We describe an accurate method for the determination of K and Ca using isotope dilution inductively coupled "cold" plasma mass spectrometry (ID ICP-MS). Measurements were applied to the certification of K and Ca in a new oyster tissue standard reference material, SRM 1566b, and the determination of Ca for an international measurement comparability exercise for serum. Measurements were made using an ICP-MS operated in the cold plasma mode. Molecular ion interferences arising from the plasma were reduced below 1000 counts per second (cps) for the isotopes of interest, while maintaining a sensitivity of better than 9 × 10^6 cps for a 1 mg L^{-1} Ca solution. Detection limits of 1.3 ng L^{-1} and 2.5 ng L^{-1} were obtained for K and Ca, respectively. Isotope ratio measurement repeatability of the 40Ca/42Ca and 39K/41K ratios for spiked samples was better than 0.2% relative (n = 5, 1 s). Though interference from background peaks was reduced, molecular ions arising from the oyster tissue and serum matrices caused spectral interference. Reduction of the matrix induced interference was successfully accomplished using cation exchange chromatography. Data that demonstrate the reproducibility and accuracy of the ID ICP-MS measurements are presented. A split sample comparison with thermal ionization mass spectrometry (TIMS) was performed.

Introduction

To be useful, analytical measurements must be accurate. When relied upon for medical diagnosis, or to determine the nutritional content of food, they must also be rapid and inexpensive. Indeed, for clinical measurements, accuracy and expense are related. The German Health Report of 1998 states that the cost of performing repeat measurements on patient samples in Germany alone amounted to 1.5 billion US dollars. Normalized to the US GDP for that year, it is estimated that this would cost the US health care consumer $7.4 billion. The clinical chemistry community has long recognized the importance of reference methods to validate the results of rapid, routine test systems. The recent European directive on in vitro diagnostics also mandates such a scheme, requiring that the results of routine methods in laboratory medicine be traceable to the highest available reference material or method. Reference methods play a critical role in establishing traceability, not only in the validation of routine methods, but also in the certification of reference materials.

The accurate determination of K and Ca is important. Both elements are essential in our diet. The Ca content must appear on the labels of all foods processed in the US, as required by the Nutrition Labeling and Education Act of 1990. An excess or deficiency of either element will result in adverse health consequences. Calcium is needed for bone strength to combat osteoporosis, for blood clotting, for proper nerve and muscle function and for strong, healthy teeth. Too much calcium may be risky because calcium can inhibit the absorption of iron, zinc and magnesium. Potassium is also required for proper nerve and muscle function, as well as for maintaining the fluid balance of cells and heart rhythm. High levels of potassium in the body can cause heart problems and even death. As a result of nutrition labeling requirements and the rapid growth of the in vitro diagnostic market, the number of methods to determine K and Ca abounds. However, the influx of new and rapid techniques provides no assurance that the accuracy of results has kept pace.

Isotope dilution thermal ionization mass spectrometry (TIMS) has traditionally provided the accuracy base for the determination of K and Ca. Isotope dilution mass spectrometry has powerful advantages over conventional methods of quantitation. First, use of an enriched isotope of the element of interest as the internal standard is an ideal situation because the chemical behavior of the standard and analyte is identical. Once equilibrated (which is easily accomplished for inorganic analytes by complete dissolution), the analyte can be isolated from the matrix without fear of systematic error due to loss. Second, intensity ratios rather than absolute signals are determined in the mass spectrometer. The measurement of ratios can be accomplished very precisely. Inductively coupled plasma mass spectrometry (ICP-MS) provides an attractive alternative to TIMS because of its rapid measurement ability and subsequent wide usage. The repeatability (precision) of ratios measured by ICP-MS is typically 0.05–0.3%, relative. However, high background from the Ar plasma has hindered the use of ICP-MS for the measurement of K and Ca isotopes. Difficulty in the determination of K and Ca by ICP-MS stems from intense background peaks at masses 39, 40, 41 and 42 from the matrix without fear of systematic error due to loss. Second, intensity ratios rather than absolute signals are determined in the mass spectrometer. The measurement of ratios can be accomplished very precisely. Inductively coupled plasma mass spectrometry (ICP-MS) provides an attractive alternative to TIMS because of its rapid measurement ability and subsequent wide usage. The repeatability (precision) of ratios measured by ICP-MS is typically 0.05–0.3%, relative. However, high background from the Ar plasma has hindered the use of ICP-MS for the measurement of K and Ca isotopes. Difficulty in the determination of K and Ca by ICP-MS stems from intense background peaks at masses 39, 40, 41 and 42 from the matrix without fear of systematic error due to loss. Second, intensity ratios rather than absolute signals are determined in the mass spectrometer. The measurement of ratios can be accomplished very precisely. Inductively coupled plasma mass spectrometry (ICP-MS) provides an attractive alternative to TIMS because of its rapid measurement ability and subsequent wide usage. The repeatability (precision) of ratios measured by ICP-MS is typically 0.05–0.3%, relative. However, high background from the Ar plasma has hindered the use of ICP-MS for the measurement of K and Ca isotopes. Difficulty in the determination of K and Ca by ICP-MS stems from intense background peaks at masses 39, 40, 41 and 42 from the matrix without fear of systematic error due to loss. Second, intensity ratios rather than absolute signals are determined in the mass spectrometer. The measurement of ratios can be accomplished very precisely. Inductively coupled plasma mass spectrometry (ICP-MS) provides an attractive alternative to TIMS because of its rapid measurement ability and subsequent wide usage. The repeatability (precision) of ratios measured by ICP-MS is typically 0.05–0.3%, relative. However, high background from the Ar plasma has hindered the use of ICP-MS for the measurement of K and Ca isotopes. Difficulty in the determination of K and Ca by ICP-MS stems from intense background peaks at masses 39, 40, 41 and 42 from the matrix without fear of systematic error due to loss. Second, intensity ratios rather than absolute signals are determined in the mass spectrometer. The measurement of ratios can be accomplished very precisely. Inductively coupled plasma mass spectrometry (ICP-MS) provides an attractive alternative to TIMS because of its rapid measurement ability and subsequent wide usage. The repeatability (precision) of ratios measured by ICP-MS is typically 0.05–0.3%, relative.
applied an ammonium oxalate precipitation at pH 8 to separate

