

Rapid and precise calcium isotope ratio determinations using the Apex-ACM desolvating inlet system with sector-field ICP-MS in low resolution



Abstract

High resolution ICP-MS is used to evaluate the APEX-ACM membrane desolvator for the determination of $^{42}\text{Ca}/^{44}\text{Ca}$ and $^{43}\text{Ca}/^{44}\text{Ca}$ isotope ratios using low resolution. The APEX-ACM has a rapid uptake and wash and lower Ca blanks than a conventional spray chamber. The APEX-ACM also reduces the ArH_2^+ interference on ^{42}Ca by more than 100x, lowers total polyatomic interferences and blanks to $<0.5\%$ of the Ca signal, and provides excellent short term and run to run precision on Ca isotope ratios.

Introduction

The determination of three calcium isotopes in biological samples is necessary for ICP-MS applications in human metabolic isotopic tracer studies. The mass spectrum obtained from an ICP-MS is subject to a number of plasma, water and matrix based interferences. This is particularly problematic in the low mass range where these interferences are both numerous and significant. As a result, it is impossible to determine 3 interference free calcium isotopes in low resolution on solutions aspirated using a conventional spray chamber. Polyatomic



interferences can be reduced sufficiently by using cool plasma¹ and hexapole² or resolved using HR-ICP-MS^{3,4,5} however precision is typically not better than 0.25% (1-std). The implementation of desolvation results in excellent precision for Ca isotope ratio determination in low resolution on single collector ($<0.1\%$, 1-std⁶) and multi-collector (0.005%, 1-std⁷) ICP-MS. Here we use oxalate precipitation of urinary calcium and the APEX-ACM sample introduction system for low-resolution determination of the 42, 43 and 44 isotopes of calcium. Stability and short term precision is excellent ($<0.04\%$ 1-std) and remained better than ($<0.10\%$ 1-std) during long runs when a 50% reduction in sensitivity due to cone deposition is observed. Spectral interference reductions with the APEX desolvating inlet system were investigated using medium resolution on the ELEMENT-1. The APEX-ACM desolvation system reduced the ArH_2^+ plasma/water based interference by 100x, less than $<0.5\%$ of ^{42}Ca signal in samples.

APEX-ACM

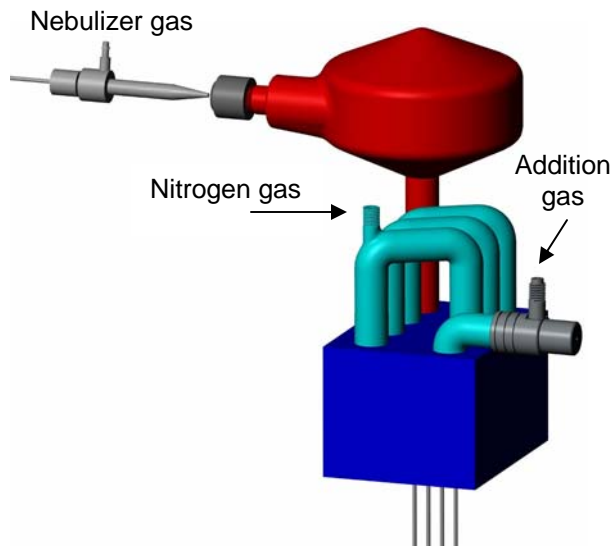


Figure 1: Apex internal flow path

The Apex is a fully-integrated inlet system that connects directly to the torch injector and incorporates ESI's MicroFlow PFA nebulizer technology. Liquid samples are nebulized into a spray chamber and desolvation system where the sample aerosol is conditioned to produce uniform aerosol that is transported to the ICP. For additional desolvation, the Actively Cooled Membrane (ACM) module is placed inline between the APEX and the injector. The ACM is a cooled Nafion® fluoropolymer membrane desolvation module that reduces the solvent load in the plasma. Solvent vapors pass into the Nafion® membrane and are removed by a counter current sweep flow of a dry gas such as nitrogen or argon. The sweep gas does not pass through the membrane or enter the sample aerosol stream, it simply serves to receive solvent vapor molecules that pass through the membrane.

