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Application News

No. AD-0096

Food Safety Analysis / Nexera X2

Development of An UHPLC Method for Simultaneous Determination of Thirteen Bisphenols in Milk Samples

❑ Introduction

Bisphenol A (BPA) and Bisphenol F (BPF) are the monomers to make polycarbonate plastics and epoxy resins. Their diglycidyl esters, i.e., BADGE and BFDGE (Figure 1), are also present in the polymeric products. These materials are made into a variety of consumer products or used as inner coatings for baby milk bottles and reusable food containers which are allowed to use in refrigerator and microwave for food storage and heating. It has been reported that polycarbonate plastics and epoxy-based coatings can release BPA, BPF BADGE and BFDGE as well as their reaction products as illustrated in Figure 1 [1]. These leached chemicals can migrate into food and become contaminants consumed by consumers eventually. Although researches indicate that the migration of these chemicals is normally extremely low [2], the specific migration limits (SMLs) of bisphenols were listed in the EU legislation No 1895/2005 on the restriction of use of certain epoxy derivatives in materials contacted with food. BPA has estrogenic effect and can disrupt normal hormone levels and development in fetuses and babies. In U.S., FDA has published food additive regulations prohibiting the use of BPA-based epoxy resins as inner coatings of containers for infant formula packaging [3]. We describe in this Application News a new UHPLC method for simultaneous determination of thirteen concerned bisphenols including BPA, BPF, BADGE, BFDGE and some structural analogues. An UHPLC system (Nexera X2, Shimadzu Corporation) with a high sensitivity fluorescence detector [4] was adopted to develop a fast and high sensitivity method to meet the requirements of regulations.

