

# Analysis of Bisphenol A, Nonylphenol, and Natural Estrogens in Vegetables and Fruits Using Gas Chromatography–Tandem Mass Spectrometry

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## S Supporting Information

**ABSTRACT:** Bisphenol A (BPA), nonylphenol (NP), and steroidal estrogens in vegetables and fruits were analyzed using gas chromatography with tandem mass spectrometry (GC-MS/MS). Isotope dilution standards were spiked before the extraction to account for extraction inefficiency and loss of analytes during sample workup. Recoveries were >90% for all of the compounds in each matrix. The limit of detection (LOD) ranged from 0.03 to 0.3  $\mu\text{g kg}^{-1}$ , whereas the limit of quantitation (LOQ) ranged from 0.1 to 1.0  $\mu\text{g kg}^{-1}$ . All analytes can be monitored in a single GC-MS/MS run with a run time of 20 min. Occurrence of these endocrine-disrupting chemicals (EDCs) in vegetables and fruits from local markets was observed using the established analytical method. BPA was detected in all vegetable and fruit samples, ranging from  $0.2 \pm 0.1$  to  $9.0 \pm 4.9 \mu\text{g kg}^{-1}$ , indicating significant exposure potential for humans. NP was detected in pumpkin, sweet potato, citrus, and apple samples. The concentration of 4-n-NP ranged from  $5.3 \pm 2.4$  to  $18.9 \pm 8.0 \mu\text{g kg}^{-1}$ , whereas that of 4-NP ranged from  $5.1 \pm 2.6$  to  $12.2 \pm 3.6 \mu\text{g kg}^{-1}$ . Concentrations of 17- $\beta$ -estradiol in vegetables and fruits ranged from  $1.3 \pm 0.4$  to  $2.2 \pm 1.0 \mu\text{g kg}^{-1}$  except those in tomato and strawberry, in which no 17- $\beta$ -estradiol was detected. The estimated daily intake of 17- $\beta$ -estradiol was beyond the recommended acceptable daily intake (ADI) for children as recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

**KEYWORDS:** endocrine-disrupting chemicals, tandem mass spectrometry, bisphenol A, alkylphenols, natural estrogens

## INTRODUCTION

There is growing interest concerning the possible health threat posed by endocrine-disrupting chemicals (EDCs), which are substances that interfere with hormone biosynthesis, metabolism, or action resulting in a deviation from normal homeostatic control or reproduction.<sup>1</sup> These compounds, either natural or synthetic, interact with estrogen receptors in organisms. Among the many EDCs, bisphenol A (BPA), alkylphenols (APs) (including nonylphenol (NP) and octylphenol (OP)), and natural estrogens (including estrone and 17- $\beta$ -estradiol) have attracted public attention because of their potential negative effects on human and environmental health<sup>1–5</sup> and wide occurrence in various environments<sup>4,6–9</sup> and foodstuffs.<sup>10–12</sup>

BPA is an industrial chemical used to make a hard, clear plastic known as polycarbonate. It has been used in many consumer products, including reusable water bottles.<sup>13</sup> BPA is also found in epoxy resins used as protective linings inside metal-based food and beverage cans.<sup>10,13</sup> These uses of BPA were approved under FDA's food additive regulations from the 1960s. NP and OP are common biodegradation products of nonylphenol polyethoxylates (NPEOs) and octylphenol polyethoxylates (OPEOs) in various environments.<sup>6,7,9,14–16</sup> NPEOs are one of the most widely used classes of nonionic surfactants, encompassing >80% of the world market.<sup>17</sup> Their biodegradation product, NP, has many isomers with a phenol group and a linear (4-n-NP) or branched carbon chain of nine carbon atoms.<sup>11,14</sup> Steroidal estrogens including estrone and 17- $\beta$ -estradiol are all essential natural endocrine chemicals in

human and animal bodies.<sup>8,18–20</sup> These hormones can be excreted with urine and feces and often are not degraded during wastewater treatment plant processes. The analysis of these contaminants is usually a challenge due to their low concentrations (in the environment and foodstuffs) and complicated sample cleanup procedures. Because consumption of foods containing EDCs through the food chain may be harmful to human health, effective analysis methods are needed.

