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► **To cite this version:**

María-Isabel Santillana. Study on the migration of bisphenol a from polycarbonate baby bottles purchased in the Spanish market by liquid chromatography and fluorescence detection. Food Additives and Contaminants, 2011, pp.1. 10.1080/19440049.2011.589036 . hal-00721230

**HAL Id: hal-00721230**

**<https://hal.science/hal-00721230>**

Submitted on 27 Jul 2012

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**Study on the migration of bisphenol a from polycarbonate baby bottles purchased in the Spanish market by liquid chromatography and fluorescence detection**

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-082.R1
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	25-Apr-2011
Complete List of Authors:	SANTILLANA, MARÍA-ISABEL; CNA-AESAN, FOOD CONTACT MATERIALS
Methods/Techniques:	Chromatography - HPLC, Chromatography - LC/MS, In-house validation
Additives/Contaminants:	Bis-phenol A
Food Types:	Baby food, Infant formulae
Abstract:	<p>During the last decade the safety in the use of BPA monomer in polycarbonate baby bottles, has drawn the attention from the public and the scientific community.</p> <p>This paper presents the results of Bisphenol A migration from polycarbonate baby-bottles bought in the Spanish market, into simulants B and E (3% acetic acid and 50% ethanol, respectively) and into real food (reconstituted infant formula). Furthermore, it was also the objective of this study to assess the suitability of 50% ethanol as simulant for infant formula. BPA was analyzed by a multianalyte liquid chromatography method with fluorescence detection and mass spectrometry confirmation. The method was in-house validated and accredited by the national accreditation body. The validation results for this analyte in the previous mentioned matrices were: LOD: 0.004-0.007 mg.kg<sup>-1</sup>; LOQ (validated): 0.03 mg.kg<sup>-1</sup>; RSD%: 3.4-5.8; recovery: 106.6-118.2%. A collection of 72 different baby-bottle samples from 12 different brands were analyzed. Baby-bottle material was identified by FTIR. The</p>

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	<p>migration test conditions used were those recommended for baby bottles in the Guidelines on testing conditions for articles in contact with foodstuffs (with a focus on kitchenware), prepared by the European network of laboratories for food contact materials. In most of the migration assays the results were under the detection limit. In four of the commercial brands there was detectable migration into the simulant 50% ethanol and only in two samples BPA was detected in infant formula (0.01 mg.kg<sup>-1</sup>). Migration results obtained were in compliance with the European Union regulation.</p>

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# Study on the migration of bisphenol A from polycarbonate baby bottles purchased in the Spanish market by liquid chromatography and fluorescence detection

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## Abstract

During the last decade the safety of Bisphenol A (BPA) monomer in polycarbonate baby bottles, has drawn the attention from the public and the scientific community. This paper presents the results of BPA migration from polycarbonate baby-bottles bought in the Spanish market, into simulants B and E (3% acetic acid and 50% ethanol, respectively) and into real food (reconstituted infant formula). Furthermore, it was also the objective of this study to assess the suitability of 50% ethanol as simulant for infant formula. BPA was analyzed by a multianalyte liquid chromatography method with fluorescence detection and mass spectrometry confirmation. The method was in-house validated and accredited by the national accreditation body. The validation results for this analyte in the previous mentioned matrices were: LOD: 0.004-0.007 mg.kg<sup>-1</sup>; LOQ (validated): 0.03 mg.kg<sup>-1</sup>; RSD%: 3.4-5.8; recovery: 106.6-118.2%. A collection of 72 different baby-bottle samples from 12 different brands were analyzed. Baby-bottle material was identified by FTIR. The migration test conditions used were those recommended for baby bottles in the Guidelines on testing conditions for articles in contact with foodstuffs (with a focus on kitchenware), prepared by the European network of laboratories for food contact materials. In most of the migration assays the results were below the detection limit. In four of the commercial brands there was detectable migration into the simulant 50% ethanol and BPA was detected in only two samples of infant formula (0.01 mg.kg<sup>-1</sup>). Migration results obtained were in compliance with the EU regulations.

