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# Analysis of Bisphenol A and Alkylphenols in Cereals by Automated On-line Solid-Phase Extraction and Liquid Chromatography Tandem Mass Spectrometry

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Ⓢ *Supporting Information*

**ABSTRACT:** An on-line solid-phase extraction (SPE) following a liquid chromatography−electrospray ionization−tandem mass spectrometry (LC−ESI−MS/MS) method was established for the simultaneous analysis of bisphenol A (BPA), nonylphenol (NP), and octylphenol (OP) in cereals (including rice, maize, and wheat). The target compounds were extracted by acetonitrile, purified by an automated on-line SPE cartridge, and analyzed by LC−MS/MS under the negative-ion mode. Mean recoveries fortified at three concentration levels ranged from 81.6 to 115.7%, and the coefficient of variation ranged from 4.6 to 19.9% ($n$ = 6). The limits of quantification (LOQs) of the method were 0.5, 0.5, and 0.25 μg/kg for BPA, NP, and OP, respectively, in both rice and maize, while the LOQs in wheat were 0.5, 1.25, and 0.5 μg/kg for BPA, NP, and OP, respectively. This method was applied in the analysis of rice, maize, and wheat from a local market. As a result, NP occurred in all cereal samples at the concentration range of 9.4−1683.6 μg/kg and BPA was detected in a few samples.

**KEYWORDS:** *Bisphenol A, nonylphenol, octylphenol, on-line solid-phase extraction, cereals, LC−ESI−MS/MS*

## ■ INTRODUCTION

Hazardous compounds in food and feed plants are of great concern because of their potential toxicity to humans and animals. Bisphenol A (BPA) and alkylphenols (APs), primarily nonylphenol (NP) and octylphenol (OP), are mimic estrogenic compounds because of their proven endocrine-disrupting properties.[1−3] BPA is widely used in the production of polycarbonate plastics and epoxy resins and as an antioxidant or stabilizer in polyvinylchloride. APs are precursors for and the dominant degradation products of alkylphenol ethoxylates (APEOs), the most commonly used non-ionic surfactants. They are also used as antioxidants in plastics and rubber products. These chemicals have been detected worldwide in air, water, sediment, soil, and biota at varying levels.[4−8] Concerns regarding food contamination are growing because the compounds are ubiquitous in the environment.

Because of their relatively high hydrophobicity, more attention was focused on foods of animal origin, such as fish,[9,10] animal tissue,[11] and milk.[12−14] However, according to the study by Güenther et al.,[15] high concentrations of NP was found not only in fatty foods but also in nonfatty foods. Cereal is an important source of energy for human beings. Lu et al.[16] analyzed 318 samples of 25 types of commonly consumed foodstuffs from Taiwan and found that rice was the most commonly consumed source of NP, with a proportion of 21.46% of the total daily intake. According to the Report of the National Diet and Nutrition Survey in China,[17] the average daily intake of cereals for residents of China is 402 g/day, which accounts for 57% of their energy intake. Thus, BPA and APs in cereal might be the major source of dietary intake. As a first step in the studies of occurrence, effects, and risk assessment of BPA and APs in cereal, reliable analytical methods are very important.

However, analytical methods for the determination of BPA and APs in cereal are scarce. Güenther et al.[15] detected NP in 60 different food products (including pasta) that are commercially available in Germany, using steam-distillation extraction and normal-phase high-performance liquid chromatography (HPLC) cleanup. The amount of NP and OP residues in 25 types of food (including rice and noodles) was performed by acetonitrile extraction, Florisil column purification, and HPLC−fluorescence detection.[16] Both methods are time- and solvent-consuming. Martínez et al.[18] employed pressurized liquid extraction (PLE) and solid-phase extraction (SPE) using Oasis hydrophilic−lipophilic balance (HLB) sorbent to analyze endocrine-disrupting compounds (EDCs) in corn samples from breakfast cereal. She et al.[19] cleaned the acetonitrile extracts using SPE with graphitized carbon black tandem primary and secondary amine (PSA) cartridges when analyzing nonylphenol ethoxylate (NPEO) and its metabolites in vegetables and crops. The methods based on off-line SPE followed by liquid chromatography−tandem mass spectrometry (LC−MS/MS) analysis are straightforward. However, quantifying trace amounts of BPA and APs in food samples is a challenging task because of widespread background contami-

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nation. Previous papers have documented the unambiguous existence of BPA and NP in various solvents, SPE sorbent, and commercial cartridges.[20,21]

On-line SPE is an emerging effective method based on column-switching techniques. One of the advantage of this method versus off-line SPE is the lower procedural contamination because of its automatic enrichment and purification process.[9,21] It was very important for the trace analysis of BPA and APs in the complex matrix. Gallart-Ayala et al.[22] developed an on-line column-switching LC−MS/MS method to analyze BPA and its chlorinated derivatives in water and found no contamination during the analyzing procedure. They also applied a similar method to determine BPA in soft drinks.[23] Ye et al.[24−26] used a unique on-line SPE−HPLC−MS/MS with peak-focusing feature, to measure the concentration of environmental phenols in human milk and serum. Yan et al.[27] established a high-speed and robust on-line SPE−HPLC−MS method using triacontyl-bonded silica (C30) as on-line SPE material for the analysis of five estrogens and BPA in bovine milk. In addition, on-line SPE techniques are more environmentally friendly and economical for saving time and costs while reducing hazardous waste consumption compared to off-line SPE methods.[28] Nevertheless, on-line SPE is primarily used in matrices, such as water, serum, urine, and milk;[22−27] little research has been reported on its use in the cereal analysis. The main objective of this work was to develop an automated method based on on-line SPE−LC−MS/MS for the simultaneous determination of BPA, NP, and OP in cereals.

