Fresenius J Anal Chem (1997) 357 : 1185–1191 © Springer-Verlag 1997

ORIGINAL PAPER

A. M. S. Nyomora · R. N. Sah · P. H. Brown
R. O. Miller

Boron determination in biological materials by inductively coupled plasma atomic emission and mass spectrometry: effects of sample dissolution methods

Received: 13 June 1996 / Revised: 17 September 1996 / Accepted: 19 September 1996

Abstract This study compares four sample dissolution methods for Boron determination in two National Institute of Standard and Technology (NIST) botanical Standard Reference Materials (SRMs) and three Agriculture Canada/NIST RMs, each having a reference (certified or best estimate) B concentration. The dissolution treatments consisted of: 1) dry ashing at 500° C, 2) wet digestion with $HNO_3 + H_2O_2$, 3) extraction with hot HNO_3 and 4) closed vessel microwave dissolution. The samples were spiked before and after imposing dissolution treatments to study B recovery by inductively coupled plasma mass spectrometric (ICP-MS) analysis. Microwave digests of NIST SRM 1515 and some in-house RMs were also used to compare the B values of ICP-MS and ICP-AES (atomic emission spectrometry). While all three digestion methods (dry ashing, wet ashing and microwave) dissolved botanical samples, only the microwave method worked well for animal tissues. In terms of B values in these materials, there was no significant difference among the three digestion treatments. Near 100% recovery of B spiked before and after the sample dissolution indicates that there may not be a significant loss of B during the dissolution process used in this study. Extraction with hot HNO_3 was as effective as the three digestion treatments, and B values for this method agreed well with reference values. For the botanical materials studied, the B values determined by ICP-AES were not significantly different from ICP-MS values. This study shows that a simple, time and labor efficient hot HNO_3 extraction is as effective as other digestion/dissolution methods for quantitative B recovery from biological materials.

Introduction

Boron is an essential element for plants [1] and its deficiency as well as toxicity threaten crop productivity in many areas [2]. Though the essentiality of B for animal is not yet fully established [3, 4], there is growing evidence that it may have a metabolic role in human and animal nutrition [5, 6]. Excess of B in food products and drinking water is considered harmful for health [7]. Radiobiological destruction of tumor tissue by Boron Neutron Capture Therapy (BNCT) involves administering ^{10}B to diseased tissues followed by irradiation with slow neutrons [8]. This technique requires targeting of ^{10}B to appropriate tissues and precise monitoring of B concentrations in tumor and surrounding tissue samples. Therefore, a method with high precision and low detection limit is required to determine the quantity and the isotope composition of B in biological materials.

Analyte introduction in an aqueous liquid phase is conventient for most analytical instruments. Boron present in biological materials is brought into the liquid phase either by destroying the sample matrix or extracting it with some solvents. The sample matrix may be destroyed by fusion, wet or dry ashing and microwave dissolution methods. The closed-vessel (high pressure) microwave dissolution method is fast and overcomes contamination and volatilization problems of open-vessel methods. However, high-pressure methods may be hazardous due to the danger of explosion [9, 10]. Boron contamination and leaching of B from borosilicate glass digestion vessels may increase the B values [9] which may explain the higher recovery of B in wet digestion compared to the microwave dissolution method found by Bañuelos et al. [11]. Boron extraction with dilute acids also overcomes contamination and volatilization problems without dealing with strong acids and high pressure.

Boron may be analyzed colorimetrically or by absorption, emission or mass spectrometry. Several colorimetric methods which make use of reagents such as azomethine-H, carminic acid, curcumin, dianthrimide, methylene blue or quinalizarine, have been used for B analysis [12]. All spectrophotometric methods however, have high limits of detection (LOD) and suffer from matrix interferences [13]. The azomethine-H method, which has been heavily used, may not be suitable for B determination in micro-

A. M. S. Nyomora · R. N. Sah (✉) · P. H. Brown · R. O. Miller
Department of Pomology, University of California, Davis, CA 95616, USA

wave digests of biological materials because the yellow color of the digest interferes with the 420 nm wavelength used for this method. Atomic absorption and emission spectroscopy methods (AAS and AES) are not sufficiently sensitive for many applications and suffer from severe interferences, memory effects, calibration drift and background noise [14, 15]. Prompt-γ spectrometry can detect B in intact tissue, but it is a time-consuming method with relatively higher limits of quantification (1.0–2.5 $\mu g \cdot g^{-1}$) and detection (0.3–0.8 $\mu g \cdot g^{-1}$) depending on the irradiation time [16]. Lower detection limits may be possible with the prompt-γ method [6]; however, as the B concentration in the sample decreases, the counting time to achieve desired precision increases drastically, often rendering this method impractical [17]. The measured values by the prompt-γ method did not well agree with the reference values for the materials having less than 5 $\mu g \cdot g^{-1}$ B concentration [16]. Inductively coupled plasma atomic emission and mass spectrometry (ICP-AES, or ICP-MS) are fast and give linear response over a wide range of boron concentrations. However, B determination by ICP-AES suffers severe interferences from Fe [18, 19]. ICP-MS offers lower LOD's and high precision over other methods of B determination and can measure ^{10}B and ^{11}B isotopes similtaneously.

