Assessment of Bisphenol A Specific Migration from Packaging Materials into Food Simulants Using UHPLC-MS/MS and LC with Fluorescence Detection

Application Note

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Abstract

Bisphenol A (2,2-bis(4-hydroxyphenyl) propane), known as BPA, is used in the production of epoxy resins for internal and external coating of food and beverage cans. It is also used in the production of polycarbonate resins. BPA has been identified as an endocrine disruptor and has been evaluated by health authorities world wide. Those regulatory agencies confirmed the safety of BPA applications; however, they limited the use of this substance in the composition of feeding bottles for infants up to 12 months. For other applications, BPA is allowed, and has a specific migration limit of 0.6 mg/kg food. Packaging for food must comply with this limit. To verify compliance with the legislation, tests of specific migration (SM) are carried out with food simulants using liquid chromatography with fluorescence detection, or UHPLC-MS/MS. The objectives of this study were to validate a method to determine the specific migration of BPA in food simulants using both types of equipment. The limit of detection (LOD) value obtained was 10–30 times lower using UHPLC-MS/MS than HPLC-FL for aqueous simulants, whereas the limit of quantification (LOQ) was approximately 10 times less for the same simulants. For the fat simulant, the LOD was almost 5 times lower for UHPLC-MS/MS, and the LOQ was 2.4 times lower than HPLC-FL. The specificity and the low LOD and LOQ obtained from UHPLC-MS/MS can support further studies related to migration of BPA into foods to establish the consumers' exposure and help the health authorities monitor the presence of BPA in food.

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Introduction

The wide use of epoxy coatings for food and beverage cans is related to their high adhesion to the substrate, high flexibility, and good chemical resistance. The epoxy-acrylate water-based varnish is one of the most commonly used varnish for aluminum two-piece soft drink cans. Epoxy-amine varnish is used for steel three-piece cans for sweetened condensed milk, while epoxy-phenolic varnish is used for steel three piece cans for milk cream, tomato products, and canned corn and peas. Due to the health concern of BPA, it has been identified as an endocrine disruptor and has been evaluated by health authorities such as EFSA, FDA, Health Ministry of Canada, and ANVISA. These regulatory agencies have confirmed the safety of BPA applications; however, they have limited the use of this substance in the composition of feeding bottles for infants up to 12 months. For other applications, BPA is allowed and has the specific migration limit of 0.6 mg/kg of food as established by European, Mercosur, and Brazilian legislation [1,2,3]. Therefore, food packaging must comply with this limit. To verify the compliance with the legislation, tests of specific migration (SM) are carried out with food simulants that are representative of different types of food. Distilled water, acetic acid solution (3 % w/v), ethanol solution (50 % v/v), and olive oil are representative of aqueous, acid, alcoholic and dairy products, and fatty foods [4].

The method developed by the European Committee for Standardization, CEN/TS 13130-13:2005 [5], is used as a reference for the determination of specific migration of Bisphenol A. The analyses are performed on HPLC with fluorescence detection. However, it has been observed that the fluorescence detector is efficient for BPA determination in food simulants, but to detect this monomer in food, or confirm its presence in food or food simulant, liquid chromatography with mass detection is essential. The knowledge of the quantity of BPA in food is vital to establish the consumers' exposure to this substance.

The objectives of this study were to validate a method to determine specific migration of BPA in food simulants using a UHPLC-MS/MS and an HPLC with fluorescence detection, and compare the results obtained.

Experimental

UHPLC-MS/MS

LC conditions

Instrument	Agilent 1290 Infinity LC System
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 50 mm, 1.8 µm (p/n 959757-902)
Column temperature	30 °C
Injection volume	10 µL
Mobile phase	Methanol:water acidified with 0.1 % acetic acid (70:30/v:v) isocratic elution
Flow rate	0.3 mL/min

MS conditions

Instrument	Agilent 6400 Series Triple Quadupole LC/MS System
Ion mode	AJS-ESI, negative ionization
Capillary voltage	3,500 V
Drying gas (N_2)	10 L/min
Drying gas temperature	250 °C
Nebulizer	45 psi
Sheath gas heater	300 °C
Sheath gas flow	10 L/min

HPLC-fluorescence

LC conditions

Instrument	Agilent 1200 Infinity Series
Column	Agilent 100 RP 18e (Lichrospher) 4.0 mm × 250 mm, 5 µm (p/n 79925ODE-584)
Column temperature	30 °C
Injection volume	50 µL
Mobile phase	Methanol:water (70:30/v:v) for aqueous simulants Methanol:water (60:40/v:v) for fat simulant Isocratic elution
Flow rate	1.0 mL/min

Fluorescence conditions

Instrument	Agilent 1200 Infinity Series
Wavelength emission	275 nm
Wavelength emission	305 nm

The ions monitored for Bisphenol A are listed in Table 1. The most intense transition was used as a quantifying ion (*m/z* 133) and the second most intense was used as a qualifying ion (*m/z* 211.1) for the confirmation of the analysis.