## ■ MATERIALS AND METHODS

**Chemicals and Reagents.** Standard BPA (98.5%) was purchased from Dr. Ehrenstorfer GmbH (Augsturg, Germany). A 4-NP mixture (compounds with branched side chains) was obtained from Tokyo Kasei Kogyo Company, Ltd. (Tokyo, Japan). The reagent 4-OP (99%) was purchased from Sigma-Aldrich (St. Louis, MO). Internal standards BPA-$d_4$, 4-*n*-NP-$d_4$, and 4-*n*-OP-$d_{17}$ (purity > 97.8%) were purchased from CDN (Quebec, Canada). All standards were stored at −20 °C. Ammonium hydroxide for analysis (28−30 wt % solution of $NH_3$ in water) was acquired from Acros Organics (Morris Plains, NJ). HPLC-grade acetone, LC−MS-grade methanol, and acetonitrile were supplied by Fisher Scientific (Fair Lawn, NJ).

Individual stock solutions (1000 mg/L) were prepared per half a year by dissolving an appropriate amount of each substance in methanol and storing at −20 °C in amber glass vessels. Working standard mixtures were prepared by combining the stock solutions and diluting them with methanol. The working standard solutions were stored at 4 °C.

**Instruments.** The on-line extraction and analysis were performed on a Dionex Ultimate 3000 ultrahigh-pressure liquid chromatography (UHPLC) system (Sunnyvale, CA), which was equipped with an Ultimate 3000 degasser, two Ultimate 3000 binary pumps, an Ultimate 3000 RS autosampler, injector-fitted with a 100 μL loop, an Ultimate 3000 RS column compartment, incorporating a six-port switching valve, and Chromeleon chromatography data system software. The SPE column was a SolEx horseradish peroxidase (HRP) reversed-phase perfusion cartridge (2.1 × 20 mm, 12−14 μm particle size, Dionex, Sunnyvale, CA). For the second-dimension chromatographic step, a 2.1 × 50 mm, 1.7 μm Acquity UPLC BEH C18 column (Waters, Milford, MA) was used. The LC system was interfaced with an electrospray ionization (ESI) probe of a triple quadrupole mass spectrometer (Waters, Milford, MA). Argon gas was used as the collision gas, and nitrogen gas (purity of 99.9%) was used as both the cone gas and the desolvation gas. Data acquisition and evaluation were performed using Masslynx 4.1 software (Micromass, Manchester, U.K.).

**Sample Preparation.** In this study, three typical varieties of cereals (rice, maize, and wheat) were tested. Samples containing approximately 200 g of cereal were ground in a laboratory mill for homogenization. The ground samples (2.0 g) were spiked with internal standards (25 ng) in glass tubes and ultrasonically extracted using 5.0 mL of acetonitrile in a ultrasonic cleaner (12 W, Cole Parmer ultrasonic cleaner, Vernon Hills, IL) for 30 min. Then, the mixtures were centrifuged at 3000 rpm for 15 min at 4 °C. Then, 1.0 mL of supernatant was pipetted out and diluted to 5.0 mL with ultrapure water. The pooled solution was subjected to on-line SPE−LC−MS/MS analysis.

**On-line SPE Conditions.** The chromatographic configuration and procedure were set up to enable a rapid on-line cleanup of the injected sample before introduction into the mass spectrometer. The on-line SPE procedure consisted of three main steps: loading, transfer, and elution. In the loading step, 100 μL of the extract from the actual samples was directly injected into the system with the six-port injector valve in the loading position. Through the SPE pump (left pump), the mobile phase A (methanol)/the mobile phase B (ultrapure water) solution (20:80, v/v) flowed at a rate of 2.0 mL/min for 2.0 min to wash out the matrix interferences, while the analytes were trapped on the SPE column. At the same time, the analytical column was equilibrated with the chromatographic pump (right pump). In the transfer step, at 2.0 min, the six-port valve was switched to the injection position to connect the SPE column and the analytical column in series. The Dionex right pump was used to provide the gradient elution to transfer the analytes from the SPE column onto the analytical column at a flow rate of 0.3 mL/min for 3 min. Sharp elution profiles were achieved using the back-flush mode. In the separation step, the six-port valve was switched back to the loading position and the analytes were separated on the analytical column. To prevent cross-contamination, the sample loop and the extraction cartridge were flushed with methanol after each sample and conditioned with the initial mobile phase prior to the enrichment of the next sample. The on-line SPE conditions are shown in Table 1.