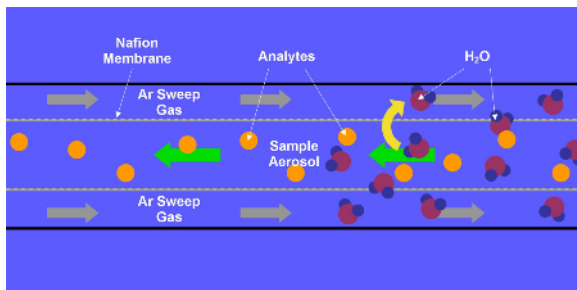


Figure 2: Nafion membrane

Operating Parameters

The most precise isotope ratios are obtained on a single collector ICP-MS when a combination of stable sample introduction and rapid scanning is used to minimize plasma noise. The APEX-ACM with a free aspirating μ Flow100 nebulizer optimizes signal stability by eliminating the peristaltic pump and reducing the water load in the plasma. The adverse affect of plasma noise is further compensated on the ELEMENT-1 by settling the magnet at the start mass (42) and electro-statically scanning rapidly the isotopes of interest without moving the magnet. Further operating parameters are outlined in Table 1.

Sample Preparation

Urine was prepared for analysis by precipitation as calcium oxalate. The procedure for the precipitation of calcium oxalate from urine is as follows:

- 1) Pipette 5.0 mL urine in acid washed pyrex test tube.
- 2) In a fume hood, add 3 drops of 25% NH_4OH .
- 3) Add 3 mL of saturated ammonium oxalate solution (1g in 20mL of Milli-Q water).
- 4) Precipitate in a fume hood overnight.
- 5) Decant off liquid then centrifuge for 15 min @ 2000 rpm.
- 6) Wash with 1 mL of a 1:4 dilution of saturated ammonium oxalate solution then vortex.
- 7) Centrifuge at 2500 RPM for 10 min.
- 8) Decant off liquid then ash in furnace at 500°C for 4 hours.
- 9) Cool and dissolve in 100 μL of 3% HNO_3 .
- 10) Dilute sample to approximately 5ppm (100X).

Table 1: Operating parameters

ELEMENT-1 with CD-1	
Gas Flow	L min ⁻¹
Sample	0.8
Auxillary	1.00
Cool	16.0
RF	1350W
Sample uptake	60 sec
Analysis time	120 sec
Sample wash	60 sec
Sample flow rate	200 $\mu\text{L min}^{-1}$
Resolution	300
Detector mode	Analog
Apex-Q	
Condenser	2° C (2)
Heater	140° C (2)
MFC	on
Gas Flows	
Nitrogen Flow	8.8 mL min ⁻¹
Additional Ar Flow	150 mL min ⁻¹

