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TWO RP-HPLC METHODS TO QUANTIFY AND IDENTIFY BISPHENOL A DIGLYCIDYL ETHER (BADGE): EUROPEAN UNION FATTY FOOD SIMULANT (OLIVE OIL)

DOS MÉTODOS RP-HPLC PARA CUANTIFICAR E IDENTIFICAR BISFENOL A DIGLICIDIL ÉTER (BADGE): SIMULANTE DE ALIMENTOS GRASOS DE LA UNIÓN EUROPEA (ACEITE DE OLIVA)

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Abstract

Two HPLC-UV methods with fluorescence detection have been applied to quantify and identify bisphenol A diglycidyl ether (BADGE: a monomer of epoxy resins used as a coating in packaging materials that contact food), in olive oil (official fatty food simulant in European Union legislation) and in food coatings. Through an extraction process in solid phase employing a Florisil cartridge, BADGE is isolated from the oil. Isocratic and gradient chromatographic methods were applied to determine this monomer at the specific migration limit (SML) (restriction proposed by European Union legislation). The calibration lines had correlation coefficients no smaller than 0.998 with a detection limit of less than 3 (µg BADGE)/L. Precision calculations yielded less than 20% deviation.

Keywords: Bisphenol A diglycidyl ether, epoxy resins, olive oil, fatty food simulant, food coatings, HPLC.

Resumen

Por medio de dos métodos de HPLC-UV y detección por fluorescencia se ha cuantificado e identificado el bisfenol A diglicidil éter (BADGE): monómero de resinas epoxi usado como recubrimiento de materiales de envasado que van a entrar en contacto con alimentos. La determinación se ha llevado a cabo en aceite de oliva (simulante oficial de alimentos legislado por la UE) y en los recubrimientos de envasado alimentario. El BADGE se extrae del aceite mediante un proceso de extracción en fase sólida utilizando un cartucho de florisil. Se utilizaron dos programas de elución cromatográfica: un método isocrático y otro en gradiente para determinar este monómero al nivel del límite de migración específico (SML) (restricción propuesta por la legislación europea). Las rectas de calibrado presentan unos coeficientes de correlación superiores a 0.998 y el límite de detección es inferior a 3 µg de BADGE/L. Las desviaciones estándar de la precisión de los métodos son siempre inferiores al 20%.

Palabras clave: Bisfenol A diglicidil éter, resinas epoxi, aceite de oliva, simulante de alimentos grasos, recubrimientos, HPLC.

Resumo

Por medio de dous métodos de HPLC-UV e detección por fluorescencia cuantificouse e identificouse o bisfenol A diglicidil éter (BADGE): monómero de resinas epoxi usado como recubremento de materiais de envasado que van entrar en contacto con alimentos. A determinación levouse a cabo en aceite de oliva (simulante oficial de alimentos lexislado pola UE) e nos recubrementos de envasado alimentario. O BADGE extraeuse do aceite mediante un proceso de extracción en fase sólida utilizando un cartucho de florisil. Utilizaron-se dous programas de elución cromatográfica: un método isocrático e outro en gradiente para determinar este monómero ó nivel do límite de migración específico (SML) (restricción proposta pola lexislación europea). As rectas de calibrado presentan uns coeficientes de correlación superiores ó 0.998 e o límite de detección é inferior a 3 µg de BADGE/L. As desviacións estándar da precisión dos métodos foron sempre inferiores ó 20%.

Palabras chave: Bisfenol A diglicidil éter, resinas epoxi, aceite de oliva, simulante de alimentos grasos, recubrementos, HPLC.

