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Open digestion under reflux for the determination of total arsenic in seafood by inductively coupled plasma atomic emission spectrometry with hydride generation

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Abstract

A method for the determination of arsenic (As) in seafood by inductively coupled plasma atomic emission spectrometry with continuous hydride generation is described. Several analytical parameters have been investigated and optimised. The analytical features of the method (recovery, precision, accuracy and limit of detection) were calculated. Practical detection limit of 3.6 μg/kg fresh weight for As has been reached. The precision of the method expressed as relative standard deviation (R.S.D.) was in the range of 2.7–3.7% and the recovery percentage ranged from 98.4 to 101.8%. The reliability of the developed method was checked by analysing several certified reference materials. A complete mineralization was obtained for arsenobetaine (AsB) containing reference material with a mixture of nitric and sulphuric acids followed by adding hydrogen peroxide in an open digestion system. This method can be applied to routine analysis without any risks of interferences.

Keywords: Seafood; Arsenic determination; Wet digestion; Hydride generation; ICP-AES

1. Introduction

Arsenic (As) is ubiquitous in our environment. Besides the toxic forms of As, arsenite and arsenate, approximately thirty organoAs compounds have been identified thus far. Arsenic and its compounds have been recognized as carcinogenic to humans by the International Agency for Research on Cancer (IARC). Epidemiological evidence supports an association between chronic exposure to inorganic As and increased risk of skin, urinary bladder, lung, liver and kidney cancer [1,2]. The continuous release of As into the environment leads to its accumulation in drinking water as well as in the food chain, of which marine food is presumably the major source of As in the diet [3].

In order to determine the total As concentration of biological tissues, it is imperative to assure complete mineralization of the sample, unless using non-destructive or As speciation techniques. In oxidative acid digestion, covalent As–C bonds of organoAs compounds should be broken to yield inorganic As. Moreover, As compounds with high number of methyl groups such as arsenobetaine (AsB), which was found to be a predominant As compound in marine animals, are supposed to be very stable [4]. Dry ashing or wet digestion procedures are commonly employed for the oxidation of organic matter. Dry ashing implies complete oxidation of the organic matter in an open system at elevated temperatures (450–800 °C), but presents a risk of analyte loss due to the formation of volatile compounds, e.g. chlorides or oxychlorides. Wet ashing, on the other hand, can be conducted in both open or closed systems. High digestion temperatures result in increased oxidation power; for AsB temperatures exceeding 320 °C are required [5]. This can be achieved in open wet digestion vessels using sulphuric acid.

The determination of total As is often based on the hydride generation (HG) technique, which makes use of the forma-

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tion of volatile arsines with $NaBH_4$. Therefore, a conversion of all organic As compounds into inorganic As is required since few As compounds such as AsB, arsenocholine and tetramethylarsonic acid do not convert into hydrides. Several papers focus on the determination of As by HG in combination with atomic absorption or atomic fluorescence spectrometry [6–17]. Little information is available on the application of inductively coupled plasma optical emission spectrometry (ICP-OES).

In this work, the optimization of the complete mineralization of seafood samples containing stable As compounds such as AsB is investigated. Additionally, the study aims at the use of ICP-OES with flow injection-hydride generation (FI-HG-ICP-OES) for total As analysis.

2. Materials and methods

2.1. Instrumentation

A Perkin-Elmer (PE) ICP Optima 4300 DV inductively coupled Ar plasma optical emission spectrometer (Norwalk, CT, USA), equipped with an autosampler (AS93 plus, PE), was used in combination with a FIAS 400 PE hydride generator with two peristaltic pumps and a regulated gas supply. The gas flow rate was set to 40–250 mL/min.

Chemical reduction to arsine (AsH_3) of acidified samples mixed with $NaBH_4$ was followed by removal of the gaseous AsH_3 with Ar and direct introduction into the inlet tube of the plasma torch.

Prior to HG, the samples were mineralized in an open digestion system (2040 Digestor, Foss Tecator), consisting of an electrically heated aluminium block for 40 test tubes (quartz, 100 mL, with air cooled condensers) and equipped with a built-in electronic temperature (from ambient to 440 °C) and time control (up to 100 h).

