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Determination of diphenylbutadiene by liquid chromatography–UV–fluorescence in foodstuffs

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Abstract

Diphenylbutadiene (DPBD) is an optical brightener incorporated into a wide range of polymeric materials. Framed in the FOODMIGROSURE project, it was chosen as a model migrant to study the migration kinetics from polymeric materials in relevant foodstuffs. An analytical method was developed and optimized for the DPBD determination in foods. The sample preparation procedure uses both hexane and acetonitrile as extraction solvents, followed by high-performance liquid chromatography (HPLC) analysis. HPLC analysis was performed using UV detection at a wavelength of 330 nm, and fluorescence detection achieved with excitation and emission wavelengths of 330 and 375 nm, respectively. Good linearity and recovery were achieved. Data are reported.
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1. Introduction

In the last years, food packaging has gained a widespread importance in the food industry. An essential investigation field within food packaging it is focused into the possibility of migration of chemicals from these materials. Food packaging materials must be safe in the sense of not releasing potentially harmful material into the food [1].

In the area of polymer chemistry, additives are often incorporated into polymer systems to improve their physical properties and to enhance their end-use performance. Plastic generally ages rapidly under the effects of light, oxygen and heat, leading to loss of strength, discoloration, scratching, etc. Typical polymer additives include antioxidants, antistatic agents, blowing agents, catalysts, colorants, fillers and reinforcements, flame retardants, impact modifiers, lubricants and slip agents, plasticizers and stabilizers, fluorescent white agents, and a large variety of chemicals with different technical functions.

A fluorescent white agent is a substance that is added to a material to absorb ultraviolet rays in sunlight and release them as blue rays. These blue rays will then interact with the yellowish color and give the plastic the appearance of being whiter. Diphenylbutadiene (DPBD) is an example of these fluorescent white agents.

Trans,trans-1,4-diphenyl-1,3-butadiene (DPBD), CAS No. [538-81-8], is a light yellow crystalline powder, soluble in many organic solvents, with high lipophilicity [Calculated Log K_{ow} (octanol–water) = 5.29] [2] and with a molecular mass of 206.3.

This compound was selected as a model migrant framed in the FOODMIGROSURE project, to study the migration kinetics from polymeric materials in relevant foodstuffs. Its choice was based on the representativeness for food contact materials migrants, so the results will be obtained can be extrapolated to other substances of similar characteristics, and based on its relevance (use frequency in food contact materials) and its analytical behavior.

The type of contact between the packaging and the food (i.e. aqueous, acidic, alcoholic or fatty) determines along with other factors the nature and the extent of the migration that occurs [3].

In order to determine the migration level into foodstuffs coming into contact with plastics, an analytical method was developed for the determination of DPBD in three selected

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foods that represent three types of foodstuffs with different physical and chemical properties: aqueous and acidic food (orange juice), non fatty food (chicken breast), and fatty food (gouda cheese).

2. Experimental

2.1. Samples

Three representative food items were chosen: orange juice (liquid, acid and medium carbohydrates content), chicken breast (solid, high protein content) and Gouda cheese (solid, high fat content). The samples were purchased in local supermarkets.

2.2. Chemicals and reagents

Acetonitrile, ethanol, and hexane were HPLC grade; all supplied by Merck (Darmstadt, Germany) and ultrapure water was prepared using a Milli-Q filter system (Millipore, Bedford, MA, USA). Trans,trans-1,4-diphenyl-1,3-butadiene (DPBD, CAS No. [538-81-8] average M_r 206.29) was from Sigma–Aldrich (Steinheim, Germany).

A stock standard solution was prepared by dissolving 100 mg DPBD in 100 ml of ethanol and was kept at 4 °C in the refrigerator. Intermediate standard solutions of DPBD were prepared by dissolution of appropriate amounts of stock standard solution in acetonitrile. These solutions were stored in amber bottles at 4 °C in the refrigerator.

2.3. Extraction of samples

About 10 g of each food sample were accurately weighed in a 40 ml Pyrex tube with a PTFE-lined screw cap. Then, 10 ml of hexane were added and the tubes were shaken manually for 10 min. To separate the organic phase the tubes were centrifuged at 1036 × *g* for 10 min. The extraction was repeated with 10 ml of hexane twice. All hexane phases were colleted in a round flask and evaporated to dryness using a rotatory evaporator.