1  
2 1 **Keywords:** Bisphenol A; polycarbonate; baby bottles; migration; simulants; infant formula; LC-  
3 MS/MS.  
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## 5 **Introduction**

6 During the last decade the use of Bisphenol A (BPA) starting-monomer in polycarbonate baby  
7 bottles, has been continuously assessed, due to the possible risk for infant population, fed using  
8 this kind of articles. Many researchers have studied the potential migration of BPA trace levels  
9 from polycarbonate into food and beverages under typical conditions of uses of polycarbonate  
10 articles. These studies include some conducted by governmental agencies in the United States  
11 (Food and Drug Administration, FDA), Canada (Health Canada), and Europe (European Food  
12 Safety Agency, EFSA), as well as studies conducted by academic researches and by industry  
13 (Biles et al, 1997b; Mounfourt et al 1997; Kawamura et al, 1998; Brede et al, 2003; Wong et al,  
14 2005; Biederman-Brem et al, 2008; Ehlert et al, 2008; Aguilar et al, 2008; Environment Canada,  
15 2008; Kubwabo et al, 2009; Nam et al, 2010; Aschberger et al, 2010). Overall they show that,  
16 under typical use conditions, the potential migration of BPA into food is extremely low and poses  
17 no Known risk to human health.

18 In January 2011, after different assessments and decisions of evaluation organizations the  
19 European Commission decided to outlaw the manufacture of polycarbonate feeding bottles  
20 containing BPA from March 2011 and ban their import and sale from June 2011. During the period  
21 2008-2010 taking into account the concern on BPA migration as well as the lack of data in Spain,  
22 we decided to carry out a study on BPA migration from polycarbonate baby bottles in the Spanish  
23 market, in an attempt to determine its behavior in conditions similar to their real use, avoiding  
24 migrations in extreme conditions.

25 Due to the fact of a great variety of conditions used in migration testing for articles intended for  
26 food contact, the European network of national reference laboratories has published Guidelines on  
27 testing conditions for articles in contact with foodstuffs (with a focus on kitchenware), to unify  
28 testing criteria and therefore, allow the comparability of results obtained in different laboratories  
29 under the same conditions (Simoneau, 2009). Therefore we have used the established simulants  
30 in the current European regulations and followed the mentioned Guidelines for the testing  
31 conditions of simulants (3% acetic acid and 50% ethanol), time and temperature. Also migration  
32 tests were performed in real food for the target population (reconstituted infant formula). The aim  
33 was to compare the behavior of polycarbonate baby bottles when using simulants and also in real  
34 food matrix because studies on the release of BPA into infant formula are scarce.

35 BPA was analyzed in the samples studied with a multianalyte method to determine BPA,  
36 Bisphenol F (BPF), Bisphenol A diglycidyl ether (BADGE), Bisphenol F diglycidyl ether (BFDGE)  
37 and derivatives in both, simulants and food, by liquid chromatography with fluorescence detection.  
38 Mass spectrometry was used for confirmation. The method was in-house validated and has been

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2 1 accredited by the national accreditation body, ENAC. Methods were based on those previously  
3 2 published in the literature (Paseiro et al, 1997; Biles et al, 1999; Theobald et al, 2000;  
4 3 Leepipatpiboon et al, 2005; Sun et al, 2006; Maragou et al, 2008; Pardo et al, 2006; Ballesteros-  
5 4 Gómez et al, 2009).  
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## 10 6 **Materials and Methods**

### 11 7 12 8 **Standards and reagents**

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16 10 Bisphenol A (BPA) standard (purity  $\geq 95\%$ ) was provided by Fluka (Germany). Acetonitrile  
17 11 (HPLC grade), glacial acetic acid and ethanol absolute (p.a) were from Merck (Darmstadt,  
18 12 Germany). Acetonitrile LC-MS grade was from Riedel-de-Haën. Ultrapure water was prepared  
19 13 using a Direct-Q system (Millipore). Sep-Pak classic C18 cartridges for solid phase extraction were  
20 14 purchased from Waters (Ireland).  
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23 15 A stock solution of BPA containing  $500 \mu\text{g}\cdot\text{ml}^{-1}$  was prepared in acetonitrile and was kept in the  
24 16 refrigerator protected from light up to 6 months.  
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### 30 18 **Identification of baby bottles material with FTIR**

31 19 Fourier-transformed Infrared Spectrometry (FTIR) was applied to a sample of each commercial  
32 20 brand using a Perkin-Elmer Spectrum One (Boston MA) equipped with a universal attenuated total  
33 21 reflectance (UATR) accessory.  
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36 22 ATR spectra were measured with a resolution of  $4 \text{ cm}^{-1}$  in the range  $4,000\text{-}650 \text{ cm}^{-1}$ . Each  
37 23 spectrum was the result of 8 co-added scans. The polymer was identified by matching the spectra  
38 24 obtained with the available IR libraries (Know It All Informatics System, IR edition v.8 and HavelAll  
39 25 IR databases, all from Bio-Rad Laboratories, Inc.).  
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### 44 27 **Liquid chromatography instrumentation**

