

# Determination of Elements in Biological and Botanical Materials by Inductively Coupled Plasma Atomic Emission and Mass Spectrometry After Extraction With a Tertiary Amine Reagent

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A simple inductively coupled plasma atomic emission and mass spectrometric (ICP-AES and ICP-MS) analysis procedure for biological and botanical materials was developed based on an extraction with a commercial, water-soluble tertiary amine solution. Standard reference materials were dispersed in a 10% v/v amine mixture at pH 8 and the resulting dispersion was agitated for 30 min at room temperature. After centrifugation, Al, Ca, Cu, Fe, Mg, Mn, Na, K and Zn were determined by ICP-AES and As, Ba, Ca, Cu, Pb, Mn, Mo, Rb, Se, Cr and Zn were determined by ICP-MS in the supernatant. Recoveries of nearly all elements in most samples were close to 100%, and no significant difference at a 95% confidence level was found between determined and certified values. Low recoveries were observed for Al and Fe in all materials and for Ca in spinach and apple leaves. The simple procedure is useful for screening purposes in the analysis of biological and botanical materials.

**Keywords:** *Inductively coupled plasma atomic emission spectrometry; inductively coupled plasma mass spectrometry; water-soluble tertiary amine reagent; biological and botanical materials; extraction*

Inductively coupled plasma atomic emission spectrometry (ICP-AES) and mass spectrometry (ICP-MS) are well-established techniques and widely applied for multi-element analysis of diverse materials. Most elements are determined in solutions, and the conversion of the solid material to a representative solution is the most critical and often slowest step in the analysis. Microwave procedures<sup>1</sup> are employed widely, but new, simpler techniques are still needed. Direct approaches, such as solid-liquid extraction with nitric acid<sup>2,3</sup> and leaching with complexing reagents,<sup>2</sup> can be effective and rapid.

De Boer and Maessen<sup>2</sup> used a dilute nitric acid solution to extract Cd, Cu, Fe, Pb, Mn and Zn from bovine liver. Their approach included a 24 h extraction at room temperature followed by centrifugation, filtration and direct measurement by ICP-AES. Minami *et al.*<sup>3</sup> also extracted Cd, Cu, Pb and Mn quantitatively from biological materials with a 1 mol l<sup>-1</sup> nitric acid solution after heating the dispersion to 40 °C for 5 min in an ultrasonic bath.

The extraction of metals with a tetramethylammonium

hydroxide (TMAH) solution was also investigated.<sup>2</sup> A sample dispersion was heated to 65 °C for 2 h in a water-bath prior to analysis. This procedure was simple, safe and suitable for a large number of samples. The feasibility of the procedure was demonstrated by the determination of Mn, Zn, Cu and Fe in bovine liver. The best results were obtained for Mn and Zn. However, owing to the instability of the TMAH reference solutions, the method of standard additions was required. The applicability of TMAH with ICP-AES was extended by Uchida *et al.*<sup>4</sup> They dispersed a botanical sample in a solution containing TMAH and EDTA, and the resulting dispersion was heated for 4 h at 120 °C in a closed vessel. The ICP-AES determination of 12 elements in botanical and zoological materials yielded acceptable results, except for low recoveries of Al and Fe. They also evaluated some problems related to the preparation of the reference solutions.

We have extended this simple extraction approach by applying a commercial reagent mixture of water-soluble tertiary amines and EDTA<sup>5,6</sup> to 17 metals in biological and botanical materials. In previous studies this reagent improved the ICP-AES determination of low Si concentration levels in foods and coral soils after dissolution with hydrofluoric acid,<sup>5</sup> and enhanced signals, and decreased polyatomic chloride interferences in the direct ICP-MS determination of As and Se in biological samples.<sup>6</sup> The main objective of this investigation was to develop a simple procedure with this amine mixture for the extraction of elements from biological and botanical materials followed by direct ICP-AES and ICP-MS determinations.

