METHOD 6800

ELEMENTAL AND MOLECULAR SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

SW-846 is not intended to be an analytical training manual. Therefore, method procedures are written based on the assumption that they will be performed by analysts who are formally trained in at least the basic principles of chemical analysis and in the use of the subject technology.

In addition, SW-846 methods, with the exception of required method use for the analysis of method-defined parameters, are intended to be guidance methods which contain general information on how to perform an analytical procedure or technique which a laboratory can use as a basic starting point for generating its own detailed standard operating procedure (SOP), either for its own general use or for a specific project application. The performance data included in this method are for guidance purposes only, and are not intended to be and must not be used as absolute quality control (QC) acceptance criteria for purposes of laboratory accreditation.

1.0 SCOPE AND APPLICATION

For a summary of changes in this version from the previously published draft method 6800, February 2007 Rev 0, please see Appendix A at the end of this document.

1.1 This method consists of two approaches: (1) isotope dilution mass spectrometry (IDMS) for the determination of the total concentrations of elements and molecules and (2) molecular speciated isotope dilution mass spectrometry (SIDMS) for the determination of elemental and molecular species. IUPAC defines speciation analysis as “analyzing chemical compounds that differ in isotopic composition, conformation, oxidation or electronic state, or in the nature of their complexed or covalently bound substituents, which can be regarded as distinct chemical species.” This method is applicable to the determination of total elements as well as elemental species and molecular species at parts per billion (ppb), parts per trillion (ppt) and sub-ppt levels in samples of various types; in waters, solids, blood, foods or in extracts or digests. In general, elements and molecules that have more than one available stable isotope or combination of enriched isotopes in molecules can be analyzed by IDMS and SIDMS. SIDMS may require more isotopes of an element and/or molecule, depending on the number of interconvertible species.

The analyses of the following elements are applicable by this method:

Element		CASRN[a]
Antimony	(Sb)	7440-36-0
Boron	(B)	7440-42-8
Barium	(Ba)	7440-39-3
Cadmium	(Cd)	7440-43-9
Calcium	(Ca)	7440-70-2
Chromium	(Cr)	7440-47-3
Copper	(Cu)	7440-50-8
Iron	(Fe)	7439-89-6
Lead	(Pb)	7439-92-1
Magnesium	(Mg)	7439-95-4
Mercury	(Hg)	7439-97-6
Molybdenum	(Mo)	7439-98-7
Nickel	(Ni)	7440-02-0
Potassium	(K)	7440-09-7
Selenium	(Se)	7782-49-2
Silver	(Ag)	7440-22-4
Strontium	(Sr)	7440-24-6
Thallium	(Tl)	7440-28-0
Vanadium	(V)	7440-62-2
Zinc	(Zn)	7440-66-6

[a] Chemical Abstract Service Registry Number

Molecules and species may be analyzed by SIDMS if appropriate performance is demonstrated for an analyte, in the matrix type and at concentration levels of interest. See examples in the list below (see Sec. 9.0).

- Glyphosate and other pesticides
- Sodium azide and other toxicant and explosives
- Molecular Species, such as oxidized and reduced forms of glutathione, and peptides and proteins,
- Toxin and toxicant species conjugated to glutathione
- Converting related species such as isoforms and biomarkers
- Enzymes in tissues and body fluids
- Toxins and toxicants in dietary supplements, food and consumer products
- Toxin and toxicant species in water and foods
- Chemical, Biological, Radiological, Nuclear, Explosives (CBRNE) for Homeland Defense and Homeland Security

1.2 IDMS is based on the addition of a known amount of enriched isotope to a sample. Equilibration of the spike isotope with the natural element/molecule/species in the sample alters the isotope ratio that is measured. With the known isotopic abundance of both the spike and the analyte in the sample (element/molecule/species), the amount of the spike added to the known amount of analyte, concentration of the spike added, and the altered isotope ratio, the concentration of the analyte in the sample can be calculated. Direct calculations independent of traditional calibration curves are described in section 12.

1.3 IDMS has proven to be a technique of high accuracy for the determination of the total concentration of elements or molecules in various matrices (Refs. 1, 9, 10, 33, 34, and 43).

