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Direct solid sample analysis with graphite furnace atomic absorption spectrometry—A fast and reliable screening procedure for the determination of inorganic arsenic in fish and seafood

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ABSTRACT

Direct solid sample analysis with graphite furnace atomic absorption spectrometry (SS-GF AAS) was investigated initially with the intention of developing a method for the determination of total As in fish and other seafood. A mixture of 0.1% Pd+0.06% Mg+0.06% Triton X-100 was used as the chemical modifier, added in solution over the solid samples, making possible the use of pyrolysis and atomization temperatures of 1200 °C and 2400 °C, respectively. The sample mass had to be limited to 0.25 mg, as the integrated absorbance did not increase further with increasing sample mass. Nevertheless, the recovery of As from several certified reference materials was of the order of 50% lower than the certified value. Strong molecular absorption due to the phosphorus monoxide molecule (PO) was observed with high-resolution continuum source AAS (HR CS AAS), which, however, did not cause any spectral interference. A microwave-assisted digestion with HNO_3/H_2O_2 was also investigated to solve the problem; however, the results obtained for several certified reference materials were statistically not different from those found with direct SS-GF AAS. Accurate values were obtained using inductively coupled plasma mass spectrometry (ICP-MS) to analyze the digested samples, which suggested that organic As compounds are responsible for the low recoveries. HPLC–ICP-MS was used to determine the arsenobetaine (AB) concentration. Accurate results that were not different from the certified values were obtained when the AB concentration was added to the As concentration found by SS-GF AAS for most certified reference materials (CRM) and samples, suggesting that SS-GF AAS could be used as a fast screening procedure for inorganic As determination in fish and seafood.

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 also absorb metals with bioaccumulative properties, such as arsenic, through the membrane surfaces, tissues and by ingestion of food and suspended material in water [3].

The toxicity of As compounds depends on their oxidation state, chemical form and solubility in the biological system. As(III) is more toxic than As(V) and the inorganic species are more toxic than the organic ones. As(III) is 10 times more toxic than As(V) and 70 times more toxic than monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) [4]. Arsenic can cause deleterious effects in the human body even at low concentration; 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) is responsible for the control of contaminants and residues in all kinds of food supplies. The National Agricultural Laboratories (LANAGRO) are part of MAPA and are recognized reference centers, acting in the development of analytical methods, as well as in 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

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Contaminant Control (NPRCC) in fish is 1 mg 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 indicate 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 used methods in the preparation of food samples are dry ashing and microwave-assisted acid digestion [14].

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 [15,16]. The conversion of all organic arsenic species into inorganic As is usually required for the determination of total arsenic by atomic spectrometry. Consequently, the high stability of AB becomes unfavorable for the determination of the total As content [7]. Wet digestions using strong oxidizing agents combined with strong acids and high temperatures, are required for complete degradation of AB [17]. In some cases, even with the use of these reagents at higher temperatures, AB is not degraded completely and the result for the total concentration of arsenic is lower than the actual value [18,19].

Unfortunately, the reports in the literature about this issue are not conclusive. Narukawa et al. [18] reported that complete decomposition of AB was achieved only in the presence of $HClO_4$ and temperatures of 320 °C. Slejkovec et al. [20] developed a digestion method using the mixture of $HNO_3+H_2SO_4+H_2O_2$ and a temperature of 300 °C. Shah et al. [21] used a microwave-assisted digestion with concentrated $HClO_4$ and $Fe_2(SO_4)_3$ prior to the determination of inorganic As in fish tissue, and Duarte et al. [22] used a microwave-induced combustion prior to total As determination in seafood samples. In contrast to this, Shah et al. [23] used a comparably mild microwave-assisted digestion with HNO_3/H_2O_2 prior to the determination of total As by HG AAS, and Carioni et al. [24] used slurry sampling GF AAS and slurry sampling HG AAS, i.e., no digestion at all, for the determination of total As in a tuna fish candidate reference material.

In spite of all these discrepancies about the best procedure for the determination of total As, this value is not of great importance because of the tremendous differences in toxicity of the different As species; however, the content of total As in various food and feed samples is the only one currently required by legal authorities. Nevertheless, research should continue to search for simple and reliable methods at least to distinguish toxic, i.e., inorganic As (iAs) and essentially non-toxic organic As compounds, mostly AB. Shah et al. [21] developed an extraction method with chloroform, followed by microwave-assisted digestion for the determination of iAs in fish tissue and Rasmussen et al. [25] used a solid-phase extraction followed by HG AAS for the same purpose. Anawar [26] published a review article about As speciation analysis in environmental samples by GF AAS and HG AAS.

