INVESTIGATION OF GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY USING DIRECT SOLID SAMPLE ANALYSIS FOR THE DETERMINATION OF ARSENIC IN FISH AND SEAFOOD

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Abstract

The determination of total arsenic in seafood is of great analytical interest due to the toxicity of some compounds of this element. A problem in the determination of total arsenic in fish and marine species is the presence of arsenobetaine (AB), which is a stable metabolic species and its chemical decomposition is very difficult. In this study, the feasibility of total As determination in seafood samples using direct solid sample (SS) analysis by graphite furnace atomic absorption spectrometry (GF AAS) was investigated. Pyrolysis and atomization temperatures of 1200°C and 2400°C,

respectively, were used. The chemical modifier was 0.1% Pd + 0.06% Mg + 0.06% Triton X-100. A matrix effect was observed when masses greater than 0.4 mg were analyzed. The results obtained for several certified reference materials using direct solid sample analysis were statistically not different from those found when using microwave-assisted digestion, but far below of the certified values. For this reason, SS-HR-CS GF AAS was used to investigate the existence of spectral interferences and inductively coupled plasma mass spectrometry (ICP-MS) was used to determine total arsenic content. Liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) was used to investigate the arsenobetaine (AB) concentration. The results obtained for the determination of total As with ICP-MS were statistically not different from the certified values. High concentration of AB was found by HPLC-ICP-MS when compared with the total As concentration. This fact suggests that the AB could be the cause of the differences observed in the determination of total As by AAS techniques.

Keywords: Arsenic; Arsenobetaine; Seafood; Direct solid sample analysis; Graphite furnace AAS;

1. Introduction

The consumption of fish has many benefits for the human health.[1] Fish provides a significant amount of polyunsaturated and highly unsaturated fatty acids and animal proteins.[2] However, fish can absorb metals (*e.g.* As) with bioaccumulative properties through the membrane surfaces, tissues and by ingestion of food and suspended material in water.[3]

The oxicity of arsenic compounds depends on their oxidation state, chemical form and solubility in the biological system. The As (III) is more toxic than As (V) and the inorganic species are more toxic than the organic ones. As (III) is ten times more toxic than As (V) and seventy times more toxic than monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA).[4] Arsenic can cause deleterious effects in the human body even at low concentrations. Increased risk of cardiovascular diseases and cancers in internal organs, skin and lung have been linked to arsenic contamination.[5,6] The organic species dominant in most seafood is arsenobetaine (AB),[7] which, in spite of the limited evidence, is considered non-toxic.[8]

In Brazil, the Ministry of Agriculture, Livestock and Food Supply (MAPA) accomplish the control of contaminants and residues in all kind of food supply. The National Agricultural Laboratories (LANAGRO) are part of MAPA and are recognized reference centers, acting in the development of analytical methods, as well in the research and monitoring programs of food contaminants.[9] Due to its high potential toxicity,[10] arsenic is included in the list of the substances controlled by MAPA. The maximum level of As established by the Brazilian National Program for Residue and Contaminant Control (NPRCC) in fish is 1000 μg kg^{-1}.[11] Thus, the monitoring of this element by highly sensitive, fast and reliable analytical methods is necessary for an efficient control of contamination and to increase the sample throughput.

Recent reviews emphasize that the main techniques used for the determination of arsenic in biological samples are: graphite furnace atomic absorption spectrometry (GF AAS), inductively coupled plasma optical emission spectrometry (ICP OES), inductively coupled plasma mass spectrometry (ICP-MS) and hydride generation atomic absorption spectrometry (HG AAS).[12,13] Appropriate selection of the sample preparation procedure in trace analysis is essential due to the integrity of chemical information that

strongly depends on the initial steps. The most frequently methods used in the preparation of food samples are dry ashing and wet digestion, preferably with microwave assistance.[14] However, direct analysis of solid samples (SS) is important due to its higher sensitivity, with no use of acids or other aggressive reagents, with the possibility of analyzing micro-samples for fast screening analysis.[14] The GF AAS technique is the most suited one for the direct SS analysis. The various stages of the electrothermal program allow the thermal transformation of the sample in the atomizer, destroying or removing the matrix.[15] The direct SS analysis in GF AAS often allows calibration against aqueous standards and the utilization of permanent chemical modifiers.[16] However, due to the complexity of biological samples, direct SS analysis requires careful investigation of the interferences using certifies reference materials (CRM), and/or conventional methods, such as acid digestion.[13]

An additional problem in the determination of total arsenic in fish and marine species by GF AAS is the presence of arsenobetaine. The AB, considered as non-toxic to humans, is a stable metabolic species and its chemical decomposition is very difficult.[17,18] The conversion of all organic arsenic species into inorganic is usually required for determination of total arsenic in the samples by atomic spectrometry. Consequently, the high stability of AB becomes unfavourable for the determination of the total amount of As.[7] Wet digestions using strong oxidizing agents combined with strong acids and high temperatures, are required for complete degradation of AB.[19] In some cases, even with the use of these reagents at higher temperatures, AB is not degraded completely and the results for the total concentration of arsenic is lower than the actual value.[20,21]

Despite the several methods proposed in the literature, the results are inconclusive for the determination of total As in seafood samples. This fact indicates the

need for careful research in developing new methods for determination of total As in seafood. To the best of our knowledge, the use of direct SS analysis for the determination of total As in seafood samples has not been described yet.

The goal of this work was, firstly, to develop an analytical method, based on SS-GF AAS, for the determination of As in seafood samples. However, the As concentrations in CRM were not in agreement with the certified values and an investigation was conducted in order to identify some possible interferences during AAS measurements. SS-HR-CS GF AAS was used to investigate the existence of spectral interferences and ICP-MS was used to determine total As concentration in seafood samples to compare obtained results with those found by AAS. HPLC-ICP-MS was used to determine the AB concentration, which could be a possible source of problems in the AAS measurements.

2. Experimental

2.1. Instrumentation

Atomic absorption measurements were carried out using an line source graphite furnace atomic absorption spectrometer model AAS Zeenit 650P (Analytik Jena AG, Jena, Germany) and a high-resolution continuum source graphite furnace atomic absorption spectrometer model contrAA 700 (Analytik Jena, Jena, Germany).

The AAS Zennit 650P is equipped with background correction by Zeeman effect (0.8 T). An As hollow cathode lamp was used as the radiation source with a lamp current of 6.0 mA. The analytical line at 193.7 was used with a spectral bandpass of 0.8 nm. The experiments were carried out using solid sampling platforms (Analytik Jena Part No. 407-152.316) and solid sampling tubes without a dosing hole (Analytik Jena

Part No. 407-152.303).