INTRODUCTION

Bisphenol A diglycidyl ether (BADGE), oxirane, 2,2'-[(1-methylethylidene)bis(4,1-phenyleneoxymethylene)]bis-, CAS Component Name (CAS Registry Number: 1675-54-3), is a epoxy resin monomer employed in the fabrication of objects and articles intended to come into contact with foods. Due to the possibility of migration of the monomer from the article (containers, vessels, tanks) to the food, it is necessary to establish a restrictive criteria. According to the European legislation the restrictions for BADGE are the following: QM=1 mg/kg (maximum residual quantity permitted of the monomer in the finished product) and SML (specific migration limit in food or food simulant) = not detectable (detection limit = 0.020 mg/kg, analytical tolerance included, (Commission of the European Communities, 1990). This leads to the need for establishing sensitive analytical methods to determine the monomer in aqueous food simulants (Paseiro-Losada *et al.*, 1997) and fatty food simulants.

The European Legislation [Directive 82/711/EEC (Commission of the European Communities, 1982), modified by 93/8/EEC (Commission of the European Communities, 1993) and 97/48/EEC (Commission of the European Communities, 1997)], has taken as official simulant rectified olive oil, also outlines the possibility of using alternative simulants if there are analytical reasons to justified it: sunflower oil and synthetic triglicerydes mixture (Figge, 1972).

The type of simulant to be used as an alternative to olive oil is controversial. Some authors propose the use of isooctane (De Kruijf and Rijk , 1988) and sunflower oil (Hamdaniand and Feigenbaum, 1996). The use of ethanol/water mixtures was also considered (Piringer, 1990). Other authors agree to use of alternative simulants when it represents an analytical advantage (Baner *et al.*, 1992). Neverthless it is clear that the choice of fatty food simulant for use in high-temperature migration tests must be carefully chosen (Rossi, 1993).

Methods for BADGE determination in fatty foods packed in polyethyleneterephthalate films containing BADGE-based epoxy-acrylic adhesive based on BADGE has been developed by other authors (Tice, 1993). In this work BADGE in olive oil has been isolated by using a florisil cartridge, concentrated by evaporation and determined by HPLC equiped with a Partisil ODS-3 column and UV detector at 228 nm (Begley *et al.*, 1991). The migration occurred at high temperatures as the food is cooked in a microwave oven. Mygliol 812 (fractioned coconut oil) was used as the fatty food simulant. A mixture hexane/acetonitrile was used as the extraction medium. The BADGE was quantified by HPLC equipped with an UV detector.

Others (Sharman *et al.*, 1995) have determined BADGE (in the container) by HPLC/UV at 280 nm and GC-MS. Chloroform was used the extractor medium. This method was developed to determine the amount of BADGE in BADGE-based epoxy resins on polymeric films. Only GC-MS was used to analyze BADGE in actual food (i. e., pizza)

BADGE was determined in fatty foods packaged in cans by employing a normal phase LC, which avoids the previous extraction process. The method utilized a two series of columns LC-LC. The first was a phase ciano and the second a silica gel column. The detection was accomplished by fluorescence, and was confirmed by GC-MS (Biedermann *et al.*, 1996).

MATERIAL AND METHODS

The BADGE used was an epoxy resin named Epikote 828 (from Shell; Zaragoza, Spain), purified (>99%) by Gairesa as described (Paz-Abuín *et al.*, 1990).

Demineralized water was obtained from a Milli-Q system (Millipore Corporation). Acetonitrile HPLC supragradient grade (Ref. Ac 331), 90% (v/v) Methanol (analytical quality) in an aqueous solution, n-heptane HPLC grade (Ref. He 131) and dichlorometane HPLC grade (Ref. Cl 335) were from Scharlau (Barcelona, Spain).

Olive oil rectified complying with EU legislation: iodine absortion number (Wijs method) = 80-88; index of refraction at 25º C = 1.4665-1.4679; acid value (expressed as oleic acid) ≤ 0.5 ; peroxide value (milliequiv. peroxide/kg sample) ≤ 10.

Cartridges Sep-Pak Plus Florisil Part No. WAT020525 and cartridges Sep-Pak Plus C18, Part No. WAT020515 (only for third confirmation option) from Waters Chromatography Division, Millipore Corporation, Milford, MA, USA.