Uptake and Wash

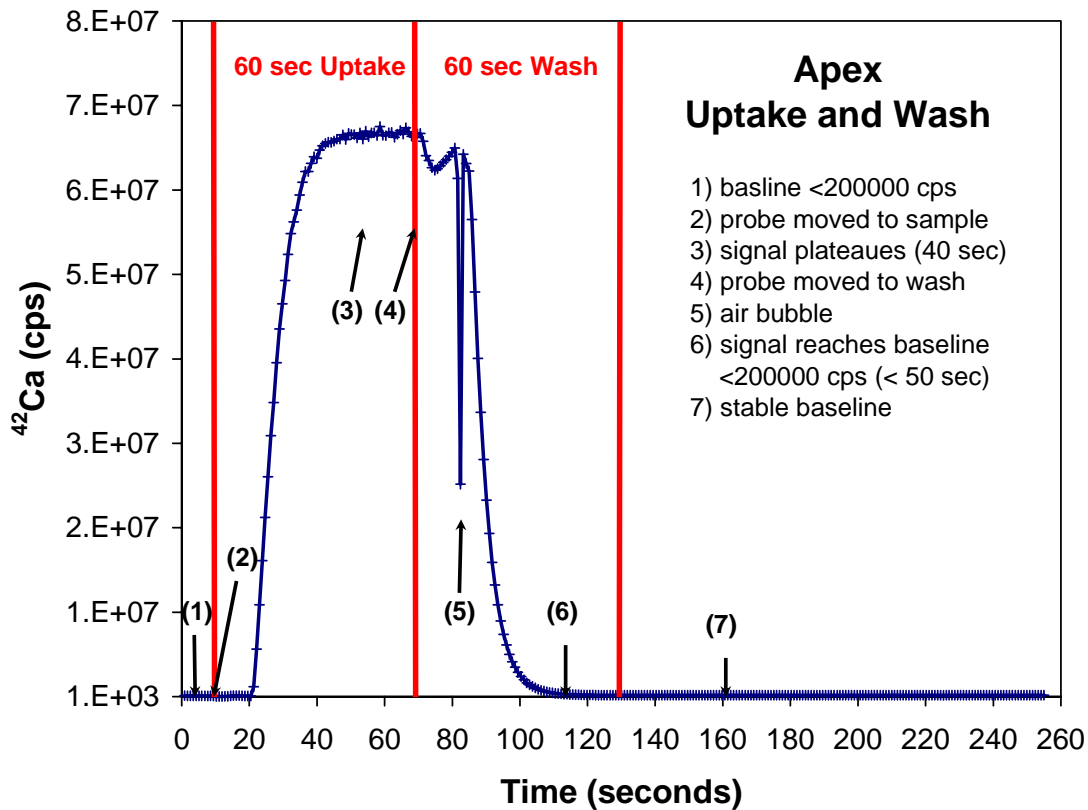


Figure 3

The uptake and wash times are determined by monitoring the ⁴²Ca signal. The sample probe, originally in a blank solution is placed in the sample solution at time 2 and returned to the blank solution again at time 4. The signal plateaus at time 3 and return to baseline again at time 6. Note that wash out is complete and no signal spiking is observed (7). Based on these observations 60 second was determined to be a sufficient uptake and wash time.

