Atomic Spectroscopy

January/February 2003 Volume 24, No. 1

In This Issue:

ASPND7 24(1) 1–36 (2003)
ISSN 0195-5373

Issues also available electronically.

(see inside front cover)

Atomic Spectroscopy
Vol. 24(1), January/February 2003

Printed in the United States and published six times a year by:

PerkinElmer Life and
Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
www.perkinelmer.com

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Panoramic Analysis for Monitoring Trace Metals in Natural Waters by ICP-MS

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ABSTRACT

A simple, rapid and accurate panoramic method based on inductively coupled plasma (quadrupole) mass spectrometry (ICP-MS) for the determination of trace metals in water samples has been developed. On the basis of an appropriate selection of the isotopes monitored and the combination of internal standardization with correction equations to overcome the matrix effects, the method permits accurate determination of up to 60 elements in a single run of less than 3 minutes. Assignment of intensities and calculation of concentrations was performed with the TotalQuant method of the PerkinElmer SCIEX ELAN 6000 software. Precision (RSD) below 5% was commonly achieved for elements present at 25 µg L^{-1}. To correct for matrix-induced ion signal variation and instrumental drift, rhodium was used as the internal standard (IS). Spectroscopic effects due to Cl, Na, Ca, Mg, P, S, and C were corrected with interference factors (IF) on the basis of a set of correction equations. The accuracy of the method was assessed based on the measurements of three certified reference materials (CRM) for trace metals in natural (SRM 1640), river (SLRS-4) and surface (SPS-SW2) water.

INTRODUCTION

For environmental applications, a survey of many trace metals is required and consequently panoramic analysis is preferred. Nowadays, inductively coupled plasma mass spectrometry (ICP-MS) provides most features of an ideal analytical technique (1,2) for panoramic analysis because of its low limits of detection, multi-element capability, and high sensitivity and selectivity.

A large number of papers deal with panoramic analysis, but the measurement accuracy of the developed procedures is not always specified. Some authors (3,4) associated the concept of panoramic with semi-quantitative analysis on the basis of the ICP-MS full mass-spectra scan method for processing the data. Panoramic analyses based on full mass-spectra scan methods were successfully applied to the analysis of various environmental (sediments, drinking, and natural waters) (4–7), industrial (8,9) and biological (10) samples.

The methodologies based on an analysis of the full mass spectra are intended for fast screening analysis. In the case of the PerkinElmer SCIEX ELAN® 6000 ICP-MS instrument, the TotalQuant™ method is used for this purpose. Internal response factors (RF) provided by the manufacturer indicate the ions' intensity per unit of concentration of each element in a sample and are used for calibration (see Figure 1). A standard calibration solution of a limited number of elements is measured prior to the analysis of the samples in order to update the response factors over the entire mass spectrum. For the analytes not specified as calibration elements in the method, the calibration is achieved by interpolation on the basis of the relative mass difference between the measured analytes and the calibration elements (11). In this context, the methods should be considered as semi-quantitative. Nevertheless, it was demonstrated that the methods provide near quantitative results (12).

Different strategies of calibration using full mass-spectra scan methods have been reported. Hu et al. (8) used six elements in order to update the response factors over the full mass range; for most elements accuracy within ±50% for three reference materials was reported. Other authors reported a better accuracy when the number of calibration elements increased. Johanson et al. (6) performed drinking water analysis by calibrating the mass spectrometer with 29 elements and suggested that the term semi-quantitative is misleading since accurate results can be achieved after applying a proper mass response calibration. In this respect, Godoy et al. (9) calibrated the mass spectrometer with 57 elements, and the agreement with quantitative results was satisfactory, indicating the possibility of using this method as a panoramic tool for environmental monitoring studies.

An interesting approach for calibration using a TotalQuant method was reported by Amarasiriwardena et al. (10). Three different repartitions of the elements in the response factor table were considered, namely all elements in one group, the elements divided over 3 and 10 groups, respectively, according to their atomic number. For each distinct group, only one calibration element was used. Most accurate results were obtained through the division of the response table into three groups and using V, Cs, and Ir for calibration.

Another enhanced panoramic method on the basis of full mass scanning was based on the correction of the degree of ionization of the elements using Saha's equation, temperature-

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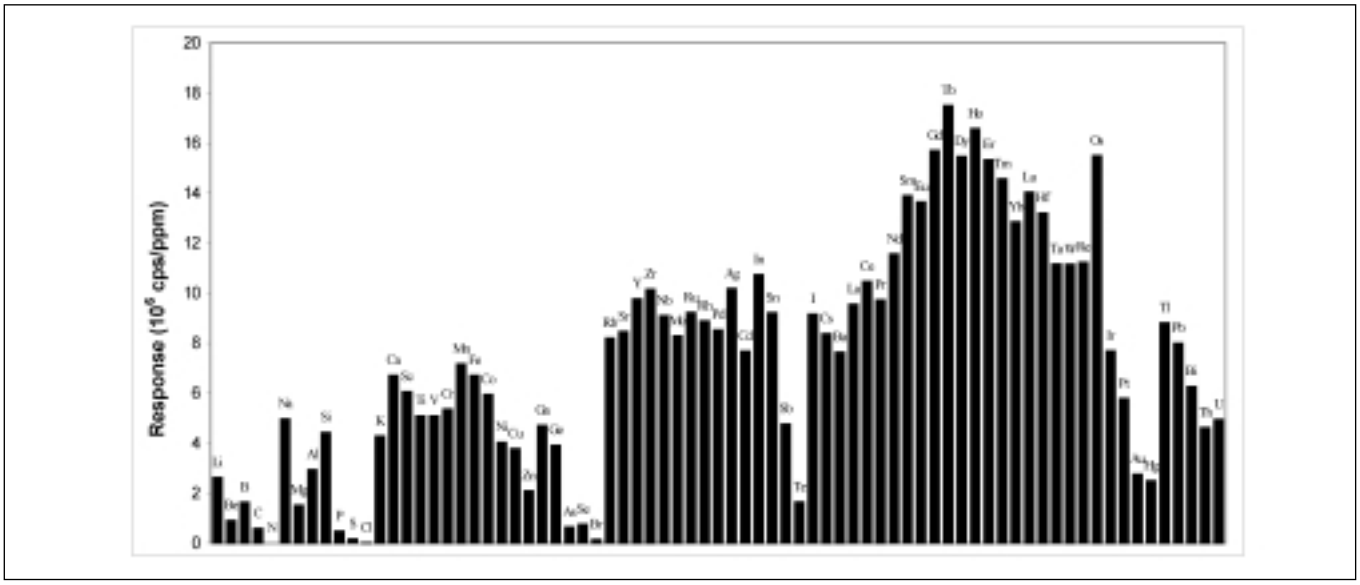

Fig. 1. The internal response factors provided by TotalQuant method.

dependent electronic partition functions, and optimized values for ionization temperature and electronic density (5,7). The proposed method provided quantitative results for both water and sediment samples.

The present work aimed at the development of a simple, fast, and accurate method as a routine tool for panoramic analysis of natural waters on the basis of full mass-spectra scan ICP-MS. The study focused on the influence of the number of calibration elements on the accuracy of the method and the robustness of interference factors of the most common polyatomic isobars formed due to Na, Mg, Ca, Cl, P, S, and C. The overall accuracy of the method was assessed by analysis of three water certified reference materials.

EXPERIMENTAL

Instrumentation

The work was carried out with the ELAN 6000 ICP-MS (PerkinElmer SCIEX, Thornhill, Ontario, Canada), using a Scott-type spray chamber and a Gem™-tipped cross-flow nebulizer. A daily optimization of both plasma and mass spectrometer settings was performed conforming to the manufacturer's recommendations. Cleaning of sampler and skimmer cones was performed daily. To minimize oxide levels, optimization of the nebulizer gas flow (using maximum intensity for rhodium) was carried out so that the ratio is equal to Ceo/Ce<3%. The operating conditions for the ICP-MS are given in Table I.

TABLE I
ICP-MS Instrumental Operating Conditions

RF power	1100 W	
Gas flow rate	Carrier	0.8–0.9 L min^{-1} (*)
	Plasma	15 L min^{-1}
	Auxiliary	1.2 L min^{-1}
Sampling cone aperture (nickel)	1.1 mm	
Skimmer aperture (nickel)	0.9 mm	
Injector id (alumina)	2.0 mm	
Sample uptake rate	1 mL min^{-1}	
Scanning mode	Peak-hopping	
Dwell time per acquisition	50 ms	
Sweeps per readings	5	
Readings per replicate	1	
Number of replicates	3	
Oxides level (CeO/Ce)	< 3%	
Measuring time	167 seconds	

(*) Depending on daily optimization, according to the formula: CeO / Ce <0.03

Reagents and Standards

All reagents used were of analytical reagent grade or higher purity. The water was deionized and further purified (18.2 MΩ) using a Milli-Q™

water purification system (Millipore, Bedford, MA, USA). A multi-element stock standard solution (10 mg L^{-1}) containing 60 elements was prepared as follows: No. 11355 ICP Multi Element Standard IV (27 elements) (Merck, Darmstadt, Germany) was mixed with a certified reference material (17 elements) consisting of rare earth elements and thorium (CLMS-1, Spex, USA). To complete the multielement solution up to sixty elements, single-element ICP-MS standard solutions (1 or 10 g L^{-1}, Merck) were used. The interference check solutions for chloride, sulphur, carbon, and phosphorus were prepared by appropriate dilution of concentrated acids (Merck) as follows: HCl supra-pur, H_2SO_4 ultra-pur, CH_3COOH pro analysi, and H_3PO_4 supra-pur. Sodium, calcium, and magnesium solutions (Merck) were used as nitrates. Appropriate dilutions with 1% (v/v) HNO_3, purified by sub-boiling HNO_3 65% (pro analysi, Merck) distillation in quartz equipment, were performed throughout for all measurements. The rinsing solution consisted of a 2% (v/v) HNO_3 solution. The certified reference materials (CRM), analyzed to evaluate the accuracy of the panoramic method, were as follows: SRM 1640 Trace Elements in Natural Water (National Institute of Standards and Technology, Gaithersburg, MD USA), SLRS-4 River Water Reference Material for Trace Metals (National Research Council of Canada, Institute for National Measurement Standards), SPS-SW2 Batch 106 Reference Material for Measurement of Elements in Surface Waters (Spectrapure Standards AS, Oslo, Norway). Prior to ICP-MS analysis, the standard solutions and water samples were stored at a low temperature (~4ºC) in polyethylene bottles.

Method

Intensities Assignment

The multi-element capability of the instrument was exploited by scanning the entire mass spectrum. To process the raw data, the TotalQuant method of the PerkinElmer SCIEX ELAN 6000 software was used (11). In order to avoid overload of the detector with oxygen and argon ions, several mass intervals were skipped and only the mass ranges 6–15, 18–39, 42–210, and 230–240 were scanned. Prior to the assignments of the intensities to the analytes, the mass spectrum was adjusted so that the intensities arising from both elemental and a series of polyatomic isobars were removed. Intensity estimation and subtraction of polyatomic species caused mainly by argon, carbon, nitrogen, and several rare earth element (REE) oxides were performed on the basis of the interference factors provided by the TotalQuant method (11).

Calibration and Concentration Calculation

The calibration was performed on the basis of the database of internal response factors correlating the ion sensitivities as represented in Figure 1. A multielement standard solution was measured prior to the samples, and then the adjustment of the response factors for all the analytes in relation to the calibration elements was performed automatically by the software. For the elements not specified for calibration in the method, the calibration was performed by an interpolation procedure on the basis of the relative mass difference between the measured analyte and the calibration elements (11) (Eq. 1):

$$a_i = a_1 + (a_2 - a_1)\frac{(M_i - M_1)}{M_2 - M_1} \quad (1)$$

where:

a_i, M_i = adjusting of the response factor (%) of the analyte not specified for calibration and its atomic mass;

a_1, a_2 = adjustment factors (%) of the response factor of the lighter and heavier analyte on the basis of external calibration;

M_1, M_2 = atomic mass of the lighter and heavier analyte, respectively.

RESULTS AND DISCUSSION

Influence of the Number of Calibration Elements

To investigate the influence of the number of calibration elements on the interpolation capability of the response factors over the entire mass range, calibrations on the basis of 3, 10, 20, and 40 elements were performed. The accuracy of the interpolation was evaluated per element by considering the ratio between the concentrations determined by means of 3, 10, 20, 40, and 60 calibration elements and the true value. When using 60 elements for calibration, no interpolation was needed as the response factor of each analyte was updated on the basis of a direct calibration. Therefore, in this case, obtained differences between the true and the measured value only reflect the variability arising from the measurement uncertainty and not from the interpolation capability of the method.

The calibration elements were not grouped according to their degree of ionization in the plasma or on the basis of atomic mass, but were selected to cover the entire mass spectrum range as follows:

3 elements: Li, In, U.

10 elements: Li, Al, Mn, Co, Zn, As, Sr, In, Pb, and U.

20 elements: Li, Al, Si, Ti, V, Cr,

Mn, Co, Ni, Cu, Zn, Ge, As, Se, Sr, Cd, In, Hg, Pb, and U.

40 elements: the latter 20 elements + Be, B, Fe, Ga, Zr, Nb, Mo, Rh, Pd, Ag, Sn, Sb, Cs, Ba, W, Pt, Au, Tl, Bi, and Th.

The calibration was performed with a solution containing the analytes at a concentration level of 50 µg L^{-1}.

The synthetic sample analyzed for the evaluation of the accuracy contained 60 analytes (each element at a concentration level of 25 µg L^{-1}) comprising the previously 40 elements listed and supplementary Na, K, Ca, Mg, and the group of rare earth elements (REEs, 16 elements). The isotopes used for calibration were selected on the basis of their abundance and minimal spectral interferences. The synthetic sample was analyzed over a period of two days, with five consecutive replicates per day. The results in terms of accuracy, expressed per element as the ratio between the concentration in the synthetic sample determined by means of 3, 10, 20, 40, and 60 calibration elements and the true value, are shown in Table II (the entry of each column corresponds to the number of calibration elements: 3, 10, 20, 40, and 60, respectively).

When using all elements in the calibration solution, no interpolation of the response factors was performed, and the accuracy of the determination of the 60 elements was in most cases better than 95%, reflecting solely the measurement uncertainty.

On the basis of three calibration elements, deviations (from the theoretical concentration value) below 15% were achieved for a number of 19 analytes as illustrated in Table II, which makes this method suitable for the accurate determination of a limited number of elements. Except for B, Mg, Al, Zn, Ga, Ge, As, Se, Cs, and Th (with errors >50%), the deviations for other elements ranged between 15% and 50%.

It must be stated that not all of the analytes showed improved accuracy by increasing the number of calibration elements. Although it is expected that better accuracy is obtained when the interpolation interval is shortened, we observed that the use of 10, 20, or 40 elements for calibration could provide similar or even worse performance for some analytes compared to three elements. As can be seen in Table II, an accuracy increase by more than 10% was only noticed for Mg, Sc, Ti, V, Cr, Fe, Ni, Cu, Ga, Ge, Se, Hg, and Bi when using 10 to 40 calibration elements compared to three elements. Moreover, for some analytes (Na, Si, K, Zr, Nb, Rh, and Pd) a decrease of accuracy occurred with the use of either 10, 20 or 40 calibration elements.

Using an interpolation procedure for concentration calculation, values of response factors (actual instrumental sensitivity) are determined based on relative mass difference between the measured analyte and the elements used for calibration. However, as discussed by Vanhaecke (13) and Houk (14), the importance of a close match in terms of both mass number and first ionization potential of analytes and internal standards can be extended to the case of interpolation procedure. In these conditions, it is clear that the increase of the number of calibration elements does not necessarily lead to an ameliorated interpolation. Based on previous consideration, an external calibration using a multielement aqueous standard solution containing all the analytes of interest (60) was used in this work for the analysis of the water samples. It must be emphasized that using 60 elements for calibration compared to 3 does not affect the total duration of the analysis, as long as the mass ranges being scanned remain the same. Moreover, since multielement standard solutions are commercially available, the influence of the number of elements on the preparation of calibration solutions is negligible.

The precision in terms of relative standard deviation (RSD, %) of each element was calculated. For most elements, the precision was below 10% on the basis of all calibration methods. Exceptions were encountered for Si, K, Ca (3 and 10 calibration elements used), Au (3–20, and 40 calibration elements) and U (3–40 calibration elements), which showed a RSD in excess of 10%.

Matrix Effects: Spectroscopic Interference Corrections and Internal Standardization

Dealing with spectroscopic interferences is an important step in any method development and plays an important role in ICP-MS. Elements commonly present in environmental samples, such as Cl, Na, Ca, Mg, S, P, and C, give rise to difficulties in the determinations of other elements at ultra-trace concentrations. Comprehensive studies with regards to polyatomic interferences and methods used to minimize them have been reported (15–26). The conventional approach to correct for spectral overlap is based on the use of interference factors (27,28). In the present work, the correction equations to subtract the potential spectroscopic effects of the matrix elements on the basis of their interference factors were computed as follows (Eq. 2):

$$Y_0 = Y - \sum_{i=1}^{n} (IF)_i \bullet x_i \qquad (2)$$

where:

Y_0 = corrected intensity of the analyte;

Y = intensity assigned at the analyte mass, which includes potential polyatomic interferences;

x_i = intensity of the interfering isotope, present in the sample matrix;

$(IF)_i$ = interference factors.