**Table 1. On-line SPE and UHPLC Conditions**[a]

| | left pump, on-line SPE | | | | right pump, UHPLC | | |
|---|---|---|---|---|---|---|---|
| time (min) | valve position | flow rate (mL/min) | A (%) | B (%) | flow rate (mL/min) | A (%) | C (%) |
| 0.0 | loading | 2.0 | 20 | 80 | 0.3 | 35 | 65 |
| 2.0 | injection | 2.0 | 20 | 80 | 0.3 | 90 | 10 |
| 2.1 | injection | 0.5 | 100 | 0 | 0.3 | 90 | 10 |
| 5.0 | injection | 0.5 | 100 | 0 | 0.3 | 90 | 10 |
| 5.1 | loading | 2.0 | 100 | 0 | 0.3 | 100 | 0 |
| 7.0 | loading | 2.0 | 100 | 0 | 0.3 | 100 | 0 |
| 7.1 | loading | 2.0 | 20 | 80 | 0.3 | 35 | 65 |
| 9.0 | loading | 2.0 | 20 | 80 | 0.3 | 35 | 65 |

[a] A, methanol; B, ultrapure water; and C, ultrapure water with 0.1% ammonium hydroxide.

**UHPLC−MS/MS Conditions.** For the second-dimension chromatographic step, the mobile phase was a mixture of ultrapure water with 0.1% ammonium hydroxide (C) and methanol (A) eluting under the gradient conditions reported in Table 1. The temperature of the column oven was set to 40 °C.

MS/MS acquisition was conducted on a Waters Xevo TQ MS triple quadrupole mass spectrometer equipped with an ESI interface operating in the negative-ion mode with multiple reaction monitoring (MRM). This method has been previously described.[29] The capillary voltage was 2.8 kV. Nitrogen gas (purity of 99.9%) was used as both the cone and desolvation gas at flow rates of 50 and 800 L/h, respectively. The electrospray source block and desolvation temperatures were held at 150 and 400 °C, respectively. Ultrahigh-purity argon was used as the collision gas. The pressure of the collision chamber was maintained at $3.2 \times 10^{-3}$ mbar. Table 2 lists the transition ions and collision energy for each compound during MRM acquisition.

### Table 2. MS/MS Parameters for the Analysis of Target Compounds

| compound | MRM transition[a] | cone voltage (V) | collision energy (eV) |
|---|---|---|---|
| BPA | **227.1 > 212.1** | 32.0 | 28.0 |
| | 227.10 > 133.0 | | 16.0 |
| NP | **219.2 > 133.0** | 34.0 | 30.0 |
| | 219.2 > 147.0 | | 28.0 |
| OP | **205.2 > 106.0** | 34.0 | 18.0 |
| BPA-$d_4$ | 231.1 > 216.1 | 36.0 | 20.0 |
| NP-$d_4$ | 223.2 > 110.0 | 38.0 | 20.0 |
| OP-$d_{17}$ | 222.2 > 108.0 | 34.0 | 20.0 |

[a] Quantitative ion transitions are in bold.

**Method Validation.** To evaluate the performance of the on-line SPE−LC−MS/MS method for the analysis of BPA, NP, and OP in cereals, quality parameters, such as calibration curves, sensitivity, accuracy, and precision, were studied in three different matrices (rice, maize, and wheat).

Calibration curves for the three target compounds were obtained by carrying out a linear regression analysis on the ratio of standard solution areas to internal standard areas versus the concentration. A multicomponent standard solution was prepared freshly by spiking NP and OP ranging from 0.01 to 20.00 μg/L, i.e., 0.01, 0.10, 0.50, 1.00, 5.00, and 20.00 μg/L, while the concentration of BPA was 4-fold accordingly. All concentrations were spiked with 1.00 μg/L internal standard. The integrated peak areas of the selected quantification MRM transitions were used to construct six-point standard calibration curves. Each point on the calibration curve represented the average of three injections.

The sensitivity of the method was evaluated by the limit of determination (LOD) and the limit of quantification (LOQ). The LOD and LOQ represent the lowest spiked concentration that can yield a signal-to-noise ratio (S/N) greater than 3 and 10 in the selected production chromatogram, respectively.

The accuracy was evaluated by the recovery of different levels of spiking. The test was performed using 2.0 g of samples (rice, maize, and wheat) spiked at three levels of BPA, NP, and OP and 25 ng of internal standards using six replicates.

The intra- and interday precisions were evaluated by spiking cereal samples at the three concentrations in six replicates, as mentioned above, within 1 day and over the course of 5 consecutive days.

**Carryover.** Carryover was evaluated for three analytes by injecting maize extracts spiked with the highest standard concentration in six samples. Two reagent blank samples were injected following the highly concentrated sample to detect the presence of the target chemicals. The peak areas of the first blank samples were divided by that of the standard at high concentration to obtain a quantitative evaluation of carryover.[30]

## ■ RESULTS AND DISCUSSION

**On-line SPE Optimization.** The composition of the mobile phase and time required for loading/washing and elution were critical parameters of an on-line SPE procedure. For the on-line SPE−UHPLC method, the contents of the organic solvent in the mobile phase should be higher than those of the loading solvent, so that the enriched compounds retained in the trap column can be eluted to the analytical column. As a result, we used methanol/water (20:80, v/v) as the loading solution. In addition, the transfer time was optimized to transfer the target analytes as much as possible and the interference as little as possible, from the SPE column to the analytical column. The total time in the range of 2.0−5.0 min was investigated. When the switching time was longer than 4.0 min, the peak area of BPA decreased (Figure 1); this may be due to a suppression of BPA ionization when the co-extracted matter washed to the analytical column over the extended time. For this reason, a 3.0 min switching time was chosen, although there was no obvious difference in the results for switching times of 3.0 and 4.0 min.

