

# Use of Inductively Coupled Plasma-Mass Spectrometry in Boron-10 Stable Isotope Experiments with Plants, Rats, and Humans

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The commercial availability of inductively coupled plasma-mass spectrometry technology (ICP-MS) has presented the opportunity to measure the boron concentrations and isotope ratios in a large number of samples with minimal sample preparation. A typical analytical sequence for fecal samples consists of 25 acid blanks, 1 digestion blank, 5 calibration solutions, 4 standard reference material solutions, 10 samples, and 4 natural abundance bias standards. Boron detection limits ( $3 \times 1\sigma$ ) for acid blanks are 0.11 ppb for  $^{10}\text{B}$ , and 0.40 ppb for  $^{11}\text{B}$ . Isotope ratios were measured in fecal samples with 20 to 50 ppb boron with <2% relative standard deviation. Rapid washout and minimal memory effects were observed for a 50 ppb beryllium internal standard, but a 200 ppb boron biological sample had a 1.0 ppb boron memory after a 6-min washout. Boron isotope ratios in geological materials are highly variable; apparently this variability is reflected in plants. The lack of a fixed natural abundance value for boron requires that a natural abundance ratio be determined for each sample or related data set. The natural abundance variability also prevents quantitation and calculation of isotope dilution by instrument-supplied software. To measure boron transport in animal systems, 20  $\mu\text{g}$  of  $^{10}\text{B}$  were fed to a fasted rat. During the 3 days after a  $^{10}\text{B}$  oral dose, 95% of the  $^{10}\text{B}$  was recovered from the urine and 4% from the feces. Urinary isotope ratios,  $^{11}\text{B}/^{10}\text{B}$ , changed from a natural abundance of 4.1140 to an enriched value of 0.9507, a 77% change. The  $^{10}\text{B}$  label in perfused rat livers peaked within 3 hr (>90% recovery, 56% change in  $^{11}\text{B}/^{10}\text{B}$ ) and returned to a natural abundance ratio within 24 hr. In summary, boron concentrations and enriched isotope ratios have been measured successfully in rat urine and feces by using ICP-MS. — Environ Health Perspect 102(Suppl 7):13–20 (1994)

Key words: ICP-MS,  $^{10}\text{B}$ , memory, stable isotopes, matrix, beryllium, boron

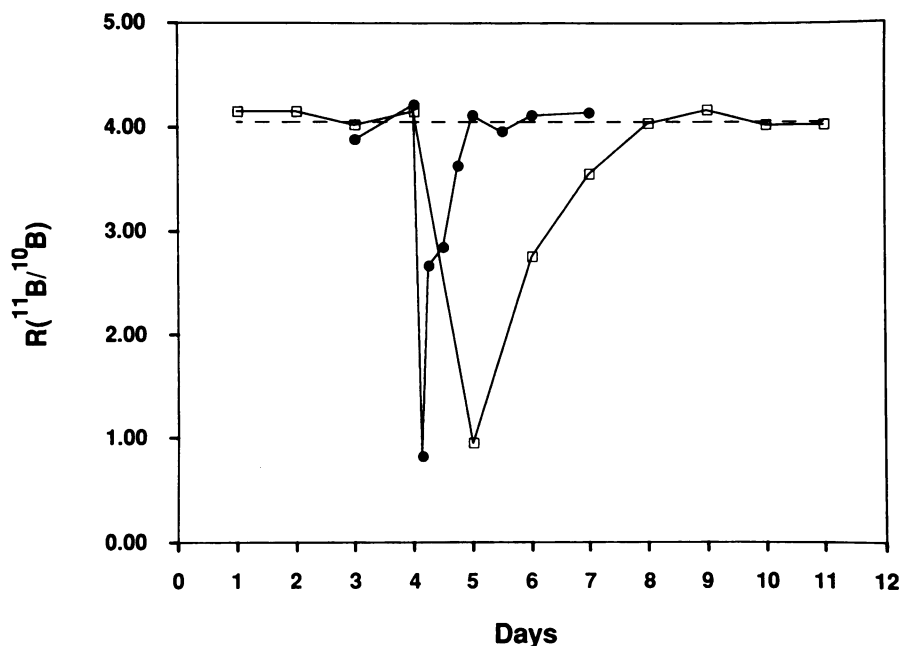
## Introduction

The lack of a suitably available radioactive isotope of boron has prevented the performance of typical experiments reported for other minerals of nutritional interest. While thermal ionization-mass spectrometry (TI-MS) could be used for stable boron isotope experiments, most kinetic work is limited by sample preparation and analysis time. However, with the introduction of commercially available inductively coupled plasma-mass spectrometry (ICP-MS) technology, boron concentrations and isotope ratios can be determined for large numbers of samples with minimal sample preparation. During the previous year, protocols have been developed to measure boron in biological samples using ICP-MS and for  $^{10}\text{B}$  stable isotope kinetics experiments with plants and animals.

## Experimental Design

Preliminary results from two  $^{10}\text{B}$  isotope experiments are illustrated in Figure 1. In a typical experiment designed to measure

boron transport and kinetics in rats, 20  $\mu\text{g}$  of  $^{10}\text{B}$  were fed to rats. Urine and fecal samples were collected in a metabolic cage, and liver samples were obtained from a



**Figure 1.** Boron (B) isotope ratios measured in microwave-digested rat urine and livers. Urine samples,  $R_{\text{corr}}$  ( $\square$ ) collected in a metabolic chamber and liver samples,  $R_{\text{corr}}$  ( $\bullet$ ) collected from individual rats given a similar  $^{10}\text{B}$  containing test meal on day four after morning urine samples were collected.

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**Table 1.** Offline calculations summary.

