A need for determination of arsenic species at low levels in cereal-based food and infant cereals. Validation of a method by LC-ICPMS

Highlights

· A method is developed and validated to determine arsenic species in cereal-based food

· The method was applied to a variety of cereal products including infant cereals

· The method is useful for regulation of inorganic arsenic content in food commodities

A need for determination of arsenic species at low levels in cereal-based food and infant cereals. Validation of a method by IC-ICPMS

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Abstract

The present study arose from the need to determine inorganic arsenic (iAs) at low levels in cereal-based food. Validated methods with a low limit of detection (LOD) are required to analyze these kinds of food. An analytical method for the determination of iAs, methylarsonic acid (MA) and dimethylarsinic acid (DMA) in cereal-based food and infant cereals is reported. The method was optimized and validated to achieve low LODs. Ion chromatography-inductively coupled plasma mass spectrometry (IC-ICPMS) was used for arsenic speciation. The main quality parameters were established. To expand the applicability of the method, different cereal products were analyzed: bread, biscuits, breakfast cereals, wheat flour, corn snacks, pasta and infant cereals. The total and inorganic arsenic content of 29 cereal-based food samples ranged between 3.7-35.6 μg As kg^{-1} and 3.1-26.0 μg As kg^{-1}, respectively. The present method could be considered a valuable tool for assessing inorganic arsenic contents in cereal-based foods.

Keywords: inorganic arsenic; food chemistry; cereal-based food; arsenic speciation; infant cereals; method validation.

1. Introduction

Humans are exposed to arsenic (As) in the environment primarily through the ingestion of food and water (Abernathy et al., 2011; EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009). Speciation of As in food products is necessary because of the varying toxicity of different As compounds. Inorganic arsenic (iAs) (arsenite or As(III) and arsenate or As(V)) is considered the most dangerous form due to its biological availability, as well as physiological and toxicological effects (iAs is classified as a non-threshold, class 1 human carcinogen) (ATSDR Toxicological profile for arsenic, 2007). Children are particularly vulnerable to the toxic effects of iAs. Other arsenic compounds, such as arsenobetaine (AB), commonly present in seafood, is non-toxic and can be consumed without health concern, while arsenosugars, usually found in edible algae, are potentially toxic (Feldmann & Krupp, 2011). Therefore, species-dependent differences in toxicity must be considered when establishing the maximum tolerated levels in food directives. Currently, no such levels have been fixed for iAs in European legislation, probably due to the lack of fully validated, standardized analytical methods and the unavailability of certified reference materials (CRM) for this measurand in food matrices (Baer et al., 2011). Only a regulatory limit of 0.15 mg iAs kg^{-1} is currently applied in China (USDA Maximum Levels of Contaminants in Foods, 2006). In 2009, the European Food Safety Authority (EFSA) (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009) reviewed the diet of the European Union population and pointed out the need to produce speciation data, particularly inorganic arsenic data, for different food commodities to estimate the health risk associated with dietary As exposure. As a general recommendation, dietary exposure to iAs should be reduced (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009). Among the conclusions from this report, cereal and cereal-based products were identified as contributors to daily iAs exposure in the general European population. Moreover, children aged less than three years were the most exposed to iAs, which was directly related to the intake of rice-based products. Several authors (Llorente-Mirandes, Calderon, Lopez-Sanchez, Centrich & Rubio, 2012; Meharg et al., 2008; Carbonell-Barrachina et al., 2012) have recently reported that some rice-based infant products have elevated levels of iAs that exceed the Chinese regulatory limit aforementioned. Therefore, iAs levels in rice-based baby food should be of concern. In addition, infants with celiac disease, who are forced to consume gluten-free products, with high percentages of rice, should be paid special attention due to the most elevated intakes of iAs. However, other infant cereals are prepared using mixtures of cereals (wheat, barley, oat and mixed cereals, among others) and their iAs contents are lower compared to rice products. The available results on arsenic speciation in infant food products are limited and confused. Thus, more studies are required to provide information that can be useful in the risk assessment of an infant's diet.

Wheat is the most widely consumed grain in Europe and in most other countries where the diet is not rice-based. For example, in Catalonia (Spain), the majority of the cereals consumed by the average adult are wheat-based. Although it is also true that the total As content of wheat is very low compared to that of other foods, arsenic is present almost exclusively as iAs (D'Amato, Aureli, Ciardullo, Raggi & Cubadda, 2011). Therefore, wheat should not be ignored as a potential contributor to the dietary iAs intake (EFSA Panel on Contaminants in the Food Chain (CONTAM), 2009) and validated methods with low limits of detection (LODs) are needed to analyze these kinds of food due to the high consumption of wheat-based products such as bread and pasta in populations with a predominantly wheat-based diet. To this end, the European Union Reference Laboratory for Heavy Metals in Feed and Food (EU-RL-HM) organized a proficiency test (PT) in 2012 for measuring total and inorganic arsenic in wheat, vegetable food and algae (de la Calle et al., 2012). The main conclusion derived from this exercise was that the concentration of iAs determined in any of the matrices covered was not method-dependent. Moreover, there was a need to consider the option of introducing possible maximum levels for iAs in wheat for risk management. Thus, analytical laboratories of food control should now be ready to determine iAs levels in food (mainly rice and cereals). They will therefore need suitable and robust methods for oncoming legislation. The use of validated methods, a requirement of the ISO-UNE-EN 17025 standard, is mandatory for analytical laboratories working on food control.

In summary, infant cereals and cereal-based food deserve special attention with respect to iAs content, and validated methods with a low limit of detection (LOD) are required to analyze these kinds of food. Therefore, the main objective of this study was to validate an analytical method for the determination of iAs, methylarsonic acid (MA) and dimethylarsinic acid (DMA) levels in cereal-based products that could be used in routine analysis for food control purposes. First, instrumental conditions for the determination of arsenic species were optimized, with the aim of improving the limits of detection (LODs). Second, the validation parameters of the method were evaluated. Finally, several samples were analyzed to establish wide applicability and provide iAs occurrence data on cereal-based food.

