Improved Certified Values for Total Mercury and Speciated Mercury in NIST Standard Reference Materials Using Isotope Dilution ICP-MS

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The negative impact of mercury on human health has been well documented, and stringent measures have been implemented to try to reduce the environmental burden of this element. Despite dramatic reductions in the industrial use of mercury, releases to the environment continue from sources such as combustion of fossil fuels and incinerator emissions. Accurate measurements of mercury are essential to informed legislative decision-making and fair regulation. The National Institute of Standards and Technology (NIST, Gaithersburg, MD) has developed a series of Standard Reference Materials (SRMs) in a variety of matrix types that are certified for mercury and methyl mercury content. These SRMs are used to validate the accuracy of methods and measurements and also provide measurement traceability to national and international standards. Until recently, NIST-certified values were based on measurements made using cold vapor atomic absorption and neutron activation analysis. Using this approach, mercury was certified with uncertainties ranging from approx. 1 to 10% relative. A new method based on isotope dilution cold vapor generation inductively coupled plasma mass spectrometry (ID-CV-ICP-MS)[1,2] has been recently developed that has revolutionized the measurement of total mercury and speciated mercury in a wide range of NIST SRMs, resulting in more certified values with lower uncertainties.

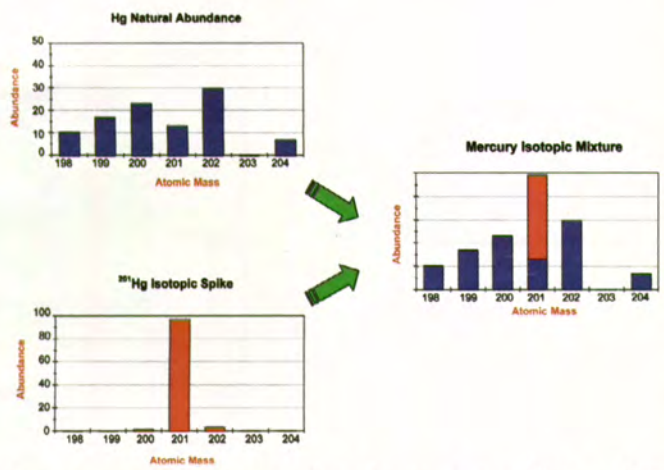

Figure 1 *Isotope dilution analysis of mercury using a ^{201}Hg enriched isotope.*

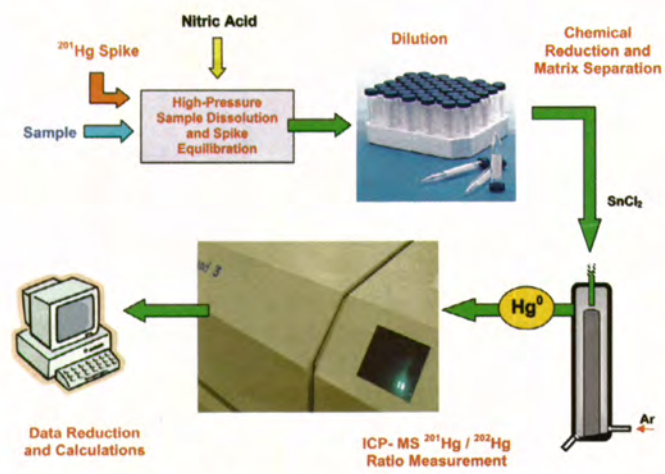

Figure 2 *Schematic of measurement system for total mercury by cold vapor isotope dilution ICP-MS.*

Isotope dilution mass spectrometry

Isotope dilution mass spectrometry is a relatively simple method in which a known amount of separated stable isotope is added to the sample and the perturbed isotopic composition is measured by a mass spectrometer. The ratio can be used to calculate very accurately the concentration of analyte in the sample. Because isotopes of the same element usually behave the same way in the measurement system, matrix-induced interferences are much lower. *Figure 1* shows an isotope dilution scheme for mercury in which the natural composition is perturbed by adding ^{201}Hg of 98% isotopic purity. The isotope ratio ^{201}Hg/^{202}Hg is measured.

