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ORIGINAL PAPER

On the certification of cadmium at trace and ultratrace levels in standard reference materials using ID ICP-MS

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Abstract Analytical methods used for the isotope dilution inductively coupled plasma mass spectrometric (ID-ICP-MS) measurement of Cd at μg kg^{-1} and sub-μg kg^{-1} levels are described and applied to the certification of new dietary supplement, blood, and serum Standard Reference Materials (SRMs). The materials are: SRM 3240 *Ephedra sinica* Stapf Aerial Parts, SRM 3241 *Ephedra sinica* Stapf Native Extract, SRM 3243 Ephedra-Containing Solid Oral Dosage Form, SRM 3244 Ephedra-Containing Protein Powder, SRM 966 Toxic Metals in Bovine Blood, Level 1 (L1) and Level 2 (L2), and SRM 1598a Animal Serum. The concentration of Cd in the materials ranges from 120 μg kg^{-1} down to 0.03 μg kg^{-1}. At these levels, the factors that most influence the accuracy of the ICP-MS data are the procedure blank and spectral and nonspectral interferences. Nonspectral interference, caused by the high concentration of dissolved solids in the matrices investigated, resulted in signal suppression. Matrix separation was used to enhance signal intensity and to reduce spectral interference for the accurate determination of Cd in SRM 1598a and SRM 3244. Chromatographic separation procedures using Chelex for SRM 1598a and anion exchange for SRM 3244 were optimized to achieve the desired separation characteristics without substantially increasing the procedure blank. Sensitivity for the determination of Cd in serum was additionally enhanced through the use of desolvation nebulization. We determined that separations were not required for the accurate ICP-MS determination of Cd in SRM 3240, SRM 3241, SRM 3243, and SRM 966 L2 under optimized analysis conditions. These samples were diluted to a minimum volume and introduced to the ICP-MS via low flow (40–100 μL/min) microconcentric nebulizers. SRM 966 L1 was also analyzed directly, but results were highly variable. The ID-ICP-MS sample preparation and ratio measurement protocols described here resulted in total expanded uncertainties of less than 1% for the determination of 90.85 μg kg^{-1} Cd in SRM 3240, and less than 10% total expanded uncertainty for the determination of 0.0468 μg kg^{-1} Cd in SRM 1598a.

Keywords Cadmium · Isotope dilution · ICP-MS · Reference materials · Dietary supplements · Blood · Serum · SRM · Trace elements

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Introduction

Cadmium (Cd) and its compounds are highly toxic and are classified as carcinogens [1]. Despite heavy regulation, the environmental burden of Cd is increasing through soil application of municipal sludge and phosphate fertilizers, and release from mining operations, manufacturing, and the burning of fossil fuels [2–4]. Data indicate that Cd bioaccumulates in all levels of the food chain [1]. The elimination half-life of Cd in humans is about thirty years [5]. Chronic exposure to Cd can result in kidney disease, lung damage, and bone damage [1]. Accurate Cd measurements in dietary sources and biological fluids are needed to assess exposure and toxicological impact. These measurements are analytically challenging due to the complexity of the samples and the low Cd levels. Reference materials representative of different sample types with certified Cd concentrations are used for analytical quality assurance. The use of a primary method of quantitation such as isotope

dilution can yield Cd concentrations with the required sensitivity, precision, and accuracy.

The Standard Reference Materials (SRMs) described in this paper are natural-matrix materials. SRM 966 is composed of bovine blood collected after the animals were orally dosed with gelatin caplets containing lead nitrate. Level 2 was prepared by spiking the collected blood with inorganic cadmium, whereas Level 1 is composed of collected blood that was not spiked and this level is therefore representative of natural levels. SRM 1598a is composed of pooled bovine (2/3) and porcine (1/3) serum and is also representative of natural levels. SRM 3240 represents a single year's harvest of *Ephedra sinica* plant material (aerial parts) from a field in China [6]. SRM 3241 was made from a portion of SRM 3240 that was extracted with hot water under pressure, filtered, concentrated, and spray-dried [6]. SRMs 3243 and 3244 were prepared from several brands of commercially available dietary supplement products [6]. SRM 3243 is composed of ground tablets and the contents of capsules, and SRM 3244 is composed of protein powder [6]. These SRMs provide well-characterized matrices relevant to assessing Cd body burden in blood (SRM 966) and serum (SRM 1598a), and Cd exposure from environmental deposition on plant material (SRM 3240), and from dietary intake via certain dietary supplements (SRM 3243 and SRM 3244).