The interference factors were calculated by ratioing the intensity of the polyatomic species formed when an interference check solution of the interfering isotope is aspirated into the plasma and the intensity measured at its mass. The blank intensity was measured and subtracted before each sample was measured; hence, the relationship for calculating interference factors (IF) is given by Eq. 3:

$$IF = \frac{I_{isobar} - I_{blank}}{I_{interferent} - I_{blank}} \quad (3)$$

The procedure for the daily determination of the interference factors is time-consuming because separate interference check solutions must be run for each interfering element, the IFs calculated and computed into the method. Hence, it is less suited for a fast panoramic analysis. In this work, the interference factors associated to the polyatomic species containing one isotope of the previously mentioned interfering elements (Cl, Na, Ca, Mg, S, P, and C) in combination with either argon, nitrogen, oxygen, and hydrogen were determined in five replicates over a period of five months (different days). The averaged value was calculated and inserted in the correction equations computed in the TotalQuant method. This approach prevents the daily determination of the interference factors and provides the possibility of increasing the sample throughput considerably. It must be noted that during the period of measurements no major modifications of the experimental setup of the mass spectrometer were done. Interference check solutions with concentrations as recommended by U.S. EPA Method 6020 (41) were used. Only for Na, a concentration of 10 mg L^{-1} was chosen because of saturation of the detector at higher concentrations. In order to minimize memory effects, the sample introduction system was rinsed for 400 s between samples with a 2% (v/v) HNO_3 solution. The correction equations are listed in Table III. It must be noted that the long-term reproducibility (standard deviation reported in parentheses) in the determination of IFs is satisfactory for a panoramic purpose, given that their determination was carried out over a period of five months. The polyatomic ions listed in Table III consist of those mostly reported in the literature (29). For simplicity, charges on species are omitted in the table.

To correct for drift and non-spectral interferences, either indium (9) or rhodium (3,11) is commonly used with a panoramic purpose. In the present study, the blanks, standards, and water samples were spiked with 50 µg L^{-1} rhodium as the internal standard.

Analysis of Water Certified Reference Materials

The accuracy of the developed panoramic method was assessed by analyzing three certified reference materials in natural (SRM 1640), river (SLRS-4), and surface (SPS-SW2) waters. The results obtained by applying correction equations and using rhodium as the internal standard are given in Table IV. The average concentration (n=6) with the corresponding two-sided confidence interval (95%) of the mean (as the certified concentration values) was calculated for each element. Attention has been paid to the long-term reproducibility of the method. In this respect, six independent replicates were performed over a period of two months.

As Table IV illustrates, the suitability of the developed method for panoramic studies is demonstrated. The metal concentration found in the certified materials analyzed agreed very well with the certified values. Errors larger than 15% were encountered only for the analytes significantly affected by spectral overlaps (Si, K, Ca, Sc, Fe, Se) and those present in an ultra-trace concentration range, mainly below 1 µg L^{-1}, as for Be, Cr, Ni, Sb, and U in the CRM SLRS 4. Nevertheless, the determination of these elements at ultra-trace levels is considered to be satisfactory for a panoramic purpose. Additionally, the results show excellent reproducibility between replicate analysis, given that for each CRM six independent replicates were carried out over a period of two months.

CONCLUSION

Application of the developed method to the monitoring of waters with relatively low matrix content is related to the possibility of measuring a large number of elements in a single run in less than three minutes with high sensitivity, accuracy, and precision. Sample preparation and knowledge with regard to the matrix composition is not necessary. An external calibration using a multielement aqueous standard solution containing all the analytes of interest (60) was used for the analysis of the water samples. Further, it was demonstrated that for panoramic analysis, the daily determination of the interference factor of subtracting the polyatomic isobars is not necessary as long as no major modifications of the

TABLE II. Accuracy and Precision Achieved by Analyzing a Synthetic Control Sample Containing Sixty Elements*

	Accuracy (%)					Precision (RSD %, n=10)				
Analyte	3	10	20	40	60	3	10	20	40	60
Li(7)	**99.4**	**101**	**101**	**99.9**	**98.3**	2.5	2.5	1.2	1.7	1.3
Be(9)	63.5	56.2	57	**100**	**101**	3.1	2.6	3.1	1.4	1.3
B(11)	27.6	22.1	22.7	**99.2**	**97.3**	5.8	2.4	3.4	2.9	4.5
Na(23)	92.2	51.9	55.8	60.3	**85.4**	3.3	3.5	6.7	1.9	3.3
Mg(24)	289	158	165	172	**97.9**	2.3	1.8	5.3	1.2	1.3
Al(27)	168	9**0.3**	**95.5**	**93.5**	**93.4**	2.3	1.8	6.5	2.8	1.6
Si(29)	99.6	53.7	**91.0**	**88.9**	**89.6**	6.1	19	4.8	3.1	3.1
K(39)	108	62.3	95.1	98.2	**79.7**	17	22	16	8.8	13
Ca(44)	85.8	83.7	111	103	**75.6**	45	20	20	5.3	8.3
Sc(45)	124	90.5	94.3	95.9	**102**	2.5	2.1	1.9	2.0	2.1
Ti(47)	134	99.0	**99.2**	**101**	**98.2**	2.1	2.6	1.3	1.8	2.9
V(51)	134	105	**101**	**100**	**99.8**	2.1	3.3	1.4	1.7	2.7
Cr(52)	125	101	**99.3**	**97.0**	**98.3**	2.8	3.6	1.3	3.1	2.3
Mn(55)	119	**98.4**	**101**	**98.6**	**101**	2.9	2.1	1.2	3.4	1.5
Fe(54)	142	116	126	**87.8**	**88.5**	7.3	4.3	3.6	4.9	3.6
Co(59)	117	**97.1**	**99.5**	**98.9**	**99.8**	3.4	3.0	1.3	3.1	2.1
Ni(60)	141	104	**99.8**	**100**	**98.9**	2.3	2.7	2.3	2.9	1.5
Cu(63)	138	92.0	**101**	**100**	**101**	3.0	3.5	2.4	2.6	1.8
Zn(66)	164	**100**	**102**	**102**	**104**	3.1	2.0	1.8	1.7	1.7
Ga(69)	256	162	158	**101**	**104**	3.6	2.4	1.2	3.8	1.3
Ge(72)	159	102	**98.7**	**100**	**103**	3.2	2.2	1.2	2.9	1.9
As(75)	152	**98.3**	**101**	**102**	**104**	3.3	2.1	1.3	1.8	1.6
Se(82)	177	117	**102**	**102**	**103**	5.3	4.3	2.3	2.2	2.3
Sr(88)	142	**96.5**	**98.6**	**101**	**100**	4.5	4.1	2.3	2.6	2.0
Y(89)	116	81.9	84.9	96.2	**101**	3.9	3.5	1.1	2.8	1.8
Zr(90)	103	76.2	80.4	**101**	**101**	3.5	3.7	1.2	3.4	3.2
Nb(93)	95.7	72.5	78.3	**101**	**100**	4.1	3.4	1.4	1.9	2.2
Mo(98)	113	88.3	97.8	**100**	**100**	4.5	3.1	1.1	2.1	1.7
Rh(103)	95.9	81.9	98.5	**99.4**	**101**	4.1	3.0	1.7	1.7	2.1
Pd(105)	95.0	83.8	104	**100**	**101**	4.6	2.3	2.1	3.2	2.5
Ag(107)	67.1	60.6	77.3	**98.8**	**101**	5.4	2.7	2.1	3.9	3.7
Cd(111)	82.3	77.0	**101**	**100**	**101**	5.0	3.1	1.8	2.9	1.8
In(115)	**100**	**97.2**	**101**	**102**	**99.2**	3.5	2.4	1.6	2.9	2.2
Sn(118)	109	105	109	**101**	**100**	3.7	2.1	1.9	2.3	2.3
Sb(123)	111	106	110	**102**	**102**	3.4	2.0	1.5	3.7	1.9
Cs(133)	160	151	156	**100**	**100**	3.8	1.7	1.7	2.4	1.8
Ba(138)	165	155	161	**99.0**	**101**	5.0	2.3	2.2	3.8	1.2
La(139)	127	118	123	78.6	**101**	5.2	2.7	2.1	2.6	1.4
Ce(140)	120	112	116	76.2	**101**	5.0	2.7	3.0	2.3	1.2
Pr(141)	136	127	131	89.1	**100**	5.5	2.5	3.1	2.6	1.4
Nd(146)	117	108	112	78.4	**100**	5.1	2.7	2.4	2.8	1.9

* The elements used for calibration are expressed in bold lettering.

TABLE II cont'd. on next page...

TABLE II (cont'd.). Accuracy and Precision Achieved by Analyzing a Synthetic Control Sample Containing 60 Elements**

Analyte	Accuracy (%) 3	10	20	40	60	Precision (RSD %, n=10) 3	10	20	40	60
Sm(147)	95.3	87.0	90.9	68.5	**99.8**	6.9	2.7	3.2	2.8	1.7
Eu(153)	94.5	85.8	89.1	69.5	**100**	7.4	2.7	3.7	2.7	1.6
Gd(158)	78.0	71.0	73.0	59.3	**99.7**	7.6	3.1	4.1	2.1	1.8
Tb(159)	68.3	61.9	64.1	54.1	**101**	7.9	3.3	3.7	2.0	1.6
Dy(163)	76.0	68.9	70.8	62.9	**99.7**	8.2	3.2	3.5	2.1	2.0
Ho(165)	69.7	63.0	64.9	59.9	**101**	8.7	3.6	3.8	2.7	1.8
Er(166)	74.4	67.3	69.3	66.9	**101**	9.1	3.4	4.3	2.1	1.5
Tm(169)	78.6	70.7	72.8	73.6	**99.9**	9.9	3.3	4.3	2.1	1.8
Yb(174)	91.9	82.6	85.1	90.2	**99.7**	9.9	3.2	3.3	3.3	1.7
Lu(175)	84.3	74.8	77.0	86.5	**98.6**	10	3.2	3.9	3.2	1.6
W(182)	82.6	73.2	74.8	**98.9**	**98.9**	10	2.1	2.7	2.7	2.5
Pt(195)	105	91.4	93.5	**101**	**100**	10	2.8	1.6	3.5	2.5
Au(197)	64.3	64.9	61.7	**86.0**	**83.9**	35	8.7	12	17	15
Hg(202)	116	98.5	**102**	**103**	**102**	10	3.1	2.2	2.1	2.5
Tl(205)	110	94.7	97.3	**101**	**102**	12	3.6	1.7	4.7	2.4
Pb(208)	114	**97.7**	**100**	**101**	**102**	12	3.3	1.5	3.3	2.5
Bi(209)	133	116	117	**101**	**102**	12	2.9	1.6	3.4	1.8
Th(232)	219	220	205	**103**	**104**	15	6.8	1.3	2.2	2.1
U(238)	107	93.5	103	115	94.1	33	50	28	33	8.8

* The elements used for calibration are expressed in bold lettering.

TABLE III. Correction Equations Based on the Experimentally Determined Interference Factors (averaged, n=5)*

Polyatomic ion	Isotope affected	Correction equation
$^{12}C^{14}N^{1}H$	^{27}Al	$^{27}Al = {}^{27}I - 0.0040(0.003)^{12}I$
$^{12}C^{16}O^{1}H$	^{29}Si	$^{29}Si = {}^{29}I - 0.013(0.003)^{12}I$
$^{13}C^{16}O^{16}O$	^{45}Sc	$^{45}Sc = {}^{45}I - 0.030(0.004)^{13}I$
$^{31}P^{16}O$ $^{33}S^{14}N$	^{47}Ti	$^{47}Ti = {}^{47}I - 0.0064(0.0007)^{31}I - 0.00015(0.0001)^{33}I$
$^{33}S^{18}O$ $^{34}S^{16}O^{1}H$ $^{35}Cl^{16}O$ $^{36}S^{15}N$ $^{37}Cl^{14}N$	^{51}V	$^{51}V = {}^{51}I - 0.00027(0.0002)^{33}I - 0.000057(0.00003)^{34}I - 0.0020(0.0008)^{35}I - 0.0010(0.0006)^{36}I - 0.0052(0.002)^{37}I$
$^{12}C^{40}Ar$ $^{34}S^{18}O$ $^{35}Cl^{16}O^{1}H$ $^{36}S^{16}O$	^{52}Cr	$^{52}Cr = {}^{52}I - 0.0032(0.001)^{12}I - 0.00021(0.0001)^{34}I - 0.00018(0.0001)^{35}I - 0.0036(0.002)^{36}I$
$^{35}Cl^{18}O^{1}H$ $^{37}Cl^{16}O^{1}H$	^{54}Fe	$^{54}Fe = {}^{54}I - 0.00024(0.0001)^{35}I - 0.00064(0.0002)^{37}I$
$^{23}Na^{16}O^{16}O$ $^{35}Cl^{18}O^{1}H^{1}H$ $^{37}Cl^{18}O$	^{55}Mn	$^{55}Mn = {}^{55}I - 0.000037(0.000004)^{23}I - 0.00015(0.00002)^{35}I - 0.00039(0.00005)^{37}I$
$^{23}Na^{36}Ar$ $^{43}Ca^{16}O$	^{59}Co	$^{59}Co = {}^{59}I - 0.0000024(0.0000001)^{23}I - 0.00086(0.0001)^{43}I$
$^{12}C^{16}O^{16}O^{16}O$ $^{24}Mg^{36}Ar$ $^{43}Ca^{16}O^{1}H$ $^{44}Ca^{16}O$	^{60}Ni	$^{60}Ni = {}^{60}I - 0.000018(0.00001)^{12}I - 0.0000018(0.0002)^{24}I - 0.0029(0.0003)^{43}I - 0.00018(0.00003)^{44}I$
$^{25}Mg^{38}Ar$ $^{31}P^{16}O^{16}O$ $^{44}Ca^{17}O$	^{63}Cu	$^{63}Cu = {}^{63}I - 0.000093(0.00001)^{25}I - 0.00044(0.0006)^{31}I - 0.000069(0.000004)^{44}I$
$^{26}Mg^{40}Ar$ $^{32}S^{16}O^{18}O$ $^{33}S^{16}O^{17}O$ $^{34}S^{16}O^{16}O$	^{66}Zn	$^{66}Zn = {}^{66}I - 0.018(0.000002)^{26}I - 0.000023(0.000004)^{32}I - 0.0020(0.0007)^{33}I - 0.00042(0.0001)^{34}I$
$^{33}S^{18}O^{18}O$ $^{34}S^{17}O^{18}O$ $^{35}Cl^{17}O^{17}O$ $^{37}Cl^{16}O^{16}O$	^{69}Ga	$^{69}Ga = {}^{69}I - 0.00013(0.0001)^{33}I - 0.000023(0.00001)^{34}I - 0.0000082(0.0000003)^{35}I - 0.000021(0.00001)^{37}I$
$^{32}S^{40}Ar$ $^{36}S^{36}Ar$ $^{37}Cl^{17}O^{18}O$	^{72}Ge	$^{72}Ge = {}^{72}I - 0.0000050(0.000002)^{32}I - 0.0000096(0.000003)^{35}I - 0.0024(0.002)^{36}I - 0.000025(0.000003)^{37}I$
$^{35}Cl^{40}Ar$ $^{43}Ca^{16}O^{16}O$	^{75}As	$^{75}As = {}^{75}I - 0.00020(0.0001)^{35}I - 0.000017(0.00001)^{43}I$
$^{34}S^{16}O^{16}O^{16}O$	^{82}Se	$^{82}Se = {}^{82}I - 0.0000020(0.000001)^{34}I$

