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Direct Analysis of Milk Powder by Axially-Viewed Simultaneous ICP-OES

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Authors

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Introduction

The elemental analysis of milk is important both as an indicator of environmental contamination and because milk is a significant pathway for toxic metal intake and a source of essential nutrients [1] for humans.

The accepted methods for elemental analysis of milk have traditionally included either wet digestion or dry ashing [3–6]. These are time consuming and involve procedures using potentially hazardous chemicals. A direct method of analysis by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-OES) would obviously be preferable, but this relies upon the instrument tolerating high levels of dissolved solids while providing sufficient sensitivity to measure toxic trace elements and the dynamic range to measure nutritional major elements.

The use of a sequential ICP-OES for direct analysis of milk has been previously described by Ryan [2]. This work describes the direct analysis of milk powder using standard quantitative calibration with aqueous standards using a simultaneous ICP-OES system. Viscosity effects of the milk powder solutions are corrected using scandium (361.383 nm—ionic line) as an internal standard. Major, minor and trace elements were determined in a single analysis. Less sensitive lines are used for the determination of major elements allowing both major and minor elements to be determined from a single solution.

Many of the major constituents in milk powder such as Na, K and Ca are Easily Ionized Elements (EIE) which can cause ionization interferences. Previous work [2] has shown that the addition of caesium as an ionization suppressant and internal standard to the standards and samples was beneficial. The ionization suppressant and internal standards are conveniently introduced into the plasma on-line, via the third channel of the peristaltic pump. The accuracy and validity of the method was assessed by the use of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 8435 Whole Milk Powder.

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Experimental

Instrumental

The Vista simultaneous ICP-OES axially viewed plasma was used for the analysis. The Vista features a free running, air cooled, 40 MHz RF generator and cooled cone interface. The Vista's optical system is based on an echelle polychromator with Charged Coupled Device (CCD) detector. The polychromator is thermostatted to 35 °C for stability, and the unique CCD detector features 70,000 pixels (detectors) arranged to exactly match the 2 dimensional echellogram. Sophisticated design of the detector has resulted in a rapid readout with excellent detection limits [4]. The instrument was controlled by an IBM computer with an Intel Pentium processor and Agilent's Vista worksheet software running under Microsoft's Windows NT operating system.

In this work a Vista with manual gas pressure regulator was used. Mass flow control of the nebulizer flow, which allows the nebulizer gas flow to be automatically adjusted, is available as an option. This work took advantage of the third channel pump to add internal standard and ionization suppressant on line. The Vista instrument is available with either the two or three channel pump option.

Table 1. Instrument Operating Conditions

Power	1.35 kW
Plasma gas flow	15.0 L/min
Auxiliary gas flow	1.5 L/min
Spray chamber type	Sturman-Masters
Torch	Standard axial torch with 2.3 mm id injector
Nebulizer	V-groove
Nebulizer pressure	240 kPa
Pump tube Inlet	PVC black-black
Outlet	PVC, blue-blue, 1.65 mm id
Polychromator purge	Boost (3.7 L/min)
Pump speed	15 rpm
Sample uptake rate	0.6 mL/min
Integration time	3 seconds
No. of replicates	3
Sample delay time	20 seconds
Fast pump	On
Stabilization time	20 seconds
Background correction	Fitted, 2 points/peak

To make sure that the milk powder sample was evenly mixed and in suspension while being aspirated, the solution was continuously stirred with a magnetic stirrer. Sodium and potassium were internally standardized with the caesium 372.328 nm line, using added caesium as both the ionization suppressant and internal standard. Scandium was used as an internal standard for all other lines.

Standard Preparation

Aqueous standards were prepared from 1000 mg/L single element standards (Spectrosol, BDH Chemicals). The standards were made up in 18 MΩ Milli-Q water with 0.5% v/v HNO_3 and 0.002% v/v Triton X100 prepared from a 1% w/v Triton X100 solution.

Table 2: Calibration Standards Prepared

Standard	Elements and concentration (mg/L)
Standard 1	Ba (0.5), Mn (0.5), Zn (0.5), Sr (0.5)
Standard 2	Ba (1.0), Fe (1.0), Mn (1.0), Zn (2.0), Sr (5.0)
Standard 3	Mg (5.0), Fe (5.0)
Standard 4	Mg (25.0), Na (25.0), Ca (25.0)
Standard 5	Na (100), Ca(100), K (100)
Standard 6	K (200)
Standard 7	S (10.0), P (50.0)
Standard 8	S (50.0), P (150.0)
Standard 9	S (100.0), P (200.0), Ca (250.0)
Standard 10	Ca (1000)
Standard 11	K (1000)
Standard 12	Na (1000)

Rinse and calibration blank solutions were prepared from 18 MΩ Milli-Q water with 0.5% HNO_3 and 0.002% Triton X100.

Sample Preparation

Solutions were prepared from samples supplied by the Ministry of Agriculture and Fisheries (MAF), New Zealand and SRM 8435 Whole Milk Powder.

Milk powder suspensions containing 2% w/v were prepared for all samples. The sample was accurately weighed and then transferred into a volumetric flask. The flask was filled approximately 3/4 full with 18 MΩ Milli-Q water and gently shaken until the milk powder was evenly mixed. Triton X100 was added to give a concentration of 0.002%. Acid was not added to the samples as it causes the precipitation of protein.

The samples were made up to the mark and placed in an ultrasonic bath for 5 minutes. The samples were then shaken vigorously for 1 minute.

A separate rinse containing 0.002% v/v Triton X100 and no acid was used for rinsing between samples.

Both caesium and scandium were used as internal standards in the analysis. Caesium also acted as an ionization suppressant. A bulk solution of 1% CsCl and scandium (0.5 mg/L) and was added to all solutions via the third channel of the peristaltic pump.

Results and Discussion

Milk Powder Analysis

The results presented in Tables 3–5 represent the concentrations of constituent elements in the milk powder on a dry weight basis. Previous work [2] had shown that the milk powder samples typically have low moisture contents and the samples were determined directly from the raw material without drying.

Table 3. Analysis of Standard Reference Material, S.R.M. 8435

Element	Wavelength (nm)	2.0% S.R.M. 8435 whole milk powder (ppm)	S.R.M. 8435 certified value (ppm)
Ba	493.408	0.72 ± 0.03	0.58 ± 0.23
Ca	373.690	8990 ± 340	9220 ± 490
K	404.721	12580 ± 210	13630 ± 470
Fe	238.204	1.07 ± 0.01	1.80 ± 1.1
Mg	285.213	838 ± 27	814 ± 76
Mn	257.610	0.17 ± 0.02	0.17 ± 0.05
Na	588.995	3810 ± 40	3560 ± 400
P	185.878	7400 ± 300	7800 ± 490
S	180.669	2320 ± 90	2650 ± 400
Sr	407.771	4.10 ± 0.20	4.35 ± 0.50
Zn	202.548	25.2 ± 0.9	28.0 ± 3.1

Table 4: Analysis of 2% Milk Powder

Element	Wavelength (nm)	2.0% Milk powder sample (wt%)
Ba	493.408	0.97
Ca	373.690	8909
K	404.721	11657
Fe	238.204	1.76
Mg	285.213	796
Mn	257.610	0.37
Na	588.995	3118
P	185.878	7729
S	180.669	2278
Sr	407.771	5.48
Zn	202.548	28.4

Table 5. Analysis of MAF#1

The "Known values" are values supplied by the New Zealand Dairy Research Institute.

Element	Wavelength (nm)	2.0% MAF#1 sample (ppm)	Known value (ppm)
Ba	493.408	3.24 ± 0.01	–
Ca	373.690	13750 ± 9	13274
K	404.721	15600 ± 120	17040
Fe	238.204	1.36 ± 0.04	2.0
Mn	257.610	0.310 ± 0.0001	–
Na	588.995	4000 ± 50	3490
P	185.878	10600 ± 140	9930
S	180.669	3280 ± 40	3280
Sr	407.771	3.99 ± 0.03	–
Zn	202.548	40.2 ± 0.8	37.33

Summary

The concentrations of various elements of both nutritional and environmental interest in milk powder samples were determined directly using the axially viewed Vista simultaneous ICP-OES. Aqueous calibration solutions were used and the scandium and caesium internal standards successfully corrected for the viscosity effects of the samples.

The addition of caesium as an ionization suppressant eliminated ionization interferences and the need for dilution, allowing both major and minor constituents to be measured in a single solution. Both trace and major element concentrations were able to be determined in the 2% w/v milk powder samples, with less sensitive analytical lines chosen for the elements present in the greatest concentrations, such as Ca. This wavelength selection flexibility, provided by the optical and detector design of the Vista, avoided the need to re-measure samples using the less sensitive radial optical configuration.

All measured values are in very good agreement with the certified values for the standard reference material, validating the accuracy of the method.

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The Direct Analysis of Milk Powder on the Liberty Series II ICP-OES with the Axially-Viewed Plasma

Application Note

Inductively Coupled Plasma-Optical Emission Spectrometers

Author

Andrew Ryan

Introduction

The analysis of milk is important because milk is an indicator of environmental contamination, a significant pathway for toxic metal intake by humans and a source of essential nutrients. Previously, milk liquid and milk powder analysis was carried out using flame and graphite furnace atomic absorption spectrometry and even anodic stripping voltammetry [1]. Inductively coupled plasma atomic emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) are now preferred for routine determinations because of the rapid multi-element analysis capabilities of these techniques.

Generally, milk samples are prepared by either wet digestion or dry ashing [1-5]. These are time consuming and involve procedures using potentially hazardous chemicals. Direct slurry nebulization combined with standard additions calibration has also been reported for ICP-MS with varying success [5,6]. The accuracy of standard additions is greatly influenced by calibration linearity and the presence of spectral interference. The ICP-MS technique has the advantage of sensitivity but is unable to analyzse high dissolved solid contents for long periods of time and the instrumentation is more expensive than ICP-OES.

This work describes the direct analysis of milk powder using standard quantitative calibration with aqueous standards. Viscosity effects of the milk powder solutions are corrected for using scandium (361.384 nm—ionic line) as an internal standard. Major, minor and trace elements were determined in a single analysis. Less sensitive lines are used for the determination of major elements allowing both major and minor elements to be determined from a single sample solution.

Many of the major constituents in milk powder such as Na, K and Ca are easily ionized elements (EIE) that have been reported to cause ionization interferences. Ionization interferences tend to cause a reduction in signal intensity with increasing concentration of EIE and the effect is prominent at interferent concentrations at or above 100 mg/L. The atomic lines of Na and, K, and to a lesser extent Ca (422.673 nm) and Li, exhibit signal enhancement with increasing concentrations of

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EIE. The effect can be easily minimized or eliminated on a radially-viewed ICP-OES by adjusting the viewing height. For the more sensitive axially-viewed ICP-OES, many reports of interferences due to EIE have been described [7,8]. In one report, scandium used as an internal standard was found to compensate for part of the signal depression [8]. Ionization interferences on the axially-viewed plasma have been found to be reduced by lowering the nebulizer pressure and increasing the power. Increasing the power increases the electron density in the plasma thus reducing the effect of electrons contributed by the EIE. Generally, when analyzsing samples that contain high levels of EIE, it is recommended that all standards have similar levels of EIE added (matrix matching).

An alternative is to saturate the plasma with a high concentration of another EIE such as caesium. Therefore, the effect of adding caesium as an ionization suppressant to the standards and samples was also investigated.

The accuracy and validity of the method was assessed by the use of National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 8435 Whole Milk Powder.

Experimental

Instrumental

An Agilent Liberty Series II ICP-OES with the axially-viewed plasma was used for the analysis.

The Liberty Series II ICP features a 40 MHz free running RF generator, a 0.75 m Czerny-Turner monochromator with a 1800 grooves/mm holographic grating used in up to four orders. The resolution of the optical system ranges from 0.018 nm in the 1st order to 0.006 nm in the 4th order.

The instrument was controlled with a Digital Equipment Corporation (DEC) Venturis computer with an Intel Pentium processor and Agilent's Plasma 96 software running under Microsoft's Windows 95 operating system.

The instrument operating conditions are listed in Table 1.

Table 1: Instrument Operating Conditions

Power	1.30 kW
Plasma gas flow	15.0 L/min
Auxiliary gas flow	1.5 L/min
Spray chamber type	Sturman- Masters
Torch	Standard axial torch with 2.3 mm id injector
Nebulizer	V-groove
Nebulizer pressure	240 kPa
Pump tube	Inlet - PVC grey-grey, 1.30 mm id
	Outlet - PVC, blue-blue, 1.65 mm id
Pump speedrate	15 rpm
Sample uptake rate	1.5 mL/min
Integration time	1 second for Ca, K, Mg, Na, P, S and Sr
	3 seconds for Ba, Fe, Mn, Zn and Sc
No. of replicates	3
Sample delay time	25 seconds
Fast pump	On
Stabilization time	20 seconds
Background correction	Polynomial plotted background
PMT voltage	600 V

For the determination of sulfur, an Auxiliary Gas Module-2 (AGM-2) is required. The AGM provides a nitrogen purge for the monochromator to extend the working wavelength range from 189 nm down to 175 nm.

To make sure that the milk powder sample was evenly mixed and in suspension while being aspirated, the solution was continuously stirred with a magnetic stirrer.

Standard Preparation

Aqueous standards were prepared from Custom-Grade Multi-element Solutions Var Cal 1, Var Cal 2 and Var Majors 1 (Inorganic Ventures, Inc.) and from 1000 mg/L single element standards (Spectrosol, BDH Chemicals). The standards were made up in 18 MΩ Milli-Q water with 0.5% v/v HNO_3 and 0.002% v/v Triton X100 prepared from a 1% w/v Triton X100 solution. Scandium was added to each solution as an internal standard with a final concentration of 0.5 mg/L.

The following calibration standards were prepared.

Table 2. Calibration Standards

Standard	Concentration (mg/L)	Elements
Standard 1	0.2	Ba, Mn, Zn, Sr
Standard 2	1.0	Ba, Mn, Zn, Sr, Fe
Standard 3	5.0	Mg, Fe
Standard 4	25	Mg, Na, Ca
Standard 5	100	Na, Ca, K
Standard 6	200	K
Standard 7	13.350 and 32.614	S and P, respectively
Standard 8	66.752	S
Standard 9	163.069	P

Rinse and calibration blank solutions were prepared from 18 MΩ Milli-Q water with 0.5% HNO_3 and 0.002% Triton X100.

Sample Preparation

Solutions were prepared from an instant full cream milk powder sample purchased at a local supermarket and SRM 8435 Whole Milk Powder.

Milk powder solutions containing 0.5% and 4% w/v were prepared for both samples. The sample was accurately weighed and then transferred into a volumetric flask. The flask was filled approximately 3/4 full with 18MΩ Milli-Q water and gently shaken until the milk powder was evenly mixed. Triton X100 was added to give a concentration of 0.002%. Acid was not added to the sample solutions as it causes the precipitation of protein. Scandium (0.5 mg/L) was added as an internal standard.

The scandium bulk standard was stabilized with acid and it was necessary to dilute the scandium by preparing a secondary standard before adding it to the milk powder solutions because even a small concentration of acid will cause the precipitation of protein. Adding dilute ammonia solution to the samples to adjust the pH to 7.5 [6] can overcome this problem, but it was not required in this case.

The solutions were made up to the mark and placed in an ultrasonic bath for 5 minutes. The solutions were then shaken vigorously for 1 minute.

A separate rinse containing 0.002% v/v Triton X100 and no acid was used for rinsing between samples.

For the study of the effect of the addition of an ionization suppressant, 1% w/v Cs as CsCl was added to all sample, standard and rinse solutions. Caesium was chosen as an ionization suppressant as it has a low energy of ionization, is not very sensitive by ICP-OES and, therefore, spectral interference is generally not a problem. Caesium chloride is available in a very pure form and does not build up in the torch injector tube as readily as other alkali salts.

Results and Discussion

Milk Powder Analysis

The results presented in Tables 3–6 represent the concentrations of constituent elements in the milk powder on a dry weight basis. Moisture content in the two milk powder samples was determined by accurately weighing the undried samples and then reweighing the samples after drying in an air oven for 4 hours at 85 °C. Moisture content was small and represented only 1.3% and 1.9% of the total weight for the full cream milk powder sample and NIST SRM 8435, respectively.

The mean results of the triplicate analyses for the determination of major constituent elements in milk powder without the addition of 1% w/v Cs are listed in Table 3.

The mean results of the triplicate analyses for the determination of minor and trace constituent elements in milk powder without the addition of 1% w/v Cs are listed in Table 4.

The mean results of the triplicate analyses for the determination of major constituent elements in milk powder with the addition of 1% w/v Cs are listed in Table 5.

The mean results of the triplicate analyses for the determination of minor and trace constituent elements in milk powder with the addition of 1% w/v Cs are listed in Table 6.

The effect of ionization interference from the EIE can be seen for K and Sr and to a smaller extent Ca, in Tables 3 and 4. Ionic lines were used for Ca and Sr and signal suppression for these lines was evident as a value lower than the certified value was found. With the addition of Cs to all solutions, Tables 5 and 6, a value very close to the certified value was found for both lines.

For K, without the addition of Cs, a lower than expected result was found. This is unexpected because signal enhancement is usually observed for K. An explanation for this could be that standard 5 contained reasonably high levels of other EIE and therefore the signal enhancement for K in standard 5 was higher than that for the sample. The sample was remeasured for K using separate standards for K. The measured K concentrations for the full cream milk powder sample and SRM 8435 Whole Milk Powder were 1.14 and 1.26 wt%, respectively. These results are closer to the certified value for the SRM, but are still unexpectedly lower. With the addition of Cs, the result for K was very close to the certified value.

Na was also expected to be affected by the presence of other EIE but this was not evident in this analysis. This is probably due to the presence of other EIE's in both the standards and samples.

The remaining elements did not appear to be greatly affected by the EIE because much of the signal depression was corrected by the internal standard.

For the 4% milk powder solutions without added Cs, EIE concentration was approximately 1000 mg/L and ionization interference is reportedly [8] quite significant at these levels. Despite this, results very close to the certified values were found for Ba, Fe and Mn. This suggests the internal standard not only successfully corrected for the different viscosity of the samples but also corrected for ionization interference for these elements.

Table 3. Major Elements in Milk Powder Without the Addition of Caesium

Element	Wavelength (nm)	0.5% Milk powder sample (wt%)	0.5%S.R.M. 8435 Whole milk powder (wt%)	S.R.M. 8435 Certified value (wt%)
Ca	315.887	0.849 ± 0.033	0.876 ± 0.008	0.922 ± 0.049
K	769.896	0.844 ± 0.044	0.975 ± 0.020	1.363 ± 0.047
Na	588.995	0.277 ± 0.010	0.376 ± 0.004	0.356 ± 0.040
P	213.618	0.761 ± 0.031	0.784 ± 0.019	0.780 ± 0.049
S	180.731	0.263 ± 0.019	0.268 ± 0.006	0.265 ± 0.035

Table 4. Minor and Trace Elements in Milk Powder Without the Addition of Caesium

Element	Wavelength (nm)	0.5% Milk powder sample (mg/kg)	4% Milk powder sample (mg/kg)	0.5% S.R.M. (mg/kg) 8435 Whole milk powder (mg/kg)	4% S.R.M. 8435 Whole milk powder (mg/kg)	S.R.M. 8435 Certified value (mg/kg)
Mg	285.213	754 ± 23		808 ± 22		814 ± 76
Sr	407.771	4.65 ± 0.04		3.77 ± 0.03		4.35 ± 0.50
Zn	213.856	27.5 ± 1.5		25.5 ± 0.8		28.0 ± 3.1
Ba	455.403		0.70 ± 0.03		0.57 ± 0.02	0.58 ± 0.23
Fe	259.940		2.30 ± 0.05		1.70 ± 0.03	1.8 ± 1.1
Mn	257.610		0.242 ± 0.001		0.151 ± 0.004	0.17 ± 0.05

Table 5. Major Elements in Milk Powder With the Addition of 1% (w/v) Caesium

Element	Wavelength (nm)	0.5% Milk powder sample (wt%)	0.5%S.R.M. 8435 Whole milk powder (wt%)	S.R.M. 8435 Certified value (wt%)
Ca	315.887	0.931 ± 0.019	0.899 ± 0.021	0.922 ± 0.049
K	769.896	1.304 ± 0.032	1.397 ± 0.024	1.363 ± 0.047
Na	588.995	0.298 ± 0.004	0.378 ± 0.004	0.356 ± 0.040
Na	330.237	0.280 ± 0.004	0.360 ± 0.007	0.356 ± 0.040
P	213.618	0.775 ± 0.017	0.758 ± 0.004	0.780 ± 0.049
S	180.731	0.252 ± 0.007	0.254 ± 0.006	0.265 ± 0.035

Table 6. Minor and Trace Elements in Milk Powder With the Addition of 1% (w/v) Caesium

Element	Wavelength (nm)	0.5% Milk powder sample (mg/kg)	4% Milk powder sample (mg/kg)	0.5% S.R.M. (mg/kg) 8435 Whole milk powder (mg/kg)	4% S.R.M. 8435 Whole milk powder (mg/kg)	S.R.M. 8435 Certified value (mg/kg)
Mg	285.213	761 ± 14		775 ± 13		814 ± 76
Sr	407.771	5.53 ± 0.05		4.40 ± 0.07		4.35 ± 0.50
Zn	213.856	30.0 0.7		26.3 ± 0.5		28.0 ± 3.1
Ba	455.403		0.79 ± 0.02		0.62 ± 0.01	0.58 ± 0.23
Fe	259.940		2.23 ± 0.07		1.76 ± 0.06	1.8 ± 1.1
Mn	257.610		0.269 ± 0.004		0.167 ± 0.007	0.17 ± 0.05

Long Term Stability

The long term stability was determined for the most concentrated milk powder solution to show that good stability can be obtained over a reasonable period of time for a solution containing a high content of dissolved and undissolved solids. The effect of internal standardization on long term stability was also studied.

Long term stability was evaluated by continuously aspirating 4% instant full cream milk powder solution over a period of time. A one minute rinse was performed at the maximum pump rate of 50 rpm (fast pump) between measurements.

The precision of the measurements over one and a half hours with internal standardization correction ranged from 1.2 and 2.0 %RSD.