Gas chromatography–mass spectrometry (GC-MS) has been a preferred technique for determination of EDCs in various environmental matrices and foodstuffs.<sup>8,10–12,14</sup> The quantification is usually performed in full scan or selected ion monitoring modes.<sup>8,10–12,14</sup> However, losses of sensitivity can be a significant problem for more complex matrices because many coexisting compounds may produce similar ions with the analytes or hinder the ionization of analytes during the ionization process.<sup>21</sup> For this reason, complicated sample cleanup procedures are usually performed for complex matrices.<sup>8,11,12</sup> Tandem mass spectrometry (MS/MS) has been a preferred technique for the determination of steroidal hormones as it is generally able to achieve improved detection limits in more complex matrices.<sup>8,22–27</sup> A number of GC-MS/MS methods have been developed for the analysis of estrogenic

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Table 1. Optimized Tandem Mass (MS/MS) Conditions

compound	retention time (min)	precursor ion	RF storage	resonant excitation voltage (V)	product ions used for quantification ( <i>m/z</i> )
OP	7.634	179	50	1.0	73
4-n-NP	8.627	179	50	1.0	73
4-NP	6.0–8.0	207 + 221 + 235	50	1.0	179
<sup>13</sup> C6-4-n-NP	8.626	185	50	1.0	73
BPA	11.326	357	130	1.2	191 + 341
<sup>13</sup> C12-BPA	11.325	369	130	1.2	197 + 353
estrone	14.979	342	130	1.2	257 + 244
<sup>13</sup> C6-estrone	14.930	348	130	1.2	260 + 246
17- $\beta$ -estradiol	15.132	416	130	1.2	285 + 326
<sup>13</sup> C6- $\beta$ -estradiol	15.141	422	130	1.2	288 + 332
E2-acetate (surrogate)	15.840	386	130	1.2	244 + 269 + 297 + 326

steroids in environmental samples.<sup>23,25,26</sup> In this study, BPA, NP, and steroidal estrogens in vegetables and fruits were analyzed using GC-MS/MS. To develop a simple, reliable, sensitive analytical, and laboratory friendly method, vegetable and fruit samples were extracted using acetone followed by GC-MS/MS analysis. No specific sample cleanup step was needed. The isotope dilution technique was applied in this study to account for extraction inefficiency and loss of analytes during sample workup according to other studies.<sup>8,28–32</sup> All analytes were monitored in a single GC-MS/MS run with a run time of 20 min. The occurrence of EDCs in vegetables and fruits from commercial sources and their potential threat to human health were evaluated using the developed analytical method.

## MATERIALS AND METHODS

### Standards, Reagents, Chemicals, and Plant Materials.

Standards of 4-n-NP ( $\geq 98\%$ ), BPA ( $\geq 99\%$ ), 17- $\beta$ -estradiol ( $\geq 98\%$ ), estrone ( $\geq 99\%$ ), and  $\beta$ -estradiol 17-acetate ( $\geq 99\%$ , served as surrogate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 4-NP (99%, Technical grade, mixture of isomers of branched NP) and 4-octylphenol (4-OP) (99%) were purchased from ACROS Organics (Morris Plains, NJ, USA). Isotope dilution standards (IDS) including <sup>13</sup>C12-BPA (ring-<sup>13</sup>C12, 99%), <sup>13</sup>C6-4-n-NP (ring-<sup>13</sup>C6, 99%), <sup>13</sup>C6-estrone (13,14,15,16,17,18-<sup>13</sup>C6, 99%), and <sup>13</sup>C6- $\beta$ -estradiol (13,14,15,16,17,18-<sup>13</sup>C6, 99%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Standards were individually dissolved in acetonitrile at concentrations ranging from 1 to 10 mg L<sup>-1</sup>. Solvents including acetone, hexane, and methylene chloride were of pesticide grade, whereas acetonitrile was of high-performance liquid chromatography (HPLC) grade (Fisher Scientific, Pittsburgh, PA, USA).