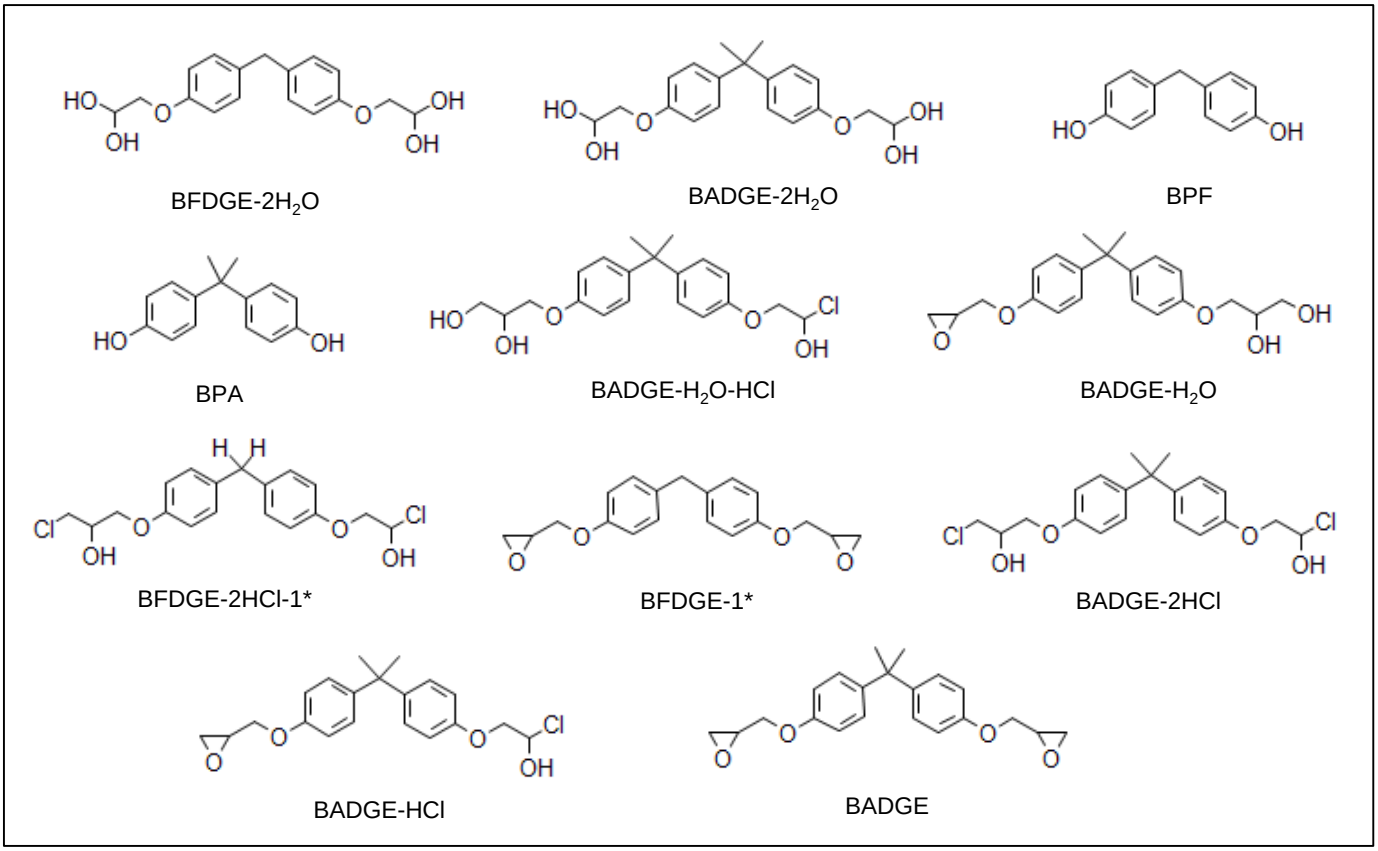

Figure 1 Chemical structures and abbreviation names of bisphenol A, bisphenol F, their diglycidyl esters and derivatives [1].
*Note: positional isomers of BFDGE-2 and BFDGE-2HCl-2 are not shown.

❑ Experimental

Instrumental and analytical conditions

An UHPLC system - Nexera X2 (Shimadzu Corporation) equipped with a fluorescence detector (RF-20Axs) was employed in this work. Separation of bisphenol A (BPA), bisphenol F (BPF) and other 11 derivatives are performed using a Shim-pack HR-ODS column (250 x 3.0mm, 3µm) with an optimized gradient elution program. Pure water and acetonitrile (ACN) were used as UHPLC mobile phases without any additive. The detailed analytical conditions of the UHPLC method are shown in Table 1.

Standards and spiked milk samples

A mixed standard stock solution of thirteen bisphenols (Refer to Table 2) containing BPA, BPF and other derivative compounds were prepared in ACN/H_2O (30:70). A serial of calibration standards of concentrations from 5 µg/L to 2,000 µg/L were prepared from the stock to set up multi-point calibration curves. Two blank milk matrix spiked with known concentrations of standards (100 and 1,000 µg/L) obtained from a third party laboratory were used for evaluation of the method performance.

Table 1: UHPLC conditions of Bisphenols and derivatives.

Column	Shim-pack HR-ODS (250 x3.0 mm, 3µm)
Mobile phase	A: Water B: Acetonitrile
Elution program	0.1min, 30% B; 13min, 45% B; 37min, 70% B; 38-43min, 85% B; 43.1min, 30% B.
Flow rate	0.40 mL/min
Detection	Ex 235 nm, Em 317nm
Oven temp.	30 ºC
Injection	10 µL

❑ Results and Discussion

Development of fast UHPLC method

For well separation of the thirteen bisphenols studied, a reference HPLC method has a long running time of 95 mins. As shown in Figure 3, the current UHPLC method was optimized to achieve fast elution for every compounds with sufficient separation resolution, especially for the separation of BADGE-H_2O and BADGE-H_2O-HCl at 19.4 and 19.9 mins. Due to the similarity in compound structure and chemical properties, separation of these two peaks was a main obstacle to achieve fast analysis speed. The results obtained show clearly the advantages of an UHPLC column with small particle size (3 µm) of the C18 stationary phase.

The main targets BPA (18.1 min) and BPF (13.4 min), BADGE (34.3 min) are separated completely without any inference. BFDGE has two positional isomers, which appeared as a pair at 28.8 min and 30.0 min, respectively. Noted that, another pair of positional isomers BFDGE-2HCl appeared just before the BFDGE peaks at 26.9 min and 27.9 min, respectively.