The long term stability plots for major constituents Ca and Mg and minor constituents Ba, Mn and Sr with and without internal standardization are displayed in Figures 1 and 2, respectively.

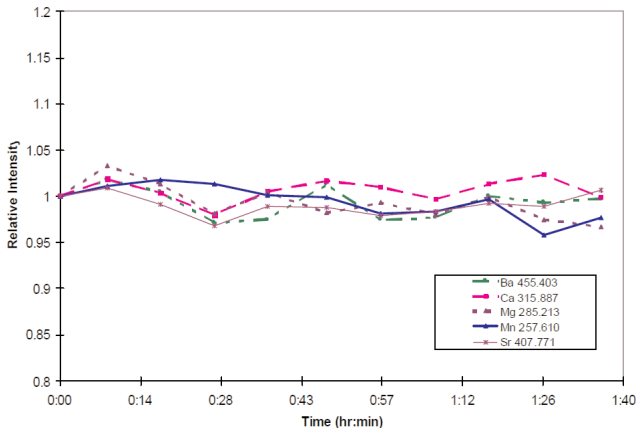

Figure 1. Signal stability over one and a half hours for a 4% full cream milk powder solution with internal standardization.

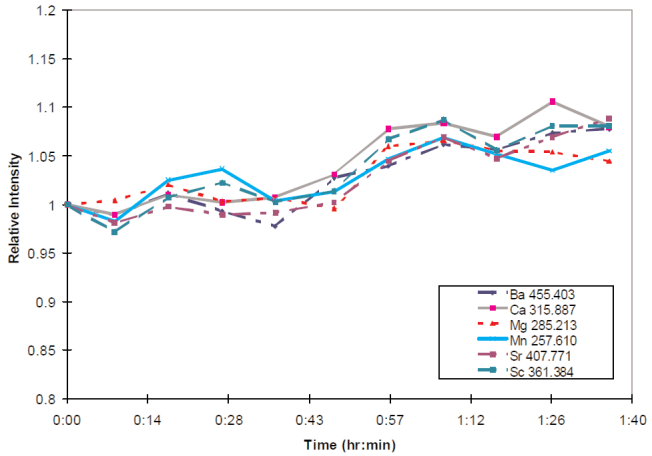

Figure 2. Signal stability over one and a half hours for a 4% full cream milk powder solution without internal standardization.

After the milk powder solution had been aspirated for over one and a half hours, the injector tube showed signs of blockage due to deposition of the milk powder. Figure 2 shows the effect of the build up of milk powder on the signal. Not only does the internal standard correct for viscosity effects and some ionization interferences, from Figure 1 it can be seen that it is also very effective in compensating for drift caused by the gradual build up of milk powder in the injector tube of the torch.

Five replicates were measured at an integration time of three seconds for each line. The precision for each measurement ranged from 0.1 to 1.6 %RSD for the major constituent elements and 0.2 to 3.0 %RSD for minor and trace constituent elements.

Sample Delay and Stabilization Times

When differing matrices are aspirated into a spray chamber, some time must be allowed so that the solution can reach the plasma and for the signal to stabilize. The time required to allow the system to stabilize when switching from aqueous solutions to the milk powder solutions was studied.

This was evaluated by firstly aspirating the rinse solution followed by a 4% full cream milk powder solution. The pump speed was set to 50 rpm (fast pump speed), which is the pump rate used during the sample delay stage, and the signal was monitored for Sc.

A plot of the signal over a short period of time is displayed in Figure 3.

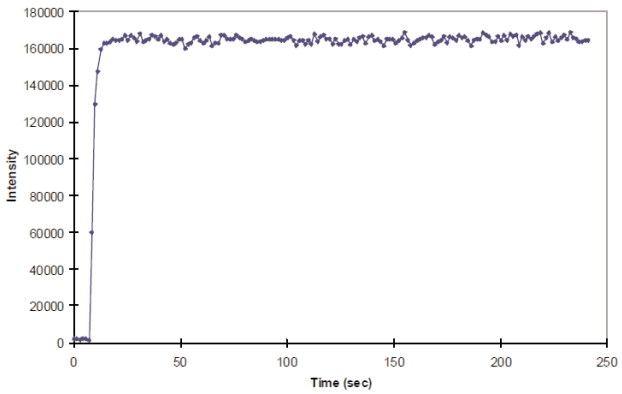

Figure 3. Stabilization profile for a 4% full cream milk powder solution.

When switching from the rinse solution to the 4% milk powder solution, the signal was found to stabilize after 20–25 seconds. Therefore, for the analysis of the milk powder solutions, a sample delay time of 25 seconds was used. The stabilization time was set to 20 seconds. The stabilization time is the time allowed for the pump to slow down from full speed to the analysis pump speed—in this case, 15 rpm.

It was observed that the presence of Triton X100 detergent, which is often used for slurry nebulization to help keep the sample in suspension, greatly reduced the stabilization time.

Summary

The concentrations of various elements of both nutritional and environmental interest in milk powder samples were determined on the Liberty Series II with the axially-viewed plasma.

Aqueous calibration solutions were used and the scandium internal standard successfully corrected for the viscosity effects of the samples, improved the long term stability of the analysis and corrected for some ionization interferences due to the high levels of EIE.

The time required for the system to stabilize after switching from aqueous solutions to the milk powder solutions was found to be very short.

The addition of caesium as an ionization suppressant eliminated ionization interferences and the need for dilution, allowing both major and minor constituents to be measured in a single solution. For the determination of trace elements such as strontium where a more concentrated milk powder solution is required, the addition of caesium becomes an important factor.

With the addition of 1% caesium, all measured values are in very good agreement with the certified values for the standard reference material, validating the accuracy of the method.

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Analysis of Shellfish Tissue for Cadmium, Mercury and Nickel

Application Note

Atomic Absorption

Author

Jonathan H. Moffett

Introduction

Huge amounts of toxic effluents are being dumped either directly or indirectly into the world's oceans. The ocean's organisms concentrate these toxic chemicals with unfortunate effects on humans. Since the tragedy at Minamata Bay, Japan, in the 1950s, the levels of toxic metals in seafood have been carefully monitored [1]. This is especially important around coastal industrial and mining areas as well as river mouths.

Shellfish such as oysters and mussels are bottom-dwelling non-mobile filter feeders. These factors mean that shellfish are very good environmental indicators and can locate sources of pollution.

The screening of fish must be rapid and accurate. A method for digesting fish for mercury determinations by cold vapor has been described [1]. As mercury is very volatile, the digestion method should be applicable to other toxic heavy metals.

A freeze dried sample of shellfish tissue was supplied and a method was required for digestion and analysis.

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Experimental

Reagents

All reagents were AR grade. Water was deionized distilled (DD) water (18 M-ohm grade).

Standards

Standards were diluted from 1000 mg/L commercial atomic absorption solutions in deionized distilled (DD) water with dilute nitric acid as stabilizer. Working concentrations used are shown in Table 1.

Table 1. Working Concentration of Standards

Cd	5.00 µg/L in 0.08 M HNO_3
Ni	50.0 µg/L in 0.08 M HNO_3
Hg	0.50 µg/L in 0.3 M HNO_3/0.04% (m/v) $K_2Cr_2O_7$

Modifier

Ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) was used as a chemical modifier for cadmium [2]. $NH_4H_2PO_4$ (1 g) was dissolved in DD water (100 mL). The solution was found to have an appreciable cadmium signal. Trace metals were removed by using an Agilent Bond Elut SCX (strong cation exchange) column (kit B). Nitric acid (30 mL of 2 M) was drawn through the column using a water vacuum pump. The column was then washed using DD water (30 mL). The column was not allowed to dry out. The modifier solution was allowed to elute through under gravity with the first 2 mL being discarded. The eluant was caught directly in a washed sample vial and placed in the modifier position of the PSD-96 carousel. The overlayed cadmium signals before and after elution can be seen in Figure 1, in which the eluted signal is at baseline level. This method of purification has not yet been fully optimized.

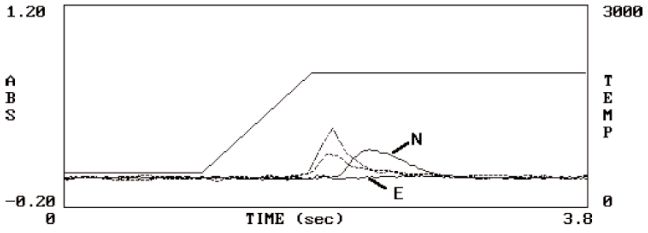

Figure 1. Noneluted (N) and eluted (E) modifier atomic (—) and background (- -) signals.

Reference Sample

A National Institute of Science and Technology (NIST) standard reference material, bovine liver (1577), was used as a reference sample.

Sample Preparation

Two digestion procedures were considered. The first digestion method was shown to be suitable for mercury in fish tissue [1]. In this method a sample (1g) was digested in concentrated nitric acid (10 mL). This method was eventually used for this study.

The second method was used to determine As, Sb and Se in various environmental matrices [3]. This method is a modified version of the first method by including hydrogen peroxide.

NIST 1577

Concentrated nitric acid (10 mL) was added to a known mass of liver (1 g) in a test tube [1]. After heating for three hours at 125 °C, the warm yellow solution was quantitatively transferred to a 50-mL volumetric flask. When made up to the mark with DD water, fat solidified as a precipitate. This did not appear to affect results.

Shellfish Sample

One fish sample was cut into halves using stainless steel scissors. Each half was digested separately. The digestion quantities above were scaled down by a factor of 10 because each half weighed about

0.1 g. Heating times were not changed. The solutions were transferred to 5-mL volumetric flasks. These were subsequently diluted as needed.

All masses and final volumes of the samples are shown in Table 2.

Table 2. Mass and Final Volume of Digested Samples

		Final Volume (mL)	
Sample	**Mass (g)**	**(Cd, Ni)**	**(Hg)**
NIST 1577	1.0026	50	
Shellfish 1	0.1373	100	125
Shellfish 2	0.1026	100	125

Instrumentation

For the determination of nickel and cadmium, an Agilent SpectrAA-300GZ atomic absorption spectrometer with Zeeman background correction, and fitted with a PSD-96 sampler, was used. Atomization for cadmium was from a pyrolytic forked platform. Atomization for nickel was from the wall of a pyrolytic coated partition tube. Argon was the inert gas.

For the determination of mercury, an Agilent SpectrAA-20ABQ atomic absorption spectrometer fitted with a VGA-76 vapor generation accessory and an MCA-90 mercury concentration accessory were used [4].

Lamps were standard Agilent hollow cathode lamps for each of the elements.

Recovery studies for nickel and cadmium were performed using Quality Control Protocol (QCP V2.0). Signal graphics were captured using Signal Graphics Library (SGL V1.01).

Instrument parameters are given in Tables 3a, 3b and 3c.

Results and Discussion

Cadmium

The aqueous calibration graph for cadmium is shown in Figure 2.

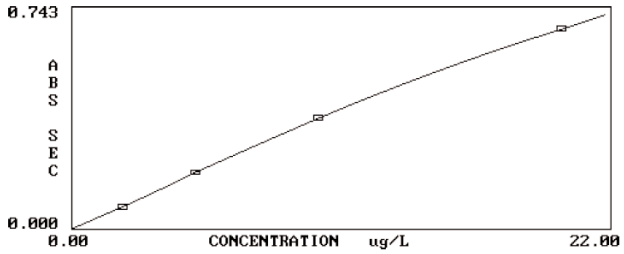

Figure 2. Cadmium calibration graph.

Replicate atomization signal peaks of NIST 1577, shellfish 1 and shellfish 2 for cadmium are shown in Figures 3, 4 and 5 respectively. The peak shapes all compare well. They are well-formed and the peak background signal is well within the capability of the instrument's corrector.

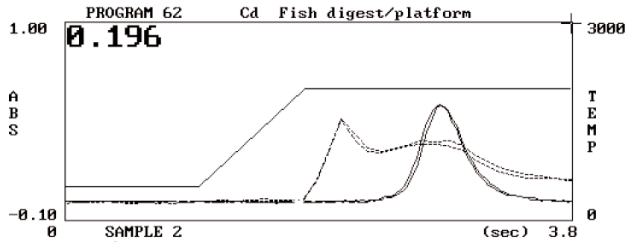

Figure 3. Cadmium signal - NIST 1577.

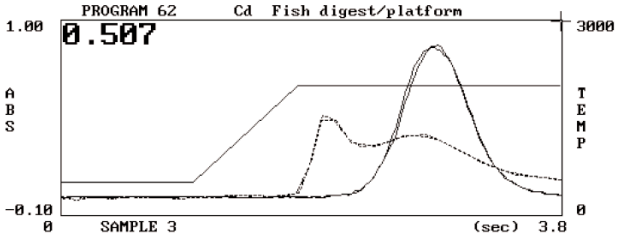

Figure 4. Cadmium signal - shellfish 1.

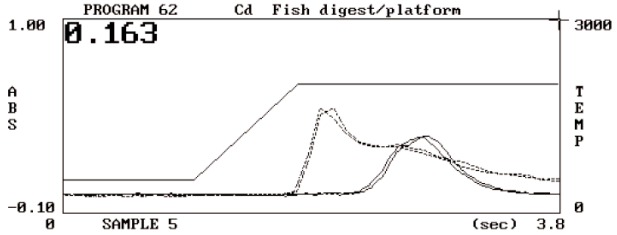

Figure 5. Cadmium signal - shellfish 2.

Table 3a. Instrument Parameters for the Determination of Cadmium

Program 62: Cd fish digest/platform

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Peak area
Lamp position	3
Lamp current (mA)	4
Slit width (nm)	0.5
Slit height	Normal
Wavelength (nm)	228.8
Sample introduction	Sampler automixing
Time constant	0.05
Measurement time (s)	1.0
Replicates	2
Background correction	On
Maximum absorbance	0.70

Furnace parameters

Step no.	Temperature (C)	Time (s)	Gas flow (L/min)	Gas type	Read command
1	350	20.0	3.0	Normal	No
2	500	15.0	3.0	Normal	No
3	500	10.0	3.0	Normal	No
4	500	1.0	3.0	Normal	No
5	2000	0.8	0.0	Normal	Yes
6	2000	2.0	0.0	Normal	Yes
7	2500	2.0	3.0	Normal	No

Sampler parameters

Volumes (µL)

	Solution	Blank	Modifier
Blank	–	20	5
Standard 1	2	18	5
Standard 2	5	15	5
Standard 3	10	10	5
Standard 4	20	5	
Sample 5	15	5	

		Recalibration rate	0		
		Reslope rate	0		
Multiple inject	No	Hot inject	Yes	Pre inject	No
		Temperature	150		
		Inject rate	5		

Table 3b. Instrument Parameters for the Determination of Nickel

Program 66: Ni fish digest/wall

Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Peak area
Lamp position	2
Lamp current (mA)	6
Slit width (nm)	0.2
Slit height	Normal
Wavelength (nm)	232.0
Sample introduction	Sampler automixing
Time constant	0.05
Measurement time (s)	1.0
Replicates	2
Background correction	On
Maximum absorbance	1.00

Furnace parameters

Step no.	Temperature (C)	Time (s)	Gas flow (L/min)	Gas type	Read command
1	150	20.0	3.0	Normal	No
2	700	15.0	3.0	Normal	No
3	700	10.0	3.0	Normal	No
4	700	1.0	3.0	Normal	No
5	2400	0.9	0.0	Normal	Yes
6	2400	2.0	0.0	Normal	Yes
7	2500	2.0	3.0	Normal	No

Sampler parameters

Volumes (µL)

	Solution	Blank	Modifier
Blank	–	20	
Standard 1	2	18	
Standard 2	5	15	
Standard 3	10	10	
Standard 4	20		
Sample	10	10	

		Recalibration rate	0		
		Reslope rate	0		
Multiple inject	No	Hot inject	Yes	Pre inject	No
		Temperature	120		
		Inject rate	5		

Table 3c. Instrument Parameters for the Determination of Mercury

Program 5: Hg	
Instrument mode	Absorbance
Calibration mode	Concentration
Measurement mode	Peak height
Lamp position	3
Lamp current (mA)	4
Slit width (nm)	0.5
Wavelength (nm)	253.7
Sample introduction	Manual
Delay time	0
Time constant	0.05
Measurement time (s)	40.0
Replicates	1
Background correction	Off
Air flow	0.00
MCA-90 parameters	
Drain (s)	20
Collect (s)	180
Heat (s)	20

The result of the NIST 1577 analysis for cadmium tends to support the validity of the digestion and calibration procedures. Table 4a shows the result is very close to the certified value.

The wide difference between shellfish 1 and shellfish 2 for cadmium cannot be explained from this study alone. The discrepancy is caused either by contamination or by inhomogeneous distribution of cadmium in the original sample, with contamination being the most likely cause.

Nickel

The aqueous calibration graph for nickel is shown in Figure 6.

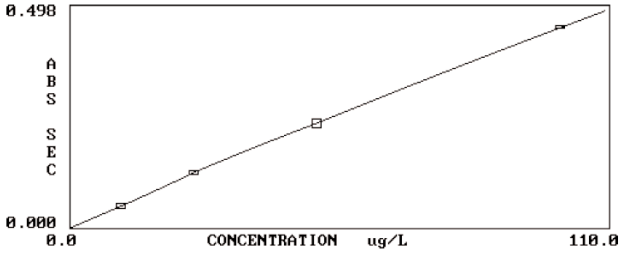

Figure 6. Nickel calibration graph.

Replicate atomization signal peaks of each of the samples for nickel are shown in Figures 7, 8 and 9. As for cadmium, peaks are well-formed, while background is negligible for nickel. NIST 1577 sample has a larger background peak because it was diluted less than the shellfish samples.

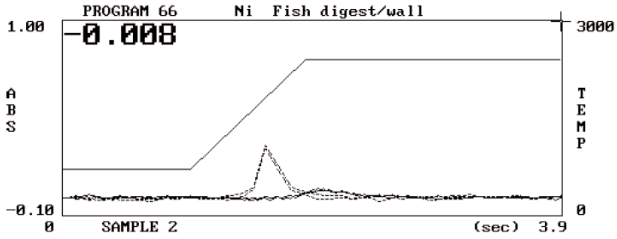

Figure 7. Nickel signal - NIST 1577.

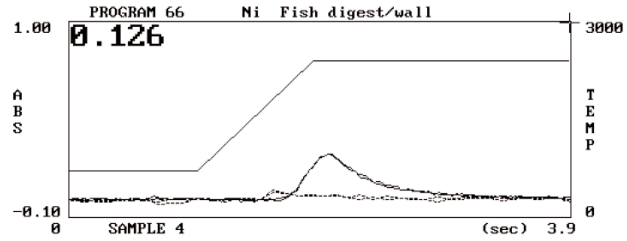

Figure 8. Nickel signal - shellfish 1.

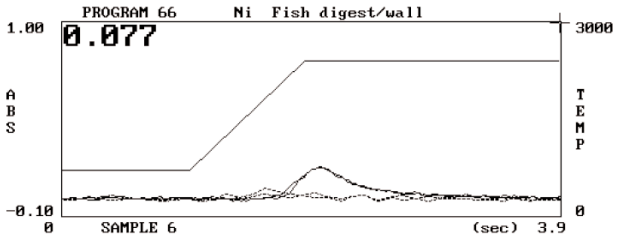

Figure 9. Nickel signal - shellfish 2.

The close result between shellfish 1 and shellfish 2 for nickel indicates homogeneous distribution in the original sample. NIST 1577 does not have a certified level of nickel.

Recovery Studies

QCP software allows a QC spike recovery by spiking the final solution with standard solution during injection into the furnace. The recovery (%R) is calculated as below:

$$\%R = (SSR-SR)/SA \times 100$$

where

SSR = Spiked sample result

SR = Sample result

SA = Spike added

If recovery is between 85% to 115% the aqueous calibration graph is normally considered to be a close match for the sample solution. A recovery of less than 40% usually indicates either a severe matrix effect or else measurement close to or greater than the maximum absorbance of the element using a Zeeman effect instrument. The recovery results for the samples are given in Table 4a, 4b and 4c.

Table 4a. Summary of Analytical Results for Cadmium in Shellfish

Sample	Soln conc (µg/L)	Conc found (µg/L)	Certif value (µg/L)	RSD (%)	recov (%)
NIST 1577	5.17	0.26	0.27 ± 0.04	0.4	94.5
NIST 1577	5.16	0.26		1.5	86.2
shellfish 1	14.08*	10.25*		0.3	28.3
shellfish 1	13.85*	10.09*		1.2	35.8
shellfish 2	4.34	4.23		3.3	85.5
shellfish 2	4.32	4.21		1.8	83.9

* is a software label to indicate the measured absorbance exceeded the maximum recommended Zeeman absorbance.

Table 4b. Summary of Analytical Results for Nickel in Shellfish

Sample	Soln conc (µg/L)	Conc found (µg/L)	Certif value (µg/L)	RSD (%)	recov (%)
NIST 1577	0.5	0.0	not certified	99.9	83.9
NIST 1577	-1.7	-0.1		47.2	102.5
shellfish 1	25.9	18.9		0.3	99.6
shellfish 1	25.2	18.4		1.0	95.1
shellfish 2	15.8	15.4		2.3	91.2
shellfish 2	15.8	15.4		6.7	79.3

Table 4c. Summary of Analytical Results for Mercury in Shellfish

Sample	Soln conc (µg/L)	Conc found (µg/L)	Certified value (µg/L)
NIST 1577	0.332	0.0166	0.016 ± 0.002
shellfish 1	0.344	0.313	
shellfish 2	0.372	0.453	

The recoveries for cadmium are good except for both aliquots of shellfish 1. The instrument software has flagged the results in Table 4a with an asterisk. This indicates that the maximum absorbance (0.7) for cadmium has been exceeded. The unexpectedly high result for shellfish 1 strongly suggested contamination of this sample and further studies (including dilution or reduced sample volumes) were not performed.

The results for shellfish 2 are below the maximum absorbance limit and the recoveries are very good.

The recoveries are also good for nickel except for one of shellfish 2 results. However, a portion of the same solution which immediately preceded this one had an excellent recovery.

A complete recovery study should also include a matrix spike which is the addition of analyte to a sample before digestion. The limited amount of sample available did not permit such study.