2.2. Chemicals and reagents

For the preparation of all solutions Milli Q water (Millipore, Milford, MA, USA; 18 Mohm.cm, Elix 100 Toc) was used. Chemicals for mineralization were sulphuric acid (98% (w/w), J.T. Baker, USA), nitric acid (70% (w/w), J.T. Baker, USA) and hydrogen peroxide (30% (w/w), J.T. Baker, Deventer, Holland). $NaBH_4$ 0.2% (w/v) solutions for the flow injection system were prepared daily by dissolving appropriate amounts of powdered $NaBH_4$ (for cold vapor analysis, J.T. Baker, Holland) in 0.05% (w/v) NaOH (Pellets, Merck). As(V) was reduced to As(III) with an aqueous solution containing 2.5% (w/v) KI (99%, Sigma–Aldrich, Steinheim, Germany) and 2.5% (w/v) ascorbic acid (minimum 90%, Sigma–Aldrich, Steinheim, Germany) added to HCl (37%, m/m, J.T. Baker, USA).

Calibration solutions (1–20 μg/L) for As were prepared daily by diluting aliquots of stock standard solutions, containing 1000 mg/L As(III) in 2% HNO_3, purchased from Perkin-Elmer, Pure Atomic Spectroscopy Standard (Shelton, CT, USA).

Ionic solutions used in the interferences' study were prepared by appropriate dilutions of stock solutions (Perkin-Elmer, Shelton, CT, USA).

Certified reference materials, DORM-2 (dogfish muscle tissue), DOLT-3 (dogfish liver) and TORT-2 (lobster hepatopancreas), were purchased from the national Research Council of Canada (NRCC, Ottawa, Ontario). BCR 278R (mussel tissue) and BCR 422 (cod muscle) were purchased from the Institute for Reference Materials and Measurement (IRMM, EC, Geel, Belgium).

2.3. Digestion procedures

Approximately 1.0 g of seafood (wet weight) or 0.1–0.25 g of CRM lyophilised powder was taken and placed in a quartz digestion tube with 5 mL of concentrated nitric acid and 3 mL of concentrated sulphuric acid. Afterwards, digestion was carried out under reflux in a temperature controlled digestor bloc. The temperature was increased stepwise from ambient temperature to 350 °C in 1 h and 30 min and kept constant at 350 °C during 3 h. After cooling the tubes, 3 mL H_2O_2 was added and temperature was raised to 400 °C and maintained during 4 h. Prior to analysis, each digested sample was transferred quantitatively into a 10 mL calibrated tube.

2.4. Analytical procedures

All experiments were performed in triplicate (instrument programmed). The As(V) compounds were reduced to As(III) prior to analyses by a mixture of concentrated HCl and a solution of KI 2.5% (w/v) (prepared in ascorbic acid 2.5%, w/v); 5 mL of previously digested sample was added to 2.5 mL of KI solutions and 2.5 mL HCl (37%, w/w). After reduction of As(V) to As(III) occurring within 15 min, the quantification of total As in seafood and CRM was started by the FI-HG-ICP-OES method. Conversion into AsH_3 resulted from transporting both the sample and $NaBH_4$ solution into the manifold block by two peristaltic pumps. Hydride was generated and separated from the gas–liquid mixture in the separator block added to the manifold, and swept with argon into the plasma for atomization and measurement of As. Detailed operating conditions of the analytical set-up are summarised in Table 1.

3. Results and discussion

3.1. Optimization of digestion procedure

The decomposition of As compounds had already been investigated [18]. Wet ashing with nitric acid and microwave assisted heating merely yielded accurate transformation and quantification of As compounds, when heating the samples for 90 min to 300 °C, which is obviously very high for Teflon

Table 1
Optimized working conditions for the FI-HG-ICP-OES method

Plasma	
Instrument	Perkin-Elmer Optima 4300 DV
Argon flow	15 L/min
Auxiliary argon flow rate	200 mL/min
Analytical wavelength	193.700 nm
Plasma view	Axial
Delay time	70 s
Hydride generation	
Sample flow rate	3.0 mL/min
HCl acidity of sample	3.0 mol/L
KI solution	2.5% (w/v) in acid ascorbic 2.5% (w/v)
$NaBH_4$ solution	0.2% (w/v) in 0.05% (w/v) NaOH
$NaBH_4$ flow rate	2.6 mL/min.
Tube coil reactor	100 cm length and 1.1 mm i.d.
Carrier gas flow rate	200 mL/min

vessels. These harsh conditions are not achievable with most commercially available microwave systems.

Other studies on the stability and oxidation pathways of As compounds such as arsenocholine (AsC), trimethylarsine oxide (TMAO) and AsB in a microwave system with nitric acid and hydrogen peroxide have been carried out [19]. It was observed that AsC and AsB could be converted to TMAO solely at 207 °C, a too low temperature to convert TMAO to As(III) or As(V), which can generate hydride. In open acid digestion, the decomposition of arsenobetaine (AsB) was incomplete, leading to underestimations of the total As concentration by HG techniques [4].

