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Effect of calibration standards on the quantification of hydroxy products from can coatings

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Abstract

A liquid chromatographic (LC) method for Bisphenol A diglycidyl ether (BADGE), Bisphenol F diglycidyl ether (BFDGE) and their derivatives is currently being validated at the level of European Committee for Standardisation (CEN). The method consists in hydrolysing the substances to their di-hydroxy forms with a quantification based on calibration using hydrolysed monomers (BADGE·$2H_2O$ or BFDGE·$2H_2O$). Considering laboratories may be tempted to use standards of BADGE·$2H_2O$ or BFDGE·$2H_2O$ now commercially available instead of performing the hydrolysis described, a comparison was made on the effect of using a calibration based on the commercial hydrolysis product versus hydrolysing the monomer on the quantification of the migration limit for enforcement purposes. In this work, the epoxy bis (diol) derivatives were quantified in three different food simulants.

Keywords: Liquid chromatography; Fluorescence; Bisphenol A diglycidyl ether dihydroxy derivatives; Bisphenol F diglycidyl ether dihydroxy derivatives; Food simulants; Calibration

1. Introduction

Bisphenol A diglycidyl ether (BADGE) and Bisphenol F diglycidyl ether (BFDGE) are commonly used in the manufacture of epoxy-based coatings for food and beverage cans [1].

As its occurrence in cans and food has been monitored in recent years, it became evident that epoxy-based lacquers could also release reaction products and consequently the legislation in force now specifies a limit including the hydroxy and chloro derivatives of BADGE and BFDGE [2].

A number of research studies have focused on migration of these compounds into food products [3–6] and food simulants [7–10], and it was shown that epoxide compounds demonstrated different stability in aqueous or fatty food simulants [11], due to the potential hydrolysis of BADGE, BFDGE and their derivatives in contact with food simulants [5,12].

A method for BADGE, BFDGE and their derivatives is currently being validated at the level of the European Committee for Standardisation (CEN). The method consists in hydrolysing the substances to their di-hydroxy forms with a quantification based on calibration using BADGE·$2H_2O$ or BFDGE·$2H_2O$. Although the method description includes a protocol to hydrolyse the monomers for calibration purposes [13], standards of BADGE·$2H_2O$ or BFDGE·$2H_2O$ are now commercially available.

In previous works the quantification of BADGE and BFDGE derivatives were expressed respectively as BADGE or BFDGE, considering derivatives had an equal response factor as the monomers [8,14]. Hammarling et al. [15] quantified the levels of BADGE·$2H_2O$ in food samples using a reference standard of this compound made at their laboratory.

Considering laboratories may be tempted to deviate from the method and use BADGE·$2H_2O$ and BFDGE·$2H_2O$ standards, a comparison was made on the effect of using a calibration based on the commercial hydrolysis product versus

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hydrolysing the monomer on the quantification of the migration limit.

In this work, the BADGE·2H_2O or BFDGE·2H_2O were quantified in three different food simulants (3% aqueous acetic acid (w/v), 10% aqueous ethanol (v/v) and sunflower oil).

2. Experimental

2.1. Chemicals and reagents

HPLC-grade acetonitrile (Carlo Erba, Milan, Italy), ultrapure water prepared using a Milli-Q filter system (Direct Q5 Millipore, Milan, Italy) were used. The standards were Bisphenol A diglycidyl ether (BADGE, purity ≥97%), Bisphenol F diglycidyl ether (BFDGE, total of three isomers purity ≥97%: *ortho–ortho*, *para–para*, *ortho–para*) and their dihydrolysed derivatives (BADGE·2H_2O and BFDGE·2H_2O, purity ≥97%) (Sigma–Aldrich, Milan, Italy). Acetic acid (purity ≥99.8%, Fluka, Milan, Italy), ethanol (HPLC-grade, Merk, Milan, Italy) and a commercial sunflower oil from a regional supermarket were used as simulants. A 3% acetic acid solution and 10% ethanol solution were separately prepared in ultrapure water as aqueous food simulants.

Spiked simulant were prepared from a mix solution in acetonitrile containing BADGE, BADGE·2HCl, BADGE·H_2O, BADGE·HCl·H_2O or alternatively from a mix solution of *o,o*-BFDGE, *o,p*-BFDGE, *p,p*-BFDGE, *o,o*-BFDGE·2HCl, *o,p*-BFDGE·2HCl, *p,p*-BFDGE·2HCl, *o,o*-BFDGE·2H_2O, *o,p*-BFDGE·2H_2O, *p,p*-BFDGE·2H_2O. For BADGE and BFDGE hydrolysis, a borate buffer (0.6 M) was prepared by dissolving 9.28 g of boric acid (purity ≥99.5%, Sigma–Aldrich, Milan, Italy) in 220 mL of water. A sodium hydroxide (purity ≥98%, Sigma–Aldrich, Milan, Italy) solution was prepared in water (4.5 M) and added to the boric acid solution to a pH of 8.5.

