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Direct analysis of trace level bisphenol A, octylphenols and nonylphenol in bottled water and leached from bottles by ultra-high-performance liquid chromatography/tandem mass spectrometry

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A high-throughput method was developed for the direct analysis of trace level bisphenol A (BPA), 4-n-octylphenol (4-n-OP), 4-*tert*-octylphenol (4-t-OP) and 4-n-nonylphenol (4-n-NP) in water samples by ultra-high-performance liquid chromatography/tandem mass spectrometry (uHPLC/MS/MS) using an isotope-labeled internal standard. Aliquots of water samples were spiked with the internal standard and analyzed without sample cleanup or enrichment. All target analytes were chromatographically separated within 3 min and detected using the highly selective multiple reaction monitoring (MRM) detection mode. The method detection limits were statistically calculated and ranged from 0.040 ppb (BPA) to 0.057 ppb (4-n-NP). Excellent correlation of determination was achieved for each analyte with r greater than 0.995. Precision was achieved within 8% relative standard deviation (RSD) for all analytes, and recoveries from spiked samples ranged from 97% to 106.2% except for 4-n-OP which may be corrected by using an isotope-labeled analog as internal standard. This method was used for analyzing eight randomly selected bottled water samples and found no detectable target analytes. This method was also used to analyze target analytes leached from bottles (6 low cost bottles and 6 brand-name baby-feeding bottles). Low levels of BPA were found in three bottles after they had been heated in a microwave oven, and a trace amount of 4-n-NP was also found in three bottles. 4-t-OP, which has not been reported as a leachable chemical, was found in two brand name baby-feeding bottles from the same manufacturer, and was confirmed with bottles from different batches. Copyright © 2010 John Wiley & Sons, Ltd.

Bisphenol A (BPA) is a widely used chemical in high-production volume manufacturing of polycarbonate plastics and epoxy resins.[1] Human exposure to BPA can come from food packaging, including food containers and plastic bottles, and the inner protective coatings of metal cans. Although studies are underway to evaluate the impact of BPA on health and the environment, and currently reported results are inconclusive, actions have been taken proactively to protect sensitive populations such as infants and children. Canada proposed banning BPA to reduce the exposure for newborns and infants[2] in 2009, the US Food and Drug Administration (FDA) is taking steps to reduce BPA exposure with respect to food supplies, and the US Environmental Protection Agency (EPA) recently added BPA to the list of target chemicals for possible regulation.[1] The US state of Wisconsin banned BPA in products intended for used by children in March 2010, joining a growing list of states that includes Minnesota, Connecticut, and Washington. Octylphenol (OP) and nonylphenol (NP) are the degradation products of widely used nonionic surfactants: alkylphenol ethoxylates. The estrogenic and toxic effects of OP and NP have been well studied and reported.[3] NP has been regulated in the EPA's *Aquatic Life Ambient Water Criteria: Nonylphenol* with 6.6 μg/L as the maximum average level[4] for fresh water. Recent reports show NP being detected in food, beverages[5,6] and food wrappers,[7] posing another concern for food safety and food packaging. It is currently unclear whether the NP found in food comes from alkylphenol ethoxylates (APEOs) which are used as dispersing or stabilizing agents during plastic manufacturing, or from the hydrolysis of tris(nonylphenyl)phosphite (TNPP) which is used as an antioxidant.[7] To monitor and reduce human exposure to BPA, nonylphenol and structurally related octylphenols, an analytical method is required for the quantitative analysis of water, food and containers.

Reported analytical methods for BPA and nonylphenol analysis may involve solid-phase extraction (SPE) for sample preparation, followed by chromatographic separation, including gas chromatography (GC) and liquid chromatography (LC), and UV, fluorescence or mass spectrometry (MS, MS/

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MS) detection.[7–14] GC/MS methods were preferred although they usually involve the derivatization of analytes and thus are not suitable for high-throughput analysis. Reported LC methods for the simultaneous analysis of BPA and phenols have greater than 30 min run times and used large sample volumes with SPE concentration,[10,14,15] and therefore are not suitable for fast analysis.

This study describes a high-throughput ultra-high-performance liquid chromatography tandem mass spectrometry (uHPLC/MS/MS) method for the simultaneous quantification of BPA and phenols using a small particle size analytical column. Total chromatographic separation was achieved within 3 min, thus significantly reducing chromatographic run time. Selective and sensitive detection was achieved using tandem mass spectrometry, allowing direct analysis of sub-ppb level target analytes without labor-intensive SPE cleanup and sample enrichment.