The contrAA 700 is equipped with a xenon short-arc lamp with a nominal power of 300 W operating in a hot-spot mode. The high-resolution double monochromator with a prism pre-monochromator and a high-resolution echelle monochromator with a linear CCD array detector with 588 pixels has a spectral resolution of 1.2 pm per pixel at the 193.69 nm As resonance line. Atomic absorption was measured using the center pixel (CP) ±1, corresponding to a spectral interval of 3.6 pm; however, the entire spectral range ±0.12 nm around the analytical line was displayed by the 200 pixels that are used for analytical purposes. Iterative background correction (IBC) mode was used throughout. The experiments were carried out using solid sampling platforms (Analytik Jena Part No. 407-152.023) and solid sampling tubes without a dosing hole (Analytik Jena Part No. 407-A81.303).

An M2P microbalance (Sartorius, Göttingen, Germany) with an accuracy of 0.001 mg has been used for weighing the samples directly onto the solid sampling platform, which was introduced into the graphite tube using a pair of pre-adjusted tweezers, which is part of the SSA 6 manual solid sampling accessory (Analytik Jena). The sample mass was transmitted to the instrument's computer to calculate the 'normalized integrated absorbance' (integrated absorbance calculated for 0.1 mg of the sample) after each measurement. The aqueous standards and modifier solution were injected manually onto the platform using a micropipette. Argon with a purity of 99.996% (White Martins, São Paulo, Brazil) was used as purge gas. The flow rate was kept at 2.0 L min^{-1} during all stages, except in the atomization, when the argon flow was stopped. The optimum parameters for the graphite furnace temperature program for the determination of As are given in Table 1.

An Agilent 7500ce ICP-MS (Agilent, Germany) with a nebuliser (BURGENER Ari Mist HP type) was used to measure total arsenic content. For arsenic speciation, HPLC-ICP-MS was used with an Agilent 1200 LC quaternary pump, equipped with an autosampler. The analytical column Zorbax 300-SCX (250mm x 4.6 mm, 5µm, Agilent, Germany) was protected by guard column filled with the corresponding stationary phase. The chromatographic system conditions used in the study was based on previous work.[22] The outlet of the LC column was connected via PEEK capillary tubing to the nebuliser of the ICP-MS system (Agilent 7500ce), which was the arsenic-selective detector. The ion intensity at m/z 75 (^{75}As) was monitored using time-resolved analysis software. Additionally, the ion intensities at m/z 77 ($^{40}Ar^{37}Cl$ and ^{77}Se) were monitored to detect possible argon chloride ($^{40}Ar^{35}Cl$) interference at m/z 75.

A microwave digestion system, Milestone Ethos Touch Control, with a microwave power of 1000 W and temperature control, was used for digestion. The fish and seafood samples were lyophilized in a lyophilizer ModulyonD Freeze Dryer (Thermo Electron Corporation, USA) and milled in a micro-mill A 11 Basic (IKA – Werke, Germany).

2.2. Reagents and solutions

Analytical grade reagents were used exclusively. Deionized water with a specific resistivity of 18 MΩ cm^{-1} from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used for the preparation of standards, modifier solutions and digestions. All containers and glassware were soaked in 3 mol L^{-1} nitric acid for at least 24 h and rinsed three times with water before use. The nitric acid (Merck, Darmstadt, Germany) used for the preparation of standards, modifier solutions and digestions was further purified by sub-boiling distillation in a quartz sub-boiling still (Kürner Analysentechnik,

Rosenheim, Germany). Arsenic stock solution (1000 mg L^{-1}) was prepared from Titrisol concentrate (Merck, Germany). The working standards were prepared by serial dilution of the stock solutions with the addition of 0.014 mol L^{-1} nitric acid (Merck, Germany). Arsenobetaine from $(CH_3)_3$ $As^+CH_2COO^-$ was supplied by NMIJ (Japan), NMIJ CRM 7901-a, standard solution. The chemical modifier utilized was 0.1% Pd + 0.06% Mg + 0.06% Triton X-100 (Pd, Mg as the nitrates from Merck, Germany, and Triton X-100 from Union Carbide; all concentrations in % m/v). The following reagents were used for sample digestion: 30% H_2O_2 and purified HNO_3 (both from Merck, Germany), NaOH, 2 mol L^{-1} (Nuclear, Brazil), TMAH 25% m/v ($C_4H_{12}NOH$, 91.15 g mol^{-1}, Aldrich, Germany).

2.3. Certified Reference Materials and samples

The following certified reference materials (CRM) were used in this work for validation and method development: DOLT-4 (Dogfish Liver), TORT-2 (Lobster Hepatopancreas) and DORM-3 (Fish Protein) from National Research Council (NRC, Canada), NIST SRM 2976 (Mussel Tissue) and NIST SRM 1566b (Oyster Tissue) both from National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA), BCR-627 (Tuna Fish Tissue) and ERM-CE278 (Mussel Tissue) both from European Commission Joint Research Centre (Belgium). The reference material (RM) 9th PT (Fish Protein) of CRL-ISS, (Italy) was also analyzed.

Four fish muscle samples of different kind of fishes were provided by the Laboratory of Trace Metals and Contaminants (LANAGRO/RS) from the Ministry of Agriculture, Livestock and Supply (MAPA/Brazil). The oyster, clam and shrimp samples were purchased from local supermarkets in Barcelona.

All samples were initially washed with Milli-Q water, cut, and homogenized

using a blender (non-contaminating kitchen mixer), and then, the samples were lyophilized during 5 hours. After this procedure, the samples were ground in a vibratory mill and sieved with a polyester sieve, mesh size of 85 μm to improve the particle size distribution. In order to avoid segregation, the part of the sample that did not pass through the sieve was ground again, until all lyophilized material passed the sieve.

2.4. Direct analysis of fish and seafood samples and CRM

The samples (0.01 to 0.25 mg) were weighed directly onto the platform, and then 15 μL of chemical modifier was added over the sample. The platform was introduced into the graphite furnace as described above, for the determination of total As, using the graphite furnace temperature program shown in Table 1.

2.5. Digestion Methods

In order to assess the accuracy the results obtained by the direct sampling method, three different digestion methods were investigated, as described below.

2.5.1. Microwave-assisted acid digestion

Initially, approximately 250 mg of CRM, lyophilized fish and seafood samples were weighed and introduced into the digestion vessels, and 8 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added. The mixtures were digested according to the following program: 10 min from room temperature to 90 °C, maintained for 10 min at 90 °C, 10 min from 90 °C to 120 °C 10 min from 120 °C to 190 °C and 10 min maintained at 190 °C. After cooling to room temperature, the digested samples were diluted with water up to 20 mL. The digested samples were analyzed by ICP-MS and by GF AAS.

2.5.2. Alkaline Digestion with TMAH and NaOH

The method used for alkaline digestion with TMAH was based on the work of Pereira *et al.*[23]. A 25% (w/v) TMAH solution (5 mL) was mixed with 0.05 g CRM TORT-2, and heated to 90–95 °C for 6 h. After cooling to room temperature, the solution was analyzed by GF AAS.