Microfilters MFS-13, PTFE membrane, diameter 13 mm, pore size 0.5 µm, from MFS (CA, USA).

Glass migration cells from Afora (Spain) as described (Simal Gándara *et al.*, 1993).

A stock solution of BADGE standard was prepared in tetrahydrofuran at a concentration of 1.0 mg/mL. 100 mg of BADGE was accurately weighed into a 100 mL volumetric flask, and then diluted to the mark with tetrahydrofuran.

Dilute stock solutions of BADGE standard at a concentration of 1.0 µg/mL. Transfer 10 mL of the stock solution of BADGE standard into a 100-mL volumetric flask and dilute to volume with 90%(v/v) methanol. Transfer 10 mL of this solution into a 100-mL volumetric flask and dilute to volume with 90%(v/v) methanol; transfer 10 mL of the resulting solution into a 100-mL volumetric flask and dilute to volume with 90%(v/v) methanol. The final 1.0 µg per mL

BADGE solution was transferred to a 250-mL cylindrical flask and store at -20 ºC. The solution should not be allowed to remain at room temperature for more than a few minutes.

Preparation of calibration solutions: 0.5, 1, 2, 3 and 4 mL of the dilute stock solution of BADGE standard at a concentration of 1 µg/mL was transferred to a series of 100-mL volumetric flasks and fill to the marks with 90%(v/v) aqueous methanol to obtain 5, 10, 20, 30 and 40 µg per litre BADGE solutions. As these solutions are not stable at room temperature they should be used immediately. Blanks were prepared by transferring 0.02 mL of THF to a 100-mL volumetric flask and fill to the mark with 90%(v/v) aqueous methanol.

50 µL of each calibration sample was injected into the chromatograph (triplicate). The area of the BADGE peak was determined in the resulting chromatograms.

Experimental conditions

The apparatus and chromatographic conditions were described by us in previous paper (Paseiro-Losada *et al.,* 1997). A description of the method is summarized below:

A liquid chromatograph system from Spectra-Physics, consisting of binary pump with helium degassing kit with fluorescence detector (excitation wavelength 225 nm and emission wavelength 305 nm) was used. Column: length 15 cm, internal diameter 4.6 mm, packed with 5 µm Spherisorb ODS 2 (Sugelabor; Madrid, Spain).

An ultraviolet detector (wavelength 225 nm) with scanning function on eluting peaks, was used for 2nd and 3th confirmation options (Paseiro-Losada *et al.,* 1997).

Elution program for Gradient Method: gradient elution consisted of a 2-min Isocratic elution with acetonitrile-water (30:70) (v/v), a 18-min linear gradient to 80% acetonitrile, a 3-min linear gradient to 100% acetonitrile and 2-min isocratic elution at 100% acetonitrile. Flow rate: 1 mL/min. Typical retention time for BADGE is 17.4 min. Elution program for Isocratic Method: isocratic elution with acetonitrile-water (65:35) (v/v). Flow rate: 1 mL/min. Typical retention time for BADGE is 5.3 min.

Procedure for extraction of BADGE in olive oil.

0.5 g of olive oil was accurately weighed (migration solution or fortified simulant) into a 10-mL beaker. Four milliliters of dichlorometane:heptane 3:1 (v/v) were added and mixed thoroughly.

The solution was transferred into a 5-mL glass syringe connected to a Florisil Sep-Pak plus cartridge, filled with a plunger and passed through the cartridge (flow rate of about 2 mL/minute). The beaker and the syringe were rinsed twice with 2 mL of dichloromethane:heptane and load into the cartridge in a similar fashion. When the syringe is empty, displace the dichloromethane:heptane solution from the cartridge by slowly pumping with approximately 5 mL of air. The eluate was discarded.

Tetrahydrofuran (3 mL) was passed through the cartridge, collecting the eluate in a 5-mL vial. Tetrahydrofuran was displaced by passing about 5 mL of air through the cartridge.