2.2. Equipment and conditions

The LC system consisted of a 1100 Hewlett Packard (Palo Alto, CA, USA) fitted with a binary pump, an autosampler, a column oven, a 1046 Hewlett Packard fluorescence detector and HP Chemstation data analysis software (version A.07.01).

A Spherisorb ODS-2 (250 mm × 4.6 mm i.d., 5 μm) with an ODS guard column (10 mm × 4.6 mm) (Waters, Milford, MA, USA) was used for the separation.

Chromatographic conditions were as in the proposed CEN method under validation and consisted in a mobile phase for elution of acetonitrile/water with a linear gradient profile as described in Table 1.

Table 1
Mobile phase gradient

Time (min)	Water (%)	Acetonitrile (%)
0	80	20
10	65	35
25	50	50
45	50	50
60	0	100
75	80	20

The flow rate was 1.1 mL min^{-1}. The injection volume was 20 μL. The column oven temperature was kept at 30 °C. Fluorescence detection was employed using 225 nm as excitation wavelength and 305 nm as emission wavelength.

2.3. Standards and quantification

Four different stock solutions of BADGE, BFDGE and their dihydrolysed derivatives (BADGE·2H_2O and BFDGE·2H_2O) containing 500 μg mL^{-1} were prepared in acetonitrile. Intermediate standards solutions of 10 μg mL^{-1} were prepared in acetonitrile for each compound.

The standards for the calibration curves were obtained by dissolving appropriate amount of 10 μg mL^{-1} stock solution separately in 3% acetic acid, 10% ethanol and acetonitrile in the range of 0.05–2 μg mL^{-1} (0.05, 0.25, 0.5, 1, 2 μg mL^{-1}).

Standards for calibration curves in fatty food simulant were prepared by adding 3 mL of each acetonitrile stock solution (range of 0.05–2 μg mL^{-1}) in 3 g of sunflower oil. The mixture was shaken for 2 min by vortex mixing, centrifuged at 1500 rpm for 5 min and acetonitrile phase was finally taken up, filtered through a PTFE 0.45 μm filter and injected.

2.3.1. Forced hydrolysis of aqueous food simulants

Three percentage acetic acid was neutralised prior to hydrolysis adding 110 μL 4.5 M sodium hydroxide solution to 1.0 mL of the BADGE and BFDGE stock solutions. 1.0 mL of the each stock solution in aqueous simulants was put in a headspace vial with 1.0 mL borate buffer (pH 8.5) and the solution was homogenised by vortex mixing. The headspace vial was hermetically closed and the solution was stored at 100 °C (±5 °C) for a period of 21 h to force hydrolysis of BADGE and BFDGE. After that period, the solutions were injected to obtain calibration curve of the hydrolysed monomers in aqueous food simulants (range of 0.05–2 μg mL^{-1}) (CEN/TC194/SC1/WG8/N024).

2.3.2. Forced hydrolysis of fatty food simulant

2.0 mL of acetonitrile phase of each stock solution in fatty food simulant were put into a headspace vial and evaporated to dryness under a nitrogen stream. The residue was re-dissolved in 400 mL acetonitrile, 1600 mL borate buffer was added and the solution was homogenised by vortex. The headspace vial was hermetically closed and the solution was stored at 100 °C (±5 °C) for a period of 21 h to force hydrolysis of BADGE and BFDGE. After that period, the solutions were filtered through a PTFE 0.45 μm filter and injected to obtain calibration curve of the hydrolysed monomers in fatty food simulant (range of 0.05–2 μg mL^{-1}).

2.4. Spiked samples

Two conical flask with 50 mL of each aqueous food simulants (3% acetic acid and 10% ethanol) and 50 g of sunflower oil were respectively added of 100 μL of the solution containing a mixture of BADGE and derivatives, and 100 μL of the solution containing a mixture of BFDGE and derivatives. The solutions were homogenised by vortex mixing. Four different samples for each simulants were forced hydrolysed as described in Sections 2.3.1 and 2.3.2.

3. Results and discussion

The protocol proposed under CEN TC194 for simulants using forced hydrolysis was effective for the total hydrolisation of all the BADGE, BFDGE substances to their bis (diol) derivatives. The hydrolysis process did not provoke the appearance of interferences for quantification.