IDMS has several advantages over conventional calibration methodologies. Partial loss of the analyte after equilibration of the spike and the sample will not influence the accuracy of the measurement. Fewer physical and chemical interferences influence the determination as they have the same effects on each isotope of the same element or molecule. The isotope ratio to be measured for quantification by IDMS can be measured at a high level of precision, typically with a relative standard deviation (RSD) of 0.25% to 1.0%. Quantification is based on direct mathematical calculation from determined isotopic ratios and known constants, and does not depend on a traditional calibration curve or sample recovery to achieve accuracy.

1.4 SIDMS takes a unique approach to speciation analysis that differs from traditional methods. Traditional methods of speciation analysis attempt to hold each species static during sample collection and preparation and while making the measurement. Unfortunately, interconversion and transformation of species can occur, resulting in biased results. SIDMS has been developed to correct for such species conversions. In SIDMS, each species is "labeled" with an isotopically enriched spike in the corresponding species form. Thus, the interconversions that occur after spiking are traceable and can be corrected. While SIDMS maintains the advantages of IDMS, it is capable of correcting for the degradation of the species or the interconversions between the species for mass spectrometer drift and other errors, such as incomplete extraction of species (Refs. 2, 3, 5 - 8, 11-22, 24-32, and 35). SIDMS is also a diagnostic tool that permits the assessment of species-altering procedures through evaluation and validation of other more traditional speciation analysis methods. SIDMS is to be used in conjunction with other methods when knowledge of species concentration, conversion and stability are necessary.

1.5 Both IDMS and SIDMS require the equilibration of the isotopically enriched species spike with the natural isotopes of the species of interest present in the sample. For IDMS, the spike and sample can be in different chemical forms, and later grouped as the same species form and only total elemental and or molecular concentrations will be measured. In general, IDMS equilibration of the spike and sample isotopes occurs during sample decomposition or transformation, which also destroys all species-specific information when the isotopes of an element or molecule are all oxidized or reduced or transformed to the same oxidation state.

For SIDMS, spikes and samples must be in the same speciated form and be equilibrated in solution (gaseous, liquid, solid), during extraction or on a separation or extraction column, as demonstrated with i-Spike cartridges (Refs. 34 and 43). This requires the analyte in spikes to be in the same molecular form as those in the sample. Efforts are taken to keep the species in their original forms after spiking as much as possible. For solutions or liquid samples, spiking and equilibration procedures can be as simple as mixing a known amount of the sample and the spikes prior to analysis. Aqueous samples such as drinking water, groundwater, etc. may be directly spiked and analyzed. Solid samples such as soils, sludges, sediments, industrial materials, biological tissues, botanicals, lysed cells, foods, including dietary supplements and consumer products, mixed samples, blood, urine and other samples containing solid matrices require spiking before, during or after extraction or prior to analysis to solubilize and equilibrate the species before introduction to the mass spectrometer. Animal and human clinical studies are areas where the higher accuracy and precision provided by method 6800 yield more statistically significant data than conventional methods. This method has been and can be used to certify reference materials, for environmental forensic analysis, and for detecting chemical and biological agents (specifically referred to as CBRNE, Chemical Biological Radiological Nuclear and Explosives) in homeland defense and homeland security applications.

1.6 Sensitivity and optimum ranges of elements may vary with the matrix, separation method, and isotope ratio measurement methods and specific ionization source and specific mass spectrometer. Using a liquid chromatography (LC) in-line with inductively coupled plasma

mass spectrometry (ICP-MS), it is convenient to separate elemental or molecular species and to measure their isotope ratios. Using a liquid chromatography (LC, or Nano-LC in-line with QTOF, triple quadrupole mass spectrometry (QQQ) or other MS instruments, it is appropriate to separate molecular species and to measure their isotope ratios. Although Method 6800 is not restricted to LC as the separation method of the species and the ICP-MS as the isotope ratio measurement MS, this document will use these techniques as examples for the most part in describing the Method's procedures. Molecular species separation methods such as extraction, precipitation, solid phase chelation, and isotope ratio measurement techniques such as thermal ionization mass spectrometry (TIMS), electrospray ionization mass spectrometry (ESI-MS), matrix assisted laser desorption ionization mass spectrometry (MALDI-MS) and other mass spectrometers such as time-of-flight mass spectrometers (TOF-MS) can also be used. Nano-ESI-MS –TOF, quadrupole time-of-flight (QTOF), QQQ, GC-MS, GC-QTOF, and GC-QQQ are mass spectrometric techniques that also separate and measure the species and isotopic ratios of molecular species and therefore are included as mass spectrometry platforms.