1	${}^9I, {}^{10}I, {}^{11}I$	Raw peak integrals
2	$I_{Nor} = I_n \times ({}^9I_1 / {}^9I_n)$	Normalize/run
3	$I_{Avg}, RSD$	Average and statistics
4	$I_{Corr} = (I_{Smp} - I_{Abik}) - (I_{Dbik} - I_{Abik})$	Blank subtraction (acid and digestion)
5	$R_{Obs} = {}^{11}I_{Corr} / {}^{10}I_{Corr}$	Observed isotope ratio
6	$R_{Corr} = SmpR_{Obs} \times ({}^{SRM}R_{lit} / {}^{SRM}R_{Obs})$	Bias correction
7	${}^{11}B = ({}^{11}I_{Corr} - b) / m$	Regression curve and nmole/ml
8	${}^{10}B_{Tot} = {}^{11}B \times ({}^{10}B / {}^{11}B)$	nmole/ml ${}^{10}B$
9	${}^{10}B_{NA} = {}^{11}B_{NA} \times ({}^{10}B / {}^{11}B)_{NA}$	nmole/ml NA ${}^{10}B$
10	${}^{10}B_{Sk} = {}^{10}B_{Tot} - {}^{10}B_{NA}$	nmole/ml ${}^{10}B$ spike

Abbreviations:  ${}^9I$ , raw peak integral,  ${}^9$ beryllium (Be);  ${}^{10}I$ , raw peak integral,  ${}^{10}B$ ;  ${}^{11}I$ , raw peak integral,  ${}^{11}Be$ ;  $I_{Nor}$ , normalized integral,  $I_{Avg}$ , average integral for each isotope;  $I_{Corr}$ , corrected integral;  $I_{Smp}$ , integral of sample;  $I_{Abik}$ , integral for acid blank,  $I_{Dbik}$ , integral for digestion blank;  $R_{Obs}$ , isotope ratios;  ${}^{11}I_{Corr}$ , corrected integral for  ${}^{11}$ boron (B);  ${}^{10}I_{Corr}$ , corrected integral for  ${}^{10}B$ ;  $R_{Corr}$ , corrected ratio;  ${}^{Smp}R_{Obs}$ , observed isotope ratios for each sample;  ${}^{SRM}R_{lit} / {}^{SRM}R_{Obs}$ , standard reference to correct for instrument bias;  $b$ , intercept of regression curve;  $m$ , slope of regression curve;  ${}^{10}B_{Tot}$ , total  ${}^{10}B$  concentration;  ${}^{10}B_{NA}$ , naturally occurring B in sample;  $B_{Sk}$ , spiked  ${}^{10}B$ ;  $n$ , sample

series of individual animals. During the first 3 days after the test meal, 95% of the  ${}^{10}B$  isotope was detected in the urine and 4% in the feces. The urinary boron isotope ratio,  ${}^{11}B / {}^{10}B$  ( $R_{corr}$ , Table 1), changed from a natural abundance of 4.11 to an enrichment of 0.951, a 77% increase in  ${}^{10}B$ . The  ${}^{10}B$  isotope, measured in rat livers after saline perfusion, peaked within 3 hr (>90% recovery, 56% change

in  ${}^{11}B / {}^{10}B$  ratio) and returned to the natural abundance ratio within 24 hr.

**Instrument**

Boron measurements were made by using a VG Elemental PlasmaQuad2+ ICP-MS. The ICP-MS system (Figure 2) consists of an autosampler with a peristaltic pump to move a sample to a nebulizer, which converts the liquid sample into an aerosol using an argon gas stream. The aerosol is

passed through a Scott double bypass spray chamber of borosilicate glass to remove larger aerosol particles and some of the water load as the spray chamber is cooled to 6°C. Boron contamination from the glass spray chamber seems to be minor when compared to boron contamination in acid blanks (see below). The aerosol then flows into a quartz torch where a radio-frequency (RF) field supports an argon plasma in which the sample is ionized in a 7500-K plasma and is extracted from the plasma with the use of two cones, a sample and a skimmer cone. The cones feed the sample into a vacuum system and through a lens stack, which focuses the ion beam into the quadrupole mass selector. The quadrupole passes ions of specific mass/charge into an electron multiplier from which the signal is passed to an amplifier, multichannel analyzer, and finally a computer for storage, retrieval, and analysis. The ICP-MS at the USDA Agriculture Research Service (ARS), Grand Forks Human Nutrition Research Center, is a standard system without special modifications.

**Operating Parameters**

Several parameters are critical for operating an ICP-MS and running a boron experiment. Operational and experimental conditions are summarized in Table 2. Data were acquired for the stable isotopes of  ${}^9Be$ ,  ${}^{10}B$ , and  ${}^{11}B$  as raw peak intervals ( ${}^9I$ ,  ${}^{10}I$ , and  ${}^{11}I$ , Table 1) in a peak jump experiment for 5 runs with 300 scans per run. Peak jump data were collected for 1280  $\mu$ sec per point and 5 points per peak. The sample was pumped to the nebulizer by a peristaltic pump at a rate of 0.64 ml/min; 5 ml of sample per analysis was used. The sample was pumped to the ICP-MS for 2.5 min before a boron analysis, and the system was rinsed with a 1% nitric acid ( $HNO_3$ ) solution for 5.0 min before the autosampler moved to the next sample.

Boron concentrations were calculated from a five point calibration curve by using 0, 5, 10, 50, 100, and 200 ppb boron standards prepared from an NIST SRM-951 (National Institute of Standards and Technology Standard Reference Material) boric acid isotope standard in 1% subboiling  $HNO_3$  and with a 50 ppb beryllium internal standard. Typically, the correlation

**Table 2.** ICP-MS system and boron experiment parameters.

System	VG Elemental PlasmaQuad2+		
Plasma	R.f. power:	Forward Reflected	1300 W (27.2 MHz) <10 W
	Gas flows:	Plasma Auxiliary Nebulizer	14 l/min 0.5 l/min 0.79 l/min
Nebulizer	Meinhard		
Spray chamber	Type	Scott-type double bypass	
	Composition	Borosilicate glass	
	Cooling	6°C	
Ion sampling	Sampling cone	Nickel, 1.0 mm orifice	
	Skimmer cone	Nickel, 0.75 mm orifice	
	Sampling distance	10 mm from load coil	
Vacuum	Expansion stage	1.7 mbar	
	Intermediate stage	<0.0 × 10 <sup>-4</sup> mbar	
	Analyzer	1.0 × 10 <sup>-6</sup> mbar	
Sample/wash	Peristaltic pump:	0.64 ml/min	
	Uptake, min:	2.5	
	Wash, min:	5.0	
Experiment $\mu$ sec	Isotopes:	${}^9Be, {}^{10}B, {}^{11}B$	Dwell: 1 2 8 0
	Type:	Peak jumping	Points/peak: 5
	Scans/run:	300	DAC steps: 5
	Time/run:	30 sec	Runs: 5
	Resolution:	3.0	$\Delta M$ : 5.7
	25 ppb B:	${}^{11}B$ 5% peak width = 0.45 amu	
Calibration	Internal standard:	50 ppb Be	
	Calibration curve:	0, 5, 10, 50, 100, 200 ppb	

Abbreviations: ICP-MS, inductively coupled plasma-mass spectrometry; B, boron; Be, beryllium.