45 28 BPA was determined using an Agilent Technologies 1200 series HPLC system equipped with a  
46 29 fluorescence detector, operating at  $\lambda$  excitation 227 nm and  $\lambda$  emission 313 nm and all controlled  
47 30 by Chemstation software. The chromatographic separation was run on a Hypersil C18 column (250  
48 31 x 4.6 mm,  $5 \mu\text{m}$ , from Phenomenex) at  $25^\circ\text{C}$ . The mobile phase was a gradient prepared by mixing  
49 32 (A) water and (B) acetonitrile delivered at  $0.9 \text{ mL}\cdot\text{min}^{-1}$ . The gradient employed was: 0-2 min, 25%  
50 33 B; 2-15 min, 25-45% B; 15-38 min, 45-70% B; 38-45 min, 70-100% B; 45-50 min, 100% B; 50-52  
51 34 min, 25% B. The injection volume was  $20 \mu\text{l}$ .  
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54 35 The analysis by liquid chromatography-mass spectrometry (LC-MS/MS) was carried out using  
55 36 an Alliance 2795 liquid chromatograph coupled to a Quatro Premier mass detector equipped with  
56 37 an electrospray ionization LC-MS interface ESI (Waters). The chromatographic separation was  
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1 performed on a column Xterra RP18 column (150 x 4.6 mm, 5 µm, from Waters) at 25 °C.  
2 Chromatographic conditions are summarized in Table 1.  
3 (Table 1)

### 4 ***Baby bottles samples and migration testing***

5 During the period 2008-2010, 72 baby bottle samples from 12 different randomly selected global  
6 market brands (A-L) were acquired. For one of the brands (A) two different types of baby bottles  
7 were included (A1-A2).

8 The kind of polymer was confirmed by FTIR, as it was not stated in all the articles. For each  
9 brand, baby bottles were distributed as follows:

- 10 - 2 samples for the migration test with 3% acetic acid
- 11 - 2 samples for the migration test with 50% ethanol
- 12 - 2 samples for the migration test with infant formula

13 Due to the lack of specimens at the retailer, for brand D the tests in infant formula could not be  
14 carried out and for brand E only was performed 50% ethanol test.

15 Table 2 summarizes information about the samples used in this study as well as migration tests  
16 performed. Simulant B (3% acetic acid in water, w/v) was used to simulate migration into acidic  
17 foods (e.g. fruit juices) and simulant 50% ethanol in water v/v to simulate migration into milk  
18 products (e.g. infant formula). Moreover, migration tests with infant formula were carried out as it  
19 represents the real use food, and furthermore, it allowed us to assess the suitability of simulant  
20 50% ethanol. In order to complete the migration tests with infant formula, second stage canned  
21 powder milk infant formula, reconstituted following the manufacturer's instructions, was prepared  
22 at the beginning of the test.. Test conditions were 2 hours at 70°C plus 24 hours at 40°C. As baby  
23 bottles are articles for repeated use, 3 exposures with each simulant and the food (reconstituted  
24 infant formula) were done. Before each exposure the baby bottle was washed with domestic  
25 washing up liquid, rinsed with tap water and left to dry. Afterwards the samples were sterilized in  
26 autoclave at 121°C for 20 min and let cooled down, trying to reproduce real use conditions at home  
27 as much as possible.

28 (Table 2)

29 After expiration of the incubation time of each migration test, the simulants or the infant formula  
30 were transferred into an Erlenmeyer flask and homogenized by manual shaking. Then, a sub-  
31 sample of the migration solutions was analyzed using the validated HPLC-FLD method.

### 32 ***Validation***

33 In-house validation was carried out following the standard protocol in our laboratory, which is  
34 based on ISO 17025 (2005). The following parameters were assessed: specificity, linearity,  
35 detection and quantification limits, precision and accuracy (expressed as recovery).

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1 Specificity was tested verifying the lack of response at the retention time of the analyte by  
2 analysing in triplicate a blank sample (simulant/food). A response was considered to be present if it  
3 was  $\geq 1/3$  the value of the quantification limit.

4 Linear regression calibration of BPA was performed using the analyte peak area vs. analyte  
5 concentration. An average calibration curve was calculated from three curves obtained in different  
6 days, each one at six concentration levels in the range 0.03 to 1.2 mg.kg<sup>-1</sup>, based on their specific  
7 migration limit (SML = 0.6 mg.kg<sup>-1</sup>).