Instrumentation and cold vapor generation for total mercury

The sample (*Figure 2*) is equilibrated with the ^{201}Hg spike and high-purity nitric acid in a high-pressure closed digestion system (such as a microwave or Carius tube) and then transferred to a polystyrene centrifuge tube, where it is diluted with deionized water. The mercury (as Hg^{2+}) is then reduced on-line with stannous chloride (cold vapor generation) in a hydride-type gas-matrix separator and transferred via Viton® fluoroelastomer tubing (**Cole-Parmer**, Vernon Hills, IL) to a commercial quadrupole ICP-MS system. *Figure 3* shows the ^{201}Hg and ^{202}Hg isotope traces from the instrument as a function of time as the sample is introduced into the instrument at approx. 30 sec into the run. *Figure 4* shows the ratio of these two isotopes as a function of time. The large variation in the isotope ratio in the first 30 sec results from the division of small signals from the instrument background. The background-corrected ratio is then used as the input variable in the standard isotope dilution equation, from which the original mercury concentration in the sample can be accurately calculated.

Instrumentation for mercury speciation

For mercury speciation measurements, a "double spike" speciated isotope dilution method has been developed in which a sample is spiked with isotopically enriched methyl mercury ($Me^{202}Hg^+$) and inorganic mercury ($^{201}Hg^{2+}$) spikes, and the mercury species are extracted into tetramethylammonium hydroxide (TMAH) utilizing a mono-mode microwave system (Explorer, **CEM**, Matthews, NC). The extract is then cooled to room temperature, buffered to pH 5, and the mercury species are then derivatized to a volatile form with sodium tetraethylborate. The derivatized mercury species are then back-extracted into hexane and the organic layer separated and placed in a GC autosampler vial. The two mercury species are separated by GC and the transient species detected by ICP-MS. *Figure 5* shows the ^{201}Hg and ^{202}Hg isotope transient signals for $MeHg^+$ and Hg^{2+} from an extracted sample of SRM 2977 Mussel Tissue (Organic Contaminants and Trace Elements), which has been double spiked. The background-corrected ratios, calculated from the peak areas, are used as the input variable for the isotope dilution equation, from which the original

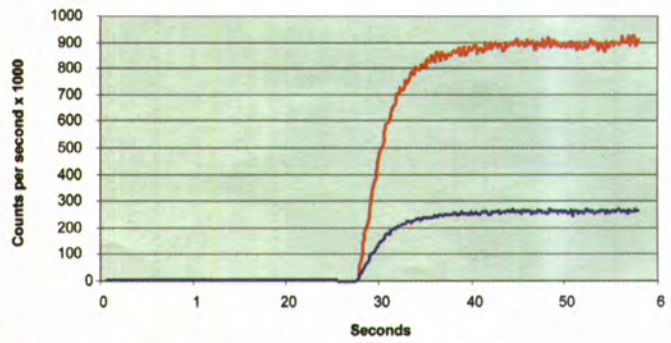

Figure 3 *Isotope–time profile output from ICP-MS system showing ^{201}Hg spiked isotope (red trace) and ^{202}Hg natural isotope (blue trace).*

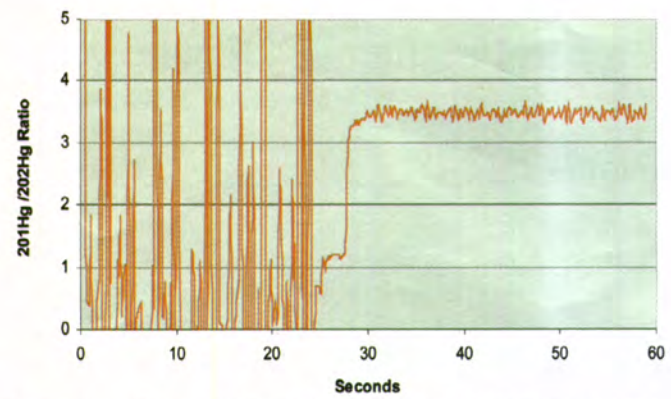

Figure 4 *Ratio of ^{201}Hg to ^{202}Hg isotopes from ICP-MS system as a function of time. The ratio (approx. 3.4) is inserted in the isotope dilution equation to calculate the Hg content of the sample.*