Interferences and Blanks

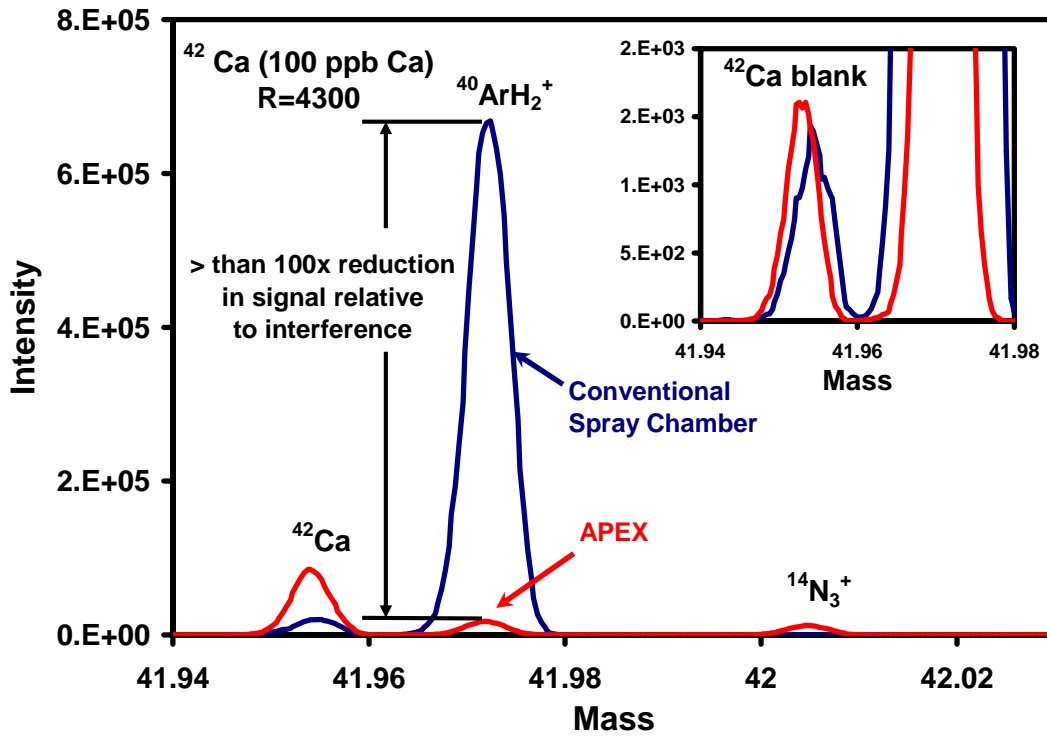


Figure 4

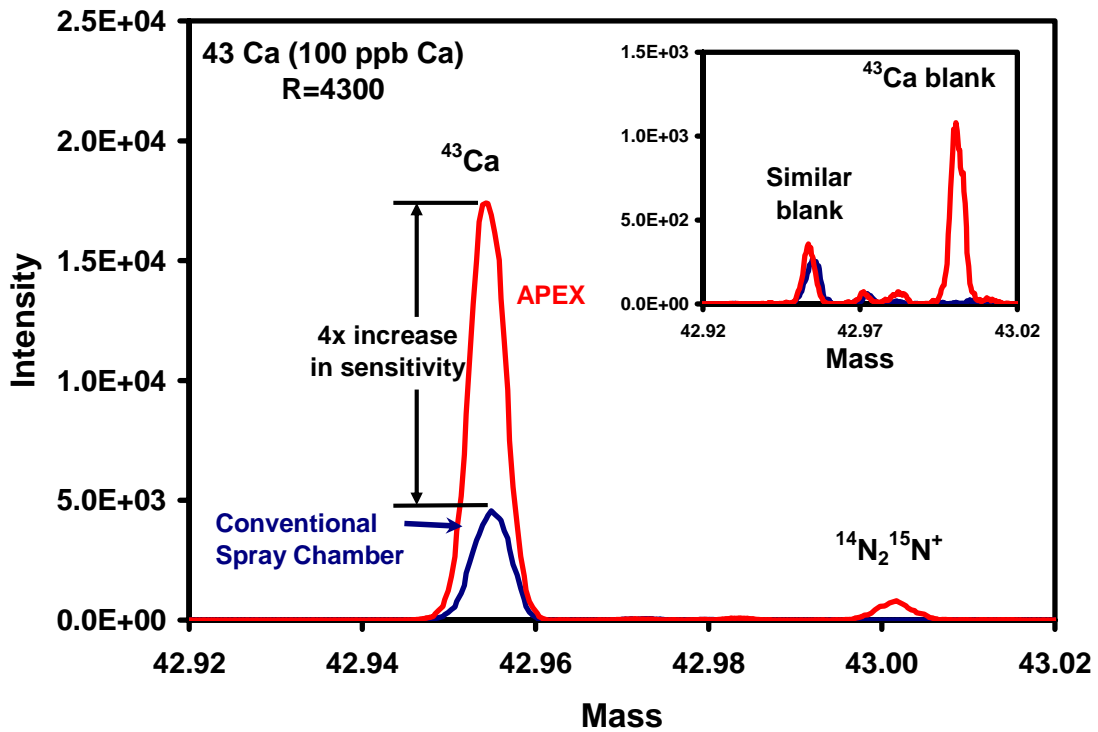