1.7 Prior to employing this Method, analysts are advised to consult the base method for each type of procedure that may be employed in the overall analysis for additional information on quality control procedures, development of QC acceptance criteria, calculations, and general guidance. Analysts also should consult the disclaimer statement at the front of the manual and the information in SW-846 Chapter Two for guidance on the intended flexibility in the choice of methods, apparatus, materials, reagents, and supplies, and on the responsibilities of the analyst for demonstrating that the techniques employed are appropriate for the analytes of interest, in the matrix of interest, and at the levels of concern.

In addition, analysts and data users are advised that, except where explicitly specified in a regulation, the use of SW-846 methods is *not* mandatory in response to Federal testing requirements. The information contained in this method is provided by EPA as guidance to be used by the analyst and the regulated community in making judgments necessary to generate results that meet the data quality objectives for the intended application.

1.8 This method is restricted to be used by, or under supervision of, properly experienced and trained personnel. Each analyst must demonstrate the ability to generate acceptable results with this method. The scientific literature should be consulted for fundamental training and for updating the fundamental of this method and for additional examples of application.

2.0 SUMMARY OF METHOD

2.1 IDMS method

2.1.1 Samples may require a variety of sample preparation procedures, depending on sample matrices and the isotope ratio measurement methods. One primary purpose of sample preparation is to solubilize the analyte of interest and equilibrate the spike isotopes with the isotopes in the sample. Solids, slurries, and suspended material must be subjected to digestion after spiking by using appropriate sample preparation methods (such as Method 3052). Water samples may not require digestion when ICP-MS is used as a detection method because ICP can destroy elemental species and thus many species may become indistinguishable for ICP-MS. Molecular species usually have the molecule-specific isotopes added during extraction to achieve equilibration of the species prior to introduction to ESI-MS, nano-ESI-TOF-MS, GC-MS, MALDI-MS, LC-QQQ, GC-TOF, GC-QQQ, LC(IC)-ICP-MS, GC-ICP-MS and other molecular mass spectrometric analysis techniques.

2.1.2 A measured representative sample is thoroughly mixed with a measured amount of the isotopic spike. If a digestion procedure is required, the spiked sample is then digested to equilibrate the spikes and samples. The sample solutions are then measured with mass spectrometry such as ICP-MS to obtain the altered isotope ratios. Method 6020 can be used as a reference method for ICP-MS detection. In addition to Method 6020, dead time correction and mass bias correction must be included in the measurement protocol. The equations described in Sec. 12.1 are used to calculate the concentrations.

Figure 2 shows an example of an IDMS determination of vanadium in crude oil (Ref. 1). IDMS determination of the restricted elements Cd, Cr, Pb and Hg in electrical components are demonstrated in Table 11 (Ref. 26).

2.2 SIDMS method

2.2.1 Speciated samples generally require sample preparation specific to the sample matrices, species, and the isotope ratio measurement method. The purpose of sample preparation is to solubilize the species of interest and to equilibrate the natural and spiked species, creating a homogeneous solution. Solids, slurries, and suspended material must be subjected to extraction before or after spiking, using appropriate sample preparation methods (such as Method 3060A for the determination of Cr(VI) in soils, and Method 3200 for the determination of mercury species in food, blood and tissues, and helix chromatography in the case of protein, peptide or proteomics). Method 3546 and other sample preparation methods in SW-845 Chapters Three and Four are applicable for environmental health and other toxicants in the environment and human health. Water samples may not need extraction and in many cases may be equilibrated and analyzed directly. In contrast to total molecular and element analysis, efforts must be taken to avoid the destruction of the species in SIDMS. For example, in molecular species analysis, reduced glutathione in blood, plasma and/or serum is transformed to oxidized glutathione (dimer species) by oxygen in the air during blood draw, storage, shipment or analysis. Sample preparation, spiking, and spike equilibration must be carried out in the absence of oxygen prior to LC-ESI-MS, nano-ESI-MS or MALDI-MS analysis. Species of mercury-glutathione complex appear to be more stable to oxygen but methylation and demethylation of these mercury species can occur during sample preparation and analysis.

