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Determination of arsenic in seagrass using inductively coupled plasma mass spectrometry ☆

Yong Cai[a,b,*], Myron Georgiadis[a], James W. Fourqurean[b,c]

[a] *Department of Chemistry, Florida International University, University Park, Miami, FL 33199, USA*
[b] *Southeast Environmental Research Center, Florida International University, University Park, Miami, FL 33199, USA*
[c] *Department of Biological Sciences, Florida International University, University Park, Miami, FL 33199, USA*

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Abstract

A study has been conducted for the determination of arsenic in seagrass using inductively coupled plasma mass spectrometry (ICP-MS) with emphasis on sample digestion procedures and interference reduction. Open-vessel digestion with four different media, $HClO_4/H_2SO_4/HNO_3$, H_2SO_4/HNO_3, HNO_3/H_2O_2, and HNO_3, was tested and compared. The HNO_3/H_2O_2 mixture was found to be the most suitable medium for sample digestion. Different inorganic and organic arsenic compounds gave similar ICP-MS responses, indicating that complete conversion from organic form to inorganic form in the sample digestion is not required. Although the $HClO_4/H_2SO_4/HNO_3$ mixture is frequently used in plant sample digestion for other analytical techniques, it is not recommended for the ICP-MS technique. The formation of the polyatomic interference ion, $^{40}Ar^{35}Cl^+$, hampers the determination of arsenic even when the interference equation is employed. The interference of $^{40}Ar^{35}Cl^+$ can be accurately corrected only at minimum chlorine concentration (< 500 ppm). The concentrations of HNO_3 in the final solutions have a significant effect on the arsenic signal. This type of interference cannot be corrected by internal standards (Sc, Y, and In) because the signal suppression due to the use of HNO_3 is dependent on the mass number. The ratios of $^{75}As/^{45}Sc$, $^{75}As/^{89}Y$ and $^{75}As/^{115}In$ vary with the concentrations of HNO_3. Standard addition has been found to be an excellent method for reducing this type of matrix effect. The analytical procedure proposed in this paper has been validated by analyzing standard reference material 1572 (citrus leaves), and successfully used for the determination of arsenic in seagrass collected from Florida Bay.

Keywords: Inductively coupled plasma mass spectrometry; Arsenic determination; Seagrass leaves

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* Corresponding author. Fax: +1-305-348-3772.
E-mail address: cai@fiu.edu (Y. Cai).

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1. Introduction

There is an increasing interest in the study of arsenic in the environment because of its toxicity to plants, animals and human beings, and because of its large numbers of natural and anthropogenic sources [1]. Arsenic cycling and its impact in estuarine and coastal marine ecosystems are especially important because of the land drainage, industrial discharge, and harbor or leisure activities in these areas. Due to the chemical similarity between arsenate, the predominant inorganic arsenic form in aquatic systems, and phosphate, arsenate interferes with important biochemical functions of phosphate, particularly phosphorylation and ATP production [2]. Nutrient cycling (P, N) in Florida Bay has been extensively studied in recent years [3–5]. However, there is no information available concerning the occurrence, transport, uptake, and transformation of arsenic in this coastal marine system. Seagrasses support complex food webs based on their physical structure and primary production capacity, and rank with mangroves and coral reefs as some of the most productive coastal habitats in the world [6]. Seagrasses are the dominant primary producer in Florida Bay [3]. In order to evaluate the behavior of arsenic in this ecosystem and its interference with phosphate, accurate and reliable arsenic concentrations in seagrass are needed.

A number of detection methods have been developed for total arsenic analysis in environmental samples [7]. Inductively coupled plasma mass spectrometry (ICP-MS) is becoming an attractive technique because it is rapid, flexible, and provides multielement analysis capability [8–10]. As with many other spectrometric techniques, ICP-MS is subject to both spectral and non-spectral interferences. Most of the prominent spectroscopic interferences and the procedures to reduce them have been described in the literature [11,12]. In many cases, spectroscopic interferences can be avoided by choosing alternative isotopes since most elements in the periodic table have two or more isotopes. This method, however, cannot be applied to arsenic analysis because arsenic is monoisotopic ($^{75}As^{+} = 74.921$ amu). The well-known polyatomic interference $^{40}Ar^{35}Cl^{+}$ at mass 74.931 amu often hampers the analysis of arsenic even at moderated chlorine concentrations in the mg/l range when using the conventional quadrupole mass analyzer system [9]. This interference can sometimes cause serious problems in the analysis of environmental and biological samples because hydrochloric acid and perchloric acid are frequently required in the sample digestion. Obviously, sample preparation procedure is closely related to the analytical method used. To date, many sample digestion procedures exist for different analytical techniques [7]. These procedures, however, may not be applicable to ICP-MS due to the different mechanisms and principles in terms of the interference and instrument operation. For example, the recent regulatory approval of USEPA Method 200.8 [13] for the analysis of water and wastewater covers all of the regulated elements, including arsenic. When following this method, difficulties have been encountered in gaining reliable results for arsenic analysis because of the use of hydrochloric acid in the sample digestion procedure. As for the analysis of arsenic in plant materials using ICP-MS, including sample preparation, very little research has been done [14].