2. Experimental procedures

2.1. Chemicals and reagents

Deionized water (18.2 MΩ cm) was used to prepare the reagents and standards. All glassware was treated with 10% v/v nitric acid (HNO_3) for 24 h and then rinsed three times with deionized water before use to reduce background As levels. Concentrated super-pure HNO_3 (Carlo Erba, Rodano, Italy) and 30% w/w hydrogen peroxide (H_2O_2) (Merck, Darmstadt, Germany) were used. Isopropyl alcohol (Merck) was used within the inductively coupled plasma mass spectrometry (ICPMS) method. A commercial solution (Agilent Technologies, Barcelona, Spain) containing 10 µg L^{-1} of lithium, yttrium, cerium, thallium and cobalt in 2% (v / v) nitric acid was used to tune the ICPMS instrument. Ammonium dihydrogen phosphate (Merck, p.a.) and aqueous ammonia solution (Panreac, p.a.) were used for speciation analysis. External calibration standards for total arsenic were prepared weekly by diluting a multi-element plasma stock solution, traceable to the National Institute of Standards and Technology, with 100 mg/L of As (J. T. Baker, Phillipsburg, NJ) in 5% (v/v) HNO_3 (Carlo Erba). A diluted solution (0.2 mg/L in 40% v/v of isopropyl alcohol) of a 100 mg/L multi-element internal standard stock solution (Agilent Technologies, Barcelona, Spain) containing Ge was used as an internal standard to correct possible instrumental drifts and matrix effects.

Stock standard solutions (1000 mg As L^{-1}) for arsenic speciation were prepared as follows: DMA, prepared from cacodylic acid $C_2H_7AsO_2$ (Aldrich, >99.0%) dissolved in water; MA, prepared from $Na_2CH_3AsO_3$ (Supelco, 98%) dissolved in water; arsenite was supplied by Fluka, As(III), as a standard solution (1000 ± 2 mg As L^{-1}); and arsenate was supplied by Merck, As(V), as a standard solution (1000 mg As L^{-1}). Arsenate, arsenite, DMA and MA, were standardized against As_2O_3 (NIST Oxidimetric Primary Standard 83d) for our internal quality control. All the stock solutions were kept at 4 °C, and further diluted solutions for the speciation analysis were prepared daily.

2.2. Samples and sample pretreatment

For the applicability study, 30 cereal-based foods, which are representative of all the types of cereal products consumed in Spain, were purchased from local supermarkets and retail stores in Barcelona, Spain, during 2011. A selection of cereal products representing different types, such as bread, biscuits, breakfast cereals, corn snacks, wheat flour, pasta and infant cereals, were analyzed for As speciation and total As. All samples were of different brands and origin, but no specific information on the origin of the cereal grain was found on the packaging and product labels. Samples were brought to the laboratory the same day of purchase and kept for not more than one day in the refrigerator until sample preparation. Samples were ground into a fine powder in a commercial coffee mill (Moulinex, Vidrafoc). Powdered samples were placed in plastic containers and stored at 4 °C until analysis.

2.3. Certified reference materials

Two certified reference materials (CRMs) were analysed throughout the study. SRM 1568a Rice Flour was purchased from the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and is certified for total arsenic. NMIJ CRM 7503a White Rice Flour was purchased from the National Metrology Institute of Japan (NMIJ, Japan) and is certified for As (III), As (V), DMA and total arsenic. All samples were used as provided, without further grinding.

2.4. Moisture determination

Aliquots of 0.5-g samples were dried, in triplicate, at 102 °C to constant weight in an oven with natural convection (Binder Inc., Bohemia, NY). Moisture ranged from 5 to 11 %, and all the results are expressed as dry mass.

2.5. Total arsenic determination

Samples were processed as described before (Fontcuberta et al., 2011). Briefly, a total of 0.5 g from every sample was weighed and 9 mL of 16% HNO_3 and 1 mL of 30% H_2O_2 were added to perform a microwave digestion using an Ethos 1 microwave system (Milestone, Gomensoro, Barcelona, Spain). The digestion method was as follows: 15 min up to 200 ºC and held for 15 min, working with a maximum power of 800 W. Finally, the digested sample was made up to 30 g with deionized water. Arsenic was measured on an Agilent quadrupole inductively coupled plasma mass spectrometer

(ICPMS) 7500 cx (Agilent Technologies, Barcelona, Spain) at 1500 kW, measuring mass at m/z 75 and using helium as a collision gas to remove $^{40}Ar^{35}Cl$ interference. The results were quantified using external calibration standards of 0.125, 0.25, 0.5, 1 and 5 µg L^{-1} prepared in 5% HNO_3 for total As. A solution of 5 µg L^{-1} of germanium was used as an internal standard and measured at m/z 72. The final solutions (standards and samples) were prepared with 2% isopropyl alcohol (or 40% if introduced within the online internal standard) to minimize the effects of the dissolved carbon on arsenic response (Pettine, Casentini, Mastroianni & Capri, 2007). Each sample was digested and analyzed in triplicate. Digestion blanks were analyzed together with samples. Quality control standard solutions at two concentrations levels were measured after every 10 samples. To assess the accuracy of total As measurements, two certified reference materials were analyzed throughout the routine sample analyses: NIST SRM 1568a Rice with a certified value of 290 ± 30 µg As kg^{-1} for total As, our method obtaining 292 ± 9 µg As kg^{-1} (n = 3, all data are expressed as mean ± standard error), and NMIJ CRM 7503a Rice with a certified value of 98 ± 7 µg kg^{-1} for total As, our method obtaining 95 ± 5 µg kg^{-1} (n = 3). The instrumental detection limit was 0.03 µg As L^{-1} (calculated as 3 times the standard deviation of a blank sample). The lowest concentration level validated was 7.5 µg As kg^{-1} for total As.