Among the tested bottles in this study, BPA was found in several baby-feeding bottles labeled 'BPA Free' when heated, and nonylphenol was also observed at low levels. It is believed that it is the first time that 4-*tert*-octylphenol has been found in baby-feeding bottles.

EXPERIMENTAL

Chemicals and reagents

Bisphenol A (Cat. No. 239658) was purchased from Aldrich (St. Louis, MO, USA), and isotope-labeled BPA internal standard (BPA-d_{16}, Cat. No. D-2151) was purchased from C/D/N Isotopes (Pointe-Claire, Quebec, Canada). 4-*tert*-Octylphenol (4-t-OP), 4-n-octylphenol (4-n-OP) and 4-n-nonylphenol (4-n-NP) (Cat. Nos. 442858, 442850, and 442873, respectively) were obtained from Supelco (Bellefonte, PA, USA). Methanol (HPLC/UV grade) was purchased from Burdick & Jackson (Honeywell, Muskegon, MI, USA). Deionized water (DI H_2O) was obtained from a Millipore water station (Billerica, MA, USA) with 18.2 MΩ-cm resistance. The chemical structures of the studied compounds and the internal standard are shown in Fig. 1.

Standard solutions

Each analyte including internal standard was weighed and dissolved in methanol/DI H_2O (50:50, v/v) at 1000 ppm (μg/mL) as primary stock solutions. Each stock solution was then diluted to 10 ppm, 1 ppm, 100 ppb, and 10 ppb as working solutions to prepare calibration standards. BPA-IS was diluted to 200 ppb to spike calibration standards and unknown water samples.

Calibration standards were prepared in DI H_2O or matrix (a bottled water sample tested free of all target analytes) with each of the four analytes (BPA, 4-n-NP, 4-t-OP, 4-n-OP) at seven levels: 0.1 ppb, 0.5 ppb, 1 ppb, 2 ppb, 5 ppb, 10 ppb, and 20 ppb with BPA-IS spiked at 2 ppb in each level.

Sample preparation

Water samples (municipal drinking water and eight randomly selected bottled water samples) were spiked with BPA-IS at 2 ppb in autosampler vials and injected directly into the uHPLC/MS/MS system for quantification.

Low-cost bottles (LCB, water bottles, baby-feeding bottles, food containers) with various volume capacities were purchased from a local store. Brand-name baby-feeding bottles (BNB) were purchased from a baby product specialty store. Each bottle was rinsed and filled with local municipal drinking water and exposed to direct sunlight for 2 days before the first testing point (Testing Point A), and microwaved for 5 min then cooled down to room temperature before testing (Testing Point B). The bottle was then microwaved for another 5 min, the water discarded, re-filled with fresh municipal drinking water, and microwaved for an additional 5 min before Testing Point C. At each testing point, 1 mL of water sample in each bottle was transferred to a 1.5 mL autosampler vial and spiked with BPA-IS at 2 ppb.

