

# A comparative study of chemical modifiers in the determination of total arsenic in marine food by tungsten coil electrothermal atomic absorption spectrometry

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## Abstract

Three platinum group elements (Pd, Ir and Rh) both in solution and in pre-reduced form, and also combined with  $\text{Mg}(\text{NO}_3)_2$  or ascorbic acid, were assessed as possible chemical modifiers on the atomization of As in digest solutions of seafood matrices (clam and fish tissue) by tungsten coil electrothermal atomic absorption spectrometry (TCA-AAS) and compared without a modifier. Of 28 modifier alternatives in study including single form and binary mixtures, and based on maximum pyrolysis temperature without significant As loss and best As absorbance sensitivity during atomization, three modifiers: Rh (0.5  $\mu\text{g}$ ), Ir (1.0  $\mu\text{g}$ ) and Rh (0.5  $\mu\text{g}$ ) + ascorbic acid (0.5  $\mu\text{g}$ ), at optimum amounts were pre-selected and compared. The definitive modifier (rhodium (0.5  $\mu\text{g}$ )) was selected by variance analysis. The mean within-day repeatability was 3% in consecutive measurements (25–300  $\mu\text{g l}^{-1}$ ) (three cycles, each of  $n=6$ ) and showed good short-term stability of the absorbance measurements. The mean reproducibility was 4% ( $n=18$  in a 3-day period) and the detection limit ( $3\sigma_{\text{blank}}/\text{slope}$ ) was 42 pg ( $n=16$ ). Quantitation was by standard additions to compensate for matrix effects not corrected by the modifier. Three sample digestion procedures were compared in fish and clam tissue samples: microwave acid digestion alone (A) or combined with the addition of 2% (m/v)  $\text{K}_2\text{S}_2\text{O}_8$  solution followed either by UV photo-oxidation (B) or re-digestion in a thermal block (C). The accuracy was established by determination of As in certified reference material of dogfish muscle (DORM-2). Procedures B and C showed good recoveries (102% ( $n=4$ ) and 103% ( $n=7$ ), respectively), whereas procedure A was not quantitative (85%). The methodology is simple, fast, reliable, of low cost and was applied to the determination of total As in lyophilized samples of clam and fish collected in the Chilean coast.

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## 1. Introduction

Arsenic (As) is a worldwide recurring pollutant of natural origin commonly associated with ores of metals like copper, lead, and gold [1]. This metalloid is found in the environment in several chemical forms [2], and is well known as a source of serious health effects by prolonged intake even at low concentrations. In particular, inorganic As (As(III) + As(V)) is extremely toxic and long-term exposure has been associated with cancers of the skin and internal organs (e.g., bladder, lung) as

well as various non-cancerous disorders (e.g., keratoses, hyperpigmentation) [3,4], whereas methylated species such as monomethylarsonic acid (MMA), di-methylarsinic acid (DMA), and tri-methylarsine oxide (TMAO) have a moderate toxic effect on humans and biota, and the cationic species arsenobetaine (AsB) and arsenocholine (AsC) are considered non-toxic [2,5]. The main exposure routes to As in humans are dietary and drinking water ingestion. Among dietary sources, seafood exposures might be of concern because As concentrations can be orders of magnitude higher than those associated with other food groups. Also, naturally occurring As is very broadly distributed in many subsurface drinking water aquifers around the globe [6,7]. In Latin America, particularly in Central and Northern Chile, copper mining and smelting is a major source of contamination by

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As, and a few smelters in this area are responsible of a large fraction of As emissions [8]. Besides, the soils and aquifers in the North of Chile are characterized by high As content due to the geomorphology exceptionally rich in As [9]. Hence, owing to natural and anthropogenic contamination and to the high mobility and bioavailability of As species, diffusion throughout the food chain including water and food for human consumption, is expected.

Actually, there is still a need of reliable and simple analytical methods and procedures for As determination in different food sources, because the determination of total As continues to be highly relevant in order to provide reliable data on the total content of As in seafood and other matrices. Among atomic spectrometric techniques, during the last decade electrothermal atomic absorption spectrometry (ET-AAS) with a tungsten coil atomizer (TCA) has shown noteworthy features as a supplementary atomization source to the flame. For small laboratories and service organizations with restricted instrumental budget the study of alternative atomization means in ET-AAS is suitable and relevant. According to a recent review [10], tungsten devices in analytical atomic spectrometry continue to provide simple and versatile atomizer alternatives. In 1988, Berndt and Schaldach [11] proposed the use of a double-layer coiled tungsten filament (150 W) which offers some advantages to enable the determination of As in solution samples, such as low power supply requirements, fast heating and low cost of the coils [12]. Though, some disadvantages limit its use, for example lack of a TCA commercial system, atomization under non-isothermal conditions, short residence time of the atomic vapor and matrix effects are produced in the solid and gaseous phase. Lately, in this research group our efforts aimed to contribute to the elucidation of these inconveniences.

One of the approaches pursued to minimize matrix effects is the use of chemical modifiers in trace metal determinations by TCA-AAS. Recently, the use of modifiers with metal atomizers was reviewed by Nóbrega et al. [13], pointing out that there is no panacea similar to the Pd + Mg mixture used in graphite furnace AAS because the chemical processes implicated during atomization are different from those occurring in a graphite tube. Both single and combined modifiers were used to thermally stabilize the analyte in a TCA without enclosure [11] operating in a reducing environment, enabling the elimination of the sample matrix and in parallel attaining a sensitivity enhancement [14–16]. It is well known that As is thermally stabilized in pyrolytic graphite surface by using W, Zr, and the platinum group metals (PGMs) as chemical modifiers, considering a thermal pre-reduction of the modifier in this surface. Among the most successful modifiers are Pd, Ir, Rh and Ru applied either (i) singly and directly onto the pyrolytic graphite surface, or (ii) on a pretreated graphite surface coated with W or Zr carbides, or (iii) combining Pd and Ir in these applications [17]. This procedure allows the trapping of hydride-forming elements and also volatile elements in the graphite surface followed by the atomization step, thereby enabling the determination of ultra-traces of metalloids and volatile elements by ET-AAS with high sensitivity, without analyte loss in molecular form and eliminating matrix effects. Regarding metal atomizers, recently Hou et al.