The solvent was evaporated using a flow of nitrogen. The residue was dissolved in 0.5 mL of 90%(v/v) methanol (30 seconds in an ultrasound bath), and filtered through the microfilter. If the filtrate was not clear, it was filtered again using a new filter. The blank was prepared as above, using simulant free BADGE that was checked previously with the same method.

RESULTS AND DISCUSSION

Calibration line

The calibration lines for both Isocratic and Gradient methods had correlation coefficients no smaller than 0.998. To verify the effective detection limit calculated from the calibration lines, a 1.5 µg/L BADGE solution was run in triplicate. The signal-to-noise ratio of the BADGE peak was about 6. Noise was measured as the maximum amplitude of the chromatogram of a blank between 4.8 and 5.8 minutes (Isocratic Method) or 16.9 and 17.9 minutes (Gradient Method). Figure 1 shows chromatograms applying both Gradient and Isocratic Method.

To establish the performance of the method for BADGE measurement, concentrations greater than 40 µg/L were tested. The experiments showed that linearity was maintained up to the concentration at which the fluorescence detector became saturated.

In previous experiments we observed that under isocratic conditions the use of pure methanol as an eluants affords double BADGE peak. However, this phenomenon disappears if we prepare the calibration with 90% (v/v) methanol.

This extraction procedure is based on the Tice method (Tice, 1993) although there are important differences:

(1) We used a solvent mixture of dichloromethane/heptane instead of heptane/diethylether/dichlorometane (used in succession). This modification simplifies the procedure and increases the retention of BADGE in the florisil cartridge. Hexane can be used instead of heptane.

(2) The solvent mixture (acetonitrile/THF) is replaced with methanol 90% (v/v). Dissolving the residue of BADGE in methanol decreases interferences.