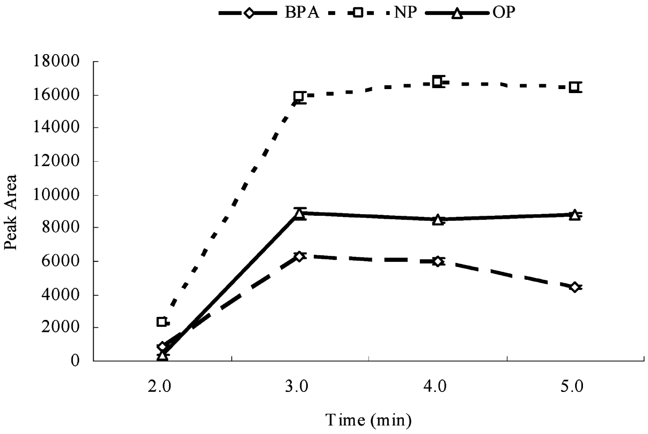

Figure 1. Effect of the washing time on the peak area of target compounds.

**Optimization of the Sample Preparation.** The maize matrix was used as the typical case to develop the pretreating method because of its complexity. The octanol−water partition coefficients (log $K_{ow}$) for the three target compounds were 3.40−4.48,[31,32] demonstrating a high hydrophobicity. Considering these properties, methanol, acetonitrile, and acetone were first used as extractants for screening purposes. Solvents (10 mL) were added to 2.0 g aliquots of spiked maize (10 μg/kg). As a result, the average recoveries for most of the target compounds when acetonitrile was used as the extractant (78−89%) were much higher than those obtained with methanol (67−74%) and acetone (65−77%). Acetonitrile was therefore selected for further use. Then, the extraction volume and cycles were optimized: 10 and 5 mL aliquots of acetonitrile, both extracted 3 times, were compared. From Figure 2, it can be observed that there was no significant difference between the recoveries of the 10 mL extract and those of the 5 mL extract during the first cycle. Although a second 5 mL aliquot of acetonitrile increased the recovery of the analytes, a 1 × 5 mL aliquot of acetonitrile was chosen to conserve the solvent and allow for further treatment.

**Quality Control.** The literature[33] reported that BPA was prone to be adsorbed by glassware; therefore, all of the glassware used in our study was tested according to the paper. However, there was no sorption of APs and BPA by glassware in our tests.

Contamination by NP and BPA must be avoided throughout the entire analytical procedure. Various quality controls were applied to ensure the accuracy of the method. Plastics were not permitted for use, and glassware was baked for 4 h at 400 °C in a muffle furnace (L9/11/B 170, Nabertherm Industrial Furnaces, Ltd., Lilienthal/Bremen, Germany) before use. However, very low NP concentrations were still measured. To evaluate possible contamination during pretreatment, a procedural blank was applied in each batch of test samples. As a result, the background contamination of NP was stable in methodological blanks. The level of NP in procedure blanks was around 0.005 μg/L, with the relative standard deviation

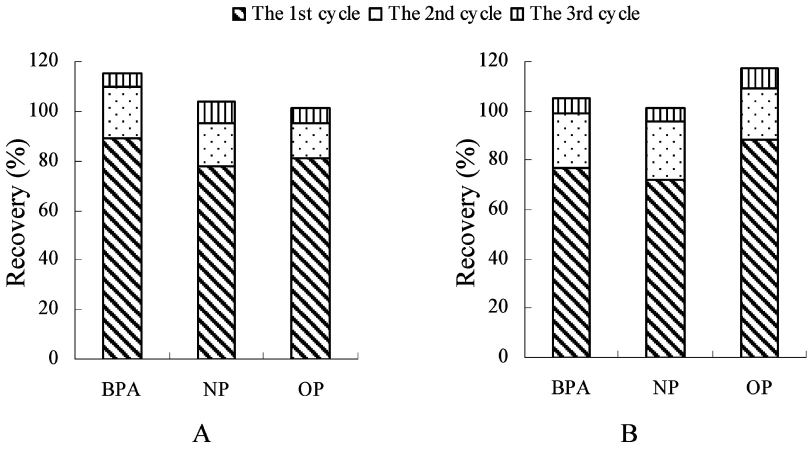

**Figure 2.** Recoveries of target compounds over 3 extraction cycles using varying acetonitrile volumes ($n = 3$): A, 10 mL; B, 5 mL.

(RSD) of 24% ($n = 6$), which was below the instrumental LOQ (0.01 μg/L).

Additionally, the carryover for all substances was below 0.04% in the first blank sample after injection of the highly concentrated spiked samples. In the second blank, no carryover could be identified for all three compounds. Therefore, in this study, to prevent any misleading results because of carryover, a blank sample was run after the injection of every positive sample.

**Method Validation.** In general, isotopic-dilution methods were employed to compensate for the loss of the target analytes during sample preparation and for ion suppression of the mass spectrometer analysis. BPA, 4-NP, and 4-OP were calibrated with BPA-$d_4$, 4-$n$-NP-$d_4$, and 4-$n$-OP-$d_{17}$, respectively. Acceptable linearities for all of the target compounds were obtained with correlation coefficients of $r^2 > 0.99$.