Mercury

The low levels of mercury in solution required the use of the MCA-90 [4,5]. This measurement consumes about 20 mg of sample. The amount of sample did not allow spike recovery studies. The close result of NIST 1577 with the certified value and the similar results for shellfish 1 and 2 suggests that the measured results are accurate.

Conclusion

The close agreement of the NIST 1577 results with the certified results indicates that the digestion procedure used was entirely satisfactory for the volatile elements mercury and cadmium. It seems reasonable that the same would be true for the shellfish. The final volumes in Table 2 should be adjusted to measure the expected levels in individual samples. They were suitable for the levels of the metals in NIST 1577 and shellfish 2.

The generally good recovery study results for the shellfish indicate the results are accurate. Certainly the calibration procedure is valid. Confirming the accuracy of any sample analysis requires a considerable amount of work. Procedures should include: long-term trend studies, inter-laboratory studies and using (if available) a certified reference fish sample.

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Using Lead Isotope Ratios to Distinguish between Samples of the Traditional Chinese Medicine Dan-shen

Geological

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Abstract

Quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) was used to determine lead (Pb) isotope ratios in Dan-shen, a type of herb used in Traditional Chinese Medicines (TCM), and in water and soil samples all taken from the same geographical location. The precision obtained for the $^{208}Pb/^{206}Pb$ ratio, the $^{207}Pb/^{206}Pb$ ratio and the $^{204}Pb/^{206}Pb$ ratio values was considerably lower than 0.5% demonstrating the applicability of the technique for Pb isotope ratio studies. The results show that it is possible to distinguish Dan-shen samples originating from different geographical areas using Pb isotope ratio measurements. As the medicinal effectiveness of a TCM is highly dependant on the source of origin of its herb components, it is useful to have a reliable, routine means of "fingerprinting" the components grown in different habitats.

Introduction

Most Traditional Chinese Medicines (TCMs) are a mixture of several different herbs that undergo special treatment to make them useful. The amounts of the effective components in the plants are influenced by the soil, water, weather conditions etc in the area where they are grown. Experienced practitioners of traditional Chinese healing know that the quality of herb medicines is strongly related to their source of origin. It is therefore important to know the specific location of a TCM herb component so ensure its effectiveness.

Many techniques have been applied to characterize various herb medicines and to correlate them with their place of origin. High Performance Liquid Chromatography, Mass Spectrometry, Nuclear Magnetic Resonance, Infrared Spectroscopy and X-ray Fluorescence have all been used to produce spectra that can be used to "fingerprint" the TCM habitat.

Lead isotope ratio measurements provide analytical information relating to the source of lead contamination in naturally occurring samples. Concentration measurements cannot provide this information. Studies of the isotopic composition of lead are therefore commonly used in environmental science as well as geological and anthropological studies. Among all the naturally occurring lead isotopes, only ^{204}Pb is non-radiogenic, whereas, ^{206}Pb, ^{207}Pb and ^{208}Pb are the daughter products from the radioactive decay of ^{238}U and ^{235}U and ^{232}Th respectively. As a consequence, small Pb isotope abundance variations occur in nature and the isotopic composition of lead in the environment is dependent on local ore deposits. If lead is present in the soil, a plant will take up small amounts and subsequent isotope ratio studies might provide a unique

means of differentiating between different plant sources of origin. Of course, local lead levels may become mixed with external sources of contamination (e.g. automobile exhaust) that vary with time depending on the anthropogenic activity. These mixing processes can be quantified as long as each lead source shows a different lead isotope abundance. The work described in this application note was undertaken to investigate if lead isotope ratios can be used to "fingerprint" the Dan-shen herb grown in different habitats. While isotope ratio studies have traditionally been undertaken with a multicollector mass spectrometer such as Thermal Ionization Mass Spectrometry (TIMS), the technique is relatively slow and the instrumentation is expensive. These experiments were carried using quadrupole Inductively Coupled Plasma Mass Spectrometry (ICP-MS) that provides a fast, convenient and precise method to determine isotope ratios.

The Agilent 7500 Series ICP-MS is an excellent tool for routine isotope ratio measurements. It is easy to operate with its fully automated optimization system (Auto-tune function) and its user-friendly software. Because of these advantages, Agilent ICP-MS systems have been widely used for isotope ratio studies for environmental monitoring applications and nuclear research projects, e.g., U isotope ratios in human urine [2], in the cooling water of nuclear plants etc., the certified values of some certified reference materials such as rock samples [3], biological samples [3,4] by isotope dilution. In Encinar et al.'s work[4,5], an older model Agilent ICP-MS (HP-4500) was used, and the performance was as good as the more expensive multi-collector ICP-MS and double focusing sector field ICP-MS especially for long term stability and isotope dilution applications.

Experimental

Instrumentation

An Agilent 7500i ICP-MS, (Agilent Technologies, Palo Alto, CA, USA), fitted with a PFA micro flow nebulizer (100 uL/min) in self-aspiration mode, a quartz spray chamber and a one-piece quartz torch was used throughout the study.

A MK-II microwave sample digestion system (Shang Hai Xin Ke Factory, China) was used for sample digestion.

Reagents

The following reagents were used during the course of the study:

- SRM981 (National Institute of Standards & Technology, USA) - for mass bias correction.
- HBr, HCl, HF, and HNO_3, (GR grade, after sub-boiling purification) - for sample digestion and dilution.
- Anion exchange resin (DOWEX 1×8, 200-400 mesh, USA), - for separating Pb from the matrix in the samples.
- Ultra Pure Water, supplied by Milli-Q water system (18.2 MOhms)

Sample preparation

Dry samples and filtered water samples were digested using a HNO_3/HF acid mixture in a microwave oven then heated to dryness in a fume cupboard to remove the HF. 1.0 mL HBr (0.5N) was used to redissolve the residue. Samples were then passed through a DOWEX 1×8 column to remove the matrix. The Pb containing eluant was dried and diluted with 3% HNO_3. In order to avoid memory effects and to keep the ICP-MS detector working in pulse counting mode only, the concentrations were controlled between 1 and 80 ug/L.

Optimization of the ICP-MS operation parameters
Since the sample volumes collected after the column pretreatment step were limited, most of them were less than 1 mL, the Agilent PFA micro flow nebulizer (100 uL type) was selected for sample introduction. Self-aspiration mode was used and the carrier gas flow rate was controlled to maintain the sample introduction flow rate at about 50 uL/min. The 1 mL sample was sufficient for 2 or 3 individual measurements so that repeat analyses could be undertaken in case of error during the determination.

Instrument sensitivity and the other parameters, such as oxide level and doubly charged ions, were optimized automatically using the AutoTune feature of ChemStation on a 10 ppb tuning solution containing Li, Y, Ce, Tl. The carrier gas was then set to 0.4 L/min to obtain a sample uptake rate of about 50 uL/min. Finally, the make-up gas was adjusted to produce the best sensitivities and lowest interferences.

Instrument operating parameters are given in Table 1.

Table 1. ICP-MS operating conditions

Plasma Conditions:

RF power:	1250 W
RF Matching:	1.7V
Sample Depth:	7 mm
Torch-H:	0 mm
Torch-V:	0.3 mm
Carrier Gas:	0.4 L/min
Make-up Gas:	0.9 mL/min
Peripump1:	0.1 rps
Spray Chamber Temperature:	2.0 degC

Data Acquisition Parameters:

"Isotope Ratio Analysis" mode, 1000 scan/sec, minimum dwell time 100 us. The integration times were 20sec for ^{204}Pb, 10sec for ^{206}Pb and ^{207}Pb, 5 sec for ^{208}Pb. Each data point is the average of 3 repetitions.

Results

Before beginning the analysis, the instrument was allowed 30 minutes following plasma ignition to reach thermal stability. A 20ppb solution of NIST SRM 981 isotope ratio standard was measured after every 5 unknowns. This data provided the 7500i short-term and long-term stability information and provided a means of making the small mass bias corrections required. The instrument demonstrated excellent precision. See Tables 2 and 3. Note that the instrument consistently delivered %RSD's at less than 0.1% over the short and long term.

Table 2: Short-term Stability of Pb Isotope Ratio Determinations of 20 ppb SRM981 using the Agilent 7500i ICP-MS (20min, 5 measurements)

File Name	PB-206A.D	PB-206B.D	PB-206C.D	PB-206D.D	PB-206E.D	RSD(%)
Acq Date	Jun 9 2001	Jun 9 2001	Jun 9 2001	Jun 9 2001	Jun 9 2001	
Acq Time	3:45 PM	3:50 PM	3:55 PM	4:00 PM	4:06 PM	
204/Total	0.01427	0.01426	0.01426	0.01426	0.01425	0.05
206/Total	0.2413	0.2414	0.2414	0.2415	0.2416	0.05
207/Total	0.2211	0.2208	0.2209	0.2209	0.2209	0.05
208/Total	0.5233	0.5235	0.5235	0.5233	0.5232	0.03
204/206	0.05913	0.05904	0.05907	0.05906	0.05897	0.10
207/206	0.9163	0.9146	0.9149	0.9146	0.9144	0.08
208/206	2.169	2.168	2.169	2.167	2.165	0.08

Table 3. Long-term Stability (10 hours) of Pb Isotope Ratio Determinations of 20 ppb SRM981 using the Agilent 7500i ICP-MS

File Name	Acq Date	Acq Time	206/Total	207/Total	208/Total	207/206	208/206
PB20-A.D	Jun 9 2001	6:14 AM	0.2411	0.2209	0.5235	0.9162	2.171
PB20-B.D	Jun 9 2001	6:26 AM	0.2412	0.2209	0.5236	0.9159	2.171
PB20-C.D	Jun 9 2001	6:33 AM	0.2412	0.2209	0.5235	0.9158	2.170
PB20-D.D	Jun 9 2001	6:40 AM	0.2411	0.2210	0.5236	0.9167	2.172
PB20_1A.D	Jun 9 2001	7:59 AM	0.2411	0.2211	0.5236	0.9172	2.172
Pb-202A.D	Jun 9 2001	9:12 AM	0.2410	0.2211	0.5236	0.9173	2.172
PB_202B.D	Jun 9 2001	9:18 AM	0.2414	0.2210	0.5234	0.9153	2.168
PB-203A.D	Jun 9 2001	11:17 AM	0.2413	0.2211	0.5233	0.9161	2.168
PB-204A.D	Jun 9 2001	12:52 PM	0.2412	0.2212	0.5233	0.9168	2.169
PB-204B.D	Jun 9 2001	12:59 PM	0.2410	0.2214	0.5234	0.9185	2.172
PB_205A.D	Jun 9 2001	2:27 PM	0.2412	0.2210	0.5236	0.9163	2.171
PB_205B.D	Jun 9 2001	2:32 PM	0.2415	0.2210	0.5232	0.9153	2.167
RSD(%)			**0.06**	**0.07**	**0.03**	**0.10**	**0.08**

The excellent results from the NIST quality assurance sample suggest that the data from the unknown samples is also very reliable and can be reported with a high degree of confidence. In this study, three types of samples were measured:

- Four samples of surface water taken from different areas in Zhong-Jiang, Si-Chuan Province
- Soil samples came from Er-Mei Mountain (2), Zhong-Jiang, Si-Chuan Province (5), Jiang-Xi Province (1)
- Dan Shen plant samples were from Zhong-Jiang, Si-Chuan Province (3), Er-Mei Mountain (2), Bei-Jing (1), Jiang-Xi Province (1), Tai Mountain, Shan-Dong Province (1), Shang-Luo, Shan-Xi Province (1), Xin-Jiang Province (1), He-Nan Province (1)

All the samples were taken from different areas even within the same province. The measured results and the standard deviations are listed in Table 4 a-c.

Table 4a: Results of Pb Isotope Ratio Measurements of Dan-shen Plant Samples

Sample Name	$^{207}Pb/^{206}Pb$ ±sd(n=3)	$^{208}Pb/^{206}Pb$ ±sd(n=3)
Jiang-Xi Province	0.8485 ± 0.0005	2.079 ± 0.002
Shan-Luo, Shan-Xi Province	0.8502 ± 0.0014	2.100 ± 0.002
Bei-Jing	0.8674 ± 0.0019	2.159 ± 0.003
Shan Dong Province	0.8570 ± 0.0018	2.107± 0.002
Xin-Jiang Province	0.8495 ± 0.0007	2.095 ± 0.001
He-Nan Province	0.8576 ± 0.0007	2.115 ± 0.002
ErMei Mountain, D1	0.8467 ± 0.0003	2.093 ± 0.001
ErMei Mountain, D2	0.8469 ± 0.0004	2.095 ± 0.004
Zhong-Jiang, D1	0.8529 ± 0.0022	2.105 ± 0.004
Zhong-Jiang, D2	0.8524 ± 0.0012	2.105 ± 0.002
Zhong-Jiang, D3	0.8537± 0.0003	2.103 ± 0.002

Table 4b: Results of Pb Isotope Ratio Measurements of Soil Samples

Sample Name	$^{207}Pb/^{206}Pb$ ±sd(n=3)	$^{208}Pb/^{206}Pb$ ±sd(n=3)
Jiang-Xi Province	0.846 3± 0.0017	2.090 ± 0.001
ErMei Mountain, T1	0.8520 ± 0.0016	2.101 ± 0.004
ErMei Mountain, T2	0.8523 ± 0.0007	2.103 ± 0.001
Zhong-Jiang, T1	0.8395 ± 0.0007	2.084 ± 0.001
Zhong-Jiang, T2	0.8417 ± 0.0004	2.089 ± 0.001
Zhong-Jiang, T3	0.8411 ± 0.0015	2.084 ± 0.002
Zhong-Jiang, T4	0.8400 ± 0.0007	2.090 ± 0.001
Zhong-Jiang, T5	0.8391 ± 0.0014	2.083 ± 0.002

Table 4c: Results of Pb Isotope Ratio Measurements of Water Samples

Sample Name	$^{207}Pb/^{206}Pb$ ±sd(n=3)	$^{208}Pb/^{206}Pb$ ±sd(n=3)
Zhong-Jiang, w1	0.8702 ± 0.0015	2.140 ± 0.003
Zhong-Jiang, w2	0.8735 ± 0.0005	2.137 ± 0.002
Zhong-Jiang, w3	0.8675 ± 0.0002	2.136 ± 0.002
Zhong-Jiang, w4	0.8693 ± 0.0014	2.138 ± 0.003

Discussion

Since ^{206}Pb, ^{207}Pb and ^{208}Pb are the daughter products from the radioactive decay of ^{238}U and ^{235}U and ^{232}Th respectively, the Pb isotope abundance varies in nature. In most of the samples, the $^{208}Pb/^{206}Pb$ ratio values are between 1.95 and 2.15, the $^{207}Pb/^{206}Pb$ ratio values are between 0.78 and 0.86 and the $^{204}Pb/^{206}Pb$ ratio values are between 0.05 and 0.06[1]. In order to distinguish between the different types of samples by their lead isotope ratio, the measurement precision is extremely important, both over the short and long term. The RSD values should be at most 0.5% in order to make a clear differentiation between the samples. As indicated in

Table 4a-c, the SD values for the determinations are considerably better (lower) than 0.5%, so it is possible to use lead isotope ratio measurements to distinguish between the different samples.

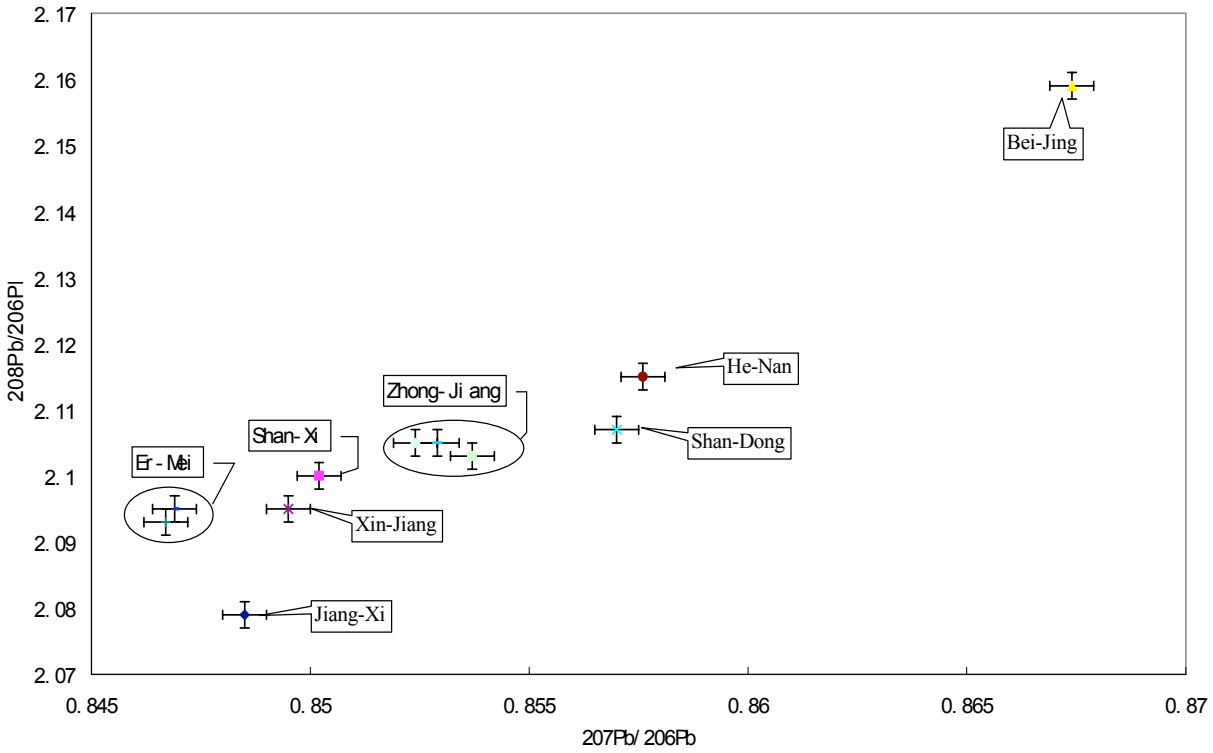

Figure 1: Pb Isotope Ratio Distribution of the Herb Medicine, Dan-Shen, from Different Sources

As a means of looking for variations/similarities in the measurements, the $^{208}Pb/^{206}Pb$ ratio values were plotted on the y-axis vs the $^{207}Pb/^{206}Pb$ ratio values on the x-axis. Figure 1 illustrates the data from the analyses of the Dan-shen plant and shows very interesting results. Obviously, the Pb isotope ratio values show their own special pattern for the Dan-shen samples from different sources. For example, Dan-shen samples grown in the Zhong-Jiang area have similar Pb isotope ratio values, although the samples were taken from different sampling areas. When compared to the Pb isotope values of the Dan-shen samples from the Er-Mei Mountain region (the two samples also have similar Pb isotope ratio values), the difference is distinct. In conclusion, it is possible to distinguish the Dan-shen samples from different sources.

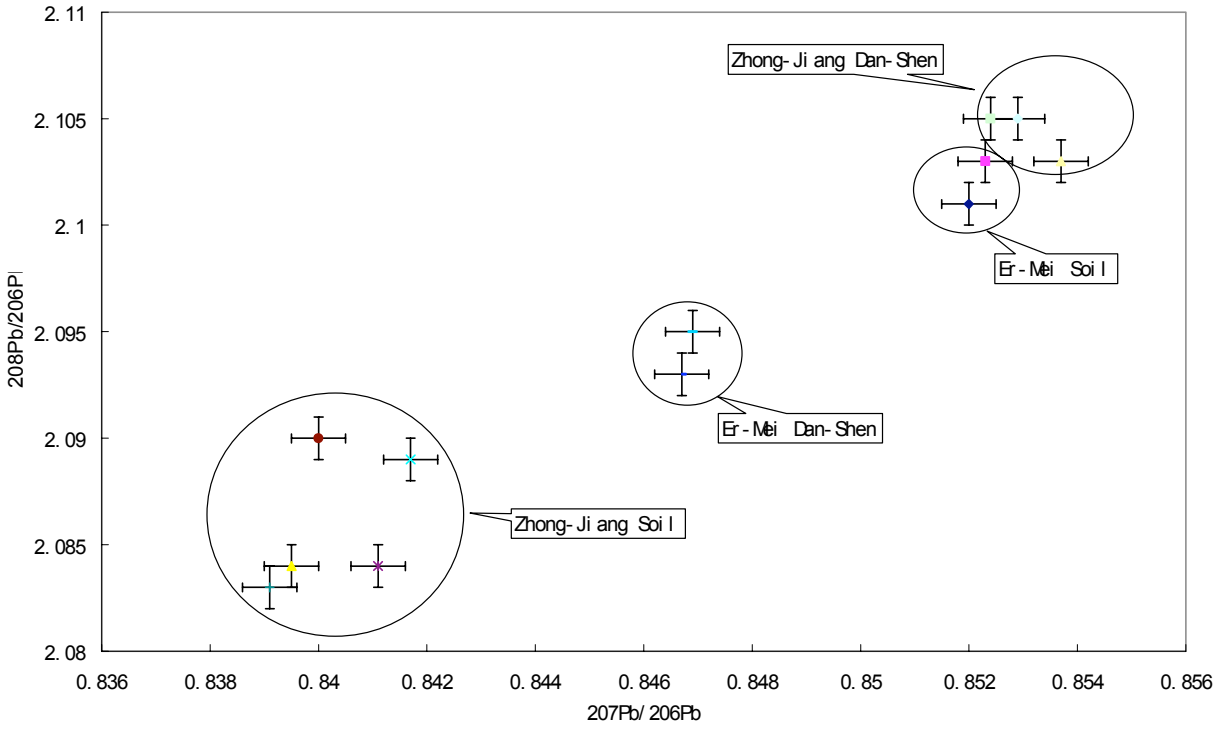

Figure 2: Pb Isotope Ratio Distribution of Soil and Dan-Shen Samples taken from Different Sources

When the soil samples from different sources are considered, it is found that the isotope values of the soil samples from different sources also have their own special pattern, as shown in Figure2. But, interestingly, the isotope values of the soils are different to the Dan-shen samples from the same place. This suggests that soil isn't the only source of lead within the plant.

When the water samples are considered, it is obvious that the Pb isotope ratio values are a combination of those found in the soil and in the water, as shown in Figure 3.