Isotope dilution mass spectrometry (IDMS) is considered a primary method of quantitation, and sources of bias have been well-characterized [7]. Isotope dilution inductively coupled plasma-mass spectrometry (ID-ICP-MS) has been used for the certification of Cd content in blood, rice, mussel tissue, and other environmental reference materials [8, 5, 9–12]. The primary advantage of isotope dilution is that it utilizes an enriched isotope as the internal standard that is chemically identical to the target analyte. When the spike and analyte isotopes are equilibrated by complete dissolution of the sample, their isotope ratio defines the analyte concentration. Matrix separation can be performed without bias due to incomplete recovery provided the chemical yield does not result in blank amplification [13]. ICP-MS mass ratios for Cd can be measured with a repeatability of 0.2% relative standard deviation (RSD). However, at low Cd concentration, spectral interference and sample loading of the plasma can play a role in limiting the accuracy of Cd measurement by ICP-MS. Spectral interference for Cd has been discussed [8, 10, 12]. The spectral resolution required to resolve the metal oxide, metal hydroxide, and Sn isobaric interferences at the Cd masses exceeds the capabilities of commercial high-resolution ICP-MS instruments, and matrix separation becomes necessary.

This paper outlines the protocols developed at NIST to accurately measure Cd in new dietary supplement, blood, and serum matrices using ID-ICP-MS. Procedures used to check for spectral interference are detailed. Various dissolution and separation schemes are compared with regard to efficiency and minimization of analytical blank. The accuracy of results is verified by processing gravimetric standards prepared at the same Cd level as the sample to provide a check on the accuracy of the procedure blank correction, and by analyzing previously certified natural-matrix reference materials to provide a check on the mitigation of spectral interference.

Experimental

Materials

Only purified reagents were used. High-purity water was prepared in-house by sub-boiling distillation using a high-purity, conditioned quartz still. High-purity nitric acid (HNO_3), hydrochloric acid (HCl) (both Optima grade, Fisher Scientific Inc., Newark, DE, USA), hydrofluoric acid (HF) (prepared in-house by sub-boiling distillation in a high-purity, conditioned Teflon still), peroxide (H_2O_2), and perchloric acid ($HClO_4$) (both Ultrex II grade, Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA.) were used. Solutions containing 1.5 mol/L HCl/1 mol/L HF, 0.02 mol/L HCl, and 1 mol/L HNO_3 were prepared in precleaned fluoroethylene–propylene (FEP) Teflon bottles. Concentrated high-purity ammonium hydroxide (NH_4OH) and glacial acetic acid ($COOHCH_3$) (Seastar Chemicals, Sidney, BC, Canada) were obtained. Solutions containing 2 mol/L NH_4OH and 2 mol/L ammonium acetate (NH_4COOCH_3) at pH 5.5 were prepared in precleaned perfluoroalkoxy (PFA) Teflon bottles.

Enriched ^{111}Cd (96.5%) as CdO obtained from Oak Ridge National Laboratory (Oak Ridge, TN, USA) was dissolved in a high-purity quartz flask and stored as a master stock solution in 0.5 mol/L HNO_3. For the dietary supplement materials, Cd was certified simultaneously with lead (Pb), and for the serum material, Cd was certified simultaneously with nickel (Ni). Spike mix solutions were prepared by diluting the master ^{111}Cd spike solution with enriched isotopes of the other elements of interest. The isotopic purity of the spike mix solution was verified by ICP-MS measurement of the pure and mixed ^{111}Cd spike solutions. The concentration of ^{111}Cd in each spike or spike mix solution was calibrated against separate primary Cd standards. Both SRM 746 Cadmium Vapor Pressure standard and SRM 3108 Cadmium Standard Solution were used.

Instrumentation

Samples were dissolved using various wet-ashing and microwave dissolution instrumentation including the M.L.

S. 1200 Mega (Milestone, Monroe, CT, USA), MarsXpress (CEM, Matthews, NC, USA), and Multiwave (Anton Paar, Graz, Austria) microwave digestion systems. The M.L.S. 1200 was equipped with 50 mL TFM-polytetrafluoroethylene Teflon (TFM) digestion vessels, the MarsXpress was equipped with 50 mL PFA Teflon digestion vessels, and the Multiwave was equipped with 50 mL quartz digestion vessels. PFA Teflon beakers (50 mL and 250 mL) and 20 mL PFA vials were used for samples processed on the hotplate.

