Collaboration

Prospective studies of bisphenol A and melamine in canned drinks

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Abstract

Bisphenol A (BPA) and melamine are substances used in the manufacture of internal coatings used in canned drink products. The potential release of these substances from this type of material into the food is regulated at national level by the Royal Decree 847/2011. This legislation includes coatings for food contact in its scope and allows the use of substances listed in the European Regulation (EU) No 10/2011 for plastic articles and materials, with the same restrictions there mentioned: 0.6 mg kg^{-1} for BPA (banned for the manufacture of polycarbonate infant feeding bottles: Regulation (EU) No 321/2011) and 2.5 mg kg^{-1} for melamine.

In the last years, controversy has been raised on the use of BPA in food contact materials, because of possible toxic health effects. This has lead the European Food Safety Authority (EFSA) to a new evaluation of this substance, which has been recently published and establishes a temporary tolerable daily intake (TDI) of 4 µg kg^{-1} b.w. day^{-1}.

In the case of melamine, EFSA issued an opinion in 2010 setting a TDI of 0.2 mg kg^{-1} b.w. day^{-1}.

Canned drinks are commonly consumed by the general population. Even though many data have been published on BPA levels found in foods, these are scarce in the particular case of canned drinks in Europe. Concerning melamine, according to our knowledge, there are not published data on melamine levels in this type of products in Spain.

This study was designed with the aim at providing data on the levels of these two substances, BPA and melamine, in canned drinks/beverages in the Spanish market. In order to perform it, the analytical methods already available in the laboratory were adapted and verified to lower the limits of quantitation. These were finally set for drinks at 2 µg kg^{-1} for BPA, and 0.5 mg kg^{-1} for melamine.

The adapted methods were used to analyse 38 samples of canned drinks, collected at the retail market, in the autonomous community of Madrid.

Melamine could not be detected in any sample, above the quantitation limit. Concerning BPA, results could be obtained for 35 out of 38 samples, and it was detected in 6 samples (17 %) at levels ranging (2-6) μg kg^{-1}, being the average concentration 3.4 μg kg^{-1}. According to these results BPA levels in canned drinks are low, which is in agreement with the studies published by several authors.

BPA and melamine found concentrations in all the analysed samples were well below the restriction limits of 0.6 mg kg^{-1} and 2.5 mg kg^{-1}, respectively, established in the regulation for plastic materials (Regulation (EU) No 10/2011) and applicable according to the Spanish Royal Decree 847/2011.

Key words

Bisphenol A, melamine, canned drinks, HPLC-FL, LC/MS-MS.

1. Introduction

Bisphenol A, (2,2-bis (4-hydroxyphenyl) propane) (BPA), is one of the chemical substances with the highest volume of production in the world (>5 million tonnes per year, forecast for 2015) (Merchant Research & Consulting, 2014). It is essentially used as a monomer or starting material in the production of polycarbonate, epoxy resins, polysulfones and as an additive in certain plastics (antioxidant, stabiliser in the production of PVC), and consequently it is found in many materials intended to come into contact with food: polycarbonate bottles and packaging, plates, cups, epoxy resins used for lining the inside of cans and food containers.

The use of BPA is authorised in plastic materials intended to come into contact with food (Regulation (EU) No 10/2011) with a specific migration limit of 0.6 mg kg^{-1} (EU, 2011a), and its use has been banned for the manufacture of plastic infant feeding bottles since June 2011 (Regulation (EU) No 321/2011) (EU, 2011b).

In recent years, there has been a major controversy regarding its possible toxicity. BPA acts as an endocrine disruptor (its exposure during the perinatal period may alter the reproductive system), and it may also produce alterations in the nervous, immunological and cardiovascular system (Geens et al., 2012) (Vanderberg et al., 2012) (Rochester, 2013). The European Food Safety Authority (EFSA) has assessed bisphenol A on various occasions. The first assessment was carried out in 2006, and a TDI (tolerable daily intake) of 50 µg kg^{-1} b.w. day^{-1} was established, based on the fact that there were not evident adverse effects in the studies using animals at a dose of 5 mg kg^{-1} b.w. day^{-1}, and applying a factor of 100. This TDI value was maintained in the subsequent two reviews made in 2008 and 2010. In the most recent review, published in 2015, in the light of the latest studies and considering the uncertainties surrounding the potential health effects of BPA, the EFSA established a temporary TDI of 4 µg kg^{-1} b.w. day^{-1} (EFSA, 2015).

