Research Note

Liquid Chromatography-mass Spectrometric Analysis of p-cumylphenol and Bisphenol A in Environmental Waters in Nagoya City

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Summary
A highly sensitive analytical method was developed for the determination of para-cumylphenol (PCP) and bisphenol A (BPA) by tandem liquid chromatography-mass spectrometry and was applied to the measurement of environmental water. The method detection limits of PCP and BPA for seawater were 2.2 ng/L and 1.9 ng/L, respectively, and the corresponding values for river water were 5.2 ng/L and 5.0 ng/L. Various water samples in Nagoya city were found to contain PCP and BPA at concentrations ranging from less than 5.2 to 160 ng/L and 33 to 290 ng/L, respectively.

Key words: para-cumylphenol, bisphenol A, liquid chromatography- tandem mass spectrometry

INTRODUCTION
para-Cumylphenol (PCP) has been widely used as an end-capping agent in polycarbonate photopolymers and copolymers, preservatives, mildew-proofing agents, and surfactant materials. Bisphenol A (BPA) has been widely used as a material for synthesizing polycarbonate plastics and epoxy resins, which are applied as lacquer to coat metal food cans, bottle tops, and water supply pipes. BPA is considered to be an endocrine-disrupting chemical (EDC) as it exhibits a xenogenic effect in vivo even at very low concentrations. When pregnant mice were treated with a dose of 20 µg/kg of BPA for 1 day, it affected the next generation, increased prostate weight, and decreased sperm quantity. In addition, BPA has been reported to affect the early sexual development in male and female rats at a dose below the non-observed adverse effect level. PCP, whose chemical structure as well as physical properties and uses are almost similar to those of BPA, binds strongly to the human-estrogen-related receptor, similar to the binding behavior exhibited by BPA. PCP also exhibits nephrotoxic action. Nakazawa et al. have investigated the susceptibility of newborn rats to PCP. They have reported that the treatment of newborn rats with 100 or 300 mg/kg of PCP for 18 days resulted in cystic tubular dilatation of the kidneys. In addition, they have also reported that PCP affected the ovary and uterus. The information about the toxicity of PCP on aquatic organisms is as follows: 72 h NOEC (No Observed Effect Concentration) = 0.33 mg/L (Pseudokirchneriella subcapitata) growth inhibition and 96 h LC50 (Lethal Dose50) = 1.2 mg/L (Oryzias latipes). Several studies have reported the environmental behavior of BPA, and effects of human exposure to, as well as analytical methods of BPA. The environmental concentrations of BPA have been reported (Table 1) for the New Orleans surface waters and Mississippi sediments, as well as for the Selangor River in Malaysia and the Seine River in France. In Japan, the BPA concentrations in water, sediments, and fish have been surveyed and reported by the local government and academic organizations. Based on the fact that both BPA and PCP have similar structures, they probably coexist widely in the environment; therefore, there is significance for examining the environmental fate of PCP and BPA; moreover, not much is known about the environmental fate of PCP. Surface water concentrations of PCP have only been reported for the Lake Dianchi in China. Other phenolic EDCs were observed at several points in the lake, while PCP was observed at a few points. The concentration of PCP was 3.88-8.96 ng/L. However, to the best of our knowledge, no study has reported the PCP concentrations in environmental media in Japan. According to PRTR (Pollutant Release
and Transfer Register) dated 2004–2014, significant consumption of BPA was observed in Nagoya city; therefore, it seemed imperative to survey PCP and BPA.

A sensitive measurement method that can simultaneously determine PCP and BPA can aid in the elucidation of their environmental behavior. In the above report, PCP and BPA were measured by gas chromatography-mass spectrometry. It is necessary to use derivatization reaction in this method. However, derivatization is not necessary with liquid chromatography-tandem mass spectrometry (LC/MS/MS), and hence, LC/MS/MS analysis is convenient. Herein, a highly sensitive LC/MS/MS method is developed and applied to the simultaneous determination of PCP and BPA in environmental water in Nagoya city.