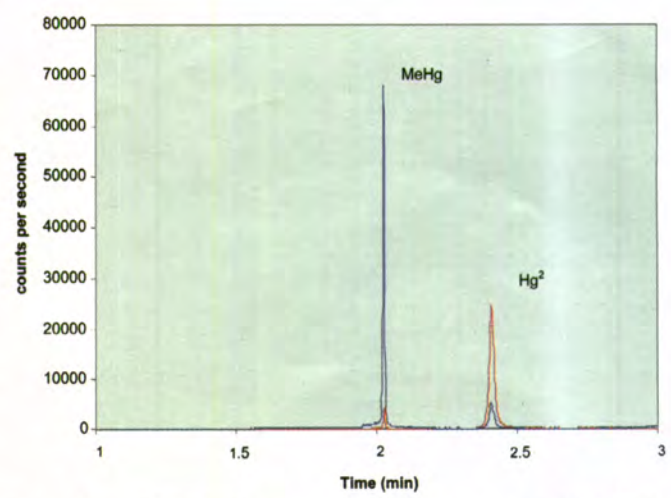

Figure 5 *GC/ICP-MS separation of mercury species in SRM 2977 Mussel Tissue (Organic Contaminants and Trace Elements) showing ^{201}Hg (red trace) and ^{202}Hg (blue trace).*

Figure 6 *Photograph of Carius combusted coal sample.*

mercury concentrations of each species in the sample can be calculated. This method allows for the simultaneous tracking and correction of any methylation or demethylation reactions that occur during sample preparation.[3]

Applications

Fossil fuels and environmental

The ID-CV-ICP-MS method has been used to determine total mercury in coals[4] and crude oils.[5] The high carbon content of this type of matrix is combusted using a Carius tube.[6] This is a truly closed system that eliminates both mercury losses and external contamination and results in extremely low analytical blanks. A Carius tube combusted coal material, which was treated at 240 °C for 12 hr in a convection oven, is shown in *Figure 6*. The headspace consists of nitrogen dioxide and carbon dioxide, while the blue color in the liquid is dimerized nitrogen dioxide. Silica from the sample, which is not dissolved using nitric acid, can clearly be seen at the bottom of the tube. This does not interfere with the determination. Using this method, NIST has recently certified eight coal SRMs, a coal fly ash, two fuel oils, and two crude oil SRMs. These results are summarized in *Table 1*. The method has also been used to determine mercury in mine-waste tailings, soil, and marine sediment (Table 1).

Biological, clinical, and marine tissues

Concern about the health impacts of mercury in seafood consumed by humans and wildlife has prompted extensive monitoring by state and federal agencies in the public health and natural resource management sectors. The corresponding need for matrix-matched mercury SRMs has led to the certification of total mercury and methyl mercury in clinical fluids such as urine and blood, and in aquatic species such as fish and shellfish that are commonly used for biomonitoring (*Tables 1* and *2*). High-accuracy measurements for both total mercury and methyl mercury have been made for several SRMs, providing appropriate reference values for less rigorous routine analyses of total Hg by regulatory agencies and speciation data for analytical or toxicological research. SRMs previously certified for mercury by other methods have also been reanalyzed using ID-CV-ICP-MS for stability testing and batch validation for other analyses (SRM 2976 Mussel Tissue, SRM 1566b Oyster Tissue, SRM 966 Toxic Metals in Bovine Blood, and SRM 2977 Mussel Tissue). These tissues are easily solubilized by microwave-assisted acid digestion, and accurate measurements are achieved even at the low mercury levels present in the clinical SRMs. The effectiveness of isotope dilution to minimize matrix-induced interferences is ideal for the complex matrices present in these biological tissues.