## EXPERIMENTAL

### Instrumentation

All ICP-AES measurements were made with a commercial system and standard ICP torch, peristaltic pump, concentric glass nebulizer and humidifier (Spectroflame, Spectro Analytical Instruments, Fitchburg, MA, USA). The optical system consists of two sequential spectrometers. One spectrometer is filled with nitrogen and its wavelength range is 162.176–462.227 nm. The second is an air-path instrument covering a wavelength range of 237.768–787.694 nm. Background corrections were made with instrument software provided (Windows version). After scanning a blank and a sample solution in the wavelength range programmed, the background correction was set selecting the background positions for each peak. The instrumental parameters are listed in

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Table 1. A variable-speed laboratory centrifuge (maximum speed 10 000 rev min<sup>-1</sup>; capacity: twelve 15 ml tubes; Fisher Scientific, Pittsburgh, PA, USA, Model 225, Cat. No. 4-978-50) and magnetic stirrers were used for extractions. All procedures were conducted in 15 ml conical-bottomed centrifuge tubes.

Trace elements were measured by ICP-MS (ELAN 5000a, Perkin-Elmer SCIEX, Norwalk, CT, USA) equipped with a standard torch, cross-flow nebulizer and Ni sampler and skimmer cones. The plasma conditions and measurement parameters used are listed in Table 2.

### Reagents and Samples

Solutions containing 10% v/v mixed amines (CFA-C, Spectrasol, Warwick, NY, USA) were prepared at pH 11, 8, 7, 5 and 4 by adding sub-boiled nitric acid. Solutions of 20 mg l<sup>-1</sup> Sc and 2.5 mg l<sup>-1</sup> Y were prepared from stock standard solutions (Spex Industries, Edison, NJ, USA; Cat. No. PLSC2-2X and PLY2-2X, respectively).

For ICP-AES measurements, reference solutions containing Al, Cu and Mn (1, 2 and 5 mg l<sup>-1</sup> of each), Ca and Mg (10, 50 and 100 mg l<sup>-1</sup> of each), Fe and Zn (5, 10 and 50 mg l<sup>-1</sup> of each), and K and Na (10, 50 and 200 mg l<sup>-1</sup>) were prepared in 10% v/v mixed amine medium from single stock standard solutions (Spex Industries). An internal reference element (1 mg l<sup>-1</sup> Sc) was added to each reference solution. All ICP-MS measurements used the standard additions method and internal standardization with Y as the reference element.

National Institute of Standards and Technology (NIST) standard reference materials (SRMs) 1577b Bovine Liver,

1566a Oyster Tissue, 1570a Spinach Leaves, 1515 Apple Leaves, 8433 Corn Bran, 1567 Wheat Flour, 8415 Whole Egg Powder, 1548 Total Diet and 1568a Rice Flour were analyzed. Extracts were prepared according to the procedure described below.

### Procedure

The main objective was to develop a simple procedure employing routine laboratory apparatus and minimum analyst effort. A 0.200 g mass of an SRM was weighed, and 0.5 ml of 20 mg l<sup>-1</sup> Sc (ICP-AES) or 0.5 ml of 2.5 mg l<sup>-1</sup> Y (ICP-MS) solutions and 9.5 ml of 10% v/v mixed amines were added. The dispersion was agitated for 30 min at room temperature with a magnetic stirrer, and the resulting extract was centrifuged at 5000 rev min<sup>-1</sup> for 5 min. Aluminum, Ca, Cu, Fe, Mg, Mn, K, Na and Zn were determined by ICP-AES in the supernatant. Extracts were diluted 10-fold before ICP-MS measurements. Arsenic, Ba, Cd, Cu, Pb, Mn, Mo, Rb, Se, Sr and Zn were determined by ICP-MS. The nebulizer flow rate was optimized with a solution containing 10 ng ml<sup>-1</sup> Li, I and Pb in 10% v/v mixed amines.

Preliminary experiments evaluated the effect of the mixed amine solution pH on the efficiency of element extraction from a biological (Oyster Tissue) and a botanical (Apple Leaves) SRM. Solutions containing 10% v/v mixed amines were prepared at pH 11, 8, 7, 5 and 4. The best recoveries for most elements were obtained at pH 11 and 8. The lower pH was adopted in all later experiments, since it would be less damaging to the quartz torch.