For orange juice and chicken breast, the solid residue was redissolved in 10 ml acetonitrile and the solution obtained was homogenized by ultrasonics. The solution was filtered through a PTFE 0.45 μm, 13 mm syringe filter, and an aliquot was transfer into a HPLC vial and then injected. Regarding Gouda cheese extraction, the fatty liquid residue obtained was extracted twice with 10 ml of acetonitrile by vortex shaking for 5 min. The acetonitrile phases were evaporated to 10 ml. The solution was filtered and then injected.

2.4. Equipment

2.4.1. UV–vis spectrophotometer

A Cary 3E UV–vis double-beam spectrophotometer (Varian, Australia) was used to perform single scans from 200 to 400 nm. Software Cary WinUV (version 3.0) was used for the acquisition of the data. A solution of 1 mg/l was used to establish the most sensitive wavelength.

2.4.2. Luminescence spectrometer

Preliminary spectrofluorimetric measurements were performed with a Perkin–Elmer LS 50 luminescence spectrometer (Buckinghamsiher, England) fitted with a xenon flash lamp, Monk Gillieson monochromators and 1 cm quartz cuvettes. Spectral data acquisition and processing were carried out by means of the program FL Winlab on a PC serially interfaced to the LS 50. In a first measure, a full scan range prescan was achieved. All scans were recorded between excitation wavelengths 200 and 800 nm and emission wavelengths 200 and 900 nm. The scan speed was 240 nm/min. Subsequently the maximum value for excitation wavelength was selected and emission scan was completed. In the same way, maximum value for emission wavelength was selected and excitation scan was completed. These measurements were carried out with solutions of 0.1 mg/l.

2.4.3. High-performance liquid chromatography

Chromatographic measurements were performed with a Hewlett-Packard (Waldbronn, Germany) system comprised of a HP1100 liquid chromatograph fitted with a quaternary pump, an autosampler, a column oven, a diode array detector, a fluorescence scanning detector and HP Chemstation data analysis software (version A.06.01). A Kromasil 100 C_{18} column (15 × 0.4 cm i.d., 5 μm particle size) from Teknokroma (Barcelona, Spain) was used for the separation.

2.4.4. Gas chromatography

Identification of selected compounds was carried out using a gas chromatograph Fisons 8000 series (Manchester, UK) coupled to a mass spectrometer (MD 800) operating in the electron impact ionization mode. The gas chromatograph was equipped with a split/splitless injector. A capillary column 30 m × 0.25 i.d., 1 μm film thickness with DB-5 MS as stationary phase from J&W Scientific (CA, USA) was used. Masslab software (version 1.4) was used for data acquisition.

2.4.5. Liquid chromatography–mass spectrometry

A high-performance liquid chromatography–mass spectrometry (HPLC–MS) system comprised a Spectra Physics series P2000 chromatograph equipped with an autosampler and a mass detector Navigator II (all from TermoQuest, Finnigan, Manchester, UK) was used to try to confirm the identity of DPBD. The column and mobile phase were as in 2.5. The detector conditions were as follows: negative and positive atmospheric pressure chemical ionisation (APCI) mode; probe temperature: 250 °C, cone voltage: ±15 V, electron multiplier voltage 650 V, full scan mode scanning a mass spectrum range of 100–400 amu (two scans for second). The system was computer-controlled using the Xcalibur (version 1.2) software.

2.4.6. Other equipment

An Ultra-turrax homogenizer (T25 basic, IKA Labortechnik, Stanfen, Germany) was used to homogenize cheese.

A rotatory evaporator (RE200, Bibby Sterilin, Staffordshire, UK), a centrifuge (Eba 12, Hettich, Krichlenger, Germany) and an ultrasonic bath (5510 Bransonic, Danbury, CT, USA) were also used in the sample preparation procedure.

2.5. Chromatographic conditions

For the HPLC system the following chromatographic conditions were applied: the mobile phase was acetonitrile–water (65:35, v/v) in an isocratic mode for 2 min, followed by a gradient to 100% acetonitrile until 17 min, and finally an isocratic elution during 13 min. The flow rate was 1.0 ml/min. The injection volume was 50 μl. The column oven temperature was kept at 30 °C. UV detection was performed at a wavelength of 330 nm. Fluorescence detection was performed with excitation and emission wavelengths of 330 and 375 nm, respectively.

In case of GC, the chromatographic conditions were as follows: column temperature program was initiated at 160 °C and held for 1 min, increased at 15 °C/min to reach 260 °C and then held at 260 °C for 54 min. Injections of 1 μl of samples were in split mode and the injector was heated to 260 °C. The carrier gas was helium at 1.0 ml/min. Mass spectra were recorded at 70 eV (EI+), in full scan mode between *m/z* 50 and 300 with a scan time of 0.45 s.