The ICP-MS signals may be affected by spectroscopic and non-spectroscopic interferences from the elements present in the sample [9, 20]. HNO_3 is an ideal acid for ICP-MS because it results in minimal corrosion of the instrument components while its components, H, O and N, are already present in air and water used as solvent [21]. Instrument noise and drift can be corrected using beryllium as internal standard [18, 22] and matrix effects may be minimized by matrix matching in the sample solutions and calibration standards [23].

Published reports comparing sample dissolution methods for B determination often contradict [11, 24, 25]. While Isaac and Jones [26] reported that the dry ashing method was as effective as the wet ashing method, others [24, 11] showed that the wet ashing method was better than the microwave or dry ashing methods. The relative effectiveness of sample dissolution methods for B extraction from biological materials is still unresolved. This study compares four sample dissolution methods for the purpose of finding a convenient method that minimizes contamination and volatilization loss, yet, quantitatively extracts B from biological materials for ICP-MS or ICP-AES analysis.

Materials and methods

Two National Institute of Standard and Technology (NIST) botanical SRMs, Agriculture Canada/NIST RMs and some in-house RMs were used for this study. Boron values for the in-house RMs were determined by ICP-AES following the microwave digestion and independently confirmed by two laboratories namely, Servi-Tech-Hastings, NE, and University of Minnesota Research Analytical Laboratory. Plant and animal materials used in this study represented a wide range in B concentration (Table 1). Dry matter contents of each material was determined and analytical values were adjusted to reflect B concentration on dry weight basis. ICP-AES (Thermo Jarrell Ash, Atom Scan 25, Menlo Park, CA) and ICP-MS (Perkin Elmer/Sciex, ELAN 500, San José, CA) were used for B determination in this study. The external calibration method was used for B determination by both methods using 0-2 $\mu g \cdot mL^{-1}$ B standards (source: 10 000 $\mu g \cdot mL^{-1}$ B Spex standard solution from Spex Industries, Inc., N. J.) in 1 mol/L HNO_3 and approximately 0.5 ppm beryllium as an internal standard. Beryllium is an excellent choice of internal standard to compensate for instrumental drift, noise and matrix interference as its mass is very close to that of boron [23, 22]. The concentration of SPEX standard was verified against NIST boric acid standard (SRM 951). The measured concentration of 0.20 μg/L NIST boric acid against the Spex standard was 0.20 ± 0.04 μg/L. Between successive measurements, the system was rinsed with 1% HNO_3 for about 30 s. For some samples, ICP-AES values determined by the external calibration method were verified by the standard addition calibration method. Trace metal grade HNO_3 (Fisher Scientific), 30% H_2O_2 (Fisher Scientific) and double deionized water (18 MΩ cm) were used throughout the study.

Effects of sample size

A preliminary study was conducted to examine the effect of sample size on B recovery using NIST botanical SRM 1515 (apple leaves). Sample sizes from 0.1 to 0.5 g were digested by the microwave dissolution method (described later) and the B concentrations in the digests were analyzed by ICP-AES using the external calibration method.

Table 1 List of biological materials used in this study and their reference B concentrations

Material	Source	Reference B Concentration[a]	
		($\mu g \cdot g^{-1}$ tissue)	Remarks
Apple SRM 1515	NIST	27 ± 2	Certified
Peach SRM 1547	NIST	29 ± 2	Certified
Tomato leaves RM 1573	NIST	30	Best estimate
Corn bran RM 8433	AGRIC CANADA/NIST	2.8 ± 1.2	Best estimate
Bovine muscle RM 8414	AGRIC CANADA/NIST	0.6 ± 0.4	Best estimate
Whole egg powder RM 8415	AGRIC CANADA/NIST	0.4 ± 0.26	Best estimate
Barley straw RM 124	UCD	9.5 ± 2.6	Best estimate
Alfalfa whole tops RM 150	UCD	27.2 ± 2.8	Best estimate
Kiwi blades RM 157	UCD	59.8 ± 4.6	Best estimate
Pistachio leaves RM 171	UCD	245 ± 14	Best estimate
Corn blades RM 184	UCD	20.3 ± 2.4	Best estimate
Alfalfa whole tops RM 483	UCD	58.1 ± 4.8	Best estimate
Sugar maple wood RM 8607	FORESTRY CANADA	6.2 ± 4.9	Best estimate