Unfortunately, most of the procedures proposed for As speciation analysis, including the determination of iAs only, are still too complicated for routine analysis. We therefore decided to explore the potential of SS-GF AAS for the determination of total As or iAs as a fast routine procedure. The advantages of direct SS-GF AAS are well known and described in several reference articles [27,28], and this technique, to the best of our knowledge, has not been described up to now for the determination of As in fish and seafood.

2. Experimental

2.1. Instrumentation

Two different atomic absorption spectrometers were used in this work: a Model AAS Zeenit 650P line source graphite furnace atomic absorption spectrometer with Zeeman-effect background correction and a Model contrAA 700 high-resolution continuum source atomic absorption spectrometer (both from Analytik Jena, Jena, Germany). Both instruments were equipped with a similar transversely heated graphite tube atomizer.

An As hollow cathode lamp was used as the radiation source for the Zeenit with a lamp current of 6.0 mA. The analytical line at 193.7 nm was used with a spectral bandpass of 0.8 nm. The experiments were carried out using solid sampling (SS) platforms (Analytik Jena Part no. 407-152.023) and SS tubes without a dosing hole (Analytik Jena Part no. 407-152.316).

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, a high-resolution echelle monochromator and a linear charge coupled device (CCD) array detector with 588 pixels has a spectral resolution of 1.2 pm per pixel at the 193.696 nm As resonance line. Atomic absorption was measured using the center pixel (CP) and the two adjacent pixels (CP $\pm$ 1), corresponding to a spectral interval of 3.6 pm; however, the entire spectral range $\pm$ 0.12 nm around the analytical line was displayed by the 200 pixels that are used for analytical purposes. The iterative background correction (IBC) mode was used throughout. The experiments were carried out using the same SS platforms (Analytik Jena Part no. 407-152.023) and SS tubes without a dosing orifice (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 SS platforms, which were introduced into the graphite tube using a pair of pre-adjusted tweezers, which is part of the SSA 6 manual SS 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 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 the purge gas. The flow rate was 2.0 L min^{-1} during all stages, except during atomization, when the argon flow was interrupted. The parameters for the graphite furnace temperature program optimized for the determination of As are shown in Table 1.

A Model 7500ce inductively coupled plasma mass spectrometer (ICP-MS, Agilent, Germany) with a BURGENER Ari Mist HP type nebulizer was used to measure the total arsenic content. A Model 1200 LC quaternary high-performance liquid chromatography (HPLC)

Table 1
Graphite furnace temperature program for the determination of As in fish samples by SS-GF AAS and HR-CS SS-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	600	0	10
Pyrolysis	1200	300	35
Atomization	2400	FP[b]	8
Cleaning	2400	1000	8

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

pump, (Agilent) equipped with an autosampler was used for AB determination by HPLC–ICP-MS. The analytical column Zorbax 300-SCX (250 mm × 4.6 mm, 5 μm, Agilent) was protected by a guard column filled with the corresponding stationary phase. The conditions used for the HPLC in the study are based on previous works [29,30]. The outlet of the HPLC column was connected to the nebulizer of the ICP-MS system (which was the arsenic-specific detector) via PEEK capillary tubing. The ion intensity at m/z 75 (^{75}As) was monitored using time-resolved analysis software. In addition, 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 Model Ethos Touch Control microwave digestion system (Milestone, Italy) with a microwave power of 1000 W and temperature control was used for digestion. The fish and seafood samples were lyophilized in a Model ModulyonD Freeze Dryer (Thermo Electron Corporation, USA) and milled in a Model A 11 Basic micro-mill (IKA-Werke, Germany).

2.2. Reagents and solutions

Analytical grade reagents were used exclusively. Deionized water with a specific resistivity of 18 MΩ cm 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 were further purified by sub-boiling distillation in a quartz sub-boiling still (Kürner Analysentechnik, Rosenheim, Germany). The arsenic stock solution (1000 mg L^{-1}) was prepared from a Titrisol concentrate (Merck). The working standards were prepared by serial dilution of the stock solution with 0.014 mol L^{-1} nitric acid. The CRM 7901-a arsenobetaine standard solution, $(CH_3)_3\ As^+CH_2COO^-$, was supplied by National Metrology Institute of Japan (NMIJ, Japan). The chemical modifier utilized was a mixture of 0.1% Pd + 0.06% Mg + 0.06% Triton X-100 (Pd, Mg as the nitrates from Merck, and Triton X-100 from Union Carbide; all concentrations in % m/v). The following reagents were investigated for sample digestion: 30% H_2O_2 and purified HNO_3 (both from Merck), 2 mol L^{-1} NaOH, (Nuclear, Brazil), and 25% m/v TMAH ($C_4H_{12}NOH$, 91.15 g mol^{-1}, Aldrich).

