



APPLICATION NOTE

Inductively Coupled Plasma – Mass Spectrometry

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The Determination of Lead in Calcium-Based Antacid and Dietary Supplements Using the NexION 300 ICP-MS System to Comply with the State of California's Proposition 65 Legislation

Summary

Compliance with California's Proposition 65 for the monitoring of lead (Pb) in calcium-based antacid medications and dietary supplements requires the analytical methodology to demonstrate detection limits below 0.05 $\mu\text{g/g}$ in the solid material. The analytical capabilities of Inductively Coupled Plasma Mass Spectrometry (ICP-MS) make it the most suitable technique for performing lead determinations at these low levels on a routine basis. However, potential problems must be overcome, which makes the analysis of these kinds of samples problematic unless certain precautions are taken. This Application Note will investigate the use of the NexION™ 300

ICP-MS system to determine lead in various antacids and dietary supplements. In particular, the Pb detection limit capability will be assessed, together with the ability to carry out spiked addition recoveries. Furthermore, long term stability data will be presented to show suitability for determining trace levels of lead in calcium-containing matrices in a high sample throughput environment.

Introduction

The California Safe Drinking Water & Toxic Enforcement Act of 1985, more commonly known as "Proposition 65", is a voter-driven initiative to address concerns regarding exposure to chemicals which might cause cancer or reproductive problems. Under this California law, companies are prohibited from discharging certain chemicals into sources of drinking water and exposing people to carcinogenic chemicals without prior warning.

Lead (Pb) is one of the elements identified by the State of California as both a cancer-causing agent and a reproductive toxin.¹ Under California Proposition 65, the "no significant risk level" (NSRL) established by the Office of Environmental Health Hazard Assessment for lead exposure is 0.5 µg/day.² Since antacids and dietary supplements are commonly ingested by people in significant amounts on a daily basis, many manufacturers are now testing these products for lead content.

Since the NSRL level for lead is given as a total exposure of 0.5 µg/day, it is necessary to determine the detection levels needed for monitoring purposes. For example, the U.S. RDA (Recommended Daily Allowance) for calcium in the adult diet is 1000 mg. If the entire RDA were to be obtained from a single calcium-containing supplement, the Pb concentration in that supplement must be <0.5 µg/g. In order to quantify <0.5 µg/g, the detection limit for Pb should be a minimum of 10 times lower. Therefore to perform reliable quantitation of Pb at the 0.5 µg/g level in the solid material, a method detection limit of 0.05 µg/g or lower would be necessary.

Extremely low detection limits have been the major attraction of ICP-MS for trace element quantitation. For example, a previous investigation showed that the IDL for lead by ICP-MS is approximately 50-100x lower than by graphite furnace AA.³ However, one of the recognized weaknesses of ICP-MS is signal suppression and long-term instability caused by the sample matrix. This is partially overcome using internal standardization, but can be particularly severe with calcium-based samples because of the potential for calcium deposits building up on the interface cones and ion optics. This issue can have a negative impact on detection limit, recovery of spiked additions and the likelihood of long-term drift when carrying out high throughput analysis. For this reason, when assessing the suitability of an instrumental technique to analyze these types of samples on a routine basis, it is very important to evaluate its long-term stability characteristics as well as assessing its detection limit and spike recovery capabilities.

The investigation was carried out using a NexION 300 ICP-MS, to determine Pb in a variety of calcium-containing materials, including calcium and calcium/magnesium-based antacids, a dietary supplement, some calcium salts and a NIST® (National Institute of Science and Technology) multivitamin tablet, using simple aqueous calibration standards.

Experimental

Six different calcium-based materials were chosen for the evaluation, which represents a typical cross section of antacids, over-the-counter mineral supplements, multivitamins and calcium salts used in the manufacturing process. Details of the samples are shown in Table 1.

Table 1. Calcium-based samples used in this study.

Sample	Description
Calcium-based antacid tablet	Over the counter antacid, containing only calcium as the active ingredient
Calcium- and magnesium-based antacid tablet	Over the counter antacid, containing both calcium and magnesium as the active ingredients
Multivitamin dietary supplement	Over the counter dietary supplement
Calcium carbonate (CaCO ₃)	Fisher® Chemical ACS grade, CAS 471-34-1
Calcium biphosphate (CaHPO ₄)	Fisher® Chemical Reagent grade, CAS 7789-77-7
NIST® 3280 SRM	Multielement vitamin tablet (Note: not certified for Pb)

Sample Preparation

Approximately 0.2 g of the crushed sample was accurately weighed into a 50 mL autosampler tube. Two mL of Veritas® double distilled, high purity concentrated nitric acid (GFS Chemicals™) was added. After the initial reaction, 2 mL of deionized water was added, together with 10 µg/L of lutetium (¹⁷⁵Lu, as an internal standard) and then diluted to 50 mL with deionized water.