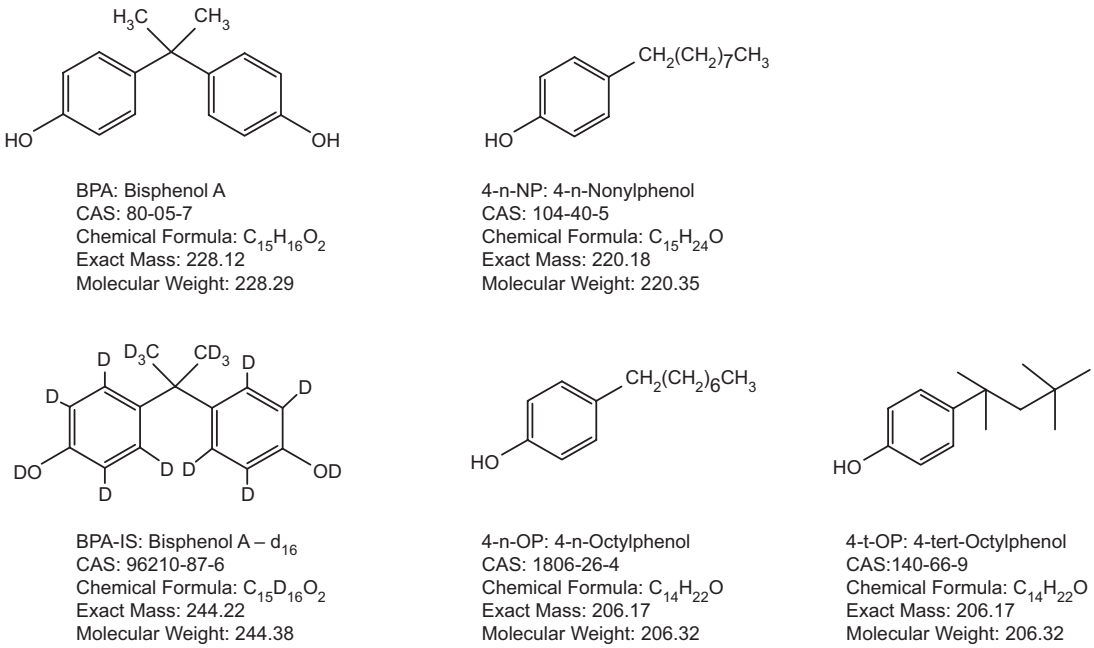

Figure 1. Chemical structures of compounds studied.

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Table 1. Scheduled MRM scan parameters

ID	Q1MS (*m*/*z*)	Q3MS (*m*/*z*)	t_R (min)	DP (V)	EP (V)	CE (eV)	CXP (V)
BPA-1	227.0	211.6	0.9	−70	−10	−26	−3
BPA-2	227.0	132.8	0.9	−70	−10	−38	−9
4-t-OP	205.1	132.8	2.1	−65	−10	−34	−9
4-n-OP	205.1	105.6	2.6	−65	−10	−26	−7
4-n-NP	219.0	105.8	2.8	−65	−10	−29	−8
BPA-IS	241.1	141.8	0.9	−65	−10	−38	−11

Q1MS: precursor ion; Q3MS: product ion; t_R: retention time; DP: declustering potential; EP: entrance potential; CE: collision energy; CXP: collision cell exit potential.

Instrument conditions

Chromatography was performed on a UltiMate® 3000 RSLC system (Dionex, Sunnyvale, CA, USA) with a Dionex Acclaim® PolarAdvantage II RSLC column (2.1 × 50 mm, 2.2 μm). Methanol (A) and DI water (B) were used as mobile phase with the following gradient program: A/B (75:25) for 0.1 min, linear gradient to A/B (95:5) in 0.8 min, and hold for 2.1 min, then return to initial condition and equilibrate for 2 min. Chromatography was performed with a column temperature of 30°C with a flow rate of 500 μL/min and an injection volume of 100 μL.

MS/MS detection was carried out on a 4000 QTRAP® triple quadrupole/linear ion trap tandem mass spectrometer (AB/Sciex, Foster City, CA, USA) in negative MRM mode. An AB/Sciex Turbo V™ ion source was used to couple the uHPLC chromatography system with the MS detector. Atmospheric pressure chemical ionization (APCI) was used as the ionization mode and the ion source conditions were set as follows: nitrogen was used as the curtain gas, source gases and collision gas; curtain gas (CUR) pressure: 15 psi; collision gas (CAD) pressure setting: medium; nebulizer current (NC): −4 μA; temperature (TEM): 400°C; and ion source gas 1 (GS1) pressure: 40 psi. The time range for each MRM scan was optimized by using the instrument manufacturer's Scheduled MRM option. The detailed MRM scan parameters are listed in Table 1.

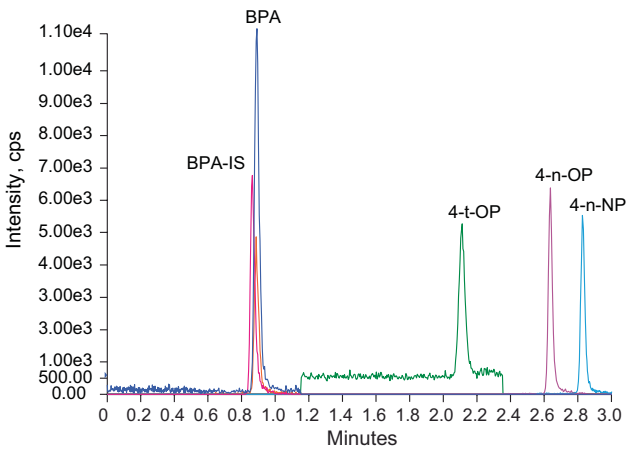

Figure 2. TIC chromatogram of BPA and phenol standards at 1 ppb.