Samples of SRM 3240, SRM 3241, SRM 3243, and SRM 966 L1 were analyzed on a Finnigan-MAT (Bremen, Germany) Element double-focusing sector-field high-resolution ICP-MS equipped with a low-flow (50 μL/min) PFA microconcentric nebulizer from ESI (Omaha, NE, USA) fitted to a water-cooled (2 °C) Cinnabar cyclonic (Glass Expansion, West Melbourne, Australia) spray chamber. This instrument was not available for the measurement of Cd in SRM 3244 and SRM 1598a, and these samples, together with SRM 966 L2, were analyzed on a VG PlasmaQuad 3 (Thermo Elemental, Winsford, Cheshire, England) equipped, in the case of SRM 1598a, with an Aridus desolvation nebulizer system (Cetac Technologies, Omaha, NE, USA). For SRM 3244, a low-flow (100 μL/min) PFA microconcentric nebulizer from ESI (Omaha, NE, USA) fitted to a water-cooled (4 °C) Cinnabar cyclonic spray chamber was used. In all cases a CETAC ASX-100 autosampler was used (Cetac Technologies, Omaha, NE, USA).

Methods

Composition sample

Prior to spiking, a single sample of each material was dissolved and analyzed. The purpose of this procedure was two-fold; first, to determine the approximate Cd level of each material so that the appropriate amount of spike could be added; and second, to check for spectral interference. Spectral interference is indicated if the natural isotopic ratios measured in the sample, and corrected for isobaric interference from Sn, differ from those measured in a standard solution of high-purity Cd.

Spiking, calibration, and sample decomposition

Sub-samples of each material were accurately weighed by difference on a balance with a readability of 0.01 mg into clean digestion vessels. One-gram sub-samples were processed for the powdered materials, but for the blood and serum samples, the entire content of each vial was analyzed, resulting in 2-g samples for the blood and 5-g samples for the serum. Sub-samples of appropriate certified reference materials (CRMs) were processed at the same time for analytical quality assurance. In addition to natural-matrix CRMs, aliquots of Cd standard solutions prepared at the same Cd level as the samples were processed as a further measure of analytical control. The spike or spike mix solution containing enriched ^{111}Cd was added by mass difference via a capped plastic syringe to all samples and controls. Samples and controls were spiked so that approximately 0.7 ng of spike was added for every 1 ng of Cd in the sample, resulting in a ^{111}Cd/^{112}Cd and ^{111}Cd/^{114}Cd ratio of 3 and 2.5, respectively. This represented a compromise between a ratio of 1, which is the best ratio to measure with a pulse-counting system, and the optimum ratio to minimize error propagation in IDMS analysis (i.e., the geometric mean), which for ^{111}Cd/^{112}Cd is 5.5 and for ^{111}Cd/^{114}Cd is 8.5 [14]. Calibration samples were prepared so as to have ratios similar to the analytical samples and were used to calibrate the concentration of ^{111}Cd in the spike solution by reverse isotope dilution ICP-MS. Four spike calibration samples were prepared from two weighed aliquots of two different primary standard solutions. Smaller amounts (≈10–15 ng) of the ^{111}Cd spike solution were added to clean Teflon vessels to determine procedure blanks.

A variety of dissolution methods were employed for these materials, enabling a comparison to be made of the procedure blank for various digestion vessels and acid combinations. In cases where the procedure blank was of primary concern, closed-vessel microwave dissolution with HNO_3 acid was the method of choice (SRM 966 L1, SRM 1598a, and SRM 3244). When materials were suspected to contain silicates, samples were treated with HF in Teflon containers (SRM 3240, SRM 3241, SRM 3243, and SRM 3244). In some cases open-beaker digestions were less cumbersome to perform because transfer to different vessels for acid evaporation or venting of over-pressurized vessels was avoided. For open-beaker digestion, H_2O_2 (SRM 3241) or $HClO_4$ (SRM 966 L2 and SRM 3243) was used to aid completeness of digestion based on the perceived complexity of the matrix. Details of the dissolution method applied to each material are summarized in Table 1. Briefly, samples of SRM 966 L 1 were decomposed in an Anton Parr Multiwave microwave digestion system using 7 g of HNO_3 acid. Digests were transferred to PFA vials and heated on a hot plate to evaporate the concentrated acid. Samples of SRM 966 L 2 were digested in 50 mL PFA beakers in a class 10 fume hood by wet ashing with HNO_3 and $HClO_4$. SRM 1598a samples were decomposed in a Milestone M.L.S. 1200 digestion system using 5 g HNO_3 acid. Digestion vessels were transferred directly to a hot plate to evaporate the concentrated acid. SRM 3240 samples were dissolved in a two-step procedure. Samples were initially decomposed in the Multiwave microwave