The method used for alkaline digestion with NaOH was based on the work of Geng *et al.*[24]. Five mL of NaOH 2 mol L^{-1} solution was mixed with 0.05 g CRM TORT-2 and heated to 90–95 °C for 4 h. After cooling to room temperature, the solution was analyzed by GF AAS.

2.6. Total arsenic determination by ICP-MS

The samples digested by microwave were diluted for the final measurements when necessary. Helium gas was used in the collision cell to remove interferences in the ICP-MS measurements. [103] The samples were quantified by means of an external calibration curve from arsenate standards. Triplicate analyses were performed for each sample. For quality control purposes, the standards of the calibration curve were run before and after each sample series. The corresponding digestion blanks (one for each sample digestion series) were also measured. Quality control standard solutions at two concentration levels were measured after the calibration curve. To assess the accuracy of the ICP-MS method, six CRM (BCR-627, DOLT-4, TORT-2, ERM-CE278, SRM 2976 and SRM 1566b) have been analyzed.

2.7. Arsenobetaine determination

The extraction procedure of As species is based on our previous study.[22] The CRM, lyophilized fish and seafood samples were weighed into the digestion vessels and

10 mL of nitric acid 0.2% (v/v) hydrogen peroxide 1% (v/v) mixture were added. The resulting mixtures were centrifuged at 3500 rpm (25 min) and the supernatants filtered through PET filters (Chromafil PET, Macherey–Nagel, pore size 0.45 μm). AB determination was carried out by HPLC-ICP-MS using the method previously applied.[22] AB in extracts was identified by comparison of retention times with the standard. An external calibration curve was used to quantify AB with the corresponding standards. Extraction blanks were also analyzed by HPLC–ICP-MS in each work session. An AB quality control standard solution was measured in each speciation run. Each sample was extracted and analyzed in triplicate. For AB accuracy, a CRM BCR-627 (Tuna fish), which has a certified content of AB, was analyzed throughout the sample analyses.

3. Results and discussion

3.1. Optimization of SS-HR-CS GF AAS and SS-GF AAS analysis conditions

The graphite furnace temperature program (Table 1) was optimized for the direct determination of As in fish and seafood samples using the CRM TORT-2 for both techniques. Two drying temperatures with slow ramp rates and long hold times were utilized as shown in Table 1. It was necessary to perform a homogenous heating, thus preventing the overflow and splash of chemical modifier. Additionally, an "ash" step was used in SS-HR-CS GF AAS measurements. In this stage, air was utilized as an alternate gas (2 L min^{-1}) to oxidize the sample and thus reduce the carbon residues on the platform.

In this work, the use of palladium and magnesium mixture as chemical modifier was investigated. This modifier is necessary for the determination of As in marine samples because, according to the literature, it stabilizes the organic and inorganic

forms of As up to temperatures of 1200 ℃ in GF AAS measurements.[25-27] A preliminary study of the amount of modifier was performed in order to find the optimum mass that thermally stabilizes As. Masses between 2.5 and 20 µg Pd and 1.5 to 12 µg Mg with a pyrolysis temperature of 1200°C and an atomization temperature of 2400°C were investigated. The highest analytical signal for the maximum amount of sample was obtained with 15 µg Pd and 9 µg Mg for As in TORT-2. Therefore, 15 µL of modifier, with the composition of 15 µg Pd + 9 µg Mg in 0.06% (v/v) Triton X-100 solution were used in further optimization studies for As for both techniques.

Pyrolysis curves obtained for the CRM TORT-2 and for 1 ng As in aqueous standards with the Pd/Mg modifier are shown in Figure 1. For the TORT-2, reliable measurement of analyte signals was only possible with pyrolysis temperatures higher than 300 °C by SS-GF AAS and higher than 200 ℃ by SS-HR-CS GFAAS. The same was observed by Giacomelli *et al.*[28] in their GF AAS measurements. A pyrolysis temperature of 1200 °C was chosen and used in all further experiments for both techniques.

Peak shapes and background absorption were also considered when choosing the proper furnace conditions for As. Typical atomization and background signals are shown in Figure 2 for SS-GF AAS. An atomization temperature of 2400 °C for As was chosen based on the sensitivity and peak shapes for both, the aqueous standard and the CRM TORT-2.

The correlation between sample mass and integrated absorbance was evaluated using the CRM TORT-2 (Figure 3). The absorbance signal does not increase any more with increasing sample mass above 0.4 mg, indicating that in this condition there is a strong influence of the matrix. For the linear part of this figure (up to 0.4 mg), there was a good linear correlation ($R^2 \cong 0.96$) between sample mass and integrated absorbance.

Similar study was also performed with other samples and good linearity was observed up to 0.25 mg for Whitefish ($R^2 \cong 0.95$), 0.28 mg for Hake-1 ($R^2 \cong 0.95$) and 0.30 for Red Porgy ($R^2 \cong 0.97$).

3.2. Figures of merit

The analytical figures of merit obtained for As by SS-GF AAS and by SS-HR-CS GF AAS are shown in Table 2. Calibration curves were established using a blank and five calibration solutions in the concentration range of 15-200 μg L^{-1} (0.15-2.0 ng).

The limits of detection and quantification have been calculated for 0.25 mg of sample, which corresponds to the maximum sample mass that could be used, as discussed above. The blank measurements were carried out according to the 'zero mass response' technique,[29] introducing repeatedly a solid sampling platform containing only the modifier into the furnace, followed by a regular atomization cycle.

The characteristic mass (m_0) values based on an aqueous standard obtained for As by SS-GF AAS are in good agreement with those reported in the literature.[15] The SS-HR-CS GF AAS technique shows higher sensitivity (LOD and m_0) and better linearity than SS-GF AAS. However, the LOQ for both techniques are far below the values established by NPRCC.[11]

The precision of the SS-GF AAS and SS-HR-CS GF AAS method for As determination was calculated from six consecutive measurements in seven real samples (SS-GF AAS), four fish samples (SS-HR-CS-GF AAS), five CRMs (SS-HR-CS-GF AAS) and seven CRMs (SS-GF AAS). The precision values were expressed as the relative standard deviation (RSD), varying between 2% and 9% (Table 3).

3.3 Analytical results

The results obtained for the determination of As in seven fish samples, six CRM and one RM using calibration with aqueous standard solutions by SS-GF AAS are summarized in Table 3. Clearly, the results obtained for the CRM were not in agreement with the certified values.