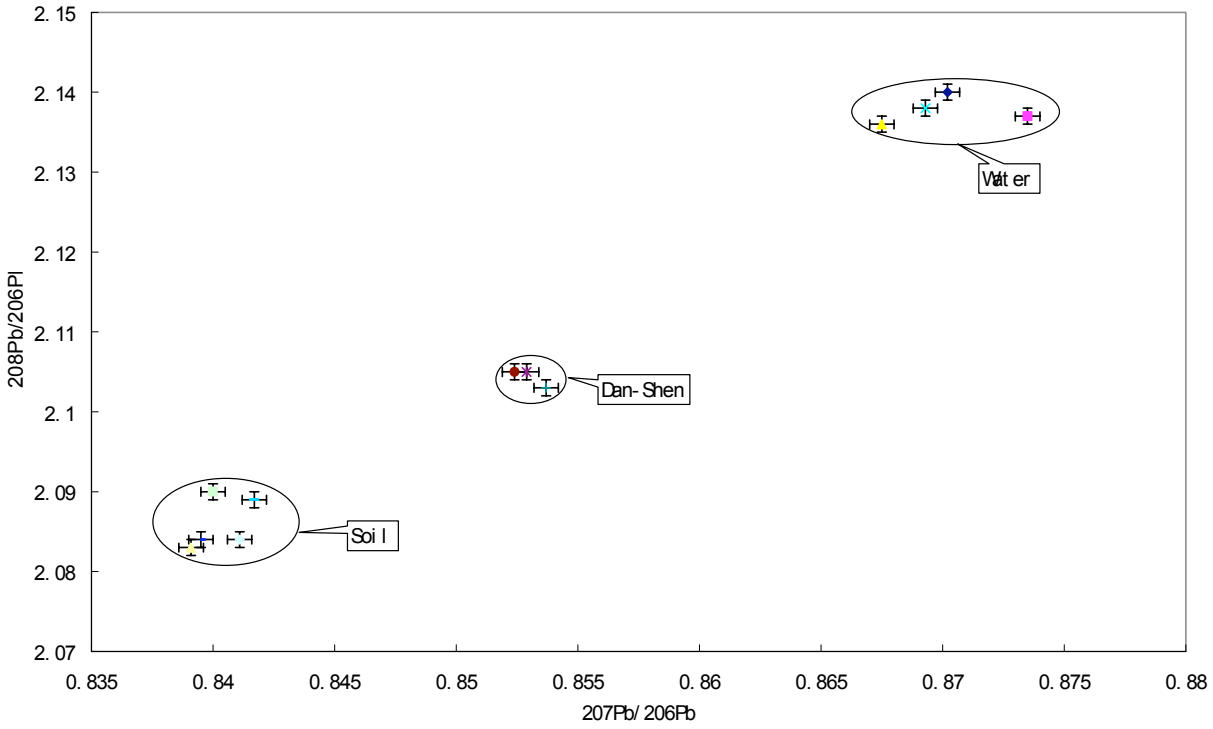

Figure 3: Pb Isotope Ratio Distribution of the Soil, Dan-Shen and Water Samples Taken from similar Sampling Area (Zhong-Jiang, Si-Chuan Province)

Conclusions

The Agilent PFA micro flow nebulizer is a good choice for isotope ratio analysis since it has very good nebulization efficiency and stability, especially when the sample amount is small. Self-aspiration mode avoids small amounts of pulsation from the peristaltic pump that could affect the precision of the isotope ratio analysis.

As this data suggests, the Agilent 7500 Series ICP-MS is well suited for routine isotope ratio analysis. Further improvements in precision may be obtained by modifying the method used in this study slightly. For instance, Tl may be added to the samples as an internal standard for lead isotope ratio analysis[5]. In addition, optimization of the dead-time correction, the stand-by mass selection, may also improve the performance of Agilent 7500 ICP-MS so that the theoretical minimum %RSD of 0.03 can be obtained. Future work at Xiamen University will look in to these potential improvements.

This preliminary research indicates that it is possible to distinguish Dan-shen samples from different areas using Pb isotope ratio measurements. Continuing these studies by running more types of samples will allow a large database to be set-up, and a chemometric model to be built to provide a convenient way to distinguish herb medicines from different sources.

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Fast and Accurate Determination of Arsenobetaine (AsB) in Fish Tissues Using HPLC-ICP-MS

Application

Foods

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Abstract

A high performance liquid chromatography-inductively coupled plasma mass spectrometry method was developed for the fast and accurate analysis of arsenobetaine in fish samples extracted by accelerated solvent extraction. The combined extraction and analysis approach was validated using certified reference materials for arsenobetaine in fish and during a European intercomparison exercise with a blind sample. Up to six species of arsenic can be separated and quantified in the extracts within a 10-minisocratic elution. The method was optimized so as to minimize time-consuming sample preparation steps and to allow for automated extraction and analysis of large sample batches. A comparison of standard addition and external calibration showed no significant difference in the results obtained, which indicates that the liquid chromatography-inductively coupled plasma mass spectrometry method is not influenced by severe matrix effects. The extraction procedure could process up to 24 samples in an automated manner while the robustness of the developed high performance liquid chromatography-inductively coupled plasma mass spectrometry approach is highlighted by the capability to run more than 50 injections per sequence which equates to a total run-time of more than 12 hours. The method can therefore be used to rapidly and accurately assess the proportion of nontoxic arsenobetaine in fish samples with high total arsenic content during toxicological screening studies.

Introduction

The element Arsenic (As) has long been thought of as poisonous and highly toxic. However, it has since been shown that the toxicity of As is largely dependent on the form or “species” the arsenic is in. Arsenic is ubiquitous in the environment due to natural and anthropogenic sources, and the relative contribution of these factors is estimated as roughly 60% and 40% respectively. In the environment, As behaves in similar ways to the Group V elements nitrogen (N) and phosphorus (P). As a result of these similarities, arsenic gets taken into the biochemical pathways of N and P. This results in the formation of compounds such as arsenobetaine (AsB) in fish and arseno-sugars, which are found in marine algae. The toxicity of the inorganic As-species (such as arsenite, As(III) and arsenate, As(V)) is far greater than the organic forms, such as monomethylarsonic and dimethylarsinic acid (MMAA and DMA) and AsB. The International Agency for Research on Cancer (IARC) has classified inorganic arsenic as a human carcinogen, whereas AsB, the predominant form of As in most marine organisms [1], is considered nontoxic to humans. Although AsB is the major form of As in many marine organisms, it is not present in all fish species [2]; therefore, an evaluation of the proportion of AsB to the total As determined can give a useful and rapid estimate of the toxicological significance of a sample. In order to determine the toxicity of seafood, the determination of the total As alone is of limited value, and the different species of As have to be extracted, separated, and determined. Fast, reliable, and practical methods are therefore required that can provide speciation information for the screening of large sample batches.

Aims and Objectives

The aim of this study was to develop a semi-automated analytical method for the extraction and determination of As-species in fish tissues. Requirements for high sample throughput analysis were the automation of the extraction procedure as well as a fully automated separation and detection method capable of analyzing large sample batches (up to 50 injections per run) during overnight runs. In order to streamline the analytical procedure, an attempt was made to develop a method with a minimal number of sample preparation steps. It was intended that the method should be established using calibration by external calibration curves, rather than the lengthy alternative of standard additions. The use of an isocratic liquid chromatography (LC) elution can be favorable in terms of time-efficiency during the liquid chromatography-inductively coupled plasma mass spectrometry (LC-ICP-MS) analysis because it negates the need for column re-equilibration between injections.

Calibration Standards

The following standards were obtained from Fluka (Sigma-Aldrich, Gillingham, UK): di-sodium hydrogen arsenate heptahydrate ($AsHNa_2O_4.7H_2O$) ≥98.5%, sodium (meta)arsenite ($AsNaO_2$) ≥99.0%, and cacodylic acid (dimethylarsinic acid, DMA, $C_2H_2AsO_2$) ≥99.0%; monomethylarsonic acid disodium salt (MMAA, $CH_3AsNa_2O_3$) >98% was obtained from Argus Chemicals (Vernio, Italy). Arsenobetaine (AsB, $C_5H_{11}AsO_2$) was obtained from BCR (Brussels, Belgium) as a solution of AsB in water at 1031 ±6 (95% C.I.) mg/kg (BCR 626).

Extraction

Accelerated solvent extraction (ASE) has been used previously for As-speciation [3, 4] and was chosen as the sample preparation method because it allows for the automated extraction and online filtration of up to 24 samples. In addition, the extraction solution is collected in glass vials, which negates further sample preparation steps such as filtration or centrifuging.

The samples were extracted using a Dionex ASE 200 accelerated solvent extractor. Sample sizes from 0.1–0.3 g were weighed accurately into 11-mL stainless steel extraction cells fitted with filter papers and PTFE liners. The extraction program was set up as shown in Table 1.

Table 1. Extraction Conditions Used for ASE

Instrument	Dionex ASE 200
Preheat	2 min
Heat	5 min
Extraction steps	5 × 2 min
Temperature	100 °C
Pressure	1500 psi
Solvent	Methanol

HPLC-ICP-MS Methodology

The HPLC-ICP-MS instrumentation consisted of an Agilent Technologies 1100 HPLC system coupled to an Agilent Technologies 7500i ICP-MS fitted with a second roughing pump, which enhances sensitivity by increasing ion transmission across the interface. The HPLC system comprised a quaternary pump module, a vacuum degasser, a temperature controlled autosampler, and column compartment. The ICP-MS instrument was tuned for sensitivity, reduced oxides, and doubly charged species prior to connection to the liquid chromatograph by performing a standard instrument tune using a 10 ng/g solution of Li, Y, Ce, and Th in 1% HNO_3. The pulse to analog (P/A) factor was adjusted on a daily basis using a solution containing ~50 ng/g Li, Mg, Mn, Cu, As, Gd, Y, Cd, Pb, and Ba. After this optimization, a 50 ng/g solution of As in 1% HNO_3 was used to specifically optimize the sensitivity for arsenic. The ICP-MS nebulizer was then connected to the HPLC-column using a length of PEEK tubing (yellow, 1/16-inch od, 0.007-inch id). See Table 2 for the ICP-MS conditions used.

Table 2. ICP-MS Conditions Used for HPLC-ICP-MS Determination of As-Species

RF Power	1430–1550 W
RF Matching	1.89–1.92 V
Sampling depth	4.0–4.8 mm
Carrier gas flow	0.89–0.93 L/ min
Make up gas flow	0.10–0.14 L/ min
Optional gas	Oxygen at 5%
Spray-chamber temperature	0 °C
Cones	Platinum
Isotopes monitored	^{75}As ^{103}Rh ^{77}Se ($^{40}Ar^{37}Cl$) to monitor Cl interferences ^{53}Cr ($^{40}Ar^{13}C$) to monitor C interferences
Other parameters	Injector diameter: 2.4 mm Nebulizer 100 µL/min PFA, Two interface pumps used

In order to develop a rapid chromatographic separation of the main As-species in fish tissues, an anion exchange column (Hamilton PRP X-100) was chosen in combination with an isocratic elution profile. Several mobile phases were tested and the best separation of AsB and As(III) as well as DMA and MMAA was achieved within 10 min using 2.2-mM NH_4HCO_3/2.5-mM tartaric acid at pH 8.2 delivered at 1 mL/min isocratic flow. This evaluation was carried out initially using matrix-free calibration standards containing the species of interest and refined using an oyster tissue extract that contained arsenocholine (AsC), two arsenosugars (As-sug. B and As-sug. D), $TMAs^+$ and several unknown species in addition [5]. The injection volume for samples and standards was 50 µL.

In order to enhance the ionization of the As-species [6, 7], methanol was added to the mobile phase at concentrations ranging from 0.5% to 5% v/v. At concentrations above 1%, the chromatographic separation degraded significantly to the degree that base-line resolution between AsB and As(III) was no longer achieved. However, the addition of 1% MeOH to the mobile phase resulted in a significant improvement in the sensitivity (3–4-fold increase in peak height) for all analytes. A chromatogram for a 5-ng/g mixed calibration standard with the final chromatography conditions is shown in Figure 1.

Variations in Signal Response for Different As-Species

The chromatogram shows that the four species analyzed here have very different response factors with this method, even when made up to contain the same concentration of As in solution. This is further illustrated by the calibration curves and their respective slopes, as shown in Figure 2. Such differences in the analyte signal intensity were reported previously in the literature [7] and appear to be due to a combination of the ICP-MS hardware used and the plasma conditions, which are in turn affected by the mobile phase composition. This points to possible differences in the nebulization, transport and/or ionization of different species by such methods. In order to determine whether this effect could be attributed to the coupling of the ICP-MS with a liquid chromatograph, aqueous standards of AsB and As(III) were made up to equivalent concentrations as As and analyzed by direct aspiration without chromatography. This indicated that the signal response of AsB was ~10%–15% higher compared to the inorganic As standard and, therefore, the difference in signal response does not appear to be related to the coupling with a liquid chromatograph.

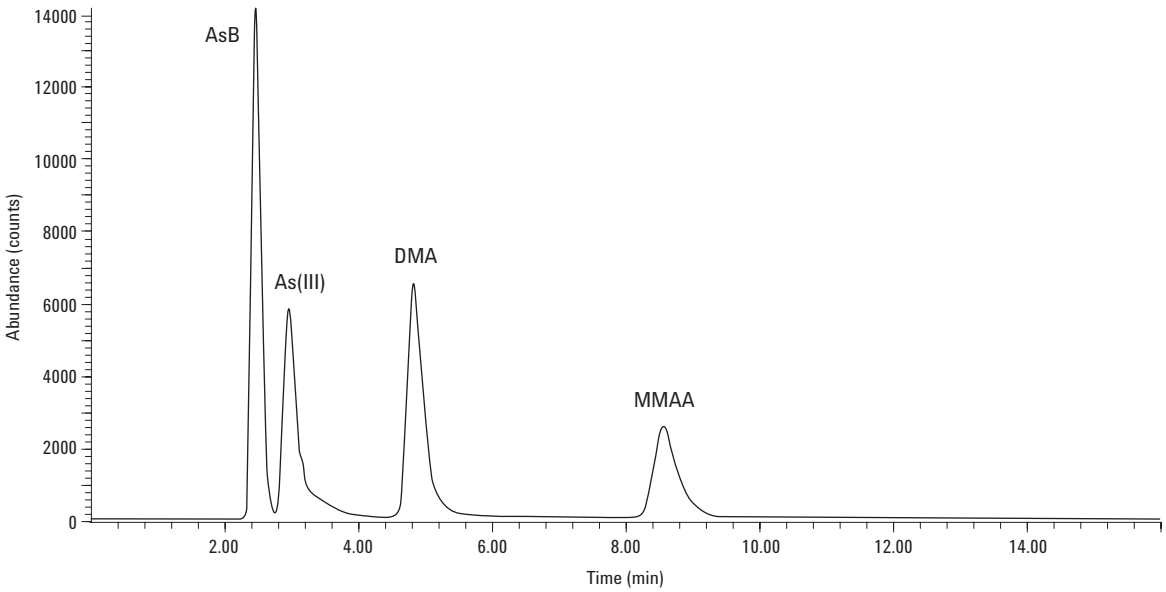

Figure 1. Chromatography A: 2.2-mM NH_4HCO_3 2.5-mM tartaric acid, 1% MeOH, pH 8.2, Hamilton PRP X-100 column. Concentration of standard ~ 5 ng/g as As.

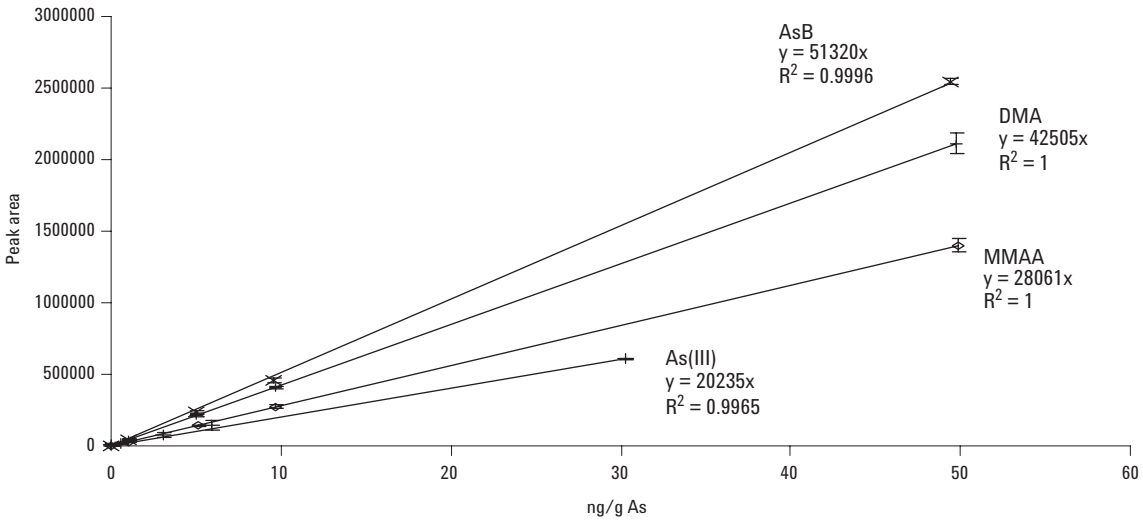

Figure 2. Calibration curves for AsB, DMA, MMAA and As(III) over a range of 0–50 ng/g as As.

In order to increase the signal intensity for species such as As(III) and MMAA by the approach described here, additional MeOH was added via a T-piece post-column so as not to impact on the chromatographic resolution. Although the relative volume of MeOH could be increased by 50%–70% in this way without deteriorating plasma stability, the relative signal responses of the four species were not influenced significantly. Because the relative signal response was stable on a day-to-day basis, no further attempts were made to equalize the signal responses.

The instrumental detection limit for AsB by this method was 0.04 ng/g as As. The linearity obtained, as indicated by the correlation coefficient of the calibration line, was 0.999–1.000 over a calibration range of 0–700 ng/g as As.

Plasma Disturbance Due to Elution of MeOH

During the analysis of fish samples, which had been extracted under the ASE conditions highlighted in Table 1, a disturbance of the plasma was observed between ~2.3 to 4.3 min after injection. This affected all of the isotopes monitored and the effect on ^{75}As and ^{103}Rh is highlighted in Figure 3. As can be seen from the chromatogram, the effect on these two isotopes is nonlinear. The ^{103}Rh signal decreases significantly during this time, whereas the ‘shoulder’ on the tailing side of the AsB peak indicates an increase in the ^{75}As signal.

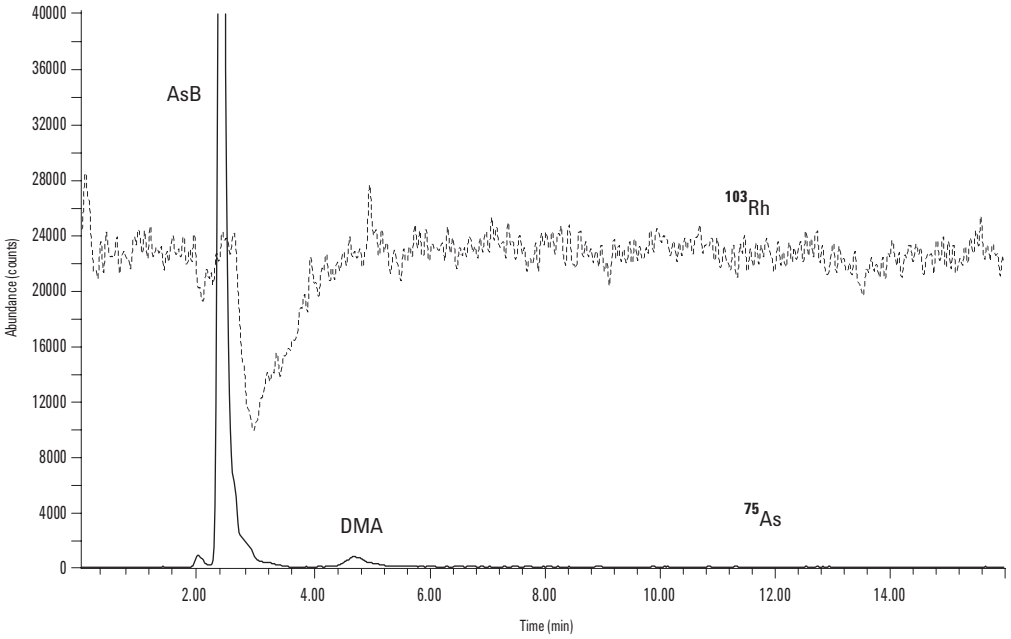

Figure 3. Signals for ^{103}Rh and ^{75}As in an undiluted fish extract. Notice the increase in the ^{75}As signal at the tailing side of the major peak (AsB) coinciding with the decrease in the ^{103}Rh signal.

The observed fluctuation in the signal intensities for the different isotopes coincides with the elution of the organic methanol fraction of the fish extracts from the analytical column. This effect could be reduced slightly by lowering the temperature of the spray-chamber from 5 °C to 0 °C, but the effect was not completely eliminated. During the injection of undiluted sample extracts, the volume of methanol that passes through the column and into the ICP-MS is ~10%. It has already been discussed that the addition of MeOH enhances the ^{75}As signal by increasing the ionization efficiency of this analyte; this effect is observed on a small scale here. Although there is no detectable As(III) in this fish material, the accurate quantitation of this compound (compared to aqueous calibration standards) could obviously lead to an overestimation if the signal of this analyte is enhanced due to the simultaneous elution of MeOH from the column. In this case, a standard addition calibration would represent a more accurate approach for quantitation. However, the spiking of each sample extract at different levels, which is necessary for this type of calibration, would make such an approach less suitable for a high sample-throughput application. In addition, the accurate integration of AsB is influenced by the signal increase on the tailing side of the peak.

In order to eliminate the effect of these signal variations on the accurate quantitation of the As-species in the methanolic extracts, the methanol fraction could either be reduced by evaporation or dilution with water. Dilution was chosen as the preferred option over evaporation in order to avoid possible analyte losses and because of time-efficiency. Whereas evaporation would either involve passing an inert gas over the solution or using rotary evaporation equipment, gravimetric dilutions were easily and quickly achieved by pipetting an aliquot of the extract into a sealed HPLC autosampler vial, weighing, and then adding the appropriate amount of water. In order to observe the effect of different dilution factors on the observed plasma disturbance, a fish extract was diluted 10-, 5-, and 2-fold in water and also injected undiluted. The effects of the different dilutions on the ^{103}Rh signal are shown by the chromatograms in Figure 4.