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 (NRCC, Ottawa, 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, Institute for Reference Materials and Measurements IRMM (Geel, Belgium). The reference material (RM) 9th PT (Fish Protein) of CRL-ISS, (Italy) was also analyzed.

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

All samples were initially washed with Milli-Q water, cut, and homogenized using a blender (non-contaminating kitchen mixer), and then lyophilized for 5 h. After this, the samples were ground in a vibratory mill and sieved through 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

About 0.01–0.25 mg sample or CRM was weighed directly onto the SS platform, and 15 μL of chemical modifier solution added over the sample. The platform was introduced into the graphite tube for the determination of As, using the graphite furnace temperature program shown in Table 1.

2.5. Digestion methods

2.5.1. Microwave-assisted acid digestion

Initially, approximately 250 mg of CRM, lyophilized fish or seafood sample was weighed and introduced into the digestion vessels, and 8 mL of concentrated nitric acid and 2 mL of hydrogen peroxide were added. The mixture was 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 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. [31]. 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 only.

The method used for alkaline digestion with NaOH was based on the work of Geng et al. [32]. 5 mL of 2 mol L^{-1} NaOH 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 also analyzed by GF AAS.

2.6. Total arsenic determination by ICP-MS

The samples digested with HNO_3 and H_2O_2 in a microwave oven were diluted for the final measurements when necessary. Helium gas was used in the collision cell to remove potential interferences in the ICP-MS measurement. Aqueous standard solutions prepared from an As(V) stock standard were used for calibration, and each sample was analyzed in triplicate. For quality control purposes, the standards of the calibration curve and the digestion blanks (one for each sample digestion series) were run before and after each sample series. Quality control standard solutions at two concentration levels were measured after the calibration curve. Six CRMs (BCR-627, DOLT-4, TORT-2, ERM-CE278, SRM 2976 and SRM 1566b) were analyzed to assess the accuracy of the ICP-MS method.

2.7. Arsenobetaine determination

The extraction procedure of As species is based on our previous study [29]. The CRM, lyophilized fish or seafood samples were weighed into the digestion vessels and 10 mL of a mixture of 0.2% (v/v) nitric acid and 1% (v/v) hydrogen peroxide was added. The resulting mixture was centrifuged at 3500 rpm for 25 min and the supernatant filtered through a PET filter (Chromafil PET, Macherey-Nagel, pore size 0.45 μm). AB determination was carried out by HPLC–ICP-MS using the method described previously [29]. The AB

in the 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. The CRM BCR-627 (Tuna fish), which has a certified content of AB, was analyzed together with the samples in order to establish the accuracy of the AB determination.

3. Results and discussion

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

3.1.1. Graphite furnace temperature program

The graphite furnace temperature program was optimized for the direct determination of As in fish and seafood samples using the CRM TORT-2 and an aqueous standard solution for both instruments. No significant differences were expected between the two data sets, because the graphite tube atomizers used in the two equipments are very similar. Significant differences could only be expected for the CRM in the case of a spectral interference that could not be handled by the Zeeman-effect background correction of the GF AAS equipment, as the background correction capability of HR-CS GF AAS is clearly superior to the latter one.

Two drying temperatures with slow ramp rates and long hold times were utilized as shown in Table 1 in order to obtain a homogenous heating, preventing an overflow or splash of the chemical modifier solution. An additional "ash" step was used in the HR-CS SS-GF AAS measurements, using air as an alternate gas ($2\ L\ min^{-1}$) to oxidize the sample and thus reduce the carbon residues on the platform. The ash step was followed by another pyrolysis stage at the same temperature of 600 °C, but with Ar as the purge gas to remove the air from the tube before further increasing the temperature in order to increase tube lifetime.

The palladium and magnesium mixture was investigated as chemical modifier in this work 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 °C in GF AAS measurements [33–35]. A preliminary study of the amount of modifier was carried out in order to find the optimum mass that thermally stabilizes the analyte. Masses of 2.5–20 μg Pd and 1.5–12 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 (Pd/Mg modifier) were used in further optimization studies.