Due to these results, an additional calibration method was investigated. Another alternative for calibration in direct solid sample analysis is the use of a correlation curve established plotting the normalized integrated absorbance for several reference materials against the certified concentration.[30] The correlation curve was established using the following CRM: DOLT-4, DORM-3, TORT-2, SRM 2976, SRM 1566b, BCR-627 and ERM-CE278 and determination by SS-GF AAS. The arsenic concentration range used was between 4.8 and 21.6 mg kg^{-1}. The Pd/Mg chemical modifier was used and SS-GF AAS measurements were performed in six replicates for each CRM. The linear correlation equation was A = 0.00198 + 0.00875c with a linear correlation coefficient (R^2) of 0.947. The linear curve is shown in the supplementary material. The R^2 value did not show good linearity and three CRM were outliers. These results could be explained by the different intrinsic characteristics of the CRM. For example, the TORT-2 has about 60% AB, while the CRM 1566b has about 16%[24] (the total concentration of AB for all CRM used in the correlation curve is shown in Table 3). As previously mentioned, the AB is a chemically stable compound[17] and the dependence of its quantity may compromise the linearity of the curve. In this case, we concluded that the correlation curve is not a good calibration technique for the determination of As in fish and seafood samples by SS-GF AAS.

3.3.1. Digestion results

Due to the difficulty of finding the certified value for the CRM, we have decided to investigate the conventional method of acid digestion. The results obtained by microwave-assisted acid digestion using GF AAS, are summarized in the Table 3. The concentrations obtained for all CRMs showed significant differences with certified values: TORT-2 (~44%), DORM-3 (~49%), SRM 2976 (~55%), SRM 1566b (~56%), DOLT-4 (~42%), BCR 627 (~46%), ERM-CE278 (~64%) and 9th PT (~43%). On the other hand, the results obtained by microwave digestion are not significantly different from the SS-GF AAS results at a 95% confidence level.

Due to this problem, other three digestions methods (according to item 2.5) were investigated for CRM TORT-2: Two alkaline digestions based on publications by Pereira *et al.*[31] and Wenhua Geng *et al.*[24]. The results for the determination of As in TORT-2 by GF AAS with two alkaline digestions were: 9.7 ± 0.42 mg kg^{-1} for digestion with NaOH and 9.6 ± 0.31 mg kg^{-1} for digestion with TMAH. In comparison with the value found by SS-GF AAS (9.4 ± 0.67 mg kg^{-1}), the Analysis of Variance (ANOVA) was applied and the values are not significantly different at the 95% confidence level. However, the concentration obtained for the CRM TORT-2 showed a significant difference of approximately 55% from the certified value.

The values obtained by the microwave-assisted acid and alkaline digestion methods were different and a possible reason could be an incomplete decomposition of AB. This problem has also been reported in the literature.[20,32,33]. Narukawa *et al.*[20] reported that complete decomposition of AB was achieved only in the presence of $HClO_4$ and temperatures of 320 °C. Slejkovec *et al.*[32] developed a digestion method using the mixture of acids (HNO_3-H_2SO_4-H_2O_2) and a temperature of 300 °C. As can be seen, the existence of certain As species in marine materials can have a significant influence on the determination of total As using SS-GF AAS.

3.3.2. Least-squares background correction in SS-HR-CS GF AAS

SS-HR-CS GF AAS was used to investigate the possible existence of spectral interferences in the determination of As in fish samples and CRM. According to Santos *et al*.,[34] arsenic has been recognized as an element of difficult determination by GF AAS due to spectral and non-spectral interferences. Structured background caused by phosphates are recognized to be the main spectral interference in the determination of As in seafood by GF AAS.[34]

Least-squares background correction (LSBC) was used to subtract the phosphate reference background spectrum (PO) from the CRM TORT-2 spectrum. The PO reference spectrum was recorded and stored with 0.4 mg of $NH_4H_2PO_4$. The salt was weighed directly onto the platform follow by the addition of 15 μg Pd + 9 μg Mg + 0.06% (v/v) Triton X-100 as chemical modifiers and introduced into the graphite furnace.

The time-resolved absorbance spectra in the vicinity of the 193.696 analytical line recorded for TORT-2 with and without correction by LSBC are shown in Figure 4. Figure 5 shows the absorption spectra with (Figure 5b) and without (Figure 5a) correction by LSBC using $NH_4H_2PO_4$ as the reference spectrum. As can be seen in Figures 4 and 5, the LSBC was applied successfully. The SS-HR-CS GF AAS measurements confirm the presence of a phosphate interference in the As determination, which is in agreement with the literature.[34,35] The use of HR-CS-GF AAS clearly shows the phosphate spectrum, which cannot be seen with the use of a line source equipment. The LSBC using $NH_4H_2PO_4$ was effective for the correction of spectral interference in CRMs and fish samples.

The results obtained for the determination of As in CRMs and fish samples by SS-HR-CS GF AAS are summarized in Table 3. The As concentration obtained by SS-GF AAS and SS-HR-CS GF AAS (Table 3) are not significantly different. In spite of the correction of the phosphate interference, the concentrations obtained for all CRM showed significant differences with certified values: TORT-2 (~44%), DORM-3 (~50%), SRM 2976 (~33%), SRM 1566b (~64%), DOLT-4 (~43%) and 9th PT (~42%). The values obtained after microwave-assisted acid digestion are not significantly different either at the 95% confidence level (student-t test) from the results obtained by HR-SS-CS GF AAS.

3.3.3. ICP-MS measurements

Due to the fact that the concentration of total As (certified value) was lower than expected by using GF AAS, ICP-MS was used to determine total arsenic, since the ICP-MS is a widely used technique for determination of As in fish and seafood.[12] The results obtained for the determination of As using microwave digestion in four fish and three seafood samples, six CRM and one RM by ICP-MS were determined in our previously work[36] and are summarized in the Table 3. The precision was calculated from three consecutive measurements and the results obtained for the CRM were in agreement with the certified values (Table 3). .

In spite of numerous attempts to obtain the certified value by GF AAS techniques, the values found did not correspond to the expected ones. The most probable cause is the presence of AB, which has a high stability and high resistance to degradation, as mentioned before. Thus, a speciation analysis was performed to determine the amount of AB in the samples.

3.3.4. HPLC-ICP-MS measurements

An extraction and determination (described in section 2.7.), based on HPLC-ICP-MS, developed in previous work,[22,36] was used in order to identify and quantify the AB in the samples and CRM investigated. For assessment of the accuracy of AB speciation by HPLC-ICP-MS, the CRM BCR-627 (which contains a certified concentration of AB) was used. The concentration of AB obtained for this CRM was in agreement with the certified value (Table 3). The results obtained for the determination of AB in CRM and samples using HPLC-ICP-MS were also determined in our previously work[36] and are summarized in the Table 3. The precision was calculated from three consecutive measurements in seven real samples, six CRMs and one RM and the values were expressed as the RSD, varying between 1% and 9%. As reported in the literature,[30,37] AB is the major arsenic species in all samples and CRM, with an exception for SRM 1566b (oyster tissue).

An interesting result was observed in all CRM: the sum of AB concentration obtained by HPLC-ICP-MS and the total As concentration determined by GF AAS techniques, is in a good agreement with the certified values (Table 4). In the case of samples, the comparison was made between the sum and the values obtained by ICP-MS. A Student's-t test was applied to the data of Table 4 and all CRM and samples are not significantly different at a 95% confidence level, except SRM 2976, BCR-627, Hake-1 Red porgy and White fish. In the case of the SRM 2976 the value obtained with SS-HR-CS GF AAS is much closer to the certified value, which indicates the presence of a spectral interference in the case of SS-GF AAS. In all the other cases, the most likely explanation for the high values after summation of the measured value and that for AB is that in these cases part of the AB could be measured using GF AAS. This obviously requires more research to be confirmed.