In our study, it was difficult to obtain the negative samples of NP; therefore, 4-$n$-NP-$d_4$ was added to the matrices to estimate the LOD and LOQ for NP. From the $k$ value of the linear equation for NP (1.0612), the response of 4-$n$-NP-$d_4$ and 4-NP was almost identical. The LOD and LOQ are listed in Table 3. These values were significantly lower than the LOD values reported in previous papers, which ranged from 3.5 to 12 μg/kg for BPA and NP in cereals and crops.[18,19]

**Table 3. LOD and LOQ for BPA, NP, and OP in Rice, Maize, and Wheat**

| | LOD (μg/kg) | | | LOQ (μg/kg) | | |
|---|---|---|---|---|---|---|
| compound | rice | maize | wheat | rice | maize | wheat |
| BPA | 0.2 | 0.2 | 0.2 | 0.5 | 0.5 | 0.5 |
| NP | 0.2 | 0.2 | 0.4 | 0.5 | 0.5 | 1.25 |
| OP | 0.1 | 0.1 | 0.2 | 0.25 | 0.25 | 0.5 |

**Table 4. Recoveries and the RSDs of the Target Compounds in Rice, Maize, and Wheat ($n = 6$)**

| sample | compound | spiked (μg/kg) | recovery (%) | RSD (%) | interday RSD (%) |
|---|---|---|---|---|---|
| rice | BPA | 0.5 | 82.5 | 17.4 | 9.7 |
| | | 1.0 | 115.4 | 12.0 | 10.9 |
| | | 5.0 | 96.8 | 8.5 | 7.4 |
| | NP (9.4 μg/kg)[a] | 9.0 | 103.4 | 14.8 | 12.8 |
| | | 18.0 | 106.6 | 15.3 | 10.6 |
| | | 90.0 | 115.7 | 5.9 | 11.2 |
| | NP-$d_4$ | 0.5 | 84.8 | 12.9 | |
| | OP | 0.25 | 98.2 | 10.1 | 7.9 |
| | | 0.5 | 95.8 | 12.8 | 5.8 |
| | | 2.5 | 105.2 | 6.1 | 8.6 |
| maize | BPA | 0.5 | 90.2 | 9.1 | 15.4 |
| | | 1.0 | 113.0 | 7.8 | 11.3 |
| | | 5.0 | 89.2 | 6.5 | 13.2 |
| | NP (20.6 μg/kg) | 20.0 | 111.5 | 5.9 | 13.7 |
| | | 40.0 | 105.4 | 4.6 | 8.8 |
| | | 200.0 | 95.4 | 6.8 | 7.7 |
| | NP-$d_4$ | 0.5 | 92.6 | 19.9 | |
| | OP | 0.25 | 110.0 | 16.3 | 10.9 |
| | | 0.5 | 98.8 | 18.6 | 11.7 |
| | | 2.5 | 81.6 | 11.3 | 9.6 |
| wheat | BPA | 0.5 | 101.2 | 10.1 | 12.9 |
| | | 1.0 | 85.1 | 5.9 | 16.8 |
| | | 5.0 | 96.8 | 9.5 | 10.9 |
| | NP (9.4 μg/kg) | 9.0 | 90.2 | 5.8 | 7.8 |
| | | 18.0 | 84.1 | 8.7 | 10.9 |
| | | 90.0 | 83.7 | 15.5 | 11.3 |
| | NP-$d_4$ | 1.0 | 85.5 | 10.5 | |
| | OP | 0.5 | 86.1 | 11.9 | 7.5 |
| | | 1.0 | 85.2 | 11.9 | 10.0 |
| | | 5.0 | 94.0 | 9.7 | 12.4 |

[a] The background concentration in the spiked sample is shown in the parentheses.

The sample preparation was evaluated using a standard spiking test at three concentrations in rice, wheat, and maize, with each condition replicated 6 times. For NP, the low level was performed at the background value of the matrices. The recovery of these compounds, spiked at different concentrations in various matrices, is summarized in Table 4. The average recoveries of each compound ranged from 81.6 to 115.7%. The reproducibility of this method is represented by the percent RSD at each fortification level for each compound (Table 4). The precision of the method was within 20%, which was fully compatible with the requirement set by the European Union and Association of Official Agricultural Chemists (AOAC). Furthermore, the interday precision was found to be 5.8−16.8% (Table 4), demonstrating good reproducibility of the method.

**Method Application.** The proposed method was applied to the analysis of real cereal samples. A total of 10 rice samples, 12 maize samples, and 20 wheat samples with different packaging materials, habitats, and brands were purchased