In this paper, we report a rapid and simple digestion procedure, followed by ICP-MS, for analysis of arsenic in seagrass that should be applicable to arsenic analysis in a broad range of marine plants. Research was focused on the comparisons of four different digestion media and reduction of interferences. Application and limitations of the elemental interference equation, which has been suggested in the EPA's methods for correcting interferences, are examined. The interference of chlorine with arsenic determination, and the influence of nitric acid concentration on the responses of both arsenic and internal standards were studied. The analytical procedure proposed was validated by analyzing standard reference material and was successfully applied to the determination of arsenic in a number of seagrass samples collected from Florida Bay. Florida Bay was chosen as a site because of the strong gradient in P production and seagrass biomass in

the bay [4]. Significant correlation between arsenic and phosphorous concentration has been found.

2. Experimental

2.1. Materials and reagents

Inorganic arsenic standard and other individual stock solutions of internal standards (ICP grade, 1000 ppm) were purchased from GFS Chemicals, Inc. Powell, OH, USA. A stock solution of monomethylarsonic acid (MAA) (1000 mg/kg) was a gift from P.S. Analytical, Kent, UK. Dimethylarsinic acid (DMAA) (98% purity) was purchased from Aldrich. An arsenobetaine standard solution (CRM 626, 1031 ± 6 mg/kg) was purchased from Community Bureau of Reference (BCR), European Commission, Brussels, Belgium. All these standards were used as received without further purification. Fresh calibration standards were prepared every week or as needed by diluting these commercial standards in 5% nitric acid. Trace metal grade hydrochloric acid and nitric acid, ACS grade hydrogen peroxide and perchloric acid were obtained from Fisher Scientific. All other chemicals used were of analytical grade or better. Distilled deionized water (DDW) was prepared using a Barnstead Fistream II Glass Still System (Barnstead Thermolyne Corp., Dubuque, Iowa) and was used in all standard and sample preparations. High purity grade (99.99%) argon was purchased from Air Products (Allentown, PA).

All glass and plasticware was cleaned prior to use by soaking in 5% nitric acid overnight, rinsing with DDW water and storing clean. The procedural blank after these cleaning steps has been found to be negligible.

Standard reference material (SRM) 1572 (citrus leaves) was obtained from National Institute of Standard & Technology (NIST), Gaithersburg, MD. The certified arsenic concentration in this SRM is 3.1 ± 0.3 μg/g dry wt. A number of seagrass samples were collected from 18 sites across Florida Bay, a subtropical lagoonal estuary on the southern tip of the Florida peninsula (approx. 25°00′N, 80°45′W). The sampling locations and procedures have been described in detail elsewhere [5]. The samples were air-dried, ground to a fine powder using a mortar and pestle, and stored in 7-ml glass vials until use.

2.2. Apparatus

The ICP-MS instrument used in this study was Model HP 4500 plus (Hewlett-Packard Co., Wilmington, DE) equipped with a Babington-type nebulizer and an ASX-500 autosampler (Cetac Technologies Inc., Omaha, NE). The Hewlett-Packard research grade quadrupole mass analyzer system is used in this device. All analyses can be performed at the same resolution setting (typically 0.70 amu at 10% peak height). Instrument configuration and general experimental conditions are summarized in Table 1.

2.3. Procedures

Open vessel digestion was used throughout this study. From the dried, ground seagrass sample, a 0.050–0.100-g aliquot was accurately weighed and transferred to a 125 ml Erlenmeyer flask. To the flask, 10 ml of digestion media was added. Based on the literature information, four different digestion media were selected and compared. The detailed procedures are illustrated in Fig. 1.

Two types of blanks were used with each batch of sample digestion to assess laboratory perfor-

Table 1
ICP-MS experimental conditions

Plasma gas flow rate	17.0 l/min
Auxiliary gas flow rate	1.1 l/min
Carrier gas flow rate	1.1 l/min
RF power	1300 W
Nebulizer	Babington-type
Spray chamber	Glass, double pass
Spray chamber temperature	4°C
Sample cone	Nickel
Skimmer cone	Nickel
Sampling depth	6.3 mm
Integration time	0.1 s/point
Points/mass	3
Analysis time/mass	0.3
Number of replicates	5