Figure 5

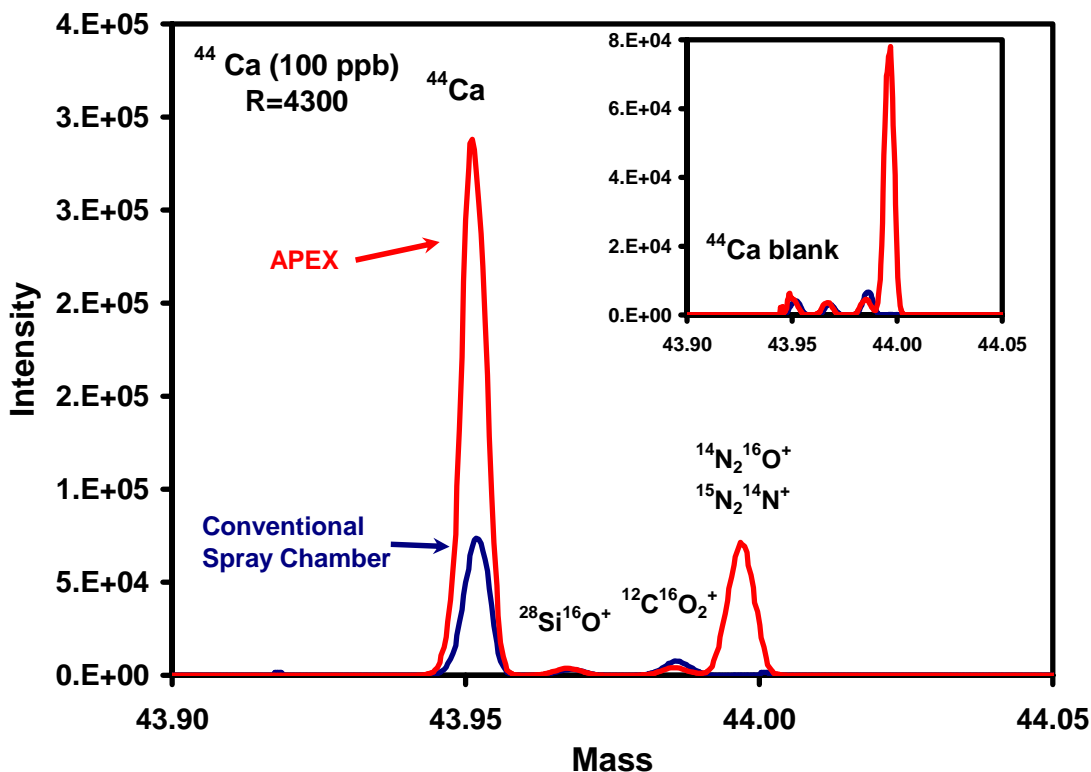
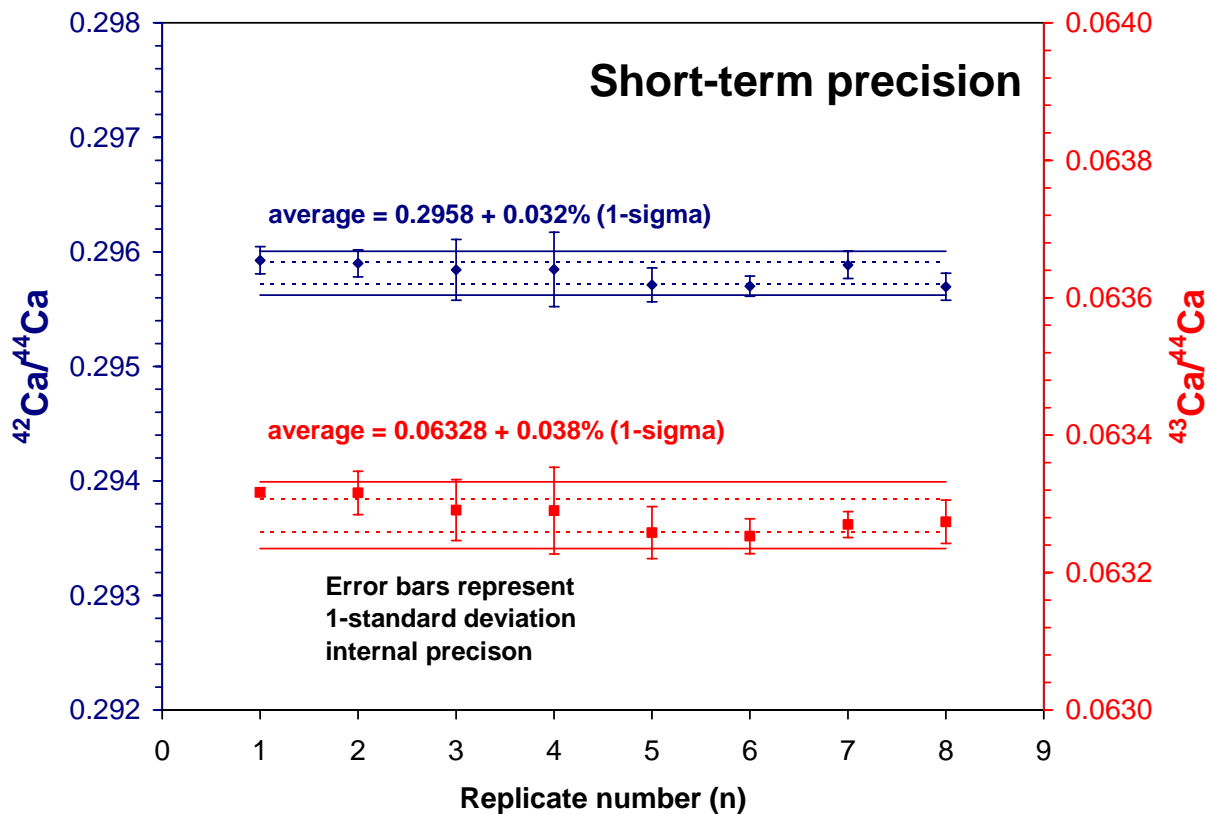