## RESULTS AND DISCUSSION

To evaluate the stability of metals in the mixed amines reagent at pH 8, synthetic solutions containing Al, Ca, Fe, Mn and Sc were prepared. As recommended by Myers and Tracy,<sup>7</sup> Sc was added to assess its suitability as an internal reference element. Successive measurements showed that these metals remained in solution even after 3 months. The concentration of each element was constant during this period, and recoveries were close to 100% when prepared and found concentrations were compared. The precision was less than 5% (relative standard deviation) for all elements measured during this period. Thus, these sample dispersions and reference solutions could be stored without metal losses as hydroxides. Additionally, the emission intensities recorded for these elements were approximately the same in the mixed amine solution medium as those obtained in a 2% v/v nitric acid solution. This stability is a considerable improvement compared with results reported for TMAH.<sup>2,4</sup>

A 10% v/v mixed amine solution (pH 8) was used to prepare extracts from biological and botanical SRMs, and metals were determined by ICP-AES (Table 3). Determined and certified values for most elements agreed well. The best results were obtained for Cu, Mn, K, Na and Zn. For most of the materials investigated no statistical difference exists between found and certified values at the 95% confidence level. Low recoveries were observed for Ca and Mg in botanical materials, however. This could be related to insoluble silicate compounds often retained in the inorganic matrix of plant tissues. The worst recoveries were observed for Al and Fe. For these elements recoveries varied from 5 to 50%, and lower results were obtained for botanical materials. Uchida *et al.*<sup>4</sup> also observed low recoveries for these elements. Another procedure involving microwave digestion of biological samples with TMAH and EDTA<sup>8</sup> also gave low recoveries. Wieteska *et al.*<sup>9</sup> recently proposed an extraction procedure for preparation of vegetable samples and demonstrated that a quantitative dissolution of Al and Fe required hydrofluoric acid addition. Therefore, our low Al and Fe recoveries are consistent with other reports.

**Table 1** Instrumental conditions for ICP-AES measurements

Rf frequency/MHz	27.12
Rf forward power/kW	1.2
Nebulizer	Meinhard Type C
Spray chamber	Scott-type double pass
Argon gas flow rates/l min <sup>-1</sup> :	
Outer	14
Intermediate	1.0
Aerosol	1.0
Read delay/s	20
Integration time/s	2
Analytical wavelength/nm	Al I 394.401 Ca II 317.933 Cu I 324.754 Fe II 259.940 K I 766.491 Mg II 279.806 Mn II 257.610 Na I 589.592 Zn I 213.856 Sc II 361.384
Reference wavelength/nm	Sc II 361.384

**Table 2** ELAN 5000a ICP-MS operating conditions and measurement parameters

<i>ICP-MS plasma conditions—</i>	
Rf frequency/MHz	40
Rf forward power/kW	1.0
Argon gas flow rates/l min <sup>-1</sup> :	
Outer	15.0
Intermediate	0.8
Nebulizer	0.92–0.96
<i>Measurement parameters—</i>	
Resolution ( <i>m/z</i> at 10% peak height)	0.8 (normal)
Scanning mode	Peak hopping
Replicate time/ms	250
Sweeps per reading	1
Readings per replicate	1
Number of replicates	10
Points per spectral peak	1

**Table 3** Found and certified values of elements determined by ICP-AES after extraction with 10% v/v mixed amines (pH 8). Numbers represent mean values and 95% confidence intervals ( $n=4$ )