0.4 ng L\(^{-1}\) for \(^{40}\)Ca and 0.35 ng L\(^{-1}\) for \(^{40}\)K.\(^{22}\) Reaction with a collision gas serves not only to reduce plasma induced interference, but is believed to cause collisional damping of ion beam fluctuations, allowing for precise isotope ratio measurement.\(^{23}\) Boulyga and Becker obtained repeatability \((s_n = 6)\) of 0.26% and 0.40% for \(^{40}\)Ca\(^{2+}\) and \(^{40}\)Ca\(^{4+}\) Ca ratios measured in NIST SRM 915, Calcium Carbonate, at a Ca concentration of 10 \(\mu\)g L\(^{-1}\).\(^{24}\) Repeatability better than 0.01% for \(^{42}\)Ca\(^{40}\)Ca has been obtained using a multicollector ICP-MS with a collision cell.\(^{25}\) Though collision cells appear as a very promising alternative to high resolution ICP-MS for the determination of analytes with spectral interference, applications to date have focused primarily on analysis of high-purity acids and matrix free samples. Feldmann found that use of H\(_2\) as a reaction gas was effective in reducing polyatomic ions caused by Ar, but matrix induced polyatomic ions were unaffected.\(^{26}\) Operation of the dynamic reaction cell can be achieved under a single set of plasma conditions; however some elements are better analyzed in a non-pressurized mode which requires switching between pressurized and vented modes for full elemental coverage. The need for additional high-purity gases and the inability to upgrade current instrumentation add to the expense of using reaction cell technology. Use of cold plasma to reduce Ar-based interference, however, requires only slight modification of existing instrumentation. Recent generation instruments usually come ready for operation in cold plasma mode.

Low power ("cold plasma") conditions along with a configuration to attenuate the secondary discharge has been shown to significantly reduce Ar-based interferences.\(^{27,28}\) Sakata and Kawabata used a forward power of 900 W, a higher carrier gas flow \((1.1 \text{ L min}^{-1})\), and a grounded metal shield inserted between the torch and the load coil to significantly reduce the polyatomic argide ion background.\(^{29}\) They obtained detection limits of 1.1 ng L\(^{-1}\) for K and 20 ng L\(^{-1}\) for Ca. Use of a large mechanical vacuum pump to lower the pressure in the expansion chamber serves to recover any loss in sensitivity due to cold plasma conditions, resulting in further improvement of detection limits.\(^{29}\) Instrumentation which allows for operation in the cold plasma mode has been commercially available for several years, yet few publications have appeared until recently.\(^{29–33}\) One reason for limited use is that the formation of matrix induced polyatomic ions is enhanced under cold plasma conditions. As a result, application of cold plasma primarily occurs in the semiconductor industry for analysis of high purity reagents. However, reduction of matrix effects can be achieved by chemical separation. Separations are more easily applied when quantifying by isotope dilution because once isotopic equilibration between the sample and the enriched isotope internal standard is achieved, total analyte recovery is not required for accurate results. Cold plasma ID ICP-MS determinations of Fe and Cr have been made after matrix separation.\(^{34}\) Patterson et al. applied an ammonium oxalate precipitation at pH 8 to separate Ca in serum, urine, feces and breast milk for measurement of Ca ratios in nutritional studies using stable isotope tracers.\(^{29}\)

We describe a reference method for the determination of calcium as well as potassium in oyster tissue and serum matrices using cold plasma ICP-MS. Application of cation exchange chromatography completely removed matrix-induced interference and allowed for the determination of both elements in a single sample aliquot. We demonstrate the comparability of cold plasma ID-ICP-MS methodology with the previously applied ID-TIMS definitive method used in this laboratory to provide traceability to the SI for chemical measurements. This demonstration is made directly using the two techniques for ratio measurements on the same isotope dilution samples. For calcium, it is also made indirectly through the analysis of SRM 956a, which was certified in 1996 by ID-TIMS. In addition, the serum calcium results have been compared with those from other National Measurement Institutes using different isotope dilution methodologies and instrumentation. The results for the oyster tissue are compared with NIST nuclear methods that were also applied in the certification of this material. The critical evaluation and application of new methodology capable of accurate measurement, as is described here, will be required to satisfy the increased need for traceability and global comparability in chemical measurement.