This study used plant materials from separate sources for construction of the calibration curve and for evaluation of the occurrence of EDCs. Plant materials included lettuce, tomato, potato, pumpkin, carrot, apple, strawberry, and citrus. The lettuce, tomato, and potato used in the spiking studies for constructing the calibration curves and developing the extraction method were grown under contaminant-free conditions in a greenhouse; whereas contaminant-free pumpkin, carrot, apple, and strawberry samples were obtained from a local supermarket and citrus samples were obtained from a citrus grove located at the U.S. Department of Agriculture (USDA) Horticulture Research Laboratory, Fort Pierce, FL, USA. All of these plant materials were confirmed by prescreening using GC-MS/MS to ensure they were residue-free. Following development of the extraction/analytical methods, vegetables and fruits were obtained from separate commercial sources (not the same sources used for method development) for evaluation of the potential occurrence of these EDCs in commercially available food crops.

**Sample Pretreatment Procedure.** The fruit and vegetable samples with peel intact were cut into small pieces and homogenized with 200  $\mu$ L of 1 N HCl in a laboratory blender (Waring Laboratory,

Torrington, CT, USA) for 5 min. For the spiking experiments, analyte standards were added before the homogenization was performed. The homogenized sample (5 g) was mixed with 100 mL of acetone after the IDS and surrogate ( $\beta$ -estradiol 17-acetate) were added. The surrogate was spiked at 50  $\mu$ g kg<sup>-1</sup>. Relative extraction efficiencies were measured by spiking IDS and surrogates into the sample before sample extraction. Spiking in this fashion accounts for extraction inefficiencies and losses of analytes during sample workup.<sup>8,28–32</sup> Acetone was used for the extraction because high recoveries were observed for extraction of estrogens from solid samples.<sup>33</sup> The samples were then blended and subjected to ultrasonic extraction for 15 min following the method of Wu et al.<sup>34</sup> Extraction was performed using a 50–60 Hz ME 2.1 ultrasonic cleaner (Mettler Electronics Corp., Anaheim, CA, USA), followed with vacuum filtration using Whatman GF/D glass fiber filters (pore size = 2.7  $\mu$ m, Fisher Scientific). The extracts were evaporated to dryness using a water bath at a temperature of 70 °C and redissolved in 0.5 mL of acetonitrile. Nonspecific acidic hydrolysis of the extract was next performed by adding 20 mL of 1 N HCl and incubating at 80 °C for 0.5 h to release the conjugated estrogens as respective free forms.<sup>35</sup> The acid solution with the target compounds was then extracted with methylene chloride (10 mL). The extract was evaporated to dryness using a RapidVap 79000-02 vacuum evaporator (Labconco Corp., Kansas City, MO, USA) before being subjected to chemical derivatization.

**Trimethylsilyl Derivatization.** In preparation for GC-MS/MS analysis, all samples were chemically derivatized by adding 50  $\mu$ L of *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (99%)–trimethylchlorosilane (TMCS) (1%) and 50  $\mu$ L of pyridine to the dried extracts. The vials were sealed and heated at 68 °C for 30 min. The derivatized extracts were allowed to cool to room temperature and were then dried using ultrahigh-purity (99.999%) nitrogen. The dried derivatized residues were dissolved into 100  $\mu$ L of hexane for analysis by GC-MS/MS.