8 Linearity was established by estimating the relative standard deviation of the mean slope,  
9 according to the following equation:

$$Cm \% = 100 \times \frac{Sm}{m}$$

12  
13 Where  $Sm$  is the standard deviation of the mean slope and  $m$  is the slope of the mean  
14 calibration curve. Values of  $Cm \leq 5\%$  are considered satisfactory. The correlation coefficient of the  
15 linear regression was also calculated (requirement  $\geq 0.996$ ).

16 The theoretical limits of quantification ( $LOQ$ ) and detection ( $LOD$ ) were calculated from the  
17 mean calibration curve using the following equations:

$$LOQ = \frac{10 \times S_b}{m\sqrt{n}}$$

$$LOD = \frac{3 \times S_b}{m\sqrt{n}}$$

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23 Where  $S_b$  is the intercept standard deviation,  $m$  is the slope of the mean calibration curve and  $n$   
24 is the number of calibration curves.

25 Precision and accuracy were assessed spiking a blank sample with BPA at three different  
26 concentrations:  $LOQ$  (1/5 of SML), SML and 2xSML. Each level was analyzed three times on three  
27 different days ( $n = 9$ ). Precision (internal reproducibility) was expressed in terms of relative  
28 standard deviation (RSD% requirement  $\leq 15$ ) and the accuracy as percentage of recovery for each  
29 concentration level (requirement 80 -120%).

## 31 Results and discussion

32 Initial BPA validation in the multianalyte method was carried out using canned natural  
33 asparagus, pickled mussels and tuna in vegetable oil.

34 An intermediate standard solution of BPA in acetonitrile was prepared by dissolving appropriate  
35 amounts of stock solution to yield a concentration of 10  $\mu\text{g}.\text{ml}^{-1}$ . Calibration solutions, in the range  
36 0.05 to 2.0 mg.kg<sup>-1</sup> food, were prepared spiking blank samples with the intermediate standard



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2 1 solution. The concentration  $0.05 \text{ mg.kg}^{-1}$  was used as the lowest level of the curve and it was the  
3 2 validated quantification limit as it resulted to be the lowest quantifiable value by mass in food. For  
4 3 this purpose, 5 g of the homogenized preserving liquid were extracted with 5 ml of acetonitrile  
5 4 containing BPA at the required concentration. The sample was then centrifuged and an aliquot of  
6 5 the upper organic layer was cleaned using a Sep-Pak C18 cartridge before HPLC analysis. The  
7 6 validation was performed as described. The results were satisfactory according to our internal  
8 7 requirements. The chromatographic conditions reported previously were found to provide good  
9 8 resolution of BA, BF, BADGE, BFDGE and derivates peaks (Figure 1). The validation results for  
10 9 BPA in the can food matrices are shown in Table 3.

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17 10 (Figure 1)

18 11  
19 12 (Table 3)

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21 14 To confirm the presence of BPA in samples in initial method validation, LC-MS/MS in ESI (-)  
22 15 mode was checked. BPA in acetonitrile was infused directly and MS parameters (cone voltages,  
23 16 probe temperature, ionization source temperature, electron multiplier voltage, desolvation and  
24 17 cone gas) were optimized. Once the precursor ion was selected, product ion scans were acquired.  
25 18 Several collision voltages were tested and the one yielding the more intense and stable fragments  
26 19 was chosen. The fragments did not differ from those found in the bibliography (López-Cervantes et  
27 20 al, 2003; Maragou et al, 2008). The multiple reaction monitoring transition (MRM) selected was  
28 21  $227.1 > 212.01$ .

29 22  
30 23 (Figure 2)

31 24  
32 25 For the present study in infant formula and in the simulants 50% ethanol and 3% acetic acid we  
33 26 considered it important to validate at the lowest concentration, as the target samples (baby bottles)  
34 27 were intended for the infant population. To perform the migration tests into real food, blank formula  
35 28 powder milk was used.

36 29 Calibration solutions in 3% acetic acid in water (w/v), 50% ethanol in water (v/v) and  
37 30 reconstituted infant formula were prepared in the range  $0.03$  to  $1.2 \text{ mg.kg}^{-1}$ .