### Experimental

#### Materials and reagents

Concentrated, high-purity nitric and hydrochloric acids (Optima grade) were obtained from Fisher Scientific, Pittsburgh, USA. Concentrated perchloric and hydrofluoric acids and de-ionized water were purified in-house by sub-boiling distillation. Solutions containing 0.3 mol L\(^{-1}\), 0.5 mol L\(^{-1}\), 1.0 mol L\(^{-1}\) and 1.75 mol L\(^{-1}\) HCl were prepared from the purified reagents. Bio-Rad AG 50W-X8, 100–200 mesh cation exchange resin in the H\(^{+}\) form was used for the separation of K and Ca. The resin was bulk-cleaned in 300 mL batches and cleaned again in individual columns by washing alternatively with water and 5 mol L\(^{-1}\) HCl. Resin was discarded after a single use. Enriched isotopes, \(^{41}\)K \((99.18\%, \text{ series #149401})\) as KCl, and \(^{42}\)Ca \((94.42\%, \text{ series #139601})\) as CaCO\(_3\) were obtained from Oak Ridge National Laboratory (Oak Ridge, Tennessee, USA). Stock solutions containing 28.6 \(\mu\)mol g\(^{-1}\) and 6.3 \(\mu\)mol g\(^{-1}\) total K and Ca, respectively, were prepared in 0.5 mol L\(^{-1}\) HNO\(_3\). For the determination of Ca in serum, a working \(^{42}\)Ca spike solution was prepared by gravimetric dilution of the master stock solution to yield a concentration of approximately 1.0 \(\mu\)mol g\(^{-1}\). The exact concentration of enriched isotope in each spike solution was calibrated against fresh, gravimetrically prepared stock solutions of SRM 918a Potassium Chloride and SRM 915a Calcium Carbonate primary standards. For Ca, SRM 3109a Calcium Standard Solution (lot #000622) was used as well. For accuracy assessment, one vial of SRM 956a Electrolytes in Frozen Human Serum, Level 2, was analyzed for Ca, and one bottle of SRM 1566a Oyster Tissue was analyzed for K as well as Ca.

#### Instrumentation

ICP-MS measurements were made using a VG PlasmaQuad 3 (Thermo Elemental, Winsford, Cheshire, England). The instrument was operated with the plasma screen and S-option pump under low power conditions. Operating parameters are listed in Table 1. Solution was introduced to the plasma via a concentric nebulizer and impact bead spray chamber water cooled to 4 °C. Solution was pumped at rate of 0.6 mL min\(^{-1}\). The auxiliary gas flow rate and torch position had a significant effect on the intensity of the background plasma-based molecular ions and these parameters were optimized to achieve the best signal to background ratio for...
Table 1 Instrument parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>K</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward power/W</td>
<td>630</td>
<td>630</td>
</tr>
<tr>
<td>Gas flows/L min⁻¹</td>
<td>1.4</td>
<td>1.0</td>
</tr>
<tr>
<td>Nebulizer flow</td>
<td>0.92</td>
<td>0.94</td>
</tr>
<tr>
<td>Torch position X,Z</td>
<td>100 units back from typical hot plasma setting</td>
<td></td>
</tr>
<tr>
<td>Lens voltages/V</td>
<td>Optimized for maximum signal to background</td>
<td></td>
</tr>
</tbody>
</table>

Sample description and preparation

Oyster tissue. One sample from each of six statistically selected bottles of the candidate SRM 1566b was analyzed. SRM 1566b is composed of oysters collected in the Gulf of Mexico. These were shucked, washed, ground, freeze-dried, re-
ground and sterilized before bottling. Subsamples of approximately 0.25 g were weighed by difference into clean Teflon™
beakers. Loss of mass upon drying was determined for each bottle using separate samples removed at the same time as the
analytical samples. Accurately weighed aliquots of enriched
⁴¹K and ⁴²Ca spike solutions were added to the analytical
samples by mass difference via capped plastic syringes. The approximate concentration of K and Ca in the new
oyster tissue was known, and spike was added to form a ⁴⁰K/⁴¹K ratio of 0.95 and a ⁴⁰Ca/⁴²Ca ratio of 1.1. These ratios represent a
compromise between the optimum ratio for measurement on
pulse counting systems and the minimization of uncertainty
due to error propagation. ⁵⁰Subsamples of SRM 1566a, SRM 918a, and SRM 915a were treated in a similar manner.

Samples were dissolved by wet-ashing with nitric (HNO₃),
hydrofluoric (HF), and perchloric (HClO₄) acids. Hydrofluoric
acid was used because it is possible that some sediment is
present with the oyster tissue. We find HClO₄ to be the most
effective acid for the decomposition of organic matter in
biological matrices and this acid was used in the decomposition
of the serum samples as well. Our intent is always to attain the
most complete dissolution of the sample and to achieve the
highest oxidation state for the metals present. This ensures
equilibration of the sample and spike isotopes. However, great
care must be exercised when using HF and HClO₄. It is
important to decompose the sample with HNO₃ prior to the
addition of HClO₄. Eight grams of nitric acid were added, and
the samples refluxed in a class 10 laminar flow hood until fumes
of NO₂ were no longer observed. The solution volume was
reduced and the samples cooled. Four grams of HF and 3 g of
HClO₄ were added, and the samples gently heated. Heating was
increased and the samples allowed to reflux again. Solution
volumes were reduced to fumes of HClO₄ and samples were
gently taken to dryness. The residue was washed several times
with H₂O to remove excess perchlorates and then converted to
the chloride by evaporating to dryness several times with
hydrochloric acid (HCl).