2.6. Arsenic speciation analysis

The extraction procedure of arsenic species was based on our previous study in rice samples (Llorente-Mirandes et al., 2012). Briefly, 0.25-g aliquots of the cereal products were weighed in PTFE vessels and then extracted by adding 10 mL of 0.2 % (w/v) HNO_3 and 1 % (w/v) H_2O_2 solution in a microwave digestion system. This extraction method completely oxidizes As(III) into As(V), without conversion of the methylated arsenic species into iAs, so we quantified iAs as As(V). Arsenic species were determined by ion chromatography IC-ICPMS. Speciation analysis by IC was performed using a Dionex ICS-3000 Ion Chromatograph. The outlet of the column was connected via polyether ether ketone (PEEK) capillary tubing to the nebulizer of the ICPMS system. Separation of As(III), As(V), DMA and MA was achieved with an anion exchange column (Hamilton PRP-X100, 150 mm x 4.1 mm, 5 μm, Hamilton, USA) and using the conditions shown in Table 1. The ion intensity at m/z 75 (^{75}As) was monitored using Agilent Chemstation ICPMS software rev. B.04.00. Additionally, the ion intensities at m/z 77 (^{40}Ar^{37}Cl) and m/z 35 (^{35}Cl) were monitored to detect possible argon chloride (^{40}Ar^{35}Cl) interference at m/z 75. Arsenic species in the chromatograms were identified by comparison of the retention times with those of the standards. External calibration curves were used to quantify MA, DMA and arsenate against the corresponding standards. Both water blanks and extraction blanks were also analyzed by IC-ICPMS in each batch of samples. Each sample was extracted and analyzed in triplicate. Sample solutions were analyzed in batches including internal quality control, such as a standard solution and two certified reference materials every ten samples and also at the end of the sequence, to control the stability of the instrument sensitivity during the analytical run.

After full validation, the method was recently accredited by ENAC (Spanish National Accreditation Entity) under the ISO/IEC 17025 standard for its applicability in cereal-based food.

3. Results and discussion

3.1 Optimization of the IC-ICPMS parameters

Some IC-ICPMS parameters were modified and optimized from our previous study to improve LODs. First, the injection volume was increased to 250 µL and an increase in arsenic sensitivity (by a factor of around 2) in IC-ICPMS measurements were achieved without this affecting the good chromatographic resolution between the peaks. The ion intensities at m/z 77 ($^{40}Ar^{37}Cl$ and ^{77}Se) and m/z 35 (^{35}Cl) were monitored to detect possible argon chloride interference at m/z 75 on the IC-ICPMS measurements. Since no interferences were found, helium was not required, resulting in a noticeable increase in As sensitivity in IC-ICPMS measurements. The ionization of arsenic may be significantly increased by the presence of carbon in the ICPMS plasma, according to the chemical ionization process (Pettine et al., 2007). Hence, isopropyl alcohol (IPA) and methyl alcohol (MeOH) solutions containing different proportions of alcohol were examined to improve sensitivity to arsenic detection. The best signal-to-noise ratio was obtained with the IPA solution. Therefore, a 10% IPA solution was added through a T-piece after the column and before the nebulizer, using a peristaltic pump and thus, ensuring a compromise between increasing As sensitivity and maintaining suitable plasma conditions. The conditions for arsenic speciation analysis are reported in Table 1.

3.2. Validation parameters

The validation parameters were established as specified elsewhere (Thompson, Ellison & Wood, 2002).

3.2.1 Linearity, Limit of Detection and Limit of Quantification

Linearity was assessed by analyses of mixed standard solutions in triplicate from 0.05 to 5 µg As L^{-1} (6 calibration points) in doubly deionized water (Table 2). It was then validated through three analytical runs on three different days.

Limits of detection (LODs) were estimated for iAs, DMA and MA with the standard error of y-intercepts of regression analysis (σ) and the slope (S) of the standard curves, using the following equation $LOD = 3\ \sigma/S$ (Table 2) (Miller JN & Miller JC, 2005). Compared to the previous method (Llorente-Mirandes et al., 2012), lower instrumental detection limits for As species were obtained (see Table 2). Limits of quantification (LOQs) were estimated in the same manner from the equation $LOQ = 10\ \sigma/S$ (Table 2) (Miller JN & Miller JC, 2005).

3.2.2 Accuracy and repeatability

To evaluate the accuracy of the speciation method, two rice CRMs were analysed throughout the study (Table 2). NMIJ 7503a rice has a certified value of 84.1 ± 3.0 µg kg^{-1} for iAs (sum of the certified values for As(III) and As(V) (the square sum of their uncertainties)) and a certified value of 13.3 ± 0.9 µg kg^{-1} for DMA. The results obtained were in agreement with the certified values. SRM NIST 1568a rice is certified only for total arsenic, but when performing speciation, our results were consistent with the literature on the presence of arsenic species in this material (D'Amato et al., 2011; Carbonell-Barrachina et al., 2012). Moreover, the sum of the As species (284.5 µg As kg^{-1}) compared well with the certified total As value of 290 µg As kg^{-1}. For within-day repeatability, six replicates of NMIJ 7503a White Rice Flour and NIST SRM 1568a Rice CRMs were analyzed within a day and by the same analyst (Table 2).

3.2.3 Intermediate precision, trueness and expanded uncertainty

Intermediate precision, trueness and expanded uncertainty were assessed for iAs, MA and DMA using spiked cereal-based products at three concentrations in triplicate. Biscuit, breakfast cereal and white bread were chosen for the spiking experiments at low and medium concentrations, while black rice, long-grain rice and infant cereal (rice-based) were selected to evaluate high concentrations. Spiking experiments were performed by adding As(III), As(V) DMA and MA standards to solid samples and then homogenized. The mixtures were then left to stand for 30 minutes before microwave extraction. Unspiked samples were also analyzed in triplicate to calculate spike recovery. The lowest concentration levels validated were 4 µg As kg^{-1} for iAs, DMA and MA. Below such concentration, the values obtained for precision and accuracy could not reach the specified limits established for further routine laboratory operating conditions.