2.2.2 Although SIDMS is a general method applicable to many elements in various species forms, for example chromium species, Cr(III) and Cr(VI), or proteomic species such as oxidized and reduced glutathione in human health and environmental samples such as water samples or soil extracts blood and serum will be considered here for demonstration of molecular species analysis. For the chromium species two isotopic spikes are prepared and characterized as follows: ^{50}Cr(III) spike enriched in ^{50}Cr and ^{53}Cr(VI) spike enriched in ^{53}Cr. The dominant natural isotope for Cr is ^{52}Cr, at 83.79% abundance (^{50}Cr, 4.35%; ^{53}Cr, 9.50%; ^{54}Cr, 2.36%). A measured amount of a representative aqueous sample is mixed well with an appropriate amount of ^{50}Cr(III) and ^{53}Cr(VI) spike solutions. Cr(III) and Cr(VI) are separated using chromatography or another separation method (Figure 3), and four isotope ratios are measured: ^{50}Cr(III)/^{52}Cr(III), ^{53}Cr(III)/^{52}Cr(III), ^{50}Cr(VI)/^{52}Cr(VI), and ^{53}Cr(VI)/^{52}Cr(VI). The concentrations of the species are determined from speciated isotope dilution calculation in section 12.2.

Figures 4 and 5 show examples of SIDMS for the determination of chromium species in an aqueous sample. Any species transformation between Cr(VI) and Cr(III) is mathematically corrected, as described in Sec. 12.2.

2.2.3 SIDMS may be called upon to analyze multiple species of interest that require selection of different isotopically enriched species and combinations of species. For example, if reduced and oxidized glutathione are being determined, the two isotopic forms of reduced glutathione and oxidized glutathione are equilibrated prior to shipment of blood samples to correct for oxidation of reduced glutathione to oxidized glutathione in shipment and during analysis to produce a statistically accurate result that is actionable by the practitioners. However, if for example the inorganic (Hg^{2+}) and methylmercury (CH_3Hg^+) conjugated forms of glutathione are being quantified, then species enriched with C-13, N-15, O-17 or O-18, or Hg-196 through Hg-204 are potential alternative spikes to quantify conjugates of glutathione and mercury species.

Figures 8 A-D show examples of SIDMS for the determination of glutathione mercury species of glutathione-Hg dimer and glutathione-methylmercury monomer. Figure 9 demonstrates nano-ESI-QTOF-MS abundance of reduced and oxidized glutathione for the analysis of Total GSH and GSH/GSSG ratios.

3.0 DEFINITIONS

See the "Glossary" at the end of this document for definitions of applicable terms. Also refer to SW-846 Chapters One and Three, and the manufacturer's documentation for definitions that may be relevant to these procedures.

4.0 INTERFERENCES

4.1 Solvents, reagents, glassware, and other sample processing hardware may yield artifacts and/or interferences to sample analysis. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by analyzing method blanks. Specific selection of reagents and purification of solvents by distillation of all-glass systems may be necessary. Refer to each method to be used for specific guidance on quality control procedures and to SW-846 Chapter Three for general guidance on the cleaning of glassware.

4.2 Sample preparation

4.2.1 Because this Method requires equilibration of the spike isotope(s) with the natural isotopes, solid samples must be digested, dissolved and/or extracted into solution. If the analyte of interest does not completely dissolve, if the spike or sample isotopes are selectively lost before equilibration, or if contamination occurs in the sample preparation process, the measured isotope ratio will not reflect the accurate ratio of the added spike atoms to sample atoms for that element or species (Refs. 1 and 3).

