Food Chemistry 141 (2013) 816–822

Contents lists available at SciVerse ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Migration of antimony from PET containers into regulated EU food simulants

María Sánchez-Martínez, Teresa Pérez-Corona, Carmen Cámara, Yolanda Madrid *

Department of Analytical Chemistry, Faculty of Chemical Science, Complutense University of Madrid, Avenida Complutense s/n, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 4 December 2012
Received in revised form 14 February 2013
Accepted 18 March 2013
Available online 26 March 2013

Keywords:
Antimony
PET containers
Sb migration
Food simulants
ICP-MS
HG-AFS

ABSTRACT

Antimony migration from polyethylene terephthalate (PET) containers into aqueous (distilled water, 3% acetic acid, 10% and 20% ethanol) and fatty food simulants (vegetable oil), as well as into vinegar, was studied. Test conditions were according to the recent European Regulation 10/2011 (EU, 2011). Sb migration was assayed by ICP-MS and HG-AFS. The results showed that Sb migration values ranged from 0.5 to 1.2 μg Sb/l, which are far below the maximum permissible migration value for Sb, 40 μg Sb/kg, (EU, Regulation 10/2011). Parameters as temperature and bottle re-use influence were studied. To assess toxicity, antimony speciation was performed by HPLC-ICP-MS and HG-AFS. While Sb(V) was the only species detected in aqueous simulants, an additional species (Sb–acetate complex) was measured in wine vinegar. Unlike most of the studies reported in the literature, migration tests were based on the application of the EU directive, which enables comparison and harmonisation of results.

1. Introduction

Polyethylene terephthalate (PET) is the most widespread polymer used for the manufacture of packaging and films in contact with food, especially for beverages and drinking water (Bach, Dauchy, Chagnon, & Etienne, 2012). From PET containers, additives and residual chemical compounds of the polymer may migrate into food. Antimony trioxide (Sb_2O_3; CAS Registry no. 0001309-64-4) is the main catalyst used in PET synthesis (Pang, Kotek, & Tonelli, 2006), resulting in an antimony content in commercial PET resins ranging from 190 to 300 μg Sb/g (Duh, 2002). Sb has also been considered as a priority pollutant by the European Union (EU) and the United States Environmental Protection Agency (US EPA) (Shotyk, Krachler, & Chen, 2006) setting the Maximum Contaminant Level (MCL) to 5 μg Sb/l (EC, 2003) and to 6 μg Sb/l (US EPA, 2009), respectively. The toxicity of antimony depends on its chemical form and oxidation state, and compounds of trivalent antimony are generally more toxic than pentavalent forms (Filella, Belzile, & Lett, 2007; Saracoglu, Soylak, Dogan, & Elci, 2003). Antimony leaching from PET into food products during storage implies a possible health hazard for humans. Different studies have already reported Sb leaching from PET containers into drinking water and juices. Shotyk et al. (2006) first provided evidence of Sb leaching from PET containers into drinking water. Sb concentration in 132 brands of bottled water from 28 countries was determined, and Sb contents ranged from 0.156 to 0.343 μg/l, which is some 100-fold higher than the blank values from unbottled water.

A similar study was performed by Westerhoff, Prapaipong, Shock, and Hillareau (2008) in nine commercially available bottled waters from the southwestern US, here the antimony content ranged from 0.095 to 0.521 μg/l. The effect of temperature and storage time on antimony release was also studied. The rate of antimony released was fitted to a power function model to obtain a temperature- and time-dependent relationship for antimony leaching. The authors concluded that below 60 °C only a small fraction of the antimony from PET is released into the water, however the situation changes when higher temperatures are reached. Keresztes et al. (2009) investigated Sb leaching from PET packaging material into 10 different brands of still (non-carbonated) and sparkling (carbonated) Hungarian mineral water. The Sb concentration of still mineral water was lower than that of sparkling under identical storage conditions. Only at storage under extreme light and temperature conditions, Sb concentration exceeded 2 μg/l. The authors also pointed out that the extent of Sb leaching from PET recipients of different mineral water brands can differ by even one order of magnitude in experiments conducted under the same experimental conditions.

Hansen and Pergantis (2006) determined Sb in a selection of different juices, mainly of red fruit juices, packed in either bottles of PET or other commonly used container materials. The study was motivated by the fact that fruit juices contain high amounts of several organic acids, such as citric acid, malic acid and ascorbic acid, known to be efficient extractants of Sb. Juices bottled in PET

* Corresponding author. Tel.: +34 913945145.
E-mail address: ymadrid@quim.ucm.es (Y. Madrid).

0308-8146/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.foodchem.2013.03.067

contained significantly much more Sb (0.28–1.05 μg/l) than those bottled in Tetra Pak (0.07 μg Sb/l), glass recipients (0.28–0.30 μg Sb/l) or aluminium cans (0.24–0.56 μg Sb/l). Speciation analysis revealed that either the more toxic inorganic Sb(III) (44 ± 17%) or a Sb(V)–citrate complex of unknown toxicity (41 ± 20%) was present as the main Sb species in the juices. In a follow-up study, the same authors (Hansen et al., 2010) found up to 17-fold higher concentrations of Sb compared to the previous reports. Antimony concentrations up to a factor of 2.7 above the EU limit for drinking water were found in commercial juices, though it remains unclear whether this amount was introduced by leaching from the packaging material or during the manufacturing process. The authors postulated the need of further studies.