\*Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the United States Department of Agriculture and does not imply its approval to the exclusion of other products that also may be suitable.

**Table 3.** Dwell and precision.<sup>a</sup>

Dwell μsec	Scans per min	Time sec	%RSD (n = 20) <sup>b</sup>			
			<sup>9</sup> Be	<sup>10</sup> B	<sup>11</sup> B	R <sub>(11/10)</sub>
10240	450	100	1.5	1.1	1.2	0.6
5120	490	60	1.6	1.5	1.3	0.6
2560	530	40	1.8	1.7	1.7	0.9
1280	560	35	2.0	1.8	1.7	1.3
80	1100	10	2.3	3.1	2.8	2.4

R, isotope ratio. RSD, Relative standard deviation. <sup>a</sup>50 ppb beryllium (Be) and 50 ppb boron (B) (National Institute of Standards and Technology Standard Reference Material 951); average of 20 accumulations per isotope for an average <sup>9</sup>Be peak integral of 16,891 ± 200. <sup>b</sup>n = 10 for 5120 μsec dwell.

**Table 4.** Inductively coupled plasma-mass spectrometry autosampler load sequence for fecal sample.<sup>a</sup>

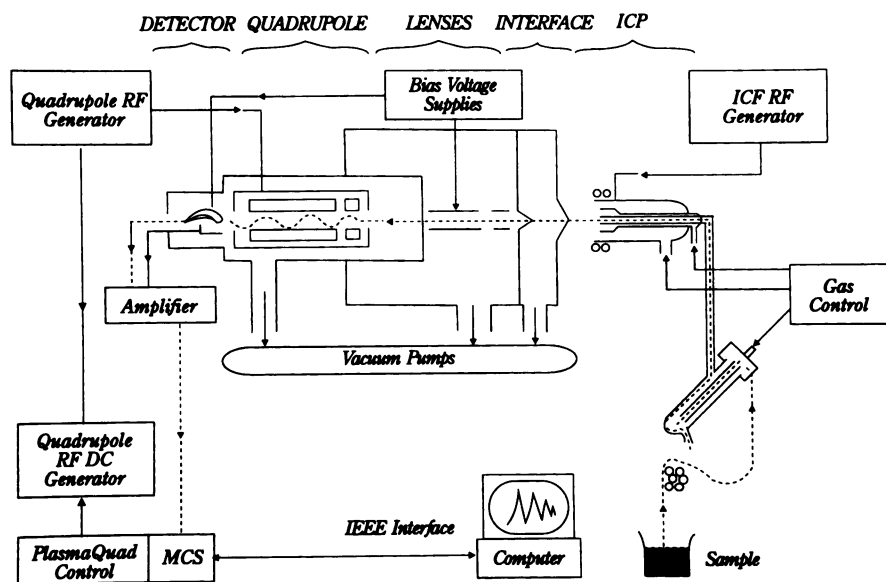
Acid blank	Acid blank	Acid blank
5 ppb standard	Digestion blank 1 <sup>a</sup>	Sample 6 <sup>a</sup>
Acid blank	Acid blank	Acid blank
10 ppb standard	Sample 1 <sup>a</sup>	Sample 7 <sup>a</sup>
Acid blank	Acid blank	Acid blank
50 ppb standard	Bias standard 1	Bias standard 3
Acid blank	Acid blank	Acid blank
100 ppb standard	Sample 2 <sup>a</sup>	Sample 8 <sup>a</sup>
Acid blank	Acid blank	Acid blank
200 ppb standard	SRM standard 1 <sup>a,b</sup>	SRM standard 3
	Acid blank	Acid blank
	Sample 3 <sup>a</sup>	Sample 9 <sup>a</sup>
	Acid blank	Acid blank
	Sample 4 <sup>a</sup>	Sample 10 <sup>a</sup>
	Acid blank	Acid blank
	Bias standard 2	Bias standard 4
	Acid blank	Acid blank
	Sample 5 <sup>a</sup>	SRM standard 4
	Acid blank	
	SRM standard 2	

<sup>a</sup> Indicates the 12 samples digested per turntable in microwave oven procedure. <sup>b</sup> Digested standard reference material (SRM) standard diluted to prepare SRM standards 1 to 4.

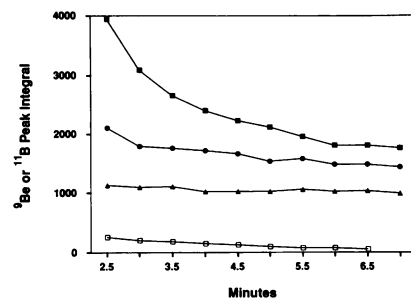
coefficient, *R*, was greater than 0.999 for the calibration curve. The 1% HNO<sub>3</sub> blank used in the calibration ranged from 3 to 5 ppb with much of the boron contamination coming from the laboratory prepared subboiling distilled HNO<sub>3</sub>. Detection limits were determined as three times the standard deviation (3 × 1σ) of an acid blank by collecting 10, 1-min counts in single ion monitoring. The detection limit for <sup>11</sup>B was calculated at 0.4 ppb and that for <sup>10</sup>B at 0.2 ppb. In practice, sample digest blanks were typically 10 ppb, so sample concentrations were prepared to be at or above 10 ppb for routine boron analysis.