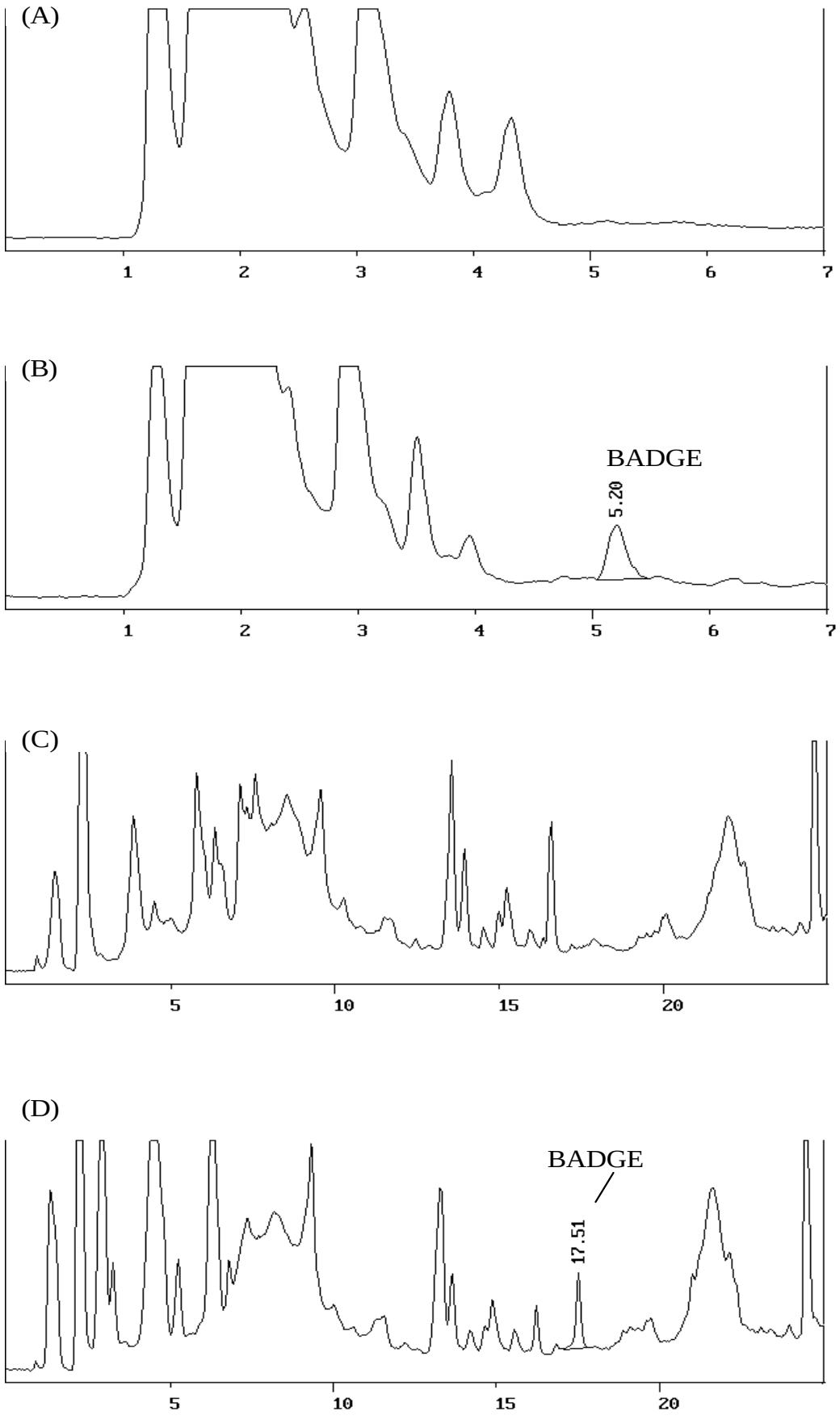

Figure 1. Chromatograms applying both Isocratic and Gradient: (A) and (C) olive oil blanks, (B) and (D) solution with 20 µg BADGE/L in olive oil.

Table 1.- Migration results of BADGE from 3 types of coating obtained under two diferent test conditions in olive oil food simulant using gradient method.

Simulant	Analyte	10 days at 40ºC (test a) Conc. measured (µg/l)			30 min. at 121ºC (test b) Conc. measured (µg/l)		
		Coating			Coating		
		Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
Olive oil	BADGE	<20	74	<20	97	266	145
	+ Other migrants*	180	1964	127	629	1731	655

* The concentrations of "Other migrants" are expressed in BADGE.

Stability of stock and dilute stock solutions

The stability of BADGE stock solutions was studied as described (Paseiro-Losada *et al.*, 1997). BADGE dilute stock solutions, content 1 µg of BADGE per mL of 90% (v/v) methanol, were stored for 3 months at -20 ºC, 0-5 ºC and at room temperature and then determined by the Gradient Method after dilution at 40 µg/L with 90% (v/v) methanol. As these solutions are not stable at room temperature, they should be prepared just prior to use.

Precision

Since the detection limit for the BADGE established by the European Legislation is 20 µg/L, we prepared several BADGE solutions using olive oil instead of 90% methanol at concentrations above to 20 µg/L following the previously procedure described. These solutions and their corresponding blanks were processed as described in "Procedure for extraction of BADGE in olive oil". Results (performed in duplicate) afford standard deviations below 20 per cent.

Recovery

Solutions of BADGE in olive oil were prepared, as above, at concentrations of 10, 20 and 40 µg/L and analysed after processing as described in "Procedure for extraction of BADGE in olive oil". Recoveries greater than 79% were achieved with standard deviations of less than 9%.

The variability of the results was due to interferences produced by the complex extraction of the olive oil. The calibration was determined in 90%(v/v) methanol, since the olive oil extracts were analysed in 90% (v/v) methanol.

Confirmation

The confirmation of the identity of BADGE was carried out as has been reported by the authors previously (Paseiro-Losada *et al.*, 1997).