[18] established the feasibility of Ir as a permanent chemical modifier for the TCA, and Barbosa et al. [19] showed that a Rh-coated tungsten coil was useful for selenium hydride collection. More recently, a systematic study was performed by TCA-AAS to establish among three PGMs (Pd, Ir, and Rh) an optimum chemical modifier to enable the determination of As at trace levels in biological materials, and Rh (2.0  $\mu\text{g}$ ) in pre-reduced form was found more effective [20]. The aim of this work is to extend the study of chemical modifiers for As determination in digest solutions of seafood samples, in particular of fish and clam tissues, including Pd, Ir, Rh both in solution and in pre-reduced form, and also combined with  $\text{Mg}(\text{NO}_3)_2$  and ascorbic acid, well known modifiers used in ET-AAS with the graphite furnace [21], comparing their performance in terms of maximum pyrolysis temperature to eliminate the sample matrix without significant As loss in this step, and best absorbance sensitivity for As in the atomization step. In addition, three sample digestion procedures are considered in this study to provide a complete conversion of As present in various organoarsenicals into As(V), bearing in mind that these species comprise the major fraction of the As content in these seafood matrices (>90%) [22–24]. The employ of microwave acid digestion alone or combined with the addition of  $\text{K}_2\text{S}_2\text{O}_8$  followed either by UV photo-oxidation [25,26] or re-digestion in a thermal block are compared in fish and clam tissue samples. Both seafood matrices are significant food items in the Chilean diet and it would be of great utility to have a reliable, simple, fast and inexpensive method that would make it possible to quantify total As therein.

## 2. Experimental

### 2.1. Apparatus

A Perkin-Elmer (PE) Model 1100 atomic absorption spectrometer (Überlingen, Germany) was equipped with a TCA [11]. The limiting time resolution of the spectrometer was 0.02 s and peak height absorbance was measured throughout [15] at the 193.7 nm As resonance line (EDL System 2 lamp, PE). The absorbance was background corrected with a deuterium arc, and time-resolved absorption signals were printed out. The measurement time was 1.0 s. Lamp current and slit-width settings corresponded to manufacturer's recommendations. Sample and reference solution aliquots of 10  $\mu\text{l}$  were manually delivered into the coil through a micropipette (Transferpette, Brand, Wertheim, Germany). The double-layer coiled tungsten filament (150 W, Osram, Munchen, Germany, Part No. 64633 HLX) was heated by a programmable power supply with a voltage feedback circuit (Anacom Equipment and System, Sao Bernardo do Campo, SP, Brazil) and run with a PC computer (Pentium 100 MHz). A purpose-built control interface was used to trigger the read command of the spectrometer. Argon–hydrogen (90:10) (Indura, Santiago, Chile) for TCA-AAS was used as purge gas at an optimum flow rate of 1.5 l  $\text{min}^{-1}$  throughout.

Marine food samples were ground and blended both in a pre-cleaned mixer (Philips HR2810/A) and a pre-cleaned chopper (Sindelen model Silhouette P-123) fitted with stainless steel blades and plastic vessels. The samples were frozen in a Frio

Lux freezer and lyophilized in a Leybold (model Lyovac GT2) freeze dryer supported by a Welch vacuum pump (model W series,  $10^{-1}$  to  $10^{-2}$  Pa). The lyophilized samples were powdered in a micro-dismembrator (B. Braun Biotech, Melsungen, Germany) equipped with PTFE containers with lid and PTFE balls by using a cryogenic approach. Liquid nitrogen (AGA, Concepcion, Chile) was used to freeze the ground mass of the fish samples within the PTFE flasks before comminution in the micro-dismembrator [27]. Microwave sample digestions were carried out in a Milestone MLS-1200 MEGA microwave system (Bergamo, Italy) by using Milestone's digestion HPR-1000/6 rotor with TFM vessels. In the first re-digestion approach assisted by UV photo-oxidation, the digest solution was passed in a continuous flow mode through a Tefzel tubing (i.d. 0.30 mm, 270 cm in length) coiled along a germicidal UV fluorescent tube (15 W). The later was fixed in a wood support under an aluminum shield containing several venting slots to dissipate minor heat produced by the low power radiation. A Ismatec MCP (model ISM 726 (2–200 rpm)) peristaltic pump was used to propel the digest solution through the coil tubing, which was held at 0.5 cm from the UV tube surface affixed throughout by four galvanized wire strips set parallel to the UV tube (equidistant to the wires) to avoid a warm up effect from the radiation. The second re-digestion approach was performed in a laboratory made aluminum thermal block supplied with five cavities drawn therein to lay the digestion tubes, a thermo regulator inserted in the side of the block and a thermometer set in a central orifice on top, and was heated at the required temperature by a portable furnace controlled through a rheostat. An analytical balance AA 200 DS (sensitivity 0.02 mg, Denver Instrument Co.); a vortex shaker, a laboratory oven and several micropipettes (Transferpette and Finnipette) were also used.

## 2.2. Reagents, materials and samples

Most reagents were of analytical-reagent grade (Merck, Darmstadt, Germany), except for  $\text{HNO}_3$  which was further purified in a quartz sub-boiling still (Kürner, Rosenheim, Germany) and stored in quartz,  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{H}_2\text{O}_2$  (30%, m/m) which was Suprapur grade (Merck), Iridium stock solution (Strem Chemicals, Inc., Newburyport, MA, USA) and Rhodium stock solution (Aldrich, Milwaukee, WI, USA). The modifiers stock solutions were  $10.0 \pm 0.2 \text{ g l}^{-1}$  Pd (as  $\text{Pd}(\text{NO}_3)_2$  in 15%, v/v  $\text{HNO}_3$ ),  $1000 \mu\text{g ml}^{-1}$  Ir (as  $\text{IrCl}_3$  in 10% HCl) and  $1000 \mu\text{g ml}^{-1}$  Rh (Rh in 5% HCl). Modifier solutions of Pd, Ir, Rh, Mg and ascorbic acid were prepared in the concentration range 5–1000  $\mu\text{g ml}^{-1}$  by dilution of the stock solutions (Pd, Ir and Rh) and by dissolving 0.2637 g of  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$  in 25 ml ( $1000 \mu\text{g ml}^{-1}$  Mg) and 0.025 g of ascorbic acid in 25 ml ( $1000 \mu\text{g ml}^{-1}$ ), respectively. The pre-selected modifiers and optimized amounts for more in depth studies in sample matrices were Rh ( $50 \mu\text{g ml}^{-1}$ ) in solution (0.5  $\mu\text{g}$ ), Ir ( $100 \mu\text{g ml}^{-1}$ ) in solution (1.0  $\mu\text{g}$ ) and the binary mixture Rh ( $50 \mu\text{g ml}^{-1}$ ) + ascorbic acid ( $50 \mu\text{g ml}^{-1}$ ) in solution (0.5  $\mu\text{g}$  + 0.5  $\mu\text{g}$ , respectively). The re-digestion procedures were performed in 2% (m/v)  $\text{K}_2\text{S}_2\text{O}_8$  solution prepared daily in 0.5% (m/v) NaOH. Ultrapure (u.p.) water

( $18.3 \text{ M}\Omega \text{ cm}$  at  $25^\circ\text{C}$ ) was used throughout. Arsenic (V) stock solutions ( $1000 \mu\text{g ml}^{-1}$ ) were prepared from Titrisol concentrate (Merck), and reference solutions of As(V) were prepared daily by stepwise dilution of a working standard solution ( $20 \mu\text{g ml}^{-1}$ ) prepared weekly. The stock and the later solutions were stored at  $4^\circ\text{C}$  until use. Standard additions (10, 20, 40 and  $60 \mu\text{g l}^{-1}$  As(V)) were applied for As determination in sample solutions.