Blanks and spike calibration mixes. Process blanks were
prepared. A small aliquot (± 100–500 ng) of ⁴¹K and ⁴²Ca spike
was added to clean Teflon™ beakers and processed in a manner
typical to the samples. In addition to the samples and process
blanks, spike calibration mixes were prepared. The purpose of
these mixes is to accurately calibrate the concentration of enriched
isotope in the spike solution against primary standards by
reverse isotope dilution mass spectrometry. Two separate,
gravimetrically prepared primary standard solutions were
prepared for each element. Standard solutions were prepared as
previously described. ⁵⁰Subsamples (± 0.25 g) of the primary
standards were weighed to +0.01 mg on a microbalance. Two
weighed aliquots of each standard solution were added to
aliquots of the spike solution to form four calibration mixes per
element.

Cation exchange separation. Samples were separated using
columns (0.7–1 cm id) which were hand-packed with 5 mL of
AG 50W-X8, 100–200 mesh cation exchange resin. The packed
columns were cleaned by washing alternatively with water and
5 mol L⁻¹ HCl, and conditioned with water. Samples were
loaded in 2 mL water. The Na fraction was eluted with 60 mL
of 0.3 mol L⁻¹ HCl, and discarded. The K fraction was eluted
with 40 mL of 0.5 mol L⁻¹ HCl, followed by Mg, which was
eluted with 30 mL of 1 mol L⁻¹ HCl. The Ca fraction was
eluted last with 20 mL of 1.75 mol L⁻¹ HCl. The elution
progress of the Na, K and Ca fractions was monitored by flame

emission using a Bunsen burner. The K and Ca fractions were taken to dryness, treated with concentrated HNO₃ to remove chloride, and diluted to the appropriate concentration with a volume fraction of 2% HNO₃ in water.

Mass spectrometric procedures

ICP-MS. Measurements were made using peak jump data acquisition with one point per peak. Five blocks of data, each 1 min in duration, were acquired per sample, and the mean ratios used for computations. Total time for uptake, stabilization, and measurement was about 8 min per sample. For spiked potassium samples, masses 39 and 41 were measured at dwell times of 10 ms each. Sample concentrations were calculated from the 39K/41K ratio. For spiked calcium samples, masses 39, 40, and 42 were measured at dwell times of 5, 10, and 10 ms, respectively. Mass 39 was measured to determine the correction for isobaric interference on 40Ca from 40K but no correction was required for either the oyster tissue or serum samples. Sample concentration data were calculated from the 42Ca/40Ca ratio. This mass bias corrected spike calibration sample was labeled with a spike calibration standard. The working isotopic standard had an isotopic ratio similar to the analytical samples and was re-measured throughout the analysis and used to correct the remaining calibration samples, analytical samples, and blanks for mass bias and any subsequent instrument drift. Any change from the mass bias factor established at the start of the analysis was assumed to be drift. A correction was applied every three samples, assuming the drift to be linear with time. Detector dead time was experimentally determined using natural Mg and Ti solutions diluted so that the major isotope spanned the count rate range of 1 \times 10⁵ to 9 \times 10⁵ counts per second. The dead time that resulted in the least variability of the measured ratios (54Mg/56Mg, 48Ti/56Ti) across the concentration range was chosen. To minimize the correction for detector dead time, samples were diluted so that the count rate of the major isotope was below 300 000 counts per second (cps). Two dilutions were required to reach the analysis concentration for each element. The final dilution was prepared fresh on the day of the analysis. Potassium was diluted with a volume fraction of 4% HNO₃ in water to an analysis concentration of 9.5 ng g⁻¹, yielding a count rate of 240 000 cps 39K for a natural composition standard. Calcium was diluted to an analysis concentration of 28 ng g⁻¹, yielding a count rate of 250 000 cps 40Ca for a natural composition standard. It was discovered that the use of LDPE bottles caused contamination problems for K and Ca at analysis concentrations, so the final dilution was prepared and analyzed using Teflon containers. Correction for instrument background was more significant for K than for Ca. Background at mass 39 for the worst case was 950 cps ± 11 cps (1 s, n = 5), amounting to 0.4% of the signal. However, the background was stable, changing by no more than 100 counts during a 4 hour analysis. The background at mass 41 was 492 ± 5 cps, indicating that there was some residual 40Ar²⁺H⁺. This too remained stable throughout an analysis. For Ca the background at mass 40 for the worst case was 290 cps ± 5 cps (1 s, n = 5) and at mass 42 it was 9 ± 0.5 cps.

TIMS. For the measurement of potassium a triple-filament procedure was used. Ten µg of potassium was loaded onto each of two Re side filaments using a micro-pipette and dried using an infrared lamp in a laminar flow clean-air enclosure. The assembly was then mounted into the mass spectrometer and the center ionizing filament set at 1150 °C. The side filaments were slowly heated at a current of approximately 0.6 A to produce a stable ion beam of approximately 2 nA. Four sets of 39K/41K isotope ratios were acquired for each sample and the average ratio used for the computations. Mass fractionation of the 41K/41Ca ratio was assessed by measurement of a potassium isotopic standard.

