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Analysis of Bisphenol A in Beverages and Food Packaging by High-Performance Liquid Chromatography

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Abstract

Food contact materials should be monitored to ensure product quality and safety, with the ultimate objective of ensuring that no damage will occur in consumer health. This issue has become very important in recent years. Bisphenol A (BPA) is present in many products for daily use. This chemical can be transferred to food from some types of materials such as polycarbonate containers and metal cans with epoxy resin coatings. In this work foods packaged in metal cans and their containers were analyzed to check if there was migration of bisphenol A from the packaging to the food. Four types of drinks were taken as study samples, including beer and energy drink. The identification and quantification of BPA was performed using an analytical method based on High Performance Liquid Chromatography (HPLC) with fluorescence detection and confirmation of the results with Liquid Chromatography coupled to tandem mass spectrometry (LC-MS/MS). The proposed method has a limit of detection appropriate, low enough for compliance with Regulation 10/2011. No detectable amounts of bisphenol A were found in the samples analyzed.

Keywords: Bisphenol A; Food; Food Packaging; HPLC-FLD; LC-MS/MS

Introduction

BPA (2,2-bis(4-hydroxyphenyl)propane) was synthesized for the first time in 1891, by means of the condensation of a molecule of acetone and two phenol groups. In past years the production of the chemical has grown significantly [1,2].

BPA is authorized to be used as monomer in the manufacture of plastic materials intended to come into contact with food with a specific migration limit of 0,6 mg/kg [3]. Some of main uses are as monomer in the manufacture of polycarbonate and epoxy resins which are commonly employed as inner coatings of food and beverage cans [1,4].

Lately great attention has been paid to BPA, the concern over BPA exposure is related to their classification as endocrine disruptor. The diet is the main source of exposure, foods become contaminated through chemical migration from the packaging and also during the processing, storage and transport. In 2006 the European Food Safety Authority (EFSA) conducted a risk assessment of BPA and established a TDI of 50 µg/kg bw per day, however, more recently in 2015 a temporary Tolerable Daily Intake (t-TDI) was fixed in 4 µg/kg bw per day and it concludes that "The dietary exposure to BPA for the highest exposed groups, which includes infants, children and adolescents, is below the t-TDI of 4 µg/kg bw per day, indicating that there is no health concern for BPA at the estimated levels of exposure" [5-8].

To determine BPA in food contact materials as well as in food samples chromatographic methods have been applied. Reversed-phase liquid chromatography with fluorescence detection has become a popular technique. In the scientific literature, also methods based on either liquid or gas chromatography coupled to mass spectrometry have been reported [2, 9-11].

Since the consumer could be exposed to BPA through the diet, analytical methods to determine the analyte in food and packaging samples are required, the objective of the present paper was to apply a high-performance liquid chromatographic method with fluorescence detection (HPLC-FLD) to quantify BPA in packaged beverages and in the packaging materials. In the second part of the work LC-MS/MS was used to confirm the results.

Materials and Methods

Chemicals and standard solutions

All reagents were of analytical grade. Acetonitrile, ethanol and methanol were from Merck (KGaA, Darmstadt, Germany). Ultrapure water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Standard of BPA (>99%) was from Aldrich-Chemie (Steinheim, Germany). The chemical structure and physico-chemical properties of BPA are shown in Table 1 [12].

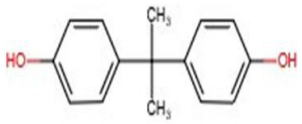
BPA	
	CAS: 80-05-7
	MW: 228.289
	Molecular Formula: C15-H16-O2
	Melting Point: 153ºC[a]
	log P (octanol-water): 3.32[a]
	Water Solubility: 120 mg/L[a]
	Vapor Pressure: 3.91E-07[b]
[a]: Experimental; [b]: Estimated	

Table 1: Physicochemical properties of BPA.

Stock standard solutions of BPA were prepared in acetonitrile and the working solutions were made by dilution. All solutions were stored at 4ºC in the fridge.

Samples

A total of four commercial drinks including three beers and an energetic beverage, all packed in cans, were purchased in local supermarkets. Samples were stored at room temperature until analysis.

Equipments

HPLC-FLD

The chromatographic system, Agilent Technologies 1200 Series (Waldbronn, Germany) consisted of a quaternary pump, a degassing device, an autosampler, a column thermostat system, a diode array detector, a fluorescence detector and an Agilent ChemStation for LC and ChemStation for LC 3D Systems software.

LC-MS/MS

The LC-MS/MS system consisted of an Accela autosampler, a column thermostat system and Accela 1250 pump fitted with a degasser, coupled to a TSQ Quantum Access Max Triple Quadrupole controlled by Xcalibur 2.1.0 (Thermo Fisher Scientific, San José, CA, USA). MS data were acquired in the negative Electrospray Ionisation (ESI) mode. The operating conditions were: nebulizer gas (N2), 35 psi; Spray voltage 2500 V; vaporizer temperature 340ºC; capillary temperature 350ºC. Argon was used as the collision gas (1.5 mTorr).

