Determination of 4-tert-octylphenol, 4-nonylphenol and bisphenol A in surface waters from the Haihe River in Tianjin by gas chromatography–mass spectrometry with selected ion monitoring

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Abstract

The estrogenic pollutants 4-tert-octylphenol (OP), 4-nonylphenol (NP) and bisphenol A (BPA) were determined in surface water samples from the Haihe River, Tianjin, China. The analytes were extracted and concentrated from 300 ml acidified water samples by liquid–liquid extractions using dichloromethane, derivatized with trifluoroacetic anhydride, and quantified by gas chromatography–mass spectrometry (GC–MS) with selected ion monitoring (SIM). Among the samples collected from 14 sampling sites, only one sample was found to have a relatively high concentration of BPA (8.30 µg l⁻¹) and NP (0.55 µg l⁻¹). The concentrations of OP, NP and BPA in the other samples were in the range of 18.0–20.2, 106–296 and 19.1–106 ng l⁻¹, respectively. Recoveries for OP, NP and BPA in the spiked water samples were all over 80%.

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1. Introduction

In the last few years, many phenolic xenoestrogens have been reported to have mimic estrogen effects, and may adversely affect the health and reproduction of animals and human (Klotz et al., 1996; Olea et al., 1996; Sonnenschein and Soto, 1998; Völk et al., 2002). Among them, 4-tert-octylphenol (OP), 4-nonylphenol (NP) and bisphenol A (BPA) deserve particular attention because of their estrogenic activity, widespread application and ubiquity in environment (Kuch and Ballschmiter, 2001; Petrović and Barceló, 2001; Lee and Peart, 2002; Meesters and Schröder, 2002). There is evidence that OP and NP can cause estrogenic effects in fish and other aquatic organisms (Jobling et al., 1996). A recent paper by Hunt et al. (2003), reported that exposure to BPA causes a chromosomal abnormality in the oocytes of female mice, and suggested that this abnormality could lead to reproductive or developmental effects.

OP and NP are used as precursors in the manufacture of non-ionic surfactants and are also degradation products of alkylphenol ethoxylates (APEOs), which are used in household detergents, pesticide formulations and...
other applications (Renner, 1997). Approximately 51% of APEOs is released to the environment in the final form of metabolic products by undergoing mechanical and biological sewage, and sewage sludge treatment (Ahel et al., 1994; Isobe et al., 2001). In 2001, the amount of NP production was 16,000 t and the total consumption quantity of NP was 24,290 t in China (Anon., 2002).

BPA was applied mainly as an intermediate to synthesize epoxy resins and polycarbonate plastics (Staples et al., 1998). Releases of BPA into the environment are mainly in wastewater from plastics-producing industrial plants and from landfill sites. In 2001, the amount of BPA production in China was 12,200 t and the total consumption quantity of BPA (36,261 t) was contributed mainly by import amount of BPA (94,243 t), epoxy resins (114,000 t) and polycarbonate (211,160 t) (Zhu and Qi, 2003).

Many published analytical methods for the sensitive determination of OP, NP and/or BPA have concentrated on quantitative analysis using liquid chromatography (LC) (Petrović and Barceló, 2001) and gas chromatography (GC) (Bolz et al., 2000, 2001). GC–MS has been applied extensively to determine these compounds without derivatization or after derivatization to various esters (Varelis and Balafas, 2000; Kuch and Ballschmiter, 2001; Rinken, 2001). Several methods have given a limit of detection (LOD) of several nanograms per liter (Heemken et al., 2001), and even down to tens pg l⁻¹ (Kuch and Ballschmiter, 2001).

In China, very limited study on environmental monitoring of OP, NP and/or BPA has been performed, and the relative reporting is rare. The Haihe River, located in Tianjin which has a long history as an industrial city, receives input of industrial, domestic and agricultural effluents. In order to evaluate the contamination status of OP, NP and BPA in surface water from the Haihe River, an investigation has been recently performed with a quantitative GC–MS–SIM method.