A certified reference material (CRM) of dogfish muscle (DORM-2, National Research Council of Canada, Canada) and a homogenized, lyophilized, powdered clam sample of *Semella solida* ("almeja") collected in natural banks from the Chilean coast [28] were used in the pyrolysis and atomization studies with all matrix modifiers and their combinations. The CRM was used too in the accuracy study. Also, besides the former clam sample, a powdered sample of clam *Tagelus dombeii* ("navajuela") and five powdered samples of fish muscle (three samples of *Merluccius gayi* ("merluza común") and two samples of *Salmo Salar* ("salmón del Atlántico")) caught in the coastal area of the Eighth Region of Chile were analyzed for total As by the proposed methodology. Both clam samples were prepared as reported elsewhere [28] and the fish samples corresponded to fresh fish obtained directly from a fisherman at the fish market, and were washed carefully with distilled water, dried externally with paper towel, skinned, filleted with a plastic knife, and cut in small slices. These slices were ground first in a pre-cleaned mixer and second, in a pre-cleaned chopper, and the ground mass obtained as a paste was transferred into a pre-weighed glass vessel; the vessel was reweighed, frozen in a freezer at  $-20^\circ\text{C}$  for 24 h, lyophilized in a freeze dryer for 120 h divided in consecutive 8-h periods (with the corresponding freezing periods overnight) to enable redistribution of the ground mass within the glass container, and reweighed to obtain the sample water content. The lyophilized sample divided in small fractions was powdered in a micro-dismembrator using a cryogenic approach with liquid nitrogen [27]. All powdered fractions were mixed together in a closed PTFE container and homogenized in a mechanical shaker. This product was screened through a set of three polyester sieves (16–32–65 mesh, respectively) being the largest particle size of the fine powder between 180 and  $250 \mu\text{m}$ . The later was re-homogenized, weighed, divided in plastic containers and stored in a desiccator at room temperature. The residual humidity was <2% in the fish samples and <5.5% in the clam samples.

Glass and plastic materials were cleaned as described elsewhere [12].

## 2.3. Sample solutions in study

Samples (500 mg lyophilized clam or fish) were digested in a microwave system; the digestion was performed in a mixture of  $\text{HNO}_3/\text{H}_2\text{O}_2$  (6:1) according to manufacturer's suggested program and to conditions indicated elsewhere [29] without an evaporation step after digestion: step 1, 1 min/250 W; step 2, 1 min/0 W; step 3, 5 min/250 W; step 4, 5 min/400 W; step 5, 5 min/650 W; and step 6, 5 min/0 W. The total digestion time was 22 min. No temperature and pressure measurements were made

due to the lack of automatic temperature or pressure control accessories in the microwave digestion unit. The digest solutions were carefully transferred into 25-ml volumetric flasks and diluted with u.p. water (sample digest solution) (A). Besides this approach, the sample digest solution was re-digested further for a complete conversion and oxidation into inorganic As(V) of the organoarsenicals still present in this solution. Thus, a photo-oxidation (B) and a re-digestion in an aluminum thermal block (C) were applied. In both approaches a 10 ml aliquot of the sample digest solution was mixed with 10 ml of 2% (m/v)  $K_2S_2O_8$  solution (in 0.5%, m/v NaOH), and this solution mixture (M) was prepared in duplicate. In approach B, the M solution was propelled through a Tefzel tubing (described previously), irradiated by a germicidal UV fluorescent tube (15 W) in continuous flow mode at a flow rate of  $1 \text{ ml min}^{-1}$  for 30 min, and was stored at  $4^\circ\text{C}$  until analysis (within 48 h). In approach C, two 6-ml aliquots of M solution were transferred into two silica digestion tubes respectively, re-digested in the aluminum thermal block between 50 and  $60^\circ\text{C}$  for 3 h, and subsequently heated up to  $100^\circ\text{C}$  and held for 1 h at this temperature, for the decomposition of  $K_2S_2O_8$  and partial evaporation of the solution [30]. Both re-digested solutions were transferred quantitatively and combined in a 25-ml volumetric flask, and diluted with u.p. water. Sample and CRM digest solutions were diluted suitably to fit within the third lowest segment of the linear working range and above the limit of quantification for As determination. Blank solutions of the acid digestion and re-digestions were prepared likewise.

#### 2.4. Procedure

A preliminary study was performed on the thermal program of the TCA with a blank and diluted digest solutions (1:25) of lyophilized fish muscle (DORM-2) and clam tissue (*Semelle solida*), all of them spiked with As(V) ( $100 \mu\text{g l}^{-1}$ ), without and with Pd, Ir, Rh, Mg and ascorbic acid (Asc.ac.) as chemical modifiers, both in single form and in binary mixtures, and applied into the tungsten coil both in solution (all of them) and in pre-reduced form (Pd, Ir, Rh and Mg) to establish the optimum pyrolysis conditions based on the highest temperature possible without significant As loss in this step, and high As absorbance sensitivity. An 8-step thermal program of the TCA was used in this study (Table 1); steps 1, 2, 4, 5 and 8 were optimized in previous work [20], and steps 3 and 6 correspond to the pyrolysis and atomization, respectively. The pyrolysis and atomization times (30 and 2.5 s, respectively) were selected based on the corresponding conditions established in previous studies performed in digest solutions of biological matrices [20]. During the pyrolysis study, the maximum atomization temperature (step 6) and the clean temperature (step 7) applied was  $1750^\circ\text{C}$  (11.5 V) in an attempt to extend the modifier effect. In each modifier and application approach was used a new coil to avoid possible memory effects. The first approach was the use of each modifier in solution at set concentration of  $50 \mu\text{g ml}^{-1}$  for Pd, Ir, Rh and Mg, based on our previous experience with elements of the PGMs as modifiers in the TCA [20], and  $100 \mu\text{g ml}^{-1}$  for Asc.ac [29]. Using the optimized pyrolysis and atomization conditions established therein, the concentration of each modifier was studied

Table 1

Thermal program for the determination of As<sup>a</sup> without and with chemical modifier by TCA-AAS

Step	Time (s)	Voltage (V)	Temperature ( $^\circ\text{C}$ )	Effect
1	60	0.50	250	Dry (1)
2	50	0.55	280	Dry (2)
3	30	In study <sup>b</sup>	In study <sup>b</sup>	Pyrolysis
4	10	0	20	Cool
5	0.20	0	20	Read
6	2.5	In study <sup>c</sup>	In study <sup>c</sup>	Atomization
7	3	11.5	1750	Clean
8	10	0	20	Cool

<sup>a</sup> Injection volume, 10  $\mu\text{l}$ .