The colour of the PET bottle is also an important parameter; Westerhoff et al. (2008) observed that the release of Sb into ultrapure water was four times higher from clear PET bottles, compared to blue-coloured ones. In their study, equally sized PET samples (clear and blue) were incubated in 1 L of ultrapure water at 60 °C for 10 days. Opposite behaviour was observed by Reimann, Birke, and Filzmoser (2010), who found that Sb leaching is higher for dark bottles compared to clear ones.

Welle and Franz (2011) investigated antimony concentration in 67 PET samples from the European bottle market. A mean value of 224 ± 32 μg Sb/g PET was found. The migration of antimony into beverages was also predicted by mathematical modelling of migration for different surface/volume ratios and antimony bottle wall concentrations. The authors postulate that consumers exposure to antimony, by its migration from PET bottles into beverages and even into edible oils, reaches approximately 1% of the current tolerable daily intake (TDI), established by World Health Organisation (WHO) (WHO, 2003).

As shown, literature results for Sb leaching from PET containers into food are still rather inconsistent, with values often scattered over a wide concentration range (Keresztes et al., 2009; Shotyk et al., 2006). These differences may be explained by the variety of exposure conditions employed. Under these conditions, it is difficult to draw realistic conclusions about the consideration of Sb as a health hazard for consumers. Factors contributing to the inadequate harmonisation of the results include, lack of application of a systematic protocol to carry out migration assays (size of material, temperature, contact time and food simulants) and lack of use of a common measurement process. In order to harmonise migration test results, the European Union (EU) has published a number of Directives setting the basic rules for migration testing of packaging materials intended to come into contact with foodstuffs. In this context, plastic materials are covered by the recent regulation 10/2011 (EU, 2011) published in the Official Journal of the European Union. The conformity of a plastic material to come in contact with food is based on migration tests. The overall migration limit should not exceed 10 mg of the total constituents released from 10 cm^2 of the packaging surface. Specific migration limits (SML) are also provided for some substances on the positive list, e.g. 40 μg/kg, in the case of Sb_2O_3, expressed as Sb. However, the determination of such low Sb concentrations requires very specific and sensitive analytical techniques. Inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry coupled to hydride generation (HG-AFS) have been widely applied for trace antimony determination (Miravet, López-Sánchez, & Rubio, 2004; Westerhoff et al., 2008). Antimony determination by HG-AFS strongly depends on its oxidation state. Nevertheless, this effect has been employed to carry out speciation studies, as an alternative to the use of hyphenated techniques based on coupling of high performance liquid chromatography (HPLC), capillary electrophoresis (CE) or gas chromatography (GC) to atomic and mass spectrometry (ICP-MS) detectors (Hansen & Pergantis, 2008; Smichowski, Madrid, & Cámara, 1998).

The aim of this work was to study antimony migration from PET bottles into food stimulants, following the mentioned updated EU regulation. Unlike most previous studies focused on bottled water, migration assays were here performed by using liquids simulating aqueous and fatty foodstuff, in order to cover as much as possible the different types of food commercially sold in PET containers. We also report Sb levels in apple and wine vinegars for the first time. The selection of these samples was motivated by the fact that acetic acid is known to be an efficient extractant of antimony (Hansen and Pergantis, 2006). Furthermore, inorganic antimony speciation in aqueous food simulants and vinegar after the migration tests was performed by HPLC-ICP-MS and HG-AFS in order to determine the nature of Sb species present and therefore to assess toxicity of the antimony leached from PET.

2. Materials and methods

2.1. Instrumentation

An inductively coupled plasma mass spectrometer (Agilent, HP-7700 Series) fitted with a Meinhard nebulizer and a Peltier cooled sample introduction system was used to measure the total antimony concentration and to quantify antimony content after chromatographic separation.

A continuous flow HG-AFS system (Excalibur P.S. Analytical Ltd.) equipped with a Sb boosted hollow cathode lamp (Super Lamp, Photron, Teknokroma) and a drying membrane (Perma Pure Products, Farmingdale, NJ) was used as an alternative method for total and Sb(III) determination, where Sb(V) was evaluated as the difference between total content and Sb(III).

A PU-2089 HPLC pump (Jasco Corporation, Tokyo, Japan) fitted with a six-port sample injection valve (Model 7725i, Rheodyne, Rohner Park, CA, USA) with a 100 μl injection loop was selected for chromatographic experiments. Antimony species separation was based on the use of an anion-exchange column (Hamilton PRP-X100). The chromatographic system was coupled to the ICP-MS by a 5 cm polytetrafluoro-ethylene capillary tube (0.5 mm id) running from the column outlet to the nebuliser inlet for online measurements. The optimum chromatographic and instrumental parameters for ICP-MS and HG-AFS measurements are shown in Table 1.

Table 1
Instrument operating conditions for Sb determination by HG-AFS and HPLC-ICP-MS.