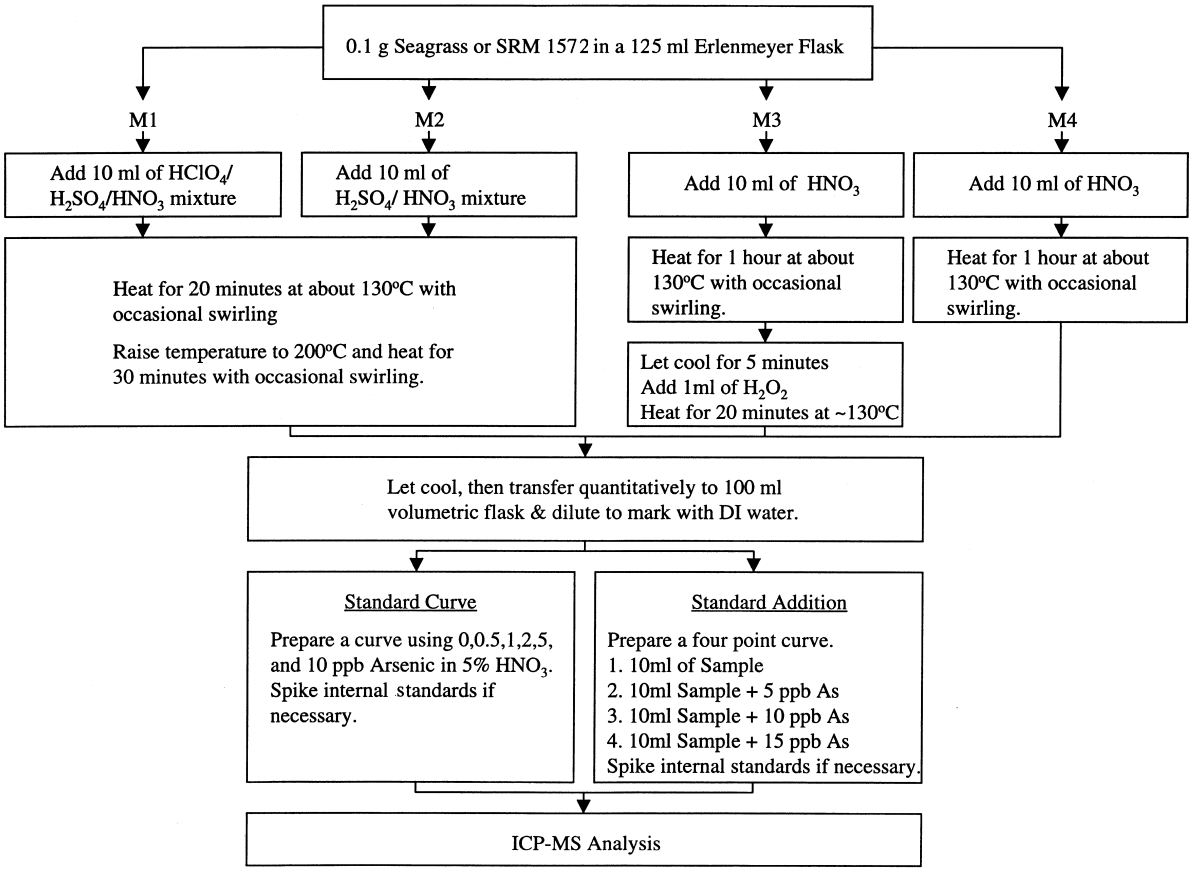

Fig. 1. Sample preparation procedures for four different digestion media.

mance. The laboratory reagent blank (LRB) consisted of an aliquot of digestion matrix water and was treated exactly like a sample, including exposure to all glassware, equipment, solvents, reagents, and internal standards that were used with other samples. LRB data was used to assess contamination from the laboratory environment and to characterize spectral background from the reagents in sample processing. The laboratory fortified blank (LFB) was the same as the LRB except a known quantity of arsenic standard was added in the digestion media to make a final concentration of 1 μg As/l in 100 ml solution. The LFB was analyzed exactly like a sample, and its purpose was to determine whether the methodology was in control and whether an accurate measurement was obtained.

3. Results and discussion

3.1. Sample digestion

Sample preparation is, in many cases, a key step in achieving accurate and reliable results for environmental analysis. Both dry ashing and wet ashing techniques have been developed for digestion of environmental samples, including marine plant samples [7]. Wet digestion is generally preferred over dry ashing because it is relatively simple and has less potential for introducing contamination. $HClO_4/H_2SO_4/HNO_3$ mix has been frequently found to be suitable for the determination of arsenic in marine biological samples [15]. In comparison with the $HClO_4/H_2SO_4/HNO_3$ mix, decomposition of marine biological tissue

with HNO_3 under pressure gave lower values for arsenic, presumably because of incomplete decomposition of the native (organic) arsenicals [15]. Those results are not surprising because arsenobetaine has been demonstrated by several studies to be stable even in hot HCl/HNO_3 mix [16]. HNO_3/H_2O_2 was evaluated as a digestion mixture for the analysis of some marine biological reference materials, DORM-1 (dogfish muscle), and DOLT-1 (dogfish liver tissue), by ICP/MS [17]. Although the procedure is relatively long, accurate results were obtained by standard addition technique for most elements tested.