**GC-MS/MS Analysis.** GC-MS/MS analysis of the derivatized extracts was achieved using a Varian 3800 gas chromatograph connected to a Varian 4000 mass spectrometer with an electron ionization (EI) source (Agilent Technologies, Santa Clara, CA, USA). The optimized EI conditions were as follows: ionization energy, 70 eV; ionization temperature, 200 °C; and scan time, 0.5 s. The target compounds were separated on an Rxi-5MS (30 m  $\times$  0.25 mm, 0.25  $\mu$ m film thickness) capillary column (Restek Co., Bellefonte, PA, USA). Helium was used as the carrier gas and was maintained at a constant flow rate of 1 mL min<sup>-1</sup>. The temperatures of the transfer line, ion trap, and manifold were set at 270, 150, and 50 °C, respectively. A sample volume of 2  $\mu$ L was injected in splitless mode. The inlet temperature was held at 280 °C, and the oven temperature program was as follows: initial temperature, 130 °C; increased to 280 °C at 10 °C min<sup>-1</sup>; held at 280 °C for 5 min. The solvent delay was set to 5 min. The quantification and confirmation of analytes were performed in multiple reaction monitoring (MRM) mode; specific precursor ions were specified for each compound, and specific product ions were monitored. Table 1 summarizes the optimized operational conditions for quantification.

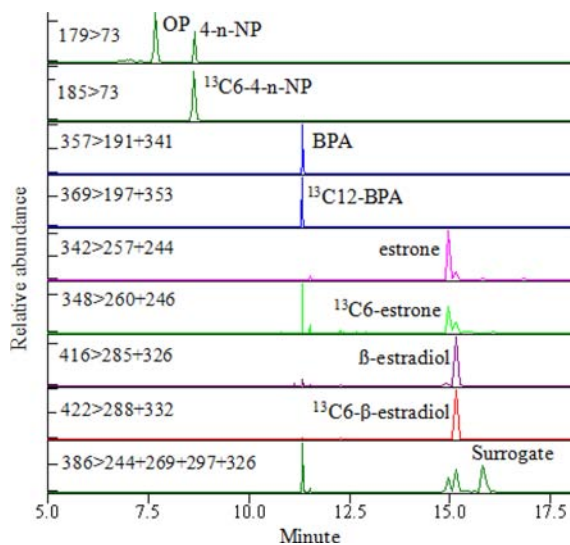
**Calibration and Method Validation.** Matrix-matched standards were employed for calibration of the GC-MS/MS system. Analyte-free matrices for each fruit/vegetable were identified by screening several sources using the procedures previously described. Calibration standards were added to raw plant materials, which were then subjected to all extraction and preparation processes. Each analyte was calibrated at 1, 5, 10, 20, 50, 100, and 200  $\mu\text{g kg}^{-1}$ . An isotopically labeled IDS for each analyte was also added to each calibration point at a concentration of 20  $\mu\text{g kg}^{-1}$  to generate a relative response ratio. Linear regression was used for generation of calibration curves for all analytes. Coefficients of determination for calibration curves were required to be at least 0.990.

Because of the multiple steps in sample preparation and extraction, many opportunities for increasing systematic errors exist. Potential systematic errors associated with this method were characterized using spiking techniques to measure relative recoveries and percent relative standard deviations (%RSD). The analytes and IDS were spiked at 20  $\mu\text{g kg}^{-1}$ , and the spiked plant samples were extracted and analyzed as previously described. The limits of detection (LOD) for the analytical procedure were estimated from the concentration of analytes that provided signals equal to 3 times the baseline noise for the analysis. The limits of quantification (LOQ) were equal to the concentration of analytes that provided signals equal to 10 times the baseline noise for the analysis.

**Occurrence of EDCs in Consumer-Available Vegetables and Fruits.** To evaluate the potential occurrence of selected estrogenic contaminants in commonly available plant materials, six samples of each material were randomly selected from local markets, extracted, and analyzed using the methods previously described.

