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Journal of Chromatography A, 1129 (2006) 145–148
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Short communication

Single laboratory validation of a method for the determination of Bisphenol A, Bisphenol A diglycidyl ether and its derivatives in canned foods by reversed-phase liquid chromatography

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Received 20 December 2005; received in revised form 31 July 2006; accepted 10 August 2006

Abstract

A method was developed and validated for the simultaneous determination of Bisphenol A (BPA), Bisphenol A diglycidyl ether (BADGE), BADGE-H_2O, BADGE-$2H_2O$, BADGE-H_2O-HCl, BADGE-HCl, and BADGE-2HCl in canned food using reversed phase high-performance liquid chromatography (HPLC) with fluorescence detection; chromatographic separation of all seven analytes was achieved ($R_s \geq 1.08$) using HPLC gradient elution technique. Acetonitrile was used to extract the analytes from the food matrix before subjecting the samples to liquid–liquid extraction, solid-phase extraction for further clean-up and preconcentration prior to HPLC analysis. Excellent inter-day precision data ($n = 10$) and intra-day precision data ($n = 5$) were obtained on a 200 μg/kg spiked sample. The RSD ranged from 0.20% to 2.96% for the inter-day precision tests, and 0.04% to 2.82% for the intra-day precision tests. Accuracy was measured at three concentration levels: 200, 1000, and 2000 μg/kg; recoveries ranged from 86.07% to 114.06%. The excellent validation data suggests that this method can be applied on canned foods for the determination of migration of BPA, BADGE and its derivatives from can coatings into food.

Keywords: Can coatings; Bisphenol A (BPA); Bisphenol A diglycidyl ether (BADGE); Migration; High-performance liquid chromatography (HPLC)

1. Introduction

Bisphenol A (2,2′bis(4-hydroxyphenyl)propane, BPA) is used in the manufacture of bisphenol A diglycidyl ether (BADGE) which is then used for can coatings. The European Union has recently adjusted the specific migration limit of these compounds in food due to migration from can coatings [1,2]. Many methods have been developed for such migration studies [3–6]. The method reported in this paper is an improvement of the method reported by Leepipatpiboon et al. [6] involving an additional analyte, BPA. In addition, an extraction method with better recoveries of analytes is also reported. This paper therefore provides a robust analytical method capable of determining seven bisphenolic analytes simultaneously, namely, BPA, BADGE, BADGE-H_2O, BADGE-$2H_2O$, BADGE-H_2O-HCl, BADGE-HCl, and BADGE-2HCl from can coatings into food.

2. Experimental

2.1. Materials and reagents

Bisphenol A (minimum purity 99%) was purchased from TCI (Tokyo, Japan); BADGE, BADGE-H_2O, BADGE-$2H_2O$, BADGE-H_2O-HCl, BADGE-HCl, and BADGE-2HCl were purchased from Fluka (Buchs, Switzerland). HPLC grade acetonitrile, and methanol, and analytical grade hexane and ethyl acetate were purchased from Labscan Asia Co. Ltd. (Bangkok, Thailand). Stock standard solutions were individually prepared in acetonitrile, and kept in the refrigerator for not more than three months. All working standard solutions were freshly prepared prior to use. Mobile phases were prepared using HPLC

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doi:10.1016/j.chroma.2006.08.018

grade acetonitrile and HPLC grade water. Oasis HLB cartridges (200 mg, 6 cc) were purchased from Waters (Milford, Massachusetts, USA) for solid phase extraction.

2.2. Apparatus

HPLC analyses were performed using a Waters 2695 separations module and a Waters 2475 fluorescence detector (Milford, Massachusetts, UK); excitation wavelength: 235 nm; emission wavelength: 317 nm; injection volume: 10 μL; run length: 42 min; column temperature: 25 °C; column: Nucleosil-100, 5 μm, C18, 250 mm × 4 mm i.d. (Hichrom Limited- Berkshire, UK); flow rate: 0.4 mL/min, where 0–1 min: 40:60 A:B (v/v); 22 min: 50:50 A:B (v/v); 35.5–36 min: 95:5 A:B (v/v); 38.5–42 min: 40:60 A:B (v/v), where A = acetonitrile, and B = HPLC grade water. Prior to each series of chromatographic separations, the analytical column was conditioned for 30 min with methanol, and equilibrated with (40:60 v/v) acetonitrile: water for 42 min to provide a stable baseline for subsequent chromatographic analysis. 10 min of equilibration was required before the next injection. Method robustness was tested using three different columns: Nucleosil-100 5 μm, C18, 250 mm × 4 mm i.d. (Hichrom Limited- Berkshire, UK); Hypurity Elite Hypersil ODS 5 μm, 250 mm × 4 mm i.d. (Alltech- Massachusetts, USA); Shim-Pack VP-ODS 5 μm, 250 mm × 4 mm i.d. (Shimadzu Corporation- Kyoto, Japan).