HG-AFS conditions	
Sample flow rate	1.5 ml min^{-1}
HCl flow rate	1.5 ml min^{-1}
$NaBH_4$ flow rate	1.5 ml min^{-1}
HCl concentration	2 mol l^{-1}
$NaBH_4$ concentration	1.5%
H_2 flow rate	540 ml min^{-1}
Ar flow rate	180 ml min^{-1}
ICP-MS conditions	
Forward power	1550 W
Plasma gas flow rate	15.0 l min^{-1}
Auxiliary gas flow rate	1.26 l min^{-1}
Carrier gas flow rate	1.1 l min^{-1}
Nebulizer type	Meinhard
Spray chamber type	Scott-double pass
Isotope monitored	^{121}Sb, ^{123}Sb
Internal standard	^{115}In
LC parameters	
Analytical column	Hamilton PRP X-100 (250 × 4.1 mm)
Mobile phase	5 mM EDTA, 1 mM phthalic acid, pH 3.5
Flow rate	1.5 ml min^{-1}
Injection volume	100 μl

For total antimony determination, samples were microwave digested in double-walled advanced composite vessels using a 1000 W MSP (Microwave Sample Preparation system) microwave oven (CEM, Matthews, NC, USA).

An incubator Heraeus D-6450 (Hanau, Germany) was used for migration tests.

2.2. Chemicals and reference materials

All chemicals and reagents used were of analytical grade and solutions were prepared with de-ionised water (18 M Ω cm) obtained from a Milli-Q water purification system unit (Millipore, Bedford, MA). 1000 mg/l stock standard solutions of Sb(III) and Sb(V) were prepared by dissolving appropriate amounts of potassium antimonyl tartrate (Sigma–Aldrich, 99% purity) in 6 M HCl and potassium hexahydroxyantimonate (Merk, 99% purity) in 2.4 M HCl, respectively. Antimony stock solutions were stored in glass bottles in the dark at 4 °C and working solutions were prepared daily by dilution.

De-ionised water and glacial acetic acid (Panreac, 99.8%) and EtOH (Scharlau, 99.9%) were used for preparing aqueous food simulants, whereas olive and sunflower oil (from local supermarkets) were employed as fatty food simulants. Samples of apple and wine vinegars were also purchased in local supermarkets.

Total antimony measurements by HG-AFS were based on stibine generation using 1.5% (w/v) $NaBH_4$ solution (Aldrich, Milwaukee, Wis., USA) in 2 M HCl (Merck, 37%). Boronhydride solution was prepared by dissolving $NaBH_4$ powder in de-ionised Milli-Q water and stabilising in 0.1% w/v NaOH (Merck). Solutions were filtered before use to eliminate turbidity. Sb(V) was pre-reduced to Sb(III) using 0.5% w/v L-cysteine in 2 M HCl (Sigma–Aldrich, 98.5% purity).

Separation of antimony species for anion-exchange chromatography was performed using 5 mM ethylenediaminetetraacetic acid (EDTA) (Merk, 99%) in 1 mM phthalic acid (Aldrich, 99% purity) at pH 3.5 as the mobile phase.

H_2O_2 (35%) from Panreac and HNO_3 (Scharlau, 60%) were used to digest the samples.

A Standard Reference Material for trace elements in natural water (SRM 1640, National Institute of Standards and Technology, Gaithersburg, MD) with a certified antimony content of 13.8 ± 0.42 μg Sb/kg was used for method validation.

2.3. Specific migration test experiments

Migration tests were carried out according to the above mentioned European regulation. The food simulants used are those listed in Table II of its annex III, in particular: (A) 10% (v/v) ethanol; (B) 3% (w/v) acetic acid; (C) 20% (v/v) ethanol and (D2) vegetable oil. Aqueous food simulants A, B and C are assigned for foods that have a hydrophilic character and are able to extract hydrophilic substances. Food simulant B is used for those foods which have a pH below 4.5, and food simulant C is set for alcoholic foods with an alcohol content of up to 20% and those foods which contain a relevant amount of organic ingredients that render the food more lipophilic. Food simulant D2 is assigned for foods that have a lipophilic character and are able to extract lipophilic substances. Although distilled water has been removed from the aqueous simulants list in the current directive, we decided to include it since bottled water is mainly sold in PET bottles.

According to the directive, the sample shall be placed in contact with the food stimulant, in a manner representing the worst of the foreseeable conditions regarding contact time and temperature. Therefore, double sided, total immersion migration tests were performed as follows: a 12 cm^2 piece of PET and 20 ml of the studied simulant (area-to-volume ratio 6 dm^2/l) were placed in a closed glassy beaker. Samples (plastic + simulant) were then introduced for 10 days in a thermostatic oven set at 40 °C. Immediately after the test, the total Sb and Sb(III) in the simulants were determined by ICP-MS and HG-AFS. The individual Sb species were measured with HPLC-ICP-MS, following the conditions described above. Three replicates were tested and analysed for each food simulant.

Simulant blanks were also prepared by placing the simulant in the appropriate container without PET and exposed under the test conditions. Specific migration tests were also performed under more severe conditions, applying a temperature of 60 °C for 10 days.

2.4. Reuse migration experiment

The conditions were the same as for specific migration but, in this case, migration tests were carried out three times on a single sample, using another portion of food simulant in each occasion, again following the procedure detailed in regulation EU10/2011. In particular, once the glassy beakers were removed from the oven (after 10 days), the simulant was decanted from the beakers, leaving behind only the PET sample. Each beaker was refilled with 20.0 ml of fresh stimulant and the entire procedure was repeated twice.