38 31 Infant formula extraction was performed in a similar way as already described for the preserving  
39 32 liquid in can foods: 10 g of reconstituted infant formula (following the manufacturer instructions)  
40 33 were extracted with 10 ml of acetonitrile in an orbital shaker for 15 minutes. The samples were  
41 34 then centrifuged for 20 minutes (8,000 g) and an aliquot from the organic layer was taken and pass  
42 35 through a Sep-Pak C18 cartridge before HPLC analysis. In the case of the food simulants (3%  
43 36 acetic acid and 50% ethanol) after the migration test, an aliquot of the migration solutions was  
44 37 filtered through PTFE filters and analyzed directly by HPLC. Detection and quantification limits  
45 38 were calculated from the intercept value (Table 4), but the lowest validated concentration level was

1 0.03 mg.kg<sup>-1</sup>, and this was considered as the quantification limit for the report of sample results.  
2 Since the method had been previously validated, though for other matrices, in the case of  
3 simulants and infant formula a verification was done only at the LOQ. Precision as internal  
4 reproducibility (RSD%) and accuracy (% recovery) results obtained for BPA in 50% ethanol, 3%  
5 acetic acid and infant formula were satisfactory and are summarized in Table 4.

6  
7 (Table 4)  
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9 The suitability of the method was also confirmed by participating in different interlaboratory  
10 trials: BPA in oil (FAPAS) and BPA in 50% aqueous ethanol (JRC). The z-scores obtained were  
11 considered satisfactory as they were in all cases < 0.5.

12 Therefore, method performance parameters assessed in the method validation showed that the  
13 analytical procedure was well applicable for the reliable determination of BPA in baby bottles with a  
14 limit of quantification suitable for the purpose of our study.

15 The method was applied to determine BPA migration from the baby bottles described in Table  
16 2. All the migration solutions and infant formula were analyzed twice and a parallel recovery  
17 analysis was done for all  
18 of them (n = 36)

19 The average recovery values were: 102.5% (RSD% = 2.1) 102.7% (RSD% = 3.1) 99.4%  
20 (RSD% = 15.4) for 3% acetic acid, 50% ethanol and infant formula, respectively. These results  
21 were in agreement with those obtained in the method validation and the HorRat, for the duplicated  
22 analysis was <1.

23 The FTIR confirmed that all the baby bottles samples were made of polycarbonate, except two  
24 brands (D and F) that were made of polypropylene; one of them, brand F, was labeled as  
25 "Bisphenol A free" and was used to confirm the absence of BPA (no BPA migration was detected).  
26 The samples results are shown in Table 2, and it can be observed that most of them were below  
27 the detection limit.

28 Concerning the migration from PC baby bottles into the acidic simulant, none of the 12 samples  
29 released detectable amounts of BPA at 70°C for 2 hours plus 40°C for 24 hours, throughout the 3  
30 cycles of the migration procedure (<0.004 mg.kg<sup>-1</sup>). Only in samples of brand L, quantities above  
31 the LOD were detected in the first (0.023 mg.kg<sup>-1</sup>) and second (0.011 mg.kg<sup>-1</sup>) exposition (not  
32 shown in table 2). However, no amount was detected in the third migration. These results are in  
33 agreement with previous works that reported non-detectable BPA when the temperature applied in  
34 the migration test was between 60-80°C and times below 2 hours (Kawamura et al., 1998;  
35 D'Antuono et al., 2001; Maragou et al., 2008), quite similar to the present used conditions.

36 In all the baby samples where a chromatographic signal ascribed to BPA in the third exposition  
37 was detected, the migration solutions were further concentrated (x10) to be able to quantitate BPA  
38 at lowest values than the LOD of the method (Figure 3). Also, for these samples it was decided to

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2 1 continue the exposures (up to 9). The aim was to check whether the continuous use could induce a  
3 2 significant increase of the analyte concentration, related to a potential degradation of the polymer  
4 3 with the repeated use, as some studies reflect in the literature (Biedermann-Brem et al., 2009;  
5 4 Maia et al., 2009). The results are presented in Table 2.  
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10 6 (Figure 3)  
11 7