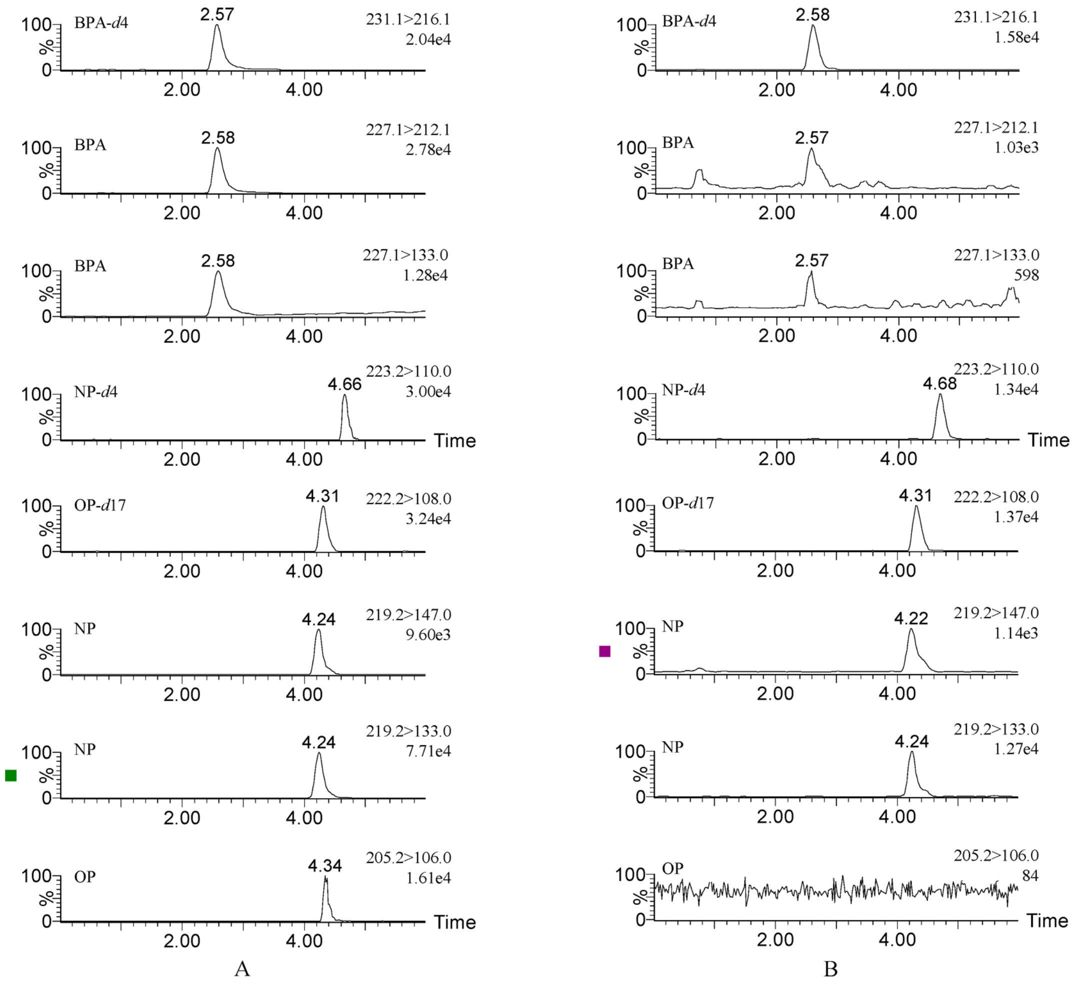

**Figure 3.** MRM chromatograms of BPA, NP, and OP and their internal standards in (A) 2 μg/L standards with 1 μg/L internal standards and (B) a rice real sample with 1 μg/L internal standards.

from supermarkets in Beijing, China, and analyzed. Figure 3 shows the MRM chromatograms of 1 rice sample. OP was not detected, but BPA was found in 9 samples, with levels ranging from 1.0 to 3.8 μg/kg, whereas NP was detected in all samples, at levels ranging from 9.4 to 1683.6 μg/kg (Table 5). The high concentrations of NP in several wheat and corn samples above the calibration range were re-evaluated by reducing the sample weighing to appropriate levels, still spiked with 25 ng of internal standards.

**Table 5. Concentrations of BPA, NP, and OP in Real Cereal Samples**

| sample | compound | frequency of detection (%) | mean (μg/kg) | median (μg/kg) | range (μg/kg) |
|---|---|---|---|---|---|
| rice, $n = 10$ | BPA | 1/10 | <LOQ | <LOQ | 1.0 |
| | NP | 10/10 | 43.4 | 33.5 | 9.4−106.0 |
| | OP | 0/12 | | | |
| maize, $n = 12$ | BPA | 3/12 | <LOQ | <LOQ | 1.0−1.7 |
| | NP | 12/12 | 139.4 | 49.3 | 20.6−1076.6 |
| | OP | 0/12 | | | |
| wheat, $n = 20$ | BPA | 3/12 | 0.6 | <LOQ | 1.1−3.8 |
| | NP | 12/12 | 396.8 | 257.3 | 9.4−1683.6 |
| | OP | 0/12 | | | |

The result of BPA was in accordance with the results by Gyllenhammar et al.[34], for they found that BPA levels were below LOQ (2 μg/kg) in all 11 cereal products. In addition, the results showed that NP was ubiquitous in various cereals, which was consistent with the findings by Güenther et al.,[15] Lu et al.,[16] and Gyllenhammar et al.[34] Besides, the concentration of NP (43.2 ± 35.2 μg/kg) in rice coincided with that reported in previous papers (39.7 ± 21.0 μg/kg in Taiwan[16] and 20−71 μg/kg in Sweden[34]). The level of NP in maize (49.3 μg/kg) was comparable with the study by She et al.[35] (72.5 μg/kg) in China. However, the occurrence of NP in wheat was quite different in different areas. For example, in Germany, pasta had a low level of NP with 1 μg/kg[15] and, in Taiwan, noodles had a level of NP with 5.8 ± 3.0 μg/kg,[16] while the level of NP in wheat analyzed in our study was 200- and 40-fold higher than that of the formers, respectively.