Given that the primary exposure to BPA is through diet, a huge number of studies have been carried out on the possible transfer of BPA from packaging into the food (Goodson et al., 2002) (Munguía-López et al., 2005) (Sajiki et al., 2007) (Santillana et al., 2011) (Liao and Kannan, 2013).

However, the data available in the literature regarding levels of bisphenol A in canned drinks in Europe is limited. Gallart-Ayala et al. (2011) observed levels between 44 ng L^{-1} and 607 ng L^{-1} in 7 out of 11 samples of drinks analysed, taken from the market in Barcelona in 2009. Geens et al. (2010) estimated a mean concentration of 1.0 µg L^{-1} in the study of 45 canned drinks from the Belgian market. The levels of BPA detected by Cuhna et al. (2011) in canned drinks in Portugal, ranged between 0.01 µg L^{-1} and 4.7 µg L^{-1}. More recently, Fasano et al. (2015), in samples taken from the Italian market found a mean value of BPA in carbonated drinks to be 1.24 µg L^{-1} and in non-carbonated drinks the mean was 0.80 µg L^{-1}; nevertheless, this study included drinks in cans and in other types of packaging. A study with a larger number of samples (72) was conducted in Canada by Cao et al. (2009), in which 85 % of the samples contained <1 µg L^{-1} of BPA and the mean concentration was 0.57 µg L^{-1}.

Melamine is a monomer used to obtain melamine/formaldehyde resins, for the manufacture of thermostable articles with a wide number of uses, commonly known as "melamine" tableware. In addition, this substance is also used as an additive, for example in epoxy-type resins for lining

tins, or in metal closures used for glass jars, acting as a catalyst in the formation of the three-dimensional network in the hardening of the resin. It is also used in adhesives.

EFSA, in their assessment report on melamine, published in 2010, established a TDI of 0.2 mg kg^{-1} b.w. day^{-1} (EFSA, 2010). In the same document, and in accordance with the data provided by the industry for the food subcategory: Soft drinks with percentage of fruits lower than nectar, excluded fruit juice, the mean value of the level of melamine (upper bound) was 0.05 mg kg^{-1} (n=1), whereas the data provided by the European countries gave a mean value (n=13) of 0.69 mg kg^{-1} (minimum 0.05 mg kg^{-1}and maximum 2.5 mg kg^{-1}).

In Spain, to our knowledge, no data have been published regarding levels of melamine in canned drinks, a product with high levels of consumption among the population.

In Spain, 50.5 % of the marketed cans contain beer (4 % alcohol-free). As regards non-alcoholic drinks, the main soft drink consumed is cola type with a percentage of 51.1 % of the total; citrus-flavoured drinks (orange, lemon) make up 21.2 % of the total, in particular orange-flavoured drinks represent the 13.7 %. For tonic-flavoured drinks the current percentage is 16.3 % (MAGRAMA, 2015).

The internal coating of cans and metal containers are not included in the scope of application of the plastics regulation; nevertheless at national level, Royal Decree 847/2011 (BOE, 2011), establishing the positive list of substances permitted for the manufacture of polymeric materials intended to come into contact with food, includes coatings in its scope. Although neither bisphenol A nor melamine are included in the positive list of monomers, additives and other starting materials of Royal Decree 847/2011, this regulation authorises the use of the substances listed in Annex 1 of the plastics Regulation (EU) No 10/2011, with the restrictions for use listed there. Consequently, it is understood that the use of BPA and melamine, is authorised in the manufacture of coatings, applying a restriction of 0.6 mg kg^{-1} and 2.5 mg kg^{-1}, respectively.

In addition, with respect to melamine, Regulation (EU) No 594/2012, (EU 2012) amending Regulation (EC) No 1881/2006 as regards the maximum levels of the contaminants in foodstuffs, establishes a maximum level of melamine of 2.5 mg kg^{-1} in foodstuffs, and of 1 mg kg^{-1} in infant formulae and follow-on formulae.