4.2.2 In general, SIDMS incorporates the requirement that all the converted species can be found in other species that are being monitored. As an example, in the interconversion between Cr(III) and Cr(VI), the lost Cr in one species must be found in the other species. Similarly, for example, in molecular species, if reduced and oxidized glutathione or inorganic glutathione and methylmercury-glutathione are found to transform among them, the isotopic signature from the enriched species spike are able to correct for these transformations. Thus, efforts should be made to keep all species in solution after equilibration with the enriched isotope species of interest.

4.2.3 Preservation of the species is required in SIDMS since interconversion degrades the precision of the measurement. Complete conversion of the species will prevent the deconvolution of the species concentration. Thus, sample decomposition applied for total element determination is inappropriate for SIDMS. However, the altered isotope ratios will indicate the conversion that has occurred and will not lead to an incorrect answer, but to a situation where the concentration cannot be determined and the species interconversions has been revealed. As little as 1 to 5% of the species have remained with correction to the original concentration based on the calculations and equations are provided (Refs. 30, 34, and 35). Approaches developed to maintain the species are applicable to SIDMS.

4.3 Isotope ratio measurement

4.3.1 Discussions about isobaric interference, doubly-charged ion interference, and memory interference in Method 6020B are applicable to this method. The discussion about the physical interference, suggesting the addition of an internal standard, does not apply. The internal standard is unnecessary because the isotope ratio measurement is free from physical interferences and each measurement is a standardized measurement without traditional calibration curves (General considerations for isotope ratio measurement can be found in Sec. 13.5.1).

4.3.2 Dead time measurement in ICP-MS must be performed daily. At high count rates, two effects cause pulse counting systems to count fewer events than actually occur (Sec. 13.5.2 and 13.5.3). The first is dead time (τ), the interval during which the detector and its associated counting electronics are unable to resolve successive pulses. If the true rate, *n,* is much less than *1/*τ, then:

$$m \approx n(1 - m\tau)$$

where *m* is the observed rate. The second effect is the loss of gain at high rates caused by the inability of the multiplier's dynode string to supply enough current to maintain constant dynode voltage drops. This effect is indicated by a sharp increase in apparent dead time at high count rates. Both effects cause the measured isotope ratios to diverge from the true isotope ratios with increasing count rate. While the dead time can be mathematically corrected, the gain loss cannot.

In molecular measurements on a TOF, QTOF or QQQ at low concentrations dead time may not be significant compared to the signal of the analytes. In these cases dead time may be neglected if it does not significantly affect the statistical uncertainty of the measurements.

A series of solutions with different concentrations can be prepared from isotopically certified standards for the determination of dead time. The concentrations may not be accurate, but the concentrations should spread out evenly, covering the blank to the highest count rate that may be used in measurements. The isotope pairs that are monitored should have large differences between their isotopic abundances, since the major isotopes suffer dead time effects much more seriously than minor isotopes; this makes the dead time correction significant. The sum of the dead-time-corrected counts is used for calculating the isotope ratios after background subtraction.

$$R_m = \frac{{}^{Isotope1}S_{sample/standard} - {}^{Isotope1}S_{background}}{{}^{Isotope2}S_{sample/standard} - {}^{Isotope2}S_{background}}$$

- R_m is the dead-time-corrected isotope ratio;
- ${}^{Isotope1}S_{sample/standard}$ and ${}^{Isotope2}S_{sample/standard}$ are the integrated dead-time-corrected counts for the sample or standard of Isotope1 and Isotope2, respectively;
- ${}^{Isotope1}S_{background}$ and ${}^{Isotope2}S_{background}$ are the integrated dead-time-corrected-counts for the background of Isotope1 and Isotope2, respectively.

As shown in Figure 1, which displays the $^{50}Cr/^{52}Cr$ ratios for SRM 979 ($Cr(NO_3)_3 \bullet 9H_2O$) as a function of the count rate, the isotope ratios are highly dependent on the number used for dead time correction. When the dead time is set to 43.5 ns, the isotope ratios are approximately constant up to the count rate of 5.8×10^5. At higher count rates, gain loss will occur and cannot be mathematically corrected. Therefore, the solutions must be diluted in the case where the count rate is higher than this value.