Chromatographic conditions

The chromatographic separation was performed on a Kromasil C18 (150 x 3.20 mm, 5 µm particle size) from Phenomenex thermostated at 25ºC. The mobile phases consisted of (A) Milli-Q water and (B) acetonitrile. The flow rate and the injection volume were 0.5 mL/min and 20 µL, respectively. The FLD detector was set at λem305 nm and λex 225 nm. For LC-MS/MS analysis the chromatographic conditions were the same as HPLC-FLD, but methanol was used instead of acetonitrile. The gradient elution conditions are shown in Table 2.

Time (min)	% A	% B*
0.00	70.0	30.0
2.00	70.0	30.0
23.00	0.0	100.0
30.00	0.0	100.0
*: B corresponds to acetonitrile in HPLC-FLC or methanol in LC-MS/MS		

Table 2: HPLC-FLD and LC-MS/MS elution conditions.

BPA Quantification

Quantification was performed on the basis of linear calibration plots of peak area against concentration. Calibration lines were constructed based on four concentration levels of standard solutions within 0.05-1 mg/L range, in acetonitrile, 90 % acetonitrile (v/v) and 10% ethanol (v/v).

Additionally, in parallel a calibration curve, based on five concentration levels of standard solutions within 0.0025-1 mg/L range was prepared in beer.

Extraction procedure

BPA was extracted from the food packaging materials using acetonitrile. A known surface of the packaging, 0.62 dm2 was extracted with 100 mL of the solvent during 24 h at 40ºC. Then, 4.5 mL of the solution were removed and made up to 5 mL with water. An aliquot of the solution was filtered through a 0.45 µm PTFE membrane filter (Advantec MFS, INC, CA, USA) and injected into the chromatograph.

Beer samples were degassed for 60 min using an ultrasonic bath, after that an aliquot was filtered through a 0.45 µm PTFE membrane filter (Advantec MFS, INC, CA, USA) and directly injected into the chromatograph. Samples were analyzed in duplicate.

Results and Discussion

In order to improve the peak shape of BPA, Milli-Q water

was added to acetonitrile in the preparation of standard solutions. Under these conditions (90% acetonitrile (v/v)) a better chromatographic peak resolution was achieved compared to that obtained when only acetonitrile was used. Identification of BPA was made by comparison of the retention time and fluorescence spectra with that of a pure standard and confirmed by LC-MS/MS.

As it has commented above the linearity of the method was tested by using a series of BPA standard solutions of known concentration. The calibration curves were constructed using four or five concentration levels and they were fitted to a linear equation. The linearity was tested in acetonitrile, 90% acetonitrile (v/v), 10% ethanol (v/v) and beer. Parameters of linearity; range of linearity, origin ordinate, slope and determination coefficients are shown in Table 3. The method showed a good linearity within the range of concentration studied with R^2 ≥0.9963.

Matrices	Linear range (mg/L)	Slope	Origin ordinate	R^2	LOQ (mg/L)	LOD (mg/L)
acetonitrile	0.05-1	223.9280	6.4029	0.9963	0.05	0.025
90% acetonitrile (v/v)	0.05-1	207.4790	1.0879	0.9999	0.05	0.025
10% ethanol (v/v)	0.05-1	199.3366	-0.0059	0.9990	0.05	0.025
beer	0.025-1	205.6439	-0.8973	0.9994	0.05	0.01

Table 3: Parameters of linearity and limits of detection and quantification.

The limits of detection and quantification, calculated according ACS guidelines [13] (defined as signal three and ten times, respectively the height of the noise level) were presented in Table 3. The limit of detection obtained in beer (0.01 mg/L) was slightly lower due to the better resolution of the peak in this solution. The method presents enough sensitivity to detect BPA at the regulatory level. The repeatability within day was determined by analyzing ten replicates of the standards at a concentration level of 1 mg/L, expressed as the percentage of RSD (RSD % (n = 10)) was 0.56%. BPA stability was evaluated in stock standard solutions in 90% acetonitrile stored at 4ºC and protected from the light for a period of 41 days. The standard solutions were analyzed at time 0, 15, 34 and 41 days. The RSD % obtained for the assay was 4.07 %, therefore BPA was stable during the period tested. Packaging and food samples were analyzed for the presence of BPA and it was found that no sample contained detectable amounts of BPA. However, in the energetic beverage sample, a suspicious peak very close to the peak corresponding to BPA was observed as it is shown in Figure 1. To confirm if it was really BPA, the sample was spiked with the standard compound and analyzed and confirmed the presence/absence of BPA in the sample by LC-MS/MS.

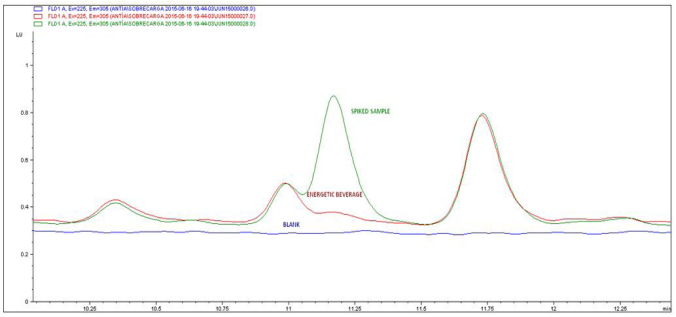


Figure 1: HPLC chromatogram with a blank (ACN 90%), the suspicious sample (energetic beverage) and the spiked sample (0.025mg/L of BPA in energetic beverage).