2. Materials and methods

2.1. Apparatus and reagents

Pesticide grade dichloromethane and n-hexane were purchased from Fisher Scientific (New Jersey, USA). Analytical reagent grade acetone was distilled before use. De-ionized water was double distilled with a quartz distiller. NP (a mixture of isomers with a branched C₉H₁₉ side chain) and OP were obtained from Tokyo Kasei Kogyo Co. Ltd, Japan. Bisphenol A (97%) was purchased from Acros Organics (NJ). Trifluoroacetic anhydride (TFAA) was obtained from Koch-Light Laboratories Ltd, England. Standard stock solutions (OP, 10 mg l⁻¹; NP, 44.6 mg l⁻¹; BPA, 10.19 mg l⁻¹) were prepared by dissolving appropriate amounts of these compounds in methanol. Analytical grade sodium chloride, 1,2,4-tribromobenzene (TBB), hydrochloric acid and anhydrous sodium sulfate were obtained from Beijing Chemicals Corporation (Beijing, China). Microporous filter membrane of cellulose acetate (diameter, 50 mm; 0.45 μm) was purchased from subsidiary factory of Beijing University of Chemical Technology. Silica gel was conditioned with methanol and dichloromethane, and heated at 160 °C for 10 h before being deactivated by adding 5% water. In the whole analytical process only glassware previously rinsed with acetone and methanol were used.

2.2. Sampling

The total population of Tianjin is over 10,000,000, and inhabitants in the city and towns are over 7,000,000. According to the Reports of Tianjin Environmental Quality in 2002, the sewage discharge was 497,000,000 t including 220,000,000 t industrial effluents and 277,000,000 t domestic wastewater. Industrial effluent was treated completely by sewage treatment plants (STP); the total treatment ratio of sewage was up to 50%. One municipal STP effluent (capacity of treatment, 260,000 t/d) discharged into the Dagupaiwu River and finally entered into the Bohai Sea. Dagupaiwu and Beitangpiaiwu River received 65% sewage effluents of Tianjin.

To investigate the contamination levels of OP, NP and BPA in water samples from the Haihe River, 14 different sampling sites were selected and shown in Fig. 1. Samples were collected in 4 l amber glass bottles on April 8 and 9, 2003. Samples were filtered through 0.45 μm microporous filter membrane in order to remove algae, zooplankton and suspended particles. Water samples should be extracted immediately after sampling and filtering.

2.3. Extraction procedure

15 g NaCl were added to 300 ml water sample and its pH was adjusted to less than 2 with several drops of 50% HCl (v/v). Extraction was performed with 3 × 20 ml of dichloromethane, shaken rigorously for 3 min each time. The combined organic phase was dehydrated through a glass column filled with 2.5 g anhydrous sodium sulfate, reduced by rotary evaporation to a volume of about 1 ml. The extracts were transferred to a reaction vial and gently evaporated to dryness under a stream of high purity nitrogen.

2.4. Clean-up and derivatization procedure

The concentrated extracts were redissolved in 1 ml n-hexane/acetone (2:1), and then purified by passage
through 1.5 g of deactivated silica gel column (1 cm i.d. × 30 cm). This column was eluted by 15 ml n-hexane/acetone (2:1), and the eluent was evaporated to dryness by high-purity nitrogen. Derivatization was rapidly performed with 100 μl of TFAA (Varelis and Balafas, 2000; Rinken, 2001). The reaction vial was sealed and kept in 30 °C water bath for 10 min, then sonicated for 10 min in 30 °C water bath. The products were evaporated to dryness under a stream of nitrogen. The residues were redissolved in 100 μl of n-hexane containing 0.4 mg/l of TBB as internal standard of quantitative analysis.

2.5. GC–MS conditions

The samples were analyzed by GC–MS using an Agilent 6890 GC coupled with a 5973 Mass Selective Detector. The GC separation was performed on a fused-silica capillary column (HP-5MS (Hewlett-Packard); film thickness, 0.25 μm; 30 m×0.25 mm i.d.). Helium, used as carrier gas, was controlled at a flow rate of 1.0 ml/min in a constant flow mode. The injection was operated in pulsed splitless mode at a temperature of 250 °C, the injection pulse pressure was 30 psi lasting for 0.70 min, and 50 ml/min of purge flow was started at 0.75 min after injection. As to the MSD parameters, electron impact ionization was set at 70 eV. The temperatures of interface, source and quadrupole were 280, 230 and 150 °C, respectively.

For analysis, 1 μl aliquot of the derivatized samples was injected. The oven temperature program was set as follows: the initial temperature was held at 80 °C for 1 min, increased to 220 °C at 10 °C min⁻¹, and ramped to 250 °C at 20 °C min⁻¹, a post-analysis baking at 300 °C for 3 min was applied to the column. Full scan mass spectra were recorded from m/z 40 to 450. The characteristic ion fragments of the derivatized OP, NP and BPA were selected and identified in the scan mode, and shown in Table 1. The mass spectrometer was operated in selected ion-monitoring (SIM) mode to detect quantitatively the trifluoroacetate derivatives of OP, NP and BPA.