NIST = National Institute of Standard and Technology, AGRIC. CANADA/NIST = Produced by Agriculture Canada and distributed by NIST, UCD = University of California, Davis,
RM = Reference material
SRM = Standard Reference Material
[a] Certified: B concentrations certified by NIST; Best estimate: B concentrations are determined by interlaboratory analysis but not certified

Comparison of sample dissolution methods

Two NIST botanical SRMs – apple leaves (SRM 1515) and peach leaves (SRM 1547) –, one botanical RM – corn bran (RM 8433) –, and two animal RMs – whole egg powder (RM 8415) and bovine muscle (RM 8414) – were used for this study (Table 1). Three replicates of each sample were spiked with 10 μg B (using a 100 μg mL^{-1} B solution of H_3BO_3, NIST SRM 951) before the sample dissolution procedure to study the effects of dissolution treatments on B losses during the dissolution process. Boron concentrations in digests/extracts were analyzed by ICP-MS. The sample dissolution treatments were as follows:

Dry ashing. Approximately 0.5 g samples were weighed into 30 mL porcelain crucibles and ashed in a muffler furnace at 300° C for 1 h followed by 500° C for 12 h. Ashed samples were allowed to cool to room temperature, digested with 10 mL 1 mol/L HNO_3 over a hot plate at 80° C for 20 min., filtered through Whatman filter paper No. 1 and diluted to 50 mL with water.

Wet digestion. About 0.5 g samples were weighed in 75 mL graduated borosilicate glass digestion tubes to which 0.1 mL ceric ammonium nitrate and 3 mL conc. HNO_3 were added and allowed to oxidize overnight under a fume hood. The digestion mixture was heated on a digestion block at 110° C for about 30 min. After the dense fumes subsided, 2 mL of 30% H_2O_2 was added and the mixture was heated for another 30 min. Four more additions of H_2O_2 and heating were repeated and the temperature was increased to 125° C. Two milliliters conc. formic acid and few drops of 5.6 mol/L HCl were added and the mixture was heated again for 30 min. The digests were cooled to room temperature, filtered through Whatman filter paper No. 1 and made up to 50 mL with water.

Microwave dissolution. Microwave vessels, CEM model MDS-81D (CEM Corp., Mathews, NC) and Microwave oven, Amana model RS8MP (Amana Refrigeration Inc., Amana, Iowa) were used for all microwave dissolutions. Approximately 0.5 g samples were weighed in 120 mL Teflon-lined digestion vessels to which 2 mL 30% H_2O_2 and 0.5 mL conc. HNO_3 were added. The vessels were closed and predigested at room temperature for 10 min, followed by microwaving for 2 min at 296 W power and 8 min at 565 W power [27]. The samples were cooled under a hood to approximately room temperature, vented and diluted to 50 mL volume with water.

Hot HNO_3 dissolution. Preliminary dissolution studies using 0.5 to 2.0 mol/L HNO_3 showed that 1.0 mol/L HNO_3 quantitatively extracted B from the biological materials and there was no additional extraction with more concentrated solution (data not shown). Ten milliliters 1 mol/L trace-metal grade HNO_3 were added to approximately 0.5 g samples weighed in 50 mL polystyrene centrifuge tubes. The tubes were capped and heated in a microwave oven (described above) to boiling (about 30 s). The samples were then filtered through a Whatman filter paper No. 1 and diluted to 50 mL volume with water.

Spiking the sample digests/extracts. The objective of this study was to examine the effects of digest/extract matrices on B recovery and compare with the recovery of B spiked before sample dissolution. The dissolution treatments utilized in this study resulted in different final matrices, which could also affect B recovery. Therefore, it was necessary to resolve these effects from the effects of the dissolution process on B recovery. A 25 mL portion of digest/extract from each treatment was spiked with 5 μg B using a 100 μg mL^{-1} B solution (H_3BO_3, NIST SRM 951). The samples were analyzed by ICP-MS using the operating parameters shown in Table 2.