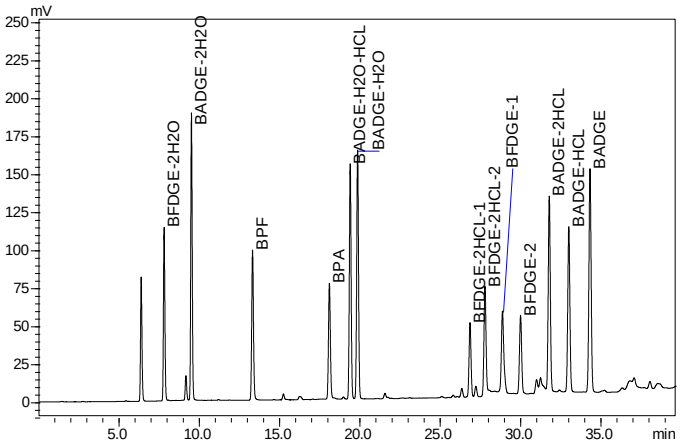

Figure 2: UHPLC-RF chromatogram of mixed standards of thirteen bisphenols at concentration of 100 µg/L each.

Table 2: Summary of UHPLC method and performance evaluation results for analysis of thirteen bisphenols

ID #	Name	Ret Time (min)	Calibration range: 5-2000 µg/L		RSD (%), n=6		Sensitivity [2]	
			R^2	Accuracy (%) [1]	5µg/L	100µg/L	LOD (µg/L)	LOQ (µg/L)
1	BFDGE-2H_2O	7.9	0.9998	100.9	1.0	0.9	0.7	2.0
2	BADGE-2H_2O	9.5	0.9991	98.7	0.4	0.4	0.4	1.2
3	BPF	13.4	0.9997	101.7	0.7	0.3	0.8	2.5
4	BPA	18.1	0.9997	101.2	0.8	0.3	1.0	3.1
5	BADGE-H_2O-HCL	19.4	0.9998	101.5	0.5	0.3	0.5	1.5
6	BADGE-H_2O	19.9	0.9997	103.2	0.3	0.3	0.5	1.5
7	BFDGE-2HCL-1	26.9	0.9996	98.8	0.6	0.1	1.5	4.6
8	BFDGE-2HCL-2	27.8	0.9997	100.8	2.3	0.2	1.2	3.6
9	BFDGE-1	28.9	0.9997	99.3	0.9	0.3	1.4	4.2
10	BFDGE-2	30.0	0.9997	101.3	1.2	0.4	1.6	4.7
11	BADGE-2HCL	31.8	0.9997	98.9	1.8	0.3	0.6	1.7
12	BADGE-HCL	33.0	0.9997	101.7	0.5	0.4	0.7	2.2
13	BADGE	34.3	0.9997	100.7	1.0	0.5	0.5	1.6

Notes: 1. Average of 12 concentration levels 5-2000 µg/L
2. Estimated using 5 ug/L mixed stds data based on S/N=3 for LOD and S/N=10 for LOQ

Calibration curves, range and linearity

Linear calibration curves of the thirteen bisphenols are established using mixed standards samples for concentrations ranging from 5 µg/L to 2000 µg/L as shown in Figure 3. A total of 12 concentration levels were used with each compound in the mixture being 5, 10, 20, 50. 100, 200, 400, 600, 800, 1200, 1500 and 2000 µg/L. All of the thirteen bisphenols peaks give excellent linearity with R^2 greater than 0.999 as tabulated in Table 2.