Trueness was expressed in terms of recovery, according to the method of (Thompson, Ellison & Wood, 2002). No As(III) was found in spiked extracts, so we calculated iAs recoveries assuming that all of the added As(III) was oxidized into As(V). Recoveries were calculated as follows: recovery (%) = (a-b)*100/c, where a is the As concentration measured in the extracts of samples which were spiked with standards solutions; b is the As concentration measured in the unspiked sample and c was the known concentration added to the sample. The values for DMA, MA and iAs are given in Table 3**,** and show that all species were recovered successfully.

To evaluate intermediate precision, various factors were changed: three different analysis days over three weeks, different analysts and different standards for spiking. Intermediate precision was expressed in terms of relative standard deviation (%RSD) of arsenic recovery and the results are shown in Table 3**.** They were consistent with the precision acceptance criterion.

The relative expanded uncertainty was estimated by a top-down method, adapted from (Maroto, Boqué, Riu, Ruisánchez & Òdena, 2005) and was calculated using a formula that combined the precision and trueness values of the spiking experiments (Llorente-Mirandes et al., 2012). The results for each species and each spiked level are shown in Table 3 and agree with the uncertainty acceptance criterion.

3.2.4 External quality control

The method was tested in two proficiency tests as external quality control. It was checked by an interlaboratory comparison of the European Union-Reference Laboratory for Heavy Metals in Feed and Food, IMEP-112, Total and inorganic arsenic in wheat, vegetable food and algae (de la Calle et al., 2012). The wheat test material was analyzed during the validation process and good results were obtained: for an assigned value of 169 ± 25 µg kg^{-1} for iAs, 170.0 ± 3.5 µg kg^{-1} was obtained. Moreover, the laboratory had previously participated in a proficiency test of the Central Science Laboratory-Food Analysis Performance Assessment Scheme (CSL-FAPAS) to determine total and inorganic As levels in rice (FAPAS round 07151 (Food Analysis Performance Assessment Scheme (FAPAS) Report 07151, 2011). The result obtained was satisfactory: for an assigned value of 390 ± 72 µg kg^{-1} for iAs, 424.3 ± 5.1 µg kg^{-1} was obtained.

There are few certified reference materials (CRMs) for arsenic species in food matrices. Recently, the JRC-IRMM released a new certified reference material, ERM-BC211 (rice). The CRM was prepared from rice destined for human consumption and is certified for total arsenic, the sum of arsenite and arsenate, and dimethylarsinic acid. The present method was employed in the certification study of ERM-BC211 and accurate results were obtained compared to the final certified values, further demonstrating its validity and reliability (Boertz et al., 2012).

3.3. Method application

A selection of 30 cereal-based food samples representing different types were analyzed for their contents of As species and total As. Table 4 summarizes the As speciation results, total As and mass balance for all analyzed samples. For quality assessment, mass balance (calculated as the ratio of the sum of As species in the extract to total As) was calculated and the results were comparable with others reported in the literature (D'Amato et al., 2011; Cubadda, Ciardullo, D'Amato, Raggi, Aureli & Carcea, 2010; Jackson, Taylor, Punshon & Cottingham, 2012b; Zhao, Stroud, Eagling, Dunham, McGrath & Shewry, 2010). Mass balance values were satisfactory notwithstanding the low arsenic concentration in cereal samples. Values ranged from 73 to 123%, averaging 96%, which indicated a full quantification of the As species that may exist in cereal-based samples. The extraction solution was suitable solvent for the extraction of As species in this type of matrix. The total arsenic concentrations in some samples were below the QL (Table 4). Nevertheless, these values were estimated and used to calculate mass balance knowing that their precision and accuracy could not reach the specified limits established for routine laboratory operating conditions.

3.3.1. Cereal-Based foods

Bread, biscuits, breakfast cereals, corn snacks, wheat flour and pasta were analyzed and the results are shown in Table 4**.** Total As content ranged from 3.7 to 23.3 µg As kg^{-1} and the mean As concentration was 7.8 µg As kg^{-1}. Total As content was below the LOD in a breakfast cereal sample. The present results are similar to others reported in the literature for total As in cereal-based food (range from 4.6 to 128.0 μg As kg^{-1}) (D'Amato et al., 2011; Fontcuberta et al., 2011; Cubadda et al., 2010; Jackson, Taylor, Karagas, Punshon & Cottingham, 2012a). A recent study on cereal bars showed that the bars not listing any rice product among the ingredients were among the lowest As-containing ones (range from 8 to 27 μg As kg^{-1}) (Jackson et al., 2012a). The As level in cereal grains (e.g., wheat, barley and maize) is typically about one order of magnitude lower than that in rice (Duxbury & Panaullah, 2007). Different factors such as soil physical conditions or water may affect As concentration in wheat grain. For example, high As content was found in wheat grown in an area with high water As concentrations in West Bengal (India) (Roychowdhury, Uchino, Tokunaga & Ando, 2002). Furthermore, another study also reported high As levels in wheat from contaminated areas, with a mean of 69 μg As kg^{-1} (range= 41 to 101 μg As kg^{-1}), at an arsenic-rich site in France (Zhao et al., 2010). The authors also found that As concentration in wheat bran was higher than that in white flour, containing only iAs and no methylated As. Cubadda and colleagues (Cubadda et al., 2010) analyzed 726 samples of wheat grains collected from 22 different locations in Italian agricultural areas over 3 consecutive years. They observed an average arsenic concentration of 9 μg As kg^{-1}, with a range of 2 to 55 μg As kg^{-1}. The authors concluded that iAs was the major As compound, highlighting the importance of wheat as a source of inorganic arsenic in the Italian diet.