### Table 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Retention time (min)</th>
<th>Selected ion (m/z)</th>
<th>Start time (min)</th>
<th>Dwell time (ms)</th>
<th>Cycles s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBB</td>
<td>9.72</td>
<td>235, 314, 316</td>
<td>4.00</td>
<td>90</td>
<td>3.13</td>
</tr>
<tr>
<td>OP</td>
<td>10.10</td>
<td>203, 231, 302</td>
<td>10.00</td>
<td>90</td>
<td>3.13</td>
</tr>
<tr>
<td>NP</td>
<td>11.10–11.85</td>
<td>203, 217, 231, 245, 259</td>
<td>10.90</td>
<td>50</td>
<td>3.03</td>
</tr>
<tr>
<td>BPA</td>
<td>14.57</td>
<td>405, 406, 420</td>
<td>13.00</td>
<td>90</td>
<td>3.13</td>
</tr>
</tbody>
</table>

3. Results and discussion

3.1. Identification and quantification by GC–MS–SIM

With the help of the NIST98 standard mass spectral library, the characteristic ions and the retention times of the target compounds were obtained and identified with full scan mass spectra. Under the above described GC conditions, 11 peaks were obtained for the derivatized NP, and one peak was resolved for the derivatized OP and BPA, respectively. In 15 min, all the target compounds could be well separated and were shown in Fig. 2. Based on their mass spectra, the characteristic ions of the derivatized OP were m/z 231 and 203, and its
molecular ion was $m/z$ 302 (Fig. 2a). From the average mass spectra of the derivatized NP, its typical characteristic ions were $m/z$ 203, 217, 231, 245 and 259, and its molecular ion was $m/z$ 316 (Fig. 2b). The characteristic ions of the derivatized BPA were $m/z$ 405, 406 and 420, while its molecular ion was $m/z$ 420 (Fig. 2c). Their molecular ions and characteristic ions all contained a same group, [CF$_3$CO–], but the cleavage of [CF$_3$CO–] did not form base peak and its abundance was very low. The electron impact fragmentation pathways of the derivatized analytes were well accord with their free phenol.

On the basis of the presence of major fragments from the mass spectra of the derivatized OP, the base peak

Fig. 2. GC–MS chromatograms of the derivatized analytes in the standard and the samples using SIM. *Denoted 1,2,4-tribromo-benzene (TBB) as internal standard. Peaks a, b and c represented the derivatized OP, NP and BPA, respectively. Mass spectra of a and c represented the derivatized OP and BPA, b was the average mass spectra of NP as typical example.
m/z 231 was formed by loss of [CH2C(CH3)3]− radical from M. While the base peak m/z 405 of the derivatized BPA was obtained by loss of a methyl group from the molecular ion. Another characteristic ion of m/z 406 was formed by [(M + 1)−CH3]+ ion. The cleavage pathways and the base peaks of the derivatized OP and BPA were shown in Fig. 2a and c. The base peak of the derivatized NP varied with different isomers of NP, Fig. 2b exhibited the average mass spectra of all the derivatized NP isomers.

The characteristic ions of the derivatized analytes were selected and listed in Table 1, and applied to quantify the levels of the analytes. Shown in Fig. 2, the derivatives of target compounds could be detected from all samples and standard solution. OP, NP and BPA were quantified by comparing their derivatives peak area with the peak area of the internal standard TBB. However, 11 peaks of the derivatized NP were attributed to the different isomers. Not all peaks represented single isomer, some of individual peaks contain several isomers. Therefore, it is difficult to identify the structure of each individual isomer and quantify the levels of them one by one. For quantifying the NP, the peak areas of the 11 selected peaks had to be integrated individually and added up, and then compared the sum area with the peak area of TBB. The responses of peak area were calculated by integrating with RTE integrator.

### 3.2. Establishment of calibration curve

Preparation of calibration standards and establishment of calibration curve were referenced by many documents (Lee and Peart, 2000; Varelis and Balafas, 2000; Heemken et al., 2001; Kuch and Ballschmitter, 2001; Rinken, 2001). Calibration standards were obtained by fortifying 300 ml redistilled de-ionized water with 1 ml serial diluted stock solutions. With the proposed procedure, calibration curves of all analytes were established and the various parameters were shown in Table 2. The LOD of the target compounds were calculated by a signal-to-noise ratio of 3 (S/N = 3). 50 μl stock solution was directly derivatized by 0.2 ml TFAA, the products were dissolved and further diluted by TBB solution. When the absolute amount of OP, NP and BPA was 0.5, 2.2, 0.5 pg, signal-to-noise ratio of them were more than 3. Taking into account an enrichment factor of 3,000. LOD of OP, NP and BPA were estimated as 0.17, 0.73, and 0.17 ng l−1, respectively.