BADGE, BFDGE stock solutions and spiked samples were analysed before and after hydrolysis in order to verify the effectiveness of alkaline forced hydrolysis. An example of BADGE spiked sample in 3% acetic acid is represented in Fig. 2. All the compounds identified as BADGE, BADGE·2HCl, BADGE·1H$_2$O, BADGE·1HCl·1H$_2$O before hydrolysis (Fig. 1A) were totally hydrolysed to BADGE·2H$_2$O (Fig. 1B). The identification of all the compounds was made comparing retention times and fluorescence spectra with the ones of commercial standards.

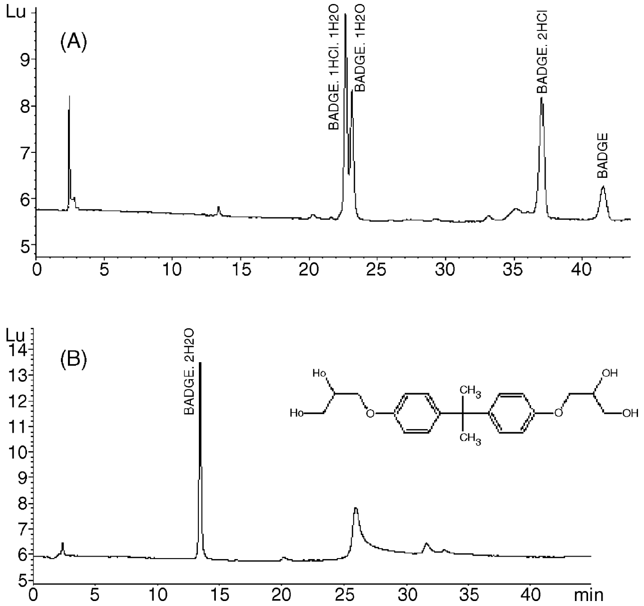

Fig. 1. Sample BADGE in 3% acetic acid before (A) and after hydrolysis (B).

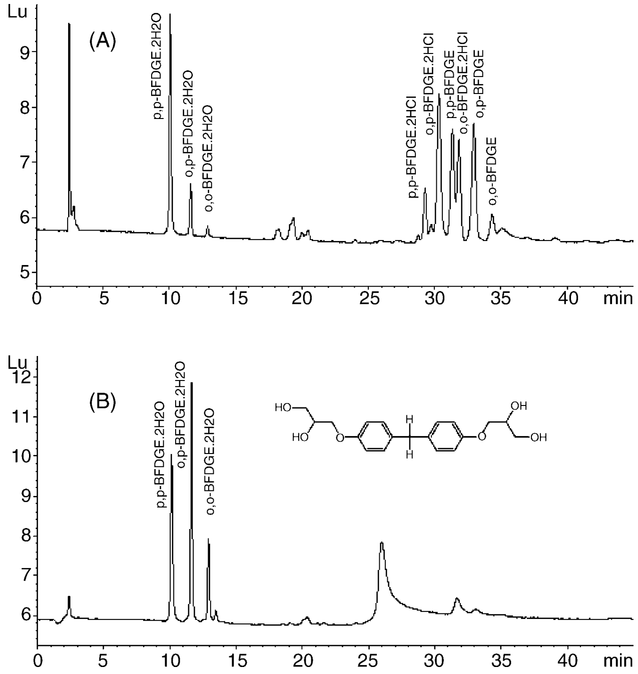

Fig. 2. Sample BFDGE in 3% acetic acid before hydrolysis (A) and after hydrolysis (B).

The analysis of BFDGE spiked samples before hydrolysis evidenced the presence of *o,o*-BFDGE, *o,p*-BFDGE, *p,p*-BFDGE, *o,o*-BFDGE·2HCl, *o,p*-BFDGE·2HCl, *p,p*-BFDGE·2HCl, *o,o*-BFDGE·2H$_2$O, *o,p*-BFDGE·2H$_2$O, *p,p*-BFDGE·2H$_2$O. All the compounds were totally hydrolysed to the BFDGE bis (diol) derivatives form after the alkaline hydrolysis. Fig. 2 shows and example of spiked BFDGE samples in 3% acetic acid before and after hydrolysis.

The calibration lines were obtained by using BADGE, BFDGE as pure standards and hydrolysing the stock solutions to their bis (diol) derivatives. A correction factor considering the differences in molecular weight of the monomers and their totally hydrolysed compounds was applied to construct the BADGE·2H$_2$O and the BFDGE·2H$_2$O calibration curves.