Table 1 Dissolution method used in the preparation of blood, serum, and dietary supplement standards for ID-ICP-MS analysis

Method	1	2	3	4	5	6	7
Material	Bovine Blood SRM 966 , L1	Bovine Blood SRM 966 , L2	Animal Serum SRM 1598a	Ephedra Plant SRM 3240	Ephedra Extract SRM 3241	Ephedra Oral Dose SRM 3243	Ephedra Protein Powder SRM 3244
System	Anton Parr Multiwave	Hot plate	Milestone M.L.S. 1200	Anton Parr Multiwave	Hot plate	Hot plate	MarsXpress
Vessel type	Quartz/PFA vial	PFA beakers	TFM Teflon	Quartz/PFA beaker	PFA beakers	PFA beakers	PFA vessels/ beakers
Acid	7 g HNO_3	5 g HNO_3+5 g $HClO_4$	5 g HNO_3	12 g HNO_3+1 g HF (after transfer to PFA)	12 g HNO_3+1 g HF+1 g H_2O_2	10 g HNO_3+1 g HF+1 g $HClO_4$	11 g HNO_3+0.6 g HF
Procedure parameters	Two-step procedure with gradual application of power from 100 W to 600 W. Vessels held at 1,000 W for 10 min	180 °C hot plate surface temperature	Multi-stage procedure involving the gradual application of power from 250 W to 500 W. Vessels held at 500 W for 20 min	First stage at 600 W power, then vessels cooled and vented. Second stage at 1,000 W power for 10 min Vessels reach 200 °C	185 °C hot plate surface temperature reached. Samples refluxed in HNO_3/ HF, then H_2O_2.	185 °C hot plate surface temperature reached. Samples refluxed in HNO_3/ HF, then $HClO_4$.	Single stage procedure at 720 W power, ramping to 190 °C over 15 min and holding at 190 °C for 20 min
Time required	20 min dissolution, 15 min cooling, 2 h evaporation	2 d	40 min dissolution, 35 min cooling, 1 h evaporation	15 min dissolution, 4 h cooling, 4 h reflux, 2 h evaporation	2.5 d	1.5 d	35 min dissolution, 35 min cooling, 2 h evaporation

digestion system using quartz vessels to obtain high decomposition temperatures, but samples required treatment with HF to dissolve remaining silicate material and so were transferred to PFA beakers and heated on a hot plate. Samples of SRM 3241 were dissolved in 50 mL PFA Teflon beakers in a class 10 fume hood by wet ashing with HNO_3, HF, and H_2O_2. Samples of SRM 3243 were wet ashed in 125 mL PFA Teflon beakers with HNO_3, HF, and $HClO_4$ in a perchloric acid fume hood. SRM 3244 samples were decomposed in a MarsXpress microwave digestion system using HNO_3 and HF. The concentrated acids can be evaporated from the MarsXpress digestion vessels with the MicroVap XpressVap accessory, but this component was not in operation at the time, and digests were transferred to Teflon beakers to evaporate the concentrated acids.

Chelex separation for Cd and Ni in SRM 1598a

A preliminary analysis, performed by comparing the signal intensity measured for Cd in a synthetic serum sample prepared to contain Cd, Ni, Na, Ca, K, Mg, and Fe at the same element concentrations as SRM 1598 with the signal intensity measured in a Cd standard solution prepared at the same Cd concentration as the synthetic serum sample, showed that signal suppression for Cd would be severe in the serum matrix. As Ni and Cd were being determined from the same sample, a separation scheme applicable to both elements was chosen. Iminodiacetate chelating resin has been successfully used to separate transition metals from alkali and alkaline earth metals [10]. In order to minimize the amount of resin and reagents used, thereby reducing the potential for contamination, columns were hand-packed, and the separation performed manually. Samples were separated using 0.052±0.001 g of dry Chelex 100 resin (Bio-Rad, Richmond, CA, USA) 200–400 mesh (40–75 μm) in the Na form, loaded in a water slurry onto 1.5 mL extract-clean reservoirs (Alltech, Deerfield, IL, USA) fitted with plastic frits (Alltech). The packed columns were cleaned with 5 mL of 2.5 mol/L HNO_3 followed by 4 mL of high-purity water (sub-boiling and distilled in-house). The plastic columns and frits had been precleaned by soaking in 20% volume fraction HNO_3 for one week. The resin bed was conditioned with 3 mL of 2 mol/L NH_4OH, followed by 3 mL of high-purity water, and 4 mL of 2 mol/L NH_4COOCH_3. The resin bed was observed to shrink at acidic pH and swell at basic pH. Digests were taken to dryness and the residual salts redissolved and loaded onto the column in 1.5 mL of 2 mol/L NH_4COOCH_3. Metals were retained by the Chelex resin, and the serum matrix was removed with 4 mL of 2 mol/L NH_4COOCH_3. The exact amount of reagent required to remove the matrix was determined by tracking the decrease

of Na in drops of the eluant with a Bunsen flame. An additional 3 mL of high-purity water was added to remove the residual 2 mol/L NH_4COOCH_3, and the metals were removed with 4 mL of 2.5 mol/L HNO_3. The separated fraction was taken to dryness and redissolved in 3 mL of 0.4 mol/L HNO_3.