## RESULTS AND DISCUSSION

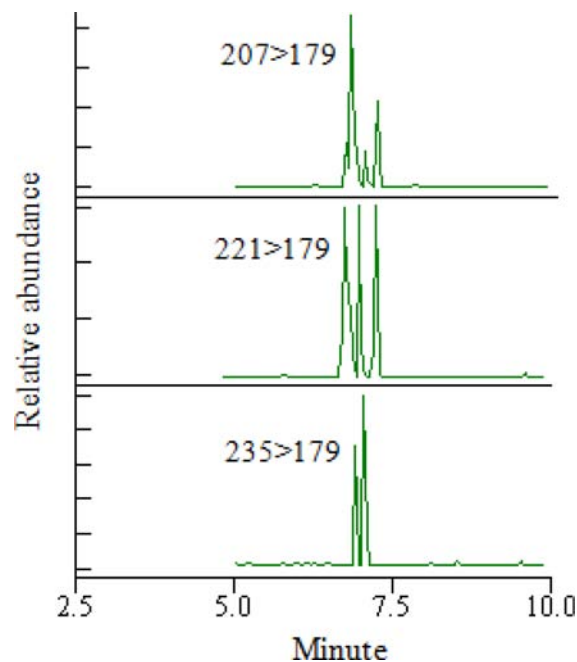
**Typical Chromatograms of Analytes.** Example chromatograms for the analytes and IDSs in tomatoes are shown in Figure 1. The 4-n-NP, BPA, estrone, and 17- $\beta$ -estradiol



**Figure 1.** Typical MS-MS chromatograms for the quantification ions under optimized conditions for analytes spiked into tomato samples. OP, 4-n-NP, BPA, estrone, and 17- $\beta$ -estradiol were spiked at 20  $\mu\text{g kg}^{-1}$ , whereas the surrogate ( $\beta$ -estradiol 17-acetate) was spiked at 50  $\mu\text{g kg}^{-1}$ . Isotope dilution standards (IDS) were spiked at 20  $\mu\text{g kg}^{-1}$ .

coeluted with their IDS. Two peaks of the alkylphenols (OP and 4-n-NP) were observed in the chromatogram with the precursor ion  $m/z$  179. The OP eluted first, followed by 4-n-NP. The primary ion for 4-n-NP was  $m/z$  179. Primary ions of  $m/z$  207, 221, and 235 (Figure S1, Supporting Information) were used for 4-NP because technical 4-NP is a mixture of

many isomers, especially the branched alkyl chain NP,<sup>11,14</sup> which tend to form these primary ions. A typical peak pattern (6–8 min) of technical 4-NP was used for quantification using the daughter ion  $m/z$  179 of the precursor ions (Figure 2).



**Figure 2.** Typical MS-MS chromatograms for 4-NP spiked into tomato samples under optimized conditions. 4-NP was spiked at 50  $\mu\text{g kg}^{-1}$ . Precursor  $m/z$  fragments were 207, 221, and 235. Product/quantification  $m/z$  is 179 for each precursor.

Sharp narrow peaks for BPA and its IDS were observed in the chromatograms with precursor ions  $m/z$  357 and 369, respectively. All natural estrogens and their IDSs were eluted near 15 min.

**Comparison of Response across Matrices.** Relative response ratios for each analyte–IDS pair were examined across different vegetable and fruit matrices (Table 2). Relative response factors within each plant matrix for the analyte pairings BPA/<sup>13</sup>C12-BPA, 4-n-NP/<sup>13</sup>C6-4-n-NP, estrone/<sup>13</sup>C6-

**Table 2.** Relative Response Factors of Each Matrix for the Following Analyte/Surrogate Pairings: BPA/<sup>13</sup>C12-BPA, 4-n-NP/<sup>13</sup>C6-4-n-NP, 4-NP/<sup>13</sup>C6-4-n-NP, OP/<sup>13</sup>C6-4-n-NP, Estrone/<sup>13</sup>C6-Estrone, and 17- $\beta$ -Estradiol/<sup>13</sup>C6- $\beta$ -Estradiol

