Simultaneous Analysis of Eight Bisphenols in Food Storage Containers Using Liquid Chromatography-Triple Quadrupole Mass Spectrometry

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1. Introduction

- Recent regulatory attention to bisphenols has intensified due to growing evidence of human exposure from food-contact materials and accumulation of toxicological data. 4,4′-Isopropylidenediphenol (bisphenol A, BPA), a key monomer used in the manufacture of polycarbonate plastics and epoxy resins that line food and beverage containers, is a well-known endocrine-disrupting chemical with effects observed at very low exposure levels. Since the late 1990s, migration of BPA from polycarbonate tableware and epoxy-coated cans into foods and drinks has raised persistent public health concerns regarding dietary exposure. Over the past two decades, risk assessments—principally conducted in the EU—have markedly lowered the tolerable daily intake (TDI) for BPA and tightened restrictions on its use in food-contact applications. Regulatory scrutiny has also broadened to encompass alternative bisphenol analogues.
- In this study, we describe a targeted analytical method for the simultaneous determination of multiple bisphenols and related compounds in polypropylene (PP) food storage containers using liquid chromatography coupled with triple quadrupole tandem mass spectrometry (LC–MS/MS) (Fig. 1). The method affords higher selectivity and sensitivity than conventional HPLC approaches and is suitable for monitoring BPA, bisphenol B (BPB), bisphenol F (BPF), bisphenol S (BPS), bisphenol AF (BPAF), 4,4′-(1,3-dimethylbutylidene)diphenol, phenolphthalein, and tetrabromobisphenol A (TBBPA). We discuss method performance, including limits of detection, matrix effects, and suitability for routine surveillance of bisphenol migration from food-contact PP materials.

Figure 1. Nexera™ X3 and LCMS-8060RX

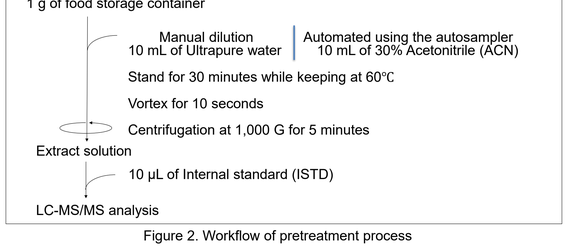

Figure 2. Workflow of pretreatment process

2. Methods

- Commercial PP food storage containers were used as sample material. Sample preparation followed the Japan Food Analysis Center guidelines for synthetic resin utensils, containers and packaging. Each PP container was cut into approximately 1 cm squares and pulverized using a forced mill (Osaka Chemical Co., Ltd.). For migration extraction, 1.0 g of the pulverized sample was weighed into a centrifuge tube, spiked with 1.0 ng Reference standards , and added 10 mL of ultrapure water for manual dilution or 30% ACN for automated dilution The mixture was incubated at 60° C for 30 minutes, vortexed for 10 seconds, and centrifuged at 1,000 × g for 5 minutes at room temperature. The supernatant was collected, spiked with 5 ng of the ISTD, and analyzed by LC–MS/MS. (Fig. 2).
- LC–MS/MS analysis conditions are summarized in Table 1, and Table 2 shows the MRM conditions. Two dilution approaches were employed: manual dilution and automated dilution using the autosampler. Table 3 lists the autosampler's automated dilution/preparation commands.

Table 1. Analytical conditions

[HPLC conditions]	(Nexera™ X3)	[MS conditions]	(LCMS-8060RX)
Analytical Column :	Shim-pack™ XR-ODS Ⅲ (75 mm × 2.0 mm I.D., 1.6 µm)	Interface :	ESI Negative mode
Delay Column :	Shim-pack Scepter™ C18-120 (50 mm × 3.0 mm I.D., 1.9 µm)	Mode :	MRM
Mobile phase A :	Ultrapure water	Interface voltage :	-3 kV
Mobile phase B :	ACN	Ion focus voltage :	0 kV
Gradient program :	[Manual dilution] B conc. 30% (0 min) → 40% (0-2 min) → 100% (5-7 min) → 30% (7.01-10 min) [Automated dilution] B conc. 30% (0 min) → 40% (0-2 min) → 100% (5-6.9 min) →30% (7 min)	Interface temp. :	300℃
		Nebulizer gas flow :	3 L/min
		Drying gas flow :	6 L/min
		Heating gas flow :	17 L/min
		Heating block temp. :	350℃
		DL temp. :	200℃
		Probe position :	+2 mm
Injection volume :	10 µL		
Flow rate :	0.3 mL/min		
Column temp. :	40 ℃		
Rinse method :	Cleaning port→Washing pump 2.0 s		
Rinse solution :	R0, R3 ACN / 2-Propanol = 50:50 (v/v)		