2.3. Samples

Food analysis proficiency assessment scheme (FAPAS®) test materials for BADGE-2HCl, BADGE-H_2O-HCl, and BADGE-HCl (series T1224) were purchased from the Central Science Laboratory (York, UK). Canned food samples of oily, aqueous or acidic media analyzed in this study were obtained from local supermarkets, and two aliquots of each sample were taken for duplicate analyses.

For each analytical run, a sample blank prepared using suitable food simulants, i.e., corn oil, 10% ethanol, or 3% acetic acid, and a fortified sample (w/w) of the appropriate food simulant, were prepared to estimate the degree of recovery. In order to ensure accurate analytical results, the food simulants were analyzed separately to ensure that they were free from any interfering contaminants.

2.4. Sample preparation

Five grams of food was shaken with 40 mL of acetonitrile in a round-bottomed flask for 25 min and filtered through a filter paper. After rinsing the round-bottomed flask with 10 mL of acetonitrile, 75 mL of *n*-hexane was added. The mixture was shaken and allowed to stand for 25 min. The acetonitrile layer was removed but retained, and the hexane layer was washed twice with acetonitrile (30 mL; 20 mL). The solvent was removed from the acetonitrile extracts using a rotary evaporator, and dissolved in 3 mL methanol: water (5:95, v/v). They were then loaded onto SPE cartridges that were previously conditioned with 5 mL of methanol and equilibrated with 4 mL methanol: water (5:95, v/v). After washing the cartridge with 4 mL methanol: water (20:80, v/v), the analytes were eluted with 2 mL of methanol twice, followed by 2 mL of methanol: ethyl acetate (50:50, v/v), and 2 mL of ethyl acetate, into screw-capped glass vials. Following that, the samples were blown dry using a stream of nitrogen gas, reconstituted with 1 mL of (90:10, v/v) acetonitrile:water, and filtered using 0.20 μm nylon filters into HPLC vials for analysis.

3. Results and discussion

3.1. Optimization of method

3.1.1. Liquid–liquid extraction clean-up efficiency

To determine the standing time (10, 15, 20, 25 and 30 min) necessary for optimum extraction efficiency of the liquid–liquid extraction, fortified corn oil samples spiked with all seven analytes at 1000 μg/kg levels, were subjected to a three-time liquid–liquid extraction ($n = 3$). The analyte concentrations were evaluated by HPLC for their efficiencies. It was found that the samples with standing times of 25 min (RSD%: 2.61%) and 30 min (RSD%: 2.03%) resulted in similar high recoveries of more than 98%. Consequently, 25 min was chosen as the standard extraction time for subsequent analyses.

3.1.2. SPE elution solvent efficiency

Due to the wide ranging analyte polarity, the optimum elution solvent system had to incorporate a series of solvents to elute all the analytes effectively. After conditioning and equilibrating the solid phase extraction cartridges, 1 mL of a mixed standard solution containing all the seven analytes at 500 μg/L level were loaded onto their respective cartridges (identified as SPE-1 to SPE-4), washed with 20% methanol solution, but eluted using different elution solvent systems. Recoveries for all the analytes (92.48%–100.14%), performed in triplicate (RSD%: 0.35%–2.88%), were superior in the eluate collected from SPE-4 which had incorporated a series of elution solvents differing in polarity (methanol, methanol: ethyl acetate (1:1, v/v), and ethyl acetate).

3.1.3. Chromatographic analysis

The use of gradient elution was necessary to achieve optimum baseline separation for all seven structurally-similar analytes, and the retention times of the various analytes were found to be 9.458, 20.853, 22.425, 23.743, 35.614, 36.474, and 37.371 min for BADGE-2H_2O, BPA, BADGE-H_2O-HCl, BADGE-H_2O, BADGE-2HCl, and BADGE-HCl, and BADGE, respectively (Fig. 1).