12 8 Regarding migration test into 50% ethanol at 70°C hours followed by 24 hours at 40°C, in the  
13 9 third exposure, brands A<sub>1</sub>, A<sub>2</sub>, C, G and H migrated in higher concentrations than the LOD. When  
14 10 these samples were subjected to a new cycle of three migrations (4<sup>th</sup>, 5<sup>th</sup>, 6<sup>th</sup> migration) only  
15 11 brands A<sub>2</sub>, C and L gave quantifiable BPA values (2<sup>nd</sup> column, Table 2). The results of a third cycle  
16 12 of three exposures (7<sup>th</sup>, 8<sup>th</sup>, 9<sup>th</sup>) of all samples (3<sup>rd</sup> column, Table 2) concluded with only one brand  
17 13 (L) giving a quantifiable value, very similar to that obtained in previous exposures, which confirmed  
18 14 the asymptotic migration tendency of brand L and the lack of migration for the rest of the brands.  
19 15 Regarding brand L samples, although in the third migration BPA was under the LOD, quantifiable  
20 16 values for the first (0.045 mg.kg<sup>-1</sup>) and second (0.035 mg.kg<sup>-1</sup>) migration were obtained. This was  
21 17 not the case for the rest of the samples.  
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23 18 The scarcity of available data from the literature (Kubwabo et al, 2009) regarding migration in  
24 19 50% ethanol are in agreement with the ones obtained in our lab. In the Kubwabo study the residual  
25 20 BPA mean value obtained from the analysis of 12 baby bottles incubated at 40°C for 24 hours was  
26 21 0.002 mg.kg<sup>-1</sup>. This study also reported that the BPA concentration release into simulant 50%  
27 22 ethanol was relatively higher than into water.  
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29 23 Some data can be found in the literature in relation to BPA content values in canned infant  
30 24 formula: 0.1 - 13 ng.g<sup>-1</sup>; (Biles et al, 1997); <0.002 mg.kg<sup>-1</sup> (Goodson, et al, 2002); 45 - 113 ng.g<sup>-1</sup>  
31 25 (Kuo, et al, 2004); 1.7 - 15.2 ng.g<sup>-1</sup> (Maragou, et al, 2006); 2.27 - 10.2 ng.g<sup>-1</sup> (Cao et al, 2008);  
32 26 0.07 - 1.29 mg.kg<sup>-1</sup> (Ferrer et al, 2011). However migration data from baby bottles into infant  
33 27 formula are very limited as established in the Joint Research Center (JRC-European Commission)  
34 28 Scientific and Technical Report (Aschberger et al, 2010). The migration results obtained in our  
35 29 study for infant formula showed that only brands A1 and A2 yielded quantifiable BPA values (0.011  
36 30 y 0.012 mg.kg<sup>-1</sup> respectively). These values are not in disagreement with those found by Mountfort  
37 31 et al, 1997: not detectable with a migration limit of 0.03 mg/kg, though the temperature and time  
38 32 conditions were different. The lack of data in the literature regarding BPA migration into infant  
39 33 formula in similar conditions as the one we used does not allow a direct comparison of results.  
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41 34 Following the testing protocol for baby bottles in the CRL-NRL-FCM Guidelines on testing  
42 35 conditions for articles in contact with foodstuffs (with a focus on kitchenware) (Simoneau, 2009), the  
43 36 obtained results indicate that BPA migration is detected in a larger number of baby bottles when  
44 37 50% ethanol was used. This could mean that the residual BPA is more easily extracted from the  
45 38 polymer into 50% ethanol solution. On the other hand, samples from brands A1 and A2 yielded  
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1 higher migrations values into the real food than into the 50% ethanol simulant, however given the  
2 fact that this samples represent a small percentage (below 10%) and also the limited number of  
3 samples analyzed, no definitive conclusions can be drawn.

4 The results are in compliance with the EU law (Directive 2002/72 CE) and therefore it doesn't  
5 seem necessary to take any additional actions in the Spanish market before the final ban of BPA  
6 use in baby bottles next June 2011.

### 7 8 **Acknowledgements**

9 The study was financially supported by the Ministerio de Ciencia e Innovación (project ref.  
10 AGL2008-04146). The authors are grateful to Ms Marta Tello for her excellent technical  
11 assistance and critical reading of the manuscript.

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1 Table 1. Experimental conditions used in the LC-MS/MS method