<sup>b</sup> In the study of pyrolysis step the atomization temperature was  $1750^\circ\text{C}$  (11.5 V). In the selected modifier (Rh (0.5  $\mu\text{g}$ )) the optimum temperature was:  $850^\circ\text{C}$  (2.5 V) (blank digest);  $760^\circ\text{C}$  (2.0 V) (clam and fish tissue digest).

<sup>c</sup> The optimum atomization temperature was:  $1860^\circ\text{C}$  (13.0 V) (blank digest);  $1800^\circ\text{C}$  (12.0 V) (clam and fish tissue digest).

separately in the range between 5 and  $250 \mu\text{g ml}^{-1}$ ; additionally, the modifier concentrations were studied too in some binary mixtures (Rh–Asc.ac.; Rh–Mg; Rh–Ir; Ir–Asc.ac.; Pd–Mg; and Pd–Asc.ac.), based on the pyrolysis curves obtained with As(V) ( $100 \mu\text{g l}^{-1}$ ). Also, the elements of the PGMs (Pd, Ir and Rh) and Mg were used as modifiers in pre-reduced form (p-r), and the optimized conditions for the pre-reduction are shown in Table 2. In this case, 2 modifier approaches were tested on the tungsten coil: (i) 10 consecutive injections of 30  $\mu\text{l}$  (or 20  $\mu\text{l}$ ) of a  $1000 \mu\text{g ml}^{-1}$  modifier solution (Pd, Ir and Rh) equivalent to 300  $\mu\text{g}$  (or 200  $\mu\text{g}$ ), each one pre-reduced as indicated in Table 2 (step 5) in the search of a permanent modifier effect; and (ii) a single injection of 10  $\mu\text{l}$  of  $200 \mu\text{g ml}^{-1}$  solution equivalent to 2.0  $\mu\text{g}$  (Pd, Ir and Rh) pre-reduced likewise, before the injection of the As reference or sample solution in study, to make sure that a modifier effect was obtained in it. The coil temperature was estimated by voltamperometric measurements according to a procedure described elsewhere [12,14] and the mean uncertainty of this temperature estimate is 6.2% [14]. New coils were conditioned as described elsewhere [20] and aligned in the radiation beam before proceeding with the measurements. The modifiers studied included 28 alternatives and were compared without a modifier. Besides the use of a single modifier, some binary combinations were considered as well, based on apparent synergic modifier effects by comparison of the pyrolysis curves with those obtained without a modifier. Since the modifier effect observed

Table 2

Thermal program for the pre-reduction of the modifier on the TCA

Step	Time (s)	Voltage (V)	Temperature ( $^\circ\text{C}$ )	Effect
1	20	0.65	315	Dry (1)
2	25	0.60	300	Dry (2)
3	40	0.55	280	Dry (3)
4	20	0.50	250	Dry (4)
5	10	x <sup>a</sup>	x <sup>a</sup>	Pre-reduction
6	2	11.5	1750	Atomization
7	10	0	20	Cool

<sup>a</sup> 3.0 V/ $950^\circ\text{C}$  (Ir); 6.0 V/ $1330^\circ\text{C}$  (Pd); 3.0 V/ $950^\circ\text{C}$  (Rh); 1.5 V/ $625^\circ\text{C}$  (Mg).

with Pd both in solution and in pre-reduced form was not significant, the binary mixtures Pd–Ir and Pd–Rh were not considered in this study. After establishing the optimum pyrolysis temperatures, the three modifiers and/or mixtures with best performance according to the criteria indicated above were selected for the study on the atomization temperature. The definitive selection of the most appropriate modifier in the aforementioned diluted digest solutions of fish muscle and clam tissue spiked with As(V) ( $100 \mu\text{g l}^{-1}$ ) was by one-way variance analysis (Tukey test) [31], comparing the pyrolysis curves and the As absorbance obtained at the maximum voltage without significant As loss.

Interference effects of the main cations and anions according to the approximate concentrations present in digest solutions of clam and fish muscle, considering both the sample amount digested and the final solution volume were assessed for As(V) ( $200 \mu\text{g l}^{-1}$ ) in aqueous reference solutions with the selected modifier Rh ( $0.5 \mu\text{g}$ ). For cations at two concentration levels were studied the effects of  $\text{Na}^+$  (10 and  $100 \mu\text{g ml}^{-1}$ ),  $\text{K}^+$  (20 and  $200 \mu\text{g ml}^{-1}$ ),  $\text{Ca}^{2+}$  (15 and  $150 \mu\text{g ml}^{-1}$ ),  $\text{Cu}^{2+}$  ( $0.1$  and  $1.0 \mu\text{g ml}^{-1}$ ),  $\text{Mg}^{2+}$  (5 and  $50 \mu\text{g ml}^{-1}$ ),  $\text{Fe}^{3+}$  (1 and  $10 \mu\text{g ml}^{-1}$ ) and  $\text{Zn}^{2+}$  (1 and  $10 \mu\text{g ml}^{-1}$ ) as nitrates. The anions studied likewise corresponded to  $\text{Cl}^-$  (15 and  $150 \mu\text{g ml}^{-1}$ ) (as  $\text{NH}_4\text{Cl}$ ),  $\text{SO}_4^{2-}$  (10 and  $100 \mu\text{g ml}^{-1}$ ) (as  $(\text{NH}_4)_2\text{SO}_4$ ) and P (15 and  $150 \mu\text{g ml}^{-1}$ ) (as  $(\text{NH}_4)_2\text{HPO}_4$ ). Blank solutions were prepared and measured for each concomitant ion at both concentration levels and As absorbance of the corresponding solution was blank corrected. In each instance, the background corrected absorbance, the peak shape and the background absorbance were compared with those obtained for As without interference in Rh ( $0.5 \mu\text{g}$ ) modifier. This study was performed with the optimized thermal program for As(V) in Rh ( $0.5 \mu\text{g}$ ) using  $10 \mu\text{l}$  injections.

The analytical performance was assessed for As by using As(V) without modifier (sensitivity and linear working range) and in the selected modifier (Rh,  $0.5 \mu\text{g}$ ) under optimized conditions by systematic evaluation of the linear working range, characteristic mass ( $m_0$ ), repeatability, reproducibility and detection limit ( $\text{D.L.}, 3\sigma_{\text{blank}}/\text{slope}$ ). The three sample pretreatment procedures (A, B, C) were applied to independent subsamples of a CRM of dogfish muscle tissue (DORM-2) and the results obtained in the determination of total As in Rh ( $0.5 \mu\text{g}$ ) modifier by TCA-AAS in the respective digest solutions – based on dry weight (following the procedures recommended by the CRM producer for establishing the sample humidity) [32] – were compared both with the certified concentration and among them. The accuracy was established by the determination of As in the CRM of fish muscle. Quantitation was performed by standard additions. The methodology including the three sample pretreatment procedures was applied to the determination of total As in typical clam and fish samples.