Several factors control accuracy, precision, and signal intensity in a boron analysis. Factors such as the degree of elemental ionization in the plasma are uncontrollable. The degree of ionization for an element in the torch plasma is related directly to the first ionization energy of the element (2). For lithium, the first ionization energy is low and ionization in the plasma is near 100%. Bromine has a much higher first ionization energy and, therefore, the ionization level in the plasma is 3%. Boron and beryllium have similar masses and ionization energies, with ionization levels in the plasma around 70%. This makes beryllium an excellent internal standard for boron analysis.

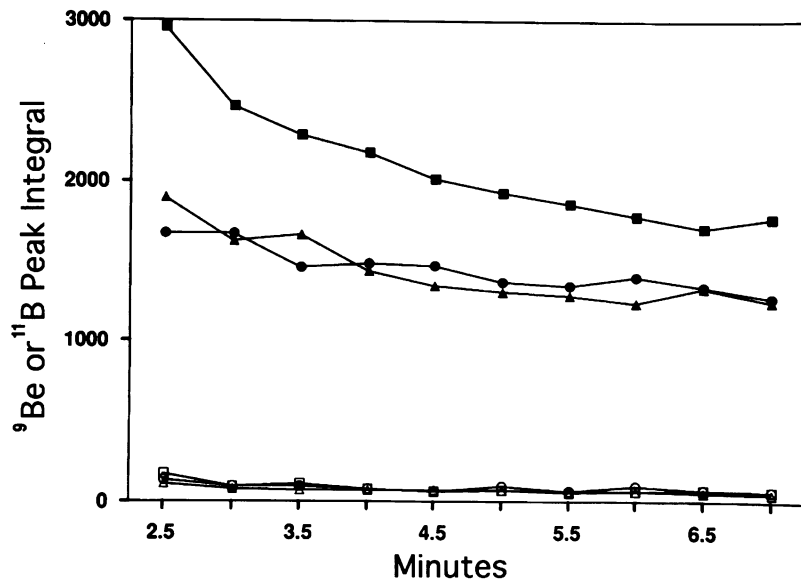
Dwell time defines the period of time that counts are collected for each point defined in the peak jump acquisition experiment (Table 2). Long dwell times translate to longer collection times and fewer scans for a set collection time period. For a 50 ppb boron solution, there were not large changes in the RSD for most dwell times other than 80 μsec for our ICP-MS system (Table 3). Therefore, the dwell time for our boron analyses was set at 1280 μsec. This finding may be



**Figure 2.** Schematic of an inductively coupled plasma-mass spectrometer (ICP-MS). Overall schematic of an ICP-MS, consisting of: a peristaltic pump, Meinhard nebulizer, spray chamber, torch, skimmer cone, sampler cone, lens stack, quadrupole, electron multiplier, associated electronics, and vacuum pumps. Reprinted with permission from VG Elemental (1).



**Figure 3.** Boron memory effects in ICP-MS. Memory effects observed in the <sup>11</sup>B signal response, <sup>11</sup>I, for boron concentrations of 200 (■), 100 (●), and 10 (▲) ppb, and a lack of observed memory effects for 50 ppb <sup>9</sup>beryllium, <sup>9</sup>I, (□) in the 200 ppb boron solution.



**Figure 4.** Boron rinse characteristics in ICP-MS. The ICP-MS <sup>11</sup>B and <sup>9</sup>Be rinse characteristics after a 10-min sample uptake of 75-ppb B and 50-ppb Be in different acid concentrations: <sup>11</sup>B and 0% nitric acid (HNO<sub>3</sub>) (■), 1% HNO<sub>3</sub> (●), 5% HNO<sub>3</sub> (▲); <sup>9</sup>Be and 0% HNO<sub>3</sub> (□), 1% HNO<sub>3</sub> (○), 5% HNO<sub>3</sub> (△).

specific to our ICP-MS system or to low-mass elements, as other literature sources recommend short dwell times for isotope ratios measurements (3).

### Memory

The length of the rinse period between samples is a significant time commitment for boron analyses. The system rinse includes the autosampler needle, transfer tubing, nebulizer, spray chamber, and torch, and requires 5 min with 1% HNO<sub>3</sub>. We found that signal intensity did not drop until 1.5 min after the start of the 1% HNO<sub>3</sub> rinse; however, rinsing was continued for 6.5 min because of the uptake time of the next sample. While the boron signal intensity decreased quickly during rinsing, an absolute rinse was never obtained. Memory, the retention of a signal from the previous sample, is a serious problem in boron analysis, especially with matrices such as fecal samples.

Figure 3 shows <sup>11</sup>B and <sup>9</sup>Be peak intensities (<sup>11</sup>I and <sup>9</sup>I) for the final minutes of rinsing for several concentrations of boron and a 50 ppb beryllium internal standard. Beryllium was rinsed from the system in 3 min with minimal memory. However, an increasing boron concentration resulted in greater degrees of boron memory, even after 7 min of rinse. A 200 ppb boron sample increased a 10 ppb boron integral by 75%, which resulted in, at a minimum, a higher blank for the next sample. However,

boron concentrations measured in biological samples in our laboratory are typically in the range of 20 to 75 ppb. Acid blanks before each sample, including the digestion blank, plus lower boron concentrations in biological samples, compensate for memory effects (Table 4).

Several rinse strategies have been examined to minimize the effects of both memory and rinse time. Investigators report that other ICP-MS systems use various HNO<sub>3</sub> concentrations for sample dilution and rinsing (4–6). We found that different acid concentrations in the rinse solution had minimal effects on either beryllium or boron rinse times (Figure 4). The exception was a longer rinse time when a 0% HNO<sub>3</sub> rinse solution was used.

Figure 5 compares the rinse characteristics of 50 ppb boron in 1% HNO<sub>3</sub> with 1% HNO<sub>3</sub>, pooled urine samples with 1% HNO<sub>3</sub>, pooled fecal samples with 1% HNO<sub>3</sub>, 50 ppb boron in 1% mannitol with 1% mannitol, and 50-ppb boron in 0.5% ammonium hydroxide (NH<sub>4</sub>OH) with 0.5% NH<sub>4</sub>OH. To use a basic wash, the ICP-MS rinse system was converted from an acid wash with a distilled water rinse before the basic rinse was started. Boron solutions were pumped through the system for 10 min before the rinse experiment was started. Rinse rates were (slow to fast): fecal pool < standard < urine pool < mannitol < NH<sub>4</sub>OH. The above results match laboratory observations that

show fecal samples and boron standards are the most difficult to rinse from the ICP-MS system. Basic washes have been reported for boron and other elements such as bromine (7). However, a basic rinse requires the ICP-MS acid rinse system be changed and the acid digested samples converted to a basic pH. Mannitol represents another option; but when using 1% mannitol in both samples and wash, a rapid buildup of a white material in the torch and connecting glassware has been a problem. The current rinse procedure is to use only a 1% HNO<sub>3</sub> wash solution.