Statistical analyses

For both ICP-MS and ICP-AES methods, the boron concentration from the five normalized signals of each sample after subtracting the average normalized signal of the blank gave the mean reading and relative standard deviation (RSD %). Recovery of the spike was based on the amount of B spiked and the difference between the mean B concentration of the spiked and respective unspiked sample. Confidence limits [sample mean ± $t_{0.05\ (df = 2)} \times SE$] were calculated to compare differences in B concentration between dissolution treatment means as well as between differences in spike recovery. All B concentration measurements and percentage recoveries were based on the signal (mass-to-charge ratio) of ^{10}B.

Table 2 Operating conditions for the ICP-AES and ICP-MS[a]

Parameter	ICP-AES	ICP-MS
Plasma gas flow (L min^{-1})	15	1.4
Auxiliary (L min^{-1})	1.5	12.0
Nebulizer (L min^{-1})	2	0.5
Sample flow rate (peristaltic pump)	3	3
Sweeps/replicate	4	1
Replicates/	3	5
No. readings/replicate	3	1
Estimated time (s)		
per replicate		5
per sample		26
Resolution	Normal	Normal
Scanning model		Peak hop

[a] ICP-AES Jarell Ash Aton Scan 25
ICP-MS Elan 500

Comparison of ICP-MS and ICP-AES values

Boron concentrations of NIST tomato leaves (RM 1573) and three in-house RMs determined by ICP-AES using external calibration method were compared with those determined by ICP-MS using the operating parameters shown in Table 2. In the case of ICP-AES, we also evaluated the effect of the emission wavelengths (249.773, 249.678, 208.959, and 182.641 nm) for the accuracy of the B analysis. A standard addition calibration method was used for the ICP-AES at 249.773 nm wavelength to verify the B values obtained from the external calibration method at different wavelengths. The wavelength 249.773 nm was found most sensitive and was therefore subsequently used in the comparative study with ICP-MS.

Results and discussion

Sample size study

The boron recovery was not affected by the microwave dissolution of 0.1 to 0.5 g sample mass of NIST SRM 1515 (Table 3). Although the B concentration in the digest

Table 3 Influence of sample size on the boron recovery of microwave digested NIST-SRM 1515 analyzed by ICP-AES at 249.773 nm

Sample size (g)	μg B · g^{-1}
0.1	28.3 ± 2.3
0.15	27.8 ± 2.2
0.2	27.4 ± 1.9
0.2	27.1 ± 1.5
0.4	27.3 ± 1.5
0.5	26.9 ± 1.4
Certified value	27.0 ± 2.0

tended to progressively increase as the sample size decreased, the differences were not significant. Some biological materials used in the sample dissolution study contained very low B concentrations (Table 1). Smaller sample sizes of these materials would result in B concentrations in the digest too low to analyze reliably. Therefore, we used 0.5 g mass of all material in the sample dissolution study.

Effects of sample dissolution methods

Plant tissues were fully dissolved by three digestion methods (dry ashing, wet digestion and microwave dissolution). The hot HNO_3 dissolution method extracted B without decomposing/dissolving the biological materials. Animal tissues were difficult to dissolve either by dry ashing or by wet digestion. Some charred materials were still visible in animal tissues at the end of dry ashing, suggesting an incomplete digestion. The wet digested animal tissues contained scum and micelle floating on top of the digest. Among the four digestion methods studied, only the microwave dissolution method worked well for dissolving animal tissues. However, microwave digests of plant as well as animal tissues were yellowish in color, possibly because of the presence of organic materials in the digest. The hot HNO_3 dissolution is a non-destructive method that prevents volatilization loss of B. However, extracts in this method were also generally light brown, possibly due to the presence of organic materials extracted with HNO_3.

B concentration

The sample dissolution treatments, in general, produced no significant difference in B concentration (Table 4). The effects of dissolution treatments were more consistent for the materials with higher B concentrations (SRMs 1515 and 1547) than those with lower B concentrations (RMs, 8414, 8415 and 8433). Biological materials used in this study were either SRMs or RMs each having a reference B value. The amounts of boron extracted by the four dissolution methods were generally within the confidence limits (sample mean $\pm$ $t_{0.05\ (df = 2)} \times$ SE) of their respective reference values, suggesting that all these sample dissolution methods were equally effective for B determination by ICP-MS. The limits of detection (3σ of the blank) were 0.004 for dry ashing and microwave, 0.006 for the hot acid and 0.01 μg B mL^{-1} for wet digestion methods. Both ICP-MS and ICP-AES share the same sample ionization device (ICP) and in both instruments, the sample digest/extract is subjected to similar conditions during the ionization process. Therefore, these results are expected to hold true for ICP-AES analysis also.