NOTE: Dead time correction in ICP-MS is performed before mass bias correction, so the dead-time-corrected isotope ratios may be different from the certified isotope ratios. Although it is unnecessary to use isotopically certified material for the determination of dead time, the certified material is still required for the measurement of mass bias factors. Thus, it is convenient to use the same certified material for both dead time and mass bias factor measurement. Dead time correction in Nano-ESI-QTOF is unnecessary due to the small sample size and low demand on detector capacity.

NOTE: It has been observed in ICP-MS that using different isotope pairs for dead time measurement may obtain different dead times. Thus, it is required to do the dead time measurement for each isotope pair that will be used. The dead time must be determined daily.

4.3.3 Instrumental discrimination/fraction effects are changes induced in the "true" isotope ratios from the ionization process or from differential transmission/detection by the mass spectrometer. This effect can bias the ratios either positively or negatively. To correct for the mass bias, mass bias factors should be determined with isotopically certified materials.

$$\text{mass bias factor} = R_t / R_m$$

where:

- R_t and R_m are the certified isotope ratio and the measured dead-time-corrected-isotope-ratios of the standard material, respectively.

The dead-time-corrected isotope ratios of the samples can be corrected using:

$$R_c = \text{mass bias factor} \times R_m$$

where:

– R_c and R_m are the corrected isotope ratio and the measured dead-time-corrected-isotope-ratios of the sample, respectively.

Mass discrimination is a time-dependent instrumental effect, therefore, the mass bias factors must be determined periodically during the measurement of the samples. Samples are run with the assumption that mass bias factors remain constant. In general, the mass bias factors are stable over several hours of ICP-MS operation. However, the measurement interval for determining mass bias factors should generally not exceed four hours.

NOTE: Some previous work observed the following relationship between the measured and the true isotope ratios for ICP-MS: $R_m=R_t(1+an)$, where *a* is the bias per mass unit and *n* is the mass difference between isotopes (12). This enables the calculation of the mass bias factors of other isotope pairs based on the measurement of one pair of isotopes. However, this must be verified experimentally. Otherwise, the mass bias factor for each isotope pair must be determined.

5.0 SAFETY

5.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of Occupational Safety and Health Administration (OSHA) regulations regarding the safe handling of the chemicals listed in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.2 Refer to Chapter Three for a discussion on safety-related references and issues.

5.3 Many chromium, mercury and molecular compounds are highly toxic if swallowed, inhaled, or absorbed through the skin. Extreme care must be exercised in handling hexavalent chromium, mercury species and many molecular reagents. These toxic and toxicant reagents should only be handled by analysts who are knowledgeable about their risks and of safe handling procedures.

6.0 EQUIPMENT AND SUPPLIES

Trade names or commercial products mentioned in this manual is for illustrative purposes only, and does not constitute an EPA endorsement or exclusive recommendation for use. The products and instrument settings cited in SW-846 methods represent those products and settings used during method development or subsequently evaluated by the Agency. Glassware, reagents, supplies, equipment, and settings other than those listed in this manual may be employed provided that method performance appropriate for the intended application has been demonstrated and documented.

6.1 ICP-MS or other mass spectrometers capable of baseline resolution (at least one unified atomic mass unit, u) are required. For example, mass spectrometers such as TOF may have 20,000 to 60,000 u resolution. Other mass spectrometers of even higher resolution are available. The data system should allow for corrections of isobaric interferences, dead time and mass bias or the equivalent, or the raw data may be exported to a computer for further processing. For quadrupole mass spectrometers, the dwell time should be adjustable since proper settings of dwell time can significantly improve the precision of the isotope ratio measurement in mass scanning instruments. Both scan mode and peak jump mode and simultaneous (i.e., TOF) systems can be used, depending on the instrumentation. The use of a mass-flow controller for the nebulizer argon and a peristaltic pump for the sample solution are recommended. When chromatography is coupled to ICP-MS for on-line detection, the ICP-MS data system must be capable of correcting interferences, dead time and mass bias, and calculating the isotope ratios in time resolved analysis mode (TRA), or the raw data can be exported for off-line processing. Other mass spectrometers may also be used, providing a precision of 0.5% or better can be obtained for the isotope ratio measurement. Examples of other ionization and mass spectrometers would be ESI, nanoESI and/or MALDI and/or microwave induced plasma (MIP) ionization coupled to quadrupole, TOF and/or ion-trap and/or QQQ mass spectrometers. Appropriate correction protocols for these mass spectrometers should be applied based on the performance specifications of the mass spectrometer.