2	1	Table 1. Experimental conditions used in the LC-MS/MS method
3	2	
4		Mobile phase
5		A: water (0.1% Acetic Acid)
6		B: Acetonitrile
7		Gradient
8		Time (min)
9		% A
10		0
11		70
12		1
13		70
14		20
15		20
16		25
17		0
18		30
19		0
20		35
21		70
22		Flow
23		0.6 mL.min <sup>-1</sup>
24		Injection volume
25		50 µL
26		Ionization mode
27		Negative electrospray
28		Probe temperature
29		400 °C
30		Ionization temperature
31		120 °C
32		Cone voltage
33		25 V
34		Electron multiplier voltage
35		650 V
36		Desolvation gas N <sub>2</sub>
37		600 L.h <sup>-1</sup>
38		Cone gas N <sub>2</sub>
39		10 L.h <sup>-1</sup>
40		Collision gas Ar
41		0.30 mL.min <sup>-1</sup>
42		Collision energy
43		22
44		Multiple reaction monitoring
45		227.1>212.0
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1 Table 2. Number of baby bottles of different commercial brands used for each BPA migration testing, IR  
2 spectra obtained and the results of BPA from migration tests  
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Brand	Number of samples	Volume (mL)	Simulants	IR id.	BPA migration (mg.kg <sup>-1</sup> ) 3 <sup>th</sup> exposure	BPA migration (mg.kg <sup>-1</sup> ) 6 <sup>th</sup> exposure	BPA migration (mg.kg <sup>-1</sup> ) 9 <sup>th</sup> exposure
A1	A11 (2) A12 (2) A13 (2)	305	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD 0.005 0.011	< LOD	< LOD
A2	A21 (2) A22 (2) A23 (2)	285	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD 0.005 0.012	0.017	< LOD
B	B31 (2) B32 (2) B33 (2)	310	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD < LOD < LOD		
C	C1 (2) C2 (2) C3 (2)	300	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD 0.005 < LOD	0.018	< LOD
D	D1 (2) D2 (2)	305	3% Acetic acid 50% Ethanol	PP	< LOD < LOD		
E	E1 (2)	310	50% Ethanol	PC	< LOD		
F	F1 (2) F2 (2) F3 (2)	180	3% Acetic acid 50% Ethanol Infant formula	PP	< LOD < LOD < LOD		
G	G1 (2) G2 (2) G3 (2)	335	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD 0.005 < LOD	< LOD	< LOD
H	H1 (2) H2 (2) H3 (2)	280	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD 0.005 < LOD	< LOD	< LOD
I	I1 (2) I2 (2) I3 (2)	385	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD < LOD < LOD		
J	J1 (2) J2 (2) J3 (2)	200	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD < LOD < LOD		
K	K1 (2) K2 (2) K3 (2)	280	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD < LOD < LOD		
L	L1 (2) L2 (2) L3 (2)	280	3% Acetic acid 50% Ethanol Infant formula	PC	< LOD < LOD < LOD	0.015	0.02

1 Table 3. BPA validation results in canned foods.

		Aqueous canned food	Pickled canned food	Oil canned food
Linearity (Cm %)		0.2	0.3	0.4
Corr.Coeff. (r)		1.000	1.000	1.000
LOD (mg.kg <sup>-1</sup> )		0.002	0.027	0.004
LOQ(mg.kg <sup>-1</sup> )		0.007	0.089	0.014
RSD %	Level 1	0.5	13.1*	5.0
	Level 2	1.8	4.7	13.9
	Level 3	0.9	3.7	2.1
Recovery %	Level 1	103.1	95.4*	120.0
	Level 2	93.9	95.9	108.9
	Level 3	97.9	95.0	100.5

3  
4 Level 1: 0.05 mg.kg<sup>-1</sup> (\*0.1 mg.kg<sup>-1</sup>)

5 Level 2: 0.6 mg.kg<sup>-1</sup>

6 Level 3: 2.0 mg.kg<sup>-1</sup>

1  
2 Table 4. Validation results for 3% acetic acid, 50% ethanol and infant formula

	3% Acetic acid	50% Ethanol	Infant formula
Linearity (Cm %)	0.4	0.5	1.2
Corr. Coeff. (r)	0.999	0.999	0.999
LOD (mg.kg <sup>-1</sup> ) estimated	0.004	0.005	0.007
LOQ (mg.kg <sup>-1</sup> ) estimated	0.012	0.018	0.025
LOQ (mg.kg <sup>-1</sup> ) validated	0.03	0.03	0.03
RSD % (0.03 mg.kg <sup>-1</sup> )	4.2	5.8	3.4
Recovery % (0.03 mg.kg <sup>-1</sup> )	106.6	109.1	118.2

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1 Equation (1):

$$2 \quad Cm\% = 100 \times \frac{Sm}{m}$$

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4 Equation (2):

$$6 \quad LOQ = \frac{10 \times S_b}{m\sqrt{n}}$$

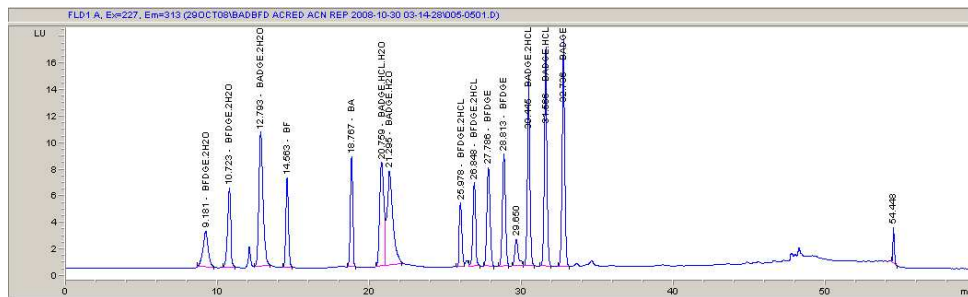
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8 Equation (3):