Calibration curves were then also obtained directly using the pure commercially available standards of already hydrolysed BADGE·2H$_2$O and BFDGE·2H$_2$O.

The results obtained are summarised in Table 2 which contain all statistical data of calibration curves in the three different simulants.

All calibration curves exhibited a correlation coefficient greater then 0.996. The concentration of BFDGE and BFDGE·2H$_2$O was calculated as sum of their three isomers (*o,o*–*o,p*–*p,p*). As seen in Table 2., the differences between slopes of calibration curves carried out using standards and hydrolysed monomers were major in aqueous simulants than in the fatty simulant.

In all simulants the slopes obtained by using commercial available standards of BADGE·2H$_2$O were always greater then the ones constructed hydrolysing BADGE while for

Table 2
Standard linearity of calibration curves in aqueous and fatty food simulants

Calibration curve	Statistical data			
	Intercept	Slope	r	Range (μg mL^{-1})
3% acetic acid				
BADGE	−2.7551	234.81	0.9999	0.05–2
BADGE·2H$_2$O HM	6.4109	174.11	0.9971	0.05–2
BADGE·2H$_2$O STD	1.5004	228.73	0.9999	0.05–2
BFDGE	3.8071	340.44	0.9993	0.05–2
BFDGE·2H$_2$O HM	−2.0214	277.93	0.9998	0.05–2
BFDGE·2H$_2$O STD	1.2144	252.27	0.9999	0.05–2
10% ethanol				
BADGE	−11.231	254.47	0.9979	0.05–2
BADGE·2H$_2$O HM	−3.5536	205.67	0.9998	0.05–2
BADGE·2H$_2$O STD	−2.4956	221.63	0.9998	0.05–2
BFDGE	0.1218	382.05	0.9993	0.05–2
BFDGE·2H$_2$O HM	0.2807	263.32	0.9991	0.05–2
BFDGE·2H$_2$O STD	−5.5993	256.18	0.9987	0.05–2
Sunflower oil				
BADGE	12.767	231.34	0.9963	0.05–2
BADGE·2H$_2$O HM	0.0022	158.26	0.9968	0.05–2
BADGE·2H$_2$O STD	−8.784	223.03	0.9994	0.05–2
BFDGE	−0.2292	344.51	0.9994	0.05–2
BFDGE·2H$_2$O HM	−1.3809	244.33	0.9986	0.05–2
BFDGE·2H$_2$O STD	−22.845	227.11	0.9982	0.05–2

HM: obtained from hydrolysed monomer, STD: standard commercially available.

Table 3
Quantification of BADGE·2H$_2$O and BFDGE·2H$_2$O in spiked simulants

Calibration curve	Simulants (mean ± S.D.) (μg mL^{-1})		
	3% acetic acid	10% ethanol	Sunflower oil
BADGE·2H$_2$O			
BADGE·2H$_2$O HM	1.025 ± 0.004	0.958 ± 0.004	1.4 ± 0.2
BADGE·2H$_2$O STD	0.802 ± 0.003	0.884 ± 0.003	1.1 ± 0.1
BFDGE·2H$_2$O			
BFDGE·2H$_2$O HM	0.92 ± 0.02	0.917 ± 0.004	0.97 ± 0.05
BFDGE·2H$_2$O STD	1.02 ± 0.02	0.966 ± 0.004	1.13 ± 0.05

Method precision ($n = 4$). HM: obtained from hydrolysed monomer, STD: standard commercially available.

BFDGE·2H$_2$O the standard lines had a slightly lower slope then hydrolysed BFDGE.

Consequently, the final resulting quantification of BADGE and BFDGE bis (diol) derivatives (BADGE·2H$_2$O and BFDGE·2H$_2$O) was also compared for the various simulants (Table 3).

The results suggest that the total quantity of BADGE·2H$_2$O was underestimated by a factor of at last 20% for both aqueous simulants and sunflower oil when using the commercial hydrolysis products whereas in the quantification of BFDGE·2H$_2$O the discrepancy did not exceed 1.2%. Therefore, for quantification of BADGE·2H$_2$O in 3% acetic acid, 10% ethanol or sunflower oil, it is more reliable to use calibration curves produced from the in-house hydrolysis of BADGE monomer, which allow an automatic correction of the hydrolysis process of real samples.

In the quantification of BFDGE·2H$_2$O there were no significant differences using commercial available standard or hydrolysed monomers in all simulants.

Further repeatability studies may allow the potential use of correction factors for BADGE·2H$_2$O quantification when the use of standards is considered.

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