As demonstrated in Figure 4, a 10-fold dilution is sufficient to eliminate the plasma disturbance sufficiently; therefore, all extracts were diluted 1:10 in water prior to injection.

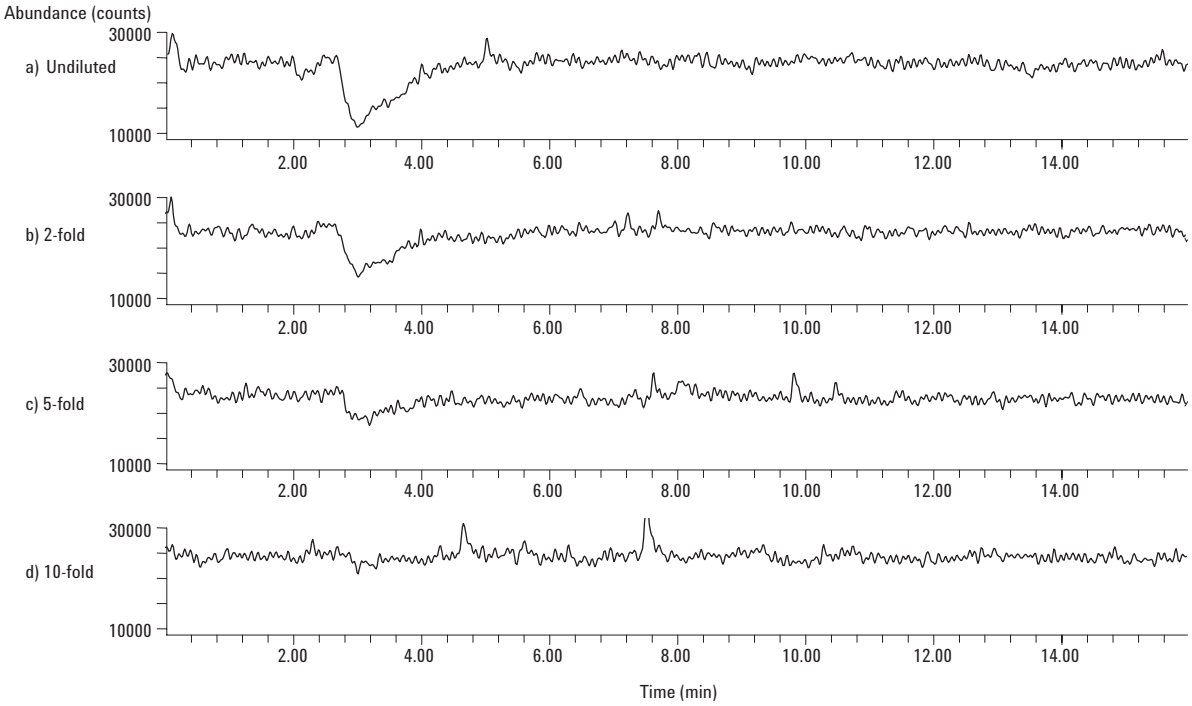

Figure 4. Signal of the internal standard ^{103}Rh, for fish sample extracts a) undiluted and diluted b) 2-fold, c) 5-fold and d) 10-fold.

Comparison of External Calibration and Standard Addition for the Quantitation of AsB in Fish Tissues

Due to the fact that arsenic is mono-isotopic, isotope dilution analysis cannot be used for the high-accuracy quantitation of this compound by LC-ICP-MS. In such circumstances, calibration by standard additions is often used in order to achieve matrix matching of standards and samples. It is also a useful technique in chromatographic applications where the possibility of retention time (RT) shifts of analytes due to matrix components exists. This can result in misidentification, and thus erroneous results. However, standard addition calibration can be very time- consuming because several aliquots of the sample require spiking with different levels of a calibration standard, and at least three levels of standard addition are needed for accurate quantitation of the same sample. External calibration by non-matrix matched standards can be used for applications where the difference in the matrix between samples and standards does not influence the accuracy of the result to a significant extent.

Standard addition calibration and non-matrix matched external calibration were compared for AsB in two certified reference materials (DORM-2, Dogfish muscle, NRC Canada and BCR 627, Tuna Fish, BCR EU) in order to assess whether the calibration technique used significantly influenced the accuracy or precision of the analytical result. The results showed that there was no significant difference in the mean results determined by the different calibration techniques with this method. The mean results for repeat analysis of both materials showed that the difference in the DORM-2 material was less than 1.4% and less than 4.5% for the BCR 627 material. When taking into account the standard deviations (SD) associated with the mean result obtained by each calibration technique, there was no statistically significant difference between the AsB results obtained by either approach in either of the fish tissue certified reference materials (CRMs).

Results of CRM Analysis

In order to test the accuracy of the developed ASE extraction and HPLC-ICP-MS method, a variety of certified and candidate reference materials of marine origin were extracted and analyzed. The samples included the certified fish reference materials DORM-2 and BCR 627, as well as an oyster tissue material (BCR 710)*, which is pending certification.

* The "MULSPOT" project has been financed by the SM&T Program (EU) (Contract SMT4-CT98-2232) and coordinated by ENEA (IT). The Project is at the certification stage and the material is not yet available on the market.

Table 3. Data Obtained for AsB in Two CRMs and a Candidate Reference Material

Expressed as mg/kg As unless otherwise stated	Measured value	Certified value
DORM-2 (Dogfish muscle)	16.3 ±0.9 (±1 SD)	16.4 ±1.1 (±95% C.I.)
BCR 627 (Tuna fish)	3.69 ±0.21 (±1 SD)	3.90 ±0.22 (±95% C.I.)
BCR 710 (Oyster tissue)† (Concentration as species)	31.8 ±1.1 (±1 SD)	32.7 ±5.1 (±1 SD)

† The data shown for this material is based on the consensus mean of the final certification round after the removal of statistical outliers.

Subsamples of the different materials (n = 4–6) were extracted, diluted in water, and analyzed as described above. The data for AsB determined in these samples is shown in Table 3. A chromatogram of the tuna fish material BCR 627 is shown in Figure 5.

The chromatogram indicates that the major species in this sample is AsB with two minor species, which were also extracted and detected. One peak was identified as DMA, and the peak labelled P1 is most likely to be AsC from RT matching. The data in Table 3 shows that the combined ASE/HPLC-ICP-MS methodology is capable of delivering accurate and reproducible results for AsB in these matrices. In addition, the extraction of other minor species, such as DMA and AsC, was achieved in the fish tissues; up to six species were extracted and separated in the oyster material, although none of these (apart from DMA) were quantified during this study. This DMA data for BCR 710 (730 ±30 ng/g DMA) showed a good agreement with the consensus mean value of the certification round (820 ±200 ng/g DMA).

Evaluation of Method Performance During a CRM Feasibility Study

The method performance was assessed in comparison to a number of European expert laboratories during the “SEAS” feasibility study organized by the The University of Plymouth Enterprise Limited and sponsored by the European Union (BCR, EU)‡. A fish material was prepared for this intercomparison by the University of Plymouth and distributed to participating laboratories. Participants were asked to determine AsB in a fish material from two different bottles using a methodology of their choice and making their determinations, at least, in duplicate on separate days.

The developed As-speciation method was used to extract and analyze the fish samples provided. A total of 12 subsamples from the two bottles were

‡The “SEAS” feasibility study was co-ordinated by The University of Plymouth Enterprise Limited (Plymouth, UK) under the EC contract: G6RD CT2001 00473 "SEAS" with the title: 'Feasibility Studies for Speciated CRMs For Arsenic in Chicken, Rice, Fish and Soil and Selenium in Yeast and Cereal'.

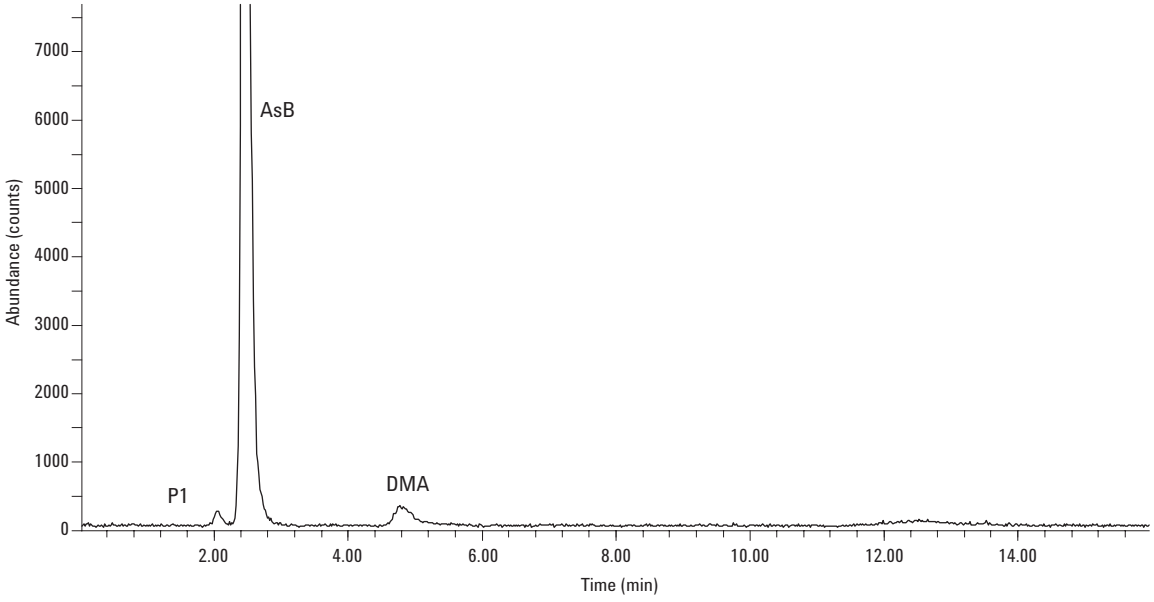

Figure 5. Chromatogram of a tuna fish extract (BCR 627) enlarged to show the detection of minor species in this material.

extracted and analyzed on 3 different days. The data were combined to provide the value labelled "LGC" in Figure 6 below. The error bars indicate the SD of the mean of individual results. The mean of all result (excluding a statistical outlier) together with 1 SD above and below the mean is indicated by the solid and dashed horizontal lines, respectively. The data provided by the combined ASE extraction and developed LC-ICP-MS methodology (94.92 ±3.95 mg/kg AsB) is in very good agreement with the mean result of all labs (95.72 ±7.79 mg/kg AsB, n = 11). The precision achieved was also satisfactory at 4.2% (RSD) for 12 subsamples from different bottles analyzed on 3 separate days. The performance of the method in this international intercomparison is highlighted by the good agreement with data provided by several European expert laboratories with longstanding expertise in As-speciation analysis. It should also be noted that the intercomparison was carried out with a blind sample of unknown concentration, rather than based on the analysis of a CRM with known certified values.

Conclusions

A robust and practical method has been developed based on accelerated solvent extraction and HPLC-ICP-MS analysis for the fast and accurate determination of AsB in fish samples. The benefits of the methods include automated extraction of up to 24 samples, minimal sample preparation steps (dilution only) after extraction, and rapid and automated analysis by HPLC-ICP-MS. The separation of four to six species of toxicological interest is achieved within 10 min using an isocratic elution. This increases the sample throughput by negating the column equilibration period needed with most gradient elution profiles.

The method was validated using commercially available CRMs and during a European intercomparison study with a fish sample of unknown concentration. The performance of the method was very satisfactory in terms of both accuracy and precision compared to several other expert laboratories.

This method can be used to rapidly determine the nontoxic proportion (AsB) in fish samples with high total As content and could therefore be used to determine whether a particular sample poses a toxicological risk in the food chain.

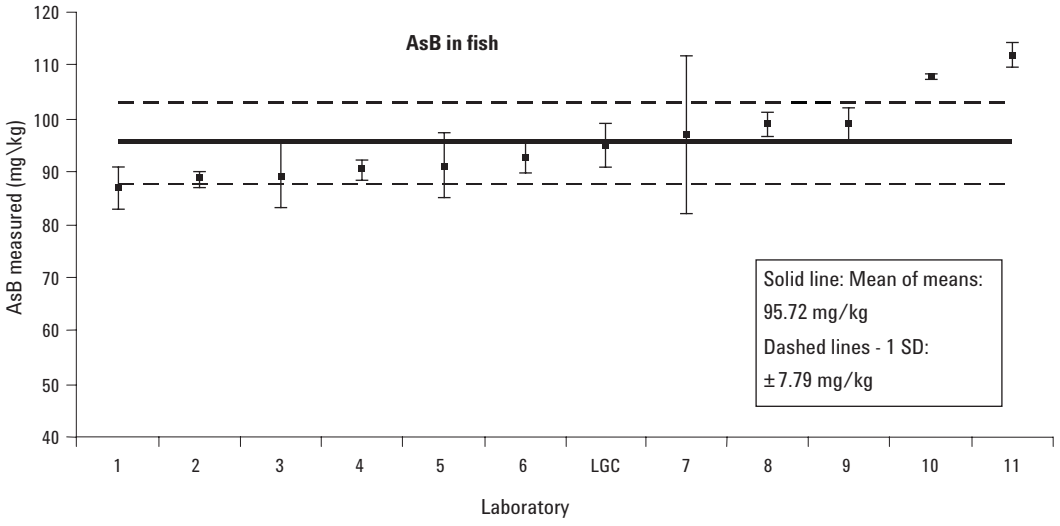

Figure 6. Comparison of data submitted by 12 participants for the determination of AsB in fish during the "SEAS" feasibility study. The error bars associated with the individual data points represent 1 SD of analysis of separate subsamples.

Acknowledgements

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Determination of Mercury in Microwave Digests of Foodstuffs by ICP-MS

Application

Food Safety

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Abstract

The quantitative determination of mercury in foodstuffs is presented using a 7500i ICP-MS. Microwave digests were prepared and then analyzed by ICP-MS. To avoid memory effects often experienced with mercury, gold was added offline to all standards/samples and wash solutions to act as a cleansing agent. The instrumental setup used a second vacuum pump, the integrated sample introduction system in the high sample throughput mode, and a micro-flow concentric nebulizer. This allowed the robust and rapid determination of mercury in the digests at the ppt range. Excellent agreement with the certified value was obtained for two certified reference materials and stability of the system was demonstrated over a 36-hour analytical run.

Introduction

The determination of sub-ppb concentrations of mercury has always been of special importance in the field of trace metal analysis. Even at trace levels, mercury is toxic and causes neurological damage, particularly in fetuses and young children. Anthropogenic sources of mercury in the environment include coal-fired power stations and chlor-alkali works. In the aquatic environment, bacteria convert elemental mercury Hg(0) to methylmercury which is accumulated and passed up the food chain. It has been reported that some whale meat contains 5000 times the Japanese legal limit of 0.4 µg/g. In addition, fish and shellfish are significant contributors to the human diet. Today mercury pollution is a global problem and extensive monitoring of foodstuffs is required. Therefore fast efficient and robust methods are needed. Mercury however is recognized as a problem element. It is known to adsorb onto the walls of storage containers and volatilize as mercury vapor. Additionally, its high first ionization potential and numerous isotopes have limited its sensitivity in ICP-MS analysis. ICP-MS allows the rapid determination of ultratrace levels of metals in food digests, however, extensive washout times have been required to reduce carryover for mercury analysis. Other workers have tried the addition of a number of chemical agents in the past. One of the most effective washout agents is gold chloride. To avoid memory effects and ensure stability, gold chloride (at the 5-ppm level) was added offline to all samples/standards and wash solutions. Extensive washout times were reduced by using by the integrated sample introduction system (ISIS). With the use of the high throughput pump, a large flush volume can be pumped through in a much shorter time. By summing the responses for multiple isotopes (199, 200, 201, and 202) and with the help of a second interface vacuum pump and a micro-flow concentric nebulizer, detection levels of between 10 and 30 ppt were routinely achieved for the digests.

Procedure

Microwave Digestion

Varying aliquots of each sample (generally between 0.2 and 0.6 g, depending on the moisture content of the sample) were weighed to the nearest 0.01 g into the digestion vessels. Wet oxidation was induced using concentrated, ultra-high purity nitric acid (10 mL, from Romil LTD, Cambridge, UK) with the addition of a 0.2 mL of concentrated hydrochloric acid (Romil LTD, Cambridge, UK). Oxidation was carried out in heavy-duty vessels (HDV) using a high-pressure microwave digestion oven (Mars 5 from CEM). Temperature control was used as opposed to pressure control. Samples were ramped to 180 °C over 20 minutes and held at 180 °C for 10 minutes before cooling to below 50 °C before venting the vessel. Both pressure and temperature were monitored by direct measurement throughout the digestion to ensure that samples attained the critical temperature of 180 °C, at which food components, such as fat, are digested. The sample digests were then made up to 100 g using ultra-high purity water (18 mega ohms, from Elga Maxima). The resultant solution was used for determination.

Operating and Acquisition Parameters

Ten milliliter portions of the sample digests were accurately pipetted into sample tubes, and using a micropipette, 20 µL of a 1000-ppm gold chloride solution (Romil LTD, Cambridge, UK) was added. This gives a final gold concentration of 5 ppm in solution. Fifty milliliters each, of blank and four standard solutions covering the range, were prepared from a 100 µg/g stock mercury solution (from SPEX CertiPrep Assurance, Metuchen, New Jersey, USA). Ten percent wt/wt nitric acid containing 5 ppm of gold was used as the wash solution for the autosampler and nebulizer. Gold is thought to have its effect by acting as an oxidizing agent ensuring that mercury stays in an ionized form in solution. Gold was added at elevated levels to ensure that any residual amounts of organic compounds in the digests would not reduce Au(III) to elemental gold and render it ineffective. A 250-ppb Thalium standard was added online as an internal standard (ISTD), using the ISIS system. There was an online dilution factor of 1:20. Gold chloride was also added to the standard solutions at 5 ppm.

Instrument Conditions

Plasma gas flow rate	16 L/min
Carrier gas flow rate	0.85 L/min
Make-up flow	0.14 L/min
RF Power	1400 Watts
Nebulizer	Agilent micro-flow 100 µL
Spray chamber	Glass double pass
Spray chamber temperature	Cooled to 2 °C
ICP Torch injector	2.4 mm
Sample tubing	0.89 mm id
Internal standard tubing	0.19 mm id
Instrument Peri pump	0.1 rps
Sample/Skimmer cones	Nickel
Rotary pumps	2
Autosampler	AX500

Acquisition Parameters

Mass	Element	Integration/Point	Time (s)/Mass
199–201	Hg	3.5	10.5
202	Hg	3.5	10.5
205	Tl	0.05	0.15

Number of points per mass:	3
Acquisition time:	43.79 s
Number of repetitions:	3
Total acquisition time:	131 s

Peristaltic Pump Program

Memory effects arise when the analyte signal is enhanced due to contributions from previous high concentration sample. This is due to adsorption/desorption of mercury in the sample introduction system. As a result, the analyst has to program long washout times. With the use of ISIS this wash-out time can be reduced.

ISIS Peristaltic Pump Program
Analysis Speed : 0.10 rps

Before acquisition	
Uptake speed	0.80 rps
Uptake time	32 s
Stabilization time (undiluted)	150 s
After acquisition (probe rinse)	
Rinse speed	0.80 rps
Rinse time (sample)	8 s
Rinse time (standard)	8 s
After acquisition (rinse)	
Rinse vial	1
Uptake speed	0.8 rps
Uptake time (undiluted)	32 s
Stabilization time	85 s

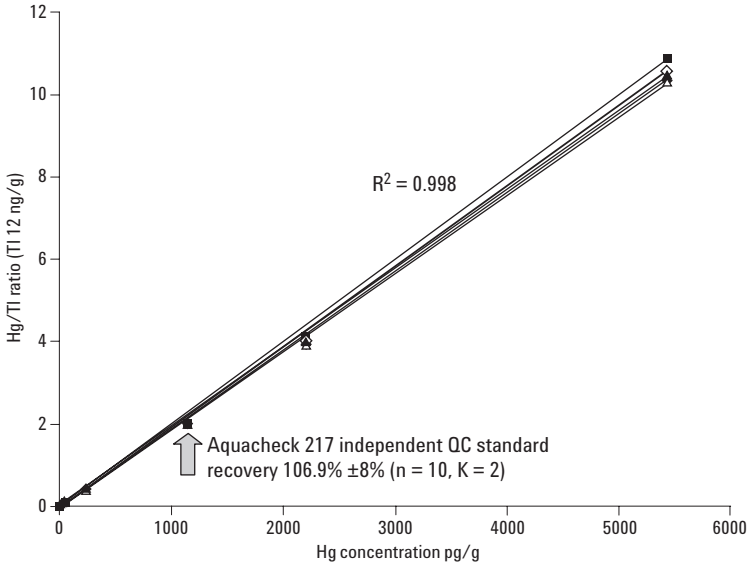


Figure 1. Calibrations over 36 hours continuous operation.