### Spectral Interferences

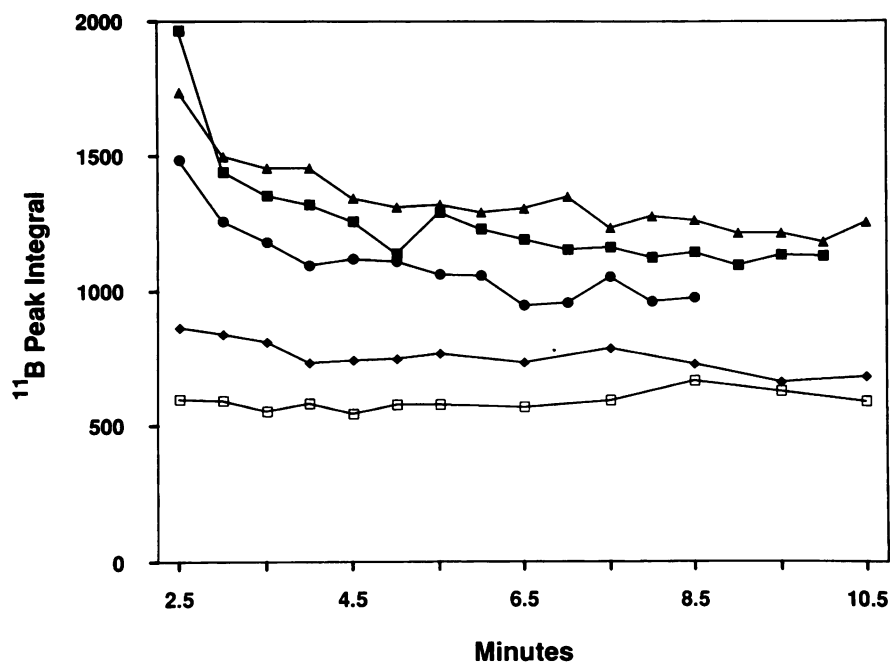
ICP-MS has fewer spectroscopic interferences than optical ICP methods. The only potential spectral interference in the low mass boron region is <sup>12</sup>C<sup>+</sup> tailing into the <sup>11</sup>B<sup>+</sup> signal (8). The <sup>12</sup>C<sup>+</sup> signal occurs naturally in the argon gas from CO<sub>2</sub> impurities, is present in the undigested portion of the sample, and is the result of the addition of mannitol during volume reduction of fecal samples. The <sup>11</sup>B<sup>+</sup> signal width was narrowed to 0.45 atomic mass units (amu) (Table 2) to reduce any potential overlap between the <sup>11</sup>B<sup>+</sup> and <sup>12</sup>C<sup>+</sup> signal.

### Matrix Interferences

Signal suppression caused by other dissolved materials in the sample is termed matrix effects (9–11). Figure 6 illustrates the effects of potassium chloride (KCl), a salt typically used to demonstrate matrix effects, and calcium chloride (CaCl<sub>2</sub>), chosen because calcium is known to interact with boron and has shown signal enhancement matrix effects (12). Note that the abscissa scale in Figure 6 is plotted with equal spacings, but not equal changes in concentration. KCl consistently lowered both <sup>9</sup>Be and <sup>11</sup>B integrals (<sup>9</sup>I, <sup>11</sup>I). The effect of calcium was similar, but the integrals for <sup>9</sup>Be and <sup>11</sup>B remained level above 0.01 M CaCl<sub>2</sub>. In comparison, a 0.1 g digested NIST citrus leaf standard diluted to 10 ml following fecal sample protocols would have a total cation concentration of 0.015 M.

### Fecal Samples

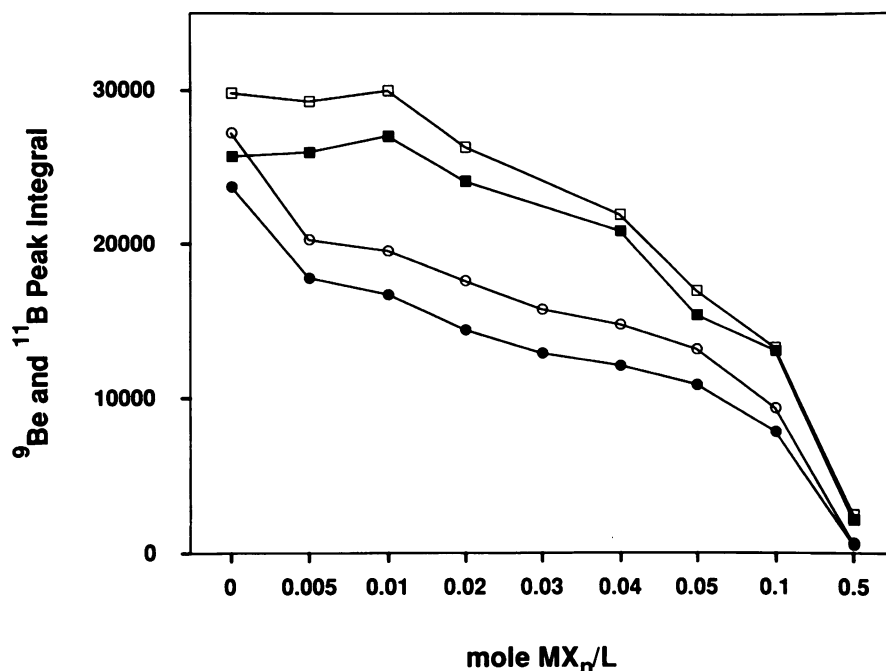
Fecal samples are one of the more difficult sample types to measure for boron concentrations and isotope ratios. As fecal samples are low in boron, mannitol was added to microwave-digested samples at a 10/1 ratio of mannitol to boron, and the sample volumes were reduced to 10 ml at 60°C. This resulted in samples that were saturated in some materials, such as silicates. Typically,