Regarding the present As speciation results, only inorganic As was quantified in cereal-based food (Table 4). Inorganic arsenic ranged from 3.1 to 23.4 μg As kg^{-1} with a mean value of 7.0 μg As kg^{-1}. DMA was found below the QL in some samples, while MA was below LOD in all samples. The finding that almost all the arsenic in cereal-based food is present as iAs is in agreement with other studies showing very low levels of methylated As species (Cubadda et al., 2010, Zhao et al., 2010). This behavior is illustrated in Fig. 1, which shows that all arsenic in the present study was in form of inorganic As in the chromatograms of macaroni (a) and wheat flour (b) extracts. Some As speciation studies have focused on wheat or wheat flour, but limited information is available in the literature about cereal-based products. Moreover, there is no study on biscuits and snack products. Other study analyzed several wheat-based food (whole grain, flour, bread and pasta) and observed that about 95% of the As in wheat-based food was in the inorganic form, whereas the remainder was mainly DMA (D'Amato et al., 2011).

There is little information of As speciation in cereal-based products in the literature, probably due to the low LOD that is required to analyze these kinds of food. Although the iAs content is much lower than that of rice, cereals and especially wheat should not be ignored as potential contributors to dietary iAs exposure in populations with a predominantly wheat-based diet. Further research on As speciation in cereal food products is required to estimate dietary exposure to inorganic As in such populations.

3.3.2. Infant cereals

Currently, there is a very broad range of infant products on the market such as infant cereals (rice-based or mixed cereals), pureed foods (meat and fish, etc.) and formulas (Carbonell-Barrachina et al., 2012; Hernández-Martínez & Navarro-Blasco, 2013). Nine infant cereal samples marketed in Spain by different manufacturers were selected. Seven of them were made with a mixture of cereals (wheat, barley, oat, corn, rye, sorghum, millet and rice) combined with fruit or honey; the other two were an organic spelt porridge and a rice-based infant cereal. The results of total arsenic and arsenic species measurements are given in Table 4**.** For non-rice-based formulations (n=8), total arsenic contents ranged from 7.7 to 35.6 µg As kg^{-1} with a mean value of 18 µg As kg^{-1}. These levels were comparable to other reported values in infant cereals and formulas, but lower than those in other studies of rice-based infant cereals displaying high As concentrations (Llorente-Mirandes et al., 2012; Meharg et al., 2008; Carbonell-Barrachina, et al., 2012; Hernández-Martínez & Navarro-Blasco, 2013; Jackson et al., 2012b). The infant cereals analyzed here had a very low rice percentage or did not contain rice (according to the labeled formulation), thus explaining the low arsenic contents found. The single rice-based infant cereal (above 90% of rice content) was analyzed and as expected, the total As content increased by an order of magnitude (267.4 ± 11.5 µg As kg^{-1}) compared to the non-rice-based infant cereals. Moreover, not only did the percentage of rice contribute to arsenic content, but also the product brand and the mode of cereal production (conventional or organic). A recent study analyzing 91 infant cereals marketed in Spain from eight different manufacturers concluded that infant cereals based on raw materials obtained in a conventional way displayed lower amounts of arsenic than those based on raw materials procured in an organic way. This study affirmed that the content of arsenic is affected by environmental conditions of the system (Hernández-Martínez & Navarro-Blasco, 2013).

We found that iAs was the major As species in all the non-rice-based infant cereals studied (mean of 93% of the extracted As), while DMA was only found in three samples as a minor species and MA was below the detection limit. Inorganic arsenic levels ranged from 8.1 to 26.0 µg As kg^{-1} with a mean value of 16.6 µg As kg^{-1}. Therefore, none of the samples exceeded the Chinese regulatory limit of 0.15 mg As kg^{-1} for iAs (USDA Maximum Levels of Contaminants in Foods, 2006). Few studies have reported As speciation results in infant cereals (non-rice-based), probably due to the low

LODs required to analyze these kinds of food. A recent study reported that As in baby food was present mainly as iAs (Jackson et al., 2012b). Similar iAs results were reported in infant cereals with gluten (wheat, oat, barley, rye and sorghum), in which the iAs content was 26 μg As kg^{-1} (corresponding to 98% of the extracted As) (Carbonell-Barrachina et al., 2012).

Additionally, recent studies have shown that rice-based infant cereals contain elevated concentrations of the toxic iAs (Meharg et al., 2008; Carbonell-Barrachina et al., 2012). Our results of the rice-based infant sample showed that DMA was the major species (accounting for 68%), while iAs accounted for 29% and MA was a minor species. Figure 2 shows, as an example, differences in the chromatograms of organic spelt infant cereal (a) and rice-based infant cereal (b) extracts.

In brief, inorganic arsenic contents were higher in products based on rice than in similar products prepared using mixtures of other cereals with gluten (wheat, barley and oat). Therefore, the potential of high iAs concentrations in rice-based products intended for infants requires special attention. A wide range of rice-based products are fed to babies, increasing the risk of dietary exposure to iAs. Thus, there is a fundamental need to reduce the rice content of baby products which would reduce the infant exposure to iAs. The elimination of rice from infant cereals or the diversification of diets by including other cereals could reduce the risk of iAs exposure. In addition, special attention should be paid to infants with celiac disease who have to eat gluten-free food that is mainly based on rice.

4. Conclusions

In summary, a straightforward method for the determination of iAs, DMA and MA in cereal-based food and infant cereals was optimized and fully validated. The optimized IC-ICPMS operating parameters provided low LODs, suitable for determining the As species present in samples. The method was successfully applied to 30 cereal-based food. Inorganic arsenic was the major As compound found in the food products studied, highlighting the importance of cereals as a possible source of iAs in wheat-based diets. The validated method is sensitive and selective for iAs and could be a valuable tool for assessing iAs in cereal-based food currently a subject of high interest in food control analysis. Moreover, the present results may contribute to the on-going discussions for establishing and implementing maximum levels on inorganic arsenic in food commodities, as it is stated within the European Union, and for further studies on risk assessment.