Pyrolysis curves obtained for the CRM TORT-2, normalized for 0.1 mg of sample, and for 1 ng As in an aqueous standard solution with the Pd/Mg modifier are shown in Fig. 1a and b. As expected, no significant difference could be found for the two equipments, and a pyrolysis temperature of 1200 °C was used in all further experiments for both instruments. However, it became already clear from these first investigations that there is something wrong with the determination of As using direct SS analysis with both equipment. The certified value for total As in CRM TORT-2 is $21.6 \pm 1.8\ mg\ kg^{-1}$, which corresponds to a mass of 2.16 ± 0.18 ng As when the signal is normalized to a sample mass of 0.1 mg. However, the integrated absorbance signal for the CRM TORT-2 in Fig. 1a and b is very close to that of the aqueous standard solution, which corresponds to 1.0 ng As. This means that less than 50% of the As in the CRM has been recovered in comparison to the aqueous standard solution.

3.1.2. Background absorption and spectral interference

Although the recovery of As from the CRM was very similar for both equipments used in this study, the potential existence of a spectral interference was investigated. Typical atomization and background signals obtained with SS-GF AAS for the CRM and an aqueous standard solution are shown in Fig. 2a and b. Obviously, the background absorption was higher for the CRM compared to the aqueous standard solution; however, no anomalies could be observed neither in the background nor in the analyte absorption signal. Nevertheless, as spectral interferences are much more difficult to detect in conventional line-source AAS compared to HR-CS AAS, similar determinations were also carried out with the latter technique.

Fig. 3a shows the wavelength-resolved absorbance spectrum for the CRM TORT-2 in the vicinity of the analytical line for As at 193.696 nm after automatic correction for continuous background absorption. There is very clearly a structured background in this spectral range, and structured background due to the diatomic molecule PO, caused by phosphates, is recognized to be the main spectral interference in the determination of As in seafood by GF AAS [36]. As HR-CS GF AAS offers the unique possibility to use least-squares background correction (LSBC) to correct for structured background, this technique was used to subtract the phosphate reference spectrum from the CRM TORT-2 spectrum. The PO reference spectrum was recorded with 0.4 mg of $NH_4H_2PO_4$, which was weighed directly onto the platform, follow by the addition of the Pd/Mg modifier and introduced into the graphite furnace. The reference spectrum was stored in the computer and subtracted from the sample spectrum, which removed essentially all the molecular absorption structures, as is shown in Fig. 3b.

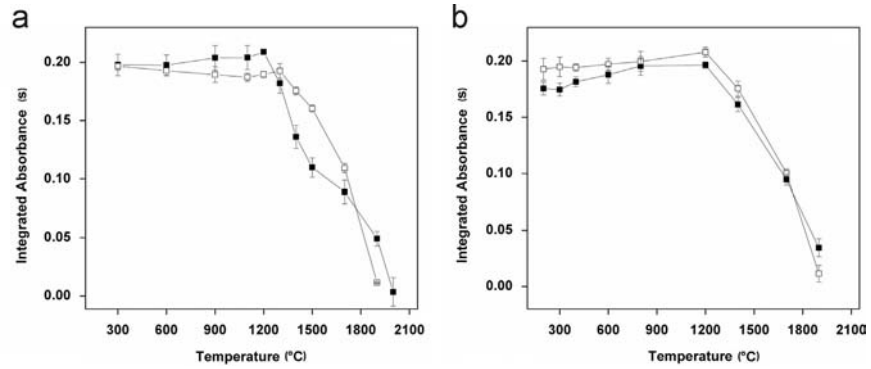

Fig. 1. Pyrolysis curves for As (a) using SS-GF AAS and (b) using HR-CS SS-GF AAS for: (□) 1 ng As in 10 μL of $0.014\ mol\ L^{-1}$ HNO_3 and (■) TORT-2 (absorbance signal normalized for 0.1 mg of the sample); atomization temperature: 2400 °C; chemical modifier: 15 μg Pd + 9 μg Mg + 0.06% (v/v) Triton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

