AOAC Official Method 2019.07
Free Bisphenol A in Commercially Packaged, Ready-to-Consume, Carbonated/Noncarbonated and Nonalcoholic Beverages

Immunoaffinity Column Purification and UPLC with Fluorescence Detector
First Action 2019

[Applicable to determination of bisphenol A (BPA) in carbonated, noncarbonated drinks, and nonalcoholic drinks.]

Caution: Wear lab coat, gloves, and safety glasses when performing experiments. Performa all BPA standard preparation and sample spiking in safety ventilation hood. Dispose of any waste containing BPA in a specified hazardous waste can. Dispose of other organic liquid waste (methanol based) in labeled liquid safety disposal can following local regulations. Dispose separately tubes or pipets and glass waste (if used) and follow the local recycling program.

A. Apparatus

(**a**) *Ultra-performance liquid chromatography*.--ACQUITY UPLC H-Class with fluorescence detector (Waters, Milford, MA, USA).

(**b**) *Column*.--CORTECS UPLC C18+, 2.1 x 100 mm, 1.6 µm (Waters Part No. 186007116).

(**c**) *Immunoaffinity columns*.—BPATest™ (VICAM, Milford, MA, USA).

(**d**) *Fluted paper filter*.--31240 (VICAM).

(**e**) *Column pump stand*.--G1104 (VICAM).

(**f**) *Phosphate buffered saline (PBS)*.--G1113 (VICAM).

(**g**) *Polypropylene tube*.—15 mL.

B. Materials and Reagents

(**a**) *Beverages*.--Carbonated regular soft drink with full calories, 100% orange juice with pulp, and dairy based coffee drink, were purchased from a local grocery store (Milford, MA, USA).

(**b**) *BPA*.—Certified reference material, TraceCERT® Bisphenol A (42088, SigmaAldrich, St. Louis, MO, USA). Bisphenol B, E, F, and S were also purchased from Sigma.

(**c**) *Acetic acid*.—Sigma (A6283).

(**d**) *Sodium hydroxide*.—Fisher, BP359-212.

(**e**) *Sodium chloride*.—Reagent grade (Fisher, S642).

(**f**) *Methanol*.—LC-MS grade (Fisher, A996-4).

(**g**) *Acetonitrile*.—LC-MS grade (Fisher, A454-4).

(**h**) *Water*.—Purified, with resistivity ≥18 MΩ at 25°C.

C. Reagent, Standard, and Sample Spiking

(**a**) *Acid, base and organic solvents*.--Prepared in the ventilation hood.

(**b**) *BPA calibrating standards*.--The stock BPA standard (for example in this study, 6400 ng/mL) was first prepared in 80% methanol and stored in refrigerator (used within 3 weeks). A standard curve was generated every day the test was performed. The current test procedure provides 10 times of enrichment. Calibrants containing 0, 5, 10, 40, 80, 160, 320 ng/mL in 80% methanol (representing 0, 0.5, 1, 4, 8, 16, 32 ng/mL samples) were prepared from stock BPA standard for recovery study. For lower level calibrants, such as LOD/LOQ study, calibrants containing 0, 0.25, 0.5, 1, 2, 4 ng/mL BPA (equivalent to 0, 0.025, 0.05, 0.1, 0.2, 0.4 ng/mL in samples) were prepared in 80% methanol. The BPA peak area of the standards was plotted against the sample equivalent BPA concentration to generate the standard curve. BPA concentrations of unknown samples were extrapolated from the standard curve by applying the peak area values into the linear regression equation.

(**c**) *BPA spiked samples*.--Beverage samples were spiked with BPA at 1, 4, 8, 16 and 32 ng/mL levels. In recovery study, 0.5 mL of BPA standard stock (6400 ng/mL) was added to 100mL sample to be tested, which produced 32 ng/mL spiked sample. The 32ng/mL sample was serially diluted to 16, 8, 4 1, 0.15 or 0.1 ng/mL using blank sample.

D. Sample Preparation

(**a**) *Carbonated full calorie regular soft drink*.—Adjust pH of 100 mL carbonated soft drink (blank and BPA spiked) to 7.4 with 4N NaOH. Dilute pH-adjusted carbonated drink with PBS (sample: PBS = 1: 1, v/v), stir for 2 min, and load 20 mL of the final diluted solution to a BPATest™ column. Volume of original sample loaded is 10 mL. Sample preparation procedure is designed for samples containing no insoluble solids, for example, carbonated diet drinks and noncarbonated drinks such as sport drinks.

(**b**) *100% Juice with pulp and dairy based coffee drinks*.—Perform BPA extraction of blank and BPA spiked juice and dairy-based coffee drink by adding and dissolving NaCl to a final concentration of 2% (w/v, g/mL), and then adding methanol at 1:1 ratio (v/v), followed by constant mixing at ambient temperature for 30 min. Filter extraction solution with fluted paper filter. Mix 10 mL filtrate with 40 mL PBS. Load 20 mL of this final solution, equivalent to 2 mL original juice or coffee sample, to a BPATest™ column. Sample preparation procedure is developed for samples containing insoluble solids, such as juice with or without pulp, dairy-based drinks, etc.

E. Immunoaffinity Column Chromatography

(**a**) *Conditions*.--Since BPA is widely present in reagents and equipment used for IA column manufacturing, it is recommended to strip the IAC to remove any potential BPA contamination in the column. After connecting IAC to a column reservoir secured on a pump stand, pass 3 mL of a freshly prepared solution of 10% acetic acid in water through the column at a flow rate of 1 drop/s. Wash column with 10 mL PBS.

(**b**) *Application*.—Pass sample solution (20 mL) through IAC at a flow rate of 1 drop/2 s. Wash column with 10 mL water at a flow rate of 1 drop/s. Elute BPA with 1 mL 80% methanol at a rate of 1 drop/2 s, and complete elution by pushing air through column. Directly inject eluate into UPLC (carbonated drink sample) or concentrated (to 200 µL for orange juice and dairy-based coffee drink) and injected to UPLC.

F. UPLC Conditions

(**a**) *Instrument*.--Waters ACQUITY UPLC H-Class System.

(**b**) *Column*.--CORTECS UPLC C18+, 2.1 x 100 mm, 1.6 µm (Waters Part No. 186007116).

(**c**) *Column temperature*.--30°C.

(**d**) *Mobile phase*.—Acetonitrile-water (v/v), 50 + 50.

(**e**) *Flow rate*.--0.4 mL/min.

(**f**) *Injection volume*.--5 µL.

(**g**) *Fluorescent detector*.--ACQUITY ULTRA Performance LC, FLR detector, excitation: 275 nm; emission: 313 nm.

(**h**) *Running time*.--2 min.

(**i**) *Retention time*.--Approximately 1.10 min.

References:

AOAC SMPR 2017.018 (BPA)
J. AOAC Int. **101**, 328(2018)
DOI: 10.5740/jaoacint.SMPR2017.018

J. AOAC Int. (future issue)

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