2.5. Total antimony determination in liquid food simulants and in PET by ICP-MS and HG-AFS

Aqueous acetic acid (3% w/v) and vinegar were directly analysed without any sample pre-treatment while aqueous ethanol (10% and 20% v/v) was diluted in deionized distilled water prior to analysis. For olive and sunflower oil, 0.5 g were microwave digested with 10.0 ml of nitric acid. About 0.2 g of sample, cut from the PET bottles, was also subjected to microwave digestion. Three replicates were prepared for each sample. The resulting digests were diluted with water up to 100 ml and further analysed by ICP-MS and HG-AFS, following the operating conditions given in Table 1. Antimony quantification was measured by ICP-MS (^{121}Sb/^{115}In), using indium as the internal standard. Total antimony determination by HG-AFS first requires the reduction of Sb(V) to Sb(III), which was done with 0.5% L-cysteine in 2 M HCl, following the conditions given by Miravet et al. (2004). Then, stibine was generated using 1.5% (w/v) $NaBH_4$ and 2 M hydrochloric acid. Standard calibration curves of Sb(III) and Sb(V) in the presence of L-cysteine were measured by HG-AFS and no significant differences between the slopes were observed. Sb(V) provides a negligible signal in the absence of L-cysteine while Sb(III) still showed the same intensity compared to the pre-reduced standard. These results show that Sb(V) reduction was complete under the selected conditions. Total antimony was determined by using both the external calibration and standard addition method.

2.6. Speciation of antimony oxidation state in aqueous food simulants and vinegar

Both Sb(III) and Sb(V) species were determined by HPLC-ICP-MS, following the experimental conditions shown in Table 1. Identification and quantification of antimony species was performed by comparing the retention time of standards and by spiking experiments, using both the external calibration and standard addition method.

Sb(III) was also determined by HG-AFS, following the same conditions as for total antimony, but without applying a pre-reduction step with L-cysteine. The concentration of Sb(V) was calculated as the difference between total antimony and Sb(III).

Table 2
Migration values for antimony after 10 days of contact at 40 ± 0.5 °C.

Food simulants	ICP-MS (^{121}Sb) (μg Sb l^{-1})	HG-AFS + L-cysteine (μg Sb l^{-1})
Distilled water	0.80 ± 0.08	0.7 ± 0.2
Acetic acid 3%	0.56 ± 0.02	0.6 ± 0.2
EtOH 10%	1.23 ± 003	1.3 ± 0.1
EtOH 20%	1.30 ± 0.04	1.2 ± 0.1
Olive Oil	<LOQ	<LOQ
Sunflower Oil	<LOQ	<LOQ

Mean ± SD. $N = 3$ replicates, confidence interval 95%.

3. Results and discussion

3.1. Quality assurance

Quality assurance steps included blanks, replicate analyses, certified reference material recoveries and calibrations.

Detection (LOD) and quantification limits (LOQ) were determined by analysing, in triplicate, ten solutions of the lowest point of calibration. They were calculated from 3σ and 10σ, respectively, of the signal and then referred to the regression line. Reproducibility was calculated as % RSD from five measurements of two independent standard solutions of 0.5 and 1.0 μg/l of Sb(III), carried on three non-consecutive days. A LOD and LOQ of 0.003 μg/l and 0.010 μg/l, respectively, was achieved for ICP-MS. These parameters rose to 0.112 μg/l and 0.375 μg/l, respectively, for HG-AFS. These values were considered to be satisfactory since they enabled the determination of Sb at trace levels. Precision was determined in terms of repeatability and reproducibility, which were better than 2% and 8% RSD, for ICP-MS and HG-AFS, respectively.

The certified antimony content in the SRM 1640 material is 13.8 ± 0.42 μg Sb kg^{-1}. This standard reference material was analysed by HG-AFS and ICP-MS in triplicate, in order to establish the precision of the measurement. The quantified antimony concentration, matched the certified value considering the associated uncertainties, achieving values of 14.0 ± 0.4 and 13.8 ± 0.8 μg/l for ICP-MS and HG-AFS, respectively.

3.2. Specific migration of antimony into aqueous and fatty-liquid simulants. Antimony content in food packed in PET: vegetable oil and vinegar

The antimony concentration in PET material, measured by ICP-MS and HG-AFS after microwave-assisted digestion, was 269 ± 39 and 276 ± 50 μg Sb/g, respectively. These results are in good agreement with those commonly found in the literature; e.g. 168–216 μg Sb/g (Nishioka, Hirahara, & Iwamoto, 2002), 213 μg Sb/g (Westerhoff et al., 2008) and 210–290 μg Sb/g (Keresztes et al., 2009).