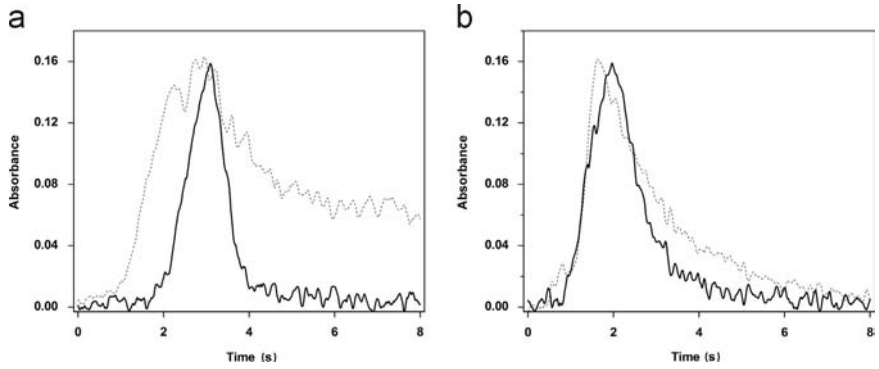

Fig. 2. Absorbance signals for As using SS-GF AAS; solid lines are atomic absorption and dotted lines background signal. (a) Tort-2 (b) 1 ng As; chemical modifier: 15 μg Pd+9 μg Mg+0.06% (v/v) Triton X-100; T_{pyr}=1200 °C and T_{at}=2400 °C.

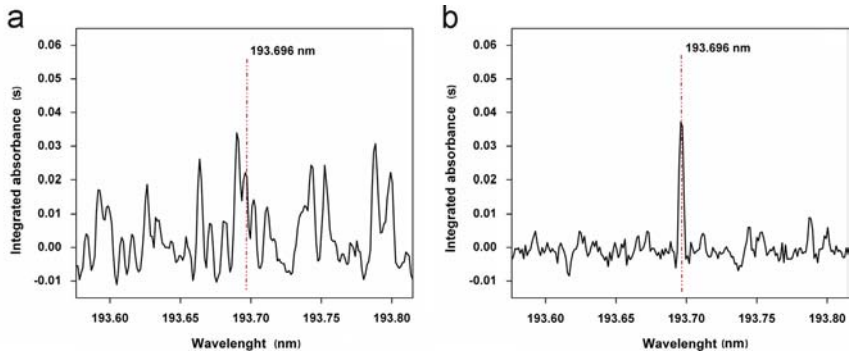

Fig. 3. Wavelength-resolved absorbance spectrum in the vicinity of the 193.696 analytical line using HR-CS SS-GF AAS recorded for TORT-2 (a) without correction and (b) after correction using LSBC and $NH_4H_2PO_4$ as a reference spectrum; chemical modifier: 15 μg Pd+9 μg Mg+0.06% Triton X-100. T_{pyr}=1200 °C and T_{at}=2400 °C .

The HR-CS SS-GF AAS measurements confirm the presence of a PO absorption spectrum in the vicinity of the As line, which is in agreement with the literature [36,37]. Nevertheless, the As concentration obtained for the CRM TORT-2 by SS-GF AAS (9.4 ± 0.7 mg kg^{-1}As) and HR-CS SS-GF AAS with or without LSBC (9.5 ± 0.4 mg kg^{-1}) is not significantly different, and still far below the certified value of 21.6 ± 1.8 mg kg^{-1}. This means that the low recovery is not due to a spectral interference.

3.1.3. Influence of sample mass

Several authors reported about a maximum sample mass that can be used in SS-GF AAS analysis [38,39], and that above this critical mass the integrated absorbance does not increase linearly any more with increasing sample mass and the precision may decrease significantly. Therefore, the correlation between sample mass and integrated absorbance was evaluated as well using the CRM TORT-2. The absorbance signal does not increase any more with increasing sample mass above 0.3 mg, as shown in Fig. 4, indicating that in this condition there is a strong influence of the matrix. For the linear part of this figure (up to 0.3 mg), there was a good linear correlation ($R \cong 0.97$) between sample mass and integrated absorbance. Similar studies were also performed with other samples and reasonably good linearity was observed up to 0.25 mg for Whitefish ($R \cong 0.95$), 0.28 mg for Hake-1 ($R \cong 0.95$) and 0.30 mg for Red Porgy ($R \cong 0.97$). The integrated absorbance values did not increase further for higher mass values. Hence, the sample mass for SS-GF AAS and HR-CS SS-GF AAS analyses was limited to a maximum of 0.25 mg sample.

3.1.4. Calibration against solid CRM

As all the above measurements were made using aqueous standard solutions for calibration, an additional calibration technique