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Table 1

Operating conditions of the LC-ICPMS system

ICPMS Parameters	
RF power	1500 W
Make up Gas flow, Ar	0.15 L min^{-1}
Carrier Gas Flow, Ar	0.95 L min^{-1}
Spray chamber (type and temperature)	Scott-type and 2 ºC
Sampler and skimmer cones	Niquel
Nebuliser	Microconcentric
Sampling depth	8.0 mm
Cell Exit	-70V
Masses	m/z 75 (^{75}As), m/z 35 (^{35}Cl) and m/z 77 (^{40}Ar^{37}Cl)
Collision cell	OFF
Dwell Time	2.0 s (m/z 75), 0.1 s (m/z 35 and m/z 77)
QP/OctP Bias difference	2 V
Organic solvent	10% isopropyl alcohol post-column
Chromatographic conditions	
Column	Hamilton PRP-X100 (150 mm x 4.1 mm, 5µm)
Mobile phase	26 mM $NH_4H_2PO_4$, pH= 6.2 (adjusted with aqueous ammonia)
Flow rate	1 mL min^{-1}
Injection volume	250 µL
Column temperature	30ºC
Pressure	95 bar
Arsenic species	As(III), DMA, MA and As(V)
Elution	Isocratic, 10min

Table 2

Linearity, LOD, LOQ, Accuracy and Repeatability of the validated method.

Analyte	**Linearity** [a]	**LOD**	**LOQ**	**Accuracy** [c]					**Repeatability** [e]	
	Range (µg As L^{-1})	(µg As kg^{-1})	(µg As kg^{-1})	NIST SRM 1568a		NMIJ CRM 7503-a			NIST SRM 1568a	NMIJ CRM 7503-a
				Measured value (n=6)	Literature value	Measured value (n=6)	Certified value	(Recovery %)	(RSD %, n=6)	(RSD %, n=6)
DMA	0.05 to 5.0	0.3	1.1	168.4 ± 8.2	160-174 [b]	13.5 ± 0.7	13.3 ± 0.9	101.5	2.5	3.7
MA	0.05 to 5.0	0.3	0.9	12.8 ± 0.5	2 -14 [b]	<LOD			3.6	
iAs	0.05 to 5.0	0.4	1.2	103.3 ± 4.6	80-110 [b]	83.7 ± 1.6	84.1 ± 3.0 [d]	99.5	2.7	1.9

[a] Acceptance criteria: $R^2 \geq 0.9990$ and residual error ≤ 15% for the lowest calibration level and ≤ 10% for the others, as recommended (Horwitz, 1982).

[b] No certified values, values reported by other studies (D'Amato et al., 2011; Carbonell-Barrachina et al., 2012).

[c] Concentrations expressed as µg As kg^{-1} on dry mass (mean ± SD).

[d] As sum of certified values for As(III) and As(V) ± the square sum of their uncertainties.

[e] Acceptance criterion: %RSD (Repeatability) ≤ 2/3* %RSD (Intermediate precision).

Table 3

Table 3

Precision, Trueness and expanded Uncertainty (k=2) values of the validated method.

Analyte	**Sample**	**Spiked levels**	**Intermediate precision** [a]	**Trueness** [b]	**Expanded Uncertainty** [c]
		(added µg As kg^{-1})	(RSD in %, n=9)	(Recovery in %, n=9)	(U in %, n=9)
DMA	biscuit, breakfast cereal and white bread	4	6.3	107.6	19.5
DMA	biscuit, breakfast cereal and white bread	40	3.4	106.6	13.0
DMA	black rice, long-grain rice, infant cereal (rice based)	125	4.2	98.2	8.9
MA	biscuit, breakfast cereal and white bread	4	6.5	108.6	20.5
MA	biscuit, breakfast cereal and white bread	40	1.7	101.5	5.0
MA	black rice, long-grain rice, infant cereal (rice based)	125	2.6	101.2	5.5
iAs	biscuit, breakfast cereal and white bread	4	5.6	95.9	11.8
iAs	biscuit, breakfast cereal and white bread	40	1.8	100.3	3.8
iAs	black rice, long-grain rice, infant cereal (rice based)	250	1.9	95.3	9.1

[a] Acceptance criterion: % RSD < 2/3 Horwitz-Thomson function (Horwitz, 1982) and is (in % RSD): 14.7% for values ≤ 100 µg kg^{-1}, 13.6% for 200 µg kg^{-1} and 12.2% for 400 µg kg^{-1} (Fryš, Bajerová, Eisner, Mudruňková & Ventura, 2011).

[b] Acceptance criterion: Rec= 85%-115%. CODEX criterion: 60-115% for 10 µg kg^{-1} and 80-110% for 0.1-10 mg kg^{-1} (Joint FAO/WHO Expert Committee on Food Additives, 2010).

[c] Acceptance criterion: $U_{max} < 2$ * %RSD Horwitz function according to (Thompson et al., 2002; Horwitz, 1982).

Table 4

Concentrations of total As and As species in cereal-based products expressed as μg As kg^{-1} on dry mass (mean ± SD, n = 3).