### 3. Results and discussion

#### 3.1. Study of pyrolysis and atomization for As in seafood samples using chemical modifiers

In previous study on chemical modifiers for As determination by TCA-AAS [20] performed in single form with pre-reduced

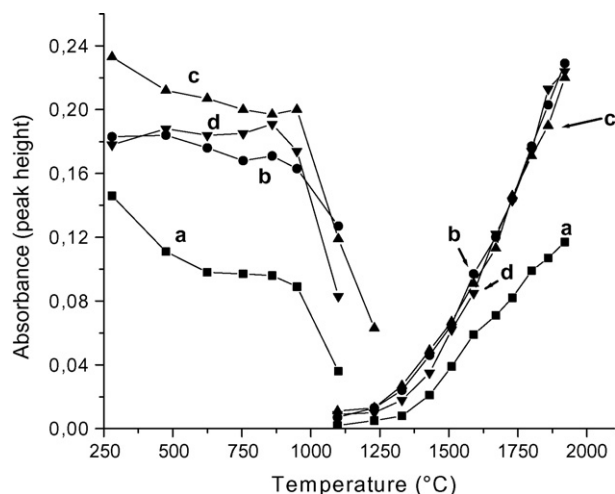


Fig. 1. (a–d) Pyrolysis and atomization temperature curves of  $1.0 \text{ ng As(V)}$  in acid-digested blank solution: (a) without chemical modifier; (b) in  $0.5 \mu\text{g Rh}$ ; (c) in  $0.5 \mu\text{g Rh} + 0.5 \mu\text{g Asc.ac.}$ ; (d) in  $1.0 \mu\text{g Ir}$ . In pyrolysis study the atomization temperature was  $1750^\circ\text{C}$ ; in atomization study the pyrolysis temperatures were  $850^\circ\text{C}$  (no modifier;  $0.5 \mu\text{g Rh}$ ;  $1.0 \mu\text{g Ir}$ ) and  $950^\circ\text{C}$  ( $0.5 \mu\text{g Rh} + 0.5 \mu\text{g Asc.ac.}$ ).

Pd, Ir and Rh, it was shown that Pd was not effective and Rh ( $2.0 \mu\text{g}$ ) was the most satisfactory modifier. Nevertheless, no further modifiers and other treatment alternatives were considered. In the present work, 28 pyrolysis studies were performed as described in Section 2.4. The pyrolysis temperature was varied between  $280^\circ\text{C}$  ( $0.55 \text{ V}$ ) and  $1100^\circ\text{C}$  ( $4.0 \text{ V}$ ) and the outcome of these studies was a pre-selection of three chemical modifiers with the best performance in terms of thermal stabilization of As (maximum pyrolysis temperature without significant As loss) and maximum As absorbance in the three aforementioned digest solutions compared to without a modifier. The pre-selected modifiers applied in solutions were: Rh ( $0.5 \mu\text{g}$ ), Ir ( $1.0 \mu\text{g}$ ) and Rh ( $0.5 \mu\text{g}$ ) + Asc.ac. ( $0.5 \mu\text{g}$ ), and subsequently their atomization temperatures were studied (at optimum pyrolysis temperature) between  $1100^\circ\text{C}$  ( $4.0 \text{ V}$ ) and  $1920^\circ\text{C}$  ( $14.0 \text{ V}$ ). As is shown in Fig. 1, in acid digested blank solution spiked with As ( $100 \mu\text{g l}^{-1}$ ) without modifier (Fig. 1a) clearly the pyrolysis curve differs from the same curves obtained with modifiers (Fig. 1b–d) in absorbance sensitivity (being significantly lower by a factor of 2) and in the profile. Also, this pyrolysis curve illustrates two effects; between 280 and  $625^\circ\text{C}$  a significant decrease in absorbance indicates apparent As loss in one molecular form (not evident in the presence of Rh modifier), whereas between  $625$  and  $850^\circ\text{C}$  a second form more stable thermally became apparent which is lost above  $850^\circ\text{C}$ . Based on these results,  $850^\circ\text{C}$  was selected as the pyrolysis temperature without modifier, which is coincident with the optimum pyrolysis temperature in the presence of Rh ( $0.5 \mu\text{g}$ ) or Ir ( $1.0 \mu\text{g}$ ) modifiers, whereas  $950^\circ\text{C}$  was the optimum pyrolysis temperature in Rh ( $0.5 \mu\text{g}$ ) + Asc.ac. ( $0.5 \mu\text{g}$ ) modifier. In the atomization curves (Fig. 1) (using a pyrolysis temperature of  $850^\circ\text{C}$  ( $2.5 \text{ V}$ ) (no modifier;  $0.5 \mu\text{g Rh}$ ;  $1.0 \mu\text{g Ir}$ ) or  $950^\circ\text{C}$  ( $3.0 \text{ V}$ ) ( $0.5 \mu\text{g Rh} + 0.5 \mu\text{g Asc.ac.}$ )), no plateau was reached in any of them but a significantly higher relative absorbance for As by a factor of 2 was observed between 1850

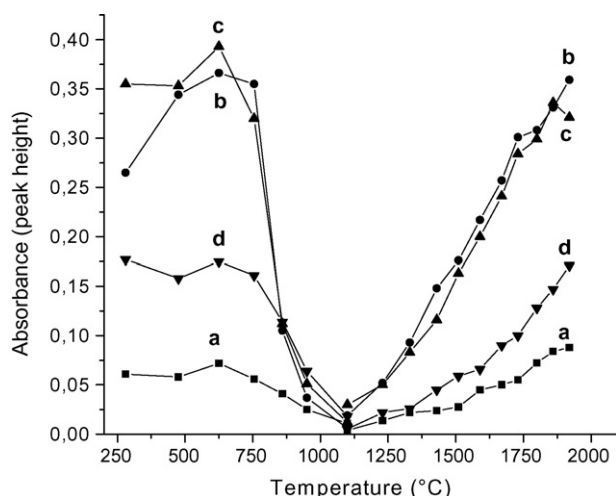


Fig. 2. (a–d) Pyrolysis and atomization temperature curves of 1.0 ng As(V) in diluted (1:25) acid-digested dogfish muscle (DORM-2) solution: (a) without chemical modifier; (b) in 0.5 µg Rh; (c) in 0.5 µg Rh + 0.5 µg Asc.ac.; (d) in 1.0 µg Ir. In pyrolysis study the atomization temperature was 1750 °C; in atomization study the pyrolysis temperatures were 625 °C (no modifier; 0.5 µg Rh + 0.5 µg Asc.ac.; 1.0 µg Ir) and 760 °C (0.5 µg Rh).

and 1950 °C (13.0 and 14.0 V) in the three modifiers, showing along this study a similar trend with steeper curves compared without a modifier. The selected atomization temperature was 1850 °C (13.0 V). The same study was performed in diluted sample digest solutions of clam and fish tissue, and the pyrolysis and atomization curves showed some significant differences compared without a modifier. Since the pyrolysis and atomization curves obtained in both matrices were quite similar, the results obtained in fish tissue will be discussed further.