The purpose of this paper is to provide data on BPA and melamine concentration levels in canned drinks. The choice of these two substances and of the type of samples used in the study was decided based on the following facts:

- BPA was being reassessed by the EFSA, and therefore there was a possibility that, depending on the results of the assessment, the migration limit (0.6 mg kg^{-1}) could be reduced or even banned from use for materials in contact with food. It was therefore of interest to obtain data regarding the presence and levels of BPA that might be found in canned food. The soft drinks group was selected in view of the high level of consumption and limited available data.
- The migration limit of melamine for plastic materials was reduced in 2011 to 2.5 mg kg $^{-1}$ (EU, 2011b), where the previous limit was 30 mg kg^{-1}. It was considered of interest to conduct tests on the same samples used in the study on bisphenol A, aimed at obtaining information regarding the levels of concentration of melamine, and to establish whether these were in line with the restriction levels established in the regulation for plastic materials.

For the determination of both melamine and of BPA, it was necessary to first adapt the available test procedures in the laboratory, accredited by the National Accreditation Body (ENAC), in order to reduce the quantification limits.

2. Materials and Methods

2.1 Samples analysed

From May to September 2014, 38 samples of canned drinks were purchased from retail outlets (large and medium-sized), in the Community of Madrid, distributed as follows: 22 samples of soft drinks/alcohol-free drinks (juices, colas, bitter, tonic, water, alcohol-free beer, etc.), 10 samples of energy drinks and 6 samples of alcoholic drinks (beers), corresponding to 23 different trade names, and in all cases the country of origin was the European Union.

Samples were presented in cans of 330 mL (25 cans), 500 mL (7 cans), 375 mL (1 can), 335 mL (1 can) and 250 mL (4 cans).

For cans with a content <500 mL an estimation of the contact surface was made, since a ratio between the contact surface and the volume of 6 dm^2 per kg of food applies for the expression of the results (mg kg^{-1}).

Samples were stored at room temperature until being opened. Once opened, the contents were divided into two portions (for the determination of BPA and of melamine) and were kept in brown flasks under refrigeration until their analysis. In the case of carbonated drinks, these were degasified in an ultrasound water bath for approximately 20 minutes.

2.2 Reagents and standards

For bisphenol A analysis, acetonitrile HPLC and LC-MS grades (Scharlab), glacial acetic acid, min. 99 % (Merck), ultra pure type I water (Direct-Q system, Millipore), 0.45 µm nylon filters (Symta) and 0.2 µm PTFE filters (Waters) were used.

Bisphenol A standard, purity ≥95 %, was obtained from Aldrich. From a concentrated solution of 500 µg mL^{-1} prepared in acetonitrile, another solution of 10 µg mL^{-1} was prepares by dilution. From this, a solution of 2 µg mL^{-1} was obtained in the same solvent, which was used to obtain the calibration solutions in the range equivalent to 2 µg kg^{-1}-20 µg kg^{-1} bisphenol in sample.

For the analysis of melamine LC-MS grade acetonitrile (Scharlab), ammonium formate (Fluka), diethylethylamine (Sigma-Aldrich), ultra pure type I water (Direct-Q System, Millipore), 0.45 µm and 0.2 µm nylon filters (Acrodisc) were used.

Standards used were melamine, purity ≥99 % (Merck), and melamine ^{13}C, 99 % amino- ^{15}N, 98 % of 100 µg mL^{-1} in water (Cambridge Isotope CNLM-8150-1) as internal standard. A melamine stock solution was prepared in water at a concentration of 1 mg mL^{-1}. From a dilution of melamine in water of 0.5 µg mL^{-1}, and from a 1 µg mL^{-1} dilution of internal standard also in water, calibration solutions were obtained in a range equivalent of 0.5 mg kg^{-1} to 5 mg kg^{-1}. For this, 100 µL of the internal standard solution and different aliquots of the 0.5 µg mL^{-1} melamine solution, were evaporated and subsequently re-dissolved in the eluent used in the chromatographic analysis.

2.3 Instruments

2.3.1 Determination of bisphenol A

For the chromatographic analysis, an Agilent Technologies 1100 series equipment connected to a fluorescence detector (Agilent G1321A) was used, operating at an excitation wavelength of 227 nm and emission wavelength of 313 nm. The column used was C18 phase, 150 mm x 3.0 mm, 3.5 μm (Zorbax Eclipse Plus), and as a mobile phase a gradient of acetonitrile/water (25 % to 100 %) at a flow rate of 0.55 mL min^{-1}. The injection volume was 50 μL.

