Application Note: 474

Determination of Bisphenol A in Infant Formula by Automated Sample Preparation and Liquid Chromatography-Mass Spectrometry

Yang Shi, Catherine Lafontaine, Matthew Berube, Francois Espourteille, Thermo Fisher Scientific, Franklin, MA, USA

Key Words

- Aria TLX-1
- TurboFlow Technology
- TSQ Vantage
- Infant Formula
- Food Safety

Introduction

2,2-bis(4-hydroxyphenyl) propane, commonly known as Bisphenol A (BPA), is one of the primary chemicals used to make plastics. It is also heavily used in the production of various types of food and drink containers. Because BPA has been known to leach from the plastic lining of metal canned food, the potential risks of exposure to BPA have been a great concern over the past few years. Higher bisphenol A levels are significantly associated with heart disease, diabetes, and abnormally high levels of certain liver enzymes. There is a consensus that infants are at the greatest risk of harm due to exposure to extremely low levels of BPA.[1] The maximum acceptable or "reference" dose for BPA is 50 µg/kg body weight/day, as established by the U.S. Environmental Protection Agency.[2]

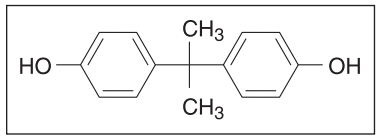

Figure 1: Chemical Structure of Bisphenol A

A liquid chromatography-mass spectrometry (LC-MS) technique has been recently described for the determination of BPA in food.[3] Current strategies for the detection of BPA in canned infant formula employ sample preparations that involve complicated extraction steps such as solid phase extraction, solvent-based extraction, and some micro-extraction techniques. All of these techniques require additional sample concentration and reconstitution in an appropriate solvent. Such sample preparation methods are time-consuming and are more vulnerable to variability due to errors in manual preparation. To offer a high sensitivity (low ppb) BPA detection method and timely, automated analysis of multiple samples, our approach is to use Thermo Scientific TurboFlow technology coupled to the detection capabilities of a high-sensitivity Thermo Scientific TSQ Vantage triple stage quadrupole mass spectrometer.

Goal

Develop a six-minute LC-MS/MS method using automated sample preparation for the assay of BPA in canned infant formula powder by negative ion atmospheric pressure chemical ionization (APCI) using a deuterated internal standard (BPA-d_{16}).

Experimental

Sample Preparation

Canned infant formula powder, used in this analysis for preparation of blanks, QCs, and standards, was obtained from a local supermarket in Massachusetts. The lid lacquer is low-density polyethylene and the body is polyester. BPA and BPA-d_{16} were obtained from Sigma-Aldrich, US (St. Louis, MO). The diluent (AmAcACN solution) was made using 3% ammonium acetate in acetonitrile-water (70:30, v/v). A BPA working solution was prepared in AmAcACN solution at 10 µg/mL. The infant formula solution was prepared by adding 10 mL of AmAcACN solution to 1 g of infant formula powder and then centrifuging at 10,000 RPM for 30 minutes. BPA standards and QC standards were serially diluted to the target concentrations with the resulting supernatant containing 25 ng/mL BPA-d_{16} as the internal standard. Target standard concentrations ranged from 0.78 ng/mL to 1000 ng/mL. The injection volume was 25 µL.

Method

The extract clean-up was accomplished using a TurboFlow™ method run on a Thermo Scientific Aria TLX-1 LC system using a TurboFlow Cyclone P polymer-based extraction column. Large molecules were not retained and were moved to waste during the loading step while the analyte of interest was retained on the extraction column. This was followed by organic elution to a Thermo Scientific Hypersil GOLD aQ end-capped, silica-based C18 reversed phase analytical column and gradient elution to a TSQ Vantage™ MS with an APCI source. The BPA precursor *m/z* 227 > 133 and 212 high-resolution selective reaction monitoring (H-SRM) transitions were monitored in negative ionization mode. The 133 *m/z* product ion for BPA was used for quantitation, and the 212 *m/z* product ion was used as confirmation. The precursor *m/z* 241 > 223 H-SRM transition was monitored for BPA-d_{16} because BPA-d_{16} is transformed into BPA-d_{14} (MW 242) in water. The total LC-MS/MS method run time was 5.6 minutes.