The values obtained for Sb migration from PET into aqueous and fatty simulants after ten days of contact, at 40 ± 0.5 °C, are given in Table 2. The Sb concentration values, ranging from 0.56 to 1.23 μg/l, were lower than the upper limit set for Sb migration by the EU (5 μg/l). However, the values found in ethanol were significantly higher compared to the corresponding migration values with distilled water and acetic acid. This may be explained by the capability of organic solvents to penetrate into plastic packaging materials, causing swelling of the polymer and thus changing its physical structure. As a result, an increase in the diffusivity of the potential migrants is produced, consequently enhancing the migration rate. It is worth noting that no statistically significant differences for Sb migration values from PET into distilled water and into 3% aqueous acetic acid were detected. This was unexpected due to the recognised capability of acetic acid for extracting Sb and stabilizing Sb(III), which could have rendered to an increase in the migration rate.

Table 3
Sb concentration in apple and wine vinegar bottled in PET.

Food	ICP-MS (^{121}Sb) (μg Sb l^{-1})	HG-AFS + L-cysteine (μg Sb l^{-1})	HG-AFS without L-cysteine (μg Sb l^{-1})
Wine vinegar	0.50 ± 0.02	0.5 ± 0.1	<LOD
Apple vinegar	0.45 ± 0.01	0.4 ± 0.2	<LOD

Mean ± SD. $N = 3$ replicates, confidence interval 95%.

Olive and sunflower oil as fatty-food simulants were also employed in the migration assays. PET bottles are usually considered suitable as containers for vegetable oils, however, very few studies have directly focused on migration from PET into these oils (Fordham, Gramshaw, Crews, & Castle, 1995; Cecchi, Passamonti, & Cecchi, 2009). Consequently, the antimony concentration was determined by ICP-MS after acid MW digestion of vegetable oils, before and after the migration assays. As it can be seen in Table 2, antimony was not detected in any of the vegetable oils, confirming the low tendency of this analyte to migrate into lipophilic compounds.

Extending this study, Sb migration into wine and apple vinegars was also tested. Both products were selected for representing acidified food and for being commercially sold in PET containers. The antimony values found in apple and wine vinegars bottled in PET are presented in Table 3. Sb concentration levels neither exceeded the European-specific migration limit of 40 μg/kg nor the European limit of 5 μg/l for drinking water, being similar to those obtained in the migration assay when using 3% acetic acid as the simulant.

Out of the five simulants employed in the migration assays, the aqueous ones were the most efficient in extracting Sb from PET. However, the results obtained are much lower than the European-specific migration limit of 40 μg/kg. In general, antimony exhibited a low tendency to migrate into all the food simulants tested. In additional experiments, migration assays, under extreme conditions (at 60 °C and 10 days storage), were performed. Temperatures up to 60 °C did not produce an increase in antimony leaching from PET (data not shown). It was considered that migration experiments, at higher temperatures were unnecessary, as these conditions are unlikely to be encountered in proper use. However, some authors consider that improper storage conditions, especially during the summertime could lead to higher temperatures. Keresztes et al. (2009) submitted still and sparkling mineral water up to 70 °C during 9 h, and found an increase of Sb concentration from 0.44 to 1.90 μg/l. In a different work, Westerhoff et al. (2008) observed that heating at 80 °C for 48 h increased antimony content up to 7 μg/l.

Since PET articles could be reused, an experiment to provide information on the migration over several refilling cycles was conducted. This was performed according to the above mentioned rules established by the UE. The data obtained (Table 4) suggested that after an initial peak value, antimony leaching from the polymer migration decreased, except in the case of acetic acid, wherein the antimony content increased from 0.56 ± 0.02 to 6.4 ± 0.9 μg/l.

Table 4
Migration values of antimony after re-use migration experiments. Measurements were performed by ICP-MS.

Food simulants	First leaching (μg Sb l^{-1})	Second leaching (μg Sb l^{-1})	Third leaching[a] (μg Sb l^{-1})
Water	0.80 ± 0.08	0.55 ± 0.03	–
Acetic acid 3%	0.56 ± 0.02	6.4 ± 0.9	5.70 ± 0.03
EtOH 10%	1.23 ± 003	0.70 ± 0.04	–
EtOH 20%	1.30 ± 0.04	0.8 ± 0.03	–

Mean ± SD. $N = 3$ replicates, confidence interval 95%.
[a] According to the EU directive, only a third assay was performed for acetic acid, the only simulant where the antimony migration level increased in the second test.