The positive confirmation of DPBD was carried out by comparing the obtained spectrum with those of the Willey Library.

3. Results and discussion

3.1. Preliminary studies

Important properties of DPBD such as ultraviolet and fluorescence were evaluated to help to decide the approach for the development of the chromatographic method that allowed determining this compound in foodstuffs. Thus, the maximum response obtained in the UV–vis spectrophotometer was at 330 nm, and in the luminescence spectrometer the maximum wavelengths of excitation and emission corresponds to 330 and 375 nm respectively. These conditions were used later to carry out the chromatographic analysis by HPLC (Fig. 1).

In order to find an analytical technique that allowed positive identification of this compound HPLC–APCI–MS was checked, both positive and negative mode, and evaluating different probe temperatures (200–400 °C) and cone voltages (10–40 V), but satisfactory results were not found. In the other hand, good results were achieved using GC–MS. The elution temperature of DPBD was 260 °C. Characteristic mass 206 corresponds to the ionisation form [M^+].

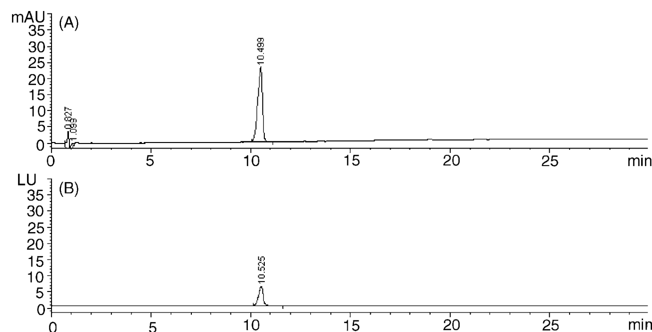

Fig. 1. HPLC chromatogram of a DPBD standard solution (1 μl/ml) (A) Signal of UV detection at a wavelength of 330 nm and (B) fluorescence detection at excitation and emission wavelengths of 330 and 375 nm, respectively.

3.2. Calibration line

The method was calibrated using series of standards in acetonitrile of known concentrations. The relationship between known concentrations and measured areas was assessed by linear regression (seven calibrations points), and the linearity obtained over the concentration range of 0.1–10 μg/ml for selected wavelengths, indicates that the method is appropriate for quantification of this compounds (Table 1). Detection limits (DLs), were calculated in accordance with the American Chemical Society [4] and are shown in Table 1.

3.3. Sample extraction

Trying to cover the analytical complexity of the food matrix, three representative food items were chosen to determine DPBD: orange juice (aqueous and acidic liquid with medium carbohydrates content), chicken breast meat (solid with a high protein content) and Gouda cheese (solid with a high lipid content) [5]. These food items were used to complete all analysis.

In order to establish a suitable sample amount, distinct quantities were assayed; good results were obtained with 10 g of sample. Besides sample amount, the sample homogenisation is of great importance once can greatly influence the extraction efficiency. Orange juice was directly extracted and it has not raised any analytical difficulty (Fig. 2). Chicken was chopped up and due to its consistency, a slurry was obtained and considered suitable for an effective extraction pro-

Table 1
Method validation parameters

Parameters of calibration curve	λ = 330 nm	λ_{ex} = 330 nm, λ_{em} = 370 nm
Slope	378.84	78.91
Intercept	−1.20	12.74
Correlation coefficient	0.9998	0.9983
Range (μg/ml)[a]	0.1–10	0.1–10
Detection limit (ng/ml)	25	12

[a] Seven calibration points in the range indicated.

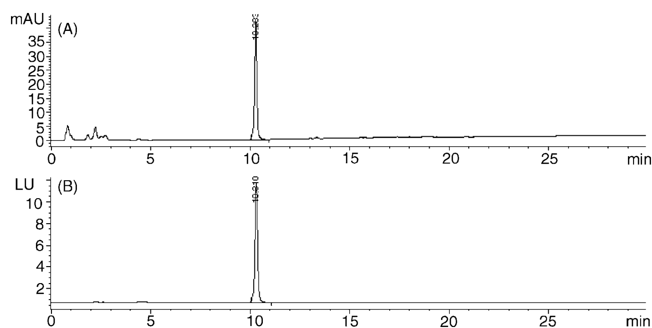

Fig. 2. HPLC chromatogram of an orange juice sample spiked with DPBD (1 μl/ml) (A) Signal of UV detection at a wavelength of 330 nm and (B) fluorescence detection at excitation and emission wavelengths of 330 and 375 nm, respectively.

cedure while cheese was homogenised with an ultra-turrax homogeniser.