As is shown in Fig. 2 in diluted acid-digested solutions of fish tissue spiked with As ( $100 \mu\text{g l}^{-1}$ ) without modifier, the optimum pyrolysis temperature was 625 °C (1.5 V) and the maximum absorbance obtained is significantly smaller (at least by a factor of 4.9–5.2 times in the pyrolysis study) compared with the maximum absorbance obtained for As in Rh (0.5 µg) and in Rh (0.5 µg) + Asc.ac. (0.5 µg), and smaller (by a factor of 3.2) compared with the maximum absorbance obtained for As in Ir (1.0 µg) modifier. Without modifier the interference effect of concomitants was similar in fish digest as in clam, causing similar loss of arsenic in both matrices. Apparently in the presence of the pre-selected modifiers the higher relative absorbance of As obtained in both sample digests compared to the case without modifier could be explained by a reduction of analyte loss due to concomitants effect [20]. The optimum pyrolysis temperature was 760 °C (2.0 V) in Rh (0.5 µg) providing some thermal stabilization of As, and was 625 °C (1.5 V) both in Rh (0.5 µg) + Asc.ac. (0.5 µg) and in Ir (1.0 µg), showing no difference with the optimum temperature obtained without modifier. In the atomization curves a significantly higher relative absorbance for As by a factor of 4.1 in Rh (0.5 µg) + Asc.ac. (0.5 µg) and 3.9 in Rh (0.5 µg), was shown up compared to the no use of modifier. Iridium (1.0 µg) modifier was somewhat less effective, and As absorbance was higher by a factor of 1.9 respect to the no use of modifier. An incipient plateau is noticed in the atomization curves obtained without

modifier and in Rh (0.5 µg) + Asc.ac. (0.5 µg) modifier. In the fish digest study like in the clam study the selected atomization temperature was 1800 °C (12.0 V), to extend the useful coil lifetime. A difference found comparing the atomization curves obtained in both sample digests and in the blank digest was the relative sensitivity for As between 1330 and 1950 °C (6.0 and 14.0 V, respectively). In the blank digest study the relative sensitivity was quite comparable between the three pre-selected modifiers and higher than without modifier, whereas in clam and fish digests the relative sensitivity was much higher and comparable between Rh (0.5 µg) and Rh (0.5 µg) + Asc.ac. (0.5 µg), being lower in Ir (1.0 µg) and much lower without modifier.

Hitherto, in both sample digests Rh modifier without or with Asc.ac. was more effective than Ir in thermally stabilizing As, and although there was a slight thermal stabilization effect in particular with Rh compared to the no use of modifier, the main effect observed in the three pre-selected modifiers was a significant increase in relative absorbance, being more favorable in Rh (0.5 µg) and in Rh (0.5 µg) + Asc.ac. (0.5 µg).

### 3.2. Selection of the modifier by variance analysis

An attempt was made to establish a systematic approach using a statistical tool to compare the pyrolysis curves obtained for As among the three pre-selected modifiers with similar performance in terms of thermal stabilization. The pyrolysis curves, the optimum pyrolysis temperature without significant As loss and the corresponding As absorbances obtained therein in the blank digest and in the diluted digest solutions of clam and fish tissue spiked with As ( $100 \mu\text{g l}^{-1}$ ) were compared by variance analysis (ANOVA). Since first order interactions in the three digest solutions were significant, comparisons between modifiers were performed in each study voltage. In blank digest the optimum pyrolysis temperature was 950 °C (3.0 V) and the mean As absorbance obtained in Rh (0.5 µg) + Asc.Ac. (0.5 µg) (0.200) was significantly higher than in the other modifiers. In this digest without sample matrix, Rh (0.5 µg) + Asc.Ac. (0.5 µg) appears as most suitable among the studied modifiers. In clam and fish digests the optimum pyrolysis temperature obtained for As in Rh (0.5 µg) was 760 °C (2.0 V) and the mean As absorbance was 0.306 and 0.355, respectively, being significantly higher compared with the other modifiers. Using Rh (0.5 µg) + Asc.ac. (0.5 µg) modifier and the same pyrolysis temperature, the mean As absorbance obtained in clam (0.306) and fish tissue (0.320) corresponded to the next alternative modifier. In fish tissue digest another alternative modifier was Rh (0.5 µg) + Asc.ac. (0.5 µg). Both in clam and fish digest solution the pyrolysis curves obtained in Rh (0.5 µg) and Rh (0.5 µg) + Asc.ac. (0.5 µg) were the most satisfactory and concurrently similar. Based on these comparisons, the definitive selected modifier for further analytical studies was Rh (0.5 µg), since the mixture Rh (0.5 µg) + Asc.ac. (0.5 µg) did not show any additional feature to persuade on this decision.

### 3.3. Analytical figures of merit

After the selection and optimization of Rh modifier (0.5 µg), the calibration curves and linear working ranges for As were

Table 3  
Calibration graphs<sup>a</sup> for As(V) obtained by TCA-AAS<sup>b</sup> without and with Rh (0.5 µg)

Modifier	Calibration range (µg l <sup>-1</sup> )	Slope ± S.D. <sup>c</sup>	Intercept ± S.D.	Standard error <sup>d</sup>	Correlation coefficient
None	10–250	0.0014 ± 0.00001	-0.0017 ± 0.0016	0.0027 (7)	0.9998
Rh (0.5 µg)	10–300	0.00186 ± 0.00002	0.0007 ± 0.0037	0.0054 (8)	0.9997

<sup>a</sup> Linear regression ( $A = mC + b$ ) representative of  $n = 6$  for each data point.

<sup>b</sup> Obtained with eight-steps thermal program.

<sup>c</sup> Standard deviation.

<sup>d</sup> Number of data points in parentheses.

