

An UHPLC-MS/MS Method for the Direct Analysis of Ultratrace Level BPA and Phenols in Water Samples and Bottles



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INTRODUCTION

Bisphenol A (BPA) is widely used in high-production-volume manufacturing of polycarbonate plastics (for example, food containers and plastic bottles) and epoxy resins.¹ Current studies evaluating the impact of BPA on health and the environment have not reported conclusive results. However, actions have been taken proactively to protect sensitive populations. Canada banned BPA to reduce the exposure for newborns and infants² in 2009 and BPA is currently listed as a toxic compound by the Canadian EPA. The US EPA recently added BPA to its list of target chemicals for possible regulation.¹ Octylphenol (OP) and nonylphenol (NP) are the degradation products of alkylphenol ethoxylates, which are widely used nonionic surfactants. The estrogenic and toxic effects of OP and NP have been well studied and reported.³ NP has been regulated in the US with a 6.6 µg/L maximum average level⁴ for fresh water. Recent reports show NP being detected in food⁵ and food wraps,⁶ posing more concerns for food safety and food packaging.

BPA and phenols were usually analyzed by gas chromatography mass spectrometry (GC-MS) after derivatization, or by liquid chromatographic (LC) separation with various detectors. GC methods involve labor- and time-consuming derivatization steps, and reported LC methods exceeded 25 min in duration, rendering them unsuitable for high-throughput analysis.

This UHPLC-MS/MS method significantly reduces run time to 5 min by using an ultrahigh performance rapid separation LC (RSLC) system with analytical columns with small particle sizes, ensuring high-throughput analysis. Selective and sensitive detection was achieved by operating the MS/MS system in multiple-reaction-monitoring (MRM) mode.

EXPERIMENTAL SECTION

Chromatographic Conditions

System: UltiMate® 3000 RSLC UHPLC system
Column: Acclaim® RSLC PA2 column
(2.1 × 100 mm, 2.2 µm)
Temperature: 30 °C
Mobile Phase: Gradient elution with A) methanol;
B) DI water: equilibrate at 75% A for 2 min,
ramp up to 95% A from 0.1 to 0.9 min,
hold for 2.1 min and then return to
original condition
Flow Rate: 0.5 mL/min
Injection Vol.: 100 µL

MASS SPECTROMETRIC CONDITIONS

System: 4000 QTRAP® (AB/SCIEX)
Interface: TurboSpray with APCI
Curtain Gas: 15 psi
Collision Gas: Medium
Nebulizer Current: -4 µA
Temperature: 400 °C
Ion Source Gas 1: 40 psi
Scan Mode: Multiple-Reaction-Monitoring (MRM)
Refer to Table 1 for MRM details

Table 1. Scheduled MRM Scan Parameters

ID	Q1MS (m/z)	Q3MS (m/z)	t _r (min)	DP (V)	EP (V)	CE (V)	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- <i>t</i> -OP	205.1	132.8	2.1	-65	-10	-34	-9
4- <i>n</i> -OP	205.1	105.6	2.6	-65	-10	-26	-7
4- <i>n</i> -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

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Chemicals and Reagents

The chemical structures of studied compounds and the internal standard are shown in Figure 1. BPA and BPA-d₁₆ were purchased from Aldrich and C/D/N Isotopes, respectively, and all phenols standards were obtained from Supelco.

Calibration standards were prepared in DI H₂O or blank matrix 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.

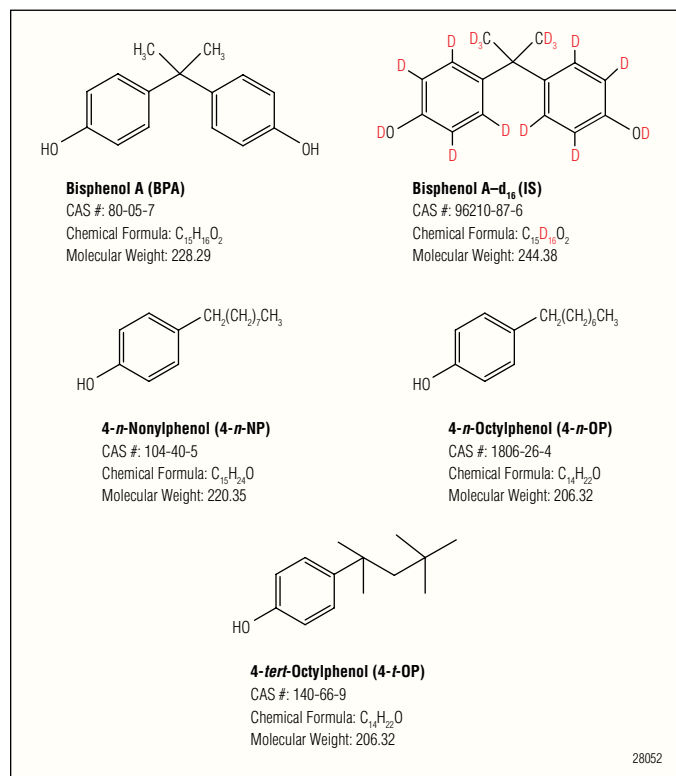


Figure 1. Chemical structures and related information of studied compounds.