RESULTS

Chromatography

A typical uHPLC/MS/MS MRM chromatogram for a mixed standard is shown in Fig. 2 with each target analyte at 1 ppb. All target analytes were chromatographically separated to avoid any potential interference. The minimum retention factor (k) was observed for BPA (retention time = 0.9, void time = 0.25) with k greater than 2.5, thus ensuring sufficient retention of all analytes from early eluting species. A fast mobile phase gradient (75% methanol to 95% methanol in 0.8 min) was used to provide good retention of BPA and effectively elute strongly retained phenols. The gradient delay volume is critical for such a fast gradient. With the standard static mixer set (placed between the proportioning valve and the analytical column) with an internal volume of 500 μL, the effective mobile phase composition was delayed for 1 min using the 500 μL/min flow rate. A 200 μL static mixer set was used to replace the standard mixer, reducing the gradient delay volume/time, while providing efficient mixing of gradient components for stable chromatography. The analytical method uses a short, small particle, small i.d. analytical column, with a high-pressure pump to provide improved chromatographic performance. As a result, the total run time was reduced from more than 25 min[10,13] to 5 min with total resolution for all target analytes, thus providing significantly improved method throughput.

Mass spectrometry

The aim of this study was to develop a selective and sensitive method for the direct analysis of trace level BPA and phenols, so MS/MS instrumentation was selected for its sensitivity and ability to provide trace level detection, and its selectivity which allows minimal sample preparation and cleanups. Although electrospray ionization (ESI) was used in reported methods,[10,15,16] an atmospheric pressure chemical ionization (APCI) source was used in this study based on our observation that APCI showed better sensitivity than ESI with the mobile phase composition used in this method. The MS/MS instrument was operated in the negative MRM mode, and scan parameters for each analyte were optimized using the 'compound optimization' procedure from the instrument operating software while infusing a 1 ppm standard solution of each individual analyte and reporting three candidate MRM transitions. These candidate transitions were further evaluated for specificity of detection, and two transitions were selected: one as BPA quantifier and the other as qualifier. Only one transition was confirmed for the specificity for each phenol. The MRM transitions and scan parameters are listed in Table 1. Optimization of the source parameters was performed by a series of runs, each with varying source parameters, and the optimum conditions are described in the Experimental section.

Deprotonated molecules $[M–H]^-$ were observed as the dominant ion species and were selected as the precursor ions in Q1. However, for the isotope-labeled internal standard, BPA-d_{16}, an ion of *m*/*z* 241 was observed instead of the expected *m*/*z* 243 (BPA-d_{16}, exact mass: 244.2). The discrepancy was caused by the facile back-exchange reaction of the two deuterated hydroxyl groups (-OD) with protons.

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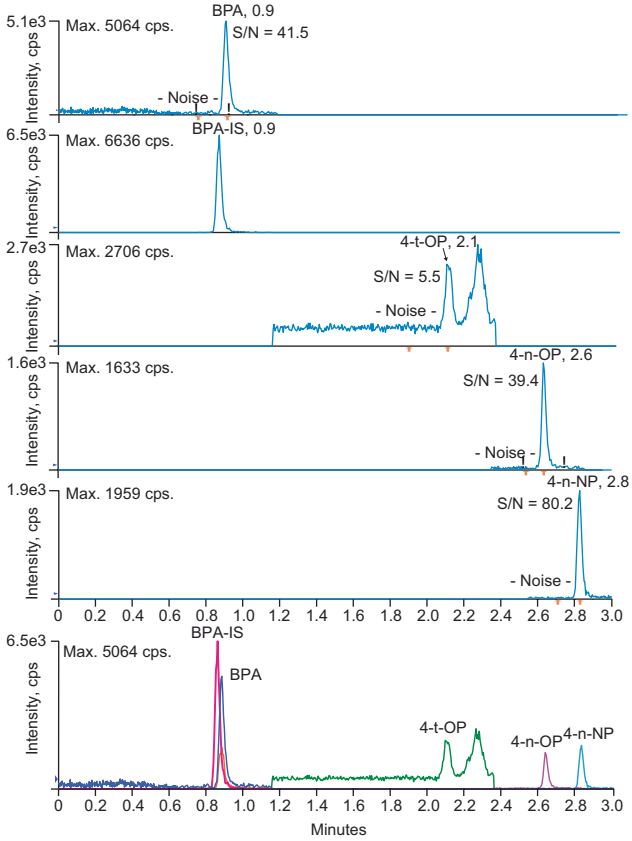