Figure 6

Spectral interferences originate from doubly charged $^{86}\text{Sr}^{++}$ on ^{43}Ca , $^{88}\text{Sr}^{++}$ on ^{44}Ca and polyatomic ions (ref). The doubly charged ions are corrected by measuring $^{87}\text{Sr}^{++}$ at half mass 43.5, but polyatomic interferences are more significant and numerous and cannot be easily corrected at the required level of precision. Most polyatomic interferences on Ca isotopes are water based (oxides and hydrides) and are significantly reduced when using the APEX-ACM desolvation unit. Using the APEX-ACM for sample introduction, interferences and blanks are evaluated at a resolution of 4300 on the ELEMENT-1 in dilute acid and a 100 ppb Ca solution. At similar sample flow rates Ca sensitivity is increased by 4X when using APEX-ACM relative to a standard Scott spray chamber, but Ca blanks and interferences on ^{43}Ca and ^{44}Ca remain similar. These data indicate the APEX-ACM provides a 4-fold increase in Ca to blank/interference for ^{43}Ca and ^{44}Ca . The most important and significant improvement is realized in the 100 fold reduction in the ArH_2^+ interference on ^{42}Ca isotope. Without the reduction of ArH_2^+ the determination of ^{42}Ca in low resolution would not be possible.

The APEX-ACM provides a significant reduction in blank and interferences relative to Ca signal when compared to a conventional Scott spray chamber. Ca isotope analyses are performed on approximately 10 ppm Ca solutions. In low resolution the APEX-ACM reduces the combined blank and interference contribution to less than 0.5% of the sample signal. For best precision these ratios can now be determined in low resolution ($R=300$) with minimal correction for blank and interferences.

Short-term Precision**Figure 7**

On a signal collector ICP-MS plasma noise contributes significantly to isotope ratio precision. As discussed above this is minimized by, 1) rapidly scanning the mass spectrum and 2) reducing instabilities associated with sample introduction. The ELEMENT-1 is configured for rapid scanning and free aspirating micro flow nebulization combined with the APEX-ACM reduces pump periodicity and plasma noise associated with variable sized water droplets. The short-term precision is determined for eight two-minute acquisitions (n=8) of Ca isotope ratios. Error bars represent the precision (1-std) of each analysis, dashed and solid lines indicate the 1 and 2 standard deviations of the data set. The data indicate that better than 0.04% (1-std) precision is obtained for data collected over 15 min.