Measured B concentrations of SRM 1547 tended to be lower than its certified value regardless of the dissolution method used. Measured B concentrations of RM 8414 (0.2 $\mu g \cdot g^{-1}$) by dry ashing and microwave dissolution techniques were lower than its reference value (0.6 ± 0.4 $\mu g \cdot g^{-1}$) but were still within the acceptable range because of high standard deviation of the reference value. Measured B values for the wet digestion method tended to be higher compared to those for other dissolution (especially dry ashing and microwave dissolution) methods regardless of the sample material. Wet digestion was performed in borosilicate glass tubes which is likely to contribute B to the digest because of B leaching from the glassware. Boron concentration in the blanks of the wet digestion method tended to be higher and more variable than of other methods (Table 4). In addition, the use of conc. HNO_3 in the wet dissolution method resulted in more acidic digest than in other methods. Being closest in the mass number of B, Be is the best internal standard for B determination by ICP-MS [23, 28, 29]. However, the acidity of the sample extract/digest suppresses the Be signal more than the ^{10}B signal [18]. Therefore, a sample matrix with higher acidity would result in a higher ^{10}B/Be signal ratio and thus higher apparent ^{10}B concentration in the sample digests. To avoid contamination, contact of sample or digests with borosilicate glass should be avoided.

Boron concentrations in RMs 8414, 8415 and 8433 (animal tissues and corn bran, originally low in B concentrations) by dry ashing and microwave dissolution methods were within the range of their respective reference values, however, those by the wet digestion and the hot HNO_3 dissolution methods tended to be more variable and generally higher. Boron tends to adhere to the sample introduction and other parts of the analytical instruments. The release of the adhered B to subsequent samples and also B volatilization in the spray chamber result in the memory effect. The memory effect of B is particularly problematic and may cause significant errors in the deter-

Table 4 Effects of sample dissolution methods on B concentration

Method	Boron concentration ($\mu g \cdot g^{-1}$)					Reagent blank (μg B mL^{-1})[a]
	Apple SRM 1515	Peach SRM 1547	Corn bran RM 8433	Bovine muscle RM 8414	Whole egg powder RM 8415	
Dry ashing	26.6 ± 1.5	25.0 ± 0.1	3.0 ± 0.6	0.2 ± 0.3	0.4 ± 0.4	0.004
Microwave	27.8 ± 2.4	26.4 ± 1.9	3.9 ± 2.5	0.2 ± 0.5	0.4 ± 0.6	0.0001
Wet digestion	31.7 ± 8.7	26.2 ± 1.2	5.8 ± 1.7	2.3 ± 3.0	1.8 ± 3.0	0.007
Hot acid	28.3 ± 2.1	27.2 ± 0.8	5.4 ± 0.8	4.5 ± 0.8	0.3 ± 0.4	0.003
Reference value	27 ± 2.0[c]	29.0 ± 2.0[c]	2.8 ± 1.2[b]	0.6 ± 0.4[b]	0.4 ± 0.3[b]	

[a] Values are at dilutions similar to those for tissue digests (100×)
[b] Best estimated concentrations
[c] Certified values

mination of a sample with low B concentration if it is run following a sample of high B concentration. High variation in these materials may have resulted from the memory effects.

The choice of the dissolution method may depend on the tissue in question, time, cost and analytical instrumentation available. The hot HNO_3 dissolution method was as effective in extracting B from these biological materials as the three digestion methods. The hot HNO_3 method is simple, fast and requires no concentrated acid or base. Since this method extracts B from the biological materials without decomposing them, the majority of the elements of the sample do not enter the extract. Thus the extract is likely to have a simpler matrix than those of the other dissolution methods. This method also minimizes cross-contamination and volatilization losses.

Bañuelos and Akohoue [11] reported lower B values in microwave digests as compared to wet digest of plant tissue when B was analyzed by ICP-AES. Findings of Bañuelos and Akohoue [11] were contrary to those of Evans and Krähenbühl [23] who reported that the measured B values in microwave digests of plant or animal RMs either by ICP-AES or by ICP-MS were in agreement with their reference B concentrations. Pennington et al. [30] also reported no difference between closed vessel microwave dissolution and dry ashing of NIST-SRM citrus leaves and a certified ammonium borate standard analyzed by ICP-AES. Some published research cited in this section was carried out with biological materials for which B values were not certified. Two biological materials used in our study had NIST certified B values (Table 4). Our measured values were in good agreement with their certified values. They are also supported by the near 100% recovery of analyte spiked before the sample dissolution procedure (will be discussed later).