Calibration

Standards of 0.1, 0.2, 0.4 and 0.6 µg/L Pb and 10 µg/L Lu in 2% nitric acid were used for the calibration, together with a 2% nitric acid blank. The calibration plot and statistical data are shown in Figure 1.

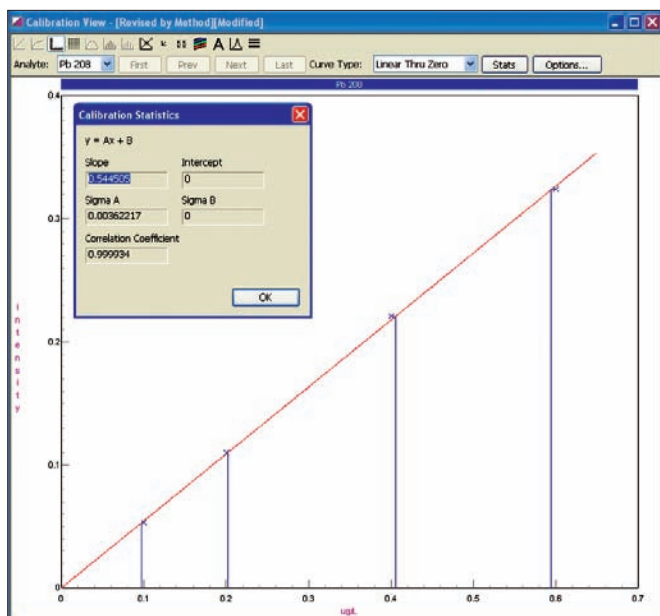


Figure 1. Calibration plot for 0-0.6 µg/L Pb in 2% nitric acid.

Instrumentation

All data in this study was generated on a NexION 300Q ICP mass spectrometer (PerkinElmer®, Inc., Shelton, CT) fitted with a low flow microconcentric glass nebulizer and a baffled, glass cyclonic spray chamber (both from Meinhard® Glass Products, Golden, CO). The instrumental operating conditions for this application are shown in Table 2.

Table 2. The ICP-MS instrumental operating conditions for this application.

Component/Parameter	Type/Value/Mode
Analyte Isotopes Measured	Sum of ²⁰⁶ Pb, ²⁰⁷ Pb and ²⁰⁸ Pb
Internal Standard	10 µg/L ¹⁷⁵ Lu
Nebulizer	Low flow glass microconcentric
Spray Chamber	Baffled glass cyclonic
Triple Interface Cone Material	Nickel
Plasma Gas Flow	16.0 L/min
Auxiliary Gas Flow	1.0 L/min
Nebulizer Gas Flow	0.92 L/min
Sample Uptake Rate	300 µL/min
RF Power	1600 Watts
Dwell Time/No. of Sweeps	50 ms/20
Total Integration Time	1 s per mass
No. of Replicates per Sample	3
Universal Cell Technology	Standard Mode

Analytical Evaluation

The analytical evaluation consisted of three different tests:

- Detection Limits:** Both the instrument detection limit (IDL) and the method detection limit (MDL) were measured. IDLs are determined by measuring the calibration blank a number of times and multiplying the resulting standard deviation by three. However, it is also important to compare these data by carrying out the detection limit test with a low-level standard (MDL) and a typical sample with matrix.
- Spike Recoveries:** Samples were first analyzed, and then a known concentration of Pb was added to each and quantified. The spike recoveries were calculated by comparing the concentration of Pb in the spiked samples to the concentration in the original sample.
- Long Term Stability:** The true test of any analytical technique for high throughput, routine analysis is the ability to run real-world samples for extended periods of time without recalibration. Therefore, to assess long-term stability, the Pb signal was monitored in one of the antacid tablets over five hours without recalibration.

Results

Detection Limits

Detection limits were determined in three different ways; these are shown in Table 3. First, the Pb Instrument Detection Limit (IDL) was determined by measuring seven reagent blanks against the calibration curve. The Pb Method Detection Limit (MDL) was determined by measuring seven reagent blanks spiked with 0.010 µg/L Pb. Table 3 shows the MDL both in solution and in the solid, based on a 0.2 g sample digested and diluted to 50 mL. Finally, the detection limit in an actual calcium- and magnesium-based antacid tablet was determined by measuring the Pb concentration from seven different digestions. Again, Table 3 shows detection limits in both solution and the solid.

Table 3. Pb Detection Limits.