	BPA	4-n-NP	4-NP	OP	estrone	17- $\beta$ -estradiol
matrix						
lettuce	2.23	2.46	3.99	2.82	2.42	2.46
tomato	2.12	2.46	4.75	2.01	2.74	1.64
pumpkin	2.34	2.29	4.46	2.37	2.58	1.49
potato	2.54	1.79	4.76	2.53	2.44	1.26
carrot	2.44	1.83	4.58	2.44	2.37	1.44
citrus	1.07	1.08	3.69	1.68	1.43	3.09
apple	1.49	1.87	4.54	2.19	2.29	1.46
strawberry	2.13	2.24	4.19	2.64	2.59	1.69
av	2.05	2.00	4.37	2.34	2.36	1.82
SD	0.51	0.46	0.38	0.37	0.40	0.63
RSD <sup>a</sup> (%)	24.8	23.1	8.7	15.6	17.0	34.6

<sup>a</sup>RSD refers to the relative standard deviation.



**Table 3. Systematic Error (Quantified as Percent Recovery), Limit of Detection (LOD), and Limit of Quantification (LOQ) for Endocrine-Disrupting Chemicals in Vegetables and Fruits ( $n = 4$ )**

	matrix	BPA	4-n-NP	4-NP	OP	estrone	17- $\beta$ -estradiol
% recovery (% RSD)	lettuce	99 (4)	96 (6)	102 (4)	98 (6)	97 (4)	99 (4)
	tomato	96 (5)	98 (5)	96 (4)	100 (8)	97 (3)	99 (3)
	pumpkin	96 (5)	98 (8)	95 (5)	102 (3)	97 (5)	98 (5)
	potato	102 (7)	98 (4)	99 (7)	97 (4)	97 (4)	97 (6)
	carrot	95 (5)	101 (4)	98 (6)	98 (3)	99 (6)	98 (6)
	citrus	98 (4)	101 (4)	96 (5)	98 (4)	96 (6)	100 (3)
	apple	96 (4)	102 (8)	98 (7)	99 (5)	98 (4)	100 (6)
	strawberry	98 (6)	96 (4)	93 (6)	97 (8)	98 (5)	101 (6)
LOD ( $\mu\text{g kg}^{-1}$ )		0.03	0.1	0.3	0.1	0.2	0.2
LOQ ( $\mu\text{g kg}^{-1}$ )		0.1	0.3	1.0	0.3	0.8	0.7

**Table 4. Concentrations of Typical Endocrine-Disrupting Chemicals in Vegetables and Fruits ( $n = 6$ )**

plant material	BPA	4-n-NP	4-NP	OP	estrone	17- $\beta$ -estradiol
lettuce	2.0 $\pm$ 1.2	nd <sup>a</sup>	nd	nd	nd	1.9 $\pm$ 1.7
tomato	0.2 $\pm$ 0.1	nd	nd	nd	nd	nd
pumpkin	4.2 $\pm$ 2.8	6.0 $\pm$ 3.1	5.3 $\pm$ 3.2	nd	<0.8	1.6 $\pm$ 0.3
potato	4.3 $\pm$ 2.1	nd	nd	nd	<0.8	1.4 $\pm$ 0.4
carrot	4.0 $\pm$ 2.3	5.3 $\pm$ 2.4	5.1 $\pm$ 2.6	nd	<0.8	1.4 $\pm$ 0.6
citrus	9.0 $\pm$ 4.9	18.5 $\pm$ 7.6	11.0 $\pm$ 3.2	nd	<0.8	2.2 $\pm$ 1.0
apple	7.5 $\pm$ 5.4	9.9 $\pm$ 4.0	7.2 $\pm$ 3.6	nd	<0.8	1.3 $\pm$ 0.4
strawberry	2.0 $\pm$ 1.4	nd	nd	nd	nd	nd

<sup>a</sup>nd, not detected.