We found acceptable spike recovery and slightly less variation in the B concentration in microwave digestion compared to wet and hot HNO_3 dissolution methods. The microwave technique is faster than other decomposition methods, requires less acid and minimizes volatilization losses and cross-contamination [10]. However, measurement of B isotope ratio in microwave digests by ICP-MS resulted in higher $^{11}B:^{10}B$ ratio than expected (data not presented). Closed vessel microwave digests have high carbon contents resulting in a large ^{12}C peak that tends to tail into the ^{11}B peak. Evans and Krähenbühl [23] observed higher spectral interference of ^{12}C on the ^{11}B peak for ICP-MS when measurements were done in normal resolution mode (0.8 u) which we used in this study. However, such interferences were minimal at high resolution (0.6 u). The error in B isotope ratio measurement in microwave digests by ICP-MS due to tailing of the ^{12}C peak may have serious implications in B tracer studies using ^{10}B enriched materials and also in B determination by the isotope dilution method. Therefore, the interference of the ^{12}C peak on ^{11}B should be seriously examined in microwave digests whenever ^{11}B values are employed. We suggest using dry ashing to decompose biological materials when the ^{11}B isotope is employed.

Recovery of spiked B

Spiking of biological materials before digestion was intended to detect possible B loss due to fixation or volatilization. Boron spiked to the samples prior to digestion was quantitatively recovered in the digests/extracts irrespective of the method of sample dissolution. Lower recovery of spiked B in corn bran (RM 8433) compared to other materials was consistent with all sample dissolution methods (Table 5). Whole egg powder (RM 8415) showed more variation in spike recovery than other materials. Bovine muscle and whole egg powder are inherently very low in B and comprised a larger error in determining their inherent B content, possibly due to uncertainty in the blank correction (Table 4) and the memory effect. We suppose that the B, spiked before the sample dissolution procedure, was fully recovered within the limits of analytical errors. Spike additions prior to sample digestion tend to have high variations since they reflect the compounded errors associated with spike addition, sample weight, digestion, dilution and analysis as well as sporadic losses.

Published reports on the merits of sample dissolution/extraction methods are ambiguous and conflicting. Bañuelos [24] reported at least 40% higher B concentration for wet digestion compared to dry ashing and attributed this to higher volatilization losses of B during dry ashing. Contrary to these results, Isaac and Jones [26] reported no B volatilization during dry ashing carried out in a muffle furnace at 500° C for 4–12 h. We followed the protocol of Isaac and Jones [26] for dry ashing. Our data show that there may not be significant loss of B in any of the four sample dissolution treatments employed in this study. In our study, measured B values of botanical SRMs 1515

Table 5 Recovery of B spiked to the samples prior to digestion. Analysis by ICP-MS

Method	Recovery (%)				
	Apple SRM 1515	Peach SRM 1547	Corn bran RM 8433	Bovine muscle RM 8414	Whole egg powder RM 8415
Dry ashing	101 ± 11	97 ± 8	94 ± 7	100 ± 15	88 ± 8
Microwave	109 ± 33	104 ± 20	90 ± 12	105 ± 20	100 ± 10
Wet digestion	103 ± 12	100 ± 35	81 ± 38	105 ± 15	90 ± 26
Hot acid dissolution	97 ± 11	103 ± 28	91 ± 7	97 ± 17	98 ± 12

and 1547 matched with their certified values for all four dissolution methods. For these SRMs, quantitative recovery of sample-B (good agreement with their certified values, Table 4) and near 100% recovery of B spiked before the sample dissolution (Table 5) show that there was no significant volatilization loss of B during the sample dissolution process. For RMs 8433, 8414 and 8415 also, the recovery of B spiked before the sample dissolution was close to 100% (Table 5) which indicates that there was no significant volatilization loss of B. However, due to uncertainties (large standard deviation) in measuring sample-B concentrations in these materials, the reliance on these findings will remain within the frame-work of analytical uncertainties. This study also does not address the possibilities of volatilization loss during sample preparation and handling processes, which may be important for some biological materials.

Spiking digests/extracts with B examined the effects of digest matrices of different sample dissolution methods on B recovery by ICP-MS. The sample matrix may affect B measurement by ICP-AES [14, 15] and ICP-MS [31]. The presence of excess dissolved solids may cause deposition on the ICP-MS interface, gradually clogging the sample orifice, leading to loss of sensitivity and memory errors. Variations in acid and salt concentrations affect nebulizer performance resulting in analytical variations [32]. In order to prevent ionization suppression in ICP-MS analysis, salt levels should be kept below 0.2% [32]. In addition, salt and acid concentrations of sample digests/extracts and calibration standards should be matched as closely as possible. All calibration standards used in this study were prepared in 1.0 mol/L HNO_3.