It should be kept in mind that for many detection systems, such as hydride generation AAS (HGAAS), the determination of total arsenic in biological samples requires the complete oxidation of the sample and organoarsenicals must be converted to inorganic arsenic compounds during the digestion procedure [15,18]. This is because different arsenic species have different efficiencies for hydride generation and some arsenic compounds commonly present in biological samples, such as arsenobetaine and arsenocholine, cannot even form hydrides. For ICP-MS, however, this organic to inorganic conversion may not be necessary because of the high energy and ionization power provided by the plasma, which may easily ionize organoarsenic compounds directly. This is demonstrated by the experimental results shown in Fig. 2. Calibration curves were made using inorganic arsenic standard and the three of the most important organic arsenic compounds present in marine organisms, namely methylarsonic acid (MAA), dimethylarsinic acid (DMAA), and arsenobetaine. Very good calibration curves were obtained for each of the compounds investigated and, most importantly, they were similar in terms of slope and intercept. This result implies that complete conversion of organoarsenic compounds to inorganic form in the sample digestion step is not required, and

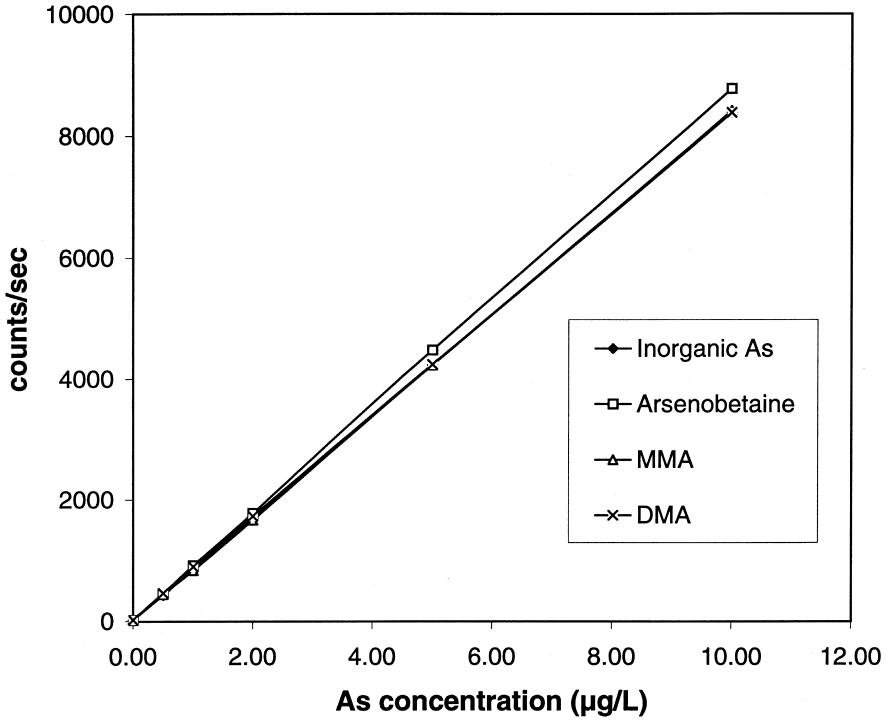

Fig. 2. Comparisons of the ICP-MS responses for inorganic arsenic and three organic arsenic compounds, arsenobetaine, monomethylarsonic acid (MMA), dimethylarsinic acid (DMA).

total dissolution of the sample is sufficient for arsenic analysis by ICP-MS.

The four different digestion media listed in Fig. 1 were studied and compared for arsenic analysis in SRM 1572 citrus leaves. Triplicate experiments were carried out and the results are summarized in Table 2. Four different quantitative methods, namely external curve with or without internal standard and standard addition with or without internal standard, were applied to these experiments. As expected extremely high concentrations of arsenic were reported by using all quantitative methods for digestions with $HClO_4/H_2SO_4/HNO_3$ acid mix, indicating strong interference from $^{40}Ar^{35}Cl^+$. Although dramatic reduction of the interference was observed after correction with interference equations (see next Section 3.2 for a detailed discussion on interferences), the results were still far off the certified value. For the H_2SO_4/HNO_3 acid mix, the results achieved using standard addition without correction by the interference equation were in good agreement with the certified value, while false data were obtained when the interference equation was applied. These results clearly indicate that the interference equation must be used with caution. For HNO_3/H_2O_2 digestion mix, satisfactory results were obtained no matter what quantitative methods were utilized. Similar to the HNO_3/H_2O_2 mix, good results were also achieved by using HNO_3 alone for most quantitative methods,except for the slightly low values obtained from the method of external curve without internal standard. This could be attributed to the matrix effect resulting from the incomplete digestion of the sample using HNO_3 alone. The results from these comparisons indicate that: (1) HNO_3/H_2O_2 mix provides the best results for digestion and analysis even though organoarsenic species may not be totally decomposed under this condition; and (2) perchloric acid should be avoided since with it analysis becomes impossible even with the use of the interference equation.