* *The standard deviation of the measurements is reported in parentheses.*

TABLE IV
Determination of Metal Content in Water Certified Reference Materials

Analyte	SRM 1640		SPS-SW2		SLRS-4	
	Certified (µg kg^{-1})	Found (µg kg^{-1})	Certified (µg L^{-1})	Found (µg L^{-1})	Certified (µg L^{-1})	Found (µg L^{-1})
Li(7)	a 50.8 ± 1.4	50.7 ± 4.45	b	-	b	-
Be(9)	34.99 ± 0.41	36.48 ± 2.37	b	-	0.007 ± 0.002	0.002 ±0.01
B(11)	301.6 ± 6.1	329.3 ± 29	250 ± 1	290 ± 56	b	-
Na(23)	a 29394 ± 310	35936 ± 5851	10000 ± 50	10940 ± 1787	2400 ± 200	2687 ±420
Mg(24)	a 5828 ± 56	6277 ± 445	2000 ± 10	2048 ± 126	1600±100	1632 ±154
Al(27)	52 ± 1.5	52 ± 2.2	250 ± 1	291 ± 75	54 ± 4	55 ±3
Si(29)	a 4737 ± 120	3070 ± 349	5000 ± 30	3063 ± 256	b	-
K(39)	a 995 ± 27	1107 ± 127	1000 ± 5	1162 ± 170	680 ± 20	739 ±54.3
Ca(44)	a 7055 ± 89	10312 ± 5381	10000 ± 50	14693 ± 6371	6200 ± 200	7106 ±4443
Sc(45)	b	-	2.50 ± 0.02	4.56 ± 0.39	b	-
V(51)	13.00 ± 0.37	13.46 ± 0.52	50.0 ± 0.3	50.8 ± 2.1	0.32 ± 0.03	0.30 ± 0.04
Cr(52)	38.7 ± 1.6	38.8 ± 2.0	10.0 ± 0.05	10.3 ± 1.3	0.33 ± 0.02	0.43 ± 0.21
Mn(55)	121.7 ± 1.1	119.2 ± 4.1	50.0 ± 0.3	50.3 ± 1.2	3.37 ± 0.18	3.05 ± 0.17
Fe(54)	34.4 ± 1.6	39.8 ± 9.6	100 ± 1	82.66 ± 6.85	103 ± 5	71.4 ± 4.32
Co(59)	20.31 ± 0.31	21.07 ± 0.92	10.0 ± 0.05	10.29 ± 0.45	0.033 ± 0.006	0.036 ± 0.008
Ni(60)	a 27.4 ± 0.8	27.0 ± 1.7	50.0 ± 0.3	49.8 ± 1.87	0.67 ± 0.08	0.87 ± 0.55
Cu(63)	a 85.3 ± 1.2	86.4 ± 1.8	100 ± 1	101 ± 4	1.81 ± 0.08	1.78 ± 0.08
Zn(66)	a 53.3 ± 1.1	46.6 ± 1.8	100 ± 1	100.9 ± 5	0.93 ± 0.10	1.21 ± 1.03
As(75)	26.71 ± 0.41	26.88 ± 1.18	50.0 ± 0.3	51.2 ± 2.2	0.68 ± 0.06	0.62 ± 0.28
Se(82)	21.99 ± 0.51	17.64 ± 0.77	10.0 ± 0.05	8.11 ± 0.45	b	-
Sr(88)	124.4 ± 0.7	127.9 ± 9.3	250 ± 1	253.5 ± 17.3	26.3 ± 3.2	28.5 ± 1.29
Y(89)	b	-	2.50 ± 0.02	2.602 ± 0.07	b	-
Mo(98)	46.82 ± 0.26	48.58 ± 1.71	50.0 ± 0.3	50.81 ± 1.62	0.21 ± 0.02	0.22 ± 0.02
Ag(107)	7.63 ± 0.25	8.84 ± 3.55	b	-	b	-
Cd(111)	22.82 ± 0.96	23.73 ± 0.99	2.50 ± 0.02	2.68 ± 0.07	0.012 ± 0.002	0.012 ± 0.006
Sb(123)	13.81 ± 0.42	13.76 ± 0.72	b		0.23 ± 0.04	0.13 ± 0.15
Cs(133)	b	-	10.0 ± 0.05	9.24 ± 0.42	b	-
Ba(138)	148 ± 2.2	149 ± 15.8	250 ± 1	239 ± 17	12.2 ± 0.6	12.6 ± 0.51
La(139)	b	-	2.50 ± 0.02	2.61 ± 0.10	b	-
Ce(140)	b	-	2.50 ± 0.02	2.67 ± 0.10	b	-
Pr(141)	b	-	2.50 ± 0.02	2.63 ± 0.11	b	-
Nd(146)	b	-	2.50 ± 0.02	2.56 ± 0.13	b	-
Sm(147)	b	-	2.50 ± 0.02	2.50 ± 0.10	b	-
Eu(153)	b	-	2.50 ± 0.02	2.63 ± 0.07	b	-
Gd(158)	b	-	2.50 ± 0.02	2.60 ± 0.12	b	-
Tb(159)	b	-	2.50 ± 0.02	2.60 ± 0.11	b	-
Dy(163)	b	-	2.50 ± 0.02	2.60 ± 0.12	b	-
Ho(165)	b	-	2.50 ± 0.02	2.65 ± 0.15	b	-
Er(166)	b	-	2.50 ± 0.02	2.57 ± 0.13	b	-
Tm(169)	b	-	2.50 ± 0.02	2.62 ± 0.13	b	-
Yb(174)	b	-	2.50 ± 0.02	2.59 ± 0.14	b	-
Lu(175)	b	-	2.50 ± 0.02	2.62 ± 0.18	b	-
Pb(208)	27.93 ± 0.14	29.45 ± 1.07	25.0 ± 0.1	26.6 ± 0.8	0.086 ± 0.007	0.094 ± 0.132
Th(232)	b	-	2.50 ± 0.02	2.73 ± 0.08	b	-
U(238)	b	-	2.50 ± 0.02	4.08 ± 3.0	0.05 ± 0.003	0.12 ± 0.04

a = Reference values. b = Not certified.

instrumental setup are performed; therefore, the method is suitable for fast routine analysis of natural waters. For certification purposes or for studies focused on a few elements, better accuracy can be achieved by updating the interference factors periodically in order to reflect the current experimental conditions more accurately.

Received December 13, 2002.

ACKNOWLEDGMENTS

We thank Prof. Dr. Freddy Adams for his assistance over the duration of the research. The study was funded by the Flemish Government as part of a bilateral scientific and technological cooperation project Belgium-Romania.

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Trace Element Characterization of High Purity Gallium: Matrix Removal as Gallium Fluoride Precipitate

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ABSTRACT

A simple method is described for the separation of 13 elements from a high purity gallium matrix. The matrix is dissolved in concentrated HNO_3 and precipitated as GaF_3 acid on treatment with HF. The precipitate is centrifuged and the supernatant analyzed for impurities by inductively coupled plasma atomic emission spectrometry (ICP-AES) and graphite furnace atomic absorption spectrometry (GFAAS). Separation of the matrix was 99.98%. The percentage recoveries of the 13 elements were in the range of 90–98%. The overall reproducibility of the method was 2–12%. The method offers a single-step matrix removal, which leads to low experimental blanks. The limits of detection values were in ng g^{-1} level. The method was applied for the trace element characterization of a high purity gallium ($4N^+$) obtained from the Special Materials Plant, NFC (Nuclear Fuel Complex), Hyderabad, India.

INTRODUCTION

High purity gallium is used in the manufacture of compound semiconductors, namely GaAs (1). Its properties are greatly influenced by the presence of impurities even at the ng g^{-1} concentration levels. Trace impurities, such as transition metals, will affect the conductivity of the material (2). Hence, characterization of high purity gallium for trace and ultratrace levels of impurities is a prerequisite for its use in the electronics industry. The growing demand for the determination of impurities at the ng g^{-1} concentration levels led to the development of various analytical methods with modern instrumental techniques.

The simplest method of analysis is the direct determination of impurities in the sample solution without any separation. The method, though simple, will lead to errors due to the high matrix content in the sample solution with >10 mg/mL of matrix. In ICP-based techniques, the high gallium matrix content in the sample solution creates spectral interferences and affects the solution flow rate due to the high salt content in the sample solution (3). Direct determinations by GFAAS are impaired by high background. Though there are methods for the direct determination of impurities in high purity gallium by GFAAS employing chemical modifiers, they are element-specific, and the selection of suitable chemical modifiers is often tedious (4,5). Hence, separation of the matrix is necessary for trace element characterization of high purity gallium. For the determination of high purity metals, matrix separation methods should involve a minimum of steps so there will be a minimum number of process blanks.

Several methods have been reported for the separation of gallium from its matrix, such as matrix evaporation, coprecipitation of impurities, solvent extraction of impurities and matrix, ion exchange, controlled dissolution, and hydride generation for gaseous hydride-forming elements (6-12). Recently, Naina Raje et al. reported an ion exchange separation method in which a set of impurities was adsorbed on an anion exchange resin in HCl medium, followed by adsorption in NaOH (13) medium. Even though controlled dissolution methods involve a single step, they are time-consuming and restricted to the determination of elements nobler than gallium (Ag, Bi, Co, Cu, Fe, Ni, and Pb) (9).

In this paper, an attempt was made to characterize high purity gallium with respect to trace impurities by separating gallium as GaF_3. Gallium is known to form an insoluble acid, GaF_3 (14) and the procedure presented here takes advantage of this fact. The recoveries of the analytes were studied using ICP-AES, and the determinations were carried out using ICP-AES and GFAAS.

EXPERIMENTAL

Instrumentation

A Model JY-2000 ICP-AES (Jobin Yvon, France), equipped with a 40.68 MHz RF generator and a Varian Model AA-875 AAS (Varian, Australia), GTA-95 graphite furnace atomizer, and programmable sample dispenser, were used for this study. A deuterium lamp was used for background correction for the GFAAS measurements. The operating parameters of both instruments are given in Table I.

Brand micropipettes and PTFE containers were used. All containers were cleaned by soaking in 20% (v/v) HNO_3 and rinsed with deionized water before use.

Reagents

Suprapur® grade 40% HF (E. Merck, Darmstadt, Germany) and sub-boiled HNO_3 were used throughout. Ultrapure water (0.05 µS) was prepared by passing

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TABLE I
Operating Parameters of ICP- AES and GFAAS

ICP-AES

Power: 1.0 kW		Plasma gas flow: 12 L/min		
Aerosol flow: 0.5 L/min		Slit width: 20 µm / 20 µm		
Wavelengths (nm):				
Au: 242.795	Ba: 455.403	Bi: 223.061	Cd: 214.438	Co: 228.616
Cr: 357.869	Cu: 327.396	Mg: 285.213	Mn: 257.610	Pb: 220.353
Pd: 340.458	Pt: 203.646	Rh: 233.477	Tl: 334.941	Zn: 213.856

GFAAS

Element	Wavelength (nm)	Slit width (nm)	Lamp current (mA)	Ashing temp. (°C)	Atomization temp. (°C)
Bi	223.1	0.2	10	500	2000
Cd	228.8	0.5	4	120	1800
Co	240.7	0.2	7	120	2300
Cu	324.8	0.5	5	120	2300
Mn	279.5	0.2	5	120	2400
Pb	283.3	1.0	5	120	2000
Tl	276.8	0.5	10	120	2200
Zn	213.9	0.2	5	---	---

potable water through a deionization system and then through a Milli-Q™ system (Millipore, Bedford, MA USA).

All stock standard solutions were prepared using high purity metal/oxide (Leico, New York, USA) in ultrapure acids.

Sampling and Sample Dissolution

The gallium sample was warmed in a water bath (in the original container as received) and completely melted. An aliquot of ~1 g of the melt was transferred to a pre-weighed PTFE container and then dissolved in 7–8 mL pure HNO_3 by heating on a halogen hot plate. After complete dissolution, the volume was made up to 10 mL using pure HNO_3. A process blank was also prepared in a similar manner.

Analytical Procedure

The sample solution of ~1 g gallium was transferred to a PTFE beaker and treated with 3 mL of HF. The solution, along with the precipitate, was evaporated to near dryness, and 1 mL of HNO_3 was added and evaporated to remove all HF. After the complete removal of HF, 2 mL of HNO_3 was added to the residue, shaken well and then centrifuged. The supernatant was transferred to a 25-mL beaker. The residue was treated with another 2 mL of HNO_3 and shaken well, then centrifuged. Both supernatants were mixed, evaporated to ~1 mL and made up to 10 mL volume with ultrapure water. The analysis of the resulting samples was carried out by ICP–AES and GFAAS using standard addition calibration.

Recovery Studies

Aqueous standard solutions of the elements under study (Au, Ba, Bi, Cd, Co, Cr, Cu, Mg, Mn, Pb, Pd, Pt, Rh, Tl, and Zn) were added to 1 g of the sample solution and treated with HF, shaken with HNO_3, and centrifuged as indicated above. The percentage recoveries were computed against aqueous standards.

Validation

The method developed was validated using the procedure as given below, which was reported in Reference (13). The metal was dissolved in a mixture of HCl and HNO_3, brought to HCl medium (0.2M), and passed through an anion exchanger. The eluate was taken into 0.2M NaOH and passed through an anion exchanger. In both cases, the adsorbed elements were eluted with 2M HNO_3 and analyzed by ICP-AES.

RESULTS AND DISCUSSION

The present procedure involves separation of the matrix by precipitation of gallium nitrate as GaF_3 using HF. The precipitation with HF has the following advantages: (a) it is available in very pure form when compared to procedures using other complexing agents; (b) precipitation is taking place at high acidity, hence, a large number of impurities are retained in solution; and (c) excess HF can easily be volatilized.

The precipitation was carried out in concentrated HNO_3 instead of water because GaF_3 is known to react with water to form $GaF_3 \cdot 3H_2O$ and is also soluble in water. Even though the recoveries for most of the elements under study were 90–98% (Table II), the recoveries for Cr and Mg were found to be ~20%, possibly due to the formation of the acid-insoluble fluorides of Cr and Mg. Addition of water may result in better recovery of these analytes, but this will also

TABLE II
Percentage Recoveries of Elements From 1 g of Sample Solution

Element	Initial amount of standard (µg)	Amount of standard recovered (µg)	Recovery (%)
Au	5.00	4.60	92
Ba	2.50	2.35	94
Bi	10.00	9.50	95
Cd	1.00	0.97	97
Co	1.00	0.90	90
Cr	2.50	0.40	16
Cu	1.00	0.90	90
Mg	1.00	0.20	20
Mn	1.00	0.96	96
Pb	5.00	4.75	95
Pd	5.00	4.80	96
Pt	5.00	4.90	98
Rh	10.00	9.50	95
Tl	5.00	4.75	95
Zn	1.00	0.98	98

TABLE III
Process Blank and Limit of Detection

Element	Process blank value (ng g^{-1})	Characteristic mass in (pg)	LOD by ICP-AES (ng g^{-1})
Au	25	ND	5.0
Ba	11	ND	2.0
Bi	50	20	31.0
Cd	12	2	5.0
Co	11	20	2.0
Cu	13.0	12	15.0
Mn	18.7	5.0	1.5
Pb	15.0	10.0	20.0
Pd	8	ND	12.7
Pt	20	ND	5.0
Rh	20	ND	5.0
Tl	20	44.0	10.0
Zn	49	0.13[a]	3.0

ND = Not Determined.
[a] μg^{-1} by FAAS.

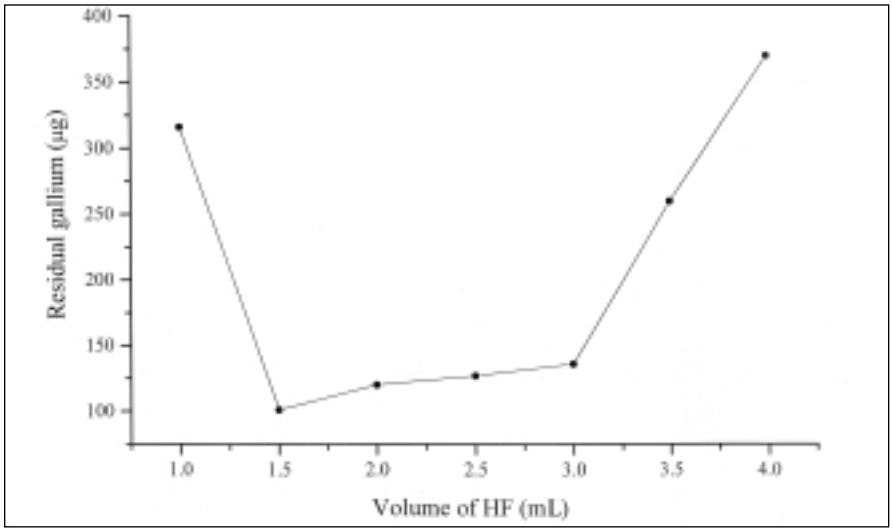

Fig. 1. Residual gallium with change in HF volume.

increase the matrix content in the final solution and, as mentioned above, lead to problems due to the matrix.

The recoveries for Pt, Rh, Au, and Pd were in the range of 92–98%; however, the concentrations of these elements (Pt, Rh, Au, and Pd) could not be reported as the concentrations of these metals in the sample are below the ICP-AES detection limits. The determinations of these elements (Pt, Rh, Au, and Pd) could not be carried out by GFAAS as these elements are used in our laboratory as chemical modifiers for our routine sample analysis.

The amount of HF required for the quantitative separation of gallium from 0.5 g of sample solution was studied by varying the volume of concentrated HF. It was found that to precipitate 0.5 g of gallium solution, 1.5 mL of HF is optimum. Even though further addition of HF decreased the removal of gallium, Figure 1 shows that there is no considerable difference in the percentage removal of gallium when the volume of HF added was between 1.5–3.0 mL. A decrease in gallium removal by adding excess HF can be due to the formation of the soluble fluorides of gallium or dissolution of GaF_3 in water which is present in excess of HF (40% solution in water). Using this procedure, a matrix removal of 99.98% was achieved.