Figure 3. MRM and TIC chromatograms of a blank water sample spiked with 0.5 ppb BPA, 4-t-OP, 4-n-OP and 4-n-NP.

Thus, for the BPA-IS we observed the $[M–H]^-$ ion of BPA-d_{14}. Our observation of this H/D exchange when using BPA-d_{16} agreed with a previous report.[16]

Method performance

Figure 3 shows the MRM and TIC chromatograms of a blank water sample spiked with 0.5 ppb of each analyte obtained under optimized conditions. To evaluate method performance, quality parameters such as selectivity, carryover, calibration, correlation of determination, detection limit, relative standard deviation (RSD), and recovery were studied. The selectivity was evaluated by analyzing blank samples, including deionized water, local municipal drinking water, and various bottled drinking waters with no quantifiable peaks. No interference was observed at the specific retention times of the target analytes. Carryover was evaluated by subsequently injecting a blank sample after the highest calibration standard at 20 ppb, and no quantifiable peaks were observed at the specific retention times. Calibration was performed using calibration standards prepared in deionized water or bottled drinking water free of target analytes. Calibration curves were generated using the peak area ratios of each target analyte and internal standard against target analyte concentrations from 0.1 ppb to 20 ppb. 1/x was used as the weighting factor to provide better quantification accuracy at lower levels. Linear regression was used for BPA and quadratic regression was used for the three phenols. The correlation of determination (r) was greater than 0.99 for each of the analytes. The recovery was evaluated by analyzing blank bottled water samples spiked at 0.5 ppb and 5 ppb and is shown as %ObservedAmount/SpecifiedAmount. The recoveries at both levels ranged from 97.0% to 106.2% except for 4-n-OP (75.8% for 0.5 ppb, and 155.4% for 5 ppb). The significant deviation indicated a strong matrix effect for 4-n-OP quantification that may be caused by interferences/suppressions. The use of isotope-labeled 4-n-OP as the second internal standard may be able to correct this deviation. (Since no quantifiable 4-n-OP was observed in any of the tested samples, the use of isotope-labeled 4-n-OP was not evaluated in this study. Isotope-labeled 4-n-OP may be purchased from C/D/N Isotopes: D-6102.) The run-to-run precision was evaluated by seven replicate assays of the 0.5 ppb spiked sample and addressed as the RSD. A value of less than 8% was achieved for each analyte RSD at 0.5 ppb. The method detection limit (MDL) was statistically calculated for each analyte using the standard deviation obtained from the seven replicate analyses of a 0.5 ppb spiked sample, following the equation: $MDL = s \times t$ where s is the standard deviation and t is the Student's t at 99% confidence interval. The calculated MDL ranged from 0.040 ppb (BPA) to 0.057 ppb (4-n-NP). The evaluation results are summarized in Table 2.

Analysis of water samples

Local municipal water samples and eight randomly selected bottled water samples were analyzed using this method. No target analytes were observed in any of the bottled water samples. Local municipal water collected from our laboratory faucets after long periods of non-use (overnight or longer) showed a low level of BPA (0.057 ppb, mean of three replicate assays), but showed no detectable BPA from the same faucet after running the water for 1 min. The detectable BPA was again confirmed in the water collected from another faucet (same model) after a long period of non-use. This may suggest that the BPA was leaching from the plastic parts of the faucets, connecting unions, or pipes.