RESULT AND DISCUSSION

Chromatography

Figure 2 shows a typical UHPLC-MS/MS MRM chromatogram for a mixed standard with each target analyte at 1 ppb. Total chromatographic resolution was observed, which is essential to avoid any potential interference. The minimum retention factor (*k*), observed for BPA, was greater than 2.5, ensuring sufficient retention of all analytes from early eluting species. Because a fast gradient was used in this method, the standard mixer (500 μ L internal volume) was replaced by a 200 μ L static mixer set in order to reduce gradient delay volume/time and to provide efficient mixing of gradient components for stable chromatography. An Acclaim RSLC analytical column featuring short length, small i.d., and small particles was used in this method with a high-pressure RSLC pump to provide better chromatographic performance.

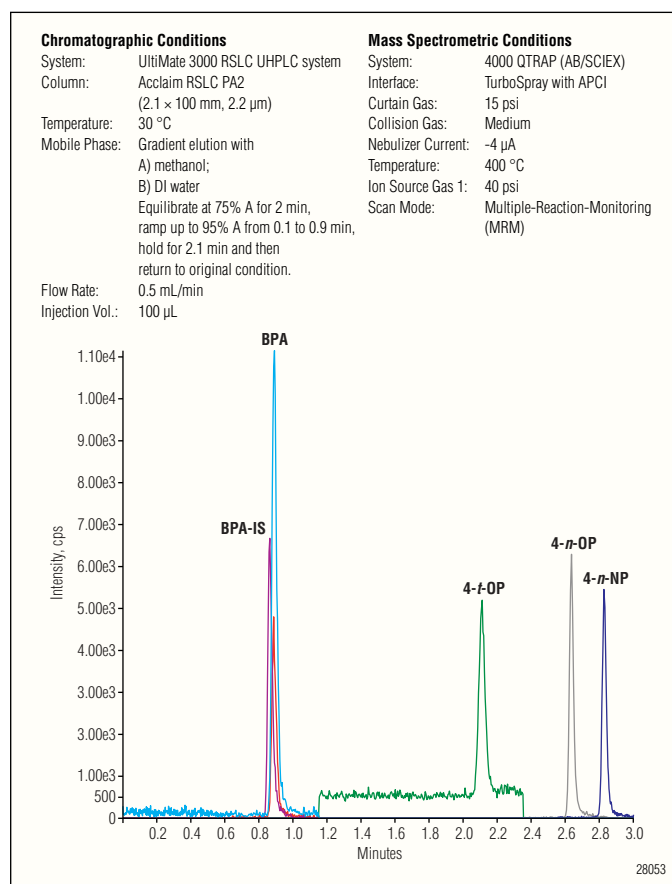


Figure 2. TIC chromatogram of BPA and phenols standards at 1 ppb.

Mass Spectrometry

To minimize time/labor/cost of intensive sample preparation and cleanup, an MS/MS instrument was selected and operated in highly selective and sensitive MRM mode, allowing direct injection of aqueous samples. An atmospheric pressure chemical ionization (APCI) source was selected in this study to provide better sensitivity than electrospray ionization. The MRM scan parameters for each individual analyte were optimized. Two transitions were selected for BPA (quantifier and qualifier MRM). Only one transition was confirmed for the specificity for each phenol. Table 1 shows the MRM transitions, scan parameters, and optimized source parameters.

Method Performance

Method performance was evaluated by studying selectivity, carryover, calibration and correlation of determination, detection limit, precision, and recovery. Selectivity was confirmed with the absence of quantifiable peaks in blank samples, including deionized water, local municipal drinking water, and various bottled drinking water samples.

A blank sample was injected following a high-concentration calibration standard at 20 ppb to evaluate method carryover, and no quantifiable peaks were observed at the specific retention times. Internal calibration with isotope-labeled internal standard was used to generate calibration curves using the peak area ratios against target analyte concentrations from 0.1 ppb to 20 ppb. $1/x$ was used as the weighting factor to achieve better quantitation accuracy at lower levels. Linear regression was used for BPA and quadratic regression was used for the three phenols with correlation of determination (r) greater than 0.99 for each of the analytes.