estrone, and 17- $\beta$ -estradiol/<sup>13</sup>C6-17- $\beta$ -estradiol ranged from 1.07 to 3.09. BPA, 4-n-NP, estrone, and estradiol fluctuated significantly in response and had correspondingly higher coefficients of variation between matrices. The relative standard deviations (RSD) ranged from 17.0 to 34.6%. Relative response factors within each plant matrix for the analyte pairing 17- $\beta$ -estradiol/<sup>13</sup>C6- $\beta$ -estradiol ranged from 1.29 to 3.09. 4-NP was analyzed through a quasi-isotope dilution method using <sup>13</sup>C6-4-n-NP as the sole "labeled" IDS in the isotopic dilution calculation, and the relative responses were relatively high, ranging from 3.69 to 4.76. OP was analyzed using the quasi-isotope dilution method based on the OP/<sup>13</sup>C6-4-n-NP pairing, resulting in relative responses ranging from 1.68 to 2.82.

**Method Validation.** Because of the multistep sample pretreatment procedures, many opportunities for increasing systematic errors may exist. Results indicate that this error was <7% for all of the compounds in each matrix (Table 3), indicating the reliability of this method for analysis of estrogenic contaminants in vegetables and fruits. The method was evaluated with respect to precision and detection limits. RSDs for target analytes were all <10%, which is similar to those reported by other researchers using isotope dilution methods for analysis of estrogenic contaminants in water and soil using GC-MS.<sup>8,36</sup> The method LOD ranged from 0.03 to 0.3  $\mu\text{g kg}^{-1}$ , whereas the LOQ ranged from 0.1 to 1.0  $\mu\text{g kg}^{-1}$ , indicating the applicability of this method for analysis of trace concentrations of these EDCs in vegetables and fruits. This LOD range is similar to that reported by Gamon et al.<sup>37</sup> for a GC-MS/MS method analyzing 80 kinds of pesticides in fruits and vegetables (0.01–35.0  $\mu\text{g kg}^{-1}$ ). Another study reported a similar LOD (0.2  $\mu\text{g kg}^{-1}$ ) for analyzing estrone and 17- $\beta$ -estradiol in other solid samples such as sediment using GC-MS/MS.<sup>38</sup> The GC-MS/MS method developed in these studies provides an effective, robust method for the simultaneous analysis of EDCs in fresh vegetables and fruits.

**Occurrence of EDCs in Consumer-Available Vegetables and Fruits.** Using the developed GC-MS/MS method, the occurrence of EDCs in vegetables and fruits was evaluated using vegetable and fruit samples obtained from local commercial sources. BPA was detected in all vegetable and fruit samples, illustrating potentially wide occurrence of BPA in fresh food (Table 4). Concentrations of BPA, ranging from 0.2  $\pm$  0.1 to 9.0  $\pm$  4.9  $\mu\text{g kg}^{-1}$ , were lower than those reported in canned beans or tomatoes.<sup>10,12</sup> NP was detected in pumpkin, carrot, citrus, and apple samples. The concentration of 4-n-NP ranged from 5.3  $\pm$  2.4 to 18.9  $\pm$  8.0  $\mu\text{g kg}^{-1}$ , whereas that of 4-NP ranged from 5.1  $\pm$  2.6 to 12.2  $\pm$  3.6  $\mu\text{g kg}^{-1}$ . Guenther et al.<sup>11</sup> reported concentrations of NP in apple and tomato of almost 20  $\mu\text{g kg}^{-1}$ . The presence of 17- $\beta$ -estradiol in vegetables and fruits was also observed in the current study. Concentrations of 17- $\beta$ -estradiol ranged from 1.3  $\pm$  0.4 to 2.2  $\pm$  1.0  $\mu\text{g kg}^{-1}$  except in tomato and strawberry. The presence of 17- $\beta$ -estradiol (2–4  $\mu\text{g kg}^{-1}$ ) in French beans has been reported.<sup>39,40</sup> The detection of BPA, NP, and natural estrogens indicated the feasibility of applying this GC-MS/MS method for the determination of trace concentrations of EDCs in vegetables and fruits. BPA, NP, and 17- $\beta$ -estradiol have been frequently detected in foodstuffs using GC-MS or HPLC analysis.<sup>10–12,39,40</sup> To our knowledge, this is the first report of the application of a tandem mass spectrometry (MS/MS) method for the analysis of EDCs in plant samples such as vegetables and fruits. The detection of BPA, 17- $\beta$ -estradiol, 4-n-NP, 4-NP, and estrone in some of the vegetables and fruits purchased from local markets supports the applicability of using this method to determine the presence of trace EDCs in vegetables and fruits.