Table 1 Total mercury measurements in NIST SRMs by isotope dilution cold vapor mass spectrometry

Fossil fuels, environmental

SRM	Type	Certified value	Units	Uncertainty*
1619b	Fuel oil, residual	3.46	ng/kg	0.74
1632c	Coal, bituminous	0.0938	mg/kg	0.0037
1633b	Fly ash, coal	0.1431	mg/kg	0.0018
1635	Coal, subbituminous	0.0109	mg/kg	0.0010
2682b	Coal, subbituminous	108.8	µg/kg	2.9
2683b	Coal, bituminous	90.0	µg/kg	3.6
2684b	Coal, bituminous	97.4	µg/kg	4.7
2685b	Coal, bituminous	146.2	µg/kg	10.6
2692b	Coal, bituminous	133.3	µg/kg	4.1
2693	Coal, bituminous	37.3	µg/kg	7.7
2702	Sediment, marine	0.4474	mg/kg	0.0069
2710	Soil, contaminated	32.6	mg/kg	1.8
2721	Crude oil (light sour)	41.7	ng/kg	5.7
2722	Crude oil (heavy sweet)	129	ng/kg	13
2724b	Diesel fuel oil	0.034	ng/kg	0.026
2780	Waste, mine	0.710	mg/kg	0.042

Biological, clinical, marine

SRM	Type	Certified value	Units	Uncertainty*
1946	Tissue, fish	0.433	mg/kg	0.009
1947**	Tissue, fish	0.2535	mg/kg	0.0053
1974b	Tissue, mussel	17.0	µg/kg	1.1
1575a	Botanical, pine needles	0.0399	mg/kg	0.0007
966	Blood, whole, level 1	0.0445	µg/L	0.0033
966	Blood, whole, level 2	31.4	µg/L	1.7
2670a	Urine, freeze-dried, low level	0.0663	µg/L	0.0058
2670a	Urine, freeze-dried, high level	95.1	µg/L	0.98
3240	Ephedra, plant	0.0167	mg/kg	0.0005
3243	Ephedra, dosage form	0.00900	mg/kg	0.00044
3244	Ephedra, protein powder	0.000253	mg/kg	0.000033

Calibration standards

SRM	Type	Certified value	Units	Uncertainty*
1641d	Standard, water	1.590	mg/kg	0.018

*Expanded uncertainty stated on SRM certificate.
**SRM 1947 Lake Michigan Fish Tissue to be released in 2007.

Table 2 Methyl mercury measurements in NIST SRMs by speciated isotope dilution

Biological, clinical

SRM	Type	Certified value	Units	Uncertainty*
1947**	Tissue, fish	0.233	mg/kg	0.010
1974b***	Tissue, mussel	6.89	µg/kg	0.25

*SRM 1947 Lake Michigan Fish Tissue to be released in 2007.
**SRM 1974b updated certified value.
***Expanded uncertainty stated on SRM certificate.

Summary

Two new methods for total mercury and speciated mercury have permitted a new generation of NIST Standard Reference Materials to be developed. The methods are highly accurate, selective, and sensitive. Sample preparation in a closed system eliminates losses of mercury and results in very low blanks.

References

1. Christopher, S.J.; Long, S.E.; Rearick, M.S.; Fassett, J.D. *Anal. Chem.* **2001**, *73*, 2190–9.
2. Mann, J.L.; Long, S.E.; Kelly, W.R. *J. Anal. At. Spectrom.* **2003**, *18*, 1293–6.
3. Rodriguez Martin-Doimeadios, R.C.; Monperrus, M.; Krupp, E.; Amouroux, D.; Donard, O.F.X. *Anal. Chem.* **2003**, *75*, 3202–11.
4. Long, S.E.; Kelly, W.R. *Anal. Chem.* **2002**, *74*, 1477–83.
5. Kelly, W.R.; Long, S.E.; Mann, J.L. *Anal. Bioanal. Chem.* **2003**, *376*, 753–8.
6. Paulsen, P.J.; Kelly, W.R. *Anal. Chem.* **1984**, *56*, 708–13.

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