4. Conclusion

The results obtained for As determination in fish and seafood samples using SS-GF AAS, SS-HR-CS GF AAS and different digestion methods are not different statistically. However, the results obtained for CRM are not in agreement with the certified values.

The use of SS-HR-CS GF AAS revealed the presence of spectral interferences caused by phosphates. These interferences were corrected, but still not allowed to obtain the certified values. The correlation coefficient obtained in the correlation curve was not satisfactory, making its use for calibration for direct solid sample analysis infeasible. This problem probably occurrs due to the different characteristics of the CRM.

The results obtained for the determination of total As, with the ICP-MS, were statistically not different from the certified values. High concentration of AB determined by HPLC-ICP-MS, was found in all CRM and samples when compared with the total As concentration. This fact suggests that the AB could be the cause of the differences observed in the determination of total As by AAS techniques, under the conditions used in this work. It should be emphasized that more investigations should be done in order to determine the total As in fish and seafood samples by GF AAS techniques. Since the AB is a non-toxic compound for the humans, the AAS techniques might be used for the determination of total toxic arsenic species in fish and seafood.

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6. References

1. O. P. Ward and A. Singh, *Process Biochem.*, 2005, **40**, 3627-3652.

2. K. V. Dhaneesh, K. M. Noushad and T. T. Ajith Kumar, *PLoS ONE*, 2012, **7**, e45439.

3. M. Alam, A. Tanaka, G. Allinson, L. Laurenson, F. Stagnitti and E. Snow, *Ecotoxicol. Environ. Saf.*, 2002, **53**, 348-354.

4. Y. Chen, J. Yao, F. Wang, Y. Zhou, H. Chen, N. Gai, H. Chen, K. Chen, T. Maskow and B. Ceccanti, *Curr. Microbiol.*, 2008, **57**, 258-263.

5. H. Lin, S. Chen, C. Shen and C. Chu, *J. Food Drug Anal.*, 2008, **16**, 70-75.

6. S. Kapaj, H. Peterson, K. Liber and P. Bhattacharya, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst.*, 2006, **41**, 2399-2428.

7. T. Narukawa, T. Kuroiwa, I. Narushima and K. Chiba, *Anal. Sci.*, 2008, **24**, 355-360.

8. J. Borak and H. D. Hosgood, *Regul. Toxicol. Pharmacol.*, 2007, **47**, 204-212.

9. M. A. Queiroz and E. S. Lins, *Food Addit. Contam., Part A*, 2012, **29**, 482-489.

10. G. F. Norderberg, B. A. Fowler, L. Friberg and M. Norderberg, *Handbook on the toxicology of metals*, Academic Press, London, third edn., 2011.

11. Amending Regulation (EC) No. 1881/2006 laying down maximum levels for certain contaminants in foodstuffs. Commission Regulation No. 629/2008, Oj No. 364, 20.12.2006 http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:364:0005:0024:EN:PDF, (accessed July 2014).

12. A. Taylor, M. P. Day, S. Hill, J. Marshall, M. Patriarca and M. White, *J. Anal. At. Spectrom.*, 2013, **28**, 425-459.

13. J. Sardans, F. Montes and J. Peñuelas, *Spectrochim. Acta, Part B*, 2010, **65**, 97-112.

14. M. G. A. Korn, E. S. B. Morte, D. C. M. B. Santos, J. T. Castro, J. T. P. Barbosa, A. P. Teixeira, A. P. Fernandes, B. Welz, W. P. C. Santos and E. B. G. N. Santos, *Appl. Spectrosc. Rev.*, 2008, **43**, 67-92.

15. B. Welz and M. Sperling, *Atomic absorption spectrometry*, Wiley-VCH, Weinheim, third edn., 1999.

16. M. G. R. Vale, N. Oleszczuk and W. N. Santos, *Appl. Spectrosc. Rev.*, 2006, **41**, 377-400.

17. J. Edmonds and K. Francesconi, in *Organometallic compounds in the environment*, John Wiley & Sons, Guildeford, 2003, ch. 195, pp. 195-222.

18. A. Leufroy, L. Noël, V. Dufailly, D. Beauchemin and T. Guérin, *Talanta*, 2011, **83**, 770-779.

19. J. Entwisle and R. Hearn, *Spectrochim. Acta, Part B*, 2006, **61**, 438-443.

20. T. Narukawa, T. Kuroiwa, K. Inagaki, A. Takatsu and K. Chiba, *Appl. Organomet. Chem.*, 2005, **19**, 239-245.

21. T. Narukawa, T. Kuroiwa, T. Yarita and K. Chiba, *Appl. Organomet. Chem.*, 2006, **20**, 565-572.

22. T. Llorente-Mirandes, J. Calderón, F. Centrich, R. Rubio and J. F. López-Sánchez, *Food Chem.*, 2014, **147**, 377-385.

23. L. A. Pereira, C. C. Windmöller, J. B. B. Silva and W. Borges Neto, *Quim. Nova*, **34**, 1167-1172.

24. W. Geng, R. Komine, T. Ohta, T. Nakajima, H. Takanashi and A. Ohki, *Talanta*, 2009, **79**, 369-375.

25. M. Deaker and W. Maher, *J. Anal. At. Spectrom.*, 1999, **14**, 1193-1207.

26. E. H. Larsen, *J. Anal. At. Spectrom.*, 1991, **6**, 375-377.

27. V. I. Slaveykova, F. Rastegar and M. J. Leroy, *J. Anal. At. Spectrom.*, 1996, **11**, 997-1002.

28. M. B. l. O. Giacomelli, M. C. Lima, V. Stupp, R. M. de Carvalho Júnior, J. B. Borba da Silva and P. B. Barrera, *Spectrochim. Acta, Part B*, 2002, **57**, 2151-2157.

29. U. Kurfürst, *Solid sample analysis: Direct and slurry sampling using GF-AAS and ETV-ICP*, Springer, Berlin, first edn., 1998.

30. Y. Shibata, J. Yoshinaga and M. Morita, *Appl. Organomet. Chem.*, 1994, **8**, 249-251.

31. L. d. A. Pereira, C. C. Windmöller, J. B. B. d. Silva and W. Borges Neto, *Quim. Nova*, **34**, 1167-1172.

32. Z. Šlejkovec, J. T. van Elteren and U. D. Woroniecka, *Anal. Chim. Acta*, 2001, **443**, 277-282.

33. W. Goessler and M. Pavkov, *Analyst*, 2003, **128**, 796-802.

34. C. Santos, F. Alava-Moreno, I. Lavilla and C. Bendicho, *J. Anal. At. Spectrom.*, 2000, **15**, 987-994.

35. R. F. Herber and M. Stoeppler, *Trace element analysis in biological specimens*, Elsevier Science, Amsterdam, 1st edn., 1994, vol. 15.