6.2 Chromatography or other separation methods are used to isolate species prior to isotope ratio measurement. Chromatography, such as ion exchange chromatography may be used to separate the species on-line in SIDMS (Figure 3). Chromatography components should be chemically inert based on the specific reagents and analytes. The eluent components and the flow rate of the chromatography system must be compatible with ICP-MS. An interface between the chromatography and ICP-MS may be required for compatibility reasons. Alternatively, any appropriate separation methods, including extraction, solid phase extraction, chelation, reverse phase, normal phase and other chromatography, precipitation, affinity -based separations and combinations of these and others can be used after validation.

7.0 REAGENTS AND STANDARDS

7.1 All reagents should be of appropriate purity to minimize the blank levels due to contamination. Whenever possible, acids should be sub-boiling distilled. All references to water in the method refer to high purity reagent water. Other reagent grades may be used if it is first ascertained that the reagent is of sufficient purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is questionable, analyze the reagent to determine the level of its impurity.

7.2 For higher precision, solutions may be prepared by weight. For IDMS, standard stock solutions with natural isotopic abundance may be purchased or prepared from ultra-high purity grade chemicals or metals. As an example, see Method 6020B for instructions on preparing standard solutions from solids. Generally, the same procedures are applicable to isotope-enriched materials. However, when a limited amount of the isotope-enriched material is used (usually due to cost considerations) to prepare the stock solutions, the solutions require calibration with inverse isotope dilution (see Sec. 7.4.1). Isotope-enriched materials with known enrichment can be purchased from several suppliers, such as the Oak Ridge National Laboratory Electromagnetic Isotope Enrichment Facility (ORNL-EMIEF). Other examples are provided below.

7.3 Currently, standard stock solutions made for speciation analysis are commercially available. Thus, in addition to the dissolution of the standard solid, the chemical conversion of the element and molecule into the desired species is usually required for SIDMS. The preparation of Cr(VI) and Cr(III) stock standards for SIDMS will be illustrated as an example and the glutathione reduced and oxidized are also illustrated for molecular examples. These two example also are specifically illustrated in the equations that follow with chromium species illustrating the elemental and glutathione species illustrating the molecular species. For other elements and molecular species, procedures must be specifically developed, obtained from the literature or obtained by the manufacture and developers. One supplier of stable isotope standards for SIDMS and IDMS is Applied Isotope Technologies (AIT) Inc. These standards are and may be covered by patents. AIT is located at 2403 Sidney St. Suite 280, Pittsburgh PA 15203, info@sidms.com, http://www.sidms.com.

7.3.1 In this example of inorganic elemental speciation of chromium species, there are four standards to be prepared for the simultaneous analysis of Cr(VI) and Cr(III): ^{nat}Cr(VI) and ^{nat}Cr(III) with natural abundance, ^{53}Cr(VI) enriched in ^{53}Cr, ^{50}Cr(III) enriched in ^{50}Cr, and isotopic-abundance-calibrated Cr standard solution. In the molecular example of reduced and oxidized glutathione these species of natural and enriched species have exact analogues and corresponding reagents. For example reduced natural glutathione (GSH) having C-12, N-14 and O-16 are the natural forms of GSH. There are isotopically enriched analogues where C-13, N-15, and/or O-17, O-18 are enriched. Oxidized glutathione (GSSG) has similar natural and isotopically enriched forms. The equations are similar to those applied to the Cr natural and enriched species with the Cr(III) corresponding to the GSH both natural and enriched and the Cr(VI) GSSG both natural and enriched. One additional factor in molecular SIDMS is that there are small percentages of natural C-13, N-15 and O-17 and O-18 that account for a 1% naturally occurring signal that can be corrected as described (Refs. 34, 35, and 36). The calculations are essentially identical using both of the iterative and determinative SIDMS equations with the exception of adding the correction for the naturally occurring isotopes of C-13, N-15, and O-17 and O18 that effect the accuracy within the range of 1 to 2% in most cases. See the referenced literature and other SIDMS literature for further details on these modifications for molecular SIDMS.