To determine optimum digestion conditions, the standard reference materials BCR 278R, BCR 422 with mineral As compounds and DORM-2 with high AsB content were submitted to digestion. The amount of H_2SO_4 added and the digestion time were investigated for both reference materials. The efficiency of the mineralization revealed that a volume of less than 1 mL H_2SO_4 is optimal for matrices without AsB, whereas 3 mL H_2SO_4 is required for the matrix containing AsB, respectively (Table 2). This is largely sufficient to give a complete mineralization of samples containing organoAs compounds. Optimal digestion time was determined with BCR 278R; the digestion was complete after 4 h. For organoAs, we decided to continue digestion for 8 h in order to make sure that conversion into mineral As was complete.

Table 2
Efficiency of total As for different amount of added concentrated sulphuric acid for BCR 278R and DORM-2; digestion time was 4 h for BCR 278R and 8 h for DORM-2

VH_2SO_4 added (mL)	Efficiency (%)	
	BCR 278	DORM-2
0.25	96	7.2
0.5	101	16
1.0	102	57
2.0	99	81
3.0	101	99

3.2. Improvement of hydride generation conditions

Many experimental parameters must be controlled in order to achieve the best performance in terms of highest analytical sensitivity and lowest signal deviation. Arsenic may be determined either in the (III) or (V) oxidation state, but the (V) state is characterized by a slowing of hydride formation rate and gives a much lower recovery [17]. Therefore, a pre-reduction to (III) is required. The pre-reduction reaction was found optimal after 15 min reaction of the sample with a solution mixture of concentrated HCl, KI and ascorbic acid. The signal corresponding to As(III) remained constant for 3 h at least.

Other parameters such as HCl acidity of the sample, $NaBH_4$ and KI concentrations, the carrier gas flow and length of reaction coil must be controlled carefully in order to assure a quantitative reaction and to produce the smallest possible signal dispersion. A reaction coil with a length of 100 cm and an internal diameter of 1.1 mm (i.d.) gave optimal response for As with a fixed flow rate of 3.0 mL/min (sample) and of 2.6 mL/min ($NaBH_4$ reagent).

3.3. Effect of HCl, KI and $NaBH_4$ concentrations on the analyte response

Fig. 1 illustrates a plot of the concentrations of total As observed (true value of 10 μg/L) versus the different HCl concentrations. This was studied in the range of 0.6–6.0 mol/L. A good recovery of As was obtained for HCl molarities comprised between 3.0 and 6.0 mol/L. A value of 3 mol/L was considered optimal for the current study.

Similar studies were performed with different concentrations of KI. Fig. 2 shows that total recovery of As was observed for a ratio of KI greater than 1.5% (w/v).

The concentration of $NaBH_4$ was also investigated and detectable amounts of total As were stable within the range from 0.2 to 0.5% (w/v). Out of this range, poor recovery gave underestimates of the As content (Fig. 3). This lower detectable amount of As for $NaBH_4$ less than 0.2% was likely due to the

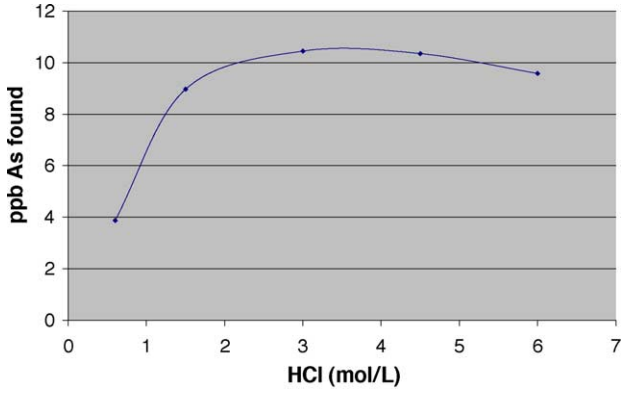

Fig. 1. Effect of HCl concentration on the As(III) response. Experimental conditions: $NaBH_4$ 0.2% (m/v) in 0.05% (m/v) NaOH at a flow rate of 2.6 mL/min; sample flow rate of 3.0 mL/min. Average corresponding to three replicates of solution containing 10 ng/mL of As.