Element	SRM 1577b Bovine Liver	SRM 1566a Oyster Tissue	SRM 1570a Spinach Leaves	SRM 1515 Apple Leaves	SRM 8433 Corn Bran	SRM 1567 Wheat Flour	SRM 1568a Rice Flour
Cu/ $\mu\text{g g}^{-1}$							
Found	140 $\pm$ 9	57.0 $\pm$ 4.9	10.9 $\pm$ 0.6	5.2 $\pm$ 0.5	1.35 $\pm$ 0.4	1.75 $\pm$ 0.7	1.90 $\pm$ 0.5
Certified	160 $\pm$ 8	66.3 $\pm$ 4.3	12.2 $\pm$ 0.6	5.6 $\pm$ 0.2	2.47 $\pm$ 0.4	2.0 $\pm$ 0.3	2.4 $\pm$ 0.3
Mn/ $\mu\text{g g}^{-1}$							
Found	9.1 $\pm$ 0.7	9.0 $\pm$ 1.1	59.5 $\pm$ 8.7	46 $\pm$ 4	2.15 $\pm$ 0.1	7.4 $\pm$ 0.4	19.0 $\pm$ 0.5
Certified	10.5 $\pm$ 1.7	12.3 $\pm$ 1.5	75.9 $\pm$ 1.9	54 $\pm$ 3	2.55 $\pm$ 0.3	8.5 $\pm$ 0.5	20.0 $\pm$ 1.6
Zn/ $\mu\text{g g}^{-1}$							
Found	110 $\pm$ 11	690 $\pm$ 53	73 $\pm$ 5	10.7 $\pm$ 0.3	12.4 $\pm$ 2.2	8.8 $\pm$ 1.0	17.8 $\pm$ 0.7
Certified	127 $\pm$ 16	830 $\pm$ 57	82 $\pm$ 3	12.5 $\pm$ 0.3	18.6 $\pm$ 2.2	10.6 $\pm$ 1.0	19.4 $\pm$ 0.5
Na (%)							
Found	0.238 $\pm$ 0.040	0.375 $\pm$ 0.009	1.756 $\pm$ 0.041	0.00249 $\pm$ 0.00013	0.0398 $\pm$ 0.0018	0.00082 $\pm$ 0.00017	0.00065 $\pm$ 0.00015
Certified	0.242 $\pm$ 0.006	0.417 $\pm$ 0.013	1.818 $\pm$ 0.043	0.00244 $\pm$ 0.00012	0.0430 $\pm$ 0.0031	0.00080 $\pm$ 0.00015	0.00066 $\pm$ 0.00008
K (%)							
Found	0.880 $\pm$ 0.017	0.727 $\pm$ 0.048	2.719 $\pm$ 0.071	1.49 $\pm$ 0.04	0.0525 $\pm$ 0.0025	0.114 $\pm$ 0.002	0.114 $\pm$ 0.005
Certified	0.994 $\pm$ 0.003	0.790 $\pm$ 0.047	2.903 $\pm$ 0.052	1.61 $\pm$ 0.02	0.0566 $\pm$ 0.0075	0.136 $\pm$ 0.004	0.128 $\pm$ 0.0008
Ca (%)							
Found	0.0127 $\pm$ 0.0006	0.139 $\pm$ 0.014	0.260 $\pm$ 0.0071	0.661 $\pm$ 0.029	0.0412 $\pm$ 0.0008	0.017 $\pm$ 0.001	0.0103 $\pm$ 0.0008
Certified	0.0116 $\pm$ 0.0004	0.196 $\pm$ 0.019	1.527 $\pm$ 0.0041	1.526 $\pm$ 0.015	0.0420 $\pm$ 0.0038	0.019 $\pm$ 0.001	0.0118 $\pm$ 0.0006
Mg (%)							
Found	0.0550 $\pm$ 0.0018	0.099 $\pm$ 0.007	0.59 $\pm$ 0.01	0.234 $\pm$ 0.004	0.0785 $\pm$ 0.0014	0.0355 $\pm$ 0.0005	0.042 $\pm$ 0.001
Certified	0.0601 $\pm$ 0.0028	0.118 $\pm$ 0.017	NA*	0.271 $\pm$ 0.004	0.0818 $\pm$ 0.0059	NA	0.056 $\pm$ 0.002

\* NA = Not available.