Sample	Total As	Arsenic species			Mass balance (%) [b]
		DMA	MA	iAs	
Bread					
Loaf	7.2 ± 0.7	<LOD	<LOD	5.4 ± 0.3	74.7
White	4.9 ± 0.3 [a]	<LOD	<LOD	5.1 ± 0.3	103.5
Whole grain	9.9 ± 0.1	<LOD	<LOD	7.2 ± 0.6	72.7
Toast	13.0 ± 0.3	<LOD	<LOD	10.9 ± 0.3	84.0
White	6.5 ± 0.5	<LOD	<LOD	5.7 ± 0.2	88.3
Biscuit					
Butter cookie	4.2 ± 0.2 [a]	<LOD	<LOD	4.8 ± 0.6	115.2
Whole grain cookie	7.0 ± 0.7	<LOD	<LOD	7.1 ± 0.6	101.8
Chocolate cookie	3.7 ± 0.1 [a]	<LOD	<LOD	3.8 ± 0.3	102.9
Breakfast cereal					
Multicereal	5.2 ± 1.1 [a]	<LOD	<LOD	4.5 ± 0.4	87.3
Corn-based	10.5 ± 0.3	<LOD	<LOD	9.9 ± 0.9	94.1
Corn-based	<LOD	<LOD	<LOD	3.3 ± 1.1	n.c.[c]
Muesli	10.1 ± 2.7	<LOD	<LOD	8.0 ± 0.5	78.9
Flour					
Wheat	4.6 ± 0.3 [a]	<LOD	<LOD	3.9 ± 0.2	84.7
Whole	5.3 ± 0.2 [a]	<LOD	<LOD	5.3 ± 0.7	99.3
Wheat	10.5 ± 1.5	<LOQ	<LOQ	10.0 ± 0.3	95.7
Snack					
Corn	4.1 ± 0.2 [a]	<LOD	<LOD	3.6 ± 0.2	87.5
Corn	4.1 ± 1.5 [a]	<LOD	<LOD	3.1 ± 0.5	76.3
Corn	9.1 ± 0.5	<LOD	<LOD	6.7 ± 0.3	73.4

Pasta					
Noodle	7.7 ± 1.3	<LOQ	<LOD	8.7 ± 0.2	112.3
Spaghetti	4.9 ± 0.2 [a]	<LOQ	<LOD	6.0 ± 0.4	122.9
Macaroni	23.3 ± 1.2	<LOQ	<LOD	23.4 ± 0.5	100.4
Infant cereal					
Multicereals (with honey and fruits)	14.4 ± 0.2	2.55 ± 0.03	<LOD	14.0 ± 0.7	115.1
Organic spelt porridge	7.7 ± 0.3	<LOD	<LOD	8.1 ± 1.3	105.6
Multicereals (8 cereals with fruits)	15.9 ± 0.3	<LOD	<LOD	15.9 ± 0.2	99.8
Multicereals (5 cereals)	21.8 ± 0.8	<LOD	<LOD	22.0 ± 0.6	100.7
Multicereals (8 cereals with honey)	9.8 ± 0.3	<LOD	<LOD	10.5 ± 1.0	106.5
Multicereals (8 cereals)	14.4 ± 0.6	<LOD	<LOD	13.5 ± 0.5	94.0
Multicereals (cereals with honey)	24.1 ± 0.7	3.56 ± 0.08	<LOD	22.5 ± 0.3	108.1
Multicereals (8 cereals with fruits)	35.6 ± 0.8	9.4 ± 0.2	<LOD	26.0 ± 1.9	99.6
Rice	267.4 ± 11.5	175.0 ± 3.7	6.3 ± 0.5	74.3 ± 0.6	95.6

[a] Values below the LOQ for total As (6.0 µg As kg^{-1}).

[b] Calculated as the ratio of the sum of As species in the extract to total As.

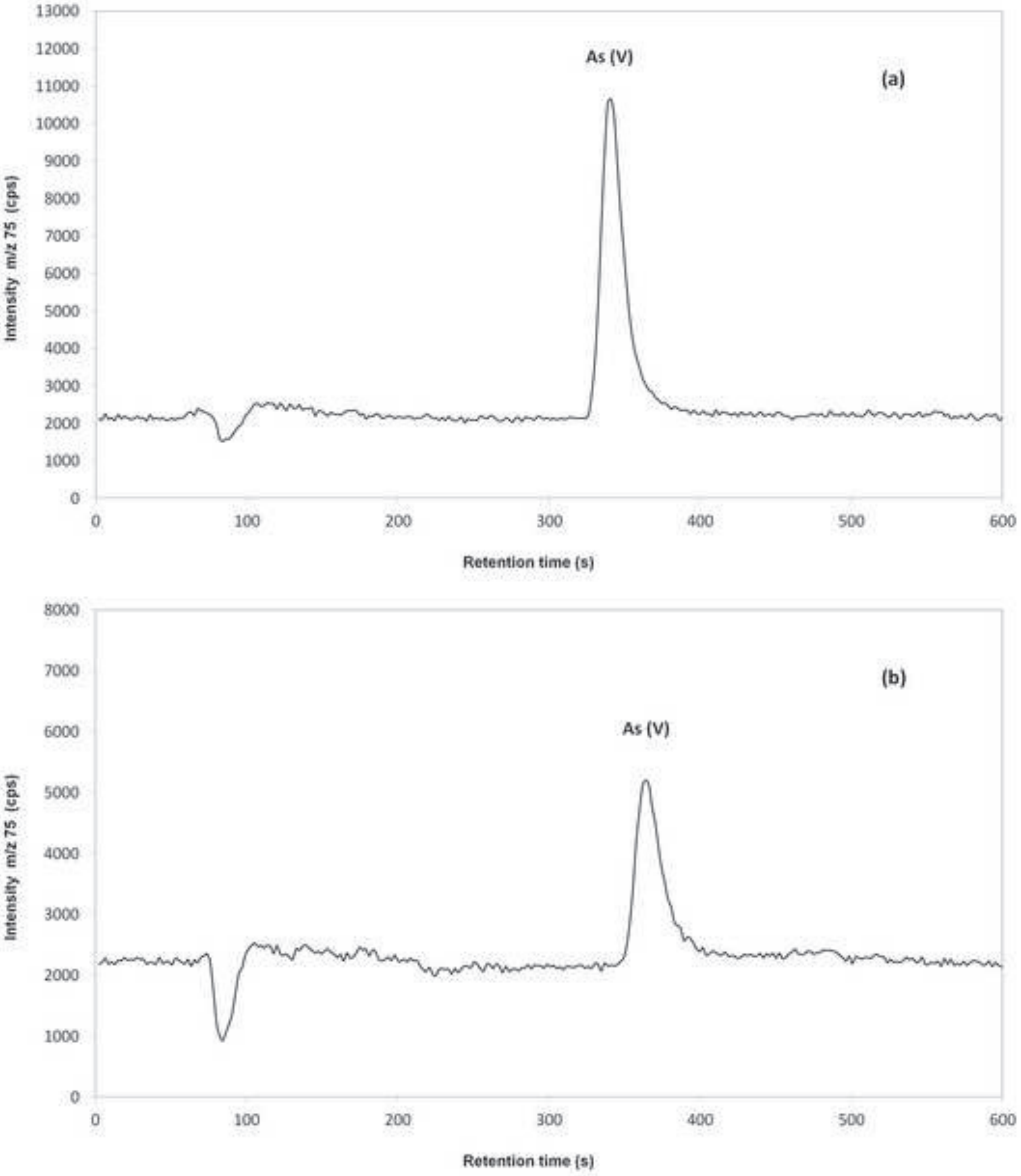
[c] No calculated.