**Figure 5.** ICP-MS rinse strategies for boron. The inductively coupled plasma-mass spectrometer <sup>11</sup>B (B) (<sup>11</sup>I) rinse characteristics after a 10-min sample uptake of: 50 ppb B in 1% nitric acid (HNO<sub>3</sub>) with 1% HNO<sub>3</sub> (■); 47.9 ppb B pooled urine samples with 1% HNO<sub>3</sub> (●); 40.7 ppb B pooled fecal samples with 1% HNO<sub>3</sub> (▲); 50 ppb B in 1% mannitol with 1% mannitol (◆); 50 ppb B in 0.5% ammonium hydroxide (NH<sub>4</sub>OH) with 0.5% NH<sub>4</sub>OH (□).

To examine the effects of signal loss, a set of 10 fecal samples was run to verify the increased RSD for the last three samples in an analysis. A pooled set of fecal samples was prepared and isotope ratios determined using samples drawn from the fecal pool. Acid blanks were run between each sample. The pooled fecal samples were run with the cones showing loss of signal caused by contamination from the previous run.

Figure 7 summarizes the results of this experiment. The initial peak integral was half of the expected signal because of the condition of the cones. The blank corrected (not normalized) <sup>11</sup>B peak integral dropped from 10,887 to 943 (a 91% loss in signal) during the acquisition period with a 68% RSD. The isotope ratios (*R<sub>obs</sub>*, Table 1, not normalized) for the pooled samples varied during the run by 3.6% RSD and 1.8% for the first seven pooled samples. For comparison (Figure 1), isotope ratios (*R<sub>obs</sub>*, Table 1) were measured in samples collected from a metabolic cage experiment, but not pooled. Data analysis demonstrated a 2.4% RSD for the run. Both sets of ratios were plotted (Figure 7) at the same scale used in Figure 1, in which enrichments have dropped the <sup>11</sup>B/<sup>10</sup>B isotope ratios below 1.00. This experiment demonstrated that isotope ratios measured in fecal samples are equivalent to results obtained under the ideal analytical conditions of clean cones, low matrix, and sequentially collected samples. However, quantitation data (ng/ml) from the pooled fecal data experiment showed a much greater variability (Figure 8). The RSD for samples 1 to 7 was 1.2 and 4.4% for the entire run. Therefore, 10 fecal samples run with clean cones would seem to be near the ICP-MS analysis limit before it is necessary to clean the cones.

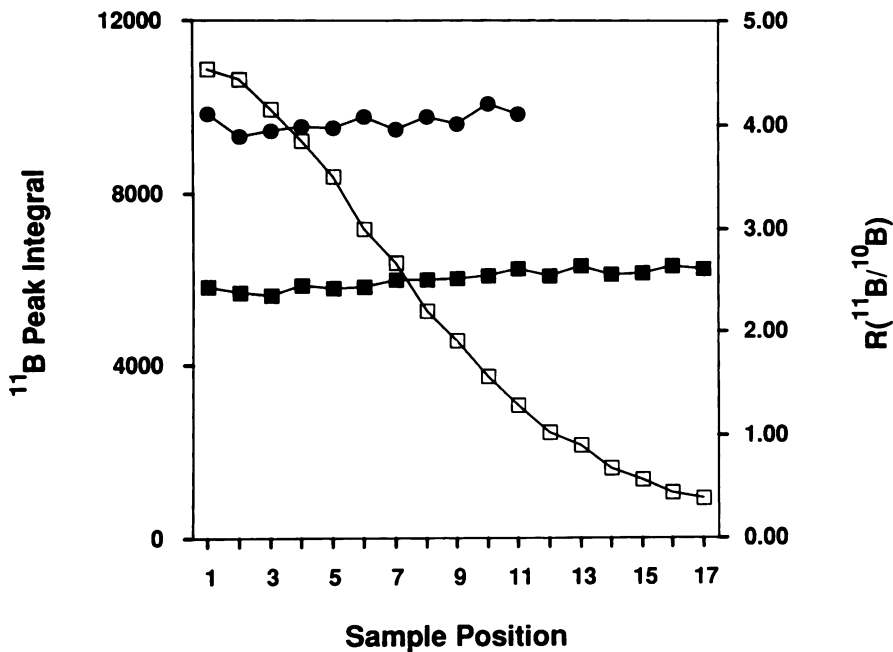


**Figure 6.** Matrix signal suppression of boron in ICP-MS. Boron (B) (75 ppb) and beryllium (Be) (50 ppb) signal, <sup>11</sup>I and <sup>9</sup>I, suppression caused by the matrix effects of potassium chloride (KCl) and calcium chloride (CaCl<sub>2</sub>); B in CaCl<sub>2</sub> (○); Be in CaCl<sub>2</sub> (■); B in KCl (●).

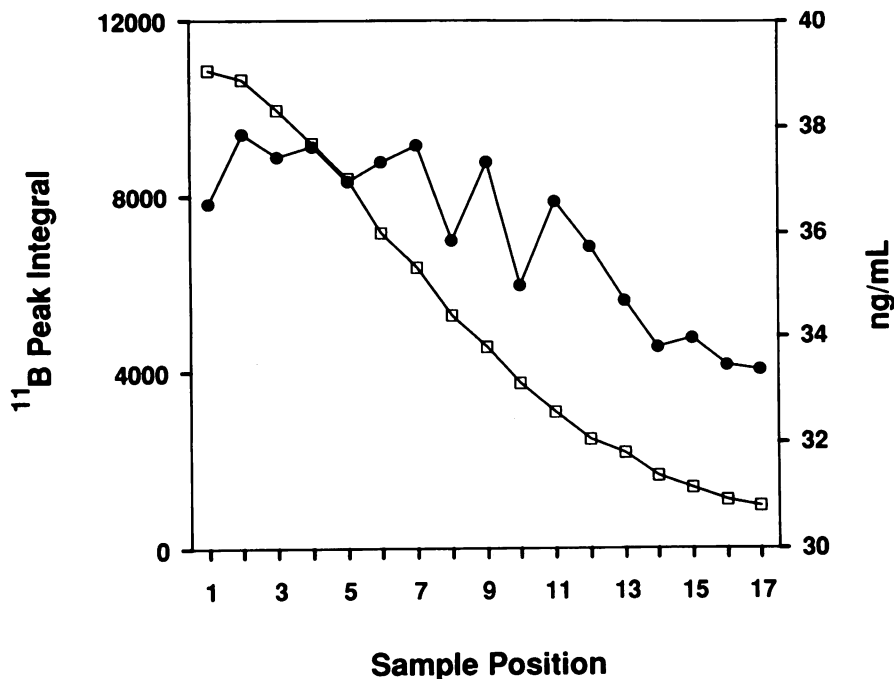
after running 10 fecal samples the last two to three samples had lower peak integrals as the result of contamination of the cones.