Migration samples

BADGE in simulant after 10 days at 40 ºC and 30 min. at 121 ºC

The stability of BADGE in olive oil was investigated by determining BADGE in simulant that had been fortified to a concentration of 20 µg/L and then stored for 10 days at 40 ºC (test "a") and 30 min. at 121 ºC (test "b"). Recovery found was in the range 77-120% by Isocratic and Gradient methods and the standard deviations 24 and 22% respectively.

Polymer

The polymer tested was an epoxy coating cured at room temperature, based on a BADGE epoxy resin with m-xylylenediamine as curing agent. Its manufacture also involves addition of benzyl alcohol (to lower the viscosity of the glass transition temperature and to increase the reactivity of the epoxy-amino system) and other products such as fillers and pigments to improve water resistance and color. Its intended use is as a coating on food storage vessels.

Migration from the polymer after 10 days at 40 ºC

Two glass discs (total surface area 2.7 dm^2) were painted with the polymer coating on one side, cured for 24 h at room temperature, and then painted with polymer coating on the other side, cured at room temperature for 5 days (thickness of the coating is aprox. 400 µm) and then immersed in 150 mL of food simulant in a glass cell especially designed for testing epoxy coatings for migration into food simulants (Simal-Gándara *et al*., 1993). The surface/volume ratio was 1.8 dm^2 per 100 mL of simulant. The usual surface/volume is 2 dm^2/100 mL.

Two discs were placed in olive oil for 10 days at 40 ºC. Migration tests were carried out (triplicate). Each was analysed in duplicate. Blanks, olive oil stored for 10 days at 40 ºC, were also analysed. The results were 7.8 µg/L by the Gradient Method and less than 5µg/L by the Isocratic Method. Typical chromatograms are showed in Figure 2 (A, B). This polymeric formulation used for large tanks for foods complies with the legal restrictions, as demonstrated by the migration of BADGE wich is lower than the SML. This formulation is therefore for use in the food industry.

Migration from other epoxy polymer formulations

We proceeded and tested the same three epoxy formulation types for use as coatings on food cans as described (Paseiro-Losada *et al.*, 1997).