Aria™ TLX-1 System Parameters

TurboFlow Cyclone P column (0.5 x 50 mm)
Hypersil GOLD™ aQ (4 x 50 mm, 3 µm particle size)

Loading Pump Mobile Phases

Mobile Phase A: 10 mM Ammonium bicarbonate pH 10
Mobile Phase B: 0.1% Formic acid in ACN
Mobile Phase C: 20:40:40 Acetone: Acetonitrile: Isopropanol

Elution Pump

Mobile Phase A: H_2O
Mobile Phase B: Methanol

MS analysis was carried out on a TSQ Vantage triple stage quadrupole mass spectrometer. The MS conditions were as follows:

Mass Spectrometer Parameters

Ion Polarity:	Negative ion mode
Discharge Current:	4.0 V
Vaporizer Temperature:	60 °C
Capillary Temperature:	275 °C
Sheath Gas Pressure (N_2):	30 units
Auxiliary Gas Pressure (N_2):	5 units
Ion Sweep Gas Pressure (N_2):	2 units
Scan Type:	Highly Selective Reaction Monitoring (H-SRM)
Chrom Filter Peak Width:	7.0 s
Collision Gas Pressure:	1.2 mTorr
Declustering Voltage:	0 V
Scan Width:	0.002 *m/z*
Scan Time:	0.05 s
Q1:	0.200 Da
Q3:	0.700 Da
S-Lens (*m/z* 321):	77 V
Collision Energy (*m/z* 227 > 133):	27 V
(*m/z* 227 > 212):	19 V

The entire experiment was controlled by Aria operating software 1.6.2. The data were processed using Thermo Scientific LCQUAN 2.5.6 quantitative software after subtracting background using Thermo Scientific Xcalibur 2.0.7 SP1 data system software.

Results and Discussion

Because BPA exists in air (2 - 208 ng/m^3), dust (0.2 - 199 ng/g), water (5 - 320 ng/L) and in many other sources, it is almost impossible to obtain a real blank of BPA in the laboratory.[3] Therefore, we subtracted the pre-standard double blank peaks from all quantified data using the Xcalibur™ built-in background subtraction tool. Figure 2 shows comparison chromatography of BPA and BPA-d_{16} at the lower limit of quantitation (LLOQ) (0.78 ng/mL) and the upper limit of quantitation (ULOQ) (100 ng/mL). The data were processed using LCQUAN™ 2.5.6 data quantitation software. Matrix-matched calibration standards of BPA showed a linear response at greater than 2 orders of magnitude with r^2 = 0.9921 (Figure 3). All %CVs (n=3) were less than 20% for the LLOQ and less than 10% for all other points of the curve. As shown in Figure 4, the comparison between the pre-blank and post-high blank (before subtraction)