Anion exchange separation for Cd and Pb in SRM 3244

Preliminary investigation showed that interferences were present at the Cd masses of interest in SRM 3244. At first a Chelex procedure similar to that listed above, but using twice the amount of resin, was tested. This was found to be unsatisfactory, and a procedure using anion resin was developed instead. The anion exchange procedure described by Nelson [15] and previously applied to environmental samples [12] was adapted for use here. The amount of resin and reagents used was minimized. Samples were taken to dryness with concentrated HCl to convert the residual salts from the nitrate to the chloride form. Samples were redissolved and loaded in 5 g of 1.5 mol/L HCl/1 mol/L HF onto clean 4 mL (1 cm×5.3 cm) resin beds of AG1x8, 100–200 mesh (75–150 μm) Biorad anion exchange resin. Impurities were eluted first with 75 g of 1.5 mol/L HCl/1 mol/L HF and then with 10 g of 0.02 mol/L HCl. Cd was removed with 10 g of 1 mol/L HNO_3. Samples were taken to dryness, treated with concentrated HNO_3, and redissolved in 0.4 mol/L HNO_3.

ICP-MS measurement

The measurement of Cd isotope ratios with a quadrupole ICP-MS system has been described [12]. In this case, the instrument was operated at 1350 W using standard gas flows. Solution was introduced via a peristaltic pump at a flow rate of 0.1 mL/min into a water-cooled (4 °C) low-volume cyclonic spray chamber. Signal intensities were measured using peak jump data acquisition with one point per peak. Intensity data for ^{111}Cd, ^{112}Cd, ^{114}Cd, and ^{118}Sn were collected at dwell times of (10, 20, 20, and 5) ms, respectively. The intensity at ^{118}Sn was measured to determine the correction for isobaric interference from ^{112}Sn and ^{114}Sn. Sn interferences on ^{112}Cd and ^{114}Cd were calculated by multiplying the average measured intensity at ^{118}Sn for each sample by the ^{112}Sn/^{118}Sn and ^{114}Sn/^{118}Sn ratios measured for a pure natural Sn solution at the start of the analysis sequence. The ^{112}Sn and ^{114}Sn intensities thus calculated were subtracted from the intensities measured at ^{112}Cd and ^{114}Cd. Five blocks of data each 1 min in duration were acquired per sample and the mean ratio was used for computations. The measurement of Cd isotope ratios using high-resolution ICP-MS has been described [16]. For high-resolution measurements, the instrument was operated using an argon sample gas flow rate of 1.06 L/min. Plasma power was set at 1325 W. Intensity data for ^{111}Cd, ^{112}Cd, ^{114}Cd, and ^{118}Sn were collected in low-resolution mode as the average of five channels on the low-resolution plateau scanned 250 times in ten separate sets. Due to the presence of variable amounts of Sn in the samples, ^{118}Sn was monitored and appropriate correction algorithms for Sn interferences on ^{112}Cd and ^{114}Cd were made as described above.

In addition to the correction for isobaric interference from Sn on the Cd isotopes, measured ratios were corrected for drift, mass bias, and detector dead-time. Detector dead-time was experimentally determined by the method of Russ [17] using natural Cd solutions with concentrations spanning the count rate range in counts per second (cps) of 1×10^5 cps to 9×10^5 cps of the major isotope. A solution of pure Cd metal was used to measure the mass bias correction factor at the beginning of an analysis sequence. This was not entirely necessary since the spike solution calibration samples were being measured at the same time under identical measurement conditions as the samples. Samples were corrected for instrument drift by measuring one of the spike calibration samples repeatedly throughout the analysis (every third sample) and correcting for changes from the initial measured ratio by assuming the change to be linear with time. Overall, the different sample types were measured in the following sequence: instrument background followed by the Sn standard, spiked procedure blanks, Cd isotopic standard, spike calibration sample used to assess drift, two of the spike calibration samples, analytical samples interspersed with controls, and finally the two remaining spike calibration samples. Cd concentrations were calculated from both the ^{111}Cd/^{112}Cd and ^{111}Cd/^{114}Cd ratios and compared. Concentrations were calculated as described by Fassett and Paulsen [14].