Because a lower RSD was observed, these samples were of questionable reliability.

To measure isotope ratios in fecal samples with the most accuracy, quality control must begin before data acquisition. We started with a clean set of cones each day, and the instrument was run for at least 2 hr for stabilization and calibration. Isotope ratios and RSD were measured on a 25 ppb boron solution by using an acquisition program, which was identical to that used for the actual boron analysis. Calculation of *R*(<sup>11</sup>B/<sup>10</sup>B) and RSD gave a daily measure of the stability of the instrument. Stability also was measured on a pooled fecal (or urine) sample to pretreat the clean cones, and this allowed the system to be reoptimized after an initial intensity loss. Typically, RSDs were 1.5 to 0.5 for fecal samples. During data acquisition, peak



**Figure 7.** Boron signal loss and isotope ratios effects. Sequential determination of 12 aliquots of pooled fecal digests with cones showing signal loss caused by contamination from previous analysis. Blank corrected  $^{11}\text{B}$  peak integral,  $^{11}I_{\text{corr}}$  ( $\square$ ) showing a 91% drop in signal; boron isotope ratios for each sample ( $\blacksquare$ ) showing a 3.6% RSD over the run and 1.8% for samples 1 to 7. For comparison the urine isotope ratios ( $\bullet$ ) collected from a metabolic cage from a single animal showing a 2.4% RSD over the entire run.



**Figure 8.** Boron signal loss and quantitation. Same experiment as in Figure 7. Blank corrected  $^{11}\text{B}$  peak integral,  $^{11}I_{\text{corr}}$  ( $\square$ ) and boron quantitation for each pooled sample in ng/mL ( $\bullet$ ) showing a 1.2% RSD for samples 1–7 and 4.4% RSD for samples 1–17.

integrals were printed to check signal intensity and stability.

Fecal samples were loaded into the autosampler as illustrated in Table 4. For

samples with high-boron concentration (e.g., 50 ppb) and high-dissolved solids such as fecal samples, acid blanks were placed between each sample, standard,

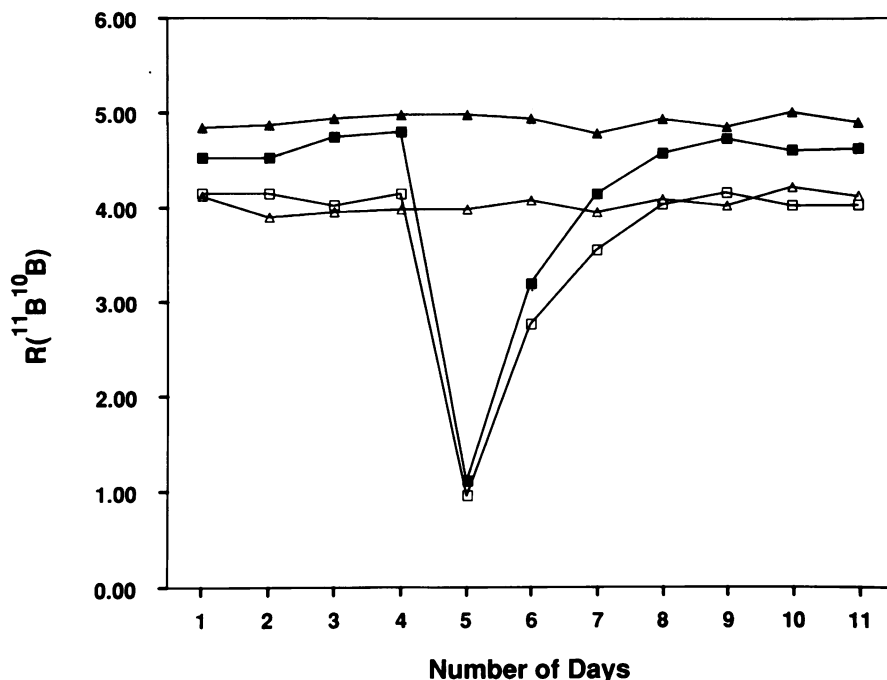
digestion blank, and bias standard. The loading sequence in Table 4 was based on samples digested in a 12-position microwave turntable. The microwave digestion procedure consisted of 10 samples, 1 digestion blank, and 1 NIST SRM biological standard. The single SRM was diluted into the 4 SRM standards shown in Table 4. For the bias standard, the 50 ppb boron standard in the calibration curve was used.

Several quality control checks were used during data analysis. The average peak integrals,  $I_{\text{avg}}$ , and RSD for each isotope were examined. Typically, the RSD within a run was consistent. An RSD of  $\leq 2\%$  for the isotope ratios indicates acceptable variability in an analysis. Finally, calculation of the boron concentration (ng/g) in the bias standard and the SRM biological standard using the mean  $^{11}\text{B}$  molar concentration and the measured isotope ratio permits a final check within the run and comparison to previous runs.