36. A. V. Zmozinski, T. Llorente-Mirandes, J. F. López-Sánchez and M. M. da Silva, *Food Chem.*, Accepted.

37. W. R. Cullen and K. J. Reimer, *Chem. Rev.*, 1989, **89**, 713-764.

Table 1. Graphite furnace temperature program for the determination of As in fish samples by SS-GF AAS and SS-HR-CS GF AAS.

Stage	Temperature / °C	Ramp / °C s^{-1}	Hold time / s
Drying 1	110	15	20
Drying 2	150	20	45
Ash[a]	600	200	30
Pyrolysis	1200	300	35
Atomization	2400	FP[b]	8
Cleaning	2400	1000	8

[a] air used as an alternate gas [b] FP: full power.

Table 2. Analytical figures of merit obtained for the determination of As using SS-GF AAS and SS-HR-CS GF AAS.

Technique	Linear Regression Equation	R^2	m_0 pg	LOD[a] (n=10) µg kg^{-1}	LOQ[a] (n=10) µg kg^{-1}
SS-GF AAS	A = 0.0078 + 0.1656m(ng)	0.9937	25	0.10	0.34
SS-HR-CS GF AAS	A = 0.0046 + 0.1954m(ng)	0.9985	20	0.05	0.16

[a] Based on 'zero mass response' technique and calculated for the 0.25 mg of sample.

Table 3. Determination of As in fish samples and CRMs using direct solid sampling (SS). GF AAS. HR-CS GF AAS and ICP-MS. Determination of AB by HPLC-ICP-MS.

Sample	Certified value (mg kg^{-1})	Concentration of As by GF AAS mg kg^{-1} (mean ± SD)						Concentration of As and AB[b] by ICP-MS mg kg^{-1} (mean ± SD)			
		SS-GF AAS (n=6)	RSD (%)	SS-HR-CS GF AAS (n=6)	RSD (%)	GF AAS (n=3)	RSD (%)	ICP-MS[d] (n=3)	RSD (%)	HPLC-ICP MS[d] (AB[b])(n=3)	RSD (%)
TORT-2	21.6 ± 1.80	9.4 ± 0.67	7	9.5 ± 0.42	4	9.4 ± 0.21	2	22.4 ± 1.10	5	13.1 ± 0.45	3
DORM-3	6.88 ± 0.30	3.4 ± 0.23	7	3.5 ± 0.19	5	2.9 ± 0.03	1	-	-	-	-
SRM 2976	13.3 ± 1.80	7.4 ± 0.40	5	4.4 ± 0.37	5	-	-	13.7 ± 0.25	1	10.3 ± 0.20	2
SRM 1566b	7.65 ± 0.65	4.3 ± 0.25	2	4.9 ± 0.33	2	-	-	7.67 ± 0.13	2	2.63 ± 0.07	3
DOLT-4	9.66 ± 0.62	4.1 ± 0.10	6	4.2 ± 0.33	6	-	-	9.64 ± 0.11	1	5.17 ± 0.51	9
BCR-627	4.80 ± 0.30 [a]	2.2 ± 0.17	7	-	-	-	-	4.84 ± 0.13	3	3.80 ± 0.07	2
ERM-CE278	6.07 ± 0.13	3.9 ± 0.27	7	-	-	-	-	6.09 ± 0.21	3	2.27 ± 0.17	7
9th PT	6.65 ± 0.71[c]	2.9 ± 0.14	5	2.8 ± 0.25	8			7.00 ± 0.32	4	4.30 ± 0.19	4
Hake-1	-	2.5 ± 0.16	6	2.0 ± 0.07	3	1.9 ± 0.07	4	7.1 ± 0.04	1	6.5 ± 0.19	3
Hake-2	-	1.2 ± 0.09	7	1.0 ± 0.07	7	1.1 ± 0.04	3	4.2 ± 0.11	3	3.2 ± 0.19	6
Red porgy	-	15.0 ± 0.92	6	13.8 ± 0.37	3	15.0 ± 0.54	4	35.0 ± 0.16	1	33.2 ± 2.70	8
White fish	-	17.2 ± 0.60	3	17.5 ± 0.80	4	17.5 ± 0.75	4	35.2 ± 1.14	3	33.5 ± 2.95	8

Shrimp	-	0.84 ± 0.07	9	-	-	-	-	2.33 ± 0.07	3	1.44 ± 0.02	1
Oyster	-	8.0 ± 0.67	8	-	-	-	-	24.6 ± 0.60	2	15.9 ± 0.65	4
Clams	-	7.30 ± 0.64	9	-	-	-	-	17.0 ± 1.40	8	11.7 ± 0.73	6

[a] 3.9 ± 0.20 is the certified value of arsenobetaine (AB);

[b] Arsenobetaine (AB) concentration;

[c] Reference value;

[d] Values obtained from reference 36.

Table 4. Determination of As in CRM, fish and seafood samples using direct solid sampling (SS) and GF AAS added with concentrations of arsenobetaine (AB) obtained by HPLC-ICP-MS. The results are expressed in mg kg^{-1} of dry mass (mean ± standard deviation. n = 3).

Samples	Certified value	ICP-MS	Techniques		
			SS-GF AAS + AB	SS-HR-CS-GF AAS + AB	GF AAS + AB
TORT-2	21.6 ± 1.80	22.4 ± 1.10	22.5 ± 1.12	22.6 ± 0.87	22.3 ± 0.66
SRM 2976	13.3 ± 1.80	13.7 ± 0.25	17.7 ± 0.60	14.7 ± 0.57	-
SRM 1566b	7.65 ± 0.65	7.67 ± 0.13	6.93 ± 0.32	7.53 ± 0.40	-
DOLT-4	9.66 ± 0.62	9.64 ± 0.11	9.27 ± 0.61	9.37 ± 0.84	-
BCR-627	4.80 ± 0.30	-	6.00 ± 0.24	-	-
ERM-CE278	6.07 ± 0.13	-	6.27 ± 0.44	-	-
9^{th} PT	6.65 ± 0.71[a]	7.00 ± 0.32	7.20 ± 0.33	7.10 ± 0.44	-
Hake-1	-	7.10 ± 0.04	9.00 ± 0.35	8.50 ± 0.26	8.40 ± 0.26
Hake-2	-	4.20 ± 0.11	4.40 ± 0.28	4.20 ± 0.26	4.30 ± 0.24
Red porgy	-	35.0 ± 0.16	48.2 ± 3.63	47.0 ± 3.08	48.2 ± 3.25
White fish	-	35.2 ± 1.14	50.7 ± 3.55	51.0 ± 3.75	51.0 ± 3.70