The HNO_3 used for the analysis was double-distilled in a quartz assembly at sub-boiling temperatures with Suprapur HF. Since these are the only reagents used for the whole process, the process blank levels were expected to be very low; the results are listed

in Table III. The limit of detection values given in Table III were computed based on the concentration corresponding to 0.0044 AU in the case of GFAAS/ FAAS and ICP-AES, with the signal corresponding to three times the standard deviation (3 σ) of six repeated measurements of a blank.

CONCLUSION

The procedure reported was applied for the trace element characterization of a high purity gallium ($4N^+$) sample obtained from the Special Materials Plant, NFC, Hyderabad, India. The concentrations were determined using the standard addition calibration. The values obtained by this procedure are cross-validated with an ion exchange procedure as reported in Reference (13). The results shown in Table IV indicate that the values obtained by the present procedure are in good agreement with the values obtained by the procedure reported in Reference (13).

The procedure described has demonstrated that it is a useful method. It is simple, involving a single-step matrix removal, when compared to reported procedures, with low process blank values for the trace element characterization of high purity gallium. Matrix removal of 99.98% was achieved in a single step. The only reagents used for the complete process were HNO_3 and HF, which are commercially easily available in purest form and will result in low experimental blank levels. Hence, the analysis can be carried out with a minimum of possible process blanks. In conclusion, this method is a suitable alternative for the determination of Au, Ba, Bi, Cd, Co, Cu, Mn, Pb, Pd, Pt, Rh, Tl, and Zn at ng g^{-1} concentrations.

TABLE IV
Comparison of Levels of Impurities Present in a $4N^+$ Pure Gallium With Ion Exchange Method (Ref. 13)

Element	Value (μg g^{-1})		Ref. (13) Value (μg g^{-1})
	ICP-AES	GFAAS	
Ba	0.115 + 0.01	ND1	ND1
Bi	0.394 + 0.050	0.327 + 0.040	0.438 + 0.050
Cd	0.050 + 0.006	0.041 + 0.005	0.054 + 0.010
Co	0.118 + 0.009	0.132 + 0.005	0.091 + 0.010
Cu	0.216 + 0.010	0.170 + 0.005	0.190 + 0.010
Mn	0.043 + 0.006	0.041 + 0.003	ND1
Pb	0.43 + 0.03	0.39 + 0.01	0.38 + 0.02
Tl	ND	0.134 + 0.014	ND1
Zn	2.70 + 0.05	2.50 + 0.05 a	2.20 + 0.05

ND = Not Detected ND1 = Not Determined.
[a]FAAS.

ACKNOWLEDGMENTS

The authors would like to thank Dr. J. Arunachalam, Head, CCCM (National Centre for Compositional Characterization of Materials), for his encouragement.

Received September 11, 2002.

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Selective Determination of Sb(III) in Drugs by Flow Injection Hydride Generation AAS

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ABSTRACT

In this work, a procedure is described for the selective determination of Sb(III) in commercial drugs based on pentavalent antimony. Antimony(III) was determined by flow injection atomic absorption spectrometry (FI-HG-AAS) in drugs injected for leishmaniasis treatment in South America. The following acids were studied: acetic, tartaric, citric, oxalic, and lactic. The optimized conditions for Sb(III) determination without interference from Sb(V) were: 20% (m/v) citric acid, 2.0% (m/v) sodium tetrahydroborate, 125 µL injection volume, and 180 cm for reactor length. Interference of As, Ni, and Pb on the Sb(III) analytical signal was also investigated. The detection limit was 0.95 ng and the characteristic mass 55 pg. The recovery values obtained using the proposed procedure for six commercial samples varied from 97.1–100.8%. The relative standard deviation was 3.8%. The proposed FI-HG-AAS procedure allows more than 60 determinations per hour. The optimized procedure was applied for quality control of pentavalent antimony-based drugs produced commercially in Brazil.

INTRODUCTION

Among tropical diseases, leishmaniasis poses a high risk factor for people in many countries. Today, leishmaniasis is endemic in five continents and is reported to affect about twelve million people, including those with overt disease and those with no apparent symptoms. It is estimated that worldwide more than three hundred million people may be at risk (1). These data may explain the increased interest in this disease by public health institutions, particularly in South America (2). Leishmaniasis, mainly the visceral type, is treated in several countries by means of N-methylglucamine antimonate (or "meglumine antimonate") via injection. These formulations contain the equivalent to 85 mg by milliliter of Sb in the pentavalent state.

Toxicity and biological effects of antimony compounds depend on the oxidation state and, in general, Sb(III) compounds are more toxic than Sb(V) compounds (3). Meglumine antimonate injections should contain only Sb in the pentavalent state. However, the presence of trivalent antimony as a contaminant has been reported in the literature (4,5). In view of this, the analytical control for Sb(III) is important to prevent risk to people under treatment for leishmaniasis. In addition, this control is important in order to ascertain the quality of the drug due to the high amount that is commercialized annually (e.g., more than 2.5 million ampoules are produced yearly in Brazil alone).

Studies for Sb speciation are increasing and so is the number of suitable analytical procedures dealing with this topic (6–8). Although various procedures have been proposed over the years, some drawbacks remain, and analytical procedures for antimony speciation need to be developed (8). Hydride generation atomic absorption spectrometry (HG-AAS) is a powerful technique for the separation and speciation of Sb. For the analytical control of meglumine antimonate, the sensitivity of this methodology can be considered sufficient. In addition, the technique is not as expensive as other spectrometry procedures such as inductively coupled plasma (9). Flow injection systems have been coupled to HG-AAS for speciation purposes to determine Sb(III) and Sb(V) in waters and biological samples (10–13).

Antimony speciation studies have been performed by controlling the pH in order to selectively generate stibine from Sb(III) and avoid SbH_3 generation from Sb(V) (14). Antimony(III) is easily reduced to stibine with tetrahydroborate, while reduction from Sb(V) is incomplete and cannot be reduced at a near-neutral pH (15). Alternatively, some complexing or buffer media have been investigated for the selective stibine generation (16–19). In the past (10,11), and more recently (20–22), the use of citric acid has been proposed for Sb speciation studies.

In this paper, we present a procedure for the selective determination of inorganic Sb(III) by flow injection hydride generation atomic absorption spectrometry (FI-HG-AAS) in samples of the meglumine

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antimonate drug used for leishmaniasis treatment. Commercial drugs studied contain nominal values of Sb(V), correponding to 85,000 mg L^{-1}.

EXPERIMENTAL

Instrumentation

A PerkinElmer Model 3030 atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT USA), equipped with a flow injection or batch hydride generation system (Model MHS-10), was used for all Sb determinations. An Sb hollow cathode lamp, operated at 15 mA, was used as the radiation source. A spectral bandwidth of 0.2 nm was selected to isolate the 217.6-nm Sb line. A deuterium lamp was used for background correction, and all determinations were made in the integrated absorbance mode (integration time of 12 s). The atomizer was a T-shaped quartz tube (1.2 cm inner diameter and 16.5 cm length) heated by an air + acetylene flame. Argon (99.996%, White Martins, Brazil) was used as the purge gas. The quartz cell was periodically cleaned by immersion in a 1.4 mol L^{-1} HNO_3 + 0.3 mol L^{-1} HF solution for 30 s.

FI Manifold

The proposed flow injection system is shown in Figure 1. An eight-channel peristaltic pump (Minipuls™-4, Gilson, Villiers le Bel, France) was used to carry the reagents and sample solutions. The following Tygon® pump tubes (Cole-Parmer, Inst. Comp., Vernon Hills, USA) were used: black-black (0.30 mm i.d.) for sample, sample carrier, and sodium tetrahydroborate; white-yellow (1.52 mm i.d.) for citric acid. Polyethylene tubes (Intramedic, Clay Adams, USA) with 0.8 mm i.d. were used to transport the reagents and sample solutions. A glass U-tube gas-liquid separator (23) was used to separate the generated hydrides and liquid phase before the atomizer. For sample injection, a sampling loop coupled to a manual injector-commutator was used. This injector has been described previously in the literature (24).

Reagents

All chemicals used were of analytical reagent grade obtained from Merck (Darmstadt, Germany). Milli-Q™ water (Millipore, Bedford, MA USA, 18.2 MΩ.cm) was used to prepare all solutions. All glassware was soaked in 5% (m/v) nitric acid and 5% (m/v) hydrochloric acid, then rinsed with water before use. Sodium tetrahydroborate solutions were prepared daily and filtered before use. Stock reference solutions of 1000 mg L^{-1} Sb(III) were prepared by dissolution of Sb_2O_3 in 6.0 mol L^{-1} hydrochloric acid. A 10-mg L^{-1} Sb(V) reference solution was prepared from a 1000-mg L^{-1} Sb(III) solution according to Reference 13; the final dilution was made with water. Working solutions of Sb(III) and Sb(V) were prepared daily just before use by appropriate dilution with water.

Samples

Commercial samples of meglumine antimonate from two manufacturers were used for the development of the proposed FI-HG-AAS procedure. These samples are commercialized in 5-mL ampoules containing the nominal value of 85,000 mg L^{-1} Sb(V) as meglumine antimonate in aqueous solution. The samples were diluted 40,000-fold with water and working solutions were prepared daily just before use. Antimony determinations in the commercial samples were carried out under the optimized conditions.

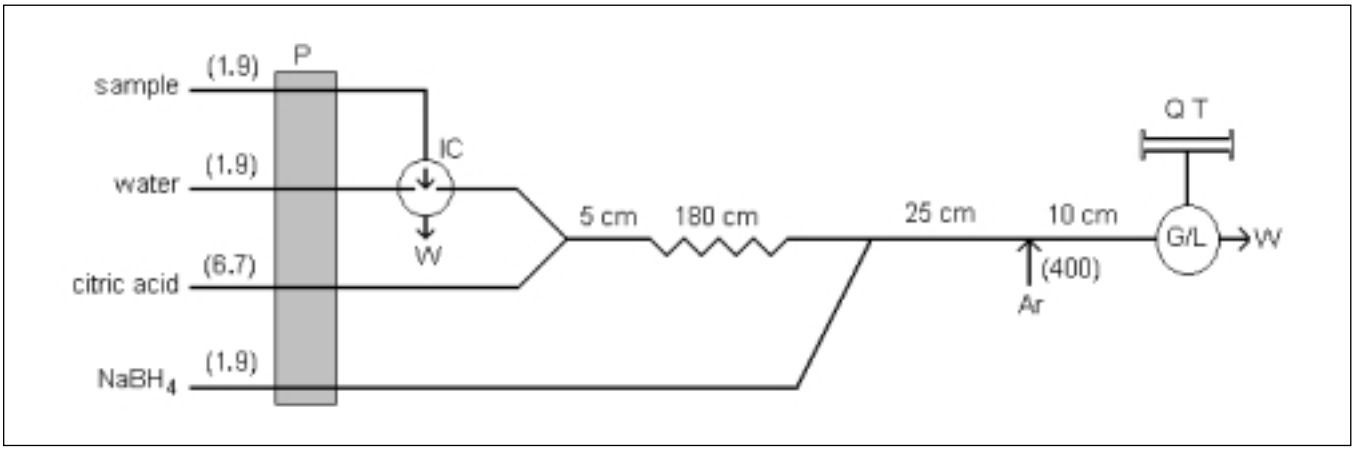

Fig. 1. Flow injection manifold for HG-AAS. P: peristaltic pump, IC: injector commutator, G/L: gas-liquid separator (U-tube), W: waste, QT: quartz tube atomizer. Numbers in parentheses are in mL min^{-1}.

Procedure

The flow injection system used for the selective determination of Sb(III) is shown in Figure 1. Reference or sample solutions were pumped (P) in order to fill up the sample loop of the injector-commutator (IC). The sample is subsequently injected into a carrier stream (water), and mixed with the chosen acid (citric, acetic, oxalic, tartaric, and lactic) and sodium tetrahydroborate. In this study, these specific carboxylic acids were chosen due to their complexing effects on Sb species (10,11,22). The stibine generated is separated in the gas-liquid separator (G/L) and carried to the quartz tube atomizer (QT) by an argon flow, while the liquid phase is carried to waste (W). Some analytical parameters for the optimization of the proposed system were investigated. In addition, the effects of potentially interfering elements such as Ni(II), Pb(II), and As(III) on the Sb(III) signal were studied.

RESULTS AND DISCUSSION

Effect of Acids and Their Concentration on the FI System

In order to carry out the initial studies for the selective determination of Sb(III) and to optimize the proposed procedure, various factors were evaluated. Signals were compared to those from Sb(V) reference solutions and diluted commercial samples. After dilution, commercial samples contain about 260 ng Sb(V) in the injected volume (125 µL). A mass of 265 ng was chosen to simulate the nominal Sb(V) concentration in commercial samples. For Sb(III), the analytical solutions were prepared so as to provide 5 ng Sb(III), corresponding to 125 µL of injected volume. This mass was arbitrarily selected for subsequent studies.

Solutions containing 20% (m/v) acetic, tartaric, citric, oxalic, and lactic acids were studied and the results are shown in Figure 2. Using the optimized conditions, no suppression of the Sb(V) analytical signal was observed with tartaric or acetic acid. For both acids, the Sb(III) signals were almost the same, but very high Sb(V) signals were observed. Oxalic and lactic acids are more effective for suppression of the Sb(V) analytical signal, but small signals can still be recorded. In addition, after mixing oxalic acid with the samples or analytical solutions, a precipitate was observed in the tubes. For the Sb(III) signal of the reference solution, no difference was observed for all acids. However, citric acid provided the best results overall for Sb(V) and Sb(III). Virtually complete suppression of the Sb(V) signals was observed, and no decrease of the Sb(III) signals was detected. The signals were more reproducible, and the background signals were very low (less than 0.05 in peak height). Due to these results, citric acid was chosen for further studies.

Figure 3 presents the results of the Sb signals when citric acid concentrations were varied from 1% up to 25% (m/v). A decrease of the signals from Sb(V) analytical

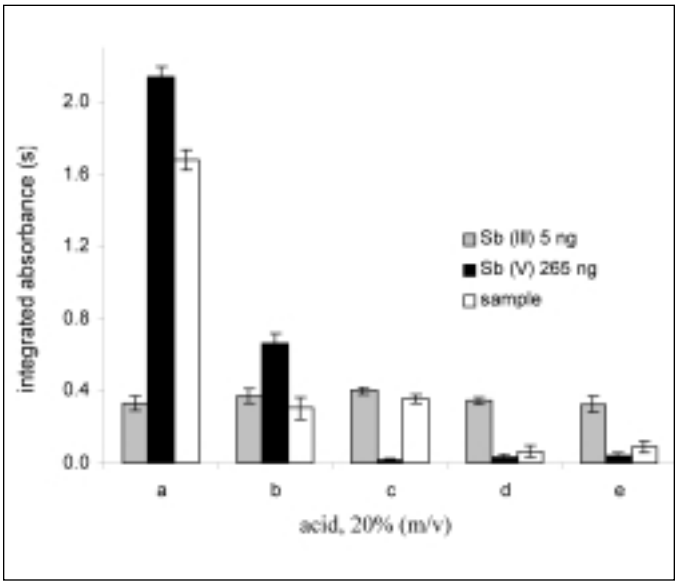

Fig. 2. Effect of acid medium (20%, m/v) on Sb(III), Sb(V), and commercial sample signals using the FI-HG-AAS system. (a: acetic acid, b: tartaric acid, c: citric acid, d: oxalic acid, e: lactic acid). Vertical bars are the relative standard deviation (n = 5).

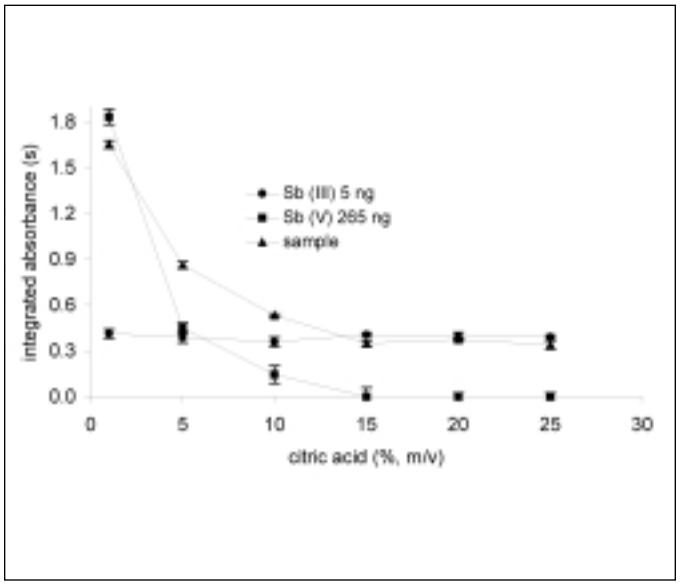

Fig. 3. Influence of citric acid concentration (%, m/v) on Sb(III), Sb(V), and commercial sample signals using the FI-HG-AAS system (2% m/v $NaBH_4$, 125-µL injection volume). Vertical bars are the relative standard deviation (n = 5).

solutions and from diluted commercial solutions was observed. Contrary to the Sb(V) signals for the analytical solutions, commercial sample solutions do not decrease down to baseline, but stabilize at about 0.3 s with a citric acid concentration higher than 15% (m/v). This is due to the presence of Sb(III) in the sample as a contaminant. For the Sb(III) analytical solution, the signals were constant during the interval studied and were maintained at around 0.4 s. Thus, a solution of 20% (m/v) citric acid was chosen and used for subsequent studies. It is important to note that suppression of the Sb(V) signals (sample or analytical solutions) is due mainly to the complexing action of citric acid and is not a question of pH control since the pH with 20% (m/v) citric acid is very low. Some authors previously reported that the pH has a minor influence on the Sb-citric acid system (10,25).