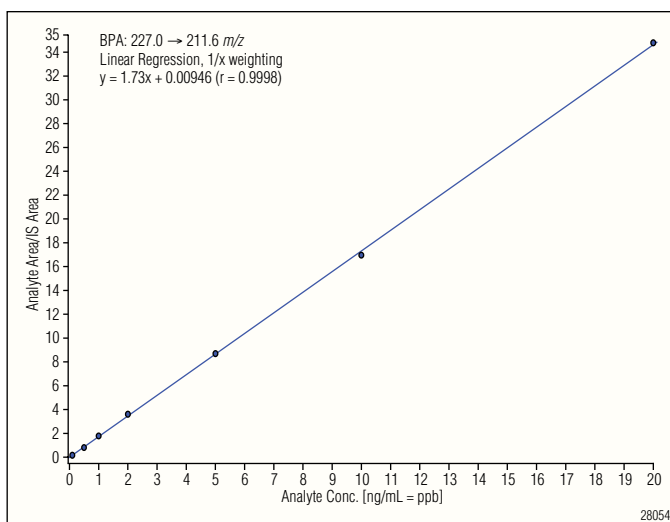


Figure 3. Calibration curve of BPA using the isotope labeled internal standard.

Figure 3 shows the calibration curve for BPA as an example. Recovery was evaluated by analyzing blank bottled water samples spiked at two levels (0.5 and 5 ppb) and calculated following the equation: observed amount/specified amount \times 100%. Precision was evaluated by seven replicate assays of the 0.5 ppb spiked sample and is shown as %RSD in Table 2. Method detection limit (MDL) was statistically calculated using the following equation: MDL = $s \times t$ where s is the standard deviation of the seven replicate assays at 0.5 ppb and t is the Student's t at 99% confidence interval. MDL was estimated to be less than 0.1 ppb for all analytes. Table 2 summarizes the performance evaluation results.

Table 2. Calibration, Recovery, Precision, and Method Detection Limit (MDL)

Analyte	r	Regression	Weighting	Recovery ^a at 0.5 ppb	%RSD ^b at 0.5 ppb	MDL	Recovery ^a at 5 ppb
BPA	0.9998	Linear	$1/x$	106.2%	5.07	0.040	99.4%
4- <i>t</i> -OP	0.9995	Quadratic	$1/x$	104.6%	6.53	0.042	97.0%
4- <i>n</i> -OP	0.9975	Quadratic	$1/x$	75.8%	7.89	0.045	155.4%
4- <i>n</i> -NP	0.9993	Quadratic	$1/x$	104.0%	7.32	0.057	99.6%

^aRecovery was calculated by observed amount/specified amount \times 100%

^b%RSD was calculated from seven replicate injections at 0.5 ppb

Analysis of Water Samples

Available water samples, including local municipal drinking water and eight randomly selected bottled water samples, were analyzed using this method. No target analytes were observed in any of the bottled water samples. Low levels of BPA were found in local municipal water collected from our laboratory faucets after long periods of non-use (overnight or longer).

Analysis of Plastic Bottles

All bottles and containers were purchased from local stores. Each of the plastic bottles or containers was filled with local municipal drinking water, exposed to sunlight, and microwaved to near or at boiling point. The bottle/water was then cooled down to room temperature for direct analysis. As seen in Table 3, BPA was detected with quantifiable amounts in two of twelve tested bottles, 4-*n*-NP was found in three bottles, and 4-*t*-OP was observed at relatively higher levels in two bottles under the same brand name.

Table 3. BPA and Phenols in Plastic Bottles and Containers

	BPA	4- <i>n</i> -NP	4- <i>t</i> -OP
LCB1	ND	ND	ND
LCB2	0.246	ND	ND
LCB3	ND	0.146	ND
LCB4	ND	ND	ND
LCB5	ND	ND	ND
LCB6	ND	ND	ND
BNB1	NQ	0.145	ND
BNB2	ND	ND	ND
BNB3	ND	0.192	ND
BNB4	ND	ND	ND
BNB5	NQ	NQ	7.09
BNB6	0.063	NQ	6.42

ppb for all concentrations

LCB: low cost bottle

BNB: brand name bottle

ND: not detected

NQ: detected but not reported because either the calculated amount is below MDL or S/N is less than 3

CONCLUSION

A high-throughput UHPLC-MS/MS method was developed, and its application successfully demonstrated for direct quantification of bisphenol A, 4-*t*-octylphenol, 4-*n*-octylphenol, and 4-*n*-nonylphenol in bottled water and plastic bottles.

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