EXPERIMENTAL

Reagents

Standards of PCP, BPA, and BPA-d14 were purchased from Wako Pure Chemical Industries (Osaka, Japan). BPA-(rings-13C12) as the internal standard was purchased from Cambridge Isotope Laboratories Inc. (Tewksbury, MA, USA). LC/MS-grade solvents such as methanol, acetonitrile, and water were purchased from Kanto Chemicals (Tokyo, Japan). Formic acid, acetone, and hexane for analyses of pesticide residues and polychlorinated biphenyl were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Materials

Out of the following eight SPE (solid phase extraction) cartridges-InertSep CH, InertSep RP1, InertSep PSA, Bond Elut DEA, Oasis HLB, Sep-Pak PS2, Sep-Pak C18, and Sep-Pak NH2, the first four were purchased from GL Sciences Inc. (Tokyo, Japan), while the last four were purchased from Waters (Milford, MA, USA). For evaluating cleanup efficiency, MF-1 and MF-2 were purchased from Showa Denko K.K. (Tokyo, Japan). InertSep GC, InertSep GC/PSA, and InertSep GC/SAX/PSA were purchased from GL Sciences Inc. (Tokyo, Japan), ENVI-Carb was purchased from Sigma Aldrich (St. Louis, MO, USA), and Sep-Pak Carbon/NH2 was purchased from Waters (Milford, MA, USA).

Instrument conditions

An Alliance 2695/QuattroMicro API LC/MS/MS system (Waters, Milford, MA, USA) was used for measurements. An Ascentis Express C18 (10 cm × 2.1 mm, 2.7 µm) (Sigma Aldrich, St. Louis, MO, USA) column was employed with a mobile phase mixture of CH3OH and water under gradient elution of 20–95% CH3OH for 10 min at a flow rate of 0.2 mL/min. PCP and BPA were measured by selected reaction monitoring (SRM) under mass spectrometric conditions listed in Table 2.

Analytical method

Figure 2 shows the scheme for analyzing PCP and BPA. First, 100 µL of formic acid was added to 200 mL of a water sample and spiked with 10 ng of BPA-ring-13C12 as the surrogate standard. Second, the sample was passed at a flow rate of 20 mL/min through a solid-phase InertSep CH cartridge, which was preconditioned with 5 mL of methanol and 5 mL of water in that order. Third, after removing the residual water from the cartridge by air suction, the cartridge was eluted using 10 mL of methanol, followed by cleaning the eluate by passing through the Sep-Pak Carbon/NH2 cartridge.

Next, the cleaned eluate was dried using a gentle stream of nitrogen at 40°C in a water bath, and then, the dried residue was dissolved in 1 mL of 50% methanol-water. After spiking with 10 ng BPA-d14 as the syringe spike, the resulting methanol-water solution is then applied to LC/MS/MS analysis.

RESULTS AND DISCUSSION

Selection and evaluation of SPE cartridges for collecting PCP and BPA in water

Two hundred milliliters of pure water spiked with 10 ng each of PCP and BPA was used for the selection and evaluation of the recoveries from the eight SPE cartridges. For this test, two spiked water

<table>
<thead>
<tr>
<th>Target compound</th>
<th>cone voltage (V)</th>
<th>capillary voltage (kV)</th>
<th>mass transition for quantitation</th>
<th>mass transition for confirmation</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>25</td>
<td>25</td>
<td>227.0 &gt; 133.0</td>
<td>227.0 &gt; 212.0</td>
</tr>
<tr>
<td>PCP</td>
<td>25</td>
<td>25</td>
<td>211.0 &gt; 196.0</td>
<td>211.0 &gt; 133.0</td>
</tr>
<tr>
<td>BPA-d14</td>
<td>30</td>
<td>25</td>
<td>241.0 &gt; 141.8</td>
<td>241.0 &gt; 223.0</td>
</tr>
<tr>
<td>BPA-ring-13C12</td>
<td>30</td>
<td>25</td>
<td>239.0 &gt; 139.0</td>
<td>239.0 &gt; 224.0</td>
</tr>
</tbody>
</table>
samples were prepared under normal pH and acidic pH (pH 3) conditions using dilute formic acid. Experiments were conducted under both neutral and acidic conditions as environmental water typically has a near neutral pH, and BPA and PCP have weakly acidic structures. Alternatively, the spiked samples were directly passed through the eight cartridges. Next, PCP and BPA collected from each of the cartridges were eluted with 10 mL of methanol. The eluted solution was analyzed according to the scheme shown in Fig. 2. Figure 3 summarizes the comparison of the recovery efficiencies.

From the results obtained by the experiment, in several solid phases, a higher recovery rate was observed under more acidic conditions than that under neutral conditions. As PCP and BPA are weakly acidic compounds having a phenolic hydroxyl group, the decrease in the recovery rate could be suppressed by dissociation under acidic conditions. Moreover, with the use of the Oasis HLB or InertSep CH cartridge, high recovery was observed. The highest recovery efficiencies of 102% for PCP and 107% for BPA were obtained with the combination of acidified water (pH 3) and InertSep CH. Thus, in this method, the CH cartridge is used under acidic conditions (pH 3).