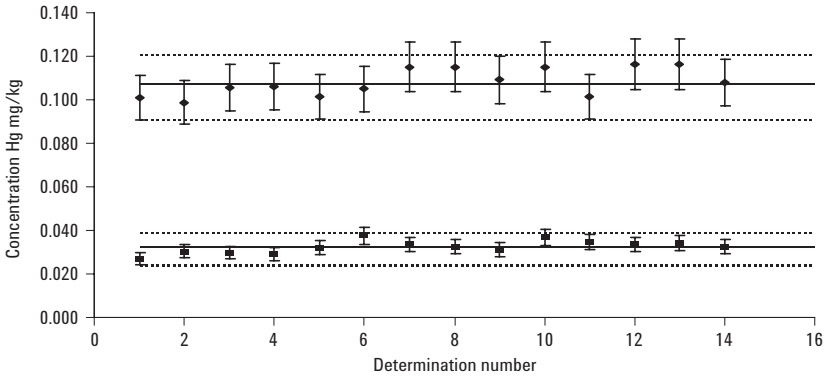
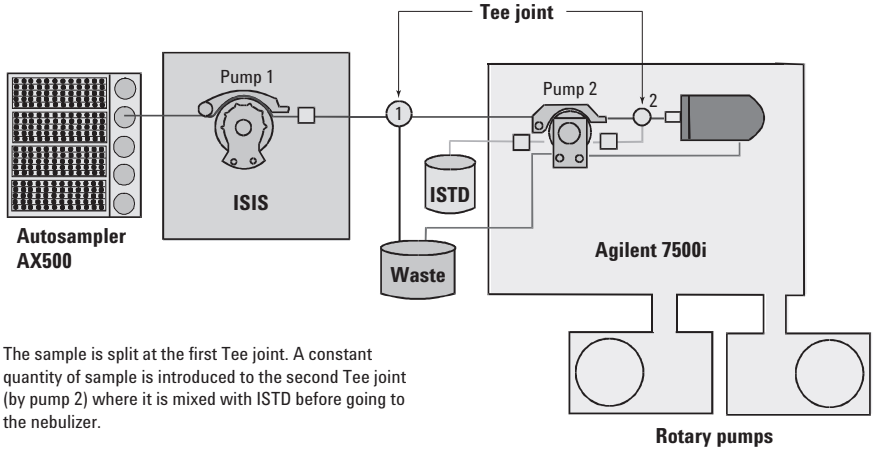


Figure 2. NIST 1547 Peach leaves (bottom trace) and LGC 7160 Crab paste (top trace) were analyzed 14 times over a period of 1 month. Each point represents a separate digest. LGC 7160 certified value 0.096 = ±0.108 mg/kg, NIST 1547 certified value 0.031 = ±0.007 mg/kg.



The sample is split at the first Tee joint. A constant quantity of sample is introduced to the second Tee joint (by pump 2) where it is mixed with ISTD before going to the nebulizer.

When going to the next sample the ISIS pump (pump 1) turns at high speed to shorten the sample and washout transfer times.

Figure 3. Schematic of ISIS in high sample throughput mode.

Results

Five calibration plots over a 36-hour period are shown in Figure 1 demonstrating excellent stability of the system. As can be seen, excellent linearity was achieved over an extended calibration range demonstrating that memory effects had been effectively eliminated. Between the calibrations over 120 various microwave foodstuff digests were analyzed.

Quality Control

An Aquacheck proficiency testing material solution from the Water Research Council (1010 ppt) was analyzed 10 times during the run and a mean recovery of 106.9% (±8%) was achieved (Figure 1). This material was analyzed throughout a run of 120 various foodstuffs samples and acted as a quality control for the quantitation.

Two certified reference materials (CRMs) were analyzed throughout a survey of 500 samples. These acted as quality control materials for the microwave digestion as well as the quantitation. The CRMs used were a crab paste—Metals LGC 7160, 0.096 mg/kg—and peach leaves—NIST 1547, 0.031 mg/kg. Each CRM was analyzed 14 times on different runs during a survey of more that 500 samples of various foodstuffs over a 1-month period. The results can be seen in Figure 2.

Conclusion

This procedure proved robust, as in excess of 500 samples were analyzed in runs lasting in excess of 36 hours without loss of sensitivity.

The 7500i ICP-MS has shown to be a robust and sensitive tool for the analysis of foodstuff for mercury. The system proved stable over an extended time period. The use of the ISIS enables the operator to take advantage of the high sample throughput possible with this technique. The use of Au(III), in particular, shortens washout time considerably, reducing the possibility of carry-over. The sensitivity of the 7500i ICP-MS gives method detection limits at low ppt levels in the foodstuff digests. The extended calibration range reduces the need for dilutions and reruns.

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Agilent Technologies

Measurement of Trace Elements in Malt Spirit Beverages (Whisky) by 7500cx ICP-MS

Application

Food

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Abstract

A method for the measurement of trace elements in malt spirits (whisky) is described with reference to six different samples. An Agilent 7500cx ICP-MS featuring the Octopole Reaction System (ORS) was used for the analysis. The 7500cx ensures simple operation as a single method and a single set of conditions can be used to remove interferences regardless of their source. Excellent spike recoveries were obtained (between 97 and 107%) following a simple dilution of the samples. A 5-hour stability test yielded excellent precision (< 2%) for almost all elements. The study shows that the 7500cx can be used for the routine measurement of trace metals in beverages.

Introduction

The measurement of trace elements in alcoholic beverages is required from a quality control standpoint and also to ensure that the final product complies with any regulatory requirements.

Metal content can originate from the raw ingredients, such as water or grain, as well as during processing, for example, from fermentation or distillation equipment. An example would be high arsenic concentration from distillation vessels manufactured from poor-quality copper. The levels of trace elements can also significantly affect the taste of the whisky. Consequently, there is a requirement to measure elemental concentrations in the final product. While ICP-MS offers high sensitivity and excellent detection limits for many elements, interferences on key elements arising from the alcohol content and required sample preparation can be problematic.

The 7500cx features the Octopole Reaction System (ORS) collision/reaction cell, which removes matrix-based polyatomic interferences using a single set of cell conditions (helium mode). For the analysis of spirits, the major interferences resulting from the sample would be carbon-based (for example, $^{40}Ar^{12}C$ on ^{52}Cr). Many elements are much more stable in a chloride matrix than simple acidification using nitric acid; for this reason, hydrochloric acid (HCl) was added to the samples. New interferences are created by the addition of HCl (for example, $^{35}Cl^{16}O$ on ^{51}V; $^{40}Ar^{35}Cl$ on ^{75}As, etc.) but they are removed by the ORS in helium mode.

An optional cell gas line is available for the 7500cx, enabling operation in hydrogen (H_2) reaction mode, which allows for the measurement of selenium at ultratrace levels. Since several of the solutions contained less than 40 ng/L Se (some significantly lower than this) in solution after dilution, H_2 reaction mode was also used during this study.

Experimental

Sample Preparation & Instrumental Conditions

Four Scottish whiskies (Highland, Speyside, Islay, and a blend), one Irish whisky, one U.S. bourbon –

as well as a further Scottish whisky and a U.S. bourbon that had been stored in lead crystal decanters – were analyzed. The samples were prepared by simple 5x dilution using 1% HNO_3 and 0.5% HCl (v/v). Using an acid mix significantly improves the stability of many elements, particularly Hg and Sn, compared to the use of nitric acid alone. Standards were prepared from 1,000 ppm stock single-element solutions to produce final mixed-element calibration solutions. In order to compensate for sample transport effects and solvent evaporation rates, the alcohol content of the standards was matched to that of the samples by adding 8% ethanol to all standard solutions (equivalent to 5x dilution of the original samples, which contained 40% v/v alcohol). This also compensates for ionization enhancement effects for As and Se in the presence of high carbon concentrations. Gold (400 µg/L) was also added to the standards and samples in order to further improve the stability of Hg.

Table 1 lists the instrumental conditions used for the analysis; sample uptake rate was approximately 150 µL/min and sampling was facilitated by the Agilent ASX-520 autosampler. The solution pump program was optimized using the preemptive rinse function in the ChemStation software in addition to a multichemistry rinse regime [1]. The 7500cx was operated under standard conditions, and internal standards (Ge, Rh, and Ir) were added automatically on line by the system's peristaltic pump. No special precautions were necessary for these sample types.

Table 1. Agilent 7500cx Operating Conditions

RF power	1550 W
Sampling depth	8 mm
Carrier gas flow	0.68 L/min
Makeup gas flow	0.33 L/min
Spray chamber temperature	15 °C
Helium cell gas flow	5.5 mL/min
Hydrogen cell gas flow	4.0 mL/min

Data Acquisition

Data was acquired operating the ORS in helium [He], hydrogen [H_2], and no-gas modes. Helium mode is the default mode of operation of the 7500cx. The inert He cell gas conditions remove interferences based on their ionic cross-section rather than relying on a reactive gas. As almost all interferences in ICP-MS are polyatomic in nature, they possess a greater cross-section than the monatomic analyte at the same mass and therefore undergo a greater number of collisions in the cell. As each collision causes energy loss, the interfering species lose more energy than the analyte and are subsequently filtered from the mass spectrum by discriminating between the two different energies (called energy discrimination). As this process takes place regardless of the analyte-interference combination, a single set of conditions can be used for all analytes.

Selenium was measured in hydrogen mode as the concentration of this element in the diluted sample was at low ppt levels. Although selenium can be measured in helium mode, hydrogen mode removes the Ar-based interference with greater efficiency, improving the detection limit for this element, and is the better option for low-ppt concentrations. Some isotopes were determined in both helium mode and no-gas mode to provide comparative data on cell performance. For routine analysis this would not be necessary, of course. All cell modes were acquired within a single acquisition and sample pass.

Results and Discussion

Table 2 summarizes the detection limits (DLs), background equivalent concentrations (BECs), and calibration regression for the isotopes studied in the different cell modes (default mode is highlighted in bold typeface). For those elements that suffer from interferences in this carbon and chloride matrix, BECs and DLs are severely compromised when operating the instrument in no-gas mode (that is, conventional ICP-MS). This can be clearly observed in the data for chromium: ^{52}Cr BEC without cell gas is 526 µg/L, and in helium mode is 0.07 µg/L. The interference is effectively reduced to background contamination levels as the BECs for both Cr isotopes are very similar. Improvements can also be observed for V, Fe and ^{65}Cu (^{63}Cu does not suffer from interferences in this relatively simple matrix), all of which were acquired in helium mode.

Figures 1 to 3 display the calibration profiles for selected interfered elements with and without cell gas applied; Figures 4 and 5 illustrate the calibration profiles for Be (low mass, difficult to ionize) and Hg (high mass, difficult to ionize, low-abundance isotope). The line does not pass through the origin in the calibrations for those elements that suffer from an interference and this offset can be seen clearly. Be and Hg are also presented to demonstrate the excellent sensitivity for these difficult-to-ionize elements. In order to obtain low detection limits, it is essential to maximize the ion-

ization efficiency of the plasma. This is done through optimization of the sample introduction system (low solution and gas flow rates and wide-bore injector torch) and plasma generator design (27.12 MHz, solid-state fixed frequency, and high-efficiency digital drive). All of these factors combine to increase the effective central channel temperature, improving ionization efficiency. This is allied to an ion lens system designed to improve low-mass ion transmission efficiency, further improving the DL of this important and relatively difficult element.

The elements Ge, Rh, and Ir were used as internal standards and were added on line.

Table 3 displays the quantitative data for all samples, including a spike recovery for the Islay whisky. Data are displayed in the preferred cell gas mode (usually helium). Although some elements were calibrated under gas and no-gas conditions, only the most appropriate cell mode is displayed to simplify the data set. Taking Cr as an example, the data for both isotopes did not match in no-gas mode and were significantly higher than the data obtained in helium mode due to the intensity of the C- and Cl-based interferences. The helium mode data for both Cr isotopes produced comparable results, which is a good indication of the accuracy of the data.

The two samples that had been stored in lead crystal decanters have obviously higher Pb concentration in comparison to the other samples. The mean Pb concentration in the noncrystal samples was about 1.3 µg/L, while the Pb content of the samples stored in the crystal decanters was almost 10x higher. As a comparison, the UK maximum permissible Pb concentration in drinking water is 25 µg/L (at the tap), which means that the Pb concentration is within this guideline; however, in 2013 this level is due to be reduced to 10 µg/L, meaning products stored in crystal would fail to meet drinking water quality standards.

Table 2. Limit of Detection, Background Equivalent Concentrations, and Regression Coefficients for the Studied Isotopes (Data are presented as ng/L [ppt] and are corrected for dilution.)

Element	Mass	Mode	r	DL	BEC
Be	**9**	**No gas**	**1**	**0.5**	**0.3**
V	**51**	**He**	**1**	**26.8**	**12.2**
V	51	No gas	0.9999	495	5220
Cr	**52**	**He**	**1**	**50.4**	**73.7**
Cr	52	No gas	0.985	48600	526000
Cr	**53**	**He**	**1**	**38.6**	**71.3**
Cr	53	No gas	0.9997	2020	52100
Mn	**55**	**He**	**1**	**7.8**	**20.8**
Mn	55	No gas	1	17.5	30.4
Fe	**56**	**He**	**0.9999**	**17.8**	**406**
Fe	56	No gas	1	1160	58300
Co	**59**	**He**	**1**	**0.5**	**3.7**
Co	59	No gas	1	5.6	6.7
Ni	**60**	**He**	**1**	**13**	**38.7**
Ni	60	No gas	1	15.2	73.6
Cu	**63**	**He**	**1**	**10.4**	**41.5**
Cu	63	No gas	1	16.6	59.6
Cu	**65**	**He**	**1**	**18.1**	**36.9**
Cu	65	No gas	1	58.6	230
Zn	**66**	**He**	**1**	**33.9**	**119**
Zn	66	No gas	1	22	171
As	**75**	**He**	**1**	**2.0**	**3.8**
As	75	No gas	1	46.1	382
Se	**78**	**H_2**	**1**	**3.6**	**13**
Se	78	No gas	1	135	1390
Cd	**111**	**He**	**1**	**5.7**	**5.3**
Cd	111	No gas	1	3.3	6.1
Sn	**118**	**No gas**	**1**	**7.8**	**50.5**
Sb	**121**	**No gas**	**1**	**7.1**	**30.8**
Ba	**137**	**No gas**	**1**	**2.8**	**5.3**
Hg	**201**	**No gas**	**1**	**1.7**	**10.7**
Pb	**208**	**No gas**	**1**	**2.1**	**10.2**
U	**238**	**No gas**	**1**	**0.1**	**0.2**

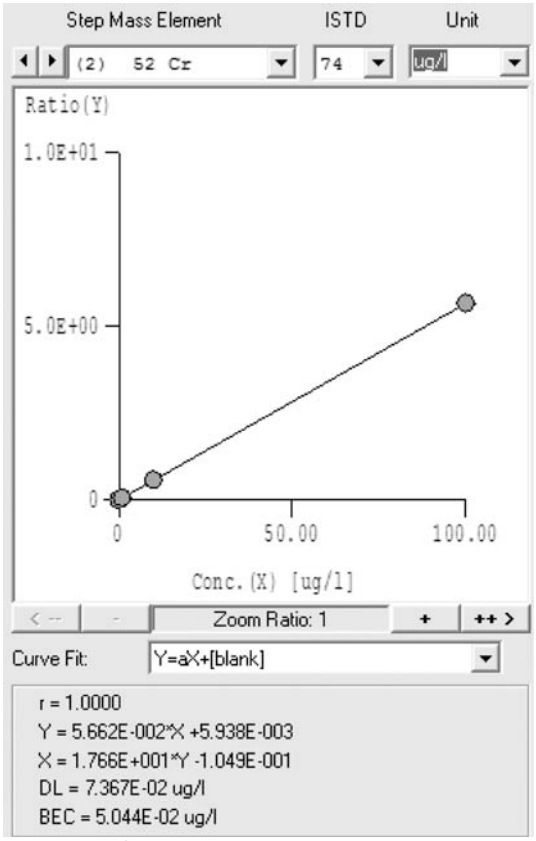

Figure1A. Chromium calibration [He mode]. Note BEC of 0.0504 µg/L.

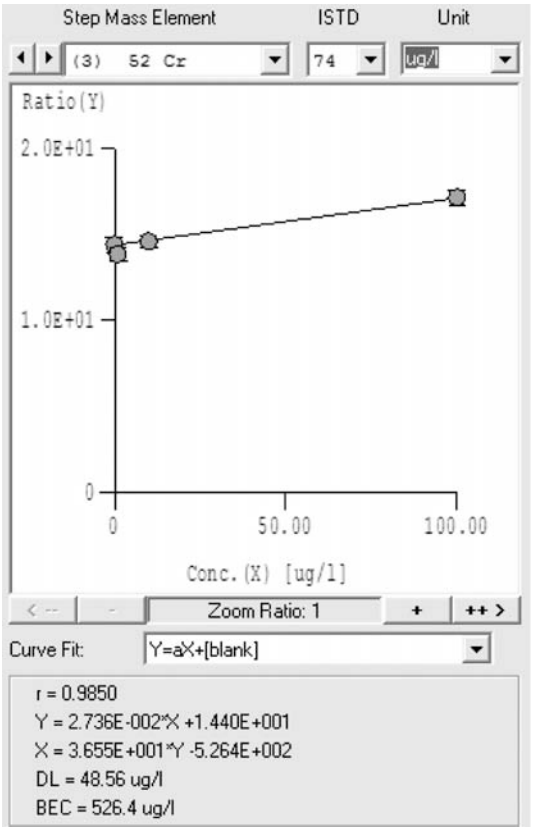

Figure 1B. Chromium calibration [no-gas mode]. Note BEC of 526 µg/L.

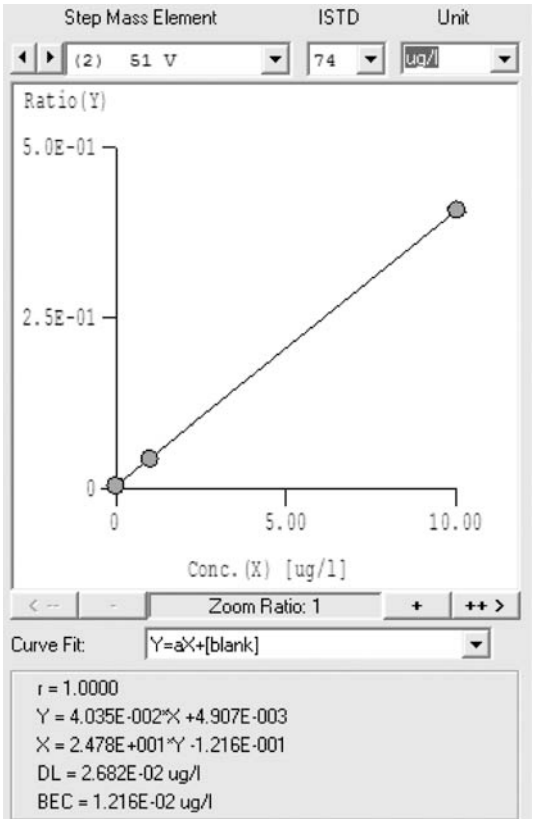

Figure 2A. Vanadium calibration [He mode]. Note BEC of 0.0122 µg/L.

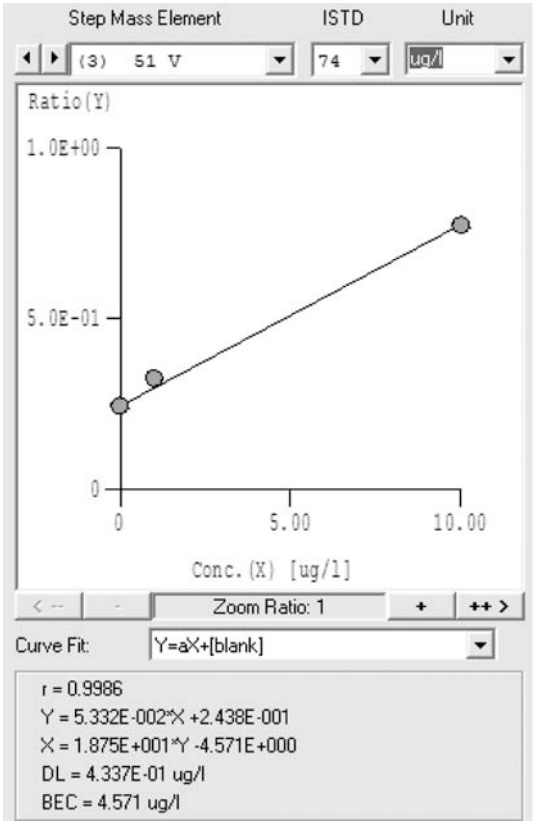

Figure 2B. Vanadium calibration [no-gas mode]. Note BEC of 4.57 µg/L.

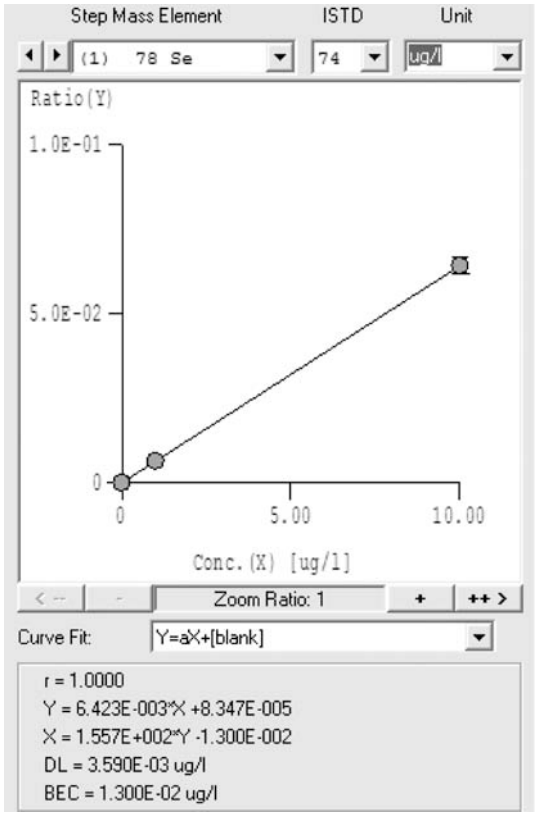

Figure 3A. Selenium calibration [H_2 mode]. Note BEC of 0.013 µg/L.

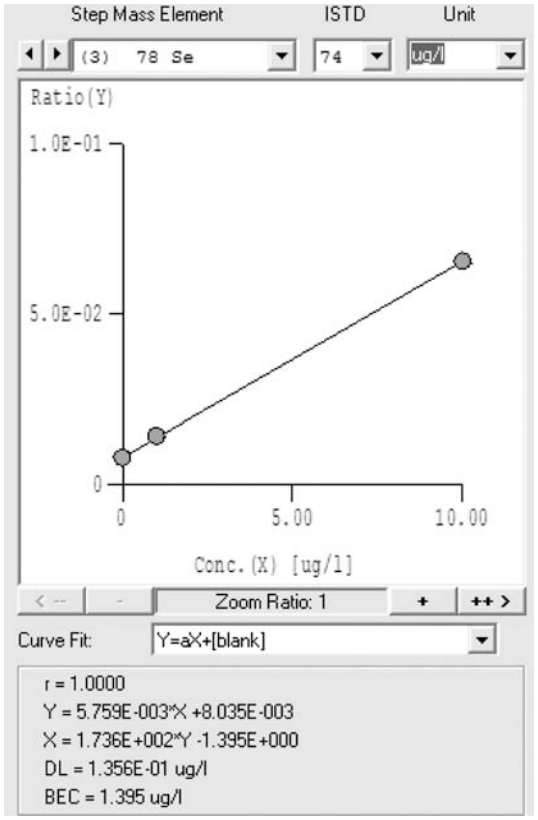

Figure 3B. Selenium calibration [no-gas mode]. Note BEC of 1.4 µg/L.