Table 2. MRM transitions

Compound	Polarity	Precursor Ion	Product Ions
BPA	—	227.00	212.20, 133.10
BPB	—	241.00	212.20, 211.20
BPF	—	199.00	93.05, 105.05
BPS	—	249.00	108.00, 92.00
BPAF	—	335.00	197.05, 265.10
4,4′-(1,3-Dimethylbutylidene) diphenol	—	269.00	212.10, 211.15
Phenolphthalein	—	317.00	93.00, 273.00
TBBPA	—	543.00	417.85, 447.80
Bisphenol A (D_{16}, 98%)	—	241.00	223.25, 142.25

Table 3. Automated dilution pretreatment program

The batch add-in allows users to specify a0–a4 in the analysis batch; these values are automatically assigned to the variables. (a0 = stock tray, a1 = stock vial, a2 = ISTD tray, a3 = ISTD vial, a4 = dilution factor).

1	a5=100/a4	14	d.rinse
2	n.drain	15	vial.n rn,sn
3	r.disp 600.0,rs,1	16	n.strk ns
4	d.rinse	17	r.disp 100.1,rs,1
5	vial.n a0,a1	18	mix 1,5,40,ss,35
6	n.strk ns	19	n.drain
7	aspir a5,ss	20	r.disp 600.0,rs,0
8	air.a 0.1,ss	21	d.rinse
9	d.rinse	22	inj.p
10	vial.n a2,a3	23	v.inj
11	n.strk ns	24	wait 2.0
12	aspir 1.0,ss	25	goto f0
13	air.a 0.1,ss	26	end

Reference standards—Bisphenol A (BPA), 4,4-Dihydroxydiphenylmethane (BPF), Bisphenol S (BPS), 2,2-Bis(4-hydroxyphenyl)hexafluoropropane (BPAF), 4,4′-(1,3-Dimethylbutylidene)diphenol, Phenolphthalein, and Tetrabromobisphenol A (TBBPA)—were obtained from Tokyo Kasei. Bisphenol B (BPB) was purchased from Fuji Film Wako Chemical. Deuterated Bisphenol A (BPA-D16, 98%) from Cambridge Isotope Laboratories was used as the ISTD.

3. Results

【 Manual dilution 】

- Figure 3 presents representative mass chromatograms and calibration curves for the simultaneous analysis of eight target analytes. All compounds eluted within 6 min, indicating efficient separation and short run times. Calibration standards (ISTD and reference standards spiked into ultrapure water) were used to construct calibration curves over the ranges 0.1–20 ng/mL for TBBPA and 0.05–20 ng/mL for the other compounds. Calibration curves based on external calibration exhibited excellent linearity (R^2 > 0.989) (Fig. 3).
- Method performance was evaluated by spike-recovery and reproducibility tests (n = 3) following EU bisphenol guidance. Recoveries were 80.8–94.6% for all analytes except TBBPA; BPA recovery improved from 80.8% to 92.6% with internal calibration. TBBPA recovery was <60%, indicating an alternative pretreatment is required. Repeatability (RSD) for all analytes except TBBPA was ≤5.9% (Table 4).