Selection and evaluation of SPE cartridges for the sample clean-up

Ten milliliters of methanol spiked with 10 ng each of PCP and BPA was used for the selection and evaluation of the recoveries from seven SPE cartridges: InertSep GC, InertSep GC/PSA, InertSep GC/SAX/PSA, ENVI-Carb, Sep-Pak Carbon/NH2, MF-1, and MF-2. For this test, two spiked methanol samples were directly passed through these seven cartridges. Next, PCP and BPA collected from each of the cartridges were eluted with 10 mL of methanol. Figure 4 shows the comparison of the clean-up recoveries obtained for each SPE cartridge.

Using Sep-Pak Carbon/NH2, the recoveries of both PCP and BPA exceeded 90%, and no contaminant peak was observed in the SRM chromatograms. However, unsatisfactory results were obtained for the other SPE cartridges. For InertSep GC, InertSep GC/PSA, InertSep GC/SAX/PSA, and ENVI-Carb, baseline drifts and impurity peaks were observed in the chromatograms; in particular, for InertSep GC/PSA and InertSep GC/SAX/PSA, the recoveries of PCP were approximately 60~75% (InertSep GC/PSA and InertSep GC/SAX/PSA). For MF-1, the recovery of BPA was approximately 50%.
For MF-2, the recovery of PCP was 300–700%, the reason for which has not yet been elucidated, because the structural information of MF-1 and MF-2 has not been published.

**Method detection limits and recovery efficiencies**

Table 3 shows the method detection limits (MDLs) and recovery efficiencies of PCP and BPA. The recoveries were determined by the analysis of the fortified control samples in conjunction with each sample set. The recovery efficiencies of BPA were compensated with those of the surrogate standard. The recovery efficiencies of PCP were 96–100% with a relative standard deviation (RSD) of less than 5% for purified water, river water, and seawater.

### Table 3  MDL *, MQL **, and recovery efficiencies *** (%) of PCP and BPA from water samples

<table>
<thead>
<tr>
<th>type of water sample</th>
<th>purified water</th>
<th>river water</th>
<th>sea water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPA</td>
<td>PCP</td>
<td>BPA</td>
</tr>
<tr>
<td>amount of spiked BPA p-CP (ng)</td>
<td>50</td>
<td>0</td>
<td>50</td>
</tr>
<tr>
<td>number of experiments</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>detected concentration (ng/L)</td>
<td>250</td>
<td>17</td>
<td>240</td>
</tr>
<tr>
<td>recovery (%)</td>
<td>97</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>surrogate recovery (%)</td>
<td>93</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>1.2</td>
<td>5.1</td>
<td>5.2</td>
</tr>
<tr>
<td>MDL (ng/L)</td>
<td>1.5</td>
<td>4.9</td>
<td>5.0</td>
</tr>
<tr>
<td>MQL (ng/L)</td>
<td>4.0</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

* MDL: method detection limit, MDL = t(n – 1, 0.05) × standard deviation × 2
  t(n – 1, 0.05): degrees of freedom n – 1, the 0.05 significance level one-tailed test

**MQL: method quantification limit, MQL= standard deviation × 10

*** Recovery efficiencies of PCP were obtained by the absolute calibration method. The recovery efficiencies of BPA were calculated by the internal standard method based on response factors using the surrogate standard.

### Table 4  Concentrations of PCP and BPA in the samples at different sampling sites

<table>
<thead>
<tr>
<th>sampling site</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA (ng/L)</td>
<td>290</td>
<td>74</td>
<td>220</td>
<td>59</td>
<td>150</td>
<td>55</td>
</tr>
<tr>
<td>PCP (ng/L)</td>
<td>5.6</td>
<td>&lt;5.2</td>
<td>160</td>
<td>46</td>
<td>21</td>
<td>6.8</td>
</tr>
<tr>
<td>PCP/BPA</td>
<td>0.02</td>
<td>-</td>
<td>0.73</td>
<td>0.78</td>
<td>0.14</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>BPA (ng/L)</td>
<td>40</td>
<td>62</td>
<td>97</td>
<td>43</td>
<td>230</td>
<td>33</td>
</tr>
<tr>
<td>PCP (ng/L)</td>
<td>&lt;5.2</td>
<td>5.7</td>
<td>8.2</td>
<td>6.3</td>
<td>5.3</td>
<td>5.8</td>
</tr>
<tr>
<td>PCP/BPA</td>
<td>-</td>
<td>0.09</td>
<td>0.08</td>
<td>0.15</td>
<td>0.02</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Sampling sites 1–11: river water, Sampling site 12: seawater; all sites were constantly monitored by the Nagoya City Government.