Influence of Sodium Tetrahydroborate Concentration

The influence of different sodium tetrahydroborate concentrations using the proposed FI-HG-AAS procedure was studied and the results are shown in Figure 4. An increase in the Sb(III) signal of the sample was observed with $NaBH_4$ concentrations of 0.1% and 0.5% (m/v). The observed signal for the sample is probably due to the presence of Sb(III) as a contaminant. With more than 0.5% (m/v) $NaBH_4$, the signal for Sb(III) stabilizes up to 3% (m/v) and starts to decrease with reductant concentrations higher than 4% (m/v). For Sb(V) analytical solutions, the signal is negligible up to 4% (m/v) $NaBH_4$. For commercial samples, the signal increases up to 2% (m/v) $NaBH_4$; after 3% (m/v) there is a small increase in the signal. A similar behavior was observed for the Sb(V) solution in 4% (m/v) $NaBH_4$. It is possible that with this high reductant concentration some stibine is generated from Sb(V). Thus, a 2% (m/v) concentration of $NaBH_4$ was chosen for the determination of Sb(III). In this condition, the relative standard deviation was about 4%.

Influence of Injected Volume on the Proposed FI System

The effect of the injected volume using the proposed FI-HG-AAS system is shown in Figure 5. As expected, a higher analytical signal was obtained by increasing the injected volume of the sample and the Sb(III) reference solution. However, with volumes higher than 250 µL, a small signal decrease was observed for the sample solutions. With all tested volumes, the analytical signals were recorded only when 378 µL of injected volume corresponded to 265 ng Sb(V). The injected volume selected was 125 µL. With this volume, analytical signals were reproducible (relative standard deviation about 4%), and the shape of the peaks from the sample and Sb(III) solutions was very similar.

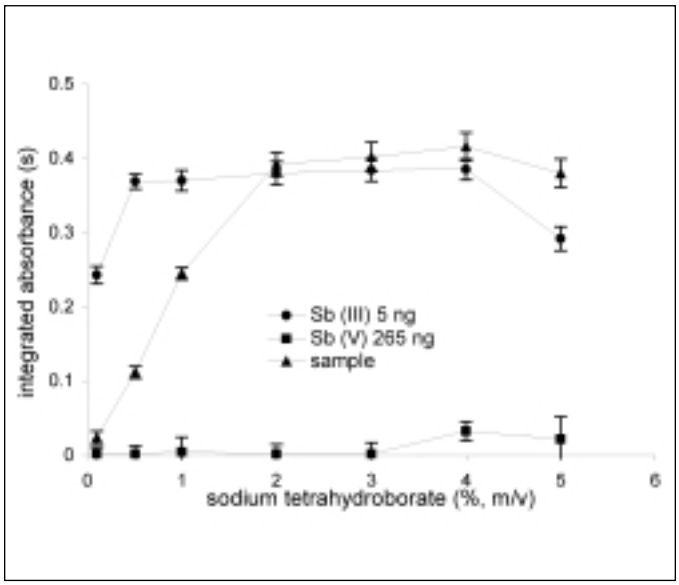

Fig. 4. Influence of $NaBH_4$ concentration on Sb(III), Sb(V), and commercial sample signals by the proposed procedure (2% m/v $NaBH_4$, 125-µL injection volume). Vertical bars are the relative standard deviation (n = 5).

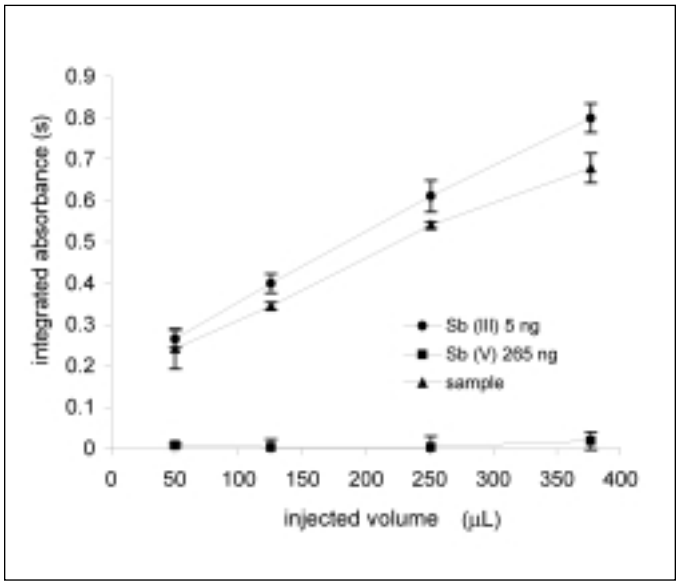

Fig. 5. Influence of injected volume on analytical signals for Sb(III), Sb(V), and commercial sample. Vertical bars are the relative standard deviation (n = 4).

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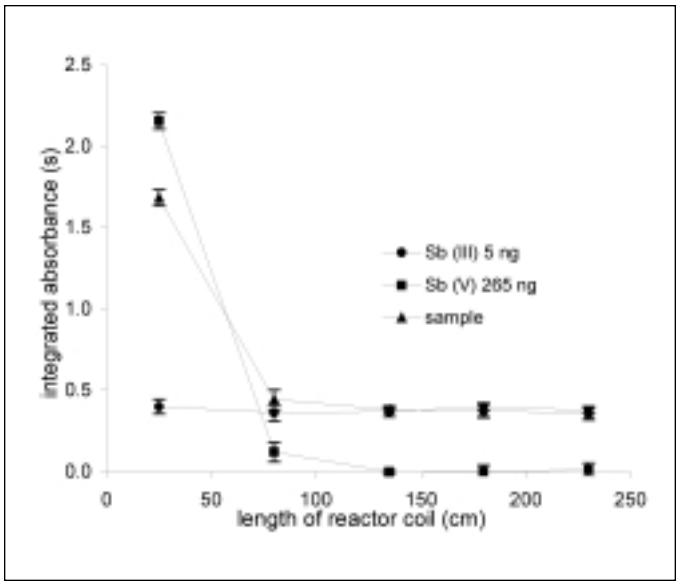

Fig. 6. Effect of length of reactor coil on Sb(III), Sb(V), and commercial sample signals by FI-HG-AAS (2% m/v $NaBH_4$, 125-µL injection volume).
Vertical bars are the relative standard deviation (n = 4).

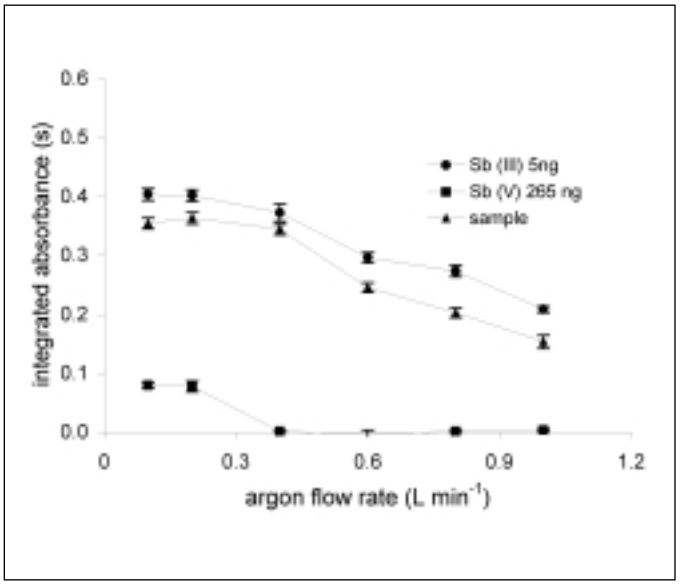

Fig. 7. Influence of argon flow rate on Sb(III), Sb(V), and commercial sample signals by FI-HG-AAS (2% m/v $NaBH_4$, 125-µL injection volume).
Vertical bars are the relative standard deviation (n = 4).

Influence of Reactor Coil Length

The influence of the length of the polyethylene reactor coil on the antimony signals is presented in Figure 6. A strong decrease of the Sb(V) signal was observed with coil lengths over 80 cm. With more than 135 cm, the Sb(V) signal suppression is complete. For the sample solution and Sb(III) analytical solution the behavior was almost the same. The signals were approximately constant with a coil length of 80 cm. This fact can be explained by the time required for the complexing action of citric acid to react on Sb(V). The coil reactor length selected for this study was 180 cm.

Effect of Carrier Gas Flow Rate on the FI System

The influence of the argon flow rate is shown in Figure 7. Argon was used to transport the generated stibine to the gas-liquid separator and then to the atomizer. The flow rate was varied from 0.1 to 1.0 L min^{-1}. The analytical signals for Sb(III) and sample decreased with an increase in the argon flow rate. This behavior is probably due to the diluting effect of the argon flow in the analytical signal of antimony. For Sb(V), the signals were negligible after 0.4 L min^{-1}. This flow rate was chosen in view of the suppression of the Sb(V) signal and a weak decrease from the Sb(III) solutions.

Influence of Interferents on the Sb(III) Analytical Signal

The effect of interferences in the determination of antimony by HG-AAS has been reported in the literature (26–29). In this work, the effect of As, Ni, and Pb on the Sb(III) signal was investigated. These elements were selected because they are present in the commercial meglumine antimonate samples that were previously analyzed. As shown in Figure 8, no interference was observed from 1 ng (total mass of the injected sample volume) up to 100 µL. The same behavior was observed for Pb for the same mass interval. However, a signal decrease was observed with more than 100 ng As, and a complete suppression of the Sb signal was observed at 100 µg As. All tests were made with injections of 125 µL, corresponding to 5 ng of Sb(III).

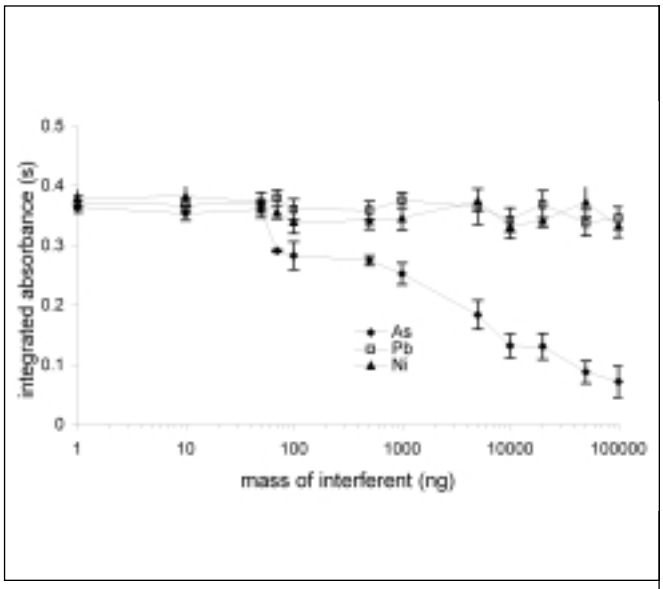

Fig. 8. Interference of Ni, As, and Pb on the Sb(III) signal using the proposed procedure (2% m/v $NaBH_4$, 125-µL injection volume). Vertical bars represent the relative standard deviation (n = 5).

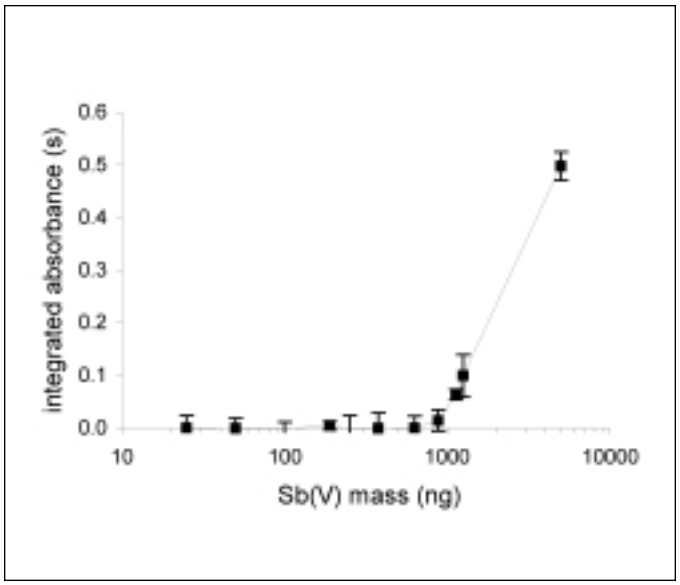

Fig. 9. Effect of Sb(V) on Sb(III) signals using the proposed FI-HG-AAS procedure (2% m/v $NaBH_4$, 125-µL injection volume). Vertical bars represent the relative standard deviation (n = 5).

CONCLUSION

A procedure is proposed to establish the maximum Sb(V) mass that can be tolerated without causing an interference in the determination of Sb(III). Figure 9 shows the analytical signals when the Sb(V) mass was varied from 30 ng to 5000 ng. No absorbance signals were observed up to 900 ng Sb(V). This limit is higher than those studied in this work (265 ng) and shows that the proposed FI-HG-AAS system allows the determination of Sb(III) in the presence of higher masses of Sb(V).

Using the optimized procedure, the detection limit (3 σ) obtained was 0.95 ng and the characteristic mass 55 pg. The typical relative standard deviation was 3.8%, and the linear working range was up to 50 ng Sb(III). More than 60 determinations can be performed per hour. Blanks were always low, and the background signals were below 0.05 in peak height. Recovery tests were made by adding Sb(III) analytical solutions to four commercial samples, corresponding to Sb(III) mass of 5 ng. The recoveries varied from 97.1% to 100.8%. Based on these figures of merit it is possible to propose the FI-HG-AAS procedure as an alternative to Sb(III) determination in routine analysis of injectable drugs containing high levels of Sb(V).

The procedure was used for the determination of Sb(III) in six commercial samples of meglumine antimonate, which is the drug currently injected into

TABLE I
Concentration of Sb(III) and Total Sb (mg mL^{-1}) in Commercial Samples of meglumine antimonate Using the Proposed FI-HG-AAS Procedure

Samples	Sb(III)	Total Sb
A (lot 1)	5.1 ± 0.2 (5.6)	91.2 ± 1.2
A (lot 2)	4.5 ± 0.2 (4.6)	97.0 ± 0.8
A (lot 3)	3.4 ± 0.1 (3.3)	102.9 ± 1.3
A (lot 4)	2.4 ± 0.1 (2.4)	100.1 ± 0.9
B (lot 1)	2.6 ± 0.1 (3.0)	85.6 ± 0.7
B (lot 2)	2.1 ± 0.1 (2.5)	83.5 ± 0.6

Results represent the mean ± standard deviation of five independent measurements. The values in parentheses represent the percentage of Sb(III) from the total Sb.

patients who are infected with leishmaniasis disease in Brazil and other South American countries. The results are presented in Table I. Total Sb determinations were performed by flame atomic absorption spectrometry using a conventional air + acetylene flame (wavelength of 217.6 nm, slit width of 0.7 nm). The Sb(III) content was between 2.4% and 5.6% from the total Sb content. The Sb(III) content in the analyzed samples is relatively high and could be a risk factor to leishmaniasis patients using meglumine antimonate which should contain no appreciable levels of Sb(III).

ACKNOWLEDGMENTS

The authors thank FAPERGS, CNPq, Farmacopéia Brasileira, ANVISA (Dr. Gonzalo Vecina Neto) for supporting this study.

Received September 27, 2002.

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Chromium Speciation by ETAAS Using 1,1,1-trifluoro-2,4-pentadione to Form a Volatile Chelate With Cr(III)

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ABSTRACT

A new method has been developed for the speciation of chromium by electrothermal atomic absorption spectrometry. The 1,1,1-trifluoro-2,4-pentadione (TFA) reacts selectively with Cr(III) to form a volatile complex. Then, the Cr(III)-TFA complex is volatilized in the graphite furnace before atomization of Cr(VI). The conditions for the complex formation were studied using acetic acid/sodium acetate as the buffer solution at pH 6 and TFA at a concentration of 4% (v/v), with subsequent heating in a microwave oven for 2 minutes at 100 W.

The optimum charring and atomization temperatures for the Cr(VI) determination were 1500ºC and 2200ºC, respectively. The volatilization temperature of the Cr(III)-TFA complex was 140ºC, where Cr(III) was completely removed. The method described for the determination of Cr(VI) is sensitive (with detection and quantification limits of 0.15 and 0.52 µg L^{-1}, respectively), accurate, and precise. The Cr(III) concentration was established by calculating the difference between total chromium and Cr(VI) concentration.