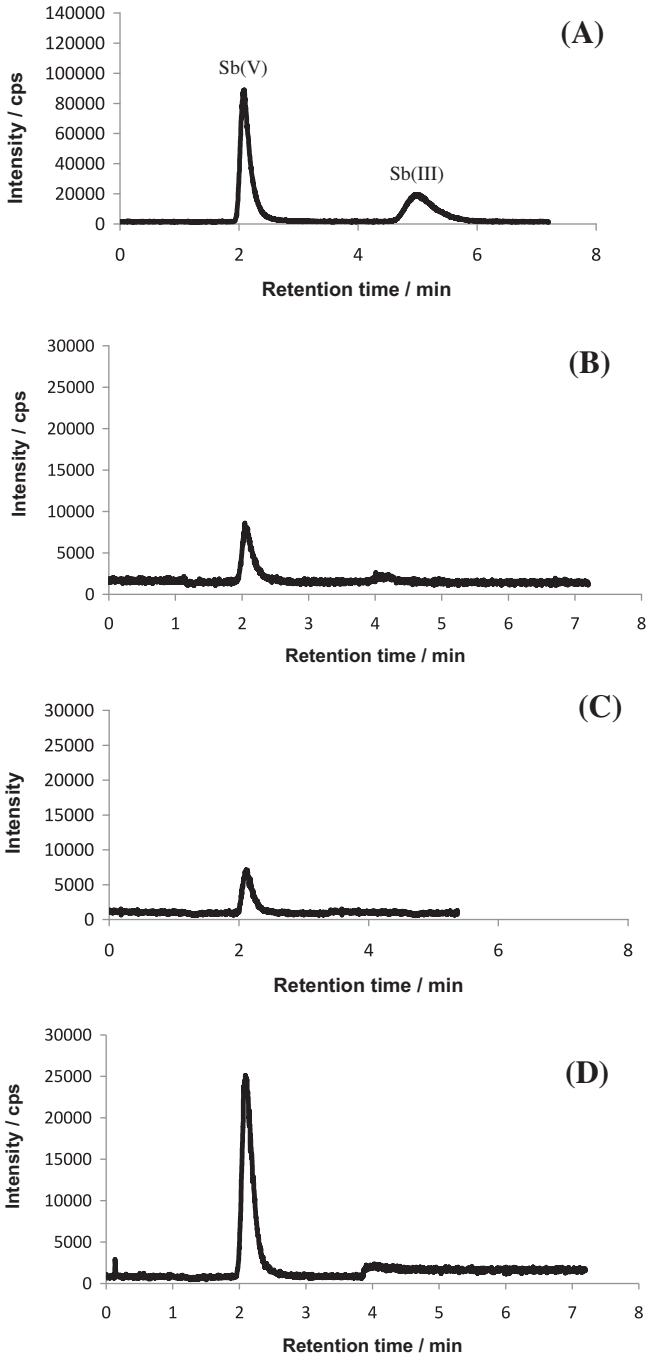

Fig. 1. HPLC-ICP-MS chromatograms achieved with 20 mM EDTA, 2 mM phthalic acid, pH 4.5 as the mobile phase for (A) standards of inorganic antimony species; and Sb leached into aqueous food simulants after 10 days of contact at 40 ± 0.5 °C such as (B) distilled water, (C) 3% acetic acid, (D) 20% ethanol. Sb-containing peaks identification was carried out by comparison of the retention time with standard solutions and by spiking experiments.

This fact could be attributed to polymer degradation produced by the acid medium of this simulant. This value exceeded the European limit of 5 μg/l for drinking water, however, refilling of PET bottles with acid food is unlikely to happen in real life applications.

3.3. Speciation of antimony oxidation state in aqueous food simulants and vinegar

As previously mentioned, antimony toxicity is strongly dependent on its chemical form and oxidation state, where Sb(III) is more toxic than Sb(V). Therefore, it is obvious that the determination of Sb species, in food and liquid food stimulants, is a key factor for assessing toxicity.

Table 5
Quantification of Sb(V) by anion-exchange HPLC-ICP-MS in food simulants after migration assays.

Food simulants	Sb found (μg Sb l^{-1})	Sb(V) (μg Sb l^{-1})	*R* (%)	Sb found (μg Sb l^{-1}) HG-AFS without L-cysteine
Water	0.80 ± 0.08	0.73 ± 0.02	93 ± 6	<LOD
Acetic acid 3%	0.56 ± 0.02	0.49 ± 0.04	98 ± 7	<LOD
EtOH 10%	1.23 ± 0.03	1.10 ± 0.02	95 ± 5	<LOD
EtOH 20%	1.30 ± 0.04	1.25 ± 0.03	96 ± 6	<LOD

Mean ± SD. *N* = 3 replicates, confidence interval 95%.

In order to determine and quantify the inorganic Sb species present in aqueous simulants and vinegar, anion exchange chromatography coupled to ICP-MS was employed for the separation and detection of trivalent and pentavalent antimony, (Fig. 1A). A combination of EDTA and phtalic acid was used as the mobile phase. One disadvantage of using EDTA as the mobile phase, is the conversion of all antimony(III) species into a Sb(III)–EDTA complex, consequently Sb(III) eluted regardless of whether Sb(III)–tartrate, Sb(III)–citrate or $SbCl_3$ was present in the sample (Hansen, Schmidt, Larsen, Gammelgaard, & Stürup, 2011). However, EDTA does not chelate Sb(V)-complexes or $Sb(OH)_6^-$. The HPLC-ICP-MS chromatograms of Sb species leached into aqueous food simulant solutions (Fig. 1B–D), only show one antimony-containing peak. Based on the retention time (t_r = 2 min), spiking experiments and the above mentioned considerations, the chromatographic peak was attributed to the presence of antimonate ($Sb(OH)_6^-$).