Figure captions

Fig. 1. Chromatograms of (**a**) macaroni and (**b**) wheat flour extracts from anion exchange by LC–ICPMS.

Fig. 2. Chromatograms of (**a**) organic spelt infant cereal and (**b**) rice-based infant cereal extracts from anion exchange by LC–ICPMS.

Figure 1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11000
10000
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8000
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6000
5000
4000
3000
2000
1000
0
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As (V)
(a)
0
100
200
300
400
500
600
Retention time (s)
8000
7000
6000
5000
4000
3000
2000
1000
0
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(b)
As (V)
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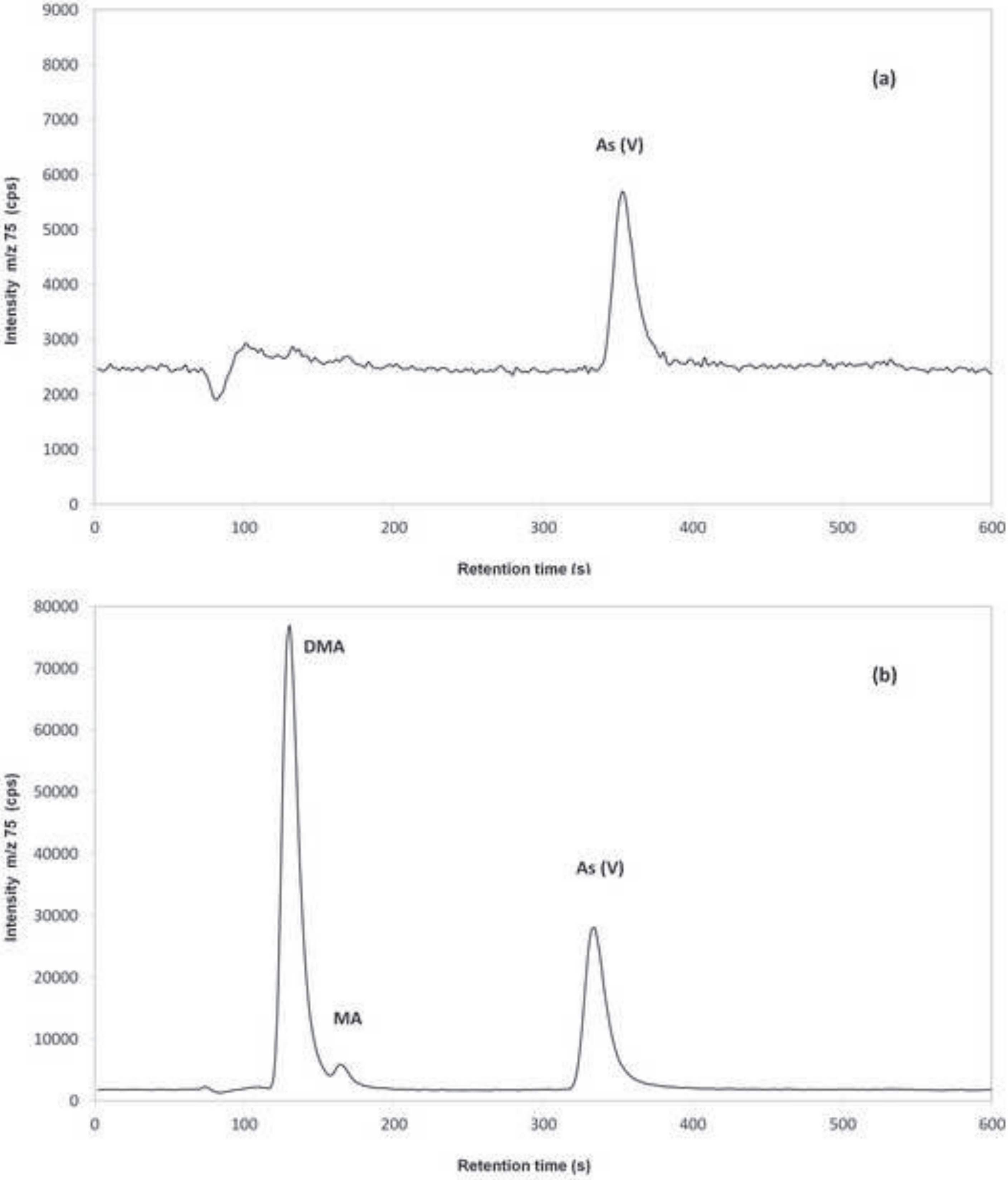
100
200
300
400
500
600
Retention time (s)

Figure 2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(a)
As (V)
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300
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Retention time (s)
80000
70000
60000
50000
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10000
0
Intensity m/z 75 (cps)
DMA
(b)
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As (V)
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400
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Retention time (s)