For the confirmation of bisphenol A, a LC-MS/MS equipment from Agilent Technologies comprising a 1200 series HPLC connected to a triple quadupole mass spectrometer (Triple Quad 6410), and a C18 column, 150 mm x 3.0 mm, 3.5 μm (X-Terra, Waters) were used. The chromatographic separation was performed with a gradient mobile phase of 0.1 % acetic acid in water/acetonitrile (30 % to 100 %) at a flow rate of 0.4 mL min^{-1} .The injection volume was 50 μL. Electrospray ionisation in negative mode was used (ESI$^-$), with a source temperature of 325 ºC, a gas flow 6 mL min^{-1}, a nebuliser pressure 45 psi and a capillary voltage of 3 500 V. MRM mode was used, monitoring the transitions 227>212 (target) and 227>133 (qualifiers).

2.3.2 Determination of melamine

Melamine instrumental determination was conducted in an equipment consisting of an Agilent 1200 series chromatograph connected to an Agilent 6330 ion trap detector. The chromatographic separation was in HILIC mode, using a silica column, 100 mm x 3.0 mm (Atlantis HILIC Silica, Waters) at a temperature of 30 ºC. As mobile phase acetonitrile/ammonium formate 10 mM pH 3 (95:5) was used in isocratic mode, at a flow rate of 0.5 mL min^{-1}. The injection volume was 15 μL. Ionisation was in ESI$^+$, drying temperature 350 ºC, nebuliser pressure 35 psi, drying gas flow of 12 L min^{-1} and capillary voltage 3 000 V. MRM mode was used, monitoring the transitions 127>85 and 133>89 for melamine and the internal standard, respectively. The confirmation of the identity of the melamine was based on the comparison of the MS(2) spectra of the sample and the standard.

2.4 Extraction of the samples

For the extraction of bisphenol A, to 10 g of sample (degasified where applicable) 10 mL of acetonitrile were added and extracted under agitation for 15 minutes. The extract was centrifuged at 3 000 rpm for 10 min, and then an aliquot was filtered through a 0.45 μm nylon filter for the determination by HPLC- FL. When the confirmation of the presence of bisphenol A by LC-MS/MS was necessary, the extract was filtered through PTFE 0.2 μm filter.

For melamine extraction, to 0.5 g of sample (degasified where applicable) 10 mL of a diethylamine/ water/acetonitrile (1+4+5) mixture were added, and extraction was carried out with agitation for 1 minute. Then, the extract was centrifuged at 10 000 rpm, 5 ºC ,10 min, and 500 μL of the filtered (nylon, 0.45 μm) supernatant were transferred, together with 100 μL of 1 μg mL^{-1} internal standard solution, into a tube for evaporation to dryness. The final residue was re-dissolved (1 minute in agitation followed by 1 minute in ultrasound water bath) in 500 μL of the mobile phase used in the chromatographic separation.

2.5 Validation

Test procedures for the determination of both, BPA at levels ≥0.05 mg kg^{-1}, based on liquid chromatography with detection by fluorescence (HPLC-FL) and confirmation by liquid chromatography connected to mass spectrometer (LC/MS-MS) with triple quadrupole; and also for melamine at levels ≥2.5 mg kg^{-1}, by LC/MS-MS (ion trap), were already available in the laboratory. The methods were adjusted and verified in order to lower the quantification limits.

2.5.1 Bisphenol A (BPA)

For the verification of the method at low levels, ≤0.05 mg kg^{-1}, a sample of orange juice contained in PET packaging (BPA content <2 μg/L^{-1}) was used.

To check whether there was matrix effect, a calibration curve was prepared in the blank sample in the range of 2 μg kg^{-1}-20 μg kg^{-1}, and was compared with the curve, in the same concentration range, prepared in acetonitrile.

The following parameters were assessed according to the criteria of the quality system followed in the laboratory, based on ISO 17025 (UNE-EN 2005):

- Selectivity/specificity: it was confirmed that in the blank sample there were no interfering peaks at the retention time of bisphenol A with a response higher than 1/3 of that obtained at the quantification limit. Bisphenol A retention time in samples should agree to within ±5 %, of retention time for standards. Moreover, in the analysis by LC/MS-MS, the ion ratios of the transitions monitored should not vary by more than 30 % with respect to those obtained with the standards.
- Response function: was assessed obtaining calibration curves (range 2 μg kg^{-1}-20 μg kg^{-1}) in acetonitrile, on different days. The linearity expressed as relative standard deviation of the slope should be ≤5 % and the correlation coefficient of the linear regression (r)≥0.996.
- Precision/accuracy: was studied by spiking the blank sample at levels of 2 μg L^{-1} and 4 μg L^{-1}, and replicate analysis were done over 2 days (n=6). Relative standard deviation (CV %) was estimated for the repeatability and internal reproducibility. The accuracy was expressed as percentage of recovery. Precision values calculated according to Hortwiz-Thompson equation were taken as reference criteria for the precision of the method. Recoveries for the studied levels should be between 40 % and 100 %, in accordance with the Guidelines for performance criteria and validation procedures of analytical methods used in controls of food contact materials (Bratinova et al., 2009).