3.2. Method validation

3.2.1. Linearity, range, limit of detection (LOD) and limits of quantitation (LOQ), and robustness

Linearity was assessed by inspecting the detection signals as a function of analyte concentration, with the aid of a regression line by the method of least-squares. The seven analytes were evaluated for linearity using concentration levels of 200, 400,

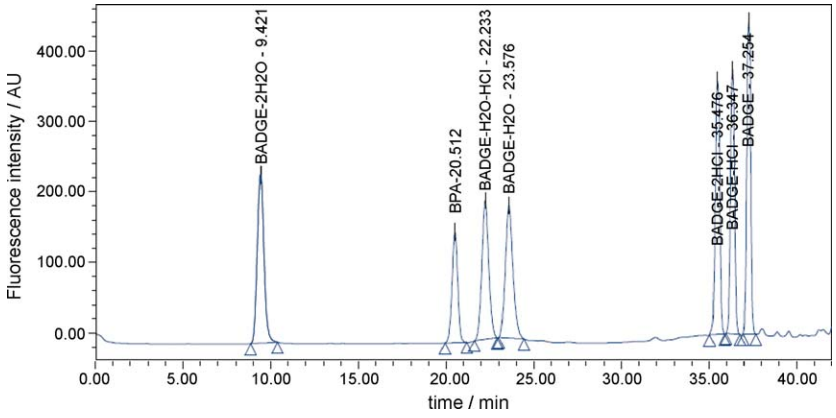

Fig. 1. Fully resolved chromatographic separation of a standard mixture containing all seven BPA and BADGE analytes at 1500 μg/L level.

600, 800, 1000, 1500 and 2000 μg/L, and duplicate injections were made for each concentration level. The correlation coefficient obtained were all ≥0.999. The limit of detection of the analytical procedure is the lowest concentration of analyte that can be measured with definable statistical certainty in a sample, and were calculated from the levels of the various analytes equivalent to three times the standard deviation of noise on analysis, while the limits of quantitation were calculated from the concentration of the analytes that provided signals equal to 10 times the signal to noise on analysis. The LODs and the LOQs of the various analytes ranged between 4.5 and 7.9 μg/kg, and 13.7 and 24.1 μg/kg, respectively. The robustness of the analytical method has been established on three different HPLC columns (Nucleosil-100, Hypurity Elite Hypersil ODS and Shim-Pack VP-ODS), and on different HPLC gradient elution programmes. Peak resolutions remained similar despite the different conditions tested.

3.2.2. Precision

Precision is the measure of how close results are to one another, and is evaluated by making repetitive measurements for the entire method. Excellent inter-day precision data ($n = 10$) and intra-day precision data ($n = 5$) were obtained on a 200 μg/kg spiked sample containing all seven analytes. The RSD ranged from 0.20% to 2.96% for the inter-day precision tests, and 0.04% to 2.82% for the 3-day intra-day precision tests. The RSD% was then calculated by dividing the standard deviation by the mean, and multiplying the value by 100%.

Table 1
Results of the analysis of various canned foods ($n = 2$)

Type of food	Concentration of analytes in food (mg/kg)							Age of canned food from manufacture date/months
	BPA	BADGE-2H$_2$O	BADGE-H$_2$O	BADGE-H$_2$O-HCl	BADGE-2HCl	BADGE-HCl	BADGE	
Young corn in brine	0.07	0.40	ND	0.08	ND	ND	ND	11
Braised peanuts	0.10	0.12	ND	ND	ND	ND	ND	9
Mushrooms	0.04	0.12	ND	ND	ND	ND	ND	12
Baked beans	0.04	0.09	ND	ND	<LOQ	ND	ND	10
Green peas	0.05	0.10	ND	0.04	ND	ND	0.11	11
Honey sea coconut	0.04	ND	ND	ND	ND	ND	ND	10
Sliced mango	0.16	0.10	ND	ND	ND	ND	ND	20
Pineapple slices	0.03	0.10	ND	0.20	0.17	ND	ND	16
Pork luncheon meat	0.14	ND	0.03	0.04	ND	ND	0.05	8
Stewed pork	0.05	0.08	ND	0.03	ND	ND	ND	13
Spiced pork cubes	0.04	ND	0.05	0.04	0.81	<LOQ	0.05	4
Sandwich tuna	0.11	ND	0.03	ND	0.08	ND	0.44	27
Sample blank	ND	ND	ND	ND	ND	ND	ND	–