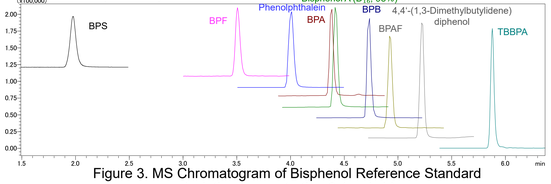

Figure 3. MS Chromatogram of Bisphenol Reference Standard (solution concentration 0.1 ng/mL)

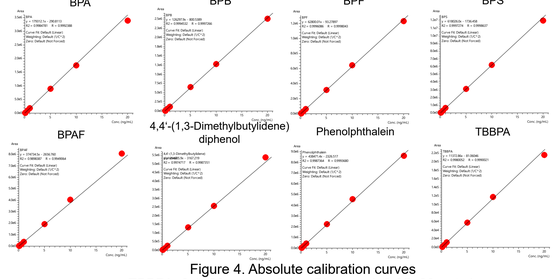

Figure 4. Absolute calibration curves
TBBPA: 0.1~20 ng/mL, Other Bisphenol: 0.05~20 ng/mL

Table 4. Recovery rate of Bisphenols in Containers (Manual dilution)

Compound	Add concentration (µg/kg)	Solution concentration (ng/mL)	Recovery rate (%)	Reproducibility (%RSD, =3)
BPA (No internal correction)	1.0	0.1	80.8	5.4
BPA (Internal correction)	1.0	0.1	92.6	4.9
BPB	1.0	0.1	89.5	3.0
BPF	1.0	0.1	94.6	2.0
BPS	1.0	0.1	87.9	3.2
BPAF	1.0	0.1	81.6	4.8
4,4′-(1,3-Dimethylbutylidene) diphenol	1.0	0.1	88.1	4.8
Phenolphthalein	1.0	0.1	82.2	5.9
TBBPA	5.0	0.5	< 60.0	-

【Automated dilution using the autosampler】

- The R0 and R3 rinse solutions are used for the needle exterior wash and are the same as those used for manual dilution; however, for automated dilution, 30% ACN was added to the R1 rinse solution as the diluent.
- All compounds eluted within 6 minutes, consistent with the manual dilution method. By programming the autosampler's preprocessing commands to perform automatic dilution and add an equal amount of ISTD, calibration curves could be generated simply by preparing a 50 ng/mL standard solution and a 500 ng/mL ISTD. Calibration curves were constructed using the absolute calibration method. BPA and BPS exhibited good linearity over 0.05–20 ng/mL, and the other compounds showed good linearity over 0.1–20 ng/mL (R^2 > 0.99).
- Method performance was evaluated by spike-recovery and reproducibility tests (n = 3). Recoveries were 88.0–100.5% for all analytes. Repeatability (RSD) for all analytes was ≤5.7% (Table 5). Using 30% ACN as the elution solvent in the pretreatment, TBBPA which did not show acceptable spike recoveries in ultrapure water following manual dilution exhibited satisfactory recoveries.

Table 5. Recovery rate of Bisphenols in Containers (Automated dilution using the autosampler)

Compound	Add concentration (µg/kg)	Solution concentration (ng/mL)	Analytical concentration (ng/mL)	Recovery rate (%)	Reproducibility (%RSD, =3)
BPA	10	1	0.4	98.8	1.4
BPB	10	1	0.4	100.5	5.7
BPF	10	1	0.4	97.7	1.0
BPS	10	1	0.4	95.6	1.2
BPAF	10	1	0.4	95.5	6.3
4,4′-(1,3-Dimethylbutylidene) diphenol	10	1	0.4	92.5	2.5
Phenolphthalein	10	1	0.4	88.0	1.3
TBBPA	10	1	0.4	98.7	3.7

4. Conclusion

- Two LC-MS/MS methods were developed: a manual dilution method and an autosampler automatic-dilution method, both capable of eluting eight bisphenols within 6 minutes.
- Using the developed sample preparation and analytical method for bisphenols in PP food storage containers, seven bisphenols can be quantified from a spiking level of 1.0 µg/kg, and TBBPA can be quantified from a spiking level of 10 µg/kg.
- Changing the elution solvent from ultrapure water to 30% ACN improved the recovery of TBBPA.
- BPA recovery was further improved by applying IS correction.
- The autosampler automatic-dilution function is applicable to quantitative analysis of bisphenols, enabling labor and solvent savings and standardization of the workflow.

5. Reference

- European Commission (2024). Regulation (EU) 2024/3190.
- European Commission (2025). Official Journal C_2025/0721.