3.2. Interferences

3.2.1. Spectroscopic interferences

For arsenic analysis the major interference is from $^{40}Ar^{35}Cl$ at mass 74.931 amu. When the conventional quadrupole ICP-MS is used, the interference caused by $^{40}Ar^{35}Cl$ can be corrected for by the use of an elemental interference equation. This equation, defined in the instrument software, ChemStation, and in various EPA methods [13], uses the naturally occurring isotope ratios of relevant elements to estimate and allows the subtraction of isobaric or polyatomic interferences.

$$\begin{aligned}\mathrm{As}(75) = {} & (1.000)(75\mathrm{C}) \\ & -(3.127)[(77\mathrm{C}) \\ & -(0.874)(82\mathrm{C})] \end{aligned} \quad (1)$$

where 75C, 77C and 82C are counts on m/z 75, 77 and 82, respectively. This equation provides a correction for chloride interference with adjustment for ^{77}Se. Detailed information about this equation can be found in both the manufacturer's application manual and the EPA method 200.8 [13]. Based on the information for other interfering ions, more complicated equations can be derived to correct for the spectroscopic interferences.

During the course of this study, difficulties were encountered in using these equations (see previous Section 3.1 on sample digestion). Therefore, the following experiments were designed to evaluate the formation of polyatomic ion $^{40}Ar^{35}Cl$ and the limitations of using the interference equation under our experimental conditions. Two different series of standards were prepared in 5% HNO_3. The series one solutions were spiked with Cl at 0.05, 0.5, 5, 50, 500 and 5000 mg/l (ppm), while series two was spiked with As at 0, 0.5, 1.0, 2.0, 5.0, and 10.0 μg/l (ppb). The solutions were measured for mass 75 and the results are shown in Table 3. At concentrations below 0.5 ppm of chloride, the count numbers recorded were similar to the reagent blank, indicating no interference caused by Cl. However, the count number increased linearly with the concentration of Cl from 5 to 5000 ppm. By comparison with the data from As solutions, an As equivalent concentration of 2 ppb for a 500 ppm Cl was observed. In our present studies on the As in seagrass, the concentration of As in the final sample solution

Table 2
Results of arsenic analysis (μg/g) in SRM 1572 with four different digestion media and different quantitative methods[a]

Digestion media	External curve without internal standards[b]		External curve with internal standards		Standard addition without internal standards		Standard addition with internal standards	
	Corrected[c,d]	Not corrected	Corrected	Not corrected	Corrected	Not corrected	Corrected	Not corrected
$HClO_4/H_2SO_4/HNO_3$	5.84 ± 0.30	65.87 ± 1.42	6.24 ± 0.33	71.15 ± 1.58	43.99 ± 2.01	58.39 ± 2.86	49.72 ± 2.29	68.21 ± 6.05
H_2SO_4/HNO_3	15.74 ± 0.84	3.27 ± 0.23	17.76 ± 1.83	3.69 ± 0.16	5.74 ± 0.41	3.07 ± 0.25	5.74 ± 0.40	3.08 ± 0.12
HNO_3/H_2O_2	2.81 ± 0.19	2.85 ± 0.07	2.89 ± 0.13	2.99 ± 0.03	3.02 ± 0.31	3.03 ± 0.31	3.31 ± 0.09	3.32 ± 0.10
HNO_3	2.59 ± 0.09	2.64 ± 0.06	2.87 ± 0.14	3.03 ± 0.06	2.91 ± 0.11	3.05 ± 0.10	2.95 ± 0.11	3.09 ± 0.11

[a] Certified value is 3.1 ± 0.3 μg/g.
[b] Yttrium was used as internal standard.
[c] Results were corrected or not corrected with the interference equations.
[d] Standard deviations were calculated based on triplicate sample digestions.