INTRODUCTION

Chromium can exist in valences from –2 to 6, but in the environment it is mainly in a trivalent or hexavalent state. Trivalent chromium [Cr(III)] is the most common and naturally occurring state, and most soils and rocks contain small amounts of chromic oxide (Cr_2O_3). Hexavalent chromium [Cr(VI)] occurs infrequently in nature; while chromates (CrO_4^{2-}) and dichromates ($Cr_2O_7^{2-}$) present in the environment are generally the result of industrial and domestic emissions. The most frequent sources of chromium are industrial processes such as plating, tanning, paint and pigment production, metallurgy, and the chemical industry (1).

The toxicity of chromium depends on its oxidation state. Trivalent chromium is an essential trace element for both humans and animals, and plays an important role in the control of glucose and the lipid metabolism. Hexavalent chromium is a well-known carcinogen that can cause serious toxic effects (2–4). For this reason, the determination of Cr(III) and Cr(VI) has become very important in environmental samples.

The techniques more commonly used for Cr determination are flame and furnace atomic absorption spectrometry (AAS) or inductively coupled plasma atomic emission spectrometry and the hyphenated techniques. Other techniques such as UV-VIS spectrometry or chromatography have also been used (5). Chromium speciation by atomic absorption requires a sample pretreatment procedure and the most commonly applied methods are complex formation (6), preconcentration (7), ionic exchange (8), processes of oxidation-reduction (9), and extraction (10).

The formation of complexes with β-diketone has been used for the determination of various elements (11-19). Some b-diketones, such as 1,1,1-trifluoro-2,4-pentadione (TFA), selectively react with certain elements and results in speciation.

The aim of this study was the selective determination of chromium in water samples by electrothermal atomic absorption spectrometry (ETAAS). By adding TFA, Cr(III) forms the complex $Cr(TFA)_3$, a chelate of high volatility. This complex volatilizes in the graphite furnace before the atomization step, and only then can Cr(VI) be atomized. The Cr(III) concentration is established by calculating the difference between total chromium and Cr(VI) concentrations.

EXPERIMENTAL

Instrumentation

The measurements were performed using a PerkinElmer Model 4100ZL atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT USA), equipped with a THGA™ transversely heated graphite atomizer and an AS-71 autosampler. The light source was a hollow cathode lamp, operating at 12 mA, using the 357.9-nm line and a spectral bandwidth of 0.7 nm. Zeeman background correction and pyrolytically coated graphite tubes with L'vov platforms were used. For all measurements, integrated absorbance was used with an integration time of 5 s. The volume injected was 20 µL.

A Model Panasonic NN-5256 domestic microwave oven was used for the complex formation.

All glassware was kept in 10% nitric acid for at least 48 hours and before use was washed three times with ultrapure water.

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Atomic Spectroscopy
Vol. 24(1), January/February 2003

Reagents

Cr(III) nitrate standard solution (1000 mg L^{-1}) (Merck, Darmstadt, Germany). Each test solution was prepared with ultrapure water immediately before use.

Cr(VI) standard solution (1000 mg L^{-1}) was prepared by dissolving $K_2Cr_2O_7$ (99%) (Aldrich Chemical, Milwaukee, WI, USA).

Sodium acetate (Panreac, Barcelona, Spain).

Acetic acid glacial, HPLC grade (Scharlau, Barcelona, Spain).

1,1,1-trifluoro-2,4-pentadione 99% (Avocado-Panreac, Sintesis, Spain).

Magnesium nitrate (Suprapur®, Merck, Darmstadt, Germany).

Reference Material, CRM 544 Lyophylized Chromium Solution, (Bureau Community of Reference, from the European Commission).

Ultrapure water, resistivity 18 MΩ.cm, obtained with a Milli-Q™ water purification system (Millipore, Bedford, MA USA).

Complex Formation

One mL of sample with 2 mL of HAc/NaAc buffer solution at pH 6 and 1 mL of 1,1,1-trifluoro-2, 4-pentadione (TFA) (4%, v/v) were transferred into a test tube and heated for 2 min in a microwave oven at 100 W. Twenty µL of this solution was injected into the pyrolytically coated graphite tube for ETAAS analysis according to the graphite furnace program shown in Table I.

TABLE I
Graphite Furnace Program for Cr(VI) Determination

Step	Temp (ºC)	Ramp time (s)	Hold time (s)	Ar flow (mL min^{-1})
1	110	1	10	250
2	130	5	30	250
3	1500	10	20	250
4	2200	0	5	0
5	2450	1	3	250

RESULTS AND DISCUSION

Complex Formation

Effect of Microwave Oven Power and Heating Time

To study the effects of power and heating time in the microwave oven, 1 mL of the sample was mixed in the test tube with 7 µg L^{-1} of Cr(III) or Cr(VI), 2 mL of HAc/NaAc buffer, and 1 mL of 1,1,1-trifluoro-2,4-pentadione (TFA) (1%). The experiments were performed using a microwave oven by varying the time of warming from 1 to 2 min and the microwave power from 100 and 200 W. It was found that the sample was lost due to vigorous boiling when it was heated for a longer period of time or at a power higher than 200 W. The optimum complex formation conditions selected for this study were 2 min at 100 W.

Effect of pH

The influence of pH on the formation of the $Cr(TFA)_3$ complex was studied by preparing a series of solutions and adjusting the pH to values from 3.5 to 7.7 with acetic acid/sodium acetate (0.5 M). A significant reduction in signal appeared at a pH above 5. The optimum pH value selected for this study was 6.

Amount of 1,1,1-trifluoro-2,4-pentadione

The TFA concentration was optimized by varying the concentration from 0.0001–10% (v/v). The results obtained (Figure 1) show that the absorbance signal diminishes when the TFA concentration is increased. For TFA concentrations higher than 1% (v/v), the signal remained constant. A TFA concentration at 4% (v/v) was selected as optimum to guarantee the volatilization of Cr(III).

Stability of the Complex

In order to study the stability of the $Cr(TFA)_3$ complex, the sample containing 7 µg L^{-1} Cr(III) was measured at time intervals from 15 min and 3 hrs. The results obtained show that the signal remains constant throughout all time intervals.

Optimization of Graphite Furnace Program

The graphite furnace temperature program was optimized for the determination of Cr(VI). Drying of the sample was carried out in two stages: 110ºC and 130ºC. The charring and atomization temperatures were optimized for a

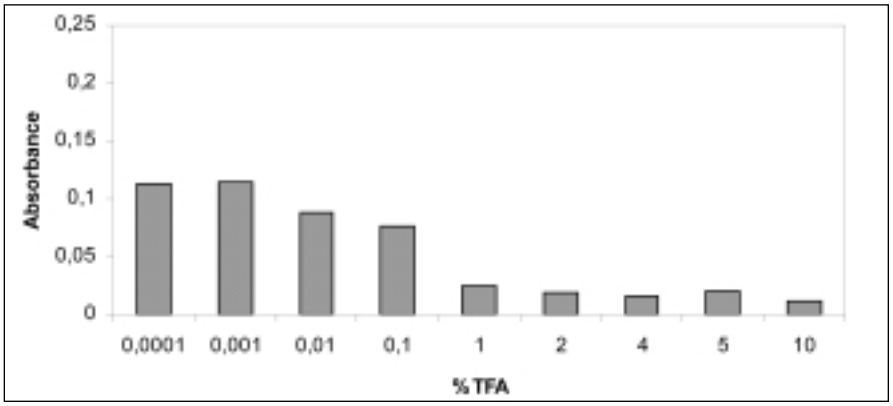

Fig. 1. Optimization of amount of 1,1,1-trifluoro-2,4- pentadione.

standard solution containing 7 µg L^{-1} of Cr(VI), and for the mixture of a standard solution with HAc/NaAc buffer and chelating agent. The optimum charring and atomization temperatures used were 1500ºC and 2200ºC, respectively. Table I lists the furnace conditions. Influence of the charring temperature on the volatilization of Cr(III) was also studied. As can be seen in Figure 2, the absorbance signal produced by Cr(III) does not vary considerably with an increase in the charring temperature since volatilization takes place at temperatures as low as 200ºC.

Chemical Modifiers

Over the last decade, different matrix modifiers have been proposed for the determination of chromium such as acid nitric (10), magnesium nitrate (10,20,21), sodium tungstate (10), ammonium nitrate (22), and calcium nitrate (21). In this work, the effect of the addition of magnesium nitrate as the chemical modifier was studied. Twenty µL of $Mg(NO_3)_2$ with a concentration ranging from 0–60 mg L^{-1} was injected into the graphite tube. No difference was observed with or without the chemical modifier $Mg(NO_3)_2$; therefore, no modifier was used for this study.

Analytical Performance

Calibration

Calibration curves and standard additions were obtained using two procedures: (a) the solutions were prepared from stock standard solutions and placed into the autosampler cups; (b) the solutions were prepared in test tubes following the same treatment as for the samples. Appropriate volumes of TFA and buffer solution were added to the standard solutions and the test tubes warmed for 2 minutes at 100 W. The calibration curves obtained from solutions exposed to microwave energy were used to calculate the concentrations of the samples, because it was observed that the sample treatment influences the calibration curves.

The concentration range used for the two procedures was between 0–8 µg L^{-1} of Cr(VI).

The equations obtained were as follows:

Calibration curve:

$A = 0.01+0.0088\ c \qquad r = 0.9944$

Standard addition curve:

$A = 0.0092+0.0091\ c \quad r = 0.9987$

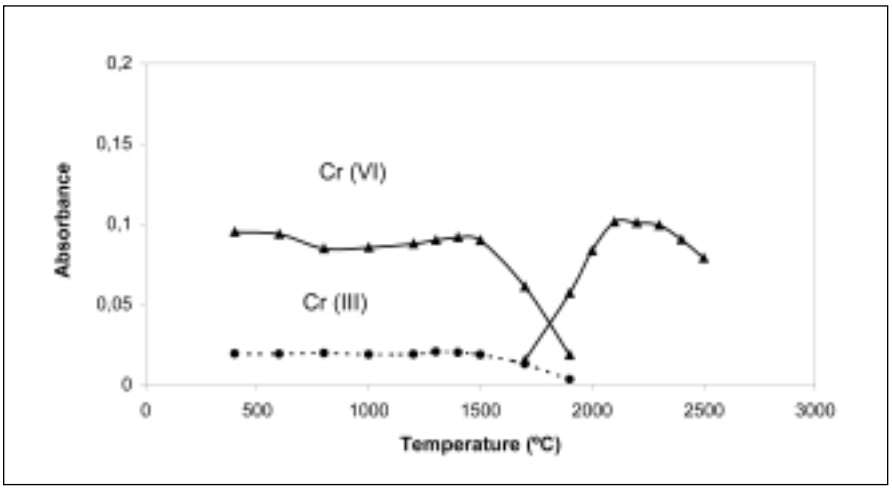

Fig. 2. Charring and atomization curves, 7 µg L^{-1} of Cr (VI).

where A is the integrated absorbance and c the Cr(VI) concentration in µg L^{-1}. No differences were observed when comparing the slopes of the calibration and the addition curves using the Student's *t*-test with a significance level of 95% (23). All measurements were made using the calibration curve.

Sensitivity

In order to express the sensitivity of a method, the following parameters were used: the limit of detection (LOD), the limit of quantification (LOQ), and the characteristic mass (m_o).

The LOD, defined as the lowest concentration level that can be determined to be statistically different from a blank, is calculated as $LOD = 3 \times SD / m$ at a 99% confidence level, where m is the slope of the calibration curve and SD is the within-run standard deviation of 10 blank replicates. The LOQ is defined as the level above which quantitative results can be obtained with a specific degree of confidence. At the 99% confidence level, the value recommended is $LOQ = 10 \times SD / m$. The values obtained using the method described were 0.15 µg L^{-1} and 0.52 µg L^{-1} for LOD and LOQ, respectively. These Cr(VI) values are better than those reported by Yingliang (24) using sequential ETV-ICP-AES (1.4 µg L^{-1}), Kingston (25) and Groll (26) using HPLC-ICP-MS (0.37 µg L^{-1}), and HPLC-FAAS (0.5–1.0 µg L^{-1}), respectively.

The characteristic mass, m_o, is defined as the mass (pg) of the analyte required to give a signal of 0.0044 s for integrated absorbance. The results obtained were 6.07 ± 0.51 pg.

Precision

The within-run precision of the method (instrumental and matrix factors), obtained for 10 replicate analyses of a single sample during

the same run, was 0.64% [for 7 µg L^{-1} of Cr(VI)].

The within-batch precision of the method, obtained for five replicates of the three samples with different concentrations of chromium added (2, 4, and 6 µg L^{-1}), was also investigated and the results obtained were 5.3, 6.3, and 5.8%, respectively.

Accuracy

The accuracy of the method was studied using the certified reference material, CRM 544 Lyophylized Chromium Solution, having a Cr(VI) certified value of 22.8 ± 1.0 µg L^{-1}. The reference material was reconstituted with 20 mL of HNO_3 /H_2CO_3 buffer, using pH 6.4 for stability reasons. In order to maintain the pH buffer at 6.4, the flask needs to be flushed with CO_2 at each use. It is recommended that the samples be analyzed as soon as possible after reconstitution. Three portions of this reference material were analyzed and the values found, expressed as the mean ± standard deviation, was 22.83 ± 1.5 µg L^{-1} of Cr(VI). No difference was observed between the certified and found values using the Student's *t*-test at a significance level of 95%. The accuracy of the method was verified by recovery studies using the method of standard addition for 2, 4, 6, and 8 µg L^{-1} of Cr(VI), resulting in 93.2, 96.6, 105.7, and 102.8%, respectively, with a mean value of 99.6%.

Futhermore, the total Cr concentration was measured in three subsamples of CRM 544 using the method proposed by Beceiro-Gonzalez (27). The certified Cr total concentration in the reference material, CRM 544 Lyophylized Chromium Solution, is 49.4 ± 0.9 µg L^{-1} of Cr and the found values were 49.19 ± 0.97 µg L^{-1} of Cr. There is no significant difference between the certified and found values using the Student's *t*-test at a significance level of 95%. Thus, the Cr(III) concentration can be exactly calculated by the difference between the total Cr and Cr(VI) concentrations; the Cr(III) concetration found was 26.36 µg L^{-1}.

CONCLUSION

Formation of the $Cr(TFA)_3$ complex resulted in the separation of Cr(III) during volatilization in the graphite furnace before atomization, with subsequent determination of Cr(VI) in the sample. The Cr(III) concentration is obtained by calculating the difference between the total Cr and Cr(VI) concentrations. The direct separation of the two species in the graphite furnace offers several advantages over other methods such as precipitation or liquid-liquid extraction, including reduced sample preparation time, minimal risk of contamination, and no loss of analyte. The proposed method is simple, rapid, and economical and provides good sensitivity, precision, and accuracy.

Received September 26, 2002.

ACKNOWLEDGMENTS

This paper is a contribution to project Ref. PGIDT00PXI20904PR, financed by Secretaría Xeral de Investigación e Desenvolvemento da Xunta de Galicia and the Consellería de Medio Ambiente, and project Ref. PGIDT01MAM20902PR, financed by Secretaría Xeral de Investigación e Desenvolvemento da Xunta de Galicia. Paloma Herbello Hermelo wishes to thank Secretaría Xeral de Investigación e Desenvolvemento da Xunta de Galicia for a predoctoral grant.

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Indirect Determination of Ciprofloxacin by Flow Injection Flame AAS Based on Forming Complex with Fe(III)

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ABSTRACT

Based on the complexation reaction of ciprofloxacin with Fe(III) in a weak acid medium, a flame atomic absorption spectrometry (FAAS) method for the indirect detemination of ciprofloxacin is proposed. In the flow injection on-line concentration and separation system, the ciprofloxacin solution reacted with Fe(III) to form the cation complex ciprofloxacin-Fe(III). This complex was then adsorbed on a cation-exchange mini column, while the excessive Fe(III) changed into anion $(FeF_6)^{3-}$ and passed through the mini column to waste. The adsorbed ciprofloxacin-Fe(III) was eluted reversely with HNO_3 to the nebulizer for measurement by FAAS. The absorbance of Fe(III) eluted from the mini column is proportional to the concentration of ciprofloxacin. With a reaction and adsorption time of 60 s and 100 s, respectively, the calibration curve was linear over the range of 5–100 µg mL^{-1} and 0.5–80 µg mL^{-1}. The relative standard deviation was 2.6% and 3.2%, at an analytical throughput of 30 and 22 samples per hour, respectively. The method was found to be suitable for the determination of ciprofloxacin in pharmaceutical preparations and superior to previously reported HPLC methods because of the simple experimental conditions required.

INTRODUCTION

Ciprofloxacin [1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolone carboxylic acid] is a third-generation quinolone carboxylic acid antibacterial agent, with high efficiency *in vitro* activity against a wide range of Gram-negative pathogens and Gram-positive cocci. It can be used clinically to combat many kinds of infections such as urinary tract, respiratory tract, gastrointestinal tract, as well as skin and soft tissue infections.