In order to maintain the good chromatographic resolution and to obtain an appropriate signal response in LC-MS, acetonitrile-water and methanol-water based mobile phases were tried. The best results were achieved when methanol-water mobile phase was used. Figure 2 show chromatograms of a BPA standard solution using both mobile phases.

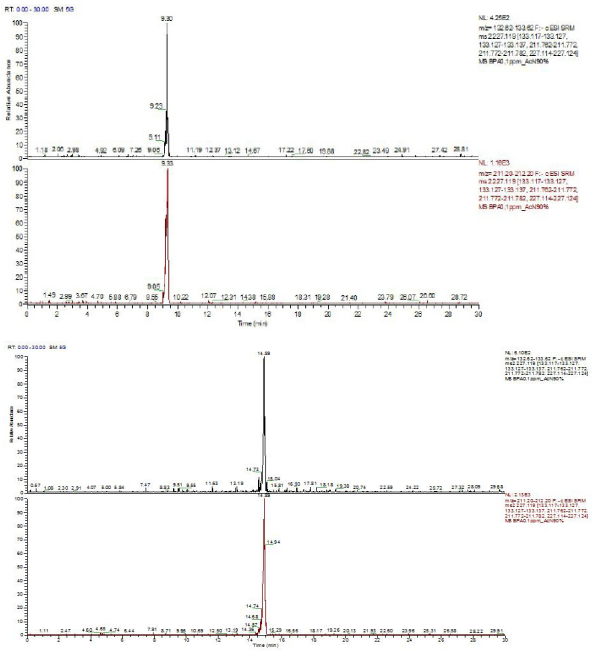


Figure 2: LC-MS/MS chromatogram of a BPA standard of 0.1 mg/L using a mobile phase based on water and acetonitrile with the transitions m/z 227→133 (A) and 227→211.77 (B), and a BPA standard of 0.1 mg/L using a mobile phase based on water and methanol with the transitions m/z 227→133 (C) and 227→211.77 (D).

MS data were acquired in Selected Reaction Monitoring (SRM) mode once the optimization of the MS/MS parameters were performed using the built-in perfusion system. Source spray voltage, sheath gas, auxiliary gas, capillary temperature and collision energy was optimized during infusion of the individual analyte. The selected precursor ion for BPA was m/z 227, the most sensitive ion in the Q1 mass spectra. Two SRM transitions of m/z

227.12→211.77 and m/z 227.12→133.13 were monitored with a collision energy of 23 and 27 V respectively. The ion m/z 227 has been assigned as the deprotonated molecule [M-H]-, the transition m/z 227.12→211.77 was related with the additional loss of oxygen [M-H-O]- and the transition m/z 227.12→133.13 with the loss of phenol group [2,14]. The results revealed that the unknown peak did not correspond to BPA as it is shown in Figure 3.

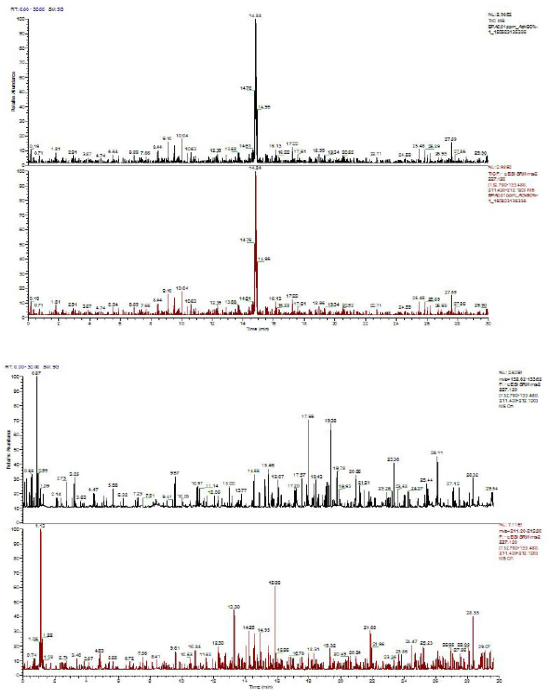


Figure 3: LC-MS/MS chromatogram BPA standard of 0.01 mg/L with the transitions m/z 227→133 (A) and 227→211.77 (B), and the suspicious sample with the transitions m/z 227→133 (C) and 227→211.77 (D) using a mobile phase based on water and methanol.

With regard to the data reported in the literature, several studies have been devoted to determine BPA in different food contact materials, in a recent review [15] about the food packaging contaminants it was reported that besides polycarbonate and canned foods, BPA has been also found in recycled paper and board used for pizza packaging.

In brief, a simple chromatographic method was applied to determine BPA in cans as well as in beverage samples. The results showed no detectable amounts of BPA in the samples analyzed. In addition, a LC-MS method was optimized for confirmation purposes.

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