was in the range of 1–3 ppb, while the concentration of Cl may be as high as several thousand ppm if perchloric acid is used. In other words, the count number at mass 75 resulting from $^{40}Ar^{35}Cl$ may be 10-fold higher than that obtained from As. Therefore, high error probability can be introduced by using the interference equation. A further experiment was designed to study the effectiveness of the elemental equation to correct for Cl interference in 5% HNO_3 solution. A series of 2 ppb As solutions were spiked with Cl (as NaCl) at 0, 0.5, 5, 50, 500, 5000 ppm and measured for As both with and without use of the interference Eq. (1). It can be seen from the results (Fig. 3) that the interference equation can correct for the influence of Cl at concentration at least up to 500 ppm. However, an over-corrected result was found at 5000 ppm of Cl and this concentration is not abnormal if HCl or $HClO_4$ is used in sample digestion. This false correction could have two sources. First, it gave a very high count number at 5000 ppm of Cl, which was approximately equivalent to 19 ppb of As. Subtraction of such high concentration for measuring As at 2 ppb clearly introduced a large error. Second, the interference equation would not be properly selected when considering the possible presence of other interfering species. Krypton (Kr), for example, can interfere with the measurement on mass 82, which is used in the equation for correcting Se interference mass 77. The second problem becomes even worse when a real sample is analyzed as demonstrated in the previous section. Although corrections could be done theoretically by involving these possible interfering species, the equation becomes so complicated that tedious pre-measurements for the interfering species have to be conducted before measuring As. Chloride content in seagrass leaves was measured in order to evaluate the potential interference of chloride present in the sample. Concentrations of chloride in the final solutions of sample extracts ranged from 0.5 to 2.4 ppm. At these concentration levels, chloride interferences can be easily corrected for by the interference equation.

A number of efforts have been carried out in reducing the Cl interference on As analysis by modifying the instrument, especially the sampling system [9,19]. Detailed discussion is beyond the scope of this study. Nevertheless, a successful correction of the interference by the elemental equation depends on the relative ratio of ^{75}As and $^{40}Ar^{35}Cl$ produced in the plasma and components of the sample matrix. Simple application of the interference equation in real sample analysis is not recommended before a careful evaluation of the system is conducted. The best way to reduce $^{40}Ar^{35}Cl$ interference is perhaps the avoidance of using perchloric or hydrochloric acids in sample digestion.

3.2.2. Matrix effects

A significant matrix effect on arsenic observed during this study was from the presence of HNO_3 in the final solutions. Since HNO_3 is utilized in seagrass analysis and also frequently in other environmental samples, the following experiment

Table 3
Comparisons of ICP-MS response from Cl and As on mass 75

From Cl		From As	
Conc. (ppm)	Mass 75 counts	Conc. (ppb)	Mass 75 counts
0.05	2.4	0	3.8
0.5	3.0	0.5	113
5	6.6	1.0	216
50	42	2.0	445
500	458	5.0	1140
500	4692	10.0	2280

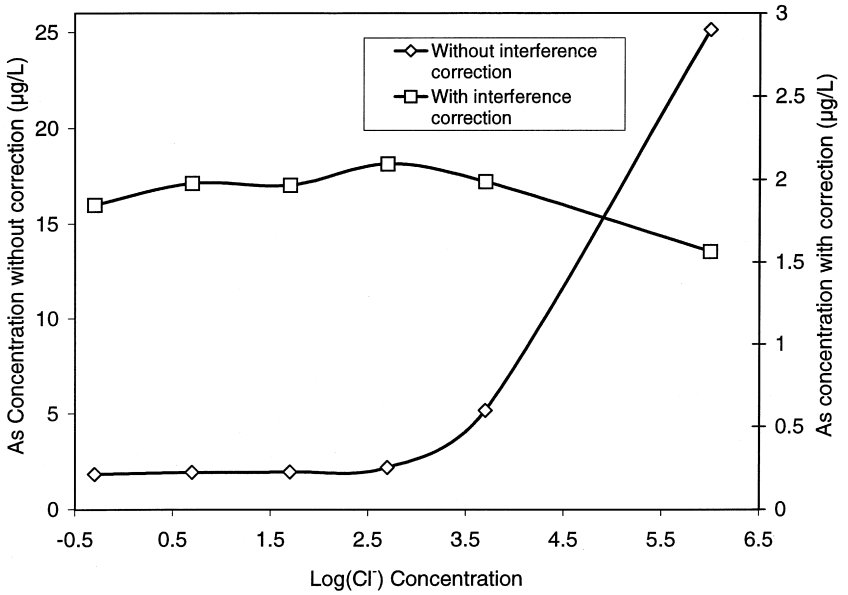

Fig. 3. Influence of Cl on arsenic signals with or without interference equation corrections.

was designed to investigate its effects on arsenic analysis. A series of 2 µg/l As solutions were prepared in 1, 2, 5, 7, and 10% of HNO_3. Internal standards (scandium, yttrium, and indium) were spiked in each of the solutions at 50 µg/l. Arsenic concentrations calculated against an external calibration curve prepared in 5% HNO_3, were 2.86 ± 0.09, 2.53 ± 0.11, 1.91 ± 0.04, 1.75 ± 0.05, and 1.44 ± 0.10 µg/l for 1, 2, 5, 7, and 10% of HNO_3 solution, respectively. This clearly indi-