The method recommended by the pharmacopoeia of the P.R. China (1) to determine the concentration of ciprofloxacin in pharmaceutical preparations (such as capsules and tablets) is high-performance liquid chromatography. Many papers have been published using this methodology (2–11), but the chromatographic conditions are very complicated. Other methods reported include spectrophotometry (12–19), fluorescence (20–22), capillary electrophoresis (23,24), polarography (25,26), luminescence (17), and immunoassay (28). However, no flame atomic absorption spectrometry (FAAS) method has been published for the direct determination of ciprofloxacin.

A number of papers reported that ciprofloxacin can form a complex with some metal ions. It is believed that the mode of action of the fluoroquinolone family of drugs is through binding the DNA-gyrase enzyme in the presence of Mg^{2+}, and this binding would induce DNA breakage (29). Sultan et al. (30) presented a sequential injection technique for stoichiometric studies, optimization and quantitative determination of some fluoroquinolone antibiotics complexed with Fe(III) in sulfuric acid media. Based on forming a complex compound with Fe(III), a FAAS method for the indirect determination of norfloxacin has been reported (31). An electrochemical method was also used to investigate the polarographic and voltammetric behavior of the complex compound of ciprofloxacin with Mg^{2+}, Mn^{2+}, and DNA (32).

In this work, a flame atomic absorption spectrometry method is proposed for the indirect analysis of ciprofloxacin. In a weak acid medium, ciprofloxacin reacted with Fe(III) to form a complex that was adsorbed on-line on a mini column filled with cation exchange resin, followed by the elution with HNO_3 and FAAS measurement. The concentration of ciprofloxacin in the samples was determined by measuring the absorbance of Fe(III) eluted from the mini column. The optimum experimental conditions and the application of the analytical system to the determination of ciprofloxacin in tablets, capsules, and drops were investigated.

EXPERIMENTAL

Instrumentation

A Model WFX-1C atomic absorption spectrometer (Beijing Ruili Analytical Instrument Plant, P.R. China), equipped with an Fe hollow cathode lamp, was used to measure the absorbance of Fe(III). The wavelength and lamp current used was 248.3 nm and 6.0 mA, respectively. 1.80 L min^{-1} of acetylene and 7.4 L min^{-1} of air was used to obtain a steady flame. A Model IFIS-C flow injector (Xi'an Ruike Electron Equipment Corporation, P.R. China) was employed as the on-line separation and concentration system.

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Reagents

All chemicals were of analytical reagent grade and doubly deionized water was used throughout.

Stock standard solutions of ciprofloxacin (1000 μg mL^{-1}) were prepared by dissolving 0.1000 g ciprofloxacin (supplied by the Institute for verifying pharmaceutical and biological reagents, P.R. China) in water and diluting to 100 mL, then storing in a refrigerator. Working solutions were prepared fresh daily by appropriate dilution of the stock solutions.

Fe(III) standard solutions (1000 μg mL^{-1}) were prepared by dissolving the appropriate amount of ammonium iron alum in water and adjusting the acidic concentration to 0.2 mol L^{-1} with nitric acid. The nitric acid solution was 3.0 mol L^{-1} and the ammonium fluoride solution was 0.2 mol L^{-1}.

Cation-exchange Mini Column

Amberlite IR-120 resin (Xi'an Electric Power Resin Plant, P.R. China, particle diameter of 0.3–1.2 mm) was first purified by soaking in a saturated solution of sodium chloride for 20 hours, then washing with water, 1 mol L^{-1} hydrochloride acid, 1 mol L^{-1} sodium hydroxide, and then with water again. The purified resin was soaked in 10% acetic acid solution for 24 hours. After washing with water, the resin was filled into a mini glass tube. Then two ends of the glass tube were blocked with glass silk.

Flow Injection On-line Separation and Concentration System

A schematic of the flow injection on-line separation and concentration system used in this work is given in Figure 1.

The analytical cycle consists of three processes: (a) reaction and adsorption, (b) washing, and (c) elution. The first two processes run when the injection valve is in the sampling position and the elution process runs when the injection valve is in the injection position.

In the reaction and adsorption process, the sample solution (S) passes through the cation exchange mini column (B) (4 mm i.d and 10 cm in length) to remove the interference, then merges and reacts with the stream of the Fe(III) solution (R) in the reaction coil (L_1) (2 mm i.d. and 100 cm in length) to form the cation ciprofloxacin-Fe(III), which merges again with the stream of the NH_4F solution in another reaction coil (L_2) (2 mm i.d. and 100 cm in length) to transform the excessive Fe(III) to anion $(FeF_6)^{3-}$.The cation ciprofloxacin-Fe(III) complex was adsorbed on-line on another cation exchange mini column (A) (4 mm i.d. and 4 cm in length), which was connected to the injection valve by PTFE tubes (0.5 mm i.d.) as a sample loop, while anion $(FeF_6)^{3-}$ passed through the mini column (A) to waste.

In the washing process (30 s), water instead of sample and the Fe(III) solution was used to rinse the whole system, especially for the mini column.

In the elution process (30 s), the ciprofloxacin-Fe(III) complex adsorbed on the mini column (A) was eluted reversely by the eluent (E) (3 mol L^{-1} nitric acid) to the nebulizer and then measured by FAAS.

RESULTS AND DISCUSSION

Optimization of Reaction and Adsorption Conditions

The optimization conditions with the maximum sensitivity investigated for the reaction and adsorption process in the determination of 50 μg mL^{-1} ciprofloxacin were pH value, concentration of Fe(III), length of the reaction coil, length and inner diameter of the mini column, flow rates of the sample and Fe(III) solutions, concentration of ammonium fluoride, and reaction and adsorption time.

The effect of the pH ranging from 0.5 to 7, which would mainly influence the formation of the ciprofloxacin-Fe(III) complex and its adsorption on the mini column (A), was studied. The results showed that the absorbance increased with an increase in pH up to 2, but remained constant at pH 2–6, then decreased slightly at pH

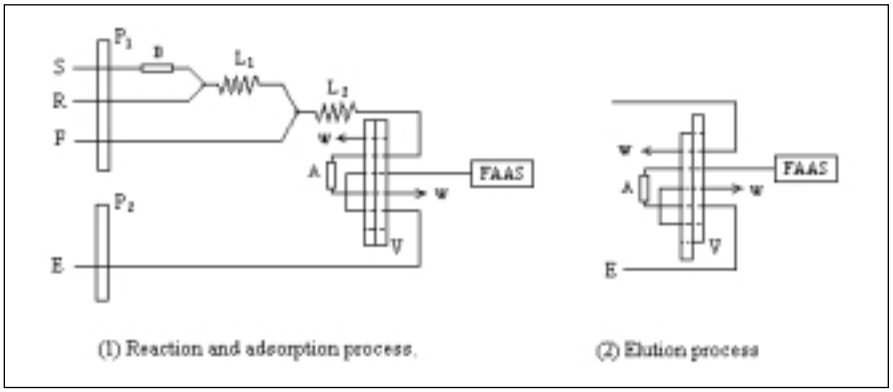

Fig. 1. Schematic of the flow injection on-line separation and concentration system used for the determination of ciprofloxacin.
P_1 and P_2, peristaltic pumps; S, sample solution; R, Fe(III) solution; F, NH_4F solution; E, HNO_3 solution; V, eight-channel rotary valve; FAAS, flame atomic absorption spectrometer; A and B, cation exchange mini-columns; L_1 and L_2, reaction coils; W, waste.

above 7. A pH of 3–4 was selected using nitric acid to adjust the sample pH value.

A series of different concentrations of Fe(III) solutions was tested. The results showed that the absorbance was basically constant when the concentration of Fe(III) was higher than 50 µg mL^{-1}. In order to ensure an excess of Fe(III) for the analysis of ciprofloxacin in higher concentrations, a 200-µg mL^{-1} Fe(III) solution was used for this work.

The absorbance increased with a reaction coil length (L_1) up to 100 cm. Thus, a reaction coil length (L_1) of 100 cm was chosen for this study.

The effect of the length and diameter of the mini column (A) was studied ranging from 30–60 mm in length with an inner diameter of 3.0 and 5.0, respectively. The results indicated that the exchange capacity increased with an increase in coil length and inner diameter, but the broadening and lowering of the absorbance peaks resulted in a decrease in sensitivity. Thus, a mini column (A) of 40×3.0 mm was chosen.

The influence of sample and Fe(III) solution flow rate was investigated from 1–7 mL min^{-1}. The absolute quantity of ciprofloxacin-Fe(III) increased with an increase in flow rates, so that the faster the flow rate, the better the sensitivity. Hence, the maximum value of 7 mL min^{-1} possible by the instrument was selected.

The concentration of the screening agent NH_4F played a conflicting role in the system. Low concentrations of NH_4F did not ensure the complete screening of excessive Fe(III), but extremely high amounts of NH_4F dissociated the ciprofloxacin-Fe(III) complex. The experimental results suggested that with a reaction coil length (L_2) of 100 cm, 0.2 mol L^{-1} of NH_4F at a flow rate of 7 mL min^{-1} could screen the excessive Fe(III) effectively and did not dissociate the complex.

The absorbance increased proportionally with an increase in reaction and adsorption time, which consequently improved sensitivity but slowed down the analytical throughput. It is suggested that the reaction and adsorption time selected should be based on the concentration of ciprofloxacin in the different samples.

Optimization of Elution Condition

The elution conditions investigated were the concentration and flow rate of the eluent, elution time, and elution mode.

The eluents considered were ammonium nitrate, sodium chloride, hydrochloric acid, and nitric acid. The experimental results proved that nitric acid was the best eluent. The concentration of nitric acid was tested in the range of 0.5–5 mol L^{-1}. The absorbance increased with a nitric acid concentration up to 3.0 mol L^{-1} and remained constant above that concentration. Therefore, 3.0 mol L^{-1} of nitric acid was used as the eluent.

The influence of elution flow rate was investigated from 1 to 7 mL min^{-1}. The optimum flow rate chosen was 5 mL min^{-1}.

In the adsorption and elution process, the acidity changed alternately, which caused swelling and shrinking of the resin and irregular filling of the mini column (A). In order to avoid this irregular filling, a reverse elution mode was adopted, in which the flow direction of the eluent stream was opposite to the reaction and adsorption stream.

Performance for Determination of Ciprofloxacin

Using the optimum experimental conditions, two calibration lines were plotted for absorbance vs. concentration of ciprofloxacin at 60 s and 100 s of reaction and adsorption time, respectively. The results of the ciprofloxacin analysis are listed in Table I. The relative standard deviation (%RSD) was obtained from seven measurements of the sample containing 50 µg mL^{-1} of ciprofloxacin.

Interference Studies

The influence of foreign substances in the samples, used as excipients and additives in the production of medicine such as starch, dextrin, sodium carboxyl methyl cellulose, carboxyl propyl cellulose, glucose, fructose, and

TABLE I
Performance in Ciprofloxacin Determination

Reaction and Adsorption Time	60 s	100 s
Regression Equation[a]	$A = 1.03\times10^{-2} + 2.1\times10^{-3}C$	$A = 1.10\times10^{-2} + 3.6\times10^{-3}C$
Regression Coefficient	0.990	0.986
Concentration Range	5–100 µg mL^{-1}	0.5–80 µg mL^{-1}
Characteristic concentrations (1% adsorption)	1.1 µg mL^{-1}	0.2 µg mL^{-1}
RSD	2.6%	3.2%
Sampling Frequency	30 h^{-1}	22 h^{-1}

[a] A is the absorbance; C is the concentration of ciprofloxacin expressed in µg mL^{-1}.

lactic acid, was investigated in the determination of 50 μg mL^{-1} ciprofloxacin. The results proved that the minimum tolerant amount of these foreign substances was 5 μg mL^{-1} which resulted in an error of less than 5%.

The cations in the sample did not interfere in the determination of ciprofloxacin, because they had already been removed by the cation-exchange mini column (B) before reaction with Fe(III).

Application

The proposed method was applied to the analysis of some pharmaceutical preparations such as tablets, capsules, and drops containing ciprofloxacin.

Tablets of ciprofloxacin (claiming to contain 100 mg of ciprofloxacin) were crushed into powder and dissolved in 4 mL glacial acetic acid, then diluted to 100 mL with water. The sample solution was obtained by appropriate dilution.

A sample solution of ciprofloxacin capsule can be prepared in the same way as tablet powder.

The solution of ciprofloxacin drops (claiming to contain 3 mg mL^{-1} of ciprofloxacin) can be determined directly by diluting it to 30 μg mL^{-1}.

The results obtained for the analysis of these three kinds of pharmaceutical preparations are summarized in Table II, which are in good agreement with the claimed content.

TABLE II
Results for the Determination of Ciprofloxacin in Pharmaceutical Preparations

Sample	This work (mg mL^{-1})	Claimed content (mg mL^{-1})
Tablet I	0.988 ± 0.033	1.0
Tablet II	0.993 ± 0.028	1.0
Capsule	0.990 ± 0.025	1.0
Eyedrops	2.97 ± 0.09	3.0

CONCLUSION

From the above results, it can be concluded that the complex reaction of ciprofloxacin with Fe(III) can be used for the indirect detrmination of ciprofloxacin by FAAS using a flow injection on-line separation and concentration system. In addition, the results show that the proposed method is suitable for the determination of ciprofloxacin in pharmaceutical products and is superior to previously reported HPLC methods because of the simple experimental conditions required. The proposed method also provides higher sensitivity, requires lower sample consumption, and offers higher sample throughput.

Received August 10, 2002.

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Flame AAS Determination of Total Chromium in Mussel Samples Using a Continuous Ultrasound-assisted Extraction System Connected to an On-line Flow Injection Manifold

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ABSTRACT

Total chromium was extracted on-line from solid mussel samples using a simple, rapid, and continuous ultrasound-assisted extraction system (CUES). CUES is connected to a flow injection manifold, which permits the on-line flame atomic absorption spectrometric determination of chromium. The manifold for total chromium determination is the simplest possible because a volume of 83 µL of acid leachate was injected into a water carrier stream. An experimental design was used for optimization of the continuous leaching procedure. Compared to off-line ultrasonic-assisted extraction methods, the sonication time was reduced by a factor of 4–28. The method allowed a total sampling frequency of 11 samples per hour, with a relative standard deviation for the complete procedure of 1.7% [for a sample containing 2.9 µg/g chromium (wet mass)]. Accuracy was verified using TORT-1 certified reference material and the procedure applied to the analysis of mussel samples.

INTRODUCTION

Cr(III) is known to be an essential trace element for living organisms, because it plays an important role in the control of the glucose and lipid metabolisms. However, Cr(VI) is toxic and carcinogenic, even at very low levels. Chromium is widely used in industry such as leather tanning, printing, and the production of dyes, pigments, primer paints, and wood preservatives. Chromium compounds are also used as slimicides in cooling towers and for audio, video, and data storage. The high concentrations of this element found in the environment are due to industrial emission, effluents from waste dumps, sewage sludges, incineration of municipal solids, and hazardous wastes (1). Mussels have been extensively used as biological monitors of coastal contamination and must also be evaluated for their suitability for human consumption (2). Hence, the determination of chromium in these types of samples is an important aspect of food and environmental analysis and monitoring.

The preparation of a solid sample is often the most time-consuming step of the analytical process and involves potential problems, such as contamination or loss of analytes. Ultrasound-assisted extraction procedures (leaching) are an attractive sample pretreatment for solid samples, because the leaching process is not a total decomposition procedure and requires low concentrations of the extractants (acid or alkaline solutions). This process increases the representative sample mass, and improves the precision and accuracy of slurry sampling. It also avoids total sample matrix introduction into the spectrometer, which occurs with solid sampling, slurry sampling, and the digestion procedures (3). However, these procedures are laborious and time-consuming, because they require long sonication times (10–120 min) to separate the liquid phase, in addition to 10–20 min for centrifugation.

Ultrasonic extraction procedures have been described by several authors. The reports published by El Azouzi et al. (4) and Amoedo et al. (5) have failed to show the quantitative chromium extraction from mussel samples by using low leaching solution concentrations [0.1 M H_2O_2 + 1.6 M HNO_3 + 1.2 M HCl, and 3% (v/v) HNO_3, respectively]. Bermejo-Barrera et al. (6), on the other hand, used more stringent ultrasound-assisted extraction conditions. They employed a high leaching solution concentration (2.7 M HCl + 4 M HNO_3) and achieved a quantitative recovery of chromium from mussel samples. However, none of the ultrasound-assisted extraction methods published applied flow injection (FI) to measure Cr continuously or to carry out on-line ultrasonic extraction.

In this paper, we describe for the first time a simple and continuous ultrasound-assisted extraction system (CUES) connected to an on-line flow injection manifold for acid leaching of Cr and its determination by flow injection flame atomic absorption spectrometry (FI-FAAS) in solid mussel samples. This procedure combines the benefits of ultrasound-assisted extraction methods with those of FI systems.