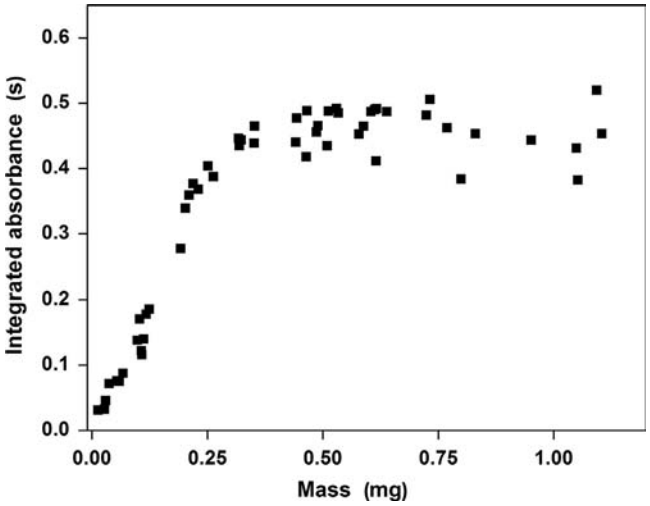

Fig. 4. Influence of the sample mass (TORT-2) on the integrated absorbance; chemical modifier: 15 μg Pd+9 μg Mg+0.06% (v/v) Triton X-100; T_{pyr}=1200 °C and T_{at}=2400 °C.

was investigated. An alternative for calibration in direct SS analysis is the use of a correlation curve established plotting the normalized integrated absorbance for several reference materials against the certified concentration [40]. 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 modifier was used and six replicates (n=6) were measured for each CRM. The linear correlation equation was A_{int}=0.00198+0.0875 m (ng) with a linear correlation coefficient (R) of 0.947. The R value did not show good linearity and three

CRMs were outliers. This result is actually not very surprising, as the recovery of As in comparison to the certified value was found to be quite different for different CRMs, as will be shown later. Hence, 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.

3.1.5. Digestion of samples

Due to the difficulty of finding the certified value for the CRM TORT-2 using SS-GF AAS, we decided to investigate the effect of acid digestion. The conversion of all organics into inorganic arsenic species is usually required for the determination of total arsenic in the samples by atomic spectrometry. Consequently, the high stability of AB, the main organic As species in fish and seafood, becomes unfavorable for the determination of the total concentration of As [7]. Wet digestions using strong oxidizing agents together with strong acids and high temperatures, are required for complete degradation of AB [17]. In some cases, even with the use of these reagents, AB is not degraded completely and the results for the total concentration of arsenic is lower than the actual value [18,19]. Narukawa et al. [18] reported that complete mineralization of AB was achieved only in the presence of $HClO_4$ and temperatures of 320 °C. Slejkovec et al. [20] developed a digestion method using the mixture of $HNO_3+H_2SO_4+H_2O_2$ and a temperature of 300 °C.

The use of perchloric acid is not allowed in our laboratory due to safety reasons, and only temperatures up to 190 °C can be used for the PTFE vessels of our microwave digestion system. Therefore, we investigated the digestion program recommended by the manufacturer for the digestion of fish, which is described in Section 2.5.1. The result obtained for the CRM TORT-2 after microwave-assisted acid digestion using GF AAS was 9.4 ± 0.2 mg kg^{-1}, which was not significantly different from the values found with SS-GF AAS and HR-CS SS-GF AAS. Similar low recoveries were obtained for As in other fish CRM, which are not shown here.

Due to this problem, two alkaline digestion procedures, based on publications by Pereira et al. [31], using NaOH, and Geng et al. [32] using TMAH (see Section 2.5.2.) were investigated as well. The results for the determination of As in TORT-2 by GF AAS were 9.7 ± 0.4 mg kg^{-1} for digestion with NaOH and 9.6 ± 0.3 mg kg^{-1} for digestion with TMAH. In comparison with the value found by SS-GF AAS (9.4 ± 0.67 mg kg^{-1}), an Analysis of Variance (ANOVA) was applied in the values obtained by NAOH and TMAH digestion and GF AAS. According to the ANOVA, the calculated F value (F_{calc}) was 0.40, while the critical F value ($F_{critical}$) was 4.10. As $F_{critical} > F_{calc}$, the values are not significantly different at the 95% confidence level. However, all the results showed a significant difference of approximately 55% from the certified value for the CRM TORT-2.

The preliminary conclusion at that point in time was that neither SS-GF AAS nor GF AAS determination after microwave-assisted acid digestion or alkaline digestion was suitable for the determination of total As in fish and seafood. A possible reason for that could be an incomplete decomposition of AB during direct SS analysis and also using different digestion methods, a problem that has already been reported in the literature [18,20,41].

3.2. Measurements using ICP-MS and HPLC–ICP-MS

In order to investigate this problem further, we decided to use the same microwave-assisted acid digestion with HNO_3 and H_2O_2 for the determination of total As using ICP-MS. We also determined AB using HPLC–ICP-MS after an appropriate extraction of the AB species.