Boron spiked to sample solutions after the dissolution process was also quantitatively recovered in dry ashing, microwave dissolution and wet digestion treatments. Recovery of spiked B in the hot HNO_3 dissolution treatment was somewhat lower (< 95%) than in other dissolution methods (Table 6). However, low recovery for this method was neither reflected in B spiked before the dissolution procedure (Table 5) nor in unspiked materials (Table 4), which should have been the case if the matrix of the extract was suppressing the B signal. The fact that the B concentrations in unspiked extracts of the hot HNO_3 dissolution method were not lower than the certified values (Table 4) also rules out matrix suppression. In fact, the B concentration in the hot HNO_3 dissolution treatment of unspiked samples, especially for bovine muscle, tended to be significantly higher than the estimated values (Table 4). Since these values are subtracted to compute spike recovery, this may have resulted in lower recoveries of B spiked to the sample extracts/digests.

Comparison of ICP-MS and ICP-AES analyses

Boron concentrations in microwave digests of four botanical materials measured by ICP-AES at four emission wavelengths are shown in Table 7. Boron concentrations were the same within SD at all emission wavelengths except at 182.641 nm, where B concentrations were higher. This was particularly evident for SRM 1515 (32.7 $\mu g \cdot g^{-1}$) and RM 157 (64.2 $\mu g \cdot g^{-1}$), possibly due to spectroscopic interferences of other elements in the digest. The reliability of B concentrations measured by ICP-AES was verified by B concentration measurements using the standard addition method at 249.773 nm wavelength (Table 7). Results of the standard addition method showed that B measurements in the microwave digests of the biological ma-

Table 6 Recovery of B spiked to the sample solution after digestion. Analyzed by ICP-MS

Method	Recovery (%)				
	Apple SRM 1515	Peach SRM 1547	Corn bran RM 8433	Bovine muscle RM 8414	Whole egg powder RM 8415
Dry ashing	107 ± 0.6	101 ± 10	96 ± 17	86 ± 5	100 ± 54
Microwave	101 ± 22	100 ± 10	95 ± 14	94 ± 13	105 ± 19
Wet digestion	101 ± 11	104 ± 22	108 ± 16	91 ± 17	92 ± 7
Hot acid dissolution	95 ± 4	91 ± 12	89 ± 13	84 ± 4	93 ± 12

Table 7 Evaluation of ICP-AES emission wavelengths for measuring B concentration in microwave digests of botanical reference materials

Material	Wavelength (nm)					Expected values
	249.773	249.773	249.678	208.959	182.641	
	Std. Add.[a]		External calibration $\mu g\ B \cdot g^{-1}$ tissue			
NIST SRM 1515	27.2 ± 1.4	28.7 ± 1.0	28.6 ± 0.9	28.8 ± 1.1	32.7 ± 1.0	26.0 ± 2.0
UCD RM 157	53.9 ± 3.6	54.6 ± 3.9	55.1 ± 3.9	54.0 ± 3.9	64.2 ± 3.9	59.8 ± 4.6
UCD RM 171	232 ± 10	229 ± 8	229 ± 9	230 ± 8	231 ± 13	245 ± 14
UCD RM 111	395 ± 18	380 ± 16	387 ± 15	393 ± 13	393 ± 13	410 ± 11

[a] Std. Add. = Standard addition method of calibration
Numbers following ± signs are standard deviations (SD)

Table 8 Boron recovery ($\mu g \cdot g^{-1}$) from microwave digests of three botanical reference materials using ICP-AES[a] and ICP-MS

Materials	ICP-AES	ICP-MS	Reference values
UCD-SRM 483	58.6 ± 2.1	60.6 ± 1.8	58.1 ± 4.8
NIST 1573	29.3 ± 1.0	32.6 ± 1.5	30
SRM 8607	8.4 ± 1.8	7.1 ± 0.9	6.2 ± 4.9

[a] ICP-AES measurements at 249.773 nm wavelength

terials under study were reliable. RM 171 showed higher variation in B concentration across all wavelengths studied, which was also confirmed by standard addition measurements (Table 7).

A comparison of ICP-MS and ICP-AES methods for microwave digests of selected UC Davis and NIST materials (Table 8) showed that ICP-MS and ICP-AES estimates of B concentrations were close to each other. Data show that both ICP-AES and ICP-MS may be used for B determination provided limitations of these methods are considered before the analysis. Two most sensitive B emission lines in ICP-AES have interference from Fe [18, 19]. However, iron levels in the materials selected for study were such that they did not interfere with the B determination. A significant advantage of ICP-MS over ICP-AES is that the ICP-MS allows B determination by the isotope dilution method. Despite the memory problem, ICP-MS appears to be the method of choice for B determination as it can measure the isotope ratio and is more precise, sensitive and suffers less interferences than ICP-AES.