Uncertainty (U%) was calculated according to the following expression:

$$U (\%) = 2 \times [U_{Reproducibility} (\%)^2 + U_{Recovery} (\%)^2]^{1/2}$$

2.5.2 Melamine

Verification of the method was carried out in parallel with the analysis of the samples. This was based on the confirmation of the linearity in the range of (0.5-5) mg kg^{-1}, of the precision and

accuracy (expressed as recovery) at the quantification limit of 0.5 mg kg^{-1}, and of the performance in the identification at this concentration level.

For this, with each series of samples a calibration curve was obtained and 2-4 recoveries were prepared on one or several drink samples, at the level of 0.5 mg kg^{-1}. In total 22 recoveries were made, distributed over 7 non-consecutive days. The criteria required for the verification of the method were: 80 %-110 % recovery; precision according to that estimated with the Hortwiz-Thompson equation; linearity should meet a correlation coefficient of the linear regression (r) ≥0,99 and the deviation or residue of the calibration levels had to be less than 15 %, except at the quantification limit where up to 20 % was allowable. With respect to the confirmation of melamine the relative ratio of the intensities of the qualifying ions, m/z 68 and m/z 110, with respect to the ion used in the quantification, m/z 85, should agree to within 50 % of ratios obtained for the standard with the closer concentration. The uncertainty was calculated using the same expression as for BPA.

3. Results and discussion

3.1 Bisphenol A (BPA)

Verification at low concentration levels of the internal method for the BPA, previously validated in the range of 0.05 to 2.0 mg kg^{-1} (Santillana et al., 2011), was satisfactory.

With respect to linearity, the correlation coefficients of the linear regression obtained were (r)≥0.999, and the relative standard deviation of the slope was less than 5 %. In addition, it was confirmed that there was no matrix effect, since a CV<1 % was obtained between the slopes of the calibration curves in the matrix and in acetonitrile. Therefore, it was decided to work with calibration curves prepared in acetonitrile.

The precision obtained, in terms of repeatability and internal reproducibility for the level of 2 μg kg^{-1}, was CV=3 % and CV=6 %, respectively. For the level of 4 μg kg^{-1}, a CV=5 % was obtained for the repeatability and internal reproducibility. The values obtained were below those estimated with the Horwitz-Thompson equation. With regard to accuracy, the mean recoveries were satisfactory: 90 % for 2 μg kg^{-1} and 95 % for the level of 4 μg kg^{-1}.

With respect to selectivity, the criteria indicated above were met as regards variations in retention time and in the ion ratio. No relevant interferences were detected in the analysis of the "blank" drink sample of drink (bisphenol A<2 μg kg^{-1}).

In summary, the obtained results were satisfactory according to the previously established criteria, and the new quantification limit for drinks was set at 2 μg kg^{-1}, with an estimated expanded uncertainty of 14 %.

Following the verification of the method, this was applied to the determination of BPA in the samples described above. All of them were analysed in duplicate and recovery tests were conducted in parallel on the sample. In 3 (2 energy drinks and 1 bitter) out of the 38 tested samples, analytical recovery problems were found and results for these are not provided.

BPA was quantified in 17 % of the samples in a concentration range of 2 μg kg^{-1}-6 μg kg^{-1} (0.26 μg dm^{-2} to 1.05 μg dm^{-2}), the mean value of the positive samples being 3.4 μg kg^{-1}, corresponding in

all cases to the soft drink group. In the case of alcoholic and energy drinks none of the samples presented detectable quantities of BPA. Results are shown in Table 1.