To the chicken sample, 2 ml of acetonitrile were added directly and homogenised by hand shaking, prior to the first extraction with hexane due to this process originated a compact mass, which not desegregate, and foam, which interfered during phases dryness in the rotary evaporator.

In what concerns to the extraction of DPBD from Gouda cheese, its high fat content provided low recovery using extraction procedure applied to orange juice and chicken. Therefore, a double extraction with pure acetonitrile was investigated, this allowed obtaining acceptable recovery data.

The accuracy of the method for determining this compound in foodstuffs was calculated by performing the extraction procedures with spiked orange juice, chicken breast and Gouda cheese at three different levels (six replicates). Recoveries data found were higher than 83% (Table 2). To orange juice the recovery found were higher than 85% (Table 2) and higher than 83% to chicken breast (Table 2), in both cases for UV detection. The recoveries obtained with the fluorescence signal were different (Table 3), which can be explained due to this signal is more affected by light. Regarding to explain this fact, a study on stability of DPBD was carried out.

Several trials were performed in order to study solutions stability. Solutions were stored in three light conditions: presence of sunlight, artificial light and darkness, all of them at room temperature. Solutions stability was studied after two hours comparing the DPBD peak of a fresh prepared stock solution with those maintained under the studied conditions.

Table 2
DPBD recoveries at different concentrations for orange juice, chicken breast and Gouda cheese obtained with an ultraviolet detector

Matrix	Level (μg/g)	Mean (μg/g)	Recovery (%)	Repeatability (R.S.D.r, %)
Orange juice	5	4.95	98.9	2.0
	1	0.852	85.2	2.3
	0.5	0.468	93.7	3.7
Chicken breast	5	4.18	83.7	8.4
	1	0.856	85.6	5.0
	0.5	0.417	83.4	3.1
Gouda cheese	5	4.20	84.1	2.3
	1	0.864	86.4	7.1
	0.5	0.5	100	9.7

Table 3
DPBD recoveries at different concentrations for orange juice, chicken breast and Gouda cheese obtained with a fluorescence detector

Matrix	Level (μg/g)	Mean (μg/g)	Recovery (%)	Repeatability (R.S.D.r, %)
Orange juice	5	5.375	107.5	2.9
	1	0.799	79.9	5.3
	0.5	0.402	80.5	6.1
Chicken breast	5	4.42	88.4	11.0
	1	0.842	84.2	12.7
	0.5	0.359	71.9	9.0
Gouda cheese	5	5.295	105.9	8.6
	1	1.057	105.7	10.7
	0.5	0.538	107.6	12.6

DPBD was more prone to degradation reaction in presence of light. It was observed that fluorescence signal was more influenced by light than UV signal. Results showed that, solutions exposed to sunlight, after two first hours, presented a 45% decrease of the DPBD peak area in case of UV signal, while for the fluorescence signal, decrease was 75%. Regarding the results obtained under artificial light, although the decrease was not so significant, in case of UV signal, this decrease was 7% and for fluorescence it was 8%. Using amber vials, only slight fluctuations were observed under artificial light as well as sunlight.

Therefore, sample preparation procedure should be performed under absence of direct sunlight and using amber glass material.

Blanks of others food items were carried out in order to establish the possibility of determine DPBD. The foods analysed were: apple sauce, milk UHT, tomato ketchup, cola, margarine, condensed milk, dark chocolate, toasted bread, wheat flour, rice and honey. In any case no interference was found at the retention time of DPBD, which indicate the suitability of the method for this foodstuffs.

DPBD identity was confirmed by GC–MS. Several assays were performed to achieve best conditions in GC–MS. SIR was selected towards full scan mode due to its sensitivity.

4. Conclusions

As DPBD is light sensitive, extraction procedures should be conducted in absence of direct sunlight, and all the material employed must be amber.

The HPLC-UV method is suitable for the determination of DPBD in foodstuffs in a range of 0.1–10 mg/kg. However, for foods with a high content of fat, the sample extraction procedure must be slightly different on order to obtain acceptable recovery data. GC–MS is an analytical technique suitable to confirm the presence of this substance.

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