3.2.1. ICP-MS measurements

The ICP-MS technique was chosen to determine total arsenic, since it is widely used for the determination of As in fish and seafood [12]. Details of the procedure are described in Section 2.6. The results obtained for the determination of As using microwave-assisted acid digestion in four fish and three seafood samples, six CRMs and one RM by ICP-MS were determined in our previous work [30] and are summarized in Table 2. The precision was calculated from three consecutive measurements and the results obtained for the CRM were in agreement with the certified values at the 95% confidence level. The precision, expressed as relative standard deviation (RSD) was between 1% and 8%.

As the same solutions that have previously been investigated by GF AAS were used in this study, the reason for the low recoveries of As with the former technique cannot be due to a loss of As during the digestion process. Spectral interferences were also excluded as a source of error (see Section 3.1.2). The only explanation that we have for the low recoveries is that organic species of As are not atomized in the graphite furnace, but lost in some molecular form, and the same loss mechanism is apparently occurring in SS-GF AAS.

Table 2
Determination of arsenobetaine (AB) using HPLC–ICP-MS and As using ICP-MS, HR-CS SS-GF AAS and SS-GF AAS in CRM and fish samples; all values in mg kg^{-1}; average ± SD; $n=3$ for ICP-MS and HPLC–ICP-MS; $n=6$ for SS-GF AAS and HR-CS SS GF AAS.

CRM or Sample	Certified value	As (ICP-MS)	RSD (%)	AB (HPLC-ICP-MS)	RSD (%)	HR-CS SS-GF AAS	RSD (%)	HR-CS SS-GF AAS+AB
TORT-2	21.6 ± 1.80	22 ± 1.1	5	13 ± 0.4	3	9.5 ± 0.2	4	22 ± 0.9
SRM 2976	13.3 ± 1.80	14 ± 0.3	1	10 ± 0.2	2	3.5 ± 0.2	5	15 ± 0.6
SRM 1566b	7.65 ± 0.65	7.6 ± 0.1	2	2.6 ± 0.1	3	4.4 ± 0.4	2	7.5 ± 0.4
DOLT-4	9.66 ± 0.62	9.6 ± 0.1	1	5.2 ± 0.5	9	4.9 ± 0.3	6	9.4 ± 0.8
BCR- 627	4.80 ± 0.30[a]	4.8 ± 0.1	3	3.8 ± 0.1[a]	2	2.2 ± 0.2[b]	7	6.0 ± 0.1
ERM-CE278	6.07 ± 0.13	6.0 ± 0.2	3	2.3 ± 0.2	7	3.9 ± 0.3[b]	7	6.1 ± 0.4
RM 9th PT	6.65 ± 0.71[c]	7.0 ± 0.3	4	4.3 ± 0.2	4	2.8 ± 0.2	8	7.1 ± 0.4
Hake-1	–	7.1 ± 0.1	1	6.5 ± 0.2	3	2.0 ± 0.1	3	8.6 ± 0.3
Hake-2	–	4.2 ± 0.1	3	3.2 ± 0.2	6	1.0 ± 0.1	7	4.2 ± 0.3
Red porgy	–	35 ± 0.2	1	33 ± 2.7	8	14 ± 0.4	3	47 ± 3.0
White fish	–	35 ± 1.1	3	33 ± 2.3	8	17 ± 0.8	4	51 ± 3.7
Shrimp	–	2.3 ± 0.1	3	1.4 ± 0.1	1	0.8 ± 0.1[b]	9	2.3 ± 0.1
Oyster	–	25 ± 0.6	2	16 ± 0.6	4	8.0 ± 0.7[b]	8	24 ± 1.3
Clams	–	17 ± 1.4	8	12 ± 0.7	6	7.3 ± 0.6[b]	9	19 ± 1.4

[a] Certified value for arsenobetaine (AB): 3.9 ± 0.20 mg kg^{-1}.
[b] Measured by SS-GF AAS.
[c] Reference material.

3.2.2. Speciation analysis using HPLC–ICP-MS

An extraction of organic As species and determination of AB based on HPLC-ICP-MS (see Section 2.7.), developed in previous work [29], was used in order to identify and quantify the AB in a variety of CRMs and samples. The results are also shown in Table 2. The concentration of 3.80 ± 0.07 mg kg^{-1} AB found for the CRM BCR-627 (the only CRM with a certified value for AB) is in good agreement with the certified value of 3.9 ± 0.20 mg kg^{-1} AB. The precision was calculated from three consecutive measurements in seven real samples, six CRMs and one RM, and given as RSD, which varied between 1% and 9%. As reported in the literature [40,42], AB is the major arsenic species in all samples and CRM, with the exception of SRM 1566b (oyster tissue).