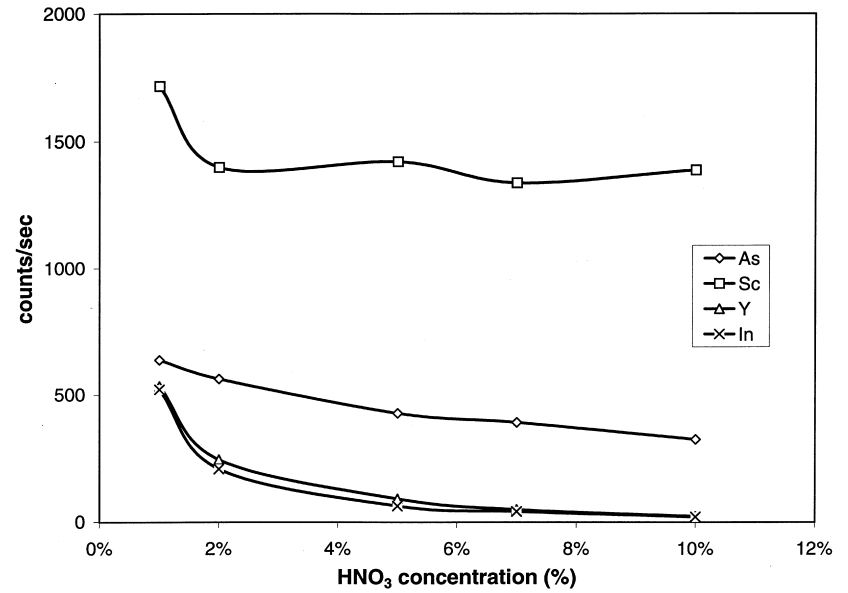

Fig. 4. Signals for arsenic, scandium, yttrium and indium as a function of HNO_3 concentration.

cated that the arsenic signal was significantly suppressed by increasing HNO_3 concentration. A plot of observed signals (counts per second) for arsenic, scandium, yttrium, and indium vs. concentrations of HNO_3 is shown in Fig. 4. The signals decreased sharply with increased concentration of HNO_3, except for the relative light element scandium. It is important to note that the ratios of $^{75}As/^{45}Sc$, $^{75}As/^{89}Y$, and $^{75}As/^{115}In$ obtained in different concentrations of HNO_3 also varied (Fig. 5). The extent of the effects of HNO_3 on the signal suppression apparently depend on the mass number. These results are in good agreement with the results obtained by the Vandecasteele's group [12]. It appears that the effect of HNO_3 concentration on arsenic determination cannot be corrected for by simple use of any of these three internal standards. Selection of a more appropriate internal standard, in terms of mass, potential, and volatility, may solve this problem. However, use of any type of internal standard for this purpose must be demonstrated before using.

In a study of matrix effects in ICP-MS, Vanhaecke et al. [12] evaluated the signal suppression and enhancement of several elements for 0.5 M H_2SO_4, 0.5 M HCl, and 0.5 M CH_3COOH solutions compared with 0.14 M HNO_3. They found that for 0.5 M H_2SO_4 the lighter mass is more suppressed than the heavy ones. However, for both 0.5 M HCl and 0.5 M CH_3COOH a mass-dependent suppression occurs with the heavier mass ions being suppressed to a larger extent than the lighter ones. Although the detailed mechanism for HNO_3 effects is not clearly understood at the present time, it has been suggested that the effects are attributed to the influence of acid on the sample uptake rate, aerosol characteristics, solution transport rate, and the excitation/ionization processes [20]. For the system used in this study, only a small percentage of the sample introduced into the nebulization system could be eventually transferred into the plasma. The changes in density, surface tension, and viscosity of the solution at different HNO_3 concentrations may discriminate the transfer process of the elements.

Based on these results, in quantitative analysis of arsenic using ICP-MS, the concentrations of HNO_3 in final sample and standards solutions must be matched if the external curve method, either with or without internal standard method, is used. When open vessel digestion is used, how-

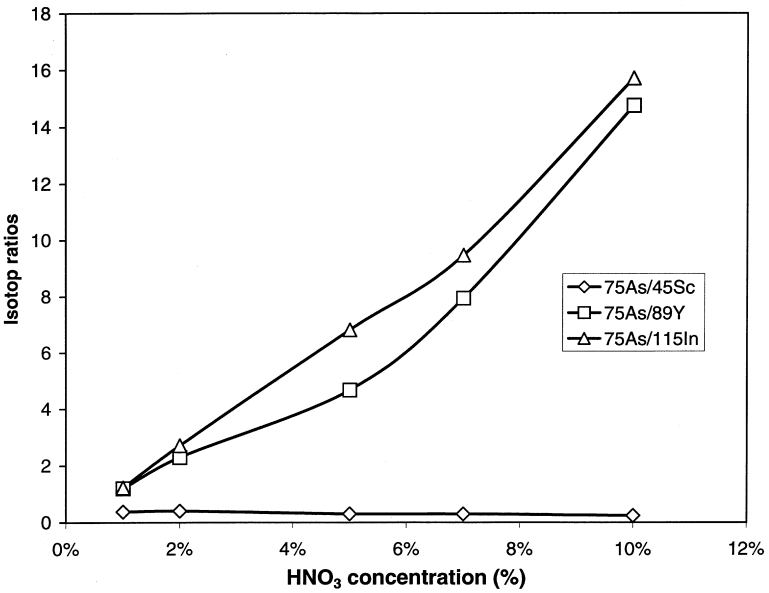

Fig. 5. Ratios of $^{75}As/^{45}Sc$, $^{75}As/^{89}Y$ and $^{75}As/^{115}In$ as a function of HNO_3 concentration.