Effect of Salt Deposition on Cones on Signal and Isotope Ratio Stability

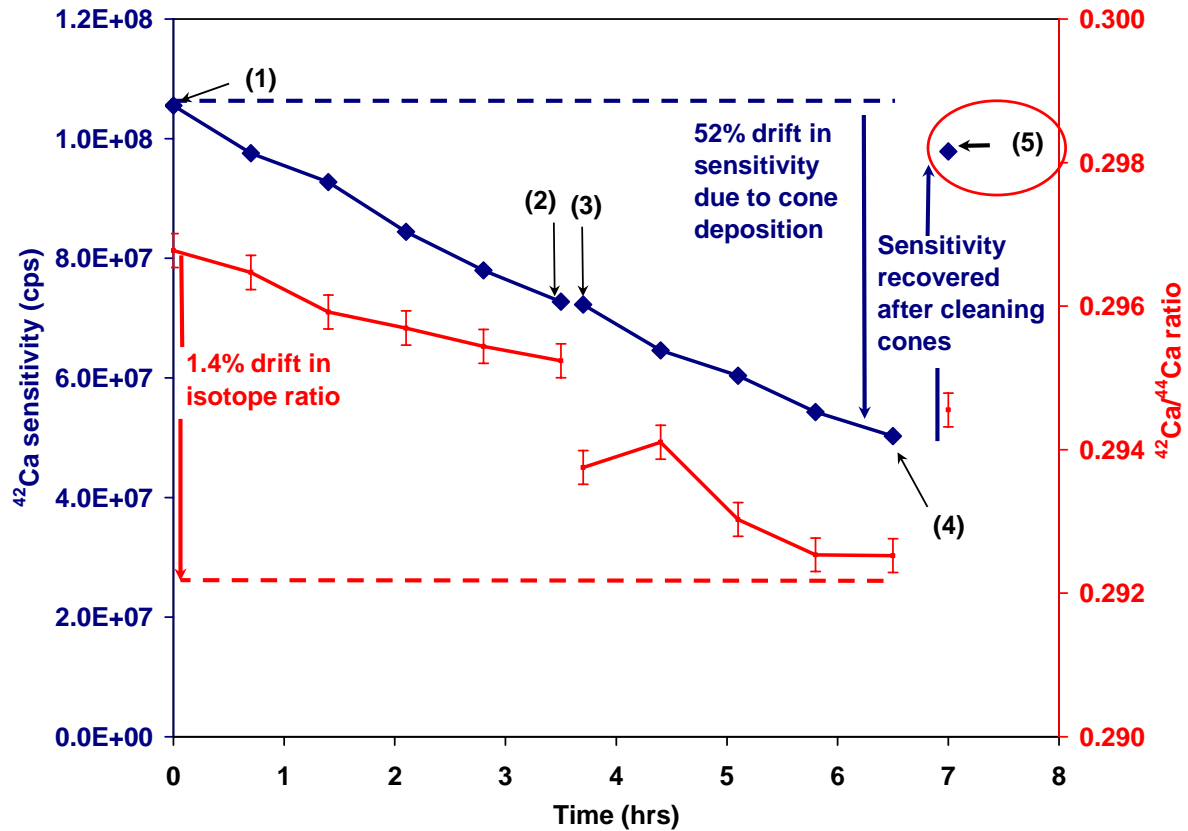


Figure 8 – Isotope Ratio Drift Corrected by Sample/Standard Bracketing

- 1) start of run 1 (24 unknowns)
- 2) end of run 1
- 3) start of run 2 (24 unknowns)
- 4) end of run 2
- 5) clean cones, start of run 3



Figure 9

During long runs, required for high sample through put, both signal intensity and isotope ratios drift with time. With this sample type this is often due to either cone deposition. Ca forms oxides that deposit on the cones and result in a gradual, but constant reduction in sensitivity. When analyzing samples containing high ppm levels of Ca this is significant and can be as much as 50% over the course of 6 to 8 hours. Two runs consisting of 24 unknowns with standards every 6th unknown indicates that isotope ratios drift only slightly (1.4%). The drift is very smooth and usually less than 0.1% between standards (every 28 min) requiring a small sample-sample correction (<0.02%). Furthermore, the precision of individual analysis remains less than 0.1% (1-std). These factors combined with the near complete recover of sensitivity (to within 10%) after cleaning the cones indicate that the APEX-ACM is a very stable sample introduction system and that drift is due to cone deposition.