For the values below MDL, half of the MDL values were used for the calculation of the average, median, and standard deviation.
than the results obtained in China. However, judging from the previously reported information about the toxicity of PCP on aquatic organisms, it is estimated to be toxic less to aquatic organisms. In addition, in comparison with the worldwide concentration of BPA, it is estimated to be toxic less to aquatic organisms. In Asia was at the same level.

**Fig. 5** Map depicting the sampling sites in Nagoya city

Site 1-11: river water, Site 12: seawater
Black circles denote all sites at which the quality of water was constantly monitored
Stars denote sewage treatment plants near the sampling point

**Fig. 6** Scatter plots between the concentrations of BPA and PCP

The concentration ratio of PCP to BPA varied from 0.02 to 0.7, and it can be classified into three groups from the calculation of the results obtained from the variation in the ratios.
- **Group 1**: concentration ratios of PCP to BPA were less than or equal to 0.02 (Sites 1 and 11)
- **Group 2**: concentration ratios of PCP to BPA were 0.02-0.2 (Sites 5, 6, 7, 8, 9, and 12)
- **Group 3**: concentration ratios of PCP to BPA were around 0.7 (Sites 3 and 4)

Fig. 6 shows the scatter diagrams of PCP and BPA concentrations at all sampling sites, except for the sites at which the PCP and BPA concentrations were below the limit of detection. The concentration ratio of PCP to BPA varied from 0.02 to 0.7, and it can be classified into three groups from the calculating the results obtained from the variation in the ratios. In six of the sites, which accounts for 50% of the total sites, the concentration ratios of PCP to BPA were around 0.1, where PCP and BPA in the water most probably originate from the use of products such as epoxy compounds, which contain some percentage of PCP and BPA. Furthermore, at some sites, the concentration ratios of PCP to BPA were less than or equal to 0.02 (Sites 1, 2, 7, and 11), attributed to sources discharging higher amount of BPA as compared to that discharged at other sites. Moreover, at other sites, the concentration ratios of PCP to BPA were around 0.7 (Sites 3 and 4), where PCP concentrations were significantly higher than those in the other sites. Effluents from sewage treatment plants, which are relatively near, might affect the PCP concentration, although further study is required to elucidate this fact.

**CONCLUSION**

An LC/MS/MS method for the highly sensitive determination of PCP and BPA was developed and applied to measure their concentration in environmental water.

The average recoveries (n=5) of PCP and BPA were 99% and 98%, respectively, in river water, while those in seawater were 100% and 99%, respectively. The MDL values of PCP and BPA in seawater were 2.2 ng/L and 1.9 ng/L, respectively, and the corresponding MDL values in river water were 5.2 ng/mL and 5.0 ng/L.

PCP and BPA were found in various water samples in Nagoya city. Their concentrations in environmental water ranged from less than 5.2 to 160 ng/L and from 33 to 290 ng/L, respectively. The PCP concentration levels in this study were higher than those obtained in China. However, PCP is estimated to be less toxic to aquatic organisms. In addition, the BPA concentration levels in the surface water investigated in this study were the same as those obtained in the Asian region. As with PCP, it appears that BPA also does not have much of an impact on aquatic organisms.

In the six sampling sites (50% of the sites) considered in this work, the concentration ratio of PCP to BPA was around 0.1. In these sites, PCP and BPA occurring in water most probably originate from the use of products containing some percentage of PCP and BPA. Four of these sampling sites might have sources discharging higher amount of BPA than PCP. In two of these sites, the PCP concentrations were significantly higher than those in the other sites, possibly attributed to the discharge of effluents from sewage treatment plants, which were relatively near.

In the future, to determine the peripheral of high-concentration point, it appears that it is necessary to conduct further investigations. There also appears to be a need to continuously monitor the PCP and BPA concentrations.

**REFERENCE**

1) FDA; U.S. Food and Drug Administration
http://www.fda.gov/food/foodborneillnesscontaminants/chemicalcontaminants/ucm166145.htm (2015.10.07.)