Shrimp	-	2.33 ± 0.07	-	-	2.28 ± 0.10
Oyster	-	24.6 ± 0.6	-	-	23.9 ± 1.44
Clams	-	17.0 ± 1.40	-	-	19.0 ± 1.40

Figure Captions

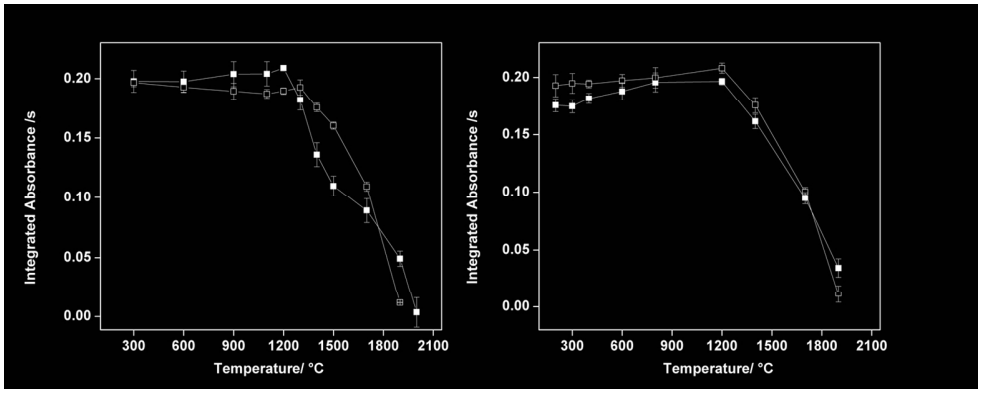
Fig. 1 Pyrolysis curves for As (a) using SS-GF AAS and (b) using SS-HR-CS GF AAS for: (□) 1 ng As in 10 µL of 0.014 mol L^{-1} HNO_3 aqueous solution and (■) TORT-2 (absorbance signal normalized for 0.1 mg of the sample. Atomization temperature = 2400 °C. In both cases, the chemical modifier used was 15 µg Pd + 9 µg Mg + 0.06% (v/v) Triton X-100. Error bars refer to the SD of 3 and 6 consecutive measurements of the standard solution and CRM TORT-2, respectively.

Fig. 2 Absorbance signals for As using SS-GF AAS. The solid lines represent atomic absorption and dotted lines represents the background signal. (a) Tort-2 (b) 1 ng As. Both in the presence of 15 µg Pd + 9 µg Mg + 0.06% (v/v) Triton X-100 as the chemical modifier. T_{at} = 2400 °C and T_{pyr} = 1200 °C.

Fig. 3 Influence of the sample mass (TORT-2) on the integrated absorbance value using 15 µg Pd + 9 µg Mg + 0.06% (v/v) Triton X-100 as chemical modifier, T_{pyr} = 1,200 °C and T_{at} = 2400 °C.

Fig. 4 Time-resolved absorbance spectrum in the vicinity of the 193.696 analytical line using SS-HR-CS GF AAS recorded for TORT-2 (a) without correction and (b) after correction using LSBC and $NH_4H_2PO_4$ as a reference spectrum. In both cases 15 µg Pd + 9 µg Mg + 0.06% Triton X-100 was used as chemical modifier. T_{pyr} = 1200 °C and T_{at} = 2400 °C.

Fig. 5 Absorption spectra in the vicinity of the 193.696 nm analytical line for the CRM TORT-2 using SS-HR-CS GF AAS (a) without correction (b) after correction with LSBC using $NH_4H_2PO_4$ as reference spectrum. In both cases 15 μg Pd + 9 μg Mg + 0,06% Triton X-100 was used as modifier and T_{pyr} = 1200 °C and T_{at} = 2400 °C.



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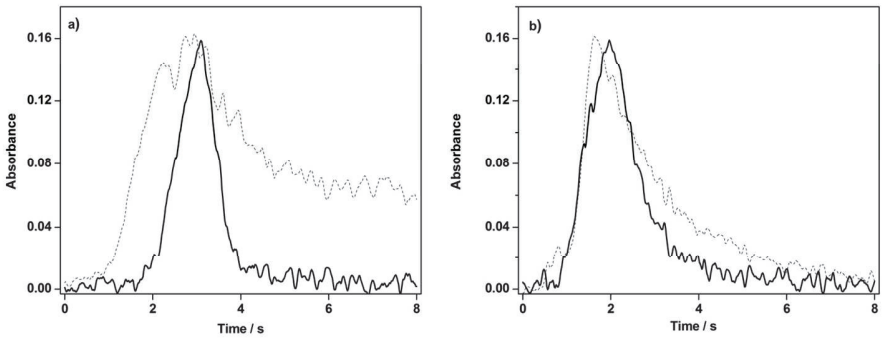

Fig. 2 Absorbance signals for As using SS-GF AAS. The solid lines represent atomic absorption and dotted lines represents the background signal. (a) Tort-2 (b) 1 ng As. Both in the presence of 15 µg Pd + 9 µg Mg + 0.06% (v/v) Triton X-100 as the chemical modifier. Tat = 2400 °C and Tpyr = 1200 °C
68x27mm (600 x 600 DPI)

