An MRM-based Assay for Bisphenol A Derivatives in Human Urine using UHPLC-MS/MS

Wei Zou, Anupama Aditham, Qi Gavin, Jianwen She*
California Department of Public Health, Richmond, CA 94804
*Corresponding author: jianwen.she@cdph.ca.gov

Introduction

Bisphenol A (BPA) has been shown to have endocrine activity and may affect the fetus and infant, including possibly causing changes in development and behavior (1-4). California enacted legislation banning the use of BPA in baby bottles and sippy cups, which was to be effective July 1, 2013 (5). However, as of July 2012, the U.S. Food and Drug Administration permanently ended the use of BPA in these products. Manufacturers are considering BPA analogs or derivatives as replacements for BPA in various applications, such as epoxy resins used as protective linings in food cans and in thermal paper. BPA analogs or derivatives could leach into food or be transferred from paper products, leading to human exposure. Targeted screening of these compounds in urine will provide an indication of human exposures to these emerging compounds.

Figure 1. Increasing exposure to substitutes for BPA in baby bottles is expected.

In the present study, bisphenol S (BPS), bisphenol B (BPB), bisphenol AF (BPAF), and other commercially available BPA analogs or derivatives were analyzed. These compounds were selected for study based on potential for exposure in the U.S., in vitro or in vivo tests showing endocrine activity, and other factors (6).

Methods

- *FIA Optimization*: An Agilent 1290 UHPLC coupled with an Agilent 6460 triple quadruple mass spectrometer was used. The FIA method for fragmentor optimization contained nine 1-min MS2 scan segments each covering m/z 100 to m/z 1000 in a dwell time of 500 msec, each had a fragmentor value of 40, 60, 80, 100, 120, 140, 160, 180, and 200, respectively. For each compound, there was a specific FIA method for CE optimization contained nine 1-min product ion scan segments each covering m/z 50 to m/z 400 in a dwell time of 500 msec, each had a CE value of 10, 20, 30, 40, 50, 60, 70, 80, and 90, respectively. In each CE optimization FIA method, the precursor ion was the molecular ion of this compound, and the fragmentor value was the optimized one during the fragmentor optimization process.
- *On column optimization:* FIA optimization results were further validated using on-column injection (Phenomenex Kinetex phenylhexyl 150x2.1 mm, 1.7 um) in modes of MS2 scan, MS2 SIM, and product ion scan. Mobile phase A was 6.5 mM (0.5 g/L) ammonium acetate in LC-MS grade water with a pH of 5.5, and mobile phase B was LC-MS grade acetonitrile (Honeywell Burdick and Jackson). The flow rate was 0.3 mL/min. The gradient started as segment 1: 0-2 min, 0% B; segment 2: 2.1-22 min, 0%-100% B; segment 3: 22.1-27 min, 100% B; 27.1 min-30 min, 0% B. Only the eluent during the segment 2 came into the ESI source of MS. The same LC method was applied in the tandem MS multiple reaction monitoring (MRM) analysis after MRM transitions of each compound were determined.

Results

In **Figure 1-4**, fragmentation patterns of BPA, BPAF, BPB, and BPS are presented. Under the current condition, the major fragments of BPA were m/z 212 (loss of CH_3) and m/z 134 (loss C_6H_6O), the major fragments of BPAF were m/z 267 (loss of CF_3) and m/z 67 (CF_3^-), the major fragments of BPB were m/z 212 (loss of CH_2CH_3) and 148 (loss of C_6H_6O), the major fragments of BPS were m/z 156 (loss of C_6H_6O) and m/2 92 ($C_6H_6O^-$). MRM transitions were determined by analyzing the fragmentation pattern of each compound.

Figure 1. BPA fragmentation pattern

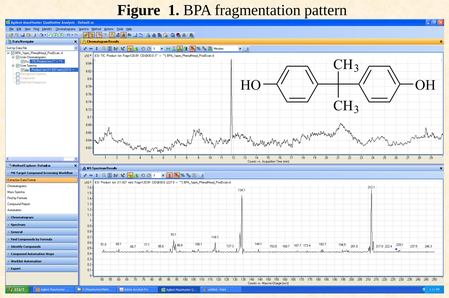


Figure 2. BPAF fragmentation pattern

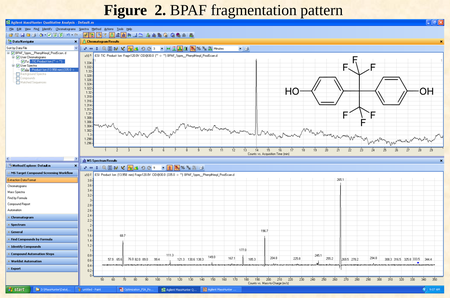


Figure 3. BPB fragmentation pattern

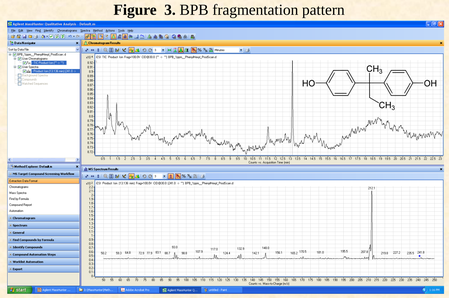
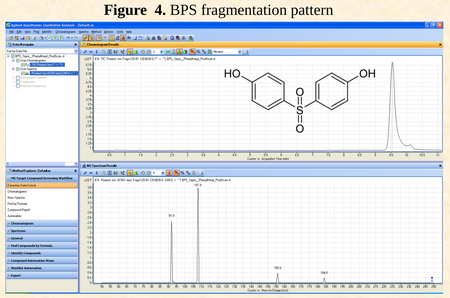


Figure 4. BPS fragmentation pattern



For each compound, standards of 10 ppb, 100 ppb, and 1 ppm were injected into the UHPLC-MS/MS to validate the specificity and sensitivity of the chosen MRM transitions. **Figure 5-8** showed that most MRM transitions at 10 ppb level had a specific and symmetric peak with a signal to noise ratio (S/N) higher than LOD (s/n = 3), except the second MRM transition of m/z 242-> m/z 148 of BPB.