Table 2. Calibration, recovery, precision and method detection limit (MDL)

Analyte	r	Regression	Equation	Weighting	Recovery[a] at 0.5 ppb	%RSD[b] at 0.5 ppb	MDL[c]	Recovery[a] at 5 ppb
BPA	0.9998	Linear	$y = 1.73x - 0.00946$	1/x	106.2%	5.07	0.040	99.4%
4-t-OP	0.9995	Quadratic	$y = 0.0146x^2 + 0.836x + 0.00567$	1/x	104.6%	6.53	0.042	97.0%
4-n-OP	0.9975	Quadratic	$y = 0.0223x^2 + 0.818x - 0.0476$	1/x	75.8%	7.89	0.045	155.4%
4-n-NP	0.9993	Quadratic	$y = 0.0312x^2 + 0.553x - 0.0128$	1/x	104.0%	7.32	0.057	99.6%

[a]: Recovery was calculated by observed amount/specified amount × 100%;
[b]: %RSD was calculated from 7 replicate injection at 0.5 ppb;
[c]: MDL was calculated by $MDL = s \times t$: s is the standard deviation of the 7 replicate injection at 0.5 ppb, and t is the student's t at 99% confidence interval.

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Table 3. BPA and phenols in plastic bottles and containers

			Testing Point A			Testing Point B			Testing Point C		
	Material	Recycle No.	BPA	4-n-NP	4-t-OP	BPA	4-n-NP	4-t-OP	BPA	4-n-NP	4-t-OP
LCB1	PP	5	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A
LCB2	PP	5	ND	ND	ND	**0.246**	ND	ND	**ND**	ND	ND
LCB3	N/A	N/A	ND	ND	ND	ND	**0.146**	ND	ND	**ND**	ND
LCB4	N/A	N/A	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A
LCB5	N/A	N/A	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A
LCB6	N/A	7	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A
BNB1	N/A	N/A	ND	NQ	ND	NQ	**0.145**	ND	ND	**ND**	ND
BNB2	PP	5	ND	ND	ND	ND	ND	ND	ND	ND	ND
BNB3	PP	5	ND	**0.163**	ND	ND	**0.192**	ND	ND	**ND**	ND
BNB4	PES	7	ND	ND	ND	ND	ND	ND	N/A	N/A	N/A
BNB5	N/A	N/A	ND	ND	**6.68**	NQ	NQ	**7.09**	ND	ND	**0.644**
BNB6	N/A	N/A	ND	NQ	**4.68**	**0.063**	NQ	**6.42**	**ND**	ND	**0.956**
BNB5-C	N/A	N/A	N/A	N/A	N/A	NQ	ND	**1.84**	ND	ND	**0.76**
BNB6-C	N/A	N/A	N/A	N/A	N/A	**0.446**	**0.090**	**2.68**	**0.129**	**NQ**	**1.09**

ppb for all concentrations.
N/A: Information (material or recycle number) was not available for bottles, or experiment was not performed.
ND: not detected; NQ: detected but not reported because either the calculated amount is below MDL or signal-to-noise (S/N) ratio is less than 3.

Analysis of plastic bottles

Each of the plastic bottles or containers was treated following the procedure described in the Experimental section. Samples were analyzed by this method at each testing point and the results are summarized in Table 3. The manufacturing material and the recycle number were read from the body or the packaging of the bottles. The designed experiments on plastic bottles were to simulate normal usage and extreme conditions. Testing Point A simulates normal usage conditions where each bottle was used as a storage container at regular temperature. Testing Point B simulates unusual usage condition, although may happen often in daily life,