Trace elements were determined by ICP-MS, and the results obtained for food and biological SRMs are given in Table 4. Three extracts prepared from Whole Egg Powder led to relative standard deviations from 2.0% (Ba) to 16% (Se) for all elements. For the determined and reference values of all elements, linear correlation coefficients were 0.9985, 0.9998,

0.9998 and 0.9999 for Bovine Liver, Oyster Tissue, Total Diet and Whole Egg Powder, respectively. These correlations indicate a close agreement between found and certified values. Preliminary measurements with botanical SRMs, such as Apple Leaves and Spinach Leaves, gave low recoveries mainly for Ba and Sr. Low recoveries were also observed for Ca in the

**Table 4** Found and certified values of elements determined by ICP-AES and ICP-MS after extraction with 10% v/v mixed amines (pH 8). Numbers in parentheses indicate non-certified values

Element	SRM 1577b Bovine Liver	SRM 1566a Oyster Tissue	SRM 1548 Total Diet	SRM 8415 Whole Egg Powder
$^{55}\text{Mn}/\mu\text{g g}^{-1}$				
Found	11.0	11.7	4.9	1.75
Certified	10.5 $\pm$ 1.7	12.3 $\pm$ 1.5	5.2 $\pm$ 0.4	1.78 $\pm$ 0.38
$^{63}\text{Cu}/\mu\text{g g}^{-1}$				
Found	174	87.2	2.8	2.65
Certified	160 $\pm$ 8	66.3 $\pm$ 4.3	2.6 $\pm$ 0.3	2.70 $\pm$ 0.35
$^{66}\text{Zn}/\mu\text{g g}^{-1}$				
Found	125	875	32.7	68.3
Certified	127 $\pm$ 16	830 $\pm$ 57	30.8 $\pm$ 1.1	67.5 $\pm$ 7.6
$^{75}\text{As}/\mu\text{g g}^{-1}$				
Found	0.049	14.2	0.11	0.01
Certified	(0.05)	14.0 $\pm$ 1.2	NA*	(0.01)
$^{82}\text{Se}/\mu\text{g g}^{-1}$				
Found	1.49	3.88	0.160	2.07
Certified	0.73 $\pm$ 0.06	2.21 $\pm$ 0.24	0.245 $\pm$ 0.005	1.39 $\pm$ 0.17
$^{85}\text{Rb}/\mu\text{g g}^{-1}$				
Found	13.5	3.0	5.0	3.1
Certified	13.7 $\pm$ 1.1	(3)	(4.8)	NA
$^{88}\text{Sr}/\mu\text{g g}^{-1}$				
Found	0.138	10.1	3.6	5.84
Certified	0.136 $\pm$ 0.001	11.1 $\pm$ 1.0	NA	5.63 $\pm$ 0.46
$^{98}\text{Mo}/\mu\text{g g}^{-1}$				
Found	3.5	0.18	0.16	0.225
Certified	3.5 $\pm$ 0.3	NA	(0.27)	0.247 $\pm$ 0.023
$^{114}\text{Cd}/\mu\text{g g}^{-1}$				
Found	0.52	3.99	0.019	0.005
Certified	0.50 $\pm$ 0.03	4.15 $\pm$ 0.38	0.028 $\pm$ 0.004	(0.005)
$^{137}\text{Ba}/\mu\text{g g}^{-1}$				
Found	0.051	0.89	1.3	3.0
Certified	NA	NA	NA	(3)
$^{208}\text{Pb}/\mu\text{g g}^{-1}$				
Found	0.124	0.340	0.06	0.020
Certified	0.129 $\pm$ 0.004	0.371 $\pm$ 0.014	(0.05)	0.061 $\pm$ 0.012

\* NA = Not available.

ICP-AES measurements. This could be related to the presence of non-easily extractable complexes containing alkaline-earth elements in the inorganic matrix of plant tissues.

As recommended by Kumina and co-workers,<sup>10,11</sup> ultrasonic treatment (60 min at 45 °C) was also tested for the same biological and botanical SRMs, but elemental recoveries were not improved.

In conclusion, the simple extraction with a 10% v/v water-soluble tertiary amine mixture at pH 8 agitated for 30 min at room temperature provides fast sample preparation for direct ICP-AES and ICP-MS analyses. The entire sample preparation step takes less than 40 min and several samples can be treated simultaneously without extra analyst's effort. This simple, rapid procedure is useful for screening purposes in the analysis of biological and botanical materials.

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