Figure 5. An MRM chromatogram of BPA standard at 10 ppb

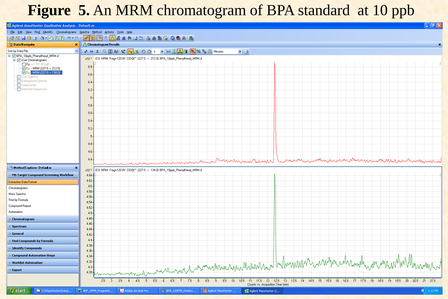


Figure 6. An MRM chromatogram of BPAF standard at 10 ppb

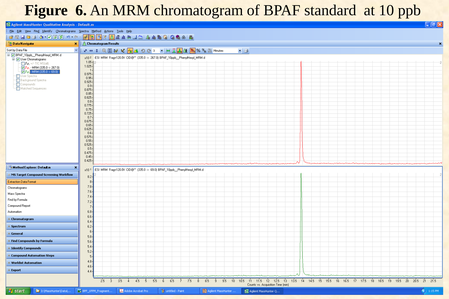


Figure 7. An MRM chromatogram of BPB standard at 10 ppb

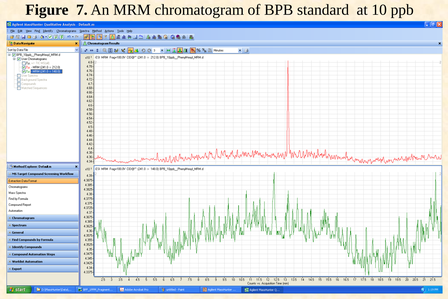


Figure 8. An MRM chromatogram of BPS standard at 10 ppb

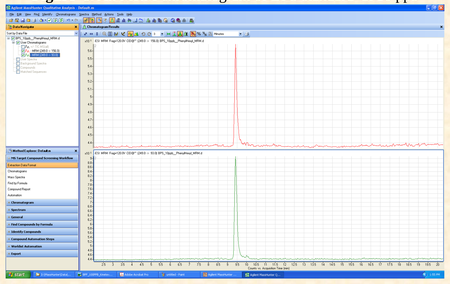


Figure 9-12 showed that every MRM transition channel at 100 ppb level had a nicely shaped peak, suggesting good confidence of detection at this level. .

Figure 9. An MRM chromatogram of BPA standard at 100 ppb

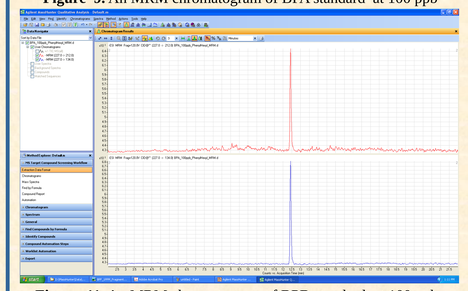


Figure 10. An MRM chromatogram of BPAF standard at 100

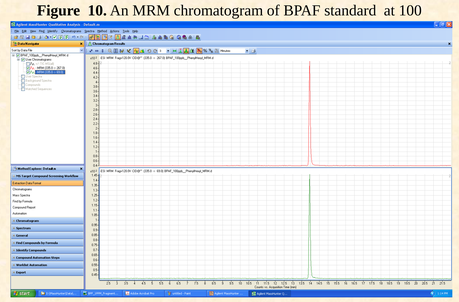


Figure 11. An MRM chromatogram of BPB standard at 100 ppb

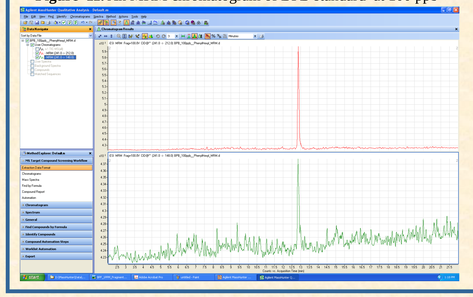
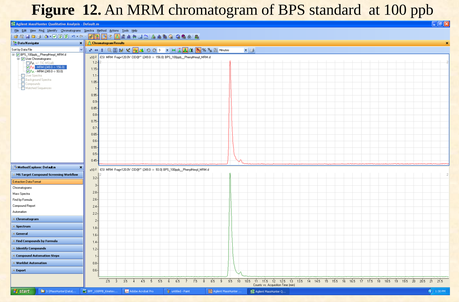


Figure 12. An MRM chromatogram of BPS standard at 100 ppb



Conclusions

- We have set up a sensitive MRM-based assay for BPA-derivatives with high priority for biomonitoring. Further experiments are needed to incorporate BADGE and BFDGE subclasses.
- For sample preparation, liquid-liquid extraction was applied but resulted in low recovery, therefore, solid phase extraction method using either C18 or pure silica cartridge will be tested to extract these BPA-derivatives.
- Initial demonstration of capability, in-house validation using control charts of 20 batches QC samples, and third-party validation using certified reference material and proficiency tests will be conducted.

References

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Acknowledgement

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