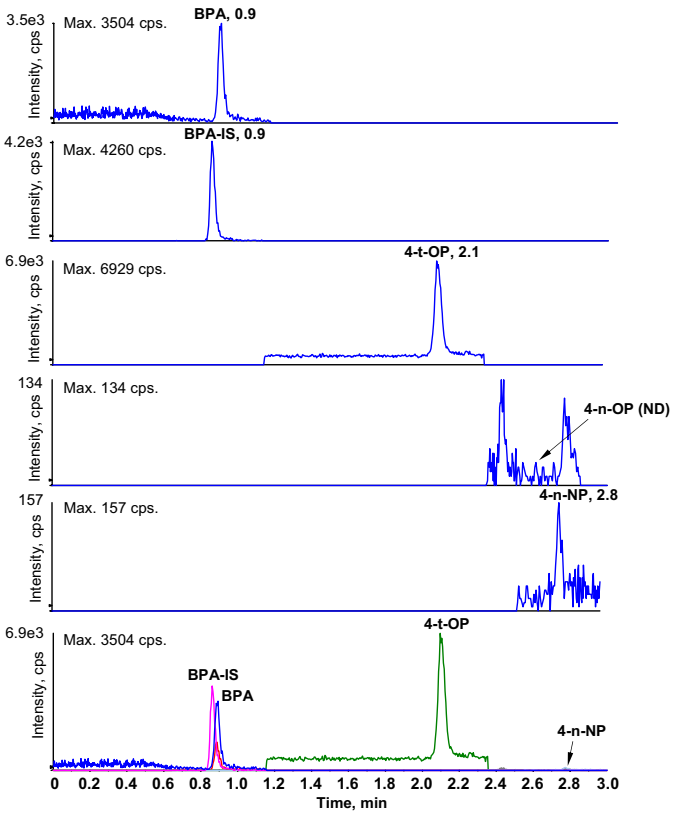

Figure 4. BPA, 4-t-OP and 4-n-NP leached from brand-name bottle 6.

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where a microwave was used to heat the water in each bottle. As a result, the water in each plastic bottle was at or near to boiling point. Testing Point C was designed to find out if the target analytes leached from the bottles after multiple usages, where the heated water was discarded and the bottles were re-filled for retesting.

As seen in Table 3, at Testing Point A, no quantifiable BPA was found in any of the tested bottles (non-heated), and low level 4-n-NP was found in one of the brand-name bottles (0.163 ppb in BNB3). However, large amounts of 4-t-OP were found in two brand-name bottles marketed under the same brand but manufactured in different countries (6.68 ppb in BNB5, and 4.68 ppb in BNB6). At Testing Point B, where bottles were heated in the microwave and the water was at or near to boiling point, quantifiable levels of BPA were observed in two bottles which had both been labeled as 'BPA-Free' (0.246 ppb in LCB2, and 0.063 in BNB6). In addition, 4-n-NP was observed in three bottles, including BNB3 with an increased amount (rising from 0.163 to 0.192 ppb). An increase in the amount of 4-t-OP was also found in both BNB5 and BNB6.

Confirmation experiments were performed on brand-name bottles 5 and 6 (BNB5-C, BNB6-C) from different lots of the same brand purchased from the baby specialty store at a different location. Here these two bottles were rinsed thoroughly and filled with local municipal drinking water and microwaved for 3 min to boiling point and cooled down to room temperature. A 1 mL aliquot from each bottle was transferred into a 1.5 mL autosampler vial and spiked with BPA-IS at 2 ppb. The presence of BPA and 4-t-OP was positively confirmed for both bottle samples, as seen in Table 3. Figure 4 shows the MRM and TIC chromatograms of a brand-name bottle (BNB6-C) with positive detection of BPA, 4-t-OP and 4-n-NP.

Each bottle/water in which one or more target analytes has been detected was microwaved and the water discarded. These bottles were rinsed thoroughly and re-filled with municipal drinking water, and microwave-heated again. The water samples were prepared as described in the Experimental section and tested for target analytes. As seen in Table 3, the observed amount of target analytes decreased significantly: no target analytes were detected in LCB2, LCB3, BNB1 and BNB3; BPA decreased from 0.063 ppb to not-detected (ND) in BNB6, and from 0.446 to 0.129 ppb in BNB6-C; 4-t-OP decreased from 7.09 to 0.644 ppb in BNB5, from 6.42 to 0.956 ppb in BNB6, from 1.84 to 0.76 ppb in BNB5-C, and from 2.68 to 1.09 ppb in BNB6-C. This observation suggests that exposure to these target chemicals from the bottles could decrease with continued usage.

Although it would be of interest and important to see if BPA and the phenol levels continued to decrease with continued usage, and to trace which part(s) of the municipal water delivery system caused the BPA leaching, they are beyond the objectives of this study and thus were not further investigated.

CONCLUSIONS

A high-throughput LC/MS/MS method was developed and used for the quantitative analysis of bisphenol A, 4-*tert*-octylphenol, 4-n-octylphenol and 4-n-nonylphenol in bottled water and plastic bottles without preconcentration sample enrichment. All bottled water appeared to be free of BPA and targeted phenols (below quantification limits). However, 4-t-OP was found in two unheated bottles, which suggests an exposure to 4-t-OP when using these bottles. Quantifiable BPA and/or 4-nonylphenol were also found in five tested bottles with 'BPA-Free' labeling after they had been heated in a microwave oven. Simulated experiments also showed that the levels of detected chemicals could decrease with continued usage.

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