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EXPERIMENTAL

Instrumentation

A PerkinElmer Model 5000 atomic absorption spectrometer (PerkinElmer Life and Analytical Sciences, Shelton, CT USA), furnished with a chromium hollow cathode lamp, was used. The instrument was set at 357.9 nm. The acetylene flow rate was 4.5 L/min. An air flow rate of 2.3 L/min was employed to obtain a clear yellow flame (reducing). The aspiration flow rate of the nebulizer was adjusted to be the same as the flow rate of the carrier (6 mL/min). The spectrometer output was connected to a PerkinElmer Model 50 Servograph Recorder with a range of 5 mV. As can be seen in Figure 1, the continuous ultra-sound-assisted extraction system (CUES) comprises a Minipuls™-3 peristaltic pump (Gilson, France) fitted with Viton tubes, an ultrasonic bath (Selecta, Barcelona, Spain), and a glass mini column (50 mm x 3 mm i.d., bed volume 350 µL) (Omnifit, UK). The ends of the mini column were plugged with filter paper (Whatman 541). The FI manifold for Cr total determination comprises a Gilson Minipuls-3 peristaltic pump fitted with PVC tubes and two Rheodyne® injection or (USA) switching valves, Models 5041 and 5301.

The numerical analysis of the experimental designs was performed by means of the STATGRAPHIC V.4.1 statistical package (Manugistic, Inc., Rockville, MD USA).

Reagents

Ultrapure water of 18.3 MΩ.cm resistivity, obtained from a Milli-Q™ water purification system (Millipore, Bedford, MA USA), was used for preparation of the samples, reagents, and standards. Hydrochloric acid, nitric acid, and trivalent chromium standards were reagent grade (Merck, Darmstadt, Germany). The certified reference material used was TORT-1, Lobster Hepatopancreas Marine, supplied by the National Research Council of Canada. All glassware used was cleaned by placing it in 4M HNO_3 for four days and rinsing with ultrapure water before use.

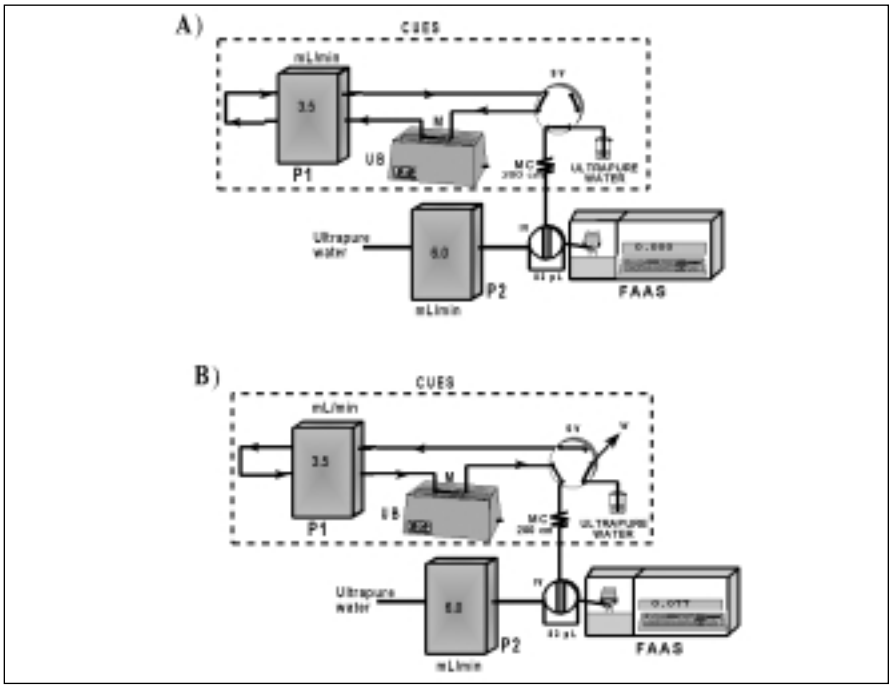

Fig. 1. Schematic diagram of CUES and optimum working conditions for the on-line total Cr determination. (a) on-line acid leaching step, (b) continuous detection of total Cr. P1 and P2= peristaltic pumps; UB= ultrasonic bath; M= minicolumn; SV = selecting valve; MC= mixing coil; IV= injection valve; W= waste; FAAS= flame atomic absorption spectrometer.

Sample Preparation and Procedure

The mussels were triturated, homogenized, and freeze-dried; then stored in clean, dry containers. The samples were pulverized in a porcelain mortar, and after sieving, fractions with particle sizes under 100 µm were taken. Before use, the samples were dried for 30 min in a stove at 105ºC.

Mussel samples of 35 mg were directly weighed into the glass mini column. Then, the mini column was connected to the CUES (see Figure 1). First, the CUES circuit (1 mL) was loaded with the acid leaching solution (3M hydrochloric acid and 3M nitric acid). Once the CUES circuit was closed, the leaching solution underwent ultrasonic treatment by circulating through the mini column at a flow rate of 3.5 mL/min for 5 min. The direction of the flow was changed each 30 s in order to avoid sample accumulation at the end of the mini column. Then, the switching valve (SV) was switched to its opposite position and the acid extract homogenized in the mixing coil. A volume of 83 µL of acid extract was injected by means of the injection valve (IV) into the water carrier stream and swept to the detector. Standard solutions containing between 0 and 5 µg/mL of Cr in the same acid medium were used as the leaching solution and introduced into the flow system. Blank determinations were performed using the acid leaching solution. In all instances, the absorbance was 0.000.

RESULTS AND DISCUSSION

Optimization of the Continuous Ultrasonic Acid Extraction of Total Cr: Factorial Design

Since optimization of the analytical procedure involves the study of a large number of variables, an experimental design was used to optimize this stage. The use of complete factorial designs does not seem to be very effective due to the large number of experiments or runs required. The Plackett Burman designs were used principally when the number of variables influencing the analytical system was very large because such designs permit the division of full factorial designs, obtaining numbers of factor combinations that are multiples of four. They are also appropriate when some of the variables could have negligible main effects and interactions or when a higher order of interactions is insignificant. The number of experimental runs can be reduced by intentionally confounding some of the experimental effects, yet they would provide knowledge about system tendencies (7). In this paper, the Plackett Burman 2^6*3/16 factorial type III resolution design with one center point was selected, allowing 4 degrees of freedom (number of runs - number of factors - number of centerpoints - 1) and involving 13 non-randomized runs. In order to test the statistical significance of the effects, an ANOVA (Statgraphic V. 4.1) was employed (8). This factorial design was applied to a mussel sample (30 mg) with a total chromium content of 2.9 µg/g (wet mass). This concentration was obtained accomplishing a conventional off-line sample digestion with concentrated nitric acid and subsequent chromium determination by FAAS. To optimize the CUES, chromium was measured on-line in the leachate by FAAS with a flow system similar to that depicted in Figure 1. The variable response (% Cr recovery) was calculated according to the following equation:

$$\%\text{Recovery} = \frac{[\text{Cr}]\ \text{continuous acid leaching}}{[\text{Cr}]\ \text{off-line acid digestion}} \times 100$$

Both Cr concentrations in the acid leachate and digest were measured twice.

Six experimental variables were optimized: HNO_3 and HCl concentrations (leaching solution), sonication time, leaching temperature, flow rate of CUES, and leaching volume. In Table I, the lower and upper levels for each studied variable are listed. These values were chosen from available data and experiences developed in previous experiments. Table II summarizes the design matrix and the extraction yield obtained in each of the experiments involved for Cr; the results are expressed as percentages. From the results of this analytical data it can be concluded that the Cr extraction appeared to be affected by three statistically significant factors: nitric acid and hydrochloric acid concentration (leaching solution) and sonication time; that is, these

TABLE I
Factor Levels in the Plackett Burman Design (2^6*3/16) for the Continuous Ultrasonic Acid Extraction of Total Cr

Variable	Key	Low (–)	High (+)
Nitric acid concentration (M)	A	0	3.5
Hydrochloric acid conc (M)	B	0	3.5
Sonication time (min)	C	0.5	5
Leaching temperature (°C)	D	20[a]	70
Flow rate of CUES (mL/min)	E	3.5	6
Leaching volume (mL)	F	1	3

[a] Room temperature.

TABLE II
Design Matrix and Response Values in the Plackett Burman Design (2^6*3/16) for the Continuous Ultrasonic Acid Extraction of Total Cr

Run No.	A	B	C	D	E	F	Recovery (%)
1	+	-	+	-	-	-	79.3
2	+	+	-	+	-	-	65.5
3	-	+	+	-	+	-	65.5
4	+	-	+	+	-	+	72.4
5	+	+	-	+	+	-	65.5
6	+	+	+	-	+	+	103.4
7	1.5	1.5	2.8	45	4.8	2	96.6
8	-	+	+	+	-	+	75.9
9	-	-	+	+	+	-	0
10	-	-	-	+	+	+	0
11	+	-	-	-	+	+	31.0
12	-	+	-	-	-	+	20.7
13	-	-	-	-	-	-	0

factors overtake the limit of statistical significance (at 95% confidence). All of them were affected by a positive sign. This means that by increasing these factors for the levels tested, Cr extraction efficiency was favored. In the case of sonication time, this behavior was logical because the contact time between sample and leaching solution was increased. All other parameters such as leaching temperature, flow rate of the CUES, leaching volume as well as the interactions between factors were not significant. For leaching, room temperature (20°C) was chosen in order to simplify the determination. A leaching volume of 1 mL was selected to obtain better sensitivity, and the flow rate of the CUES was fixed at 3.5 mL/min because it was affected by a negative sign. The response cube of the Plackett-Burman design also provided additional information about system behavior or tendency, principally when the most significant factors are considered. Figure 2 shows the response cube for the experimental model using the three significant factors. As can be seen, the extraction efficiency was directly proportional to these factors, and it peaked at the highest levels tested.

Based on these results, it seemed that higher values of HNO_3 and HCl concentrations and a higher sonication time would be desirable. However, higher acid concentrations could cause serious damage to the nebulizer of the FAAS, and quantitative chromium recoveries are obtained with the high level of significant factors. Since 3M is an easily available concentration of HNO_3 and HCl solutions, and a sonication time of 5 min is enough to obtain close contact between sample and leaching solution to achieve a high sampling frequency, we decided to work with the operational conditions as listed in Table III.

The two variables that can affect the acid extraction process were studied separately, namely mussel particle size and amount of sample. By using the optimum conditions of the CUES, particle sizes smaller than 30 µm and between 30–100 µm were tested. The results obtained show that this variable does not affect the extraction process. This can be explained by the high energy supplied by the ultrasound energy (frequency of 40 KHz), which increases the contact between sample and leaching solution. Since 30 mg was the optimum amount required to obtain good results with the CUES system, we suppose that smaller quantities would also be suitable. However, in order to increase the sensitivity of the method, it is also important to know the maximum amount of sample capable of quantitatively leaching the system. For this reason, we studied sample amounts between 30–60 mg. The results obtained demonstrated that in spite of the change in the direction of the flow, amounts of samples greater than 35 mg produced great pressure in the CUES. Therefore, the maximum sample amount that can be used is 35 mg.

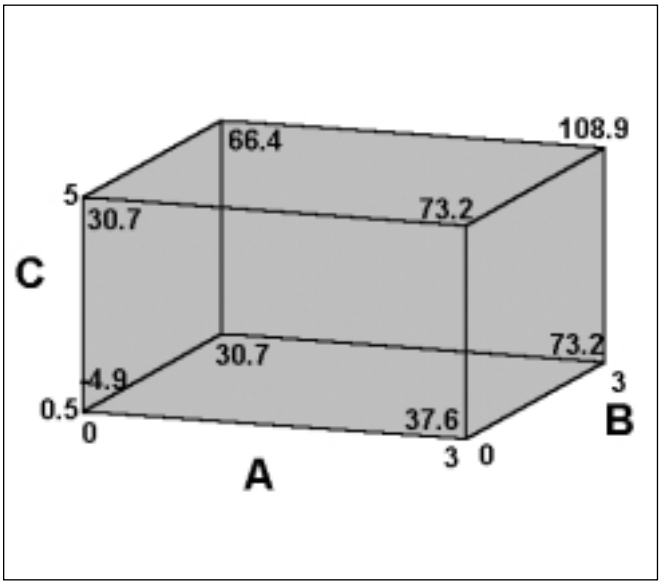

*Fig. 2. Response cube estimated for Plackett Burman design (2^6*3/16) for the continuous ultrasonic acid extraction of total Cr.*

TABLE III
Optimum Conditions for Continuous Acid Ultrasonic Extraction of Total Cr

Variable	Optimum
Nitric acid conc	3 M
Hydrochloric acid conc	3 M
Sonication time	5 min
Leaching temperature	20°C (room temperature)
Flow rate of CUES	3.5 mL/min
Leaching volume	1 mL

Optimization of the Remaining Flow Parameters Involving Total Chromium Determination

In the Plackett Burman design only the variables that affect the CUES have been considered. The mixing coil length to homogenize the acid extract was fixed to 200 cm (equivalent to 1 mL, the same volume of the acid extract). There are two reasons why not all of the acid extract is directly introduced into the detector. The most important reason is that highly acid solutions can damage the detector, and the second reason is control of the aspiration flow rate in order to achieve the maximum analytical signal. The variable studied for this flow system (Figure 1) was the carrier flow rate (water), because the injected volume of the acid extract was fixed as the minimum (83 µL, inner volume of injection valve). The carrier flow rate between 3–6 mL/min was studied and the aspiration flow rate of the nebulizer was adjusted to be the same as the flow rate of the carrier stream. It was found that the higher aspiration flow rate provides better sensitivity. Therefore, a carrier flow rate of 6 mL/min was chosen.

Features of the Method

The calibration graph was run (n=8) under optimum chemical and flow conditions for the global process, using the absorbance equation = $7.3 \times 10^{-5} + 0.051$ X (X: 0–5 µg/mL).

The precision of the continuous analytical method for the real samples was verified using a sample containing 2.9 µg/g Cr (wet mass) (n=11). The result obtained and expressed as the relative standard deviation was 1.7%.

The limit of detection (LOD) based on three times the standard deviation (n=30) of the blank was found to be 0.12 µg/g (wet mass). The sample throughput, taking into account the global process, was about 11 samples/h.

Validation of the method was performed using the certified reference material, TORT-1 Lobster Hepatopancreas with a Cr content of 2.4±0.6 µg/g. The validation was performed by the standard addition method (adding to the acid leaching solution 0, 1, 2, and 3 µg/mL Cr). The Cr content obtained for the certified reference material (mean ± SD, n=3) was 2.3±0.1 µg/g, which agrees with the certified value.

Analysis of Samples

The method was applied to determine total Cr in various mussel samples collected from Galicia, Spain. This area is subjected to intensive traffic and waste from industrial activity. The concentration of Cr in the mussel samples ranged from 0.44–2.94 µg/g (wet mass). The results obtained for total Cr with the proposed method were compared with those achieved by a conventional off-line sample digestion with concentrated nitric acid and subsequent chromium determination by FAAS. To compare the results obtained by both methods, the Paired *t*-Test was applied (9). As shown in Table IV, since the calculated *t* value is smaller than that obtained from the *t*-distribution table, the null hypothesis is retained: both methods do not give significantly different values for the total chromium concentration and thus, the agreement between the two methods is satisfactory. Table IV shows that the recovery of total chromium is satisfactory.

CONCLUSION

A simple, continuous ultrasound-assisted extraction system (CUES) connected to an on-line flow injection manifold for the acid leaching of total Cr and its determination by FAAS in solid samples is described here for the first time. This procedure combines the benefits of ultrasound-assisted extraction with those obtained with FI systems. The main goals achieved with the proposed method are reduction of sample contamination as well as analyte loss because less manipulation of the sample is required; reduction of sample amount and reagents; reduction of sample preparation time because compared to off-line ultrasonic-assisted extraction methods, sonication time is reduced by a factor of 4–28; and elimination of the centrifugation step to separate the liquid phase. The two last advantages significantly increase sample throughput.

TABLE IV
Paired *t*-Test

Sample No.	Total Cr (µg/g, wet mass)		Difference	(%) Recovery
	Off-line acid digestion and FAAS determination	Present method		
1	0.41	0.44	–0.03	96.7
2	0.96	0.93	+0.03	99.6
3	0.68	0.66	+0.02	96.7
4	0.80	0.82	–0.02	101.2
5	0.64	0.62	+0.02	98.3
6	0.56	0.55	+0.01	101.4
7	2.90	2.94	–0.04	101.7

X (mean difference): -1.4×10^{-3}; SD: 2.8×10^{-2}; n: 7; t = X /n / SD = 0.14; critical value of *t* (P=0.05): 2.45.

The same principles for system design adopted in this work may be readily transferred to other on-line systems with ETAAS detection, which would increase the sensitivity of the method.

Received October 31, 2002.

ACKNOWLEDGMENTS

The authors are grateful for the financial support provided by the Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Ministerio de Ciencia y Tecnología (Project No. CAL01-043). A.M.C.B. acknowledges a grant from the Ministerio de Educación, Cultura y Deporte, AP 2001-0367.

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