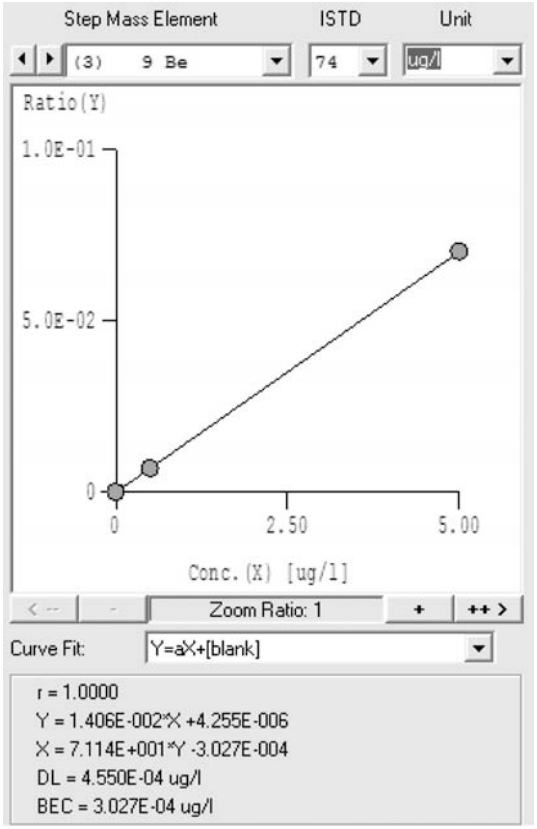

Figure 4. Beryllium calibration [no-gas mode]. Note detection limit of 0.303 µg/L.

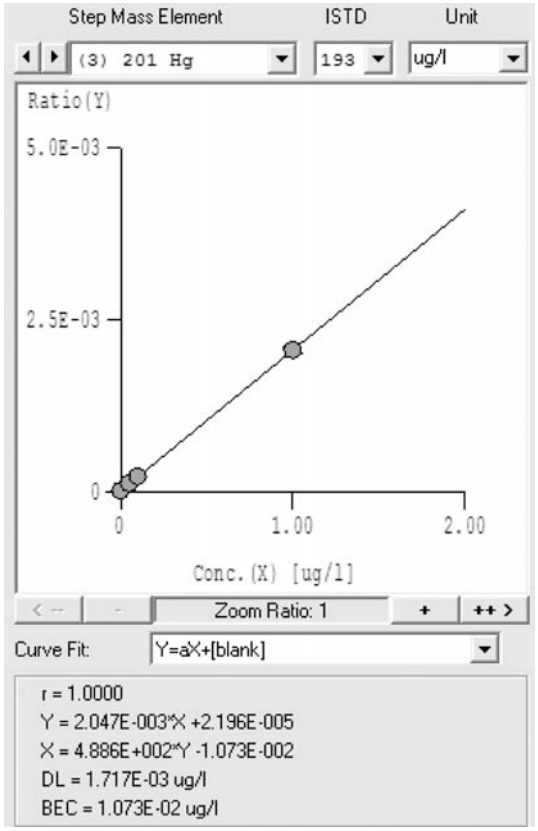

Figure 5. Mercury calibration [no-gas mode]. Note detection limit of 0.0107 µg/L.

Table 3. Quantitative Data Obtained in the Preferred Cell Mode for the Spirit Samples with Spike Recovery for the Islay Sample (Recoveries were generally excellent. All results presented as dilution-corrected µg/L.)

Sample			Highland	Speyside	Islay	Blend	Irish	Bourbon	Bourbon decanter	Whisky decanter	Islay spike	Spike qty	% recovery
Be	9	No gas	0.140	0.052	0.037	0.008	0.035	0.015	0.042	0.048	13.09	12.5	104.4
V	51	He	1.564	0.443	0.344	0.073	0.431	6.321	1.693	0.14	25	25	98.6
Cr	52	He	27.39	14.05	4.064	12.62	22.71	4.077	31.87	4.331	30.95	25	107.5
Cr	53	He	26.15	13.7	3.955	12.57	22.61	3.661	31.52	4.114	30.81	25	107.4
Mn	55	He	54.51	31.76	13.52	12.22	26.95	9.753	90.4	30.54	38.19	25	98.7
Fe	56	He	1125	191.2	99.76	583.8	250.5	131.4	1114	67.03	232.2	125	106.0
Co	59	He	1.097	0.376	0.180	0.130	0.336	0.172	0.323	0.368	12.83	12.5	101.2
Ni	60	He	14.02	3.586	1.442	5.065	3.078	2.274	12.88	1.992	25.91	25	97.9
Cu	63	He	542.9	370.8	454.4	258	38.45	22.2	445.5	367.6	579.8	125	100.3
Cu	65	He	525.5	359.2	441.6	251.4	37.4	21.43	430.8	355.9	568.6	125	101.6
Zn	66	He	21.02	18.54	8.414	14.18	8.149	13.69	68.27	21.9	137.5	125	103.3
As	75	He	0.503	0.427	0.272	0.256	0.164	2.192	0.434	0.424	25.72	25	101.8
Se	78	H_2	0.458	0.357	0.190	0.073	0.045	0.497	0.069	0.293	26.54	25	105.4
Cd	111	He	0.036	0.024	0.012	0.010	0.024	0.036	0.193	0.028	12.55	12.5	100.3
Sn	118	No gas	9.18	14.82	16.68	5.161	2.245	1.681	0.239	15.12	41.3	25	98.5
Sb	121	No gas	0.817	0.514	0.397	0.308	0.311	0.765	0.316	0.188	24.87	25	97.9
Ba	137	No gas	3.282	3.05	1.426	2.001	3.37	3.303	1.396	2.41	25.71	25	97.1
Hg	201	No gas	0.013	0.011	0.010	0.011	0.010	0.018	0.008	0.009	0.252	0.25	97.0
Pb	208	No gas	1.13	0.898	0.903	1.902	1.21	0.912	12.59	11.15	25.33	25	97.7
U	238	No gas	0.295	0.049	0.051	0.026	0.060	0.104	0.028	0.049	24.38	25	97.3

The benefit of operating the instrument in helium mode can clearly be observed for those isotopes suffering from interferences. As helium is a totally inert gas, no side reactions or new product interferences are formed – this lends itself to full mass acquisition allowing interference-free qualitative or semiquantitative analysis. The samples were prepared in an identical way as above (although a separate preparation on a different day) and Table 4 displays the semiquantitative data obtained for the samples analyzed under identical helium cell conditions as with the previous data set.

The full mass spectrum (Figure 6) is from the crystal-stored bourbon sample. The graphic includes an inset, zoomed-in region to demonstrate the excellent isotopic fit for those elements suffering most from interferences. The fit for Cr is particularly important as all three isotopes (50, 52, and 53) demonstrate good agreement with the expected natural ratio in this carbon-based matrix (^{50}Cr suffers interferences from $^{38}Ar^{12}C$, $^{13}C^{37}Cl$, $^{36}Ar^{14}N$, and $^{35}Cl^{15}N$; ^{52}Cr has interferences from $^{36}Ar^{16}O$, $^{40}Ar^{12}C$, $^{35}Cl^{17}O$, and $^{37}Cl^{15}N$; ^{53}Cr has interferences from $^{40}Ar^{13}C$, $^{37}Cl^{16}O$, $^{35}Cl^{18}O$, and $^{35}Cl^{17}O^{1}H$). Several other interferences are also possible, but each is polyatomic in nature and so is removed by the same process and using a single set of helium mode conditions.

To demonstrate instrument stability (Figure 7), 54 separate measurements were made of a spiked Highland malt whisky sample; total measurement time was 5 hours 18 minutes. Stability for the majority of elements was < 2% RSD over the run, indicating applicability of the method to routine analysis.

Table 4. Semiquantitative Data for Spirit Samples Using Helium Mode (Data are presented as µg/L [ppb] unless indicated and are corrected for dilution.)

		Highland	Speyside	Islay	Blend	Irish	Bourbon	Bourbon decanter	Whisky decanter
7	Li	0.24	0.2	0.049	0.084	0.16	0.16	0.34	0.26
9	Be	0.15	N/D	N/D	N/D	N/D	N/D	N/D	N/D
11	B	46	44	42	49	62	69	75	51
12	C	53000 ppm	55000 ppm	55000 ppm	55000 ppm	54000 ppm	76000 ppm	55000 ppm	57000 ppm
23	Na	1400	2100	1600	1600	1000	12000	540	2100
24	Mg	61	48	23	30	63	120	45	48
27	Al	3.9	1.8	1.8	2.3	2.4	1.9	2.5	1.6
29	Si	1300	1400	1300	1300	1300	2000	1500	1400
31	P	13	9.7	6.2	11	86	200	33	18
34	S	100	190	120	240	250	520	210	250
35	Cl	450 ppm	440 ppm	420 ppm	420 ppm	420 ppm	430 ppm	410 ppm	430 ppm
39	K	150	150	100	130	320	470	460	160
43	Ca	60	49	5.5	10	37	18	26	22
47	Ti	0.51	1.6	0.77	0.51	1.5	1.7	0.99	0.8
51	V	0.8	0.54	0.11	0.6	0.6	6.5	1.2	0.21
52	Cr	26	14	5.1	13	23	5.1	27	5.4
55	Mn	54	32	13	14	27	11	90	32
56	Fe	1200	210	100	630	260	150	1100	76
59	Co	1.1	0.47	0.16	0.18	0.36	0.24	0.4	0.4
60	Ni	13	3.5	1.8	5.7	3.4	2.4	13	2.3
63	Cu	530	380	450	260	39	22	440	380
66	Zn	22	18	9	15	8.9	14	71	25
69	Ga	0.65	0.45	0.27	0.39	0.66	0.52	0.3	0.51
75	As	0.44	0.3	0.21	0.2	0.19	2	0.4	0.42
78	Se	N/D	0.48	N/D	N/D	N/D	N/D	N/D	0.47
79	Br	160	150	140	130	120	150	150	150
85	Rb	0.9	1.4	0.92	1	2.2	8.1	6.1	1.5
88	Sr	1.3	0.84	0.22	0.36	1.4	1.7	0.57	0.78
89	Y	0.046	0.03	0.024	0.0084	0.1	0.034	0.0056	0.035
90	Zr	0.18	0.019	0.12	0.064	0.22	0.093	0.069	0.0097
93	Nb	0.0024	0.0076	0.005	0.0051	0.091	0.014	0.0077	0.005
95	Mo	0.7	0.31	0.37	0.41	0.33	1.5	0.76	0.13
101	Ru	0.01	0.02	N/D	0.021	N/D	N/D	0.01	0.02
105	Pd	0.0072	N/D	N/D	0.0075	N/D	0.025	N/D	N/D
107	Ag	0.017	0.027	0.0034	0.0035	0.014	0.0039	0.021	0.01
111	Cd	N/D	0.047	0.047	0.024	0.024	0.1	0.14	0.071
118	Sn	10	18	20	6	3.7	2.2	0.6	17
121	Sb	0.32	0.3	0.27	0.3	0.39	0.8	0.3	0.21
125	Te	N/D	0.38	0.38	0.19	0.19	N/D	N/D	0.18
127	I	0.46	0.42	0.65	0.41	0.5	0.89	0.45	0.47
133	Cs	0.052	0.15	0.026	0.0092	0.063	0.24	0.045	0.15
137	Ba	4.6	3.2	1.3	2.2	3.8	3.8	1.3	2.4
139	La	0.16	0.07	0.063	0.086	0.28	0.15	0.035	0.087
140	Ce	0.47	0.24	0.17	0.11	0.61	0.24	0.04	0.36
141	Pr	0.042	0.028	0.019	0.02	0.063	0.02	0.0044	0.024
146	Nd	0.21	0.12	0.045	N/D	0.3	0.14	0.022	0.14
147	Sm	0.074	N/D	0.01	0.033	0.098	0.037	0.032	0.032
153	Eu	0.0027	0.0083	0.0055	0.011	0.016	0.0063	0.0056	N/D
157	Gd	0.071	0.04	0.048	0.016	0.11	0.056	0.016	0.024
159	Tb	0.0024	0.005	0.0025	N/D	0.012	0.0029	N/D	0.0037
163	Dy	0.071	0.0096	0.024	0.029	0.083	0.028	0.0049	0.029
165	Ho	0.0045	0.0057	0.0057	0.0011	0.012	0.0013	0.0046	0.0034
166	Er	0.1	0.081	0.081	N/D	0.046	0.0075	0.0032	0.078
169	Tm	0.0059	0.003	0.004	0.001	0.0061	0.0046	0.001	0.001
172	Yb	0.055	0.013	0.0043	0.0089	0.013	0.02	0.0044	0.017
175	Lu	0.0018	0.00096	N/D	N/D	N/D	N/D	0.00097	0.00096
178	Hf	0.0096	0.0032	0.0032	0.0033	0.019	N/D	0.0066	0.0032
181	Ta	0.0038	N/D	0.0019	N/D	0.021	0.0011	0.0019	0.00098
182	W	0.11	0.065	0.11	0.13	0.07	0.31	0.07	0.077
185	Re	0.012	0.0049	0.012	0.0051	0.0025	0.0058	0.0076	N/D
189	Os	0.0049	N/D	0.01	0.0051	0.005	0.011	0.005	0.015
195	Pt	0.021	0.026	0.0044	0.018	0.013	0.0051	0.017	0.017
202	Hg	0.057	0.019	0.029	0.029	0.059	0.022	0.039	0.058
205	Tl	0.1	0.084	00.064	0.04	0.041	0.039	0.038	0.052
208	Pb	1.2	0.95	0.94	2	1.3	0.93	12	10
232	Th	0.02	0.0088	0.012	0.0079	0.009	0.027	0.0056	0.0067
238	U	0.26	0.045	0.044	0.023	0.073	0.094	0.032	0.05

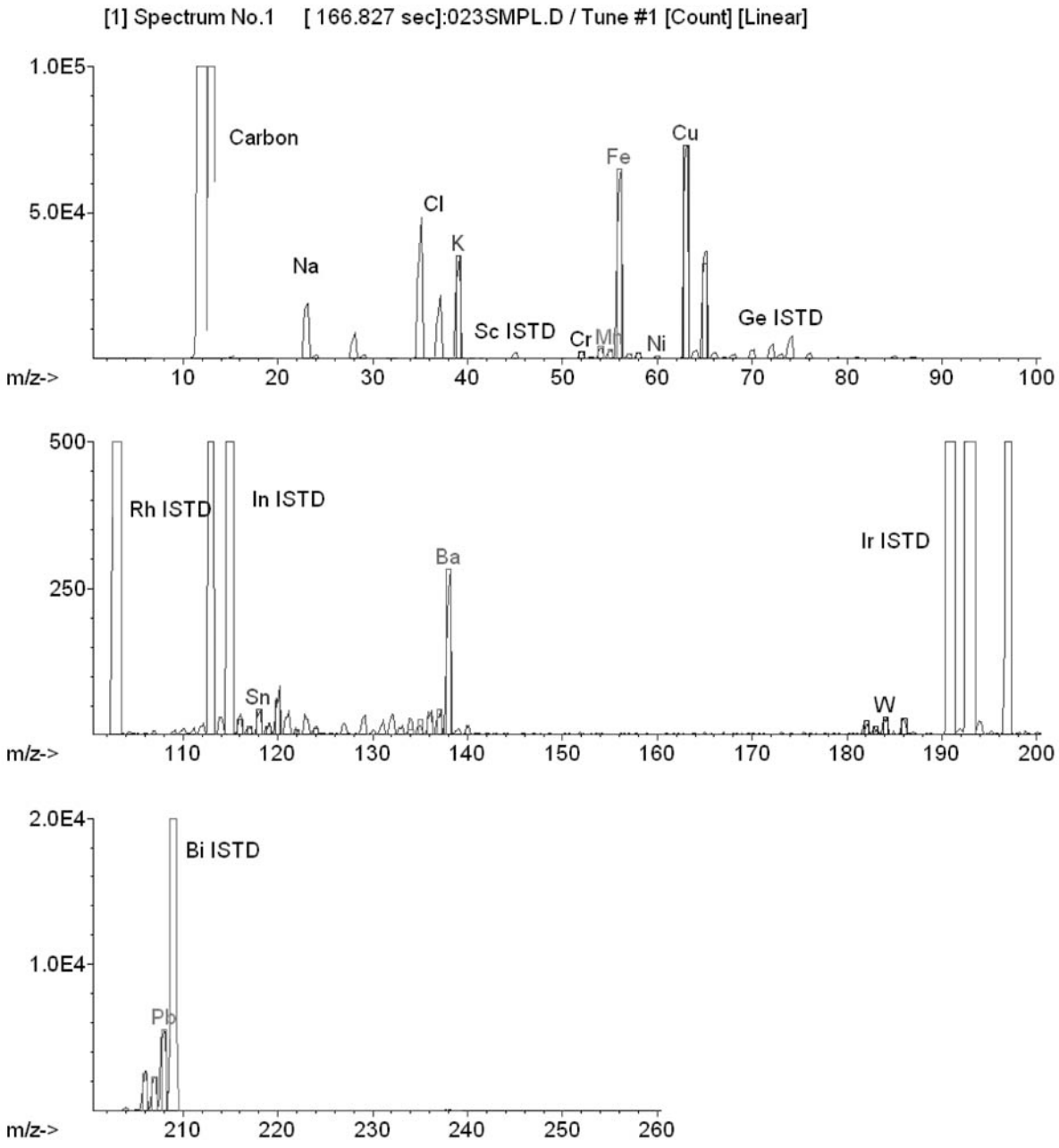

Figure 6a. Full scan (in He mode) of lead-crystal stored bourbon sample. The "major" peaks are indicated, including spectral fit for higher intensity peaks.

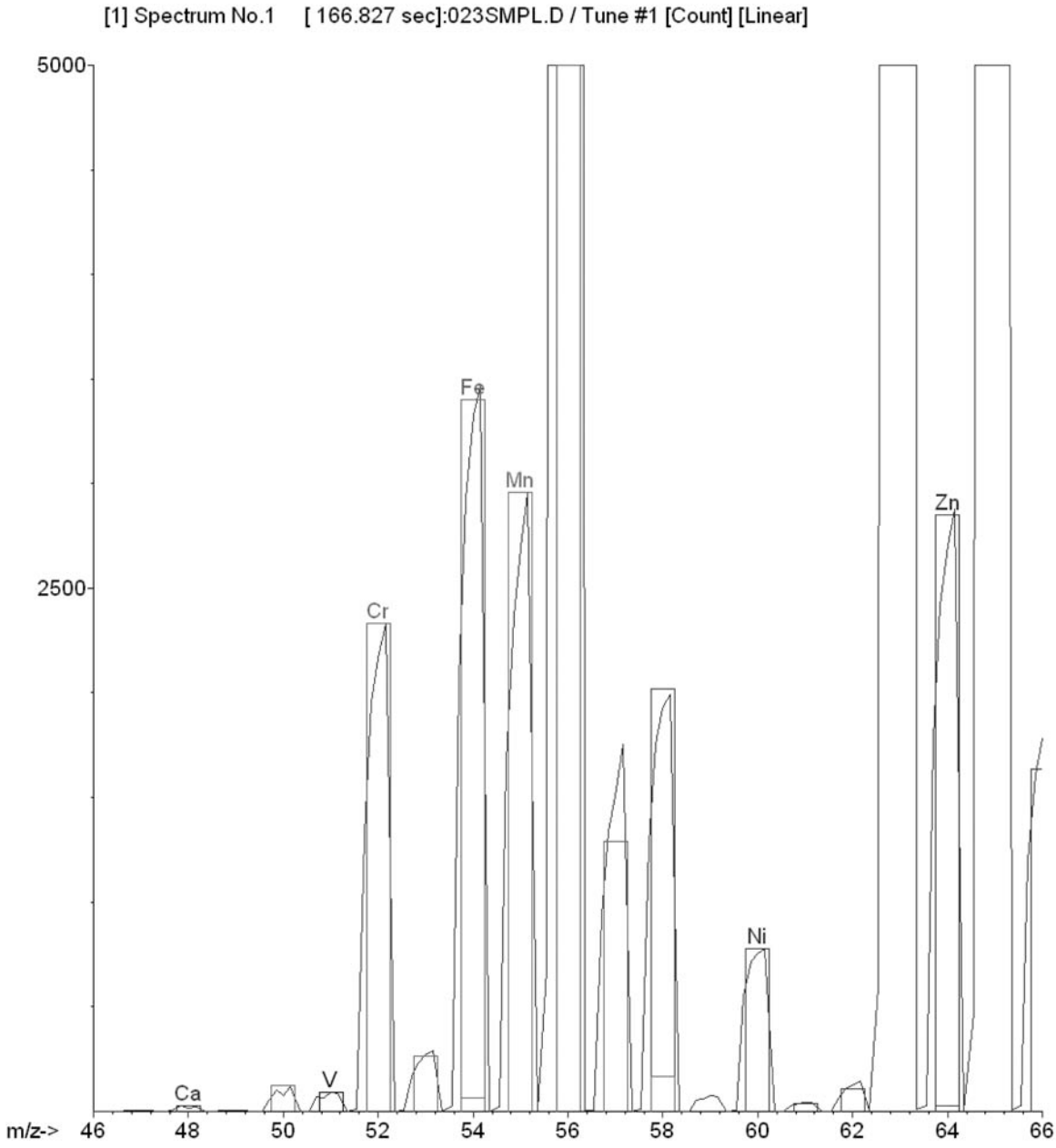

Figure 6b. Zoomed spectrum for those elements suffering from interferences in this matrix. Note good spectral fit, particularly for Cr (suffers from ArO, ArC, and ClO interferences).