Results and discussion

Spectral and nonspectral Interference

Preliminary measurements of the natural isotopic ratios of single samples of SRM 966 L1 and L2 and SRMs 3240, 3241, and 3243 did not indicate significant interferences at the Cd isotopes of interest because the measured isotope ratios were within 1% of those measured in a pure Cd standard solution. For SRM 3244, the measured ^{111}Cd/^{112}Cd ratio was 21% lower and the ^{111}Cd/^{114}Cd ratio was 5% higher than the respective ratios measured in a pure Cd standard after approximate 2.3% and 1.8% corrections for ^{112}Sn and ^{114}Sn. These results indicate interferences at Cd masses 111 and 112, but it is entirely possible that interference is present at mass 114 and is being masked

Table 2 Comparison of Cd concentration results for SRM 3244 measured with and without matrix separation

Bottle	Cd concentration (μg/kg) calculated using specified ratio					
	Without matrix separation			With matrix separation		
	$^{111}Cd/^{112}Cd$	$^{111}Cd/^{114}Cd$	% dif.[a]	$^{111}Cd/^{112}Cd$	$^{111}Cd/^{114}Cd$	% dif.[a]
1	14.09	13.61	3.5	12.43	12.50	−0.60
2	13.58	13.11	3.6	12.43	12.39	0.34
3	14.88	13.07	13.8	12.84	12.85	−0.05
4	15.19	15.18	0.0	12.57	12.60	−0.26
5	13.37	13.07	2.3	13.07	13.05	0.17
6	13.77	13.43	2.5	12.45	12.56	−0.81
AVG	14.15	13.58	4.2	12.63	12.66	−0.20
SD	0.73	0.82		0.27	0.25	
%RSD	5.18	6.01		2.11	1.94	
N	6	6		6	6	

[a] Percent difference between $^{111}Cd/^{112}Cd$ and $^{111}Cd/^{114}Cd$ results

only by the larger interference at mass 111. Spectral interference could not be assessed in this way for SRM 1598a because signal suppression was severe. For example, only 25 counts per second (cps) and 45 cps were measured at masses 111 and 114 for an unseparated sample of SRM 1598a, whereas 650 cps and 1,500 cps were measured at masses 111 and 114 in a separated sample, indicating over 20-fold signal suppression.

As an added measure to assess the presence or absence of spectral interference, data were compared for samples processed through the ID procedure and calculated using both the $^{111}Cd/^{112}Cd$ and $^{111}Cd/^{114}Cd$ ratios. This approach was critical to discovering a potential bias for the SRM 3240 and 3243 samples initially measured on the quadrupole instrument. Results for both SRMs calculated using the $^{111}Cd/^{112}Cd$ ratio were an average of 8% higher than results calculated using the 111/114 ratio and indicated interference at mass 112. To confirm the absence of interference at masses 111 and 114, several samples were separated using anion exchange chromatography. Good agreement was obtained for concentrations calculated using the $^{111}Cd/^{114}Cd$ ratio with and without matrix separation. Results calculated using the $^{111}Cd/^{112}Cd$ ratio agreed with the $^{111}Cd/^{114}Cd$ results only with matrix separation, confirming the interference at mass 112 for samples not subject to matrix separation. The interference at mass 112 is most likely due to $^{40}Ca_2O_2$. Beary and Paulsen note the same interference for the determination of low-level Cd in soil [10]. Interestingly, the bias between results for the two ratios did not exist when the unseparated samples were measured on the sector instrument, or when the natural isotopic ratios were initially measured on the quadrupole instrument. In the first case, this may be due to differences in the structure of the interface region of the two instruments, resulting in different signal behaviors in the presence of matrix [18]. In both cases, subtle differences in gas flows may have influenced oxide formation.

The effect of matrix separation on the accuracy and repeatability of the results for the determination of Cd at low levels in complicated matrices is illustrated in Table 2. Results are presented for the measurement of Cd in SRM 3244. Cd concentrations are calculated using both the $^{111}Cd/^{112}Cd$ and $^{111}Cd/^{114}Cd$ ratio for the same sample preparation measured with and without matrix separation.