Calcium measurements were made using a single-filament procedure employing a Ta ionizing filament. Approximately 5 µg of calcium was loaded onto the filament using a micro-pipette and heated to dryness under an infrared lamp in a laminar flow clean-air enclosure. Five µL of phosphoric acid was then added to the filament and heated to near dryness. The filament was next covered with a glass trough and the filament heated electrically at a current of 1 A for a few seconds. The filament was mounted into the mass spectrometer and initially heated at 1150 °C for 20 min to burn off any residual potassium, which would otherwise interfere with 40Ca measurements. Finally, the filament was ramped to a temperature in the range 1350 to 1400 °C, at which point an extremely stable ion beam of approximately 0.1 nA was produced. Multiple blocks of 40Ca/39Ca isotope ratios were then acquired. All of the ratios were internally normalized by concurrent measurement of the 43Ca/40Ca isotope ratio. This normalization procedure was very effective in correcting for fractionation effects, which tended to be more severe than the more traditionally used method employing a Re triple-filament assembly.

Results and discussion

Concentration and between sample variability

The cold plasma ID ICP-MS determination of K and Ca in six bottles of SRM 1566b, Oyster Tissue, is presented in Table 2. Our results for the determination of Ca in CCQM-P14 serum samples are presented in Table 3 and are normalized to the average ICP-MS result determined in the first run. The results are presented in this manner in order not to reveal the absolute concentration because the same material is being used in an ongoing interlaboratory study. The concentration is within the range of calcium in the serum of healthy adults, 2.25 to 2.65 mmol L⁻¹. Concentrations were calculated using the ID equation described by Fassett and Paulsen. Results for K in oyster tissue and Ca in serum highlight the repeatability obtainable with cold plasma ICP-MS. The relative standard deviation of six determinations of K in SRM 1566b was 0.078% (1 s). For five determinations of Ca in CCQM-P14, the relative standard deviation was 0.16% (1 s). ICP-MS ratio measurement repeatability (% relative standard deviation of five, 1-min measurements) for both the 39K/39Ca and 40Ca/39Ca ratios was typically between 0.1 and 0.2%, similar to the between sample repeatability obtained for K in oyster tissue and Ca in serum. This indicates that the variability in sample preparation did not exceed variability due to ICP-MS ratio measurement. Completeness of dissolution is also indicated by agreement in concentration results between samples of different mass. For the oyster tissue samples the mass of SRM 1327 is 0.06 g greater than the mass for S # 5, an 18% difference, yet the measured concentrations differ by less than 0.1%. Complete dissolution ensures that equilibration between sample and spike isotopes occurred because potassium and calcium each have only one oxidation state in solution.

Results for Ca in oyster tissue, however, do not show the good between-sample agreement obtained for K. The percent relative standard deviation (1 s, n = 6) for Ca in oyster tissue is 1.1%, primarily due to one high result for sample # 196. Since Ca was determined from the same sample aliquot as the K, it is unlikely that an error in sample preparation is the cause of the high result. Ca is an abundant element and contamination during preparation may have occurred. However, no blank of
Ca. For serum samples, the correction for Ca blank was less than 0.02% for K and 0.05% for Ca. Also listed in Tables 2 and 3 are results for replicate mass spectrometric measurements. These data were collected on different days and in a different run order. Instrument conditions were optimized each day. Average between day results differ by 0.09% for K in oyster tissue, and 0.01% and 0.1% for Ca in oyster tissue and serum, respectively. The magnitude of mass bias and the pattern of drift were different between days. The good between day agreement verifies that corrections were accurately applied.

Procedure blank
Blank resulting from the analytical procedure was assessed by adding small amounts of spike to clean Teflon beakers and manipulating and measuring them in a manner exactly the same as the samples. Sample preparation was performed in a class 10 clean environment. The average procedure blank determined for K was 29 ng ± 13 ng (n = 3). The average procedure blank determined for the Ca in oyster tissue analysis was 101 ng ± 25 ng (n = 2), and for the serum analysis it was 280 ng ± 160 ng (n = 4). For oyster tissue samples the correction for blank was less than 0.02% for K and 0.05% for Ca. For serum samples, the correction for Ca blank was less than 0.15%. A determination of the amount of Ca contributed from the cation resin yielded a result of less than 12 ng. It is believed that the beakers, which were cleaned, but had been used for previous analyses, were the primary source of Ca blank.

Spice calibrations
The concentration of the enriched isotopes in each spike solution was calibrated by reverse isotope dilution for each analysis, regardless of whether a calibration had been previously performed. The reason for this is two-fold. First, the concentration of the enriched isotope solution could change between analyses due to evaporation or adsorption. An undetected change in the concentration of the calibrating solution would result in a bias. Second, mass bias of the instrument can change dramatically from day to day due to changes in optimum operating conditions. By analyzing the spike calibration samples and analytical samples under the same operating conditions, uncertainty in the correction for mass bias is minimized and only instrument drift between samples needs to be monitored. Additionally, when preparing spike calibration samples, they are spiked so the resulting ratio is similar to the spiked ratio of the analytical samples, and they are diluted to the same count rate. This reduces uncertainty in the correction for detector dead time.

Calibrations were performed using two separately prepared solutions of primary samples. Two aliquots of two gravimetrically prepared solutions were added to aliquots of the enriched isotope solution, forming four calibration mixes. Variation between the results for the four mixes is a measure of the uncertainty associated with gravimetric sample preparation and ICP-MS (or TIMS) ratio measurement without complications caused by sample matrix. Relative standard deviations of 0.086% for K and 0.062% for Ca were obtained for the calibration of the enriched isotope solutions used for the oyster tissue analysis.