Table 1. Results of BPA in samples of canned drinks

Type of drink	Number of samples	Positive samples (≥ 2 µg kg^{-1})	Levels of BPA observed		
			µg kg^{-1}	µg dm^{-2}	Type of sample
Soft drinks/alcohol-free drinks	21	6	4.6	0.77	Orange soft drink
			2.0	0.26	Pineapple soft drink
			2.5	0.41	Lime-lemon soft drink
			6.3	1.05	Bitter
			2.0	0.27	Lime-lemon soft drink
			3.0	0.50	Lemon-grape-red wine
Alcoholic drinks	6	0	-	-	Beer
Energy drinks	8	0	-	-	-
Total	**35**	**6**	**Concentration range: (2-6) µg kg^{-1}** **Mean value: 3.4 µg kg^{-1}**		

Positive samples positive in liquid chromatography with fluorescence detection (HPLC-FL) were confirmed by mass spectrometry (LC/MS-MS) under the above described conditions. It should be noted that, of the 35 samples for which results were provided, 3 false positives were obtained in the HPLC-FL analysis, 2 of these corresponding to the energy drinks group and 1 to a soft drink sample (lemon tea). These samples were not included in the group of positive samples, as the identity of the BPA was not confirmed.

Figure 1 shows chromatograms (both transitions) of bisphenol A standard and of a soft drink sample, obtained by liquid chromatography coupled to mass spectrometry (LC/MS-MS).
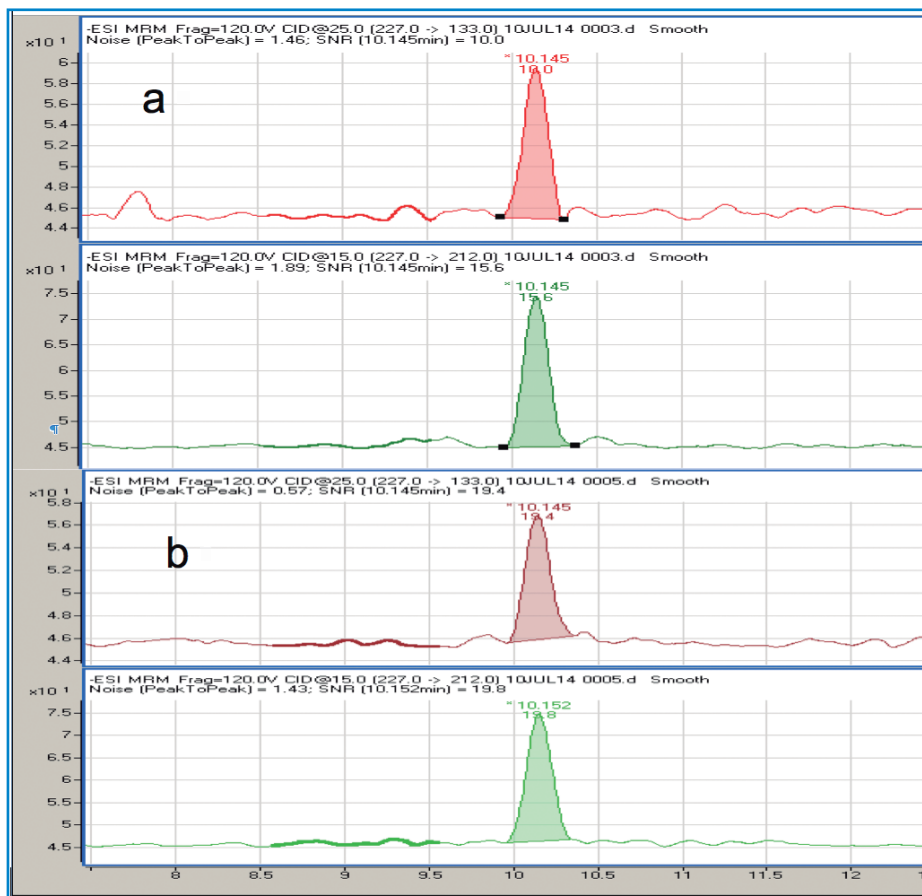
Figure 1. Chromatograms (LC/MS-MS) of: a) standard of BPA, 6 µg L^{-1}, b) drinks sample, BPA concentration 5 µg L^{-1}.

The results of BPA in canned drinks in this paper, although limited in number, indicate that the levels observed are very low, which is in line with the results published by other authors, mentioned at the introduction of this paper.

The highest level of BPA detected in this study was 6 µg kg^{-1}. The only study conducted in Spain, to the best of our knowledge, to determine the transfer of BPA in canned soft drinks was published by Gallart-Ayala et al. in 2011. In this study a range of concentration of (0.044-0.607) µg L^{-1} was observed in 7 out of 11 samples taken in Barcelona, in 2009.