Table 1. Retention Time and MRM Conditions of Selected Compounds

RT (min)	Precursor ion (*m/z*)	Product ion (*m/z*)	Dwell (ms)	Fragmentation (V)	CE (V)	CellAcc (V)
1.0	227.1	211.1	200	139	24	4
		166.8			52	
		133			24	

The limit of detection (LOD) and limit of quantification (LOQ) were obtained by injecting different solutions of the same concentration (80 µg/L for the mass detector and 188 µg/L for the fluorescence detector) seven times, as established by INMETRO DOQ-CGCRE-008 [6]. The limits were determined using four food simulants: distilled water, acetic acid solution (3 % w/v), ethanol solution (50 % v/v), and olive oil.

The recovery preparation was based on migration determination [4]. Triplicate samples of food simulants spiked with the analyte (90, 600, and 1,200 µg/L for aqueous simulants and 300, 600, and 1,200 µg/L for the fat simulant) were placed for 10 days at 40 °C, simulating prolonged contact of the package with food at room temperature. After this period, the samples were analyzed. The experiment was carried out with four simulants.

The linearity was determined by a series of six duplicate injections of standard solutions ranging from 0 to 6,000 µg/L for aqueous and fat simulants.

Results and Discussion

LODs and LOQs

Tables 2 and 3 show the comparison of LOD and LOQ obtained using UHPLC-MS/MS and a HPLC-fluorescence detector, respectively. The LOD value was 2.6–30 times lower for UHPLC-MS/MS than HPLC-FL for aqueous simulants, whereas the LOQ was approximately 10 times less for the same simulants. For the fat simulant, the LOD was almost 5 times lower for UHPLC-MS/MS and the LOQ 2.4 times lower than HPLC-FL.

Table 2. LODs and LOQs Using UHPLC-MS/MS

Simulants	LOD (µg/L)	LOQ (µg/L)
Distilled water	0.76	4.33
3 % w/v aqueous acetic acid	0.20	3.57
50 % v/v aqueous ethanol	0.95	4.41
Olive oil	4.40	95.7

Table 3. LODs and LOQs Using HPLC-Fluorescence

Simulants	LOD (µg/L)	LOQ (µg/L)
Distilled Water	5.4	43.6
3 % w/v aqueous acetic acid	6.2	38.5
50 % v/v aqueous ethanol	2.5	34.3
Olive oil	20.7	227.9

Recovery

Tables 4 and 5 present the results of recovery obtained using UHPLC-MS/MS and HPLC-FL, respectively. The recovery range accepted for concentration from 300 to 1,200 µg/L, according to Huber, is between 80–110 %. For 90 µg/L, 60–115 % is accepted [7].

Table 4. Recovery Using UHPLC-MS/MS

Simulants	Reference concentration (µg/L)	Experimental concentration* (µg/L)	Standard deviation	Recovery % (After 40 °C/10 days)
Distilled water	90	103	0.01	115
3 % w/v aqueous acetic acid		64	0.01	71
50 % v/v aqueous ethanol		101	0.01	112
Olive oil	300	330	0.01	110
Distilled water	600	601	0.05	100
3 % w/v aqueous acetic acid		490	0.04	82
50 % v/v aqueous ethanol		552	0.10	92
Olive Oil		544	0.11	91
Distilled water	1,200	1,109	0.08	92
3 % w/v aqueous acetic acid		992	0.13	83
50 % v/v aqueous ethanol		1,233	0.21	103
Olive oil		1,124	0.11	94

*Average of three determinations

Table 5. Recovery Using HPLC-Fluorescence

Simulants	Reference concentration (µg/L)	Experimental concentration* (µg/L)	Standard deviation	Recovery % (After 40 °C/10 days)
Distilled water	90	91	0.01	101
3 % w/v aqueous acetic acid		74	0.01	82
50 % v/v aqueous ethanol		92	0.01	102
Olive oil	300	300	0.03	100
Distilled water	600	630	0.02	104
3 % w/v aqueous acetic acid		490	0.04	98
50 % v/v aqueous ethanol		530	0.07	88
Olive oil		520	0.12	87
Distilled water	1,200	1,070	0.03	89
3 % w/v aqueous acetic acid		1,136	0.11	95
50 % v/v aqueous ethanol		1,059	0.21	88
Olive oil		1,125	0.17	94

*Average of three determinations

Linearity and calibration curve

The linearity of the analytical curve was studied using standard solutions in six concentrations ranging from 0 to 300 µg/L and 300 to 6,000 µg/L for aqueous simulants and 0 to 6,000 µg/L for a fat simulant. For BPA, the coefficient of determination (R^2) calculated by linear regression presented values greater than 0.996 using both systems, UHPLC-MS/MS and HPLC-FL. According to the standard method CEN/TS 13130-13:2005, the R^2 has to be higher than 0.99 [5]. Figure 1 shows examples of the calibration curves in aqueous simulants obtained by UHPLC-MS/MS.

