio-samples such as blood and urine samples to evaluate the metabolism of bisphenol in humans in further study.

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