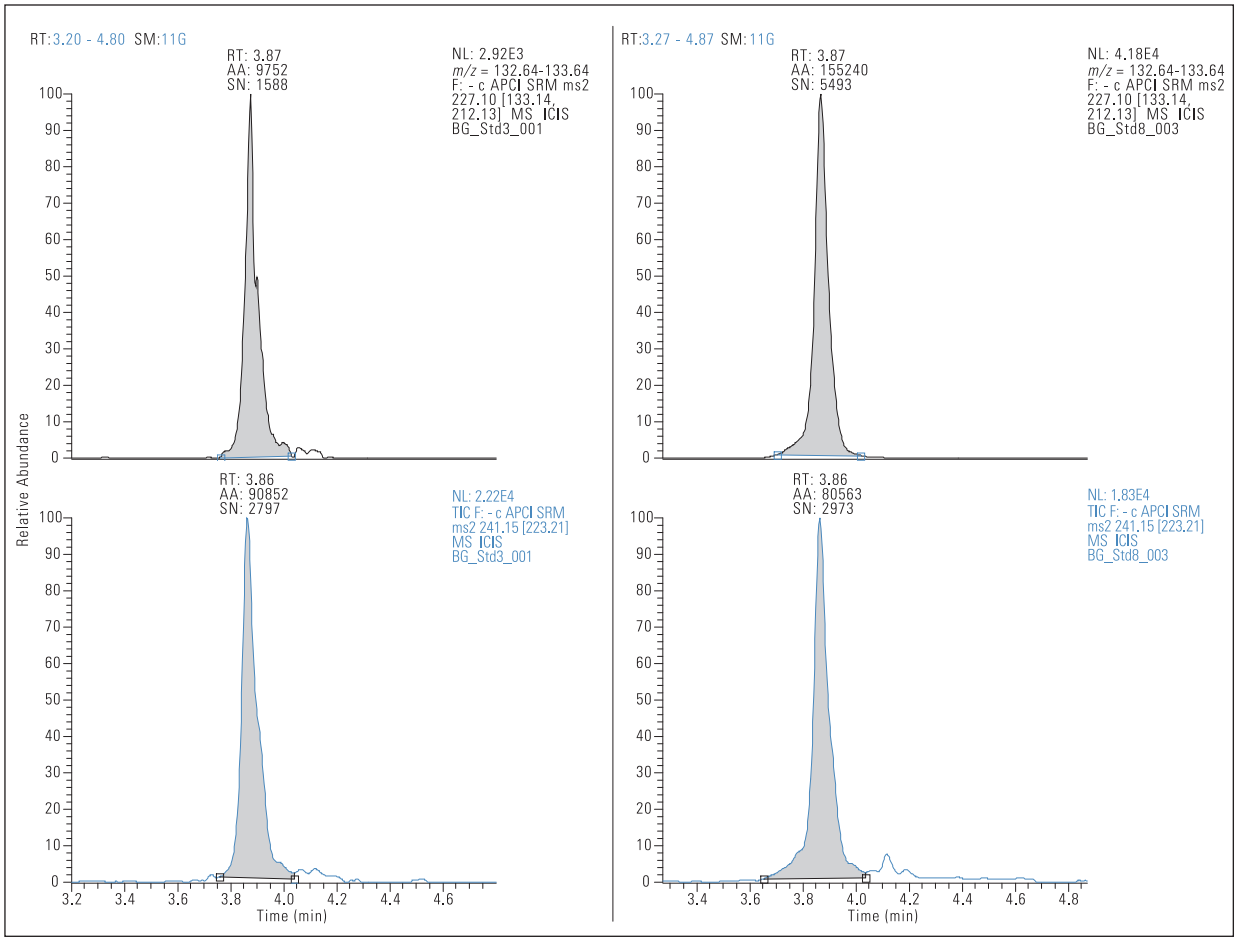

Figure 2: Chromatography comparison of BPA H-SRM *m/z* 133 transition (upper traces) and BPA-d_{16} (lower traces) at LLOQ of 0.78 ng/mL (left panel), and at ULOQ of 100 ng/mL (right panel)

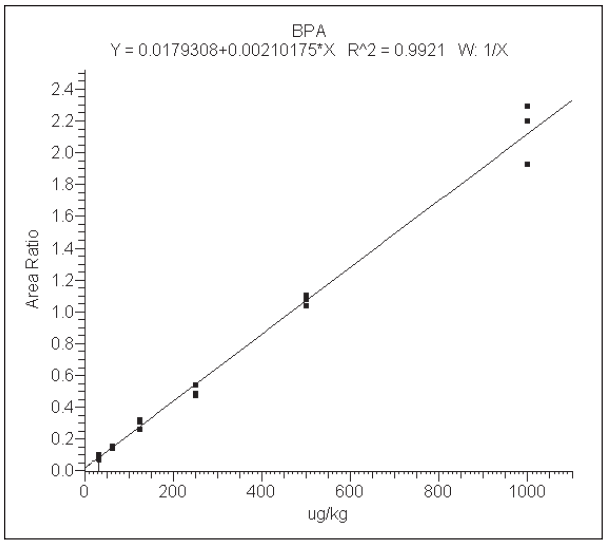

Figure 3: Linear regression curve of BPA standards based on area ratio with internal standard BPA-d_{16}

demonstrated the carryover could be ignored. The matrix interference was evaluated by comparing the chromatogram of the same concentration of BPA spiked in matrix and water. Figure 5 shows such a comparison at 12.5 and 25 ng/mL. As illustrated, the matrix interference was minimal.

We also compared the results of this TurboFlow technology LC-MS/MS study to another popular online solid phase extraction method.[4] Sample preparation times were very close due to few required offline sample treatment steps. The TurboFlow LC-MS/MS method run time, though, was four times faster. Because of differences in food matrices and the number of analytes, it is hard to compare the detection and quantitation limits directly. However, this comparison shows the benefits of using TurboFlow technology in the determination of BPA in food matrices.