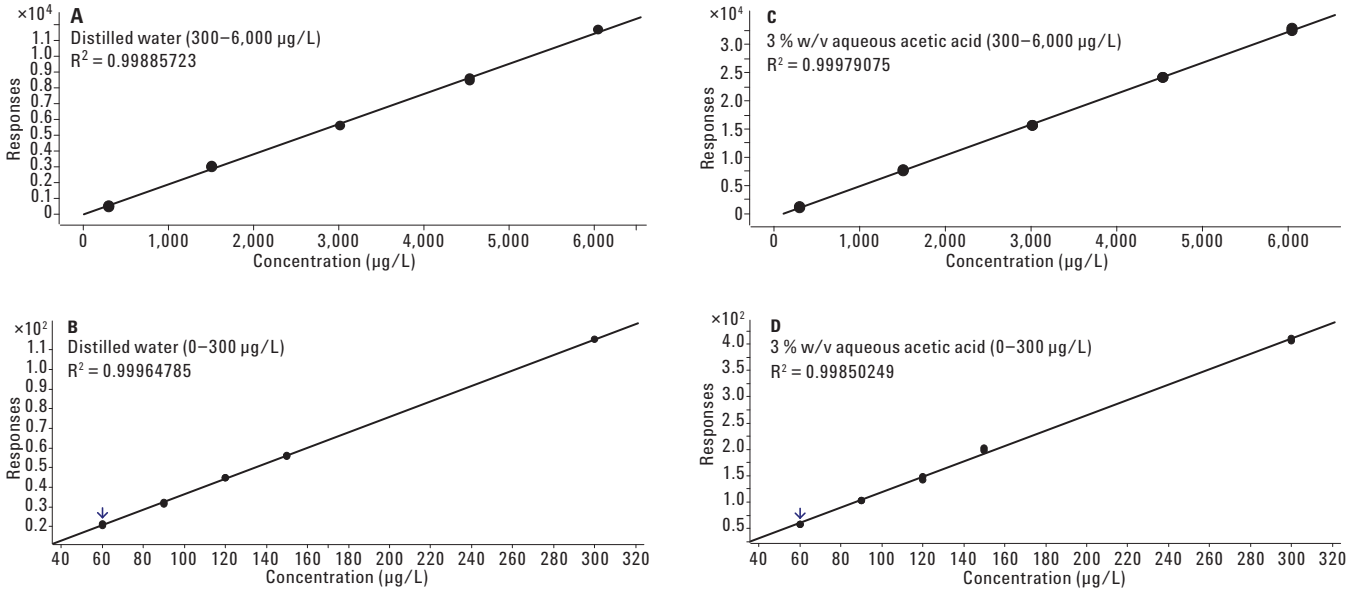

Figure 1. Calibration curves of Bisphenol A. A) distilled water simulant (300–6,000 µg/L), B) distilled water simulant (0–300 µg/L), C) 3 % w/v aqueous acetic acid simulant (300–6,000 µg/L), and D) 3 % w/v aqueous acetic acid simulant (0–300 µg/L) .

Tables 6 and 7 show the determination coefficient (R^2) obtained for all simulants.

Figure 2 shows the MRM chromatogram obtained for Bisphenol A at 600 µg/L, in 3 % w/v aqueous acetic acid.

Table 6. Coefficient of Determination Using UHPLC-MS/MS

Simulants	Determination coefficient (R^2) (0–300 µg/L)	Determination coefficient (R^2) (300–6,000 µg/L)
Distilled water	0.999	0.999
3 % w/v aqueous acetic acid	0.998	0.999
50 % v/v aqueous ethanol	0.998	0.999
Olive oil	0.999	

Table 7. Coefficient of Determination Using HPLC-Fluorescence

Simulants	Determination coefficient (R^2) (0–300 µg/L)	Determination coefficient (R^2) (300–6,000 µg/L)
Distilled water	0.999	0.997
3 % w/v aqueous acetic acid	0.991	0.999
50 % v/v aqueous ethanol	0.991	0.999
Olive oil	0.997	