In addition, the results obtained in the positive samples in our study are compatible with data published on BPA extraction levels (with acetonitrile) from epoxy coatings used in the manufacture of drink cans: between 1 µg dm^{-2} and 4 µg dm^{-2} (Oldring et al., 2014).

3.2 Melamine

Results of the method verification at the 0.5 mg kg^{-1} level were satisfactory, with a mean recovery of 95 %. The precision, expressed as coefficient of variation was 9 %, both for the repeatability and the internal reproducibility. Calibration curves met the above mentioned requirements; the correlation coefficients (r) were >0.997. Similarly, in relation to the confirmation of the identity of melamine, the pre-established criteria for ion ratios were fulfilled. Uncertainty was estimated at 18 % at the level of 0.5 mg kg^{-1}. Figure 2 shows the chromatograms of a drink sample and the same sample spiked with 0.5 mg kg^{-1} melamine.
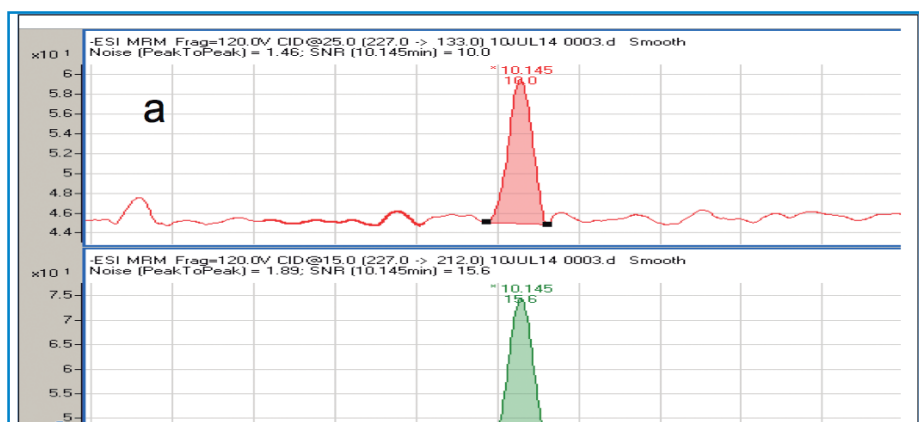
Figure 2. Chromatograms (LC/MS-MS) of a canned drink sample and the same sample spiked with 0.5 mg kg^{-1} melamine. Spectrum of melamine in the added sample (lower trace).

Concerning the results of the analysed drink samples (38), the levels of melamine were always below the quantification limit: 0.5 mg kg^{-1}.

In all the samples analysed, the levels of BPA and of melamine were well below the limits of 0.6 mg kg^{-1} and 2.5 mg kg^{-1}, respectively, included in the plastics materials regulation (Regulation (EU) No 10/2011), and applicable to coatings in accordance with Royal Decree 847/2011.

Acknowledgements

References

BOE (2011). Real Decreto 847/2011, de 17 de junio, por el que se establece la lista positiva de sustancias permitidas para la fabricación de materiales poliméricos destinados a entrar en contacto con alimentos. BOE n° 164 de 11 de julio de 2011, pp: 76316-76330.

Bratinova, S., Raffael, B. and Simoneau, C. (2009). Guidelines for performance criteria and validation

procedures of analytical methods used in controls of food contact materials. 1st edition 2009. Publication Office of the European Union, Luxembourg, *Joint Research Centre,* Scientific and Technical Report, EUR 24105 EN.

Cao, X.L., Corriveau, J. and Popovid, S. (2009). Levels of Bisphenol A in canned soft drink products in canadian markets. *Journal of Agricultural and Food Chemistry,* 57, pp: 1307-1311.

Cunha, S.C., Almeida, C., Mendes, E. and Fernandes, J.O. (2011). Simultaneous determination of bisphenol A and bisphenol B in beverages and powdered infant formula by dispersive liquid-liquid micro-extraction and heart-cutting multidimensional gas chromatography-mass spectrometry. *Food Additives and Contaminants. Part A,* 28, pp: 513-526.

EFSA (2010). European Food Safety Authority. Scientific Opinion on Melamine in Food and Feed. *The EFSA Journal,* 8 (4):1573.

EFSA (2015). European Food Safety Authority. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: Executive summary. *The EFSA Journal,* 13 (1): 3978.

EU (2011a). Regulation (EU) No 10/2011 of 14 january 2011 on plastic materials and articles intended to come into contact with food. OJ L 12 of 15 January 2011, pp: 1-89.