Run-to-Run Precision and Accuracy

To determine run-to-run reproducibility one Ca oxalate precipitated urine sample was analyzed at the beginning and end of three runs over two days. The precision, based on the reproducibility of this sample is better than 0.1% (1-std) between runs. The sample is from a person not involved in enrichment studies and should therefore exhibit a natural isotopic signature. The determined ratio for the unspiked individual is within 2 standard deviations of the expected natural ratio⁸. These data indicate that the APEX-ACM produces both precise and accurate data for Ca isotope ratios using low resolution ICP-MS.

Determination of Percent Enrichment

The percent enrichment of urinary Ca isotope ratios is simply determined by comparing the post spiking isotopic ratio to a baseline sample collected before spiking of the patient.

Summary

High resolution ICP-MS is used to evaluate the APEX-ACM for the determination of $^{42}\text{Ca}/^{44}\text{Ca}$ and $^{43}\text{Ca}/^{44}\text{Ca}$ isotope ratios in low resolution. We find that the APEX-ACM

- Has a rapid uptake and wash (<50 seconds).
- Exhibits Ca blanks 4x lower than a conventional spray chamber.
- Reduces the ArH_2^+ interference on ^{42}Ca by more than 100x.
- Lowers total polyatomic interferences and blanks to <0.5% of the Ca signal.
- Provides excellent short term precision <0.04% (1-std) on Ca isotope ratios.
- Provides excellent run to run precision <0.1% (1-std) on Ca isotope ratios.

Table 2: Run to run precision and accuracy

n=6	$^{42}\text{Ca}/^{44}\text{Ca}$	$^{43}\text{Ca}/^{44}\text{Ca}$
start of run 1	0.3119	0.06486
end of run 1	0.3115	0.06472
start of run 2	0.3116	0.06469
end of run 2	0.3117	0.06474
start of run 3	0.3116	0.06476
end of run 3	0.3120	0.06473
average	0.3117	0.06475
%RSD	0.06	0.09
natural ⁸	0.3121	0.06486
% difference	0.12	0.17

Conclusion

The APEX-ACM is a stable sample introduction system that reduces interferences on the 42 43 and 44 isotopes of Ca. The reduction of interferences combined with signal stability allows for the precise determination of Ca isotope ratios in low resolution. These results indicate that this method should be easily adapted to the precise determination of Ca isotopes on low-resolution ICP-MS instruments.

References

- 1) K. Y. Patterson, C. Veillon, A. D. Hill, P. B. MoserVeillon and T. C. O'Haver, *J. Anal. At. Spectrom.*, 1999, **14**, 1673
- 2) S. F. Boulyga and J. S. Becker, *Fresenius J. Anal. Chem.*, 2001, **370**, 618.
- 3) S. Sturup, M. Hansen and C. Molgaard, *J. Anal. At. Spectrom.*, 1997, **12**, 919.
- 4) S. Sturup, *J. Anal. At. Spectrom.*, 2002, **17**, 1.
- 5) Z. Chen, I. J. Griffin, Y.L. Kriseman, L.K. Liang, S.A. Abrams, *Clin. Chem.*, 2003, 49, 2050.
- 6) M. P. Field, S. Shapses, M. Cifuentes, and R.M. Sherrell *J. Anal. At. Spectrom.*, 2003, **18**, 727.
- 8) W. A. Russell, D. A. Papanastassiou and T. A. Tombrello, *Geochim. Cosmochim. Acta.*, 1978, **42**, 1075.