The highest concentration of BPA in vegetables was observed in potato, whereas that of natural estrogens was observed in lettuce. The highest concentrations of APs in vegetables were observed in pumpkin. Assuming a per capita consumption of 534 g day<sup>-1</sup> fresh vegetables<sup>41</sup> and that the

highest detected concentrations of EDCs in vegetables are representative of all vegetables, the daily intakes of BPA, NP (4-n-NP and 4-NP), and 17- $\beta$ -estradiol through the consumption of vegetables would be 2.3, 6.0, and 1.0  $\mu\text{g}$ , respectively. The highest concentrations of BPA, NP, and 17- $\beta$ -estradiol in fruit were observed in citrus. Assuming a per capita consumption of 383 g day<sup>-1</sup> fruit<sup>42</sup> and that highest concentrations of EDCs in citrus are representative of all fruit, the daily intake of BPA, NP, and 17- $\beta$ -estradiol would be 3.4, 11.3, and 0.8  $\mu\text{g}$  day<sup>-1</sup>, respectively. On the basis of these data, the combined (vegetables and fruits) daily intake of BPA, NP, and 17- $\beta$ -estradiol through consumption of fresh foodstuff would be 5.7, 17.3, and 1.8  $\mu\text{g}$  day<sup>-1</sup>, respectively. According to Guenther et al.,<sup>11</sup> the daily intake of NP via food for an adult was 7.5  $\mu\text{g}$  day<sup>-1</sup>, which was much lower than the estimated daily intake of NP. The tolerable daily intake (TDI) proposed by the European Commission Scientific Committee on Food (ECSCF) is 10  $\mu\text{g}$  kg<sup>-1</sup> body weight day<sup>-1</sup>,<sup>12</sup> which means that the TDI of BPA for a 60 kg adult is <60  $\mu\text{g}$  day<sup>-1</sup> provided that only 10% of the TDI is allocated to ingestion of soil.<sup>43</sup> The preliminary TDI value for NP derived by the Danish Institute of Safety and Toxicology (DIST) is 5  $\mu\text{g}$  day<sup>-1</sup> kg<sup>-1</sup> body weight, with 10% of the TDI being allocated to ingestion of soil.<sup>43</sup> Thus, the TDI of NP for a 60 kg adult would be <30  $\mu\text{g}$  day<sup>-1</sup>.<sup>43</sup> According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the acceptable daily intake (ADI) of 17- $\beta$ -estradiol for a 60 kg adult is 3.0  $\mu\text{g}$  day<sup>-1</sup> and that for a 10 kg child is 0.5  $\mu\text{g}$  day<sup>-1</sup>.<sup>44</sup> The estimated daily intakes of BPA and NP via vegetables and fruits in this study are lower than the maximum allowable values established by the ECSCF and DIST, whereas that of 17- $\beta$ -estradiol was above the JECFA recommended ADI for a 10 kg child. More attention should be paid to the monitoring of EDCs in vegetables and fruits to better characterize potential human exposures through ingestion. The method outlined in this study may facilitate the study of EDCs in food sources.

## ■ ASSOCIATED CONTENT

### ● Supporting Information

Mass spectra of 4-n-NP and 4-NP. 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.

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