Table 2 Determination of calcium in international intercomparision CCQM-P14 Serum Samples by cold plasma-ID-ICP-MS

<table>
<thead>
<tr>
<th>Bottle#</th>
<th>Dry sample mass/g</th>
<th>K concentration/mg kg⁻¹</th>
<th>Ca concentration/mg kg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 2ᵦ</td>
<td>Day 1</td>
</tr>
<tr>
<td>5</td>
<td>0.24808</td>
<td>6528.9</td>
<td>829.6</td>
</tr>
<tr>
<td>196</td>
<td>0.25232</td>
<td>6528.5</td>
<td>853.4</td>
</tr>
<tr>
<td>1327</td>
<td>0.30308</td>
<td>6533.0</td>
<td>832.7</td>
</tr>
<tr>
<td>2759</td>
<td>0.25410</td>
<td>6523.7</td>
<td>837.2</td>
</tr>
<tr>
<td>2926</td>
<td>0.24830</td>
<td>6538.3</td>
<td>829.3</td>
</tr>
<tr>
<td>3605</td>
<td>0.25251</td>
<td>6534.1</td>
<td>833.7</td>
</tr>
<tr>
<td>Avg</td>
<td></td>
<td>6531.3</td>
<td>836.0</td>
</tr>
<tr>
<td>s</td>
<td></td>
<td>5.1</td>
<td>9.0</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.078</td>
<td>0.09</td>
<td>1.1</td>
</tr>
</tbody>
</table>

*Day 2 results are provided to indicate ICP-MS measurement repeatability only. Day 2 data were not used to calculate the overall average concentration.

Table 3 Determination of calcium in international intercomparision CCQM-P14 Serum Samples by cold plasma-ID-ICP-MS

<table>
<thead>
<tr>
<th>Vial#</th>
<th>Sample mass/g</th>
<th>Normalized Ca concentrationᵦ</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 14-1</td>
<td>2.02345</td>
<td>1.0015</td>
</tr>
<tr>
<td>P 14-2</td>
<td>2.01469</td>
<td>0.9990</td>
</tr>
<tr>
<td>P 14-3</td>
<td>2.01908</td>
<td>0.9979</td>
</tr>
<tr>
<td>P 14-4</td>
<td>2.02490</td>
<td>1.0000</td>
</tr>
<tr>
<td>P 14-5</td>
<td>2.02297</td>
<td>1.0017</td>
</tr>
<tr>
<td>Avg</td>
<td></td>
<td>1.0000</td>
</tr>
<tr>
<td>s</td>
<td></td>
<td>0.0016</td>
</tr>
<tr>
<td>n</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>RSD (%)</td>
<td>0.16</td>
<td>0.01</td>
</tr>
</tbody>
</table>

*Results are normalized to the average day 1 value. This is done so as not to reveal the absolute value determined. The Ca in the serum is at a normal physiological level. Day 2 results are provided to indicate ICP-MS measurement repeatability only. Day 2 data were not used to calculate the overall average concentration.

to compare isotope ratios measured in a matrix-free standard with those measured in an unspiked sample of the oyster tissue and serum. The results of this comparison for an unseparated oyster tissue sample showed the measured $^{39}$K/$^{40}$K and $^{44}$Ca/$^{42}$Ca ratios to be 1.3% and 30% lower than the same ratios measured for the pure standards. These results indicate a larger interference at masses 41 and 42, though in both cases these masses are more sensitive to interference because of their lower relative abundance. It is possible that interference occurs at all the isotopes of interest. The same comparison performed on unspiked oyster tissue samples that were separated using cation exchange chromatography showed agreement with the pure standards to within 0.3%, well within the repeatability of the measurement, thereby verifying the successful removal of interferences with the applied separation scheme. Measurement repeatability ($1/s$, $n = 5$) for natural composition ratios averaged 0.3% for the $^{39}$K/$^{40}$K ratio and 0.6% for the $^{44}$Ca/$^{42}$Ca ratio. Unseparated serum samples showed a negative bias of 7% relative to pure standards for the $^{40}$Ca/$^{42}$Ca ratio. After cation exchange separation, measured ratios agreed within 0.3%.

Identification of the exact source of matrix-induced polyatomic interferences is difficult. The most probable sources are the oxides and hydroxides of Na and Mg. Analysis of the separated fractions for both matrices showed that separation of Na, Mg, K and Ca was complete. Strontium is difficult to separate from Ca on cation exchange resin and some Sr was observed in the Ca fraction. However, doubly charged Sr ions were not observed under cool plasma conditions in agreement with the finding of Patterson et al.\textsuperscript{27} Based on signal intensity of the separated fractions, recovery of K and Ca was estimated at over 95%.

### Split sample comparison with thermal ionization mass spectrometry

The same sample preparations were split and analyzed by both ICP-MS and TIMS. The TIMS method for both K and Ca has been accepted as definitive for clinical analyses.\textsuperscript{12–14} These mass spectrometric methods are indeed distinct—in ionization, mass filtering and detection. A sample-to-sample comparison of ICP-MS and TIMS results for K and Ca in oyster tissue and Ca in serum is presented in Figs. 1–3, respectively. Concentration is shown on the right axis and percent difference from the mean is shown on the left axis. Error bars are 1/s of the ratio measurement, multiplied by the error multiplication factor, 1.05 for K and 1.04 for Ca.\textsuperscript{36} In general the TIMS measurement repeatability is a factor of two better than ICP-MS measurement repeatability: excellent agreement between

---

**Fig. 1** Split sample comparison of cold plasma-ID-ICP-MS and ID-TIMS for the determination of Ca in six bottles of SRM 1566b, Oyster Tissue. Concentration is shown on the left axis and percent difference from the mean is shown on the right axis. Error bars are 1/s of the ratio measurement, multiplied by the error multiplication factor (1.05). Average ICP-MS and TIMS results are less than 0.06% different.