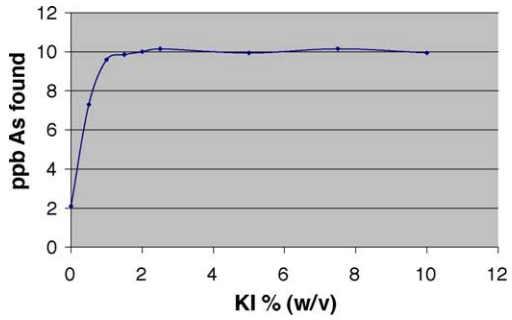

Fig. 2. Effect of KI percentage on the As(III) response. Experimental conditions: HCl 3.0 M; $NaBH_4$ 0.2% (m/v) in 0.05% (m/v) NaOH at a flow rate of 2.6 mL/min; sample flow rate of 3.0 mL/min. Average corresponding to three replicates of solution containing 10 ng/mL of As.

incomplete formation of AsH_3. For higher concentrations of $NaBH_4$, the decrease of detectable As was due possibly to the dilution of AsH_3 caused by large amounts of H_2 gas. A value of 0.2% was retained for subsequent experiments.

3.4. Effect of the argon flow rate

Argon was used as the carrier gas, transporting volatile hydrides to the plasma, but acting as an analyte diluent. The effect of its flow was evaluated in the range from 50 to 250 mL/min.

Increasing the argon flow rate resulted in enhanced responses of the analyte as evidenced by the trend in Fig. 4. An optimal flow rate of 200 mL/min was selected in order to have most reproducible and near maximal calibration slopes.

3.5. Interference study

The interference of different ions is, in general, attributed to the strength of the interaction between the element under study and the matrix. Many papers report on chemical interferences, especially for samples containing high concentrations of transition metals [20–23]. Interferences are more severe for methods in which concentrations of borohydride are relatively high and concentrations of hydrochloric acid are low. It was reported that using a hydrochloric acid concentration ranging from 0.5 to 5 mol/L improved the range of interference-free determination for As in the presence of cobalt, copper or nickel by factors of 5–100. Decreasing the sodium tetrahydroborate concentration from 3 to 0.5% (m/v) increased the range of interference-free determination for As by factors of 4–50 [21,24].

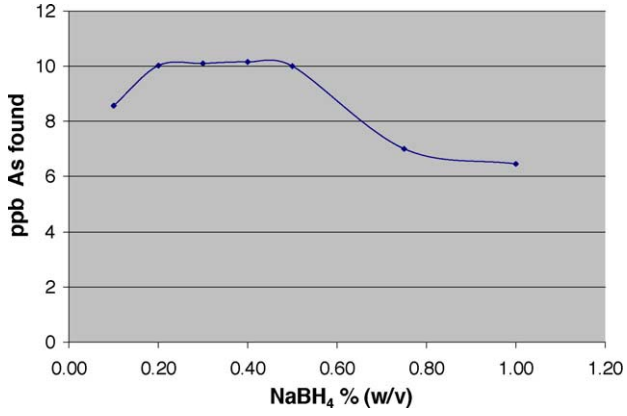

Fig. 3. Effect of $NaBH_4$ percentage on the As(III) response. Experimental conditions: HCl 3.0 M; $NaBH_4$ flow rate of 2.6 mL/min; sample flow rate of 3.0 mL/min. Average corresponding to three replicates of solution containing 10 ng/mL of As.

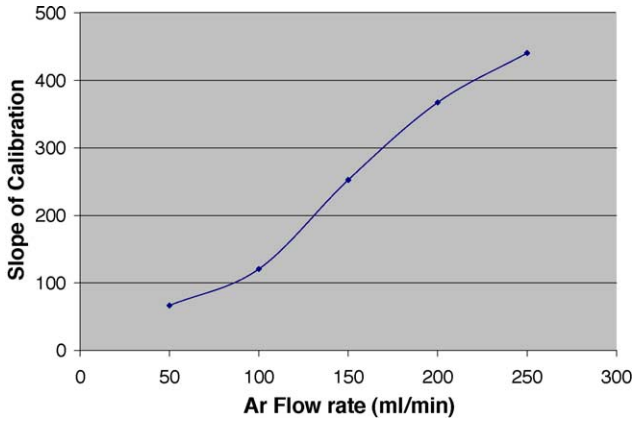

Fig. 4. Effect of Argon flow rate on the sensitivity of the procedure.

The effect of several ions on As detection was evaluated to improve the selectivity of the method. All tests were carried out under optimum operating conditions and following the general procedure. Experiments were realized with 10 mg/L of foreign ions and 10 μg/L As, and the results were the average of three replicated measurements.