Table 4  
Analytical figures of merits obtained for As in Rh (0.5 µg) by TCA-AAS

Reciprocal sensitivity (µg l <sup>-1</sup> )	Characteristic mass (pg)	Linear working range (µg l <sup>-1</sup> )	Within-day repeatability CV%	Reproducibility CV%	Detection limit (pg)
2.3	23	10–300	3	4	42

established with and without modifier for comparison, and are reported in Table 3. In Rh modifier the slope sensitivity for As was higher by 33% and the linear working range was more extensive compared without a modifier; the correlation coefficients were quite satisfactory in both conditions. In Table 4 are shown the analytical figures of merit obtained in Rh (0.5 µg) modifier; the characteristic mass was 23 pg obtained with 10-µl injections of As solutions, and was somewhat higher than in previous study with pre-reduced Rh (2.0 µg) [20] mainly due to a lower atomization temperature selected in the present work which enables a more extended linear working range and convenient coil lifetime. The mean within-day repeatability obtained in three cycles, each of six consecutive measurements and each cycle performed with new independent coil, turning off and on the spectrophotometer and EDL lamp between cycles was 3% (1% (300 µg l<sup>-1</sup>) to 8% (25 µg l<sup>-1</sup>)). The mean reproducibility obtained in 18 measurements performed per day in 3 different days with newly prepared solutions of the same concentrations was 4% (2% (300 µg l<sup>-1</sup>)–8% (25 µg l<sup>-1</sup>)), which indicates that the intralaboratory variability was small for As reference solutions in Rh modifier. The detection limit (D.L.,  $3\sigma_{\text{blank}}/\text{slope}$ ) of As in the presence of Rh (0.5 µg) was established in consecutive measurements with 10-µl injections of two As(V) reference solutions (10 and 20 µg l<sup>-1</sup>) alternated with a blank solution and

was 4.2 µg l<sup>-1</sup> (42 pg) ( $n = 16$ ). The CV% obtained in these consecutive measurements was 13% and 8% for 10 and 20 µg l<sup>-1</sup> As(V), respectively. This detection limit is comparable with the detection limit obtained in previous work for As in pre-reduced Rh (2.0 µg) [20]. Compared to the detection limit obtained by ET-AAS with the graphite furnace and integrated platform using longitudinal Zeeman background correction is higher by a factor of 4 [33]. Nevertheless, the detection limit obtained for As with Rh (0.5 µg) modifier by TCA-AAS is suitable for its direct determination in diluted digest solutions of the seafood matrices in study. The method detection limit by TCA-AAS was assessed for As in acid digested fish sample matrix with Rh modifier and was 0.2 µg g<sup>-1</sup> dry weight (dw) and the quantification limit was 0.7 µg g<sup>-1</sup> (dw). This figure of merit is satisfactory to determine total As in fish and clam samples according to the total As levels reported in the literature [34,35].

The effect of concomitants on As (200 µg l<sup>-1</sup>) in Rh modifier was assessed at two different concentration levels for the main cations and anions present in the study matrices, and was expressed with respect to As absorbance normalized to 100% (Table 5) measured without interferent. In the presence of Na<sup>+</sup>, K<sup>+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cl<sup>-</sup> and SO<sub>4</sub><sup>2-</sup> there were no significant interference effects on As (>91%) in both concomitant concentration levels. As is shown in Table 5 the main effects

Table 5  
Interference effects<sup>a</sup> on As (200 µg l<sup>-1</sup>) in Rh (0.5 µg) modifier by various concomitant ions by TCA-AAS

Interferent	C <sub>1</sub> (µg ml <sup>-1</sup> )	% Absorbance <sup>b</sup>	C <sub>2</sub> (µg ml <sup>-1</sup> )	% Absorbance <sup>b</sup>
Na <sup>+</sup>	10	92	100	94
K <sup>+</sup>	20	97	200	96
Ca <sup>2+</sup>	15	122	150	79
Mg <sup>2+</sup>	5	92	50	148
Fe <sup>3+</sup>	1	81	10	44
Zn <sup>2+</sup>	1	96	10	93
Cu <sup>2+</sup>	0.1	95	1	91
SO <sub>4</sub> <sup>2-</sup>	10	99	100	94
HPO <sub>4</sub> <sup>2-</sup>	15	21	150	1
Cl <sup>-</sup>	15	95	150	92

<sup>a</sup> C<sub>1</sub> and C<sub>2</sub> are concentration levels.

<sup>b</sup> Respect to As absorbance without interferent.

corresponded to great signal depressions produced by P (as  $\text{HPO}_4^{2-}$ ) at  $15 \mu\text{g ml}^{-1}$  (21%) and  $150 \mu\text{g ml}^{-1}$  (1%), followed in strength by  $\text{Fe}^{3+}$  at  $1 \mu\text{g ml}^{-1}$  (81%) and  $10 \mu\text{g ml}^{-1}$  (44%), and by  $\text{Ca}^{2+}$  at  $150 \mu\text{g ml}^{-1}$  (79%), whereas the main opposite effects with enhancement on As absorbance were observed in the presence of  $\text{Mg}^{2+}$  at  $50 \mu\text{g ml}^{-1}$  (148%) followed in strength by  $\text{Ca}^{2+}$  at  $15 \mu\text{g ml}^{-1}$  (122%). In the presence of Mg (0.5  $\mu\text{g}$ ) as modifier it is possible to observe full compensation of the interferences by  $\text{Ca}^{2+}$  and  $\text{Fe}^{3+}$ , however not for the effect by P as phosphate. The effect of  $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$  on As absorbance (As  $200 \mu\text{g l}^{-1}$ ) without Rh modifier was studied at two concentrations (15 and  $150 \text{ mg l}^{-1}$  as P). The results showed drastic signal depressions in As absorbance: with  $\text{H}_2\text{PO}_4^-$ , 88% and 96% drop, respectively; and with  $\text{HPO}_4^{2-}$ , 92% and 99% drop, respectively. Thus, the depression effect of both phosphate species is similar. In the presence of Rh modifier as shown in Table 5, the depression effect with  $\text{HPO}_4^{2-}$  is still quite significant, suggesting that Rh at the used concentration was too low to overcome this effect.

Simulating a sample matrix solution by mixing all cations and anions at the high concentration level considered in this study, As absorbance was 48% respect to the normalized signal. In order to reduce these interference effects not corrected by Rh modifier with the thermal program in use, the sample digest solutions were diluted properly and quantitation was performed by the standard addition method.