Conclusions

Within the stated uncertainties, there was no significant difference in the four sample dissolution methods used in this study. These findings were reinforced by near quantitative recovery of sample-B and good agreement of the measured values with the reference values. Near 100% recovery of B spiked before the sample dissolution indicates that there may not be a significant loss of B during either dry ashing (if the protocol is as stated) or other methods of sample dissolution used in this study. This study also shows that simply heating samples in 1.0 mol/L HNO_3 is adequate to fully extract B from botanical materials. The hot HNO_3 dissolution method is time and labor efficient and avoids volatilization and cross-contamination.

References

1. Mengel K, Kirby EA (1987) Boron. In: Mengel K, Kirby EA (eds) Principles of plant nutrition. International Potash Institute, Bern, Switzerland, p 559–572
2. Gupta UC (1993) Boron and its roles in crop production. Boca Raton, Fla, CRC Press
3. Nielsen FH (1988) Nutr Today 23:4–7
4. Nielsen FH (1990) Biol Trace Elem Res 27:599
5. Nielsen FH (1992) Nutr Today 27:6–12
6. Anderson DL, Cunningham WC, Lindstom TR (1994) J Food Comp Anal 7:59–82
7. Lanza P, Mortera G (1983) Anal Chim (Rome) 73:371–384
8. Pollman D, Broekaert JAC, Leis F, Tschopel P, Tolg G (1993) Fresenius J Anal Chem 346:441–445
9. Durrant SF (1992) Trends Anal Chem 11:68–73
10. Ferrando AA, Green NR, Barnes KW, Woodward B (1993) Biol Trace Elem Res 37:17–25
11. Bañuelos GS, Akohoue S (1994) Commun Soil Sci Plant Anal 25:1655–1670
12. Bingham FT (1982) Boron. In: Methods of soil analysis-part 2. Page AC, Miller RH, Keeney DR (eds) Amer Soc Agronomy Inc/Soil Sci Soc Amer Inc, p 431–446
13. McGeehan SL, Topper K, Taylor D (1989) Commun Soil Sci Plant Anal 20:1777–1786
14. Pougnet MAB, Orren MJ (1986) Intern J Environ Anal Chem 24:253–265
15. Pougnet MAB, Orren MJ (1986) Intern J Environ Anal Chem 24:267–282
16. Anderson DL, Cunningham WC, Mackey EA (1990) Fresenius J Anal Chem 338:554–558
17. Rogus R, Harling OK, Olmez I, Wirdzek S (1992) Boron-10 prompt-γ analysis using diffracted neutron beam. In: Allen BJ et al (eds) Progress in neutron capture therapy for cancer. Plenum Press, New York, p 301–304
18. Evans S, Krähenbühl U (1994) Fresenius J Anal Chem 349:454–459
19. Zarcinas BA (1995) Commun Soil Sci Plant Anal 26:713–729
20. Wickner B (1986) Commun Soil Sci Plant Anal 1:1–25
21. Sah RM (1995) Appl Spectr Reviews 30:35
22. Vanhaecke F, Vanhoe H, Dams R, Vandecasteele C (1992) Talanta 39:737–742
23. Evans S, Krähenbühl U (1994) J Anal Atomic Spectrom 9:1249–1253
24. Bañuelos GS, Cardon GC, Pflaum TP, Akohoue S (1992) Commun Soil Sci Plant Anal 23:2383–2397
25. Hunt CD, Shuler TR (1989) J Micronutrient Analysis 2:161–174
26. Isaac RA, Jones JB (1972) Commun Soil Sci Plant Anal 3:261–269
27. Sah RN, Miller RO (1992) Anal Chem 64:230–232
28. Vanhaecke F, Vanhoe H, Vandecasteele C, Dams R (1991) Anal Chim Acta 244:115–122
29. Vanhoe H, Dams R, Vandecasteele C, Versieck J (1993) Anal Chim Acta 281:401–441
30. Pennington HD, Finch CR, Lyons CC, Littau SA (1991) Hort Sci 26:1496–1497
31. Gregoire DC (1987) Anal Chem 59:2479–2484
32. Brotherton TJ, Shen WL, Caruso A (1989) J Anal Atomic Spectrom 4:39–44