A further quantification of the Sb-containing peak in all aqueous food stimulants, provided recovery values around 100% with

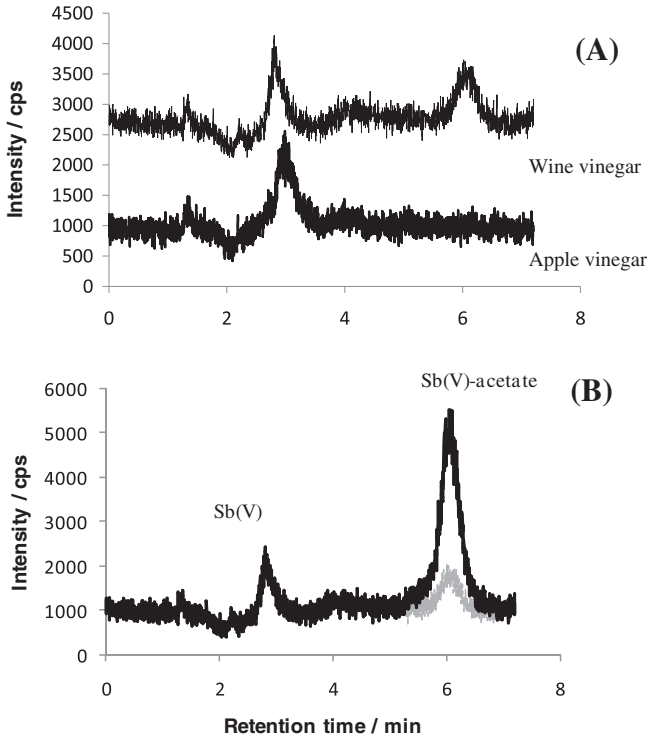

Fig. 2. HPLC-ICP-MS chromatograms achieved with 20 mM EDTA, 2 mM phthalic acid, pH 4.5 as the mobile phase for antimony redox speciation in (A) apple and wine vinegars and (B) in spiked (black line) and non spiked (gray line) wine vinegar.

respect to the antimony leached into each simulant (Table 5). These results were also verified by HG-AFS. Stibine generation without the addition of L-cysteine in each liquid simulant and detection by AFS does not provide any analytical signal, suggesting that the leached antimony is likely to be Sb(V).

With regard to the antimony species in vinegar samples (Fig. 2), both apple and wine vinegars showed a Sb-containing peak at 2.8 min, which matched with the retention time of the $Sb(OH)_6^-$ standard. Furthermore, wine vinegar also presented a Sb-containing peak at 6.1 min. Because of the high content of acetic acid (6%) in wine vinegar, this peak might be attributed to Sb(V)–acetate. Following the procedure detailed by Hansen et al. (2011), a solution of Sb(III) or Sb(V) prepared in 6% acetic acid was added to the wine vinegar sample and injected into the applied chromatography technique. As expected, the Sb(III)–acetate complex peak matched the retention time of Sb(III)–EDTA (4 min), due to the conversion of Sb(III)–acetate into the EDTA complex within the column (data not shown). However, Sb(V)–acetate had a similar retention time to the peak appearing at 6 min (Fig. 2B).

Quantification of Sb in both vinegars by HG-AFS in the absence of L-cystine (Table 3), also showed only a negligible antimony signal, confirming that antimony is present mostly as Sb(V). It is interesting to note, that a peak associated to Sb(V)–acetate was not detected, neither in 3% acetic acid after the migration test, nor in apple vinegar. The reason for that might be the low concentration of acetic acid present in these matrices (3% acetic acid w/v).

zAntimony is added to PET during its manufacture as Sb_2O_3, and consequently Sb(III) is expected to be present in the samples. However, in the current study all antimony leached into the samples was in the Sb(V) form. The results reported in the literature are, at this point, somehow contradictory, Takahashi, Sakuma, Zheng, and Mitsunobu (2008) found large differences between different bottles, and in some bottles only Sb(III) was present, whereas the Sb(V) fraction reached 50% in others. In a study on leaching of Sb from PET bottles into citrus fruit juices, it could be shown that both a Sb(V)–citrate complex and inorganic Sb(III) were present. The high concentration of the trivalent oxidation state (44% of total Sb) in juices was attributed to its stabilization by complexation with citric acid, which preserves the oxidation state of the leached Sb(III) (normally prone to oxidation) (Hansen and Pergantis, 2006). Possible explanations for discrepancies in results might be related to the nature of the PET material, the type of food under study and the experimental set-up selected. Therefore, it remains unclear whether the presence of Sb(V) is due to partial or complete oxidation of Sb_2O_3 during the PET polycondensation reaction (where this element acts as a catalyst), during storage or during performance of the migration assays.

In conclusion, the results obtained in this work show that specific migration values for antimony, into standardised aqueous and fatty food stimulants, are much lower than the upper limit set by the EU (40 μg/kg) and in all cases below the European limit of 5 μg/l for drinking water. These results prove the low tendency of Sb to migrate from PET into food under normal conditions of use. Antimony oxidation state speciation studies show that Sb leached in the simulants was mainly in the Sb(V) form. Therefore, the often addressed question regarding toxic effects caused by antimony from PET bottles, appears to be groundless. Consequently, PET packaging can be considered safe concerning antimony migration for all types of food simulants. While previously reported information on Sb migration is mostly derived from antimony leached into drinking water under a broad variety of experimental conditions (some of them very difficult to find in real use), the current study was based on performing a migration assay according to the current European Union directive, which enables us to compare and harmonise results, and to establish the real hazard caused by antimony leached from PET bottles. The obtained results were validated using two independent methodologies based on ICP-MS and HG-AFS, both with satisfying analytical characteristics and detection limits in the sub μg/kg range, as well as the application of the proposed method to a matrix matched Certified Reference Material.