Table 3 Procedure blanks measured for the certification of Cd in blood, serum, and dietary supplement standards by ID-ICP-MS

Material	Bovine Blood SRM 966, L1	Bovine Blood SRM 966, L2	Animal Serum SRM 1598a	Ephedra Plant SRM 3240	Ephedra Extract SRM 3241	Ephedra Oral Dose SRM 3243	Ephedra Protein Powder SRM 3244
Procedure:							
[a]Method:	1	2	3	4	5	6	7
Separation:	–	–	Chelex	–	–	–	Anion
Procedure blank (ng Cd)							
AVG	0.0120	0.29	0.0044	0.0715	0.0711	0.0985	0.0176
SD	0.0064	0.11	0.0020	0.013	0.093	0.029	0.0018
N	4	2	4	3	3	3	2
% Correction	27	3	3	0.08	0.1	0.08	0.14

[a] Number refers to dissolution method listed in Table 1

Table 4 Certification of Cd in blood, serum, and dietary supplement standards by ID-ICP-MS

Material	Bovine Blood SRM 966, L1	Bovine Blood SRM 966, L2	Animal Serum SRM 1598a	Ephedra Plant SRM 3240	Ephedra Extract SRM 3241	Ephedra Oral Dose SRM 3243	Ephedra Protein Powder SRM 3244
Cd (μg/kg) ID-ICP-MS	0.0303	4.88	0.0468	90.85	60.68	123.5	12.66
SD	0.0076	0.11	0.0032	0.47	0.93	1.8	0.25
%RSD	25	2.3	6.8	0.52	1.5	1.4	2.0
N	9	10	5	6	6	6	6
Type A uncertainty (% rel.)							
Sample/measurement	8.4	2.4	3.1	0.22	0.62	0.59	0.79
Spike cal.	0.36	0.039	0.25	0.15	0.24	0.19	0.25
Blank corr.	18.7	0.85	1.9	0.009	0.081	0.014	0.061
Type B uncertainty (% rel.)							
Calibrant	0.16	0.058	0.12	0.058	0.059	0.058	0.12
Sn correction	–	0.12	0.58	0.29	0.029	0.029	0.12
Dead time/mass bias	–	–	0.29	0.12	0.12	0.12	0.17
Dry mass correction	–	–	–	–[a]	–[a]	–[a]	0.55
Coverage factor	2.8	2.3	2.6	2.0	2.3	2.2	2.1
Expanded U (μg/kg)	0.017	0.28	0.0044	0.75	1.0	2.0	0.26
Expanded U (% rel.)	56	5.7	9.5	0.83	1.7	1.6	2.1
Certified value	0.030±0.017[b]	4.95±0.15[b]	0.047±0.004[b]	90.6±3.9	58.7±3.6	121.8±3.3	12.66±0.69

[a] Initial ID-ICP-MS results were based on analysis of samples "as-received." A component of uncertainty for drying was included in the final certified value, based on a universal dry mass correction factor that was subsequently applied.
[b] Values listed here have been converted to μg/kg using the densities provided on the certificate of analysis

Results for samples measured without matrix separation show on average a 4% difference between concentrations calculated using $^{111}Cd/^{112}Cd$ and $^{111}Cd/^{114}Cd$. In addition, the percent RSD between the samples was 5–6%. In contrast, separated samples show on average a 0.2% difference between concentrations calculated using $^{111}Cd/^{112}Cd$ and $^{111}Cd/^{114}Cd$, and an RSD of 2% between samples.

Procedure blank

Procedure blanks were determined by subjecting small amounts of the highly enriched ^{111}Cd to the same processing treatment as the samples. Table 3 lists the average and standard deviation of the procedure blank measured during the analysis of each material. Processing occurred in a class 10 clean room and only high-purity reagents were used. Results are listed as nanograms of Cd. A description of the dissolution process and vessels used can be found in Table 1. Procedure blanks for SRM 1598a and SRM 3244 include a matrix separation step. By carefully optimizing the amount of resin and reagents used, the matrix separation did not substantially add to the total procedure blank. Use of perchloric acid, though helpful in obtaining complete oxidation of organic matter, did result in higher Cd blanks. Cd blanks for TFM Teflon vessels

Table 5 Results for standard solution controls and Certified Reference Materials

Processed with:	Bovine Blood SRM 966 , L1	Bovine Blood SRM 966 , L2	Animal Serum SRM 1598a	Ephedra Plant SRM 3240	Ephedra Extract SRM 3241	Ephedra Oral Dose SRM 3243	Ephedra Protein Powder SRM 3244
Standard solution controls							
Processed (ng Cd)	–	–	0.179±0.017	62.18±0.52	71.7±1.1	90.0±1.4	17.29±0.36
Measured[a] (ng Cd)	–	–	0.169	62.39	71.5	90.6	17.40
% Difference	–	–	−5.2	0.33	−0.21	0.69	0.60
Matrix controls							
Control	SRM 966, L2	SRM 909[b]	SRM 1598	SRM 1547	SRM 1547	SRM 1547	SRM 1515
Measured[a] (μg/kg)	4.73±0.15	1.237±0.072	0.0984±0.0093	25.01±0.21	24.97±0.38	25.75±0.41	13.31±0.28
Certified (μg/kg)	4.95±0.15	1.24±0.1	0.089±0.016	26±3	26±3	26±3	13±2