	Instrumental Detection Limits	Method Detection Limits	Detection Limits in a Sample
Solution (µg/L)	0.0014	0.0025	0.006
Solid (µg/g)	–	0.0006	0.0015

These data indicate Pb detection limits in solution range from 1-6 ppt (ng/L), which is typical of an aqueous-based instrument detection limit for Pb by ICP-MS. However, a Pb MDL of 0.0015 µg/g in the solid indicates it is sufficiently low enough to reliably quantitate Pb in a calcium matrix at

0.05 µg/g as defined under Proposition 65. It should also be noted that these detection limit studies were done using a 1-second measurement time per mass. The benefit of such a short integration time is that the methodology can be applied to a high sample-throughput environment. If lower detection limits are required, the method could easily be modified to use a longer integration time.

Spike Recoveries

Five of the six samples were spiked with 0.2 µg/L Pb, while the CaHPO₄ was spiked with 2.0 µg/L since Pb was present at a higher level than in the other samples. The spikes were added post-digestion. The quantitative results for all the samples, together with the spike recovery data, are shown in Table 4. It can be seen that all the recoveries are within the generally accepted guidelines in the food and environmental monitoring industries of ±15% of the actual value.⁴ It should also be emphasized that Pb was not certified in NIST® 3280.

Table 4. Quantitative results for Pb in all the calcium-based samples, together with the spike recovery data.

Sample Type	Pb Conc. in Solid (µg/g)	Standard deviation (µg/g)	Spike (µg/L)	% Spike Recovery
NIST® 3280	0.029	0.002	0.2	86.4
CaCO ₃	0.093	0.001	0.2	97.8
CaHPO ₄	0.342	0.003	2.0	94.3
Antacid Tablet (Ca)	0.063	0.001	0.2	84.5
Antacid Tablet (Ca & Mg)	0.025	0.001	0.2	85.5
Multivitamin Supplement	0.039	0.001	0.2	101.1

Long Term Stability

For this test, the Ca/Mg antacid tablet was spiked with 0.5 µg/L Pb and the signal monitored every 30 minutes over approximately five hours, with a rinse cycle between the measurements. It should be emphasized that no recalibration was carried out over the time period of the stability run. The 5-hour stability plot is shown in Figure 2. It shows that even after almost 5 hours of measuring the Ca/Mg matrix, the 0.5 µg/L Pb signal has only drifted by 3%.

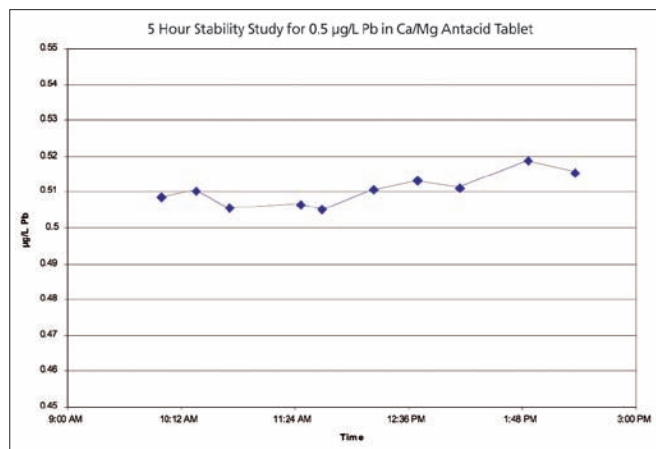


Figure 2. Five-hour stability plot for 0.5 µg/L Pb in the Ca & Mg antacid tablet.

Conclusion

The study has shown that the NexION 300 ICP-MS can handle the determination of Pb in calcium-based samples with relative ease. The Pb detection limit determined under routine analytical conditions is more than 30-fold lower than what is required to determine the levels needed under the State of California's Proposition 65. When this performance is combined with excellent long term stability and spike recovery data that is consistent with guidelines defined by federal regulatory agencies, it clearly shows the instrument is ideally-suited for the high sample throughput analysis of calcium-based antacid medications and dietary supplements.

References

1. Safe Drinking Water and Toxic Enforcement Act of 1986, California Code of Regulations, Sections 25249.5 and 25249.5
2. List of Chemicals Known to the State of California to Cause Cancer or Reproductive Toxicity, California Code of Regulations, Title 22, Section 12000, August, 1997.
3. Analysis of Lead (Pb) in Antacids and Calcium Compounds for Proposition 65 Compliance, R. Wolf, Atomic Spectroscopy, 18 (6), 1997.
4. Determination of Trace Elements in Waters and Wastes by ICP-MS: U.S. EPA Method 200.8 – Version 5.4, 1994; J. T. Creed, C.A. Brockhoff, T.D. Martin – http://www.epa.gov/waterscience/methods/method/files/200_8.pdf