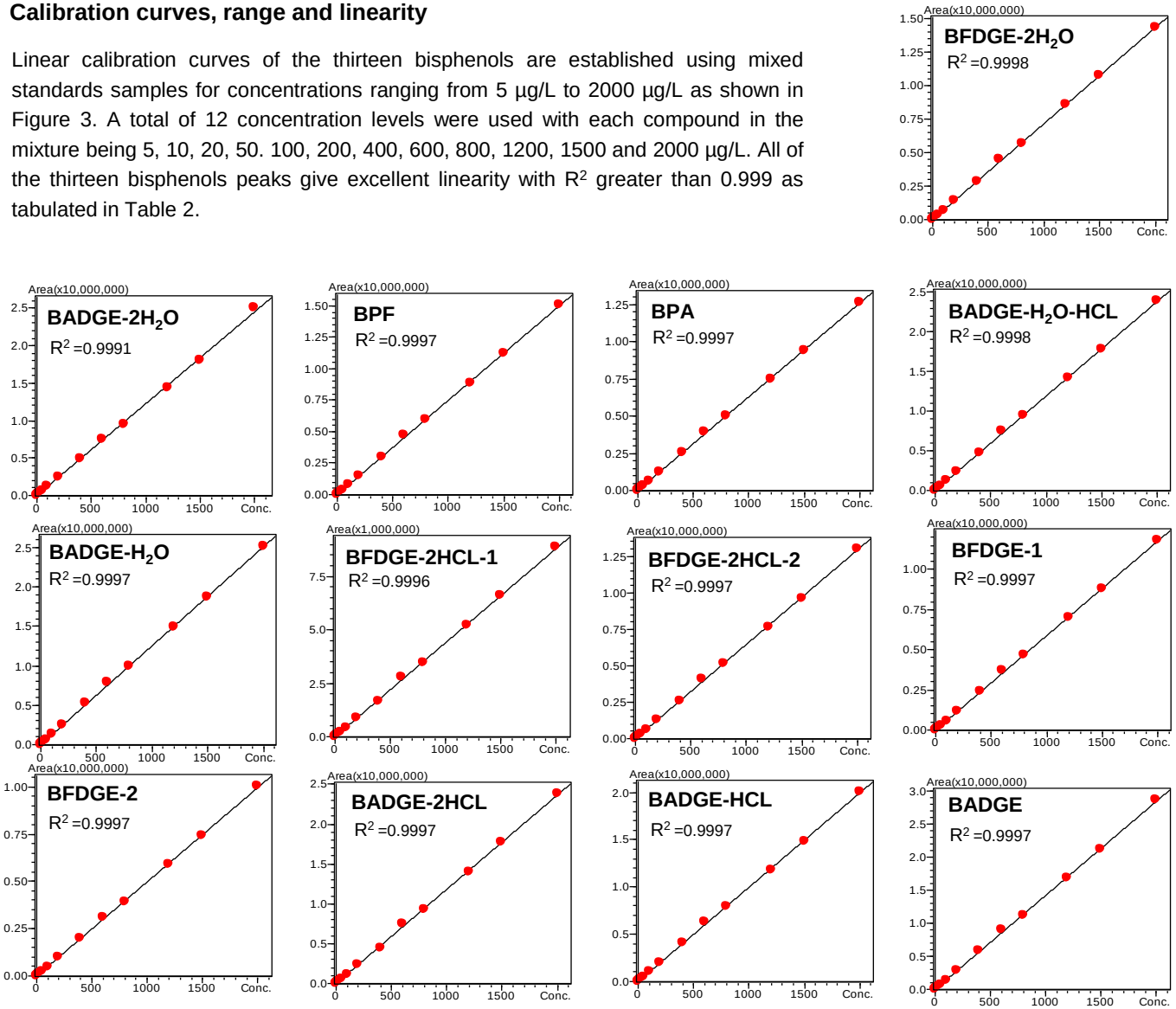

Figure 3: Calibration curves of the thirteen bisphenols with concentration range from 5 µg/L to 2,000 µg/L.

Evaluation of method performance

The accuracy of the method at every calibration levels were calculated and the average accuracy values for every compounds are presented in Table 2. To evaluate repeatability of the method, six consecutive runs of the lowest concentration mixed standard sample (5 µg/L) and a mixed standard sample of 100 µg/L were performed. The RSD values for the 5µg/L mixed standards are less than 2.3%, while RSD values for 100µg/L concentration level are less than 1%, as can be seen in Table 2. The limit of detection (LODs) and limit of quantification (LOQs) were determined from the chromatogram of the lowest concentration mixed standards (5µg/L) as shown in Figure 4, following the rule of S/N=3 for LOD and S/N=10 for LOQ. The obtained LODs and LOQs are at 0.4~1.6 µg/L, and 1.5~4.7 µg/L for the thirteen bisphenols (Table 2).

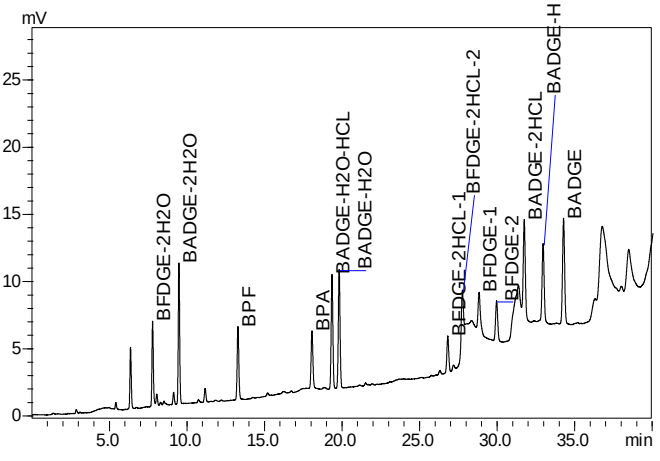

Figure 4: UHPLC-RF chromatogram of mixed standards of thirteen bisphenols, 5 µg/L each compound.