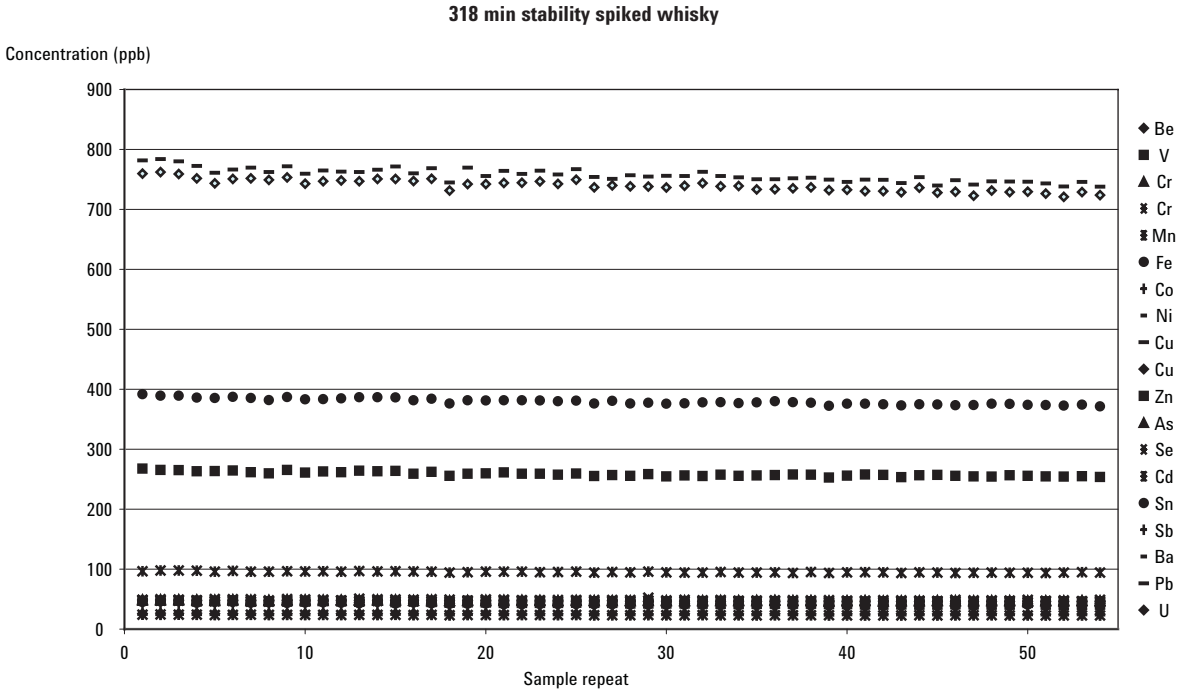

Figure 7. Stability for a spiked Highland malt sample (dilution corrected) taken over 5 hours 18 minutes (54 repeat measurements). Measurement precision was < 2% for almost all elements.

Conclusions

The analysis of high percentage alcoholic beverages using the 7500cx ICP-MS is routine after a simple acidification/dilution. The use of the ORS in the appropriate gas mode efficiently removes the plasma-based and matrix-based interferences, improving detection limits and reliability of the analysis with a simple set of conditions. The use of helium mode also allows interference-free semi-quantitative analysis, permitting greater elemental coverage and rapid screening.

References

1. Achieving Optimum Throughput in ICP-MS Analysis of Environmental Samples with the Agilent 7500ce ICP-MS, Agilent ICP-MS Journal 27, page 4; May 28, 2006, 5989-5132EN

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Speciated Isotope Dilution for the Determination of Methylmercury in Tuna Fish by GC-MS

Application Note

Environmental

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Abstract

A GC-MS with electron impact ionization was used for the development of a speciation method for the determination of methylmercury in fish samples. The method is based on isotope dilution using a spike containing 201Hg-enriched methylmercury. The spike was applied to the determination of methylmercury in tuna samples with excellent results.

Agilent Technologies

Introduction

Among the various mercury species, methylmercury is the most hazardous because of its accumulative and persistent character in the environment. Sensitive, specific, and precise analytical methods are needed to perform studies at ambient levels. Already the first step in the analysis, the isolation of methylmercury from the sample matrix can be troublesome. Since recovery of the analyte from some matrices is not always quantitative, recovery factors during isolation must be determined. This is usually done by standard addition techniques or recently by using isotope dilution mass spectrometry (IDMS).

Isotope dilution (ID) methodologies provide superior accuracy and precision compared to more common calibration strategies. ID for trace element speciation has been widely applied using ICP-MS and, recently, using GC-MS, a routine technique in testing laboratories.

Experimental

Reagents

Monomethylmercury chloride (96%) was obtained from Aldrich (Steinheim, Germany). Stock solutions were prepared by dissolving the salt in a 3:1 mixture of acetic acid (Merck, Darmstadt, Germany) and methanol (Merck). All standard solutions were kept in the dark at –18 °C and diluted working solutions were prepared by weight daily before the analysis. Acetic acid (Merck) and methanol (Merck) were used for the extraction of the organotin compounds from the solid matrices.

Sodium tetraethyl borate (Galab, Geesthacht, Germany) solutions of 2% (w/v) were prepared daily in 0.1 M sodium hydroxide solution (Merck).

A buffer solution at pH 5.3 was prepared by mixing appropriate volumes of 0.2 M acetic acid (Merck) and 0.2 M sodium acetate (Merck) solutions.

The spike solution (^{201}Hg-enriched monomethylmercury) was obtained from ISC-Science (Oviedo, Spain), diluted by weight with a mixture of methanol and acetic acid (3:1), and stored in the dark at –18 °C. Table 1 shows the isotopic composition as well as the concentration of the butyltin species in the spike solution.

Table 1. Isotopic Composition (Content %) and Concentration of the ^{201}Hg-Enriched Monomethylmercury (Uncertainty Corresponds to 95% Confidence Interval)

Hg-196	**Hg-198**	**Hg-199**	**Hg-200**
<0.01	0.043 (2)	0.109 (5)	0.890 (10)
Hg-201	**Hg-202**	**Hg-204**	
96.495 (29)	2.372 (22)	0.091 (5)	
Concentration: 5.49 ± 0.02 µg g-1 as Hg			

Additional information on www.isc-science.com

Instrumentation

GC/MS: Chromatographic analysis was performed with an Agilent (Agilent Technologies, Santa Clara, CA) gas chromatograph, model 6890N, fitted with a split/splitless injector and an HP-5MS capillary column (cross-linked 5% phenyl methyl siloxane, 30 m × 0.25 mm id × 0.25 µm coating). The gas chromatograph was equipped with an Agilent (Agilent Technologies) mass spectrometric detector, model 5973, network MSD (quadrupole based).

Helium was employed as carrier gas with a constant flow of 1.2 mL min^{-1}. The column temperature was initially held at 60 °C for 1 minute, increased at 30 °C min^{-1} to a final temperature of 300 °C. Injection was performed using a split/splitless injector in splitless mode. The transfer line and ion source temperatures were at 280 and 230 °C, respectively. Electron impact ionization was performed at an electron energy of 70 eV. The measurement of isotope ratios for methylmercury was performed on the molecular ion using 10-ms dwell-time per mass.

The solid-phase microextraction (SPME) device used for manual extraction, a holder assembly and several replaceable divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/PDMS, 50 µm/30 µm) fibers were purchased from Supelco (Madrid, Spain).

Extraction and Derivatization of Methylmercury from Tuna Fish Samples

For extraction, approximately 0.4 g of sample was spiked with a solution of the ^{201}Hg-enriched methylmercury and mixed with 15 mL of a saturated sodium chloride solution and 100 µL of concentrated hydrochloric acid. The mixture was shaken mechanically at room temperature for 5 hours.

Three milliliters of extract was adjusted to pH 5.3 with 3 mL of acetic acid/sodium acetate buffer in SPME glass vials; 1 mL of sodium tetraethyl borate was added and the vial

was then immediately closed with a PTFE-coated silicon rubber septum. The SPME needle pierced the septum and the fiber was exposed to the solution headspace for 15 minutes at room temperature. The solution was intensively stirred with a PTFE-coated magnetic stirring bar with constant velocity. Finally, the fiber was withdrawn into the needle and transferred to the GC injector for thermal desorption for 1 minute at 260 °C. During headspace solid-phase microextraction (HS-SPME), the temperature was controlled by immersing the sample vials in a water bath.

Results and Discussion

Isotope Ratio Measurements by GC-MS

While elemental isotope ratios can be easily obtained with ICP-MS, in GC/MS the isotopic pattern in molecular ions is different from that of the naturally occurring elements due to the contributions from the organic groups attached to the metal because of the presence of ^{13}C. The contribution of ^{13}C to the observed *m+1* ions can be calculated in a fairly straightforward way, by applying equation 1:

$$I_{m+1} = I_m \bullet n x_{13C} \quad (1)$$

where x_{13C} is the relative abundance of ^{13}C with respect to ^{12}C (0.0111/0.9899), n is the number of C atoms in the molecular ion, and I is the intensities of the ions *m* and *m+1*, respectively. The measured signal intensities were corrected by monitoring five molecular clusters, corresponding to the ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, and ^{202}Hg isotopes, taking into account the ^{13}C contributions to *m+1*. The intensity (I) correction equations used were:

$$^{198}Hg = {}^{198}I \quad (2)$$
$$^{199}Hg = {}^{199}I - x(^{198}Hg) \quad (3)$$
$$^{200}Hg = {}^{200}I - x(^{199}Hg) \quad (4)$$
$$^{201}Hg = {}^{201}I - x(^{200}Hg) \quad (5)$$
$$^{202}Hg = {}^{202}I - x(^{201}Hg) \quad (6)$$

where x is the contribution factor *m+1*. The selected molecular clusters for the measurement of methylmercury by GC-MS and the contribution factor x are given in Table 2.

Table 2. Monitored Masses and Contribution Factors for Methylmercury

Corresponding Hg isotopes	*m/z* selected for SIM mode ($MeEtHg^+$)
198	242
199	243
200	244
201	245
202	246

X (*m* + *1*) = 0.034

Analysis of Reference Materials

Methylmercury was determined in the reference material BCR 464 (tuna fish) by the proposed ID procedure. Three independent spiking experiments were made on each certified reference material and each sample was injected three times in GC-MS systems. The overall results obtained for the reference material by GC-MS are given in Table 3.

Table 3. Determination of Methylmercury in BCR 464 Using the 202/201 Isotope Ratio for Quantitation (Data in µg g^{-1} as Hg)

Replicate	Methylmercury
1	5.09 ± 0.06
2	5.02 ± 0.09
3	5.04 ± 0.05
Average	**5.05 ± 0.04**
RSD (%)	0.71
Certified value	***5.12 ± 0.16***

The concentration values obtained for methylmercury in the certified reference material BCR 464 show an excellent agreement between the certified and found values.

Conclusions

A precise and accurate method for the determination of methylmercury in fish samples has been developed. A single injection allows the concentration of methylmercury in the samples to be computed quickly, without the need for time-consuming calibration, standard addition, or recovery correction procedures. The method corrects for all possible errors in the speciation of methylmercury, provides low detection limits, and is fast and simple to apply by untrained personnel.

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Agilent Technologies

Analysis of Plant Materials by VaporGeneration AA

Application Note

Atomic Absorption

Author

Keith G. Brodie

Introduction

Arsenic and antimony are widely used in modern industries although they are toxic to both man and animals. Significant levels of these elements can be found throughout our environment in such diverse sources as soils, plants, fish and animals. Selenium, on the other hand, is an essential trace element but can be toxic at higher levels. The essential role of selenium in animal nutrition was first demonstrated in 1957 [1] but man's requirements are not yet fully defined.

The concentration of these elements in foods is dependent on the soil conditions and ultimately on the methods of preparation of the food. The levels found in animal products is dependent on the plant material or other animal food source.

It is of considerable interest therefore to establish the level of these elements, as well as other toxic metals, in soils, plants and animal tissues.

Arsenic, antimony and selenium have been determined at very low levels by atomic absorption for many years, and vapor generation atomic absorption offers the most sensitive means by which they can be measured.

The results of extensive collaborative studies on acid digestion techniques, and hydride generation AA for the determination of arsenic and selenium in foods have been reported [2,3]. Several laboratories were involved in those reports.

Sample digestion procedures have ranged from ashing in a furnace with a magnesium nitrate solution [4], to hot digestion in acids usually comprising nitric and perchloric mixtures [5]. Agilent instruments have been used for the vapor generation measurement of selenium levels in plant and biological material after suitable acid digestion [5,6].

Many laboratories are reluctant to use perchloric acid with digestions of organic materials because of the potential risks involved [7,8]. For this reason a number of digestion procedures have been devised which have eliminated perchloric acid. Sulphuric acid increases the boiling point of an acid digestion mixture and improves

the action of the other oxidizing acids such as nitric. The only disadvantage of sulphuric acid is the tendency to form insoluble compounds but the presence of nitric acid avoids the precipitation of trace metals with major components of the sample. Digestion mixtures of sulphuric, nitric and hydrogen peroxide have been used successfully for plant materials [7].

The results of the determination of arsenic, antimony and selenium in NBS orchard leaves (No 1571) by the vapor generation technique are reported here. A digestion procedure was prepared which did not contain perchloric acid.

Instrumentation

An Agilent AA-1475 with background correction and the VGA-76 were used in this study. The background signal was examined for all solutions, but no background was found. Measurements were therefore reported with the background corrector off. Hollow cathode lamps were operated at the recommended conditions and an Agilent 9176 strip chart recorder displayed the traces.

For development of this analytical technique samples were presented manually.

Absorbance mode was used and five 3-second integrations were taken.

The printer was an HP 82905A model. The reagents used for the VGA-76 were as follows:

Acid channel	Concentrated hydrochloric acid.
Sodium borohydride channel	0.6% NaBH4in 0.5% NaOH and 10% KI.

Sample Treatment

NBS orchard leaves (No. 1571) were dried at 80 °C and approximately 0.5 g samples were accurately weighed and prepared for analysis. The digestion procedure consisted of the addition of 5 mL concentrated sulphuric with swirling in a beaker. A 5 mL volume of 30% v/v hydrogen peroxide was added slowly and the reaction allowed to proceed. This addition of hydrogen peroxide was repeated, and when the reaction had subsided, the digestion mixture was heated on a hot plate.

After cooling, 2 mL of concentrated nitric acid was added and the mixture heated for about 2 hours to remove excess nitric acid. A further addition of nitric acid with heating may be required until the solution is a light straw color.

The digested mixture was made up to 25 mL to give a final solution in 7 M hydrochloric acid. This stock solution was used directly for the determination of selenium, but was diluted 1 in 10 with 7 M hydrochloric acid for the determination of arsenic and antimony.

Some quantities of powdered orchard leaves were also spiked with known amounts of arsenic, antimony and these samples were taken through the same digestion procedure. A standard additions calibration was thus established. Replicate samples were taken for each. Blank reagents were also prepared.

Samples were prepared in 7 M hydrochloric acid to ensure that any Se^{VI} formed in the digestion procedure, was reduced to the required Se^{IV} prior to analysis. This treatment is commonly used [5,6,9,10].

The reduction of As^{V} (or Sb^{V}) to As^{III} (or Sb^{III}) was achieved by the inclusion of potassium iodide in the sodium borohydride solution [9].

Results and Discussion

Figures 1 and 2 show chart recorder traces for the standard additions measurement of the digests for arsenic and antimony respectively. Approximately 40 seconds are required for the signal to reach an equilibrium value after introduction of the sample. Replicate absorbance readings were taken in the plateau region. It is apparent on Figure 2 that a spike appears on completion of the signal, and this is due to a brief introduction of air before the next solution is pumped. Such an occurrence is not uncommon.

Standard additions calibrations for arsenic and antimony are featured in Figures 3 and 4 respectively. The mean absorbance is shown and two values are represented by the error bars on the Figures.

That the standard additions technique was necessary, is demonstrated by aqueous calibrations for those elements on the same graphs. The calibration slopes are clearly different.

Table 1 shows the results obtained for arsenic and antimony in the orchard leaves. Agreement with the certificate was good.

Table 1 NBS Orchard Leaves No. 1571

Element	Certificate value µg/g	Found* µg/g
As	10 ± 2	11 ± 2
Sb	2.9 ± 0.3	2.7 ± 0.2

* Mean of replicate determinations.

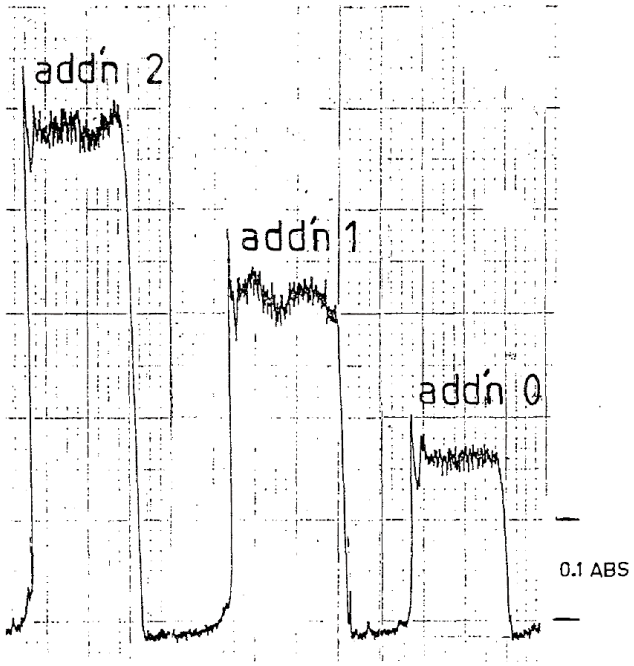

Figure 1. Chart recorder traces for the measurement of arsenic in digested leaves.

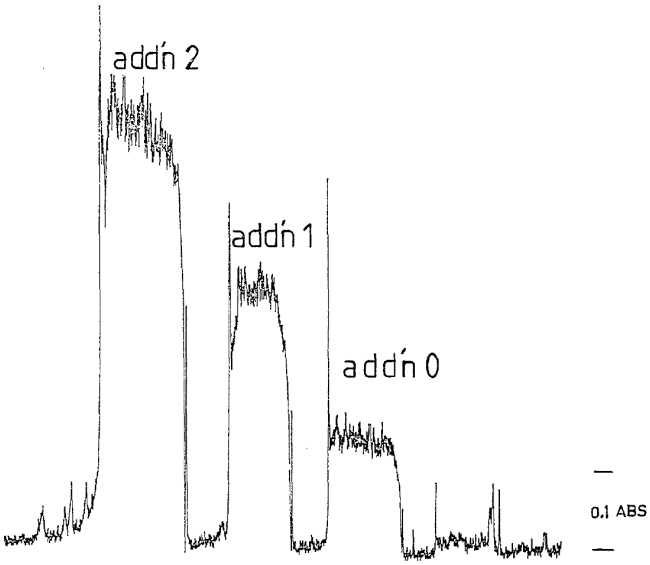

Figure 2. Chart recorder traces for the measurement of antimony in digested leaves.

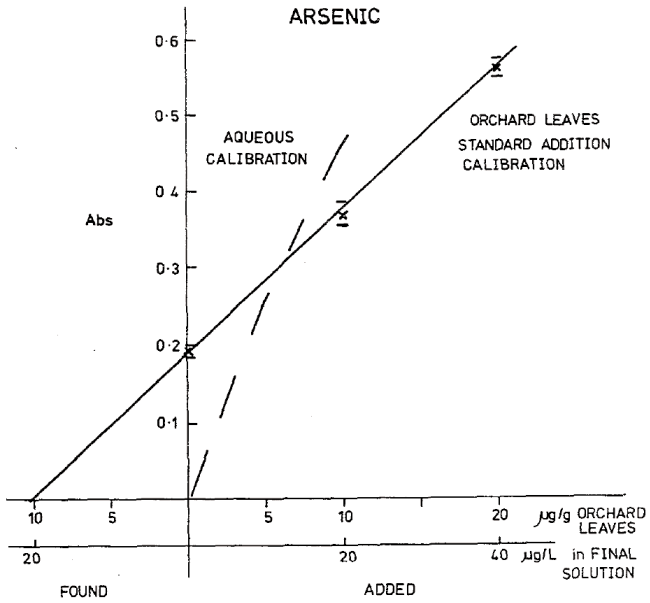

Figure 3. Calibration for the determination of arsenic.

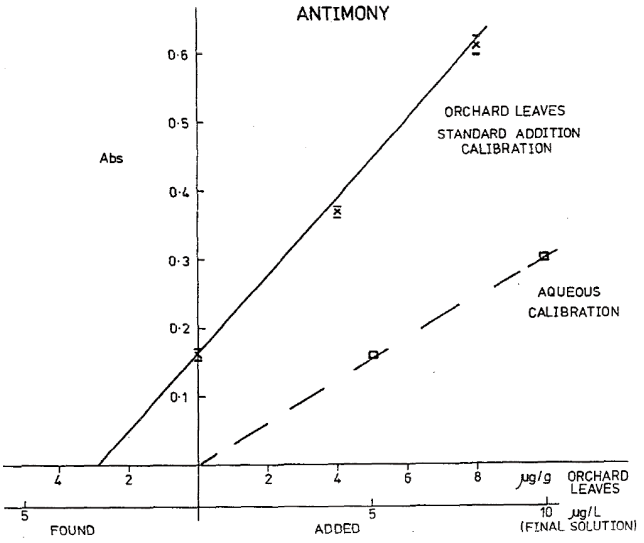

Figure 4. Calibration for the determination of antimony.

The level of selenium in the leaves was quite low (0.08 µg/gm) and a value of 0.05 was obtained from just one measurement by the standard additions technique.

No further study was made on selenium here but details of extensive work on selenium determination in agricultural materials by the VGA-76 has been recently reported [10].

An alternative digestion procedure was also attempted in which nitric and sulphuric acids only were used. The mixture was heated to remove excess nitric acid and the final solution was a light straw color. The recoveries from some samples analysed by this procedure were low, and this procedure was not pursued further.

Summary

The VGA-76 has been used to determine the amount of arsenic and antimony in digested orchard leaves. A digestion procedure was adopted which avoided the use of perchloric acid and it was necessary to use the standard additions calibration procedure.

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