**Fig. 2** Split sample comparison of cold plasma-ID-ICP-MS and ID-TIMS for the determination of Ca in six bottles of SRM 1566b, Oyster Tissue. Concentration is shown on the left axis and percent difference from the mean is shown on the right axis. Error bars are 1/s of the ratio measurement, multiplied by the error multiplication factor (1.04). The pattern of variance between bottles is replicated by the two techniques, indicating that material heterogeneity is the source of variance in the results. Average ICP-MS and TIMS results are less than 0.06% different.

**Fig. 3** Split sample comparison of cold plasma-ID-ICP-MS and ID-TIMS for the determination of Ca in five vials of international intercomparison CCQM-P14 serum samples. Percent difference from the mean is shown on the left axis. Error bars are 1/s of the ratio measurement, multiplied by the error multiplication factor (1.04). Average ICP-MS and TIMS results are less than 0.02% different.

---

The expanded analytical uncertainty for the ID-ICP-MS determination of K and Ca in SRM 1566b and Ca in CCQM-P14 is composed of individual Type A and Type B uncertainty components combined according to ISO guidelines.\textsuperscript{42} Individual uncertainty components, expressed as
relative uncertainties, and the expanded uncertainty, expressed as a 95% confidence interval, are given in Table 4. Coverage factors were determined based on the effective degrees of freedom calculated using the Welch–Satterthwaite formula.\(^4\) Minimization of potential systematic uncertainty in sample preparation, isotope ratio measurement, calibration of the spike and correction for analytical blank was discussed above. Uncertainties in these components were treated as Type A, and based on the standard uncertainties of the sample, spike calibration and blank results. Type B components were assumed to have a rectangular distribution of stated magnitude. Standard uncertainties for Type B components were derived by dividing by the square root of 3. The Type B component estimating uncertainty in instrument factors affecting ratio measurement includes uncertainty due to drift, dead time, and mass bias. Though experimental design to minimize uncertainties in these parameters was discussed, they are difficult to completely eliminate. Based upon consideration of count rate and isotope ratio differences, range of drift, and the difference in calculated concentration results for the range of experimentally determined dead times, this uncertainty component was estimated to be 0.3% relative. Uncertainty in the calibrator assay was based on the largest uncertainty reported on the certificates for SRMs 918a, 915a and 3109a. Weighing uncertainty was estimated to be of magnitude 0.06% relative, based on the smallest sample mass, the balance readability, and its linearity. The remaining Type B uncertainty component, an estimate for the uncertainty in the correction to dry mass, pertains only to the oyster tissue samples and would be common to any analytical procedure. The dry mass correction averaged 4% for the oyster tissue.

The magnitude of the correction depended strongly on the drying procedure used and an uncertainty estimate of relative magnitude 1.5% was derived based on the differences measured for samples dried using the vacuum oven procedure and the desiccator procedure.\(^4\) The uncertainty for the correction to dry mass is the largest uncertainty component for the oyster tissue determinations, but its magnitude based on experimental data is in keeping with what is observed for biological reference materials.\(^4\) Without the uncertainty component for the dry mass correction, the expanded uncertainties for the cold plasma ID ICP-MS determinations of K and Ca in oyster tissue are 0.38% and 1.1% relative, respectively, the latter being dominated by sample heterogeneity. When the uncertainty component for correction to dry mass is included, the expanded uncertainty becomes 1.7% and 1.9% relative for K and Ca, respectively. The expanded uncertainty for the determination of Ca in CCQM-P14 by cold plasma ID ICP-MS is 0.41% relative.

<table>
<thead>
<tr>
<th>Element</th>
<th>K</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>SRM 918a(^a)</td>
<td>SRM 915a(^a)</td>
</tr>
<tr>
<td>Measured value/mg kg(^{-1})</td>
<td>998.050</td>
<td>390.953</td>
</tr>
<tr>
<td>Difference (%)</td>
<td>-0.055</td>
<td>-0.010</td>
</tr>
</tbody>
</table>

\(^a\)Prepared and measured during the certification of SRM 1566b Oyster Tissue. \(^b\)Prepared and measured during the analysis of CCQM P-14, serum.

Results for control samples

Two types of accuracy validation samples were processed, gravimetrically prepared primary standards and previously certified SRMs with matrices similar to the analytical samples. The gravimetric samples do not provide a measure of matrix-related uncertainties, but method accuracy can be calculated by comparing the measured values to the gravimetric concentrations. Uncertainty on the gravimetric concentrations is small (less than 0.05%) based on the linearity and readability of the six-place microbalance and the certified assay. Gravimetric controls for K were prepared from SRM 918a and those for Ca were prepared from SRM 915a. Gravimetric control samples were treated exactly as the analytical samples and similar amounts were processed. Results are given in Table 5. Samples were measured on two different days to assess reproducibility. The average result and 1 s for the two determinations is reported. In all cases the measured concentrations agree with the gravimetric concentration to better than 0.1%.