Acknowledgements

The Research Group for Trace and Speciation Analysis of the Complutense University thank the Spanish Commission of Science and Technology (CTQ2011-22732), the Community of Madrid (Spain) and European Community for funding in the frame of the FEDER programme (project S2010/AGR-1464, ANALYSIC II). María Sánchez-Martínez would also like to thank the Spanish Government for a doctoral fellowship (CTQ2008-05925).

References

Bach, C., Dauchy, X., Chagnon, M. C., & Etienne, S. (2012). Chemical compounds and toxicological assessments of drinking water stored in polyethylene terephthalate (PET) bottles: A source of controversy reviewed. *Water Research, 46*, 571–583.

Cecchi, T., Passamonti, P., & Cecchi, P. (2009). Is it advisable to store olive oil in PET bottles? *Food Reviews International, 25*, 271–283.

Commission of the European Communities (2003). Official Journal of the Eurpean Union. Directive 2003/40/EC.

Duh, B. (2002). Effect of antimony catalyst on solid-state polycondensation of poly(ethylene terephthalate). *Polymer, 43*, 3147–3154.

Environmental Protection Agency (2009). National Primary Drinking Water Regulations: Drinking Water Regulations for Aircraft Public Water Systems.

Filella, M., Belzile, N., & Lett, M. C. (2007). Antimony in the environment: A review focused on natural waters. III. Microbiota relevant interactions. *Earth-Science Reviews, 80*, 195–217.

Fordham, P. J., Gramshaw, J. W., Crews, H. M., & Castle, L. (1995). Element residues in food contact plastics and their migration into food simulants, measured by inductively-coupled plasma-mass spectrometry. *Food Additives and Contaminants, 12*, 651–669.

Hansen, H. R., & Pergantis, S. A. (2006). Detection of antimony species in citrus juices and drinking water stored in PET containers. *Journal of Analytical Atomic Spectrometry, 21*, 731–733.

Hansen, H. R., & Pergantis, S. A. (2008). Analytical techniques and methods used for antimony speciation analysis in biological matrices. *Journal of Analytical Atomic Spectrometry, 23*, 1328–1340.

Hansen, C., Schmidt, B., Larsen, E. H., Gammelgaard, B., & Stürup, S. (2011). Quantitative HPLC-ICP-MS analysis of antimony redox speciation in complex sample matrices: New insights into the Sb-chemistry causing poor chromatographic recoveries. *Analyst, 136*, 996–1002.

Hansen, C., Tsirigotaki, A., Bak, S. A., Pergantis, S. A., Stürup, S., Gammelgaard, B., et al. (2010). Elevated antimony concentrations in commercial juices. *Journal of Environmental Monitoring, 12*, 822–824.

Keresztes, S., Tatár, E., Mihucz, V. G., Virág, I., Majdik, C., & Záray, G. (2009). Leaching of antimony from polyethylene terephthalate (PET) bottles into mineral water. *Science of the Total Environment, 407*, 4731–4735.

Miravet, R., López-Sánchez, J. F., & Rubio, R. (2004). Comparison of pre-reducting agents for antimony determination by hydride generation atomic fluorescence spectrometry. *Analytica Chimica Acta, 511*, 295–302.

Nishioka, K., Hirahara, A., & Iwamoto, E. (2002). Determination of antimony in polyethylene terephthalate bottles by graphite furnace atomic absorption spectrometry using microwave sample preparation. *Bulletin of Faculty of Human Life and Environmental Science, 8*, 35–42.

Pang, K., Kotek, R., & Tonelli, A. (2006). Review of conventional and novel polymerization processes for polyesters. *Progress in Polymer Science, 31*, 1009–1037.

Reimann, C., Birke, M., & Filzmoser, P. (2010). Bottled drinking water: Water contamination from bottle materials (glass, hard PET, soft PET), the influence of colour and acidification. *Applied Geochemistry, 25*, 1030–1046.

Saracoglu, S., Soylak, M., Dogan, M., & Elci, L. (2003). Speciation of antimony using chromosorb 102 resin as a retention medium. *Analytical Sciences, 19*, 259–264.

Shotyk, W., Krachler, M., & Chen, B. (2006). Contamination of Canadian and European bottled waters with antimony from PET containers. *Journal of Environmental Monitoring, 8*, 288–292.

Smichowski, P., Madrid, Y., & Cámara, C. (1998). Analytical methods for antimony speciation in waters at trace and ultratrace levels. A review. *Journal of Analytical Chemistry, 360*, 623–629.

Takahashi, Y., Sakuma, K., Zheng, G., & Mitsunobu, S. (2008). Speciation of antimony in PET bottles produced in Japan and China by X-ray absorption fine structure spectroscopy. *Environmental Science & Technology, 42*, 9045–9050.

Welle, F., & Franz, R. (2011). Migration of antimony from PET bottles into beverages: Determination of the activation energy of diffusion and migration modeling compared with literature data. *Food Additives and Contaminants, 28*, 115–126.

Westerhoff, P., Prapaipong, P., Shock, E., & Hillareau, A. (2008). Antimony leaching from polyethylene terephthalate (PET) plastic used for bottled drinking water. *Water Research, 42*, 551–556.

World Health Organization (2003). Guidelines for Drinking-water Quality.