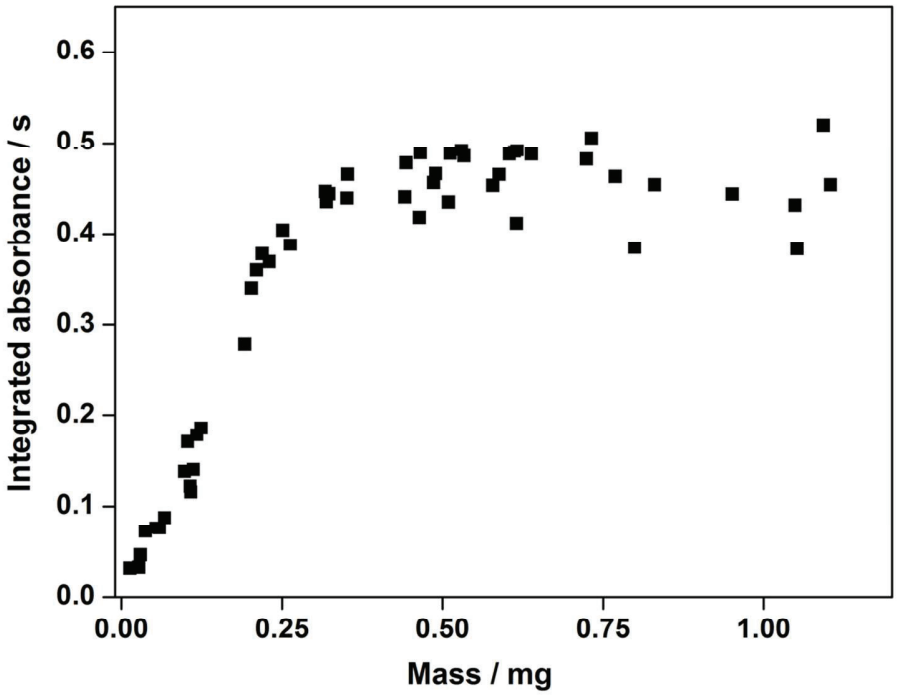

Fig. 3 Influence of the sample mass (TORT-2) on the integrated absorbance value using 15 µg Pd + 9 µg Mg + 0.06% (v/v) Triton X-100 as chemical modifier, Tpyr = 1,200 °C and Tat = 2400 °C
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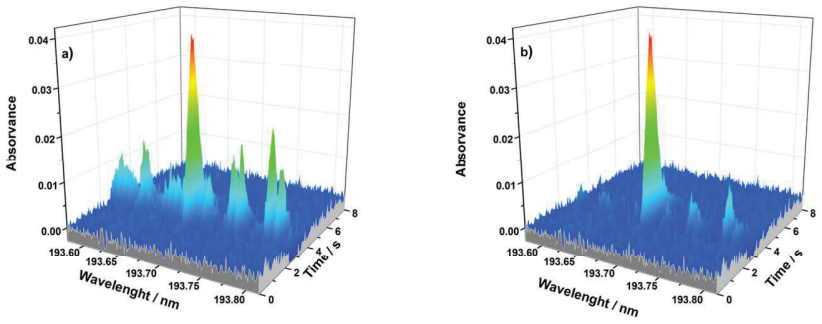

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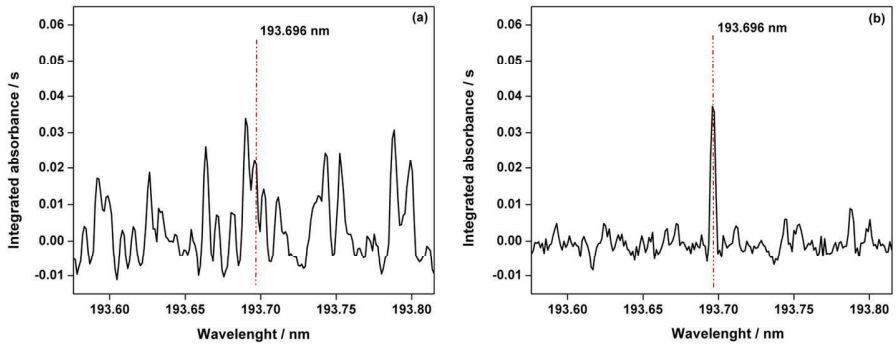
68x27mm (600 x 600 DPI)



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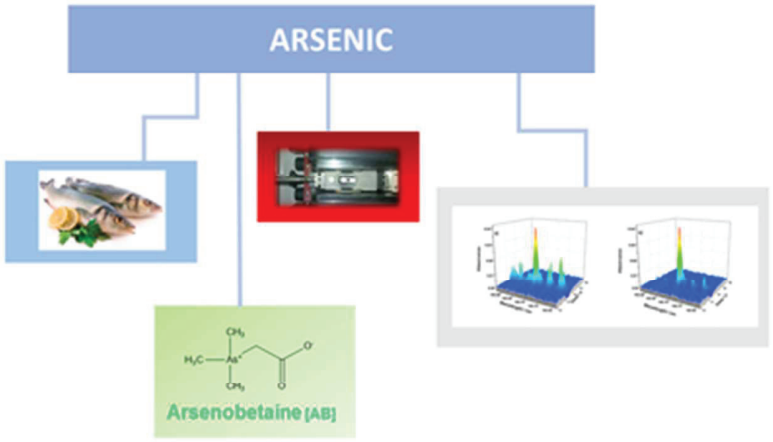
70x27mm (600 x 600 DPI)

INVESTIGATION OF GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY USING DIRECT SOLID SAMPLE ANALYSIS FOR THE DETERMINATION OF ARSENIC IN FISH AND SEAFOOD

Table of contents entry

Due to underestimation of certified values in the determination of total arsenic in seafood and fish CRMs by SS-GF AAS, other techniques were used to investigate the existence of interferences.



Due to underestimation of certified values in the determination of total arsenic in seafood and fish CRMs by SS-GF AAS, other techniques were used to investigate the existence of interferences.
39x22mm (300 x 300 DPI)

Supplementary Material

INVESTIGATION OF GRAPHITE FURNACE ATOMIC ABSORPTION SPECTROMETRY USING DIRECT SOLID SAMPLE ANALYSIS FOR THE DETERMINATION OF ARSENIC IN FISH AND SEAFOOD

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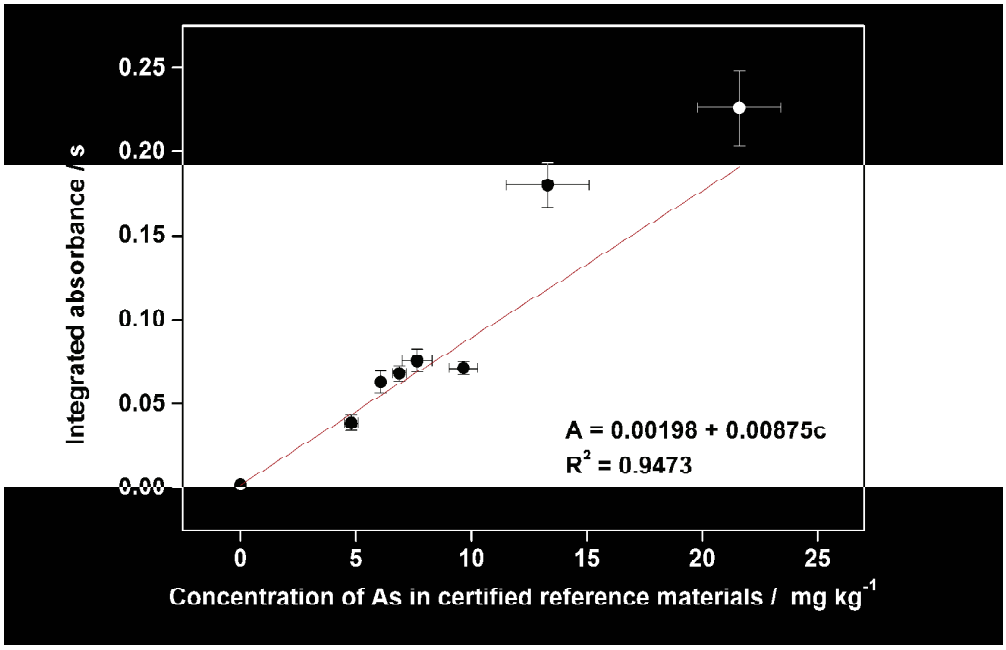

Online Resource 1: Correlation curve for As using certified reference materials and 15 µg Pd + 9 µg Mg + 0.06% (v/v) Triton X-100 as chemical modifier. T_{pyr} = 1200 ° C, T_{at} = 2400 ° C.