Spiked milk samples and recovery

A blank milk sample and two spiked samples of 13 mixed standards in the same blank were obtained from an analytical laboratory, labeled as Blank, S1 (spiked 100 µg/L) and S2 (spiked 1,000 µg/L). The blank milk matrix was analyzed first and the result showed no any detection of the 13 bisphenols studied. The chromatogram of spiked sample S1 is shown in Figure 5. The quantitative results and recovery data of the 13 bisphenols in both samples are tabulated in Table 3. In both samples, the measured concentrations of BADGE-H2O-HCl are higher than the expected levels with a recovery around 130%. On the other hand, three compounds with longer retentions (peaks 11~13) exhibit much lower concentrations as expected and low recovery of about 40% and 60%.

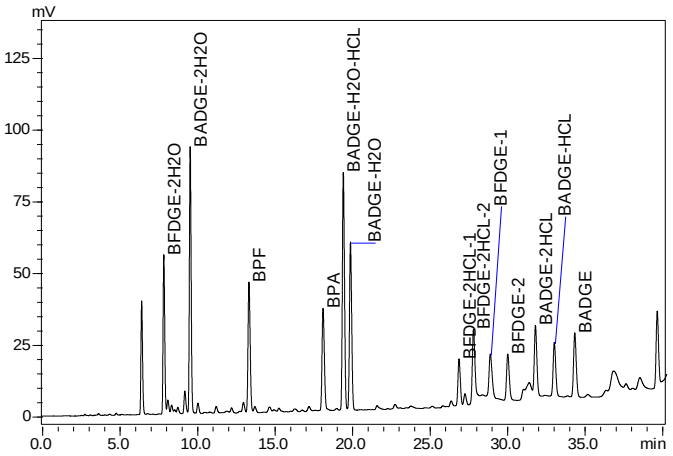

Figure 5: UHPLC-RF chromatogram of spiked milk sample S1 (100 µg/L). The sample was diluted with water for two time prior to injection (10 uL)

Table 3: Analysis results of spiked milk samples for 13 bisphenols determined by the UHPLC-RF method established

ID #	Name	Ret Time (min)	Spiked S1		Spiked S2	
			Conc. (µg/L)	Recovery %	Conc. (µg/L)	Recovery %
1	BFDGE-2H_2O	7.9	115.0	115	1140.8	114.1
2	BADGE-2H_2O	9.5	114.5	114.5	1123.6	112.4
3	BPF	13.4	114.9	114.9	1152.4	115.2
4	BPA	18.1	114.1	114.1	1162.9	116.3
5	BADGE-H_2O-HCL	19.4	131.3	131.3	1284.9	128.5
6	BADGE-H_2O	19.9	91.1	91.1	983.1	98.3
7	BFDGE-2HCL-1	26.9	79.3	79.3	947.6	94.8
8	BFDGE-2HCL-2	27.8	90.5	90.5	983.0	98.3
9	BFDGE-1	28.9	68.6	68.6	823.8	82.4
10	BFDGE-2	30.0	77.2	77.2	824.2	82.4
11	BADGE-2HCL	31.8	47.3	47.3	668.9	66.9
12	BADGE-HCL	33.0	43.3	43.3	635.7	63.6
13	BADGE	34.3	37.4	37.4	563.7	56.4

❑ Conclusions

An UHPLC method with using a high sensitivity fluorescence detector was developed for fast, well-separation and high sensitivity analysis of thirteen bisphenols, including the most concerned BPA, BPF, BADGE and BFDGE, in milk samples. This new method shows high sensitivity to low µg/L levels, high accuracy and excellent repeatability. The method was applied to spiked milk samples and the results indicated the good feasibility, high sensitivity and reliability in simultaneous determination of thirteen bisphenols in milk samples.

❑ References

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2. Ivana Poustkov, Jaroslav DobiD, Ingrid Steiner, Jan Poustka, Michal Voldr˘ich, Eur Food Res Technol (2004) 219:534–539.
3. United States Food and Drug Administration web site: Bisphenol A (BPA) for Use in Food Contact Applications
4. Application News No. L408, Shimadzu Corporation.

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