According to the Report of the National Diet and Nutrition Survey in China,[17] the mean daily intake of cereals for one adult (60 kg) is 402 g/day. Consequently, NP exposure was estimated at 1.6 μg/kg of body weight (bw) per day at the mean and 11.2 μg/kg of bw per day at the maximum.[36] The daily intake of NP was above the tolerant daily intake (TDI, 5 μg/kg of bw per day) recommended by the Danish Environmental Agency[37] when the maximum level was used, indicating that there was potential risk of NP exposure from consumption of cereals for Chinese adults.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Occurrences of BPA, NP, and OP in cereals (Table A). This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

## ■ REFERENCES

(1) Matozzo, V.; Gagné, F.; Marin, M. G.; Ricciardi, F.; Blaise, C. Vitellogenin as a biomarker of exposure to estrogenic compounds in aquatic invertebrates: A review. *Environ. Int.* **2008**, *34*, 531−545.

(2) Jobling, S.; Sumpter, J. P.; Sheahan, D.; Osborne, J. A.; Matthiessen, P. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. *Environ. Toxicol. Chem.* **1996**, *15*, 194−202.

(3) Tyl, R. W.; Myers, C. B.; Marr, M. C.; Sloan, C. S.; Castillo, N. P.; Veselica, M. M.; Seely, J. C.; Dimond, S. S.; Van Miller, J. P.; Shiotsuka, R. N. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. *Toxicol. Sci.* **2008**, *104*, 362.

(4) Jin, X.; Jiang, G.; Huang, G.; Liu, J.; Zhou, Q. 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. *Chemosphere* **2004**, *56*, 1113−1119.

(5) Sharma, V. K.; Anquandah, G. A. K.; Yngard, R. A.; Kim, H.; Fekete, J.; Bouzek, K.; Ray, A. K.; Golovko, D. Nonylphenol, octylphenol, and bisphenol-A in the aquatic environment: A review on occurrence, fate, and treatment. *J. Environ. Sci. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.* **2009**, *44*, 423−442.

(6) Wilson, N. K.; Chuang, J. C.; Lyu, C. Levels of persistent organic pollutants in several child day care centers. *J. Exposure Anal. Environ. Epidemiol.* **2001**, *11*, 449.

(7) Wilson, N. K.; Chuang, J. C.; Lyu, C.; Menton, R.; Morgan, M. K. Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. *J. Exposure Sci. Environ. Epidemiol.* **2003**, *13*, 187−202.

(8) Rudel, R. A.; Camann, D. E.; Spengler, J. D.; Korn, L. R.; Brody, J. G. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. *Environ. Sci. Technol.* **2003**, 37, 4543−4553.

(9) Meier, S.; Klungsoyr, J.; Boitsov, S.; Eide, T.; Svardal, A. Gas chromatography−mass spectrometry analysis of alkylphenols in cod (*Gadus morhua*) tissues as pentafluorobenzoate derivatives. *J. Chromatogr., A* **2005**, *1062*, 255−268.

(10) Tsuda, T.; Suga, K.; Kaneda, E.; Ohsuga, M. Determination of 4-nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate and other alkylphenols in fish and shellfish by high-performance liquid chromatography with fluorescence detection. *J. Chromatogr., B: Biomed. Sci. Appl.* **2000**, *746*, 305−309.

(11) Shao, B.; Han, H.; Li, D.; Ma, Y.; Tu, X.; Wu, Y. Analysis of alkylphenol and bisphenol A in meat by accelerated solvent extraction and liquid chromatography with tandem mass spectrometry. *Food Chem.* **2007**, *105*, 1236−1241.

(12) Otaka, H.; Yasuhara, A.; Morita, M. Determination of bisphenol A and 4-nonylphenol in human milk using alkaline digestion and cleanup by solid-phase extraction. *Anal. Sci.* **2003**, *19*, 1663−1666.

(13) Shao, B.; Han, H.; Tu, X.; Huang, L. Analysis of alkylphenol and bisphenol A in eggs and milk by matrix solid phase dispersion extraction and liquid chromatography with tandem mass spectrometry. *J. Chromatogr., B: Biomed. Sci. Appl.* **2007**, *850*, 412−416.

(14) Ackerman, L. K.; Noonan, G. O.; Heiserman, W. M.; Roach, J. A.; Limm, W.; Begley, T. H. Determination of bisphenol A in US infant formulas: Updated methods and concentrations. *J. Agric. Food Chem.* **2010**, *58*, 2307−2313.

(15) Guenther, K.; Heinke, V.; Thiele, B.; Kleist, E.; Prast, H.; Raecker, T. Endocrine disrupting nonylphenols are ubiquitous in food. *Environ. Sci. Technol.* **2002**, *36*, 1676−1680.

(16) Lu, Y. Y.; Chen, M. L.; Sung, F. C.; Wang, P. S. G.; Mao, I. Daily intake of 4-nonylphenol in Taiwanese. *Environ. Int.* **2007**, *33*, 903−910.

(17) Jin, S. *China National Nutrition and Health Survey Report: Nutrition and Health Data Set*, 2008.

(18) Carabias-Martinez, R.; Rodriguez-Gonzalo, E.; Revilla-Ruiz, P. Determination of endocrine-disrupting compounds in cereals by pressurized liquid extraction and liquid chromatography−mass spectrometry: Study of background contamination. *J. Chromatogr., A* **2006**, *1137*, 207−215.