$$10 \quad LOD = \frac{3 \times S_b}{m\sqrt{n}}$$

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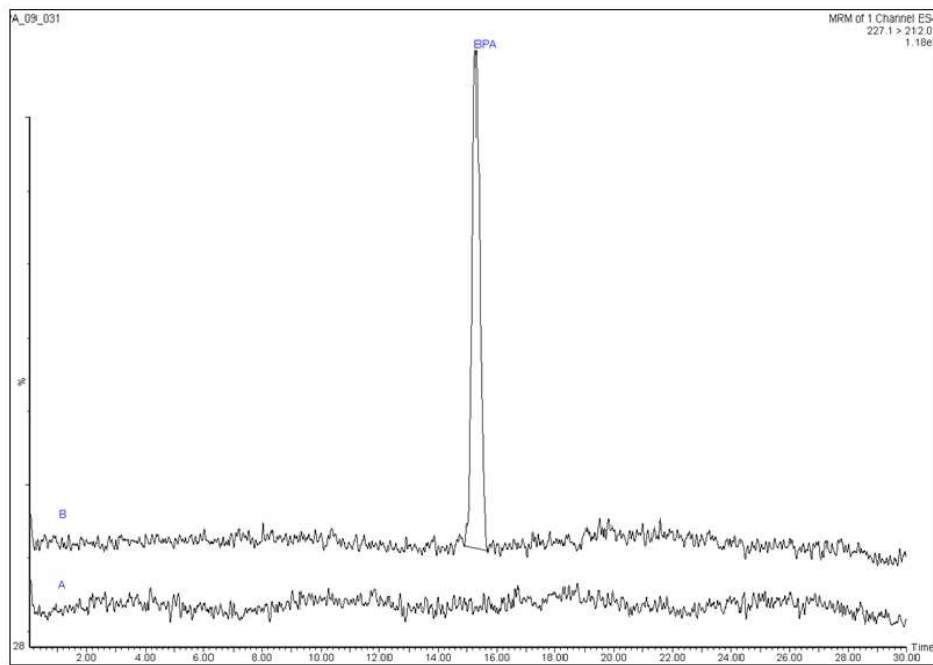


Figure 2. LC-MS-MS chromatogram of a “blank” infant formula sample (A) and the same sample spiked with BPA at  $0.03 \text{ mg kg}^{-1}$  (B)

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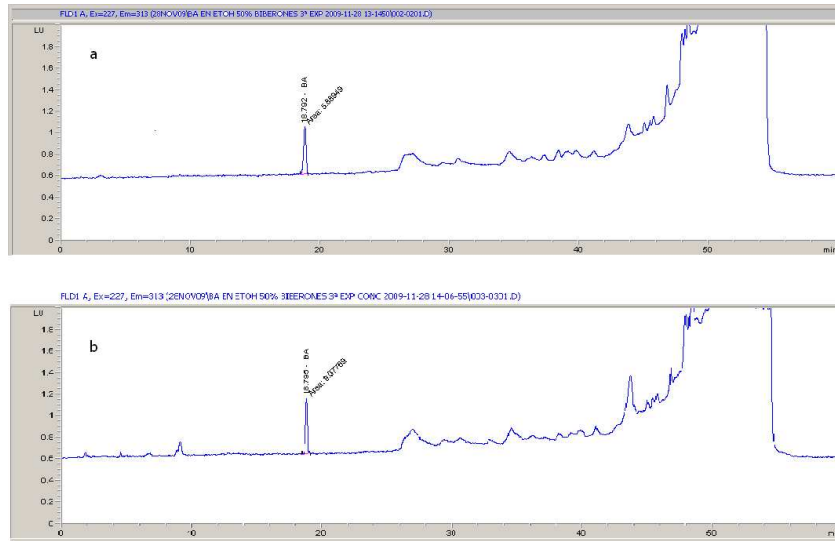


Figure 3. Chromatograms of a) 0.03 mg.kg-1 BPA standard solution in 50% Ethanol and b) concentrated migration solution in 50% Ethanol from a baby bottle (0.034 mg.kg-1)

337x238mm (96 x 96 DPI)

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