Type of food	Percentage recovery of analytes in fortified food simulants (w/w, %)						
	BPA	BADGE-2H$_2$O	BADGE-H$_2$O	BADGE-H$_2$O-HCl	BADGE-2HCl	BADGE-HCl	BADGE
Oily food simulant	105.2	94.5	89.9	93.6	99.8	94.0	90.0
Aqueous food simulant	90.2	92.5	88.6	89.9	91.9	87.3	88.5
Acidic food stimulant	87.3	92.2	91.8	96.9	103.9	93.1	87.4

Analytes that were found below the limit of detection were labeled as ND. Fortified samples (w/w) were prepared by pipetting a small volume of stock standard solution into the round bottomed flask, and gently evaporating off the solvent using a stream of nitrogen gas. Five grams of the appropriate food simulant was then weighed into the same vessel for recovery studies using the sample preparation method described.

3.2.3. Accuracy

The accuracy of the method was assessed at three concentration levels—200, 1000 and 2000 μg/kg. Ten fortified oil samples at each concentration level were extracted and analyzed using the optimized conditions. Excellent percentage recoveries (86.07%–114.06%) were obtained with acceptable variation (RSD%: 2.63%–5.15%).

3.3. Analysis of canned food samples

The fully optimized and validated analytical method was later applied for the analysis of the levels of BPA and BADGE-related analytes in canned food samples (Table 1). Detectable amounts of BPA (0.0328–0.1645 mg/kg) were found. However, these concentrations were far below the current specific migration limits of 3 mg/kg of food for BPA, 9 mg/kg of food for the sum of BADGE, BADGE-H_2O, and BADGE-$2H_2O$, and 1 mg/kg for the sum of BADGE-HCl, BADGE-2HCl and BADGE-H_2O-HCl, respectively, as imposed by the European Commission [1,2]. The other major contaminants present in the foods were BADGE-$2H_2O$ (detected in 67% of food samples), BADGE-H_2O-HCl (detected in 50% of the food samples), and BADGE-2HCl (detected in 34% of the food samples). Judging from the significantly lower concentrations of the monosubstituted BADGE-related compounds determined in these food samples, it suggests that the monosubstituted BADGE-related compounds may have undergone further hydrolysis within the food matrix to form the more thermodynamically stable disubstituted BADGE-related compounds [7]. BADGE was detected in only four samples—one aqueous green pea sample, and three oily meat samples. The oily nature of the three meat samples may have reduced the rate of BADGE hydrolysis and hydrochlorination reactions. The occurrence of BADGE in the green pea sample may have been due to a combination of factors such as its higher food-to-aqueous content, and a longer length of shelf display time.

The analysis of the food samples were performed together with fortified food simulants to assess the recovery of the analytical procedure using recommended standard food simulants [8]. The mean values obtained from the FAPAS test material analysis performed in duplicate were 453.63 μg/kg for BADGE-HCl-H_2O; 428.76 μg/kg for BADGE-2HCl; and 76.07 μg/kg for BADGE-HCl (CV 0.34%, 1.05% and 3.54%, respectively). These values were in agreement with the assigned values of 491 μg/kg for BADGE-HCl-H_2O; 477 μg/kg for BADGE-2HCl; and 90 μg/kg for BADGE-HCl.

4. Conclusions

The developed method for the simultaneous determination of BPA, BADGE, BADGE-H_2O, BADGE-$2H_2O$, BADGE-H_2O-HCl, BADGE-HCl, and BADGE-2HCl has been shown to be a more suitable method for the extraction and separation of the various bisphenolic analytes at low limits of detection. More importantly, the method can be applied to a wide range of food, which is vital for analyzing the broad range of complex food matrices for the undesirable food contaminants. Quantitative results indicated that the levels of BPA, BADGE, and all the hydrolysed and hydrochlorinated derivatives of BADGE detected in the variety of canned foods tested were below the specific migration limits imposed by the European Commission [1,2].

Acknowledgements

The author wishes to express her gratitude to the National University of Singapore for awarding the research scholarship, the Centre for Analytical Sciences at the Health Sciences Authority for providing the opportunity for this collaborative study, and Ms Lee Chooi Lan for her technical assistance.

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