ever, the final concentration of HNO_3 in the sample digest may be difficult to determine because some HNO_3 is evaporated during digestion. In this case, standard addition can be a good alternative. In standard addition, a known amount of the element of interest is added to the sample, and suffers the same matrix effects as the analyte. This quantitative method has been used in this study for arsenic analysis in SRM and a number of seagrass samples and satisfactory results were obtained.

3.3. Overall performance of the analytical method

With HNO_3/H_2O_2 as digestion medium, both citrus leaves and seagrass samples were easily digested and a clear solution was quickly obtained. The background from 5% HNO_3, which was used generally in standard and sample solutions, was typically below 10 cps. The method detection limit (MDL) was established using 10% HNO_3 fortified at a concentration of 1 μg/l of arsenic. Seven replicate aliquots of the fortified solution were processed through the entire analytical method. MDL of 0.078 μg/l was calculated as follows:

$$\mathrm{MDL} = (t) \times (S) \quad (2)$$

where t is Student's t-value for 99% confidence level ($t = 3.14$ for seven replicates), and S is the standard deviation of the replicate analyses.

When 0.1 g seagrass is used, the calculated concentration detection limit is 0.078 μg/g. This sensitivity is sufficient for arsenic analysis in marine plants where arsenic is often found at μg/g level. Reproducibility was calculated based on triplicate sample digestions and analyses, and was generally less than 10% (R.S.D.). Standard reference material 1572, citrus leaves, was used in the investigation of sample digestion and interferences. As shown in Table 2, the data obtained were in good agreement with the certified value when HNO_3/H_2O_2 was utilized.

3.4. Analysis of seagrass samples

Finally, using the ICP-MS method proposed in this paper, the arsenic content was measured in a number of seagrass (*Thalassia testudinum*) samples collected from Florida Bay. Samples were digested with HNO_3/H_2O_2 and quantitative analyses were carried out with both external curve and standard addition. As can be seen from Table 4, slightly low concentrations of arsenic were observed with the external curve method for all samples analyzed. This can be explained by the matrix effect, since 10 ml of HNO_3 was used for digestion and approximately 7–8 ml were normally left over. The calibration curve was established using 5% HNO_3. Since a higher concentration of HNO_3 in the sample causes signal suppression, lower As concentration is expected to be obtained with the external curve method. However, such a matrix effect can be reduced or eliminated by using the standard addition method.

Table 4
Arsenic contents in seagrass samples from Florida Bay

Sample ID	As concentration (μg/g)	
	External curve	Standard addition
1	2.90 ± 0.34	3.10 ± 0.06
2	2.83 ± 0.01	3.22 ± 0.02
3	1.05 ± 0.02	1.24 ± 0.04
4	2.22 ± 0.03	2.53 ± 0.05
5	2.36 ± 0.01	2.73 ± 0.02
6	3.27 ± 0.06	3.36 ± 0.18
7	1.99 ± 0.08	2.12 ± 0.01
8	1.25 ± 0.01	1.58 ± 0.02
9	0.83 ± 0.01	1.07 ± 0.05
10	1.24 ± 0.03	1.61 ± 0.05
11	1.39 ± 0.01	1.77 ± 0.02
12	1.13 ± 0.01	1.44 ± 0.04
13	0.92 ± 0.01	1.16 ± 0.02
14	1.93 ± 0.08	2.19 ± 0.22
15	0.79 ± 0.01	0.97 ± 0.02
16	0.93 ± 0.01	1.22 ± 0.03
17	0.92 ± 0.03	1.11 ± 0.01
18	1.36 ± 0.02	1.52 ± 0.08

We also analyzed all the seagrass samples for phosphorus content. Detailed results and discussion will be presented elsewhere. Briefly, there was a strong linear relationship between P and As content of the leaves; as P content increased, so did As content (linear regression, $\mathrm{As} = 0.48 + 0.00115\ \mathrm{P}$, $r^2 = 0.53$, $P < 0.001$). P content of seagrass leaves in South Florida is a function of the availability of P in the environment; hence there

is a strong gradient in P content of *T. testudinum* such that the P content is minimum in north-east Florida Bay and increases to the west and south [4]. The content of *T. testudinum* leaves mirrors this pattern. Apparently, there is a relatively constant As:P ratio in seagrass tissues over a wide range of P availability in Florida Bay.

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