In Table 3 are summarised the recovery of As in the presence of the potentially interfering elements. We noticed that the method is highly selective because no significant variation on As recovery was observed, except for the presence of Sb and Se at concentrations exceeding 100 μg/L, which can

Table 3
Effect of foreign elements on the As response, As concentration: 10 ppb

Elements	Element concentration (mg/L)	As recovery (%)
Ag	10	103.5
Cr	10	102.5
Cu	10	104
Fe	10	97.7
Hg	10	100.5
Mo	10	98.8
Ni	10	103.5
Pb	10	101
Sb	10	131
Sb	0.1	108
Sb	0.075	108.5
Se	10	75.9
Se	0.1	104
Se	0.075	109
Sn	10	104.5
V	10	100.5

Table 4
Certified and found values for reference materials

Reference material	Matrix	Certified value (mg/kg)	Found value (mg/kg)
BCR 278R	Mussel tissue	6.1 ± 0.1	6.1 ± 0.2
BCR 422	Cod muscle	21.1 ± 0.5	21.0 ± 0.3
DORM-2	Dogfish muscle	18 ± 1	18.7 ± 0.7
DOLT-3	Dogfish liver	10.2 ± 0.5	9.8 ± 0.4
TORT-2	Lobster hepatopancreas	22 ± 2	21.4 ± 0.5

be eliminated by dilution of the sample. At concentrations of Sb and Se less than 100 μg/L, no interference on the As determination was observed, owing to the high sensitivity of the hydride technique for As. This is particularly true for seafood as its As content was very higher compared to Sb and Se.

3.6. Validation of method

Calibrations were carried out under operating conditions (Table 1) for four standards solution of As (1, 5, 10 and 20 ng/mL).

Typical regression relation between emission peak area and As concentration is represented by equations:

$$I_e = (504 \pm 33)C_{As}\ \ (ng/mL) + 13,$$

with a correlation coefficient of $R^2 = 0.99989$.

The detection limit was calculated on the basis of the procedural blank mean concentration, added to three times the standard deviation of 14 procedural blanks. The LOD obtained amounts to 3.6 μg/kg wet weight.

The precision of the method, as applied to sample weights of 0.2–0.4 g for powder and 1.0 g wet weight (fresh food), was determined as the relative standard deviation (R.S.D.) obtained with six aliquots, representing the same sample. Values ranged from 2.7 to 3.7%.

Accuracy was assessed by the analysis of several CRMs. The found As concentrations, shown in Table 4, are averages of six different preparations, replicated three times. It was observed that As concentrations found were in good agreement with the certified values for both matrix types.

The calculation of recoveries for five reference materials completed the accuracy study. The values obtained (Table 5) range from 98.4 to 101.8% for As added at 5 μg/L. These results evidence the absence of analyte losses or contaminations during the digestion of sample and the effectiveness of the digestion and hydride generation measurement.

Table 5
Recoveries of arsenic spiked in seafish matrix

Matrix	As spiked concentration (ng/mL)	Recovery (%)
BCR 278R	5	99.5
BCR 422	5	101.8
DORM-2	5	101.0
TORT-2	5	98.4
DOLT-3	5	99.0

Table 6
Arsenic levels in seafood from local market (mg/kg, fresh weight)

Species	Found value (mg/kg)	Literature data range (mg/kg)[a]
Cod	6.57	3.1–7.0
Plaice	11.24	5.9–26.0
Ray	14.96	6.2–35.9
Scallop	1.04	0.99–3.6
Shrimp	6.54	4–10
Shark	8.60	–
Trout	0.96	–
Tuna	0.60	–
Sole	1.34	–
Mussel	1.68	–
Oyster	1.04	–
Squid	0.29	–
Cuttle fish	0.98	–

[a] Reference [9].

3.7. Application to seafood samples

Several frozen seafood samples obtained from the local market were analysed for total As determination using the conditions established in the present study. Analytical results are shown in Table 6. The As levels detected in seafood were higher than in other food items, but compared well to the ranges described in literature [7,9,14,25,26].

4. Conclusions

The FI-HG-ICP-OES method is highly suitable for the determination of total As in seafood. It is an improvement over other procedures in terms of sample mineralization and automation. The good analytical features of the method allow for its application to routine analysis for large sample numbers and a variety of foodstuff since there are no risks for interferences or matrix effect. The complete digestion of the samples contributed to good accuracy and quantification of total As.

This method has been applied successfully to other foodstuff matrix such as beverages and dairy products, where the As levels are very low.

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