Conclusion

A quick, automated sample preparation LC-MS/MS method has been developed that is sensitive enough to detect 7.80 µg/kg (ppb) dry powder (limit of detection) and quantify 31.3 µg/kg (ppb) dry powder (LLOQ) of BPA (background-adjusted) in infant formula powder for screening purposes. Compared to offline liquid/liquid or solid phase extractions, this method eliminates the need for time-consuming sample preparation procedures. The TurboFlow method also shows the advantage of fast separation over other online sample treatment techniques. The LC-MS/MS method run time is only 5.6 minutes, and the sample throughput can be improved by multiplexing on an Aria TLX-2 (or TLX-4) system.

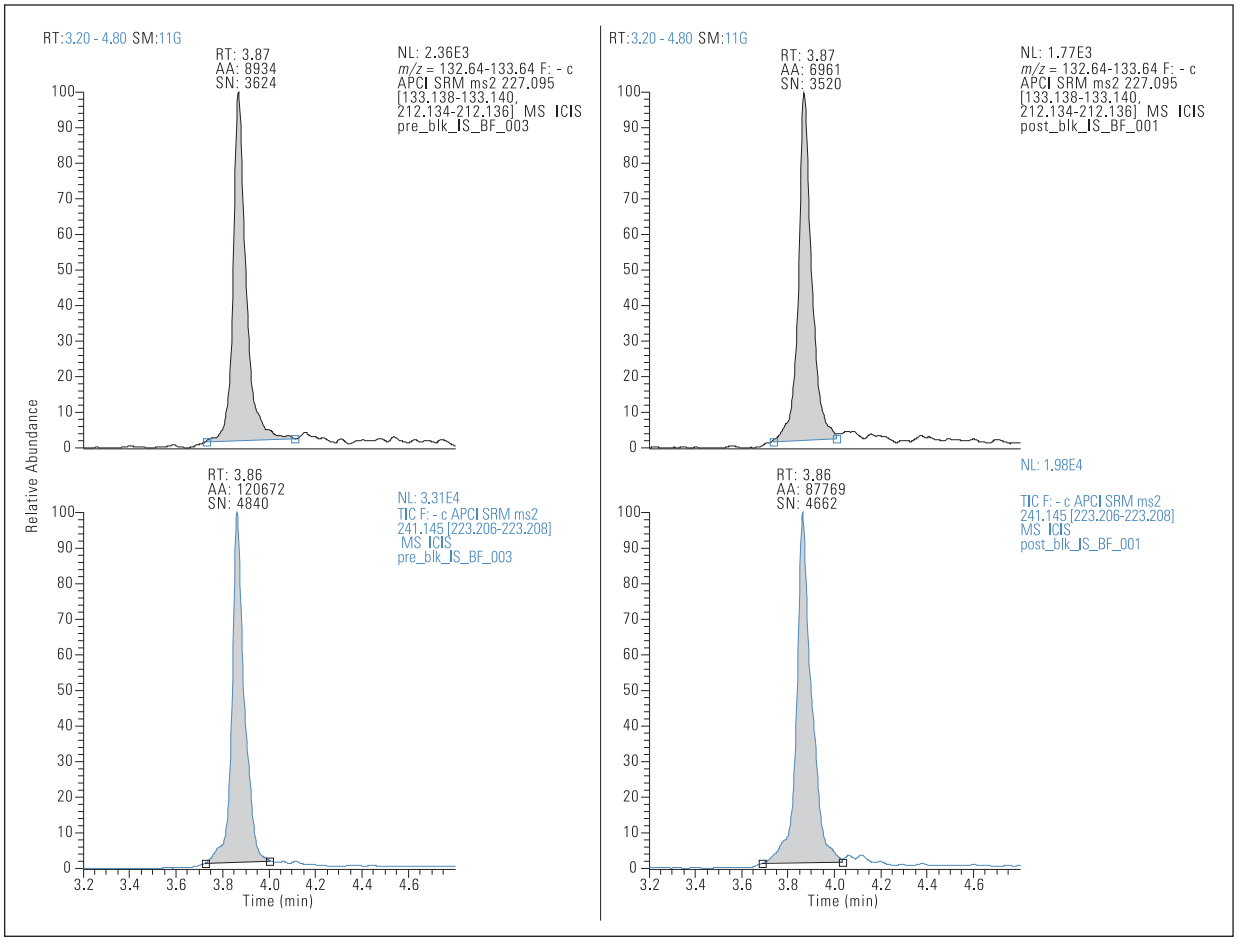

Figure 4: Chromatography comparison of BPA H-SRM *m/z* 133 transition (upper traces) and BPA-d_{16} (lower traces) in pre-blank infant formula matrix (left panel), and in post-high blank (right panel)