(19) She, Y.; Wang, J.; Zheng, Y. Q.; Cao, W.; Wang, R.; Dong, F. S.; Liu, X. G.; Qian, M.; Zhang, H.; Wu, L. Determination of nonylphenol ethoxylate metabolites in vegetables and crops by high performance liquid chromatography tandem mass spectrometry. *Food Chem.* **2011**, *132*, 502−507.

(20) Inoue, K.; Kato, K.; Yoshimura, Y.; Makino, T.; Nakazawa, H. Determination of bisphenol A in human serum by high-performance liquid chromatography with multi-electrode electrochemical detection. *J. Chromatogr., B: Biomed. Sci. Appl.* **2000**, *749*, 17−23.

(21) Watabe, Y.; Kubo, T.; Nishikawa, T.; Fujita, T.; Kaya, K.; Hosoya, K. Fully automated liquid chromatography−mass spectrometry determination of 17 β-estradiol in river water. *J. Chromatogr., A* **2006**, *1120*, 252−259.

(22) Gallart-Ayala, H.; Moyano, E.; Galceran, M. On-line solid phase extraction fast liquid chromatography−tandem mass spectrometry for the analysis of bisphenol A and its chlorinated derivatives in water samples. *J. Chromatogr., A* **2010**, *1217*, 3511−3518.

(23) Gallart-Ayala, H.; Moyano, E.; Galceran, M. Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography−tandem mass spectrometry. *Anal. Chim. Acta* **2011**, *683*, 227−233.

(24) Ye, X.; Tao, L. J.; Needham, L. L.; Calafat, A. M. Automated on-line column-switching HPLC−MS/MS method for measuring environmental phenols and parabens in serum. *Talanta* **2008**, *76*, 865−871.

(25) Ye, X.; Bishop, A. M.; Needham, L. L.; Calafat, A. M. Automated on-line column-switching HPLC−MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk. *Anal. Chim. Acta* **2008**, *622*, 150−156.

(26) Ye, X.; Kuklenyik, Z.; Needham, L. L.; Calafat, A. M. Measuring environmental phenols and chlorinated organic chemicals in breast milk using automated on-line column-switching−high performance liquid chromatography−isotope dilution tandem mass spectrometry. *J. Chromatogr., B: Biomed. Sci. Appl.* **2006**, *831*, 110−115.

(27) Yan, W.; Li, Y.; Zhao, L.; Lin, J. M. Determination of estrogens and bisphenol A in bovine milk by automated on-line $C_{30}$ solid-phase extraction coupled with high-performance liquid chromatography−mass spectrometry. *J. Chromatogr., A* **2009**, *1216*, 7539−7545.

(28) Rodriguez-Mozaz, S.; Lopez de Alda, M. J.; Barcelo, D. Advantages and limitations of on-line solid phase extraction coupled to liquid chromatography−mass spectrometry technologies versus biosensors for monitoring of emerging contaminants in water. *J. Chromatogr., A* **2007**, *1152*, 97−115.

(29) Niu, Y.; Zhang, J.; Wu, Y.; Shao, B. Simultaneous determination of bisphenol A and alkylphenol in plant oil by gel permeation

chromatography and isotopic dilution liquid chromatography−tandem mass spectrometry. *J. Chromatogr., A* **2011**, *1218*, 5248−5253.

(30) Mueller, D. M.; Duretz, B.; Espourteille, F. A.; Rentsch, K. M. Development of a fully automated toxicological LC−MS$^n$ screening system in urine using on-line extraction with turbulent flow chromatography. *Anal. Bioanal. Chem.* **2011**, *400*, 89−100.

(31) Ahel, M.; Giger, W. Aqueous solubility of alkylphenols and alkylphenol polyethoxylates. *Chemosphere* **1993**, *26*, 1461−1470.

(32) Staples, C. A.; Dorn, P. B.; Klecka, G. M.; O'Block, S. T.; Harris, L. R. A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere* **1998**, *36*, 2149−2173.

(33) Berkner, S.; Streck, G.; Herrmann, R. Development and validation of a method for determination of trace levels of alkylphenols and bisphenol A in atmospheric samples. *Chemosphere* **2004**, *54*, 575−584.

(34) Gyllenhammar, I.; Glynn, A.; Darnerud, P. O.; Lignell, S.; van Delft, R.; Aune, M. 4-Nonylphenol and bisphenol A in Swedish food and exposure in Swedish nursing women. *Environ. Int.* **2012**, *43*, 21−28.

(35) She, Y.; Wang, J.; Zheng, Y. Q.; Cao, W.; Wang, R.; Dong, F. S.; Liu, X. G.; Qian, M.; Zhang, H.; Wu, L. Determination of nonylphenol ethoxylate metabolites in vegetables and crops by high performance liquid chromatography tandem mass spectrometry. *Food Chem.* **2012**, *132*, 502−507.

(36) Reuss, R.; Leblanc, J. C. Background paper on bisphenol A exposure assessment. *Proceedings of the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Meeting on Bisphenol A (BPA)*; Ottawa, Ontario, Canada, Nov 2−5, 2010.

(37) Cunny, H. C.; Mayes, B. A.; Rosica, K. A.; Trutter, J. A.; Van Miller, J. P. Subchronic toxicity (90-day) study with *para*-nonylphenol in rats. *Regul. Toxicol. Pharmacol.* **1997**, *26*, 172−178.