EU (2011b). Regulation (EU) No 321/2011 of 1 april 2011 amending Regulation (EU) No 10/2011 as regards the restriction of use of bisphenol a in plastic infant feeding bottles. OJ L 87 of 2 April 2011, pp: 1-2.

EU (2012). Regulation (EU) No 594/2012 of 5 july 2012 amending Regulation (EC) 1881/2006 as regards the maximum levels of the contaminants ochratoxin a, non dioxin-like pcbs and melamine in foodstuffs . OJ L 176 of 6 July 2012, pp: 43-45.

Fasano, E., Esposito, F., Scognamiglio, G., Di Francesco, F., Montuori, P., Cocchieri, R.A. and Cirillo, T. (2015). Bisphenol A contamination in soft drinks as a risk for children´s health in Italy. *Food Additives and Contaminants. Part A,* 32 (7), pp: 1207-1214.

Gallart-Ayala, H., Moyano, E. and Galceran, M.T. (2011). Analysis of bisphenols in soft drinks by on-line solid phase extraction fast liquid chromatography-tandem mass spectrometry. *Analytica Chemica Acta,* 683, pp: 227-233.

Geens, T., Apelbaum, T.Z., Goeyens, L., Neels, H. and Covaci, A. (2010). Intake of bisphenol A from canned beverages and foods on the Belgian market. *Food Additives and Contaminants. Part A*, 27, pp: 1627-1637.

Geens, T., Aerts, D., Berthot, C., Bourguignon, J.P., Goeyens, L., Lecomte, P., Maghuin-Rogister, G., Pironnet, A.M., Pussemier, L., Scippo, M.L., Van Loco, J. and Covaci, A. (2012). A review of dietary and non-dietary exposure to bisphenol-A. *Food and Chemical Toxicology,* 50 (10), pp: 3725-3740.

Goodson, A., Summerfield, W. and Cooper, I. (2002). Survey of bisphenol A and bisphenol F in canned foods. *Food Additives and Contaminants,* 19, pp: 796-802.

Liao, C. and Kannan, K. (2013). Concentrations and profiles of bisphenol A and other bisphenol analogues in foodstuffs from the United States and their implications for human exposure. *Journal of Agricultural and Food Chemistry*, 61, pp: 4655-4662.

MAGRAMA (2015). Ministerio Agricultura, Alimentación y Medio Ambiente. Informe del consumo de Alimentación en España 2014.

Merchant Research & Consulting (2014). Bisphenol A (BPA): 2014 World Market Outlook and Forecast up to 2018. Market Publishers Ltd., January 2014.

Munguía-López, E.M., Gerardo-López, S., Peralta, E., Bolumen, S. and Soto-Valdez, H. (2005). Migration of bisphenol A from can coatings into fatty-food simulant and tuna fish. *Food Additives and Contaminants,* 22, pp: 892-898.

Oldring, P.K.T., Castle, L., O'Mahony, C. and Dixon, J. (2014). Estimates of Dietary Exposure to Bisphenol A (BPA) from Light Metal Packaging using Food Consumption and Packaging usage Data: A Refined Deterministic Approach and a Fully Probabilistic (FACET) Approach. *Food Additives and Contaminants. Part A,* 31 (3), pp: 466-489.

Rochester, J.R. (2013). Bisphenol A and human health: a review of the literatura. *Reproductive Toxicology,* 42, pp: 132-155.

Sajiki, J., Miyamoto, F., Fukata, H., Mori, C., Yonekubo, J. and Hayakawa, K. (2007). Bisphenol A and its source in foods in Japanese markets. *Food Additives and Contaminants,* 24, pp: 103-112.

Santillana, M.I., Ruiz, E., Nieto, M.T., Bustos, J., Maia, J., Sendón, R. and Sánchez, J.J. (2011). Migration of bisphenol A from polycarbonate baby bottles purchased in the Spanish market by liquid chromatography and fluorescence detection. *Food Additives and Contaminants,* 28 (11), pp: 1610-1618.

Vanderberg, L.N., Colborn, T., Hayes, T.B., Heindel, J.J., Jacobs, D.R.Jr., Lee D.H., Shioda, T., Soto, A.M., Vom Saal, F.S., Welshons, W.V., Zoeller, R.T. and Myers, J.P. (2012). Hormones and endocrine-disrupting chemicals: Low-dose effects and nonmonotonic dose responses. *Endocrine Reviews,* 33 (3), pp: 378-455.