An interesting result was observed for the CRM TORT-2, which was used in Section 3.1 for method development: the sum of the value of 9.5 ± 0.21 obtained in Section 3.1.2. Using HR-CS SS-GF AAS and the value of 13.1 ± 0.45 mg kg^{-1} AB obtained by HPLC–ICP-MS gives a value of 22.6 ± 0.66 mg kg^{-1} As. This value is very close to the certified value of 21.6 ± 1.8 mg kg^{-1} for total As and the value of 22.4 ± 1.1 mg kg^{-1} determined by ICP-MS. This finding stimulated us to go back to the determinations of As using SS-GF AAS and HR-CS SS-GF AAS and to see if this approach could be used for a direct determination if inorganic As (iAs) instead of determining this value from the difference of total As and AB.

3.3. Determination of iAs using HR-CS SS-GF AAS and SS-GF AAS

All the CRMs and samples that were analyzed by ICP-MS and HPLC–ICP-MS were also analyzed by HR-CS SS-GF AAS and SS-GF AAS. The results are also shown in Table 2 together with the certified values for total As and the results obtained previously by ICP-MS. Only the values obtained by HR-CS SS-GF AAS are shown in Table 2, as, except for one sample, the values obtained by SS-GF AAS did not show any significant difference to the former ones. A Student's *t*-test was applied to the data of Table 2 and all CRMs and samples are not significantly different at a 95% confidence level, except BCR-627, Hake-1, Red porgy and White fish. In the case of the CRM and the RM the comparison was with the certified value and in the case of real samples with the average values obtained by ICP-MS. The most likely explanation for the high values after summation of the values measured with HR-CS SS-GF AAS and HPLC–ICP-MS is that in these cases part of the AB could be measured also with the former technique. This assumption obviously requires more research to be confirmed.

3.4. Figures of merit

The analytical figures of merit obtained for iAs by SS-GF AAS and HR-CS SS-GF AAS are shown in Table 3. 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).

Table 3
Analytical figures of merit obtained for the determination of As using SS-GF AAS and HR-CS SS-GF AAS. For definitions see text.

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

[a] Based on the 'zero mass response' technique [37] and calculated for 0.25 mg of sample.

The limits of detection (LOD) and quantification (LOQ) 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 [38] introducing repeatedly a solid sampling platform, containing only the modifier, into the furnace, followed by a regular atomization cycle (see Table 1).

The characteristic mass (m_0), which is defined as the mass of analyte that corresponds to an integrated absorbance (A_{int}) of 0.0044 s, obtained for As by SS-GF AAS is in good agreement with the values reported in the literature [43]. The SS-HR-CS GF AAS technique shows higher sensitivity (lower m_0) and better linearity than SS-GF AAS. However, the LOQ for both techniques are far below the value of 1 mg kg^{-1} for fish, established by the Brazilian NPRCC [11].

The precision of the SS-GF AAS and HR-CS SS-GF AAS method for As determination was calculated from six consecutive measurements ($n=6$) in seven real samples and seven CRM. The precision values, expressed as the relative standard deviation (RSD), varied between 2% and 9% and are also shown in Table 2. These values are quite typical for direct SS analysis of small sample masses, and are actually not too much different from those obtained for ICP-MS in the same samples after digestion (Table 2).

4. Conclusion

Neither SS-GF AAS nor HR-CS SS-GF AAS appears to be capable to determine total As in fish and seafood samples under the conditions used in this work. The relatively mild microwave-assisted digestion with HNO_3/H_2O_2 equally does not solve the problem, as the results are essentially identical with those obtained using direct SS analysis. The recovery of As obtained with these techniques is in average some 50% lower than the certified values or those determined by ICP-MS.

However, it was found, after the determination of AB using HPLC–ICP-MS that summing up the values for As obtained with SS-GF AAS with the values for AB resulted in most cases in a perfect agreement with the certified value of total As or the value determined by ICP-MS. This means that direct SS-GF AAS could be used as a fast and reliable screening technique for the determination of iAs, i.e., the most toxic species of As, which should be of primary concern for the authorities. The fact that in a few cases a higher value for total As was obtained after summation is not of major concern, as the purpose of a screening method is only to identify samples that exceed the legal limits. In this case, the sample is taken out of the routine and analyzed more carefully using independent techniques. Hence, in case that one day legislation should be discussed about the determination of iAs instead of or in addition to total As, the method developed in this study should be considered as a simple, fast and inexpensive option.

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