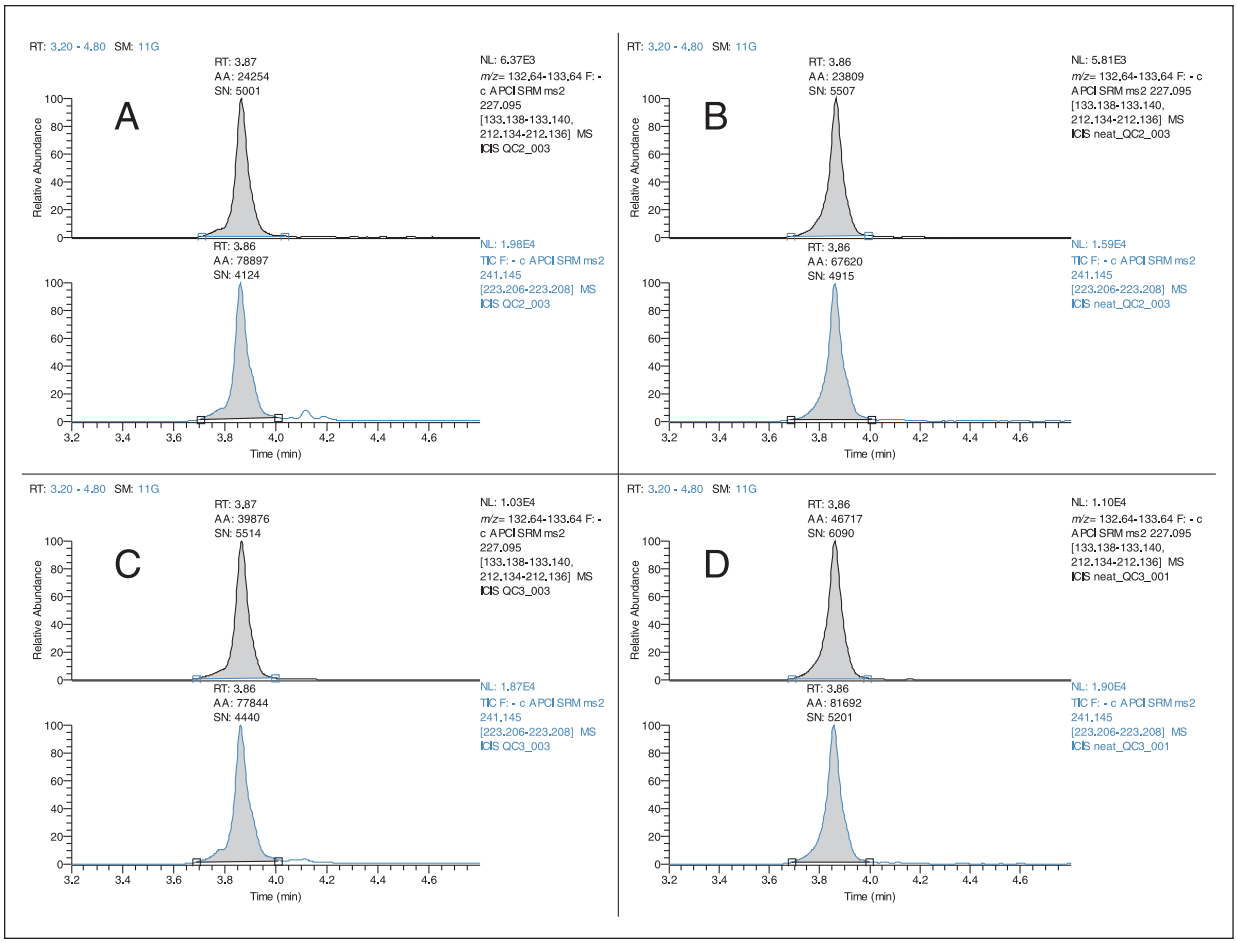

Figure 5: Chromatography comparison of BPA H-SRM *m/z* 133 transition (upper traces) and BPA-d_{16} (lower traces) at 12.5 ng/mL in matrix (panel A), at 12.5 ng/mL in water (panel B), at 25 ng/mL in matrix (panel C), and at 25 ng/mL in water (panel D)

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