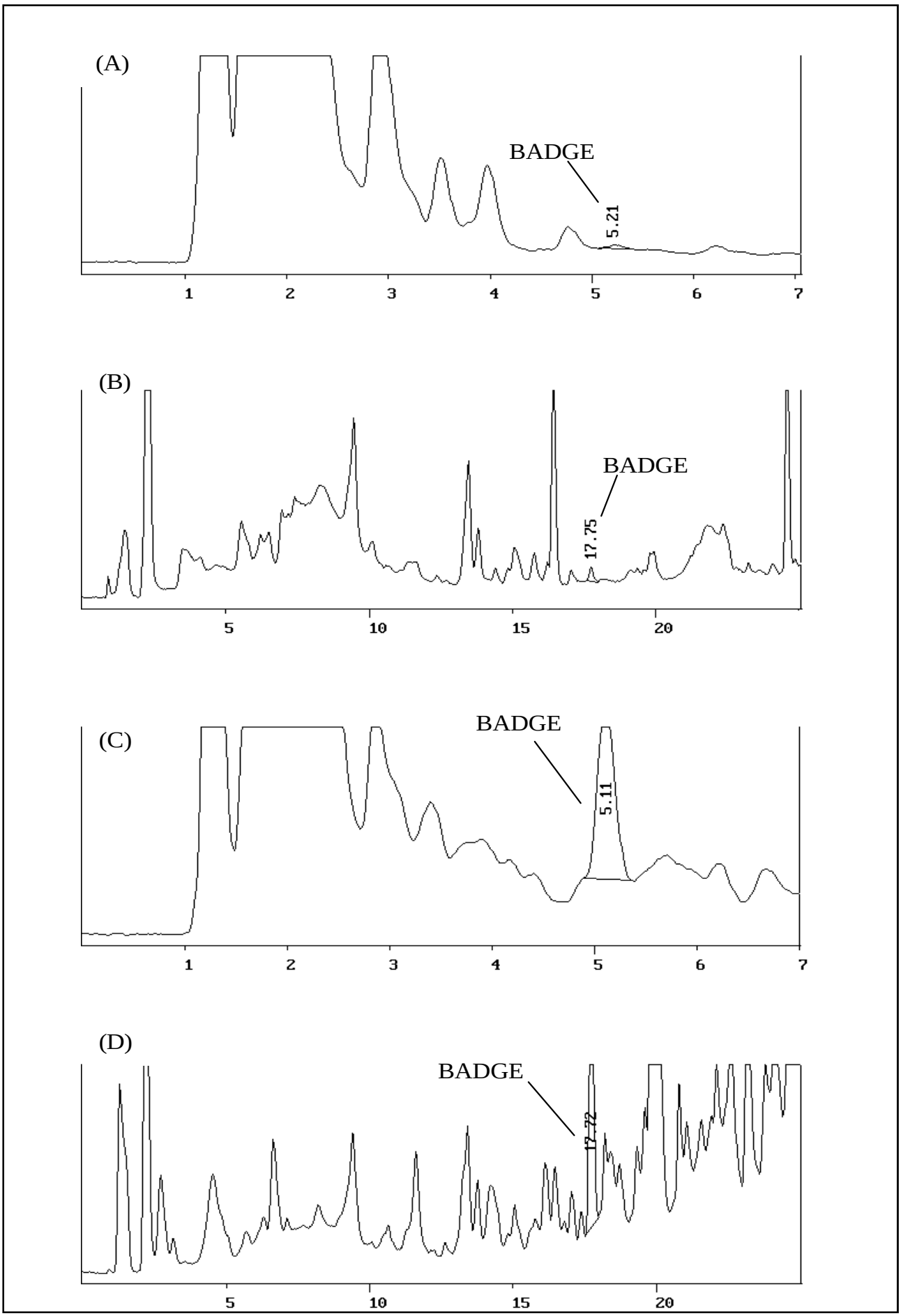

Figure 2. Chromatograms of polymer food coating after test "a" (10 days at 40ºC), (A) by Isocratic Method and (B) by Gradient Method and chromatograms of can coating Type 3 after test "b" (30 min. at 121ºC), by Isocratic Method (C) and by Gradient Method (D).

The results of analyses are listed in Table 1 and typical chromatograms are shown in Figure 2 (C, D). BADGE content determined by the Gradient method was greater than SML in the formulation Type 2 for test "a" and in all three formulations for test "b". Furthermore, the migration of other substances ("other migrants") is observed. These have been quantified in the 15 and 20 minutes range because there is no interference in this range.

None of the three types of polymeric formulations used for food can coatings complies with the legal restrictions at 30 min. for 121 ºC (test "b"), therefore they are not appropriate for use in the food industry under these conditions. Under conditions of the test "a" (10 days at 40 ºC) formulations type 1 and 3 comply with the legislation, and therefore may be used in the food industry provided they will not be submitted to a sterilization process.

CONCLUSIONS

Despite the case of calibration and use of the HPLC methods, analysis of BADGE from olive oil extracts provided dates with large standard deviations. This is due to the complex composition of olive oil, which requires a series of extractions with solvents prior to HPLC analysis.

Consequently these methods should be considered by others prior to developing an alternative method for extraction of BADGE from olive oil.

It is difficult to say whether BADGE undergoes degradation in olive oil at level of 20 µg/L. Though mean recovery after 10 days at 40 ºC was only 77% (Isocratic method), the standard deviation was very high (24%).

The results obtained from the migration test with the food cans confirm the migration of BADGE is greater in test "b" than in test "a" for the three types of coatings. This is due to the fact that in test "b" higher temperatures are used. The migration test with aqueous simulants, it is observed that the transfer of BADGE and other migrants is much greater in test "b" than in the other two types of coatings.

It is clear that the analytical difficulties with olive oil are derived from its complex composition. In our opinion, olive oil, a fatty food simulant, should be replaced by chemically pure oil.

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