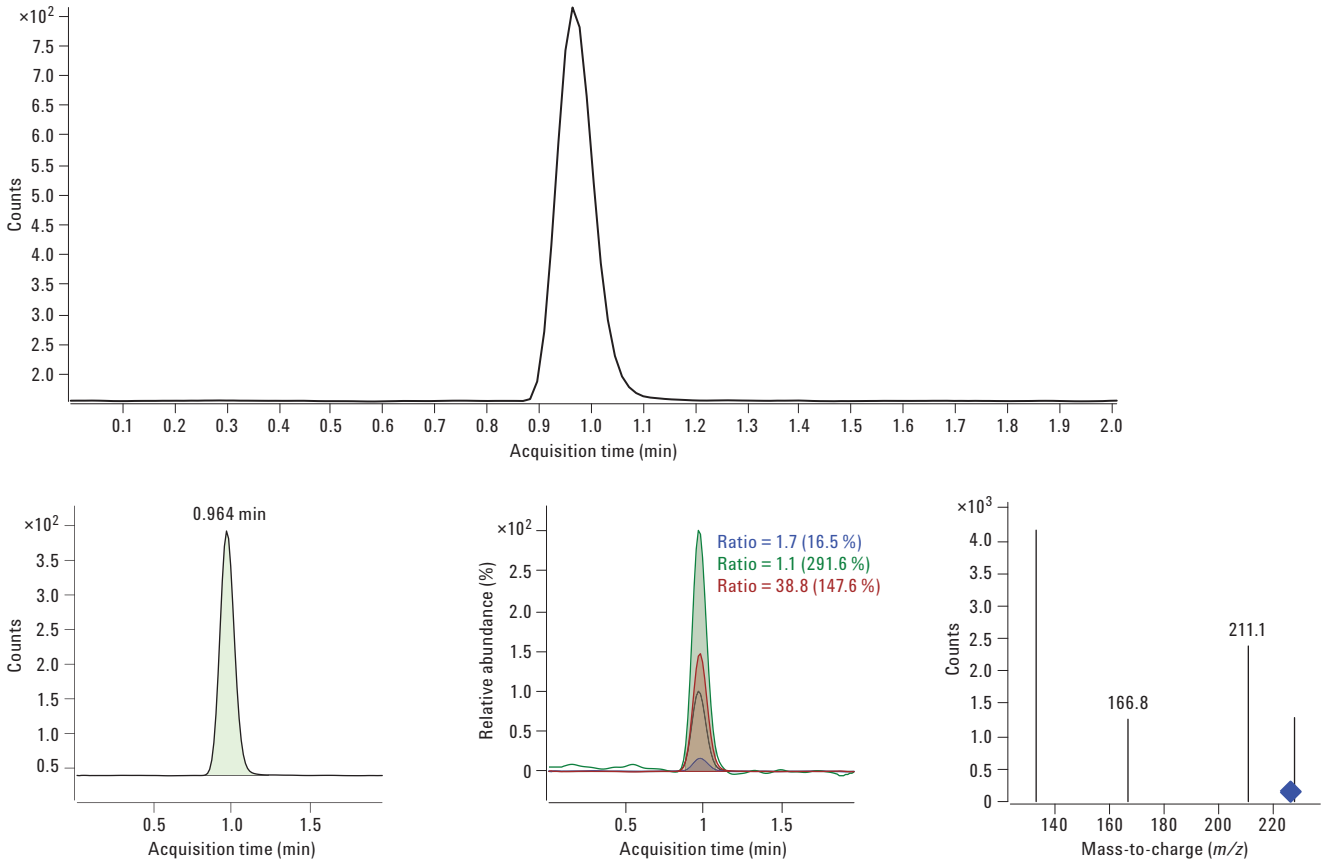

Figure 2. UHPLC-MS/MS chromatogram of Bisphenol A in 3 % aqueous acetic acid simulant.

Conclusion

The methods validated can be applied to study the specific migration of BPA from plastic materials and varnishes used as internal coating of cans in all food simulants. The method takes 2 minutes for BPA analysis using UHPLC-MS/MS, and 7 minutes (aqueous simulant) or 12 minutes (fat stimulant) with HPLC with a florescence detector. The sensitivity of the method is suitable to meet the limit of BPA established in the Mercosur [2] and European Union legislation [1]. The proposed methodology is simple, quick, and shows linear calibration curves. The recovery of BPA is within the range expected. The specificity and the low LOD and LOQ obtained from UHPLC-MS/MS can support further studies related to migration of BPA to establish the consumers' exposure and assist the health authorities to monitor the presence of BPA in foods.

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