In order to identify the efficiency and reproducibility of the proposed method for each of the analytes, study of recoveries was determined by the spiked sample R1. The recoveries of OP, NP and BPA was 80.0%, 111%, 109%, respectively, and their RSD was 7.6%, 1.6%, 9.9%, respectively. To obtain reliable results, duplicates of each water sample were processed and two aliquots of each derivatized sample were analyzed with GC–MS–SIM. The small derivation represented by error bars in Fig. 3 also indicated good reproducibility of the proposed procedure.

### 3.3. Quantitative analysis of surface water samples

The sampling sites of R14, R13 and R12 were located at Beiyun, Ziya and Nanyun River, respectively. They

![Fig. 3. Determination of the levels of the analytes in water samples from the Haihe River using GC–MS–SIM.](image)
were three source rivers of the Haihe River. From R8 to R11, were in urban of Tianjin, R3 to R7 were between Tianjin and Tanggu, R2 was in urban of Tanggu, and R1 was at estuary of the Haihe River. Except for R1, all the others contained large amounts of algae and zooplanktons.

The results shown in Fig. 3 indicated that OP, NP, BPA were ubiquitous in all samples. With the exception of sample R1, the observed concentration of the analytes in the Haihe River were similar to the results reported by references in other surface waters around the world (Bolz et al., 2000; Azevedo et al., 2001; Belfroid et al., 2002). The OP, NP an BPA in sample R2–R14 (amounting to 13 points) were determined in the range of 18.0–20.2, 106–296 and 19.1–106 ng l\(^{-1}\), with a mean value of 19.2, 200 and 41.9 ng l\(^{-1}\), and a median value of 19.3, 191 and 34.8 ng l\(^{-1}\), respectively. The low levels of OP in samples suggested that octylphenol ethoxylates are minor components in the formulation of APEOs. This was well accord with documents that octylphenol ethoxylates accounted for 20% of APEOs (Renner, 1997). The higher levels of NP related to the widespread application of APEOs and persistence of NP in the environment. The levels of BPA varied with the sampling sites. From R11 to R7, the level of BPA decreased gradually, which indicated that BPA be degraded within the stream current. Staples et al. (1998) reviewed BPA might be decomposed in surface water, with a half-time of between 2.5 and 4 days. The tendency of NP level in this section was similar to that of BPA, indicated that the decrease BPA level from sample R11 to R7 could also be a result of an enhanced hydraulic load of the river. But that of OP was different, which indicated the OP level of the increased water was similar to its level in the Haihe River, while the BPA and NP level of the increased water was far below that of the Haihe River. The increase of BPA level from R7 to R1 demonstrated that new pollution sources discharged the contaminants into the Haihe River. These results were well in agreement with the fact that a thermoelectrical plant and a paper mill were located near the site of sample R6, and the Haihe River flowed through an industrial district, Tanggu.

The BPA level detected in R1 was 8.30 μg l\(^{-1}\), which is higher than the value considered to cause estrogenic effects in fish. It was found that 2–5 μg l\(^{-1}\) of BPA was hormonally active when studied in yeast culture medium (Krishnan et al., 1993). Moreover, the OP and NP concentrations in R1 were 31.9 and 553 ng l\(^{-1}\), respectively, which obviously were the highest one in this study. Several reasons might be responsible for the high level pollution at R1: (1) Contrary to sample from other sites which containing planktons, R1 was lack of algae and zooplanktons, and thus the biodegradation of analytes in R1 was not conducted quickly. (2) The levels of the analytes increased from R2 to R1, which likely indicate that there were contaminant sources near the site of R1. It was found that many chemical industries including chemical plant of Dagu were near the estuary of the Haihe River. Our study also found that the high level of vitellogenin, a biomarker of estrogen, could be detected in all the male sea catfish collected from the site R1, which indicated the heavy pollution status of this site (Shao et al., 2004). (3) At the south of R1 there was a sewage outlet of the Dagupaiwu River, and the effluents can easily pollute the estuary of the Haihe River during the period of flood tide. Therefore, the levels of R1 were higher than the others.

4. Conclusions

In this work, the occurrence of OP, NP and BPA in surface water of the Haihe River in Tianjin was successfully determined by GC–MS–SIM method after derivatizing by TFAA. In one of the 14 samples, the BPA levels were remarkably higher than the others and also greater than the value that could cause estrogenic effects on fish. The OP, NP and BPA levels in the other aqueous samples were determined in the range of 18.0–20.2, 106–296 and 19.1–106 ng l\(^{-1}\), respectively. Further study is required to monitor the contaminated sites in order to reveal the pollution source and support the results by more reliable evidences.

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References