[a] Uncertainties listed for the measured values are based on the expanded ID-ICP-MS uncertainties derived for each analysis and listed in Table 4.
[b] Results listed as ng/mL

were lower than those obtained in PFA Teflon and quartz. In all cases the magnitude of the correction for the blank did not exceed 3%, with the exception of SRM 966 L1, for which the correction was 27%, but the variability was a factor of two lower and so it only partially explains the overall variability observed for SRM 966 L1 samples (listed below.)

Determination of Cd in blood, serum, and dietary supplement SRMs and certified values

Table 4 summarizes the measured Cd mass fractions, uncertainty components and expanded uncertainty for the ID-ICP-MS analysis of the blood, serum, and dietary supplement SRMs. Reported concentrations were calculated with the $^{111}Cd/^{114}Cd$ ratio. The results are listed as the average of single samples of 5–10 statistically selected bottles from the entire population of each material. Expanded uncertainties are based on a 95% confidence interval and are derived from the individual Type A and Type B uncertainty components listed in Table 4. Type A components of uncertainty include the standard uncertainty of the sample measurements, the standard uncertainty of the spike calibration samples, and the standard uncertainty of the procedure blank determinations. The assay of the calibrant, and the corrections for ^{114}Sn, dead-time, mass bias, and loss in mass upon drying (where applicable) were treated as Type B uncertainties. Coverage factors were computed based on the effective degrees of freedom in each case, calculated using the Welch–Satterthwaite formula [19]. Overall, for concentrations in the range 10–100 μg/kg Cd, expanded uncertainties of 1–2% (relative) were obtained for ID-ICP-MS analysis. For concentrations an order of magnitude lower than this, expanded uncertainties were still less than 10% (relative), with one exception.

Certified values for a NIST SRM must be composed of at least one NIST measurement method and can be established by a single primary method or by two or more independent methods under the conditions described in [20]. Certified values for each of the new materials are listed in Table 4. Value assignments for SRM 966 L1, SRM 1598a, and SRM 3244 were based solely on the ID-ICP-MS results. For SRM 966 L2, the certified value was based on the average of measurements from ID-ICP-MS and radiochemical neutron activation analysis performed at NIST. Certified values for the remaining dietary supplement SRMs were based on the equally weighted mean of the NIST ID-ICP-MS results combined with results from collaborating laboratories. In all cases where a second measurement result was available, agreement was obtained within the expanded uncertainties (based on a 95% confidence interval) of the ID-ICP-MS results.

Method validation samples

A known amount of Cd primary standard, close in level to the Cd concentration in the certification samples, was processed along with the samples. This provided a measure of the uncertainty associated with the measurement process and, in particular, the accuracy of the blank correction. Results for these standard solution controls are listed in Table 5. The smallest amount of Cd processed was 0.179 ng Cd, and the measured result, corrected for the corresponding procedure blank listed in Table 3, is within 5.2% of the true amount of Cd. This result is well within the estimated expanded ID-ICP-MS uncertainty of the measurement at this level. Results for the remaining amounts, which ranged from 17.2 to 89.95 ng Cd, were within 0.7% of the true value.

Table 5 lists the measured Cd concentrations and expanded uncertainties for the natural-matrix SRMs analyzed to validate the established procedures. The certified values of each SRM are also included in the table. Five different materials were analyzed. These were: SRM 909 Human Serum, SRM 1598 Inorganic Constituents in Bovine Serum, SRM 1547 Peach Leaves, SRM 1515 Apple Leaves, and SRM 966 L2 Bovine Blood. In all cases the measured values with their expanded uncertainties were within the certified interval.

Traceability

Traceability to the mole was achieved by the use of a primary method based on isotope dilution mass spectrometry, and correction for all known sources of analytical bias. The spike employed for the isotope dilution measurement was calibrated by reverse isotope dilution ICP-MS using the primary standards SRM 746 Cadmium Vapor Pressure Standard and SRM 3108 Cadmium Standard Solution.

Disclaimer Certain commercial equipment, instruments or materials are identified in this work to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for this purpose.

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