ID-ICP-MS determinations in matrix matched control materials, SRM 1566a, Oyster Tissue, and SRM 956a, Level II Serum, are presented in Fig. 4. Results are plotted as percent difference from the certified value. The certified interval is depicted as the line on the left of each plot. The average measured result is depicted by the symbol. Error bars on the measured values for the oyster tissue samples are the 95% confidence interval for 3 separate sample determinations (2 degrees of freedom). The coverage factor for the 95% confidence interval was calculated based on the effective degrees of freedom for the combined standard uncertainties outlined in the Uncertainty section. The K and Ca results for SRM 1566a are both within the certified intervals. One determination was made for the serum sample. The Ca content of SRM 956a was certified in 1996 using ID-TIMS. The ID-ICP-MS determination reported here is 0.25% different from the certified value.

### Table 4 Uncertainty components for the determination of K and Ca in Oyster Tissue and Ca in Serum by cold plasma ID-ICP-MS

<table>
<thead>
<tr>
<th>Source</th>
<th>Potassium, SRM 1566b</th>
<th>Calcium, SRM 1566b</th>
<th>Calcium, CCQM P-14</th>
</tr>
</thead>
<tbody>
<tr>
<td>u_i (% rel.)</td>
<td>d.f.</td>
<td>u_i (% rel.)</td>
<td>d.f.</td>
</tr>
<tr>
<td>Type A uncertainties—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample/measurement</td>
<td>0.035</td>
<td>5</td>
<td>0.44</td>
</tr>
<tr>
<td>Spike calibration</td>
<td>0.043</td>
<td>3</td>
<td>0.031</td>
</tr>
<tr>
<td>Blank correction</td>
<td>0.0011</td>
<td>2</td>
<td>0.036</td>
</tr>
<tr>
<td>Combined type A</td>
<td>0.055</td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Type B uncertainties—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moisture correction</td>
<td>0.87</td>
<td>∞</td>
<td>0.87</td>
</tr>
<tr>
<td>ICP-MS factors (drift, dead time, mass bias)</td>
<td>0.17</td>
<td>∞</td>
<td>0.17</td>
</tr>
<tr>
<td>Calibrant purity</td>
<td>0.058</td>
<td>∞</td>
<td>0.058</td>
</tr>
<tr>
<td>Weighing uncertainty</td>
<td>0.035</td>
<td>∞</td>
<td>0.035</td>
</tr>
<tr>
<td>Combined type B</td>
<td>0.89</td>
<td></td>
<td>0.89</td>
</tr>
<tr>
<td>Combined type A and B</td>
<td>0.89</td>
<td></td>
<td>0.98</td>
</tr>
<tr>
<td>Coverage factor</td>
<td>1.96</td>
<td></td>
<td>1.98</td>
</tr>
<tr>
<td>Expanded uncertainty</td>
<td>1.7(^a)</td>
<td></td>
<td>1.9(^a)</td>
</tr>
</tbody>
</table>

\(^a\)95% Confidence Interval.
well within the ±0.6% relative uncertainty on the certified value. Our ability to achieve comparability with a well-defined national standard validates the accuracy of our protocol and demonstrates that ID TIMS and cold plasma ID-ICP-MS results for the determination of Ca in serum are commutable.

Interlaboratory comparison CCQM-P14

The NIST result in the CCQM-P14 inter-laboratory comparison for Ca in serum was in excellent agreement with those of four other institutes that also applied the isotope dilution technique, including three that used TIMS. Our laboratory was the only one to use cold plasma, quadrupole ICP-MS.

Certified concentrations for SRM 1566b

The certified values for SRM 1566b are the weighted means of four other institutes that also applied the ID-ICP-MS technique, including three that used TIMS. Our laboratory was the only one to use cold plasma, quadrupole ICP-MS.

Conclusion

Isotope dilution inductively coupled “cold” plasma mass spectrometry is an accurate method for the quantitation of K and Ca. An expanded ID-ICP-MS uncertainty of 0.4% relative was achieved for the determination of K in oyster tissue (excluding the uncertainty in the dry mass correction) and Ca in serum. The level of heterogeneity of Ca in the oyster tissue was achieved for the determination of K in oyster tissue (excluding the uncertainty in the dry mass correction) and Ca in serum. The error bar for the oyster tissue samples is the 95% confidence interval for 3

Fig. 4 Cold plasma ID-ICP-MS determinations in control materials, SRM 1566a, Oyster Tissue, and SRM 956a, Level II Serum. The error bar for the oyster tissue samples is the 95% confidence interval for 3 separate determinations (see text). One determination was made for the serum sample. All results are within the certified interval.

Certified concentrations for SRM 1566b

The certified values for SRM 1566b are the weighted means of two or more analytical methods. Results of the different analytical methods and the final certified values for K and Ca in SRM 1566b are given in Table 6. For Ca the certified result is the weighted mean of the NIST cold plasma-ID-ICP-MS result and the NIST instrumental neutron activation result. For K the certified result is the weighted mean of the NIST cold plasma ID-ICP-MS result and NIST instrumental neutron activation and prompt gamma activation results. For both elements, excellent agreement was obtained between the mass spectroscopic results for K and Ca. An expanded ID-ICP-MS uncertainty of 0.4% relative was achieved for the determination of K in oyster tissue (excluding the uncertainty in the dry mass correction) and Ca in serum. The level of heterogeneity of Ca in the oyster tissue was achieved for the determination of K in oyster tissue.