## Fractionation

Geochemists have shown boron isotope ratios are variable; therefore, there is not a single natural abundance ratio for boron, as is the case for most other stable isotopes. Boron isotope ratios ( $^{11}\text{B}/^{10}\text{B}$ ) have been reported from 3.815 for meteorites to 4.248 in borax samples (13). Variability also has been demonstrated in plants, with boron isotope ratios ranging from 4.013 for flour to 4.162 for cabbage (14). The absence of a fixed natural abundance ratio for boron implies that for any boron experiment involving ICP-MS, the isotope ratio must be determined before the boron concentration can be calculated. This natural abundance problem was solved for the kinetics studies by collecting four samples (e.g., fecal or urine) before the labeled  $^{10}\text{B}$  test meal was fed to the test animals (Figure 1). The natural abundance ratio used for the data in Figure 1 was the average of the four isotope ratios prior to the test meal (Figure 1, broken line).

While this technique may be used for any related data set with the same natural abundance history, individual samples, such as a test meal, present a greater challenge. We prepared test meals by modifying an animal's basal diet, so the isotope ratio in the basal diet could be used as the natural abundance value. This was not a complete solution, as a test meal may not contain all the basal diet ingredients and may have other materials added.



**Figure 9.** Boron bias correction in inductively coupled plasma-mass spectrometry data. Urinary boron isotope ratios in a kinetics experiment where a <sup>10</sup>B-labeled test meal was fed (■, □) or not fed (▲, △). Data for ratios which have not been bias corrected,  $R_{Obs}$  (■, ▲) and the same data which have been bias corrected,  $R_{Corr}$  (□, △).

### Data Analysis

Because of the variability in the natural abundance of nonenriched samples and the variable enrichments in <sup>10</sup>B-enriched kinetic samples, data analysis was performed off line; the equations are summarized in Table 1. Raw peak integrals for <sup>9</sup>Be, <sup>10</sup>B, and <sup>11</sup>B (<sup>9</sup>I, <sup>10</sup>I, <sup>11</sup>I, in Table 1) were normalized for each run relative to the beryllium intensity in the first sample, which was not a digestion or acid blank. Integrals were normalized by using  $I_{Nor} = I_n \times (^9I_1 / ^9I_n)$ , where  $I_n$  is any integral in run  $n$ , <sup>9</sup>I<sub>1</sub> is the integral for <sup>9</sup>Be in sample 1, run 1, and <sup>9</sup>I<sub>n</sub> is the integral for <sup>9</sup>Be in the  $n^{th}$  run. After normalizing each intensity in each run, the runs were averaged,  $I_{Avg}$ , for each isotope and the RSD calculated. Corrected integrals,  $I_{Corr}$ , for each isotope were calculated by subtracting the integral for the acid blank,  $I_{Ablk}$ , and the digestion blank  $I_{Dblk}$ . Isotope ratios were calculated,  $R_{Obs}$ , by using the corrected integrals for <sup>11</sup>B and <sup>10</sup>B,  $^{11}I_{Corr} / ^{10}I_{Corr}$ . Observed isotope ratios for each sample,  $^{SRM}R_{Obs}$ , were multiplied by the factor  $^{SRM}R_{Lit} / ^{SRM}R_{Obs}$  to correct for instrument bias. A 50 ppb boron standard was used throughout the analysis and was prepared from NIST SRM-951, boric acid isotope standard with  $R(^{11}B / ^{10}B) = 4.04362$ . The value used for  $^{SRM}R_{Lit}$  was 4.04362, and  $^{SRM}R_{Obs}$  was the

observed ratio for the bias standard nearest the sample to be bias corrected.

Figure 9 illustrates the effects of bias correction on two kinetic experiments. The bias-corrected data for the two experiments overlap, even though the two data sets were collected several months apart. Although there may be concern about the isotope ratio drift within a run, uncorrected data in Figure 9 do not show a significant deviation from linearity. Bias standards may not be required for kinetic data sets and only may be required for isolated samples such as test meals. Bias standards, however, are still added to all boron analytical runs in our laboratory.

To measure total <sup>11</sup>B in the sample, a linear regression was calculated from the calibration curve based on the calculated <sup>11</sup>B integral,  $^{11}I_{Corr}$  versus the calculated concentration of <sup>11</sup>B in nmole/ml (Table 1). Concentrations of <sup>11</sup>B in nmole/ml were obtained by using the linear regression parameters  $b$  and  $m$ ,  $(^{11}I_{Corr} - b) / m$ , where  $b$  and  $m$  are the intercept and slope of the regression curve, respectively. The total <sup>10</sup>B concentrations,  $^{10}B_{Tot}$ , in nmole/ml, were obtained from the concentrations of <sup>11</sup>B times the inverse of the isotope ratio for the sample,  $^{11}B \times (^{10}B / ^{11}B)$ . If the sample had been enriched with <sup>10</sup>B, as in the case of a test meal or urine sample in a kinetics experiment, the spiked <sup>10</sup>B in

nmole/ml of <sup>10</sup>B<sub>Sk</sub> would be simply the difference between the total nmole/ml of <sup>10</sup>B in the sample and nmole/ml of naturally occurring <sup>10</sup>B in the sample ( $^{10}B_{Tot} - ^{10}B_{NA}$ ). Because the <sup>11</sup>B in the sample was not spiked, it was considered natural abundance, <sup>11</sup>B<sub>NA</sub>, and when multiplied by the inverse of the sample's natural abundance isotope ratio,  $(^{10}B / ^{11}B)_{NA}$ , it gave the <sup>10</sup>B natural abundance concentration. For a kinetics series, the natural abundance ratio was obtained by averaging the four ratios prior to the enriched test meal. However, for single samples such as an enriched test meal, other assumptions were required.

### Summary

After considering the variability in isotope ratios, typical boron concentrations in plants, human blood volumes, and blood, our calculations indicate that a typical food serving could have a major impact on an individual's blood boron isotope ratio ( $I_3$ ). Therefore, human <sup>10</sup>B kinetics studies will require close attention to standardization of diets to reduce variability caused by both boron intake and isotope ratios.

In summary, we have been able to follow <sup>10</sup>B kinetics in rat urine, feces, and liver samples. Sample preparation has been either minimal or very simple. While memory effects have been a concern, enrichment levels have been sufficient that these effects are of no practical consequence with proper care in sample loading and in ICP-MS rinse time. In the future, we plan to complete the <sup>10</sup>B kinetics in our rat studies, and we are currently planning human studies as well.

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