### 3.4. Study of sample pretreatment and evaluation of accuracy

In order to select and evaluate an appropriate sample pretreatment approach, independent subsamples of a CRM of dogfish muscle tissue (DORM-2) (certified total As =  $18.0 \pm 1.1 \mu\text{g As g}^{-1}$ ) were processed by the three procedures described before, and the results obtained in the determination of As ( $n$  = number of independent determinations) are:  $15.3 \pm 0.69 \mu\text{g g}^{-1}$  ( $n=5$ ) (procedure A);  $18.3 \pm 1.34 \mu\text{g g}^{-1}$  ( $n=4$ ) (procedure B); and  $18.6 \pm 0.74 \mu\text{g g}^{-1}$  ( $n=7$ ) (procedure C). Compared with the certified As concentration, the result obtained by procedure A was poor with a recovery of 85% of total As present in this material, and statistically significant difference

was established by Student's  $t$ -test at a 95% confidence level ( $p=0.0002$ ). By procedures B and C the recoveries in CRM were quite satisfactory (102% and 103%, respectively), and no statistically significant differences became evident by  $t$ -test compared with the certified value ( $p=0.6344$  (B);  $p=0.2296$  (C)). Also, the results obtained in this CRM by procedure A compared with procedures B and C showed statistically significant differences: A versus B,  $p=0.0032$ ; A versus C,  $p=0.00001$ ; whereas the comparison between procedures B and C revealed no statistical difference at a 95% confidence level ( $p=0.6375$ ). Most probably, the microwave acid digestion procedure in  $\text{HNO}_3\text{--H}_2\text{O}_2$  was not effective in full conversion of As present in organoarsenicals into As(V), and these species could be lost during the pyrolysis step of the thermal program in the TCA. The re-digestion of the sample digest solution in  $\text{K}_2\text{S}_2\text{O}_8$ , assisted either by UV photo-oxidation (B) or by heating in a thermal block (C), was effective to the complete conversion purpose to As(V). Hence, procedures B and C were selected for the analytical applications of this determination of total As in seafood by TCA-AAS. The accuracy expressed by the mean relative percent error ( $E_{\text{rel}}\%$ ) for total As determination in the CRM DORM-2 was 2% in procedure B and 3% in procedure C, and precision expressed by the relative standard deviation (R.S.D.) was 7% by procedure B and 4% by procedure C. Both figures are satisfactory and provide confidence on the feasibility of this methodology with sample pretreatment by procedures B and C.

### 3.5. Analytical application in seafood samples

The TCA was applied to the determination of total As in samples of lyophilized clam (*Semelle solida* and *Tagelus dombeii*) and fish tissue (*Merluccius gayi* and *Salmo Salar*) by the three sample pretreatment procedures and the results are shown in Table 6. Procedure A was included to verify if As recoveries in samples of clam and fish tissue denote similar figures as those observed in the CRM. In general, the concentration (dw) of As was in the range  $3.2\text{--}6.8 \mu\text{g g}^{-1}$  by procedure A,  $3.7\text{--}8.6 \mu\text{g g}^{-1}$  by procedure B and  $3.8\text{--}8.5 \mu\text{g g}^{-1}$  by procedure C, and assuming a mean water content of 85% in the original samples, the highest As concentration (fresh mass, fm) would be  $1.3 \mu\text{g g}^{-1}$  which can be considered relatively low by comparison with

Table 6  
Determination of total As concentration ( $\mu\text{g g}^{-1}$ ) (dry weight) in clam and fish muscle samples by TCA-AAS in Rh (0.5  $\mu\text{g}$ ) modifier after three sample pretreatment procedures<sup>a</sup>

Sample	Procedure A		Procedure B		Procedure C	
	Mean	S.D. <sup>b</sup>	Mean	S.D.	Mean	S.D.
<i>Semelle solida</i>	6.8	0.19 ( $n=6$ ) <sup>c</sup>	8.6	0.70 ( $n=3$ )	8.4	0.50 ( $n=4$ )
<i>Tagelus dombeii</i>	5.8	0.37 ( $n=3$ )	7.4	0.35 ( $n=3$ )	7.0	0.10 ( $n=3$ )
<i>Merluccius gayi</i> I	5.8	0.30 ( $n=3$ )	6.6	0.10 ( $n=3$ )	7.2	0.40 ( $n=3$ )
<i>Merluccius gayi</i> II	5.6	0.10 ( $n=3$ )	6.5	0.34 ( $n=6$ )	6.9	0.35 ( $n=3$ )
<i>Merluccius gayi</i> III	5.7	0.18 ( $n=3$ )	6.8	0.11 ( $n=3$ )	6.8	0.26 ( $n=3$ )
<i>Salmo Salar</i> I	3.2	0.10 ( $n=5$ )	3.8	0.10 ( $n=5$ )	3.9	0.10 ( $n=5$ )
<i>Salmo Salar</i> II	3.3	0.21 ( $n=5$ )	3.7	0.10 ( $n=5$ )	3.8	0.13 ( $n=5$ )

<sup>a</sup> A = microwave digestion (M.D.); B = M.D. +  $\text{K}_2\text{S}_2\text{O}_8$  + UV photo-oxidation; C = M.D. +  $\text{K}_2\text{S}_2\text{O}_8$  + re-digestion in thermal block at controlled temperature.

<sup>b</sup> Standard deviation.

<sup>c</sup>  $n$  = number of independent determinations.

As levels (fm) reported by the USFDA in bivalve mussels and crustacean ( $1.1\text{--}30\ \mu\text{g g}^{-1}$ ) [36]. Hence, the As levels found in seafood samples within this work are safe and do not represent any sanitary risk to consumers by exposure through the diet. In the clam samples, as a mean the recovery of As by procedure A is 75% and 82% with respect to the As content determined by procedures B and C correspondingly, and it correlates well with the recovery (85%) obtained by procedure A in the CRM (DORM-2). In the three samples of *Merluccius gayi* tissue, the results obtained by procedure A are quite similar ( $5.6\text{--}5.7\ \mu\text{g g}^{-1}$ ), and consistently lower with respect to the results by procedures B ( $6.5\text{--}6.8\ \mu\text{g g}^{-1}$ ) and C ( $6.7\text{--}7.1\ \mu\text{g g}^{-1}$ ). As a mean the As content determined by procedure A was 85% and 82% with respect to procedures B and C correspondingly, and it correlates well with the recovery obtained by procedure A in DORM-2. Also, in the two samples of *Salmo Salar* tissue, the results obtained by procedure A are quite similar ( $3.2\text{--}3.3\ \mu\text{g g}^{-1}$ ), and consistently lower with respect to the results by procedures B ( $3.7\text{--}3.8\ \mu\text{g g}^{-1}$ ) and C ( $3.8\text{--}3.9\ \mu\text{g g}^{-1}$ ). As a mean the As content determined by procedure A was 87% and 84% with respect to procedures B and C correspondingly, and also it correlates well with the recovery obtained by procedure A in DORM-2. Thus, these results confirm that procedure A does not render quantitative recovery of As; however, by procedures B and C the As content provides quantitative recovery.

The proposed methodology is simple and reliable, the sample readout frequency is  $21\ \text{h}^{-1}$ , and by keeping the nitric acid concentration low (<1%) the coil performance is unaffected (no absorption signal degradation) at least in 300 heating cycles. The tungsten coil atomizer is a good alternative for laboratories with limited funds and the use of chemical modifiers could expand the analytical applications by TCA-AAS.

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