7.3.2 1 mg/mL Cr(VI) and Cr(III) standards are commercially available. ^{nat}Cr(VI) and ^{nat}Cr(III) can also be prepared from $K_2Cr_2O_7$ and Cr metal, respectively.

7.3.2.1 ^{nat}Cr(VI) standard solution, stock, 1 g = 1 mg Cr: Dissolve 0.2829 g of $K_2Cr_2O_7$ in about 80 mL of reagent water and dilute to 100 g with reagent water.

7.3.2.2 ^{nat}Cr(III) standard solution, stock, 1 g = 1 mg Cr: Dissolve 0.1 g of Cr metal (accurately to at least 4 significant figures) in a minimum amount of 6M HCl and dilute the solution with 1% HNO_3 to 100 g.

7.3.3 ^{53}Cr(VI) standard solution, 1 g $\approx$ 10 μg Cr: The following procedure describes chromium oxide as the source material. A 150-mL glass or quartz beaker is used for the dissolution. Weigh 5.8 mg of ^{53}Cr-enriched oxide (the exact amount should be calculated based on the content of Cr in the material) into the beaker and add 8 mL of concentrated $HClO_4$. Slowly heat the beaker on a hot plate until bubbles form on the bottom; the solution should not boil. Keep heating the solution for up to 6 hrs until all solids are dissolved and only 1 to 2 mL of the solution remains. Turn off the hot plate and wait until the beaker cools down. Rinse the beaker and watch glass with 10 mL of reagent water; the solution should turn intense yellow. Add 50 μL of 30% H_2O_2 and 4.5 mL of concentrated NH_4OH. Slowly heat the vessel until the solution gently boils to oxidize all Cr

to Cr(VI). Allow the solution to boil for at least 15 minutes to remove the excessive H_2O_2. Transfer the solution to a 500-mL polymeric (e.g., polytetrafluoroethylene [PTFE], polyethylene, polypropylene, etc.) bottle and dilute the solution to 400 g. The exact concentration of the $^{53}Cr(VI)$ spike must be calibrated with $^{nat}Cr(VI)$ standard as described in Sec. 7.4.

NOTE: The procedure may be simpler when the isotope-enriched materials are available in other molecular forms. For example, when $K_2Cr_2O_7$ enriched in ^{53}Cr is available, the solid can be dissolved in reagent water without further conversion; when Cr metal is available, the metal can be dissolved in 6M HCl as described in Sec. 7.3.2.2, followed by the addition of H_2O_2 and NH_4OH to oxidize Cr(III) to Cr(VI) as described above.

NOTE: The molecular forms of GSH and GSSG have similar analogues. To prevent the oxidation of GSH to GSSG in the published application, N-ethylmaleimide (NEM) is added to prevent oxidation of GSH (Ref. 35). NEM blocks the cysteine fictional group and slows down oxidation. However addition of NEM is not completely effective and therefore correction by SIDMS might be required in some cases (Ref. 35)

WARNING: Concentrated $HClO_4$ is a very strong oxidizer. Safety protocols require this reagent only be used in a perchloric acid hood or equivalent solution and vapor handling system.

7.3.4 $^{50}Cr(III)$ standard solution, 1 g ≈ 10 μg Cr: The following procedure describes chromium metal as the source material. Weigh 4 mg of the metal into a 30-mL PTFE vessel. Add 4 mL of 6M HCl and gently heat the solution but do not boil it until the solid is dissolved. Continue to heat the solution until only 1 to 2 mL of the solution remains. The solution is then cooled and transferred to a 500-mL polymeric bottle. Dilute the solution with 1% HNO_3 to 400 grams. The exact concentration of the $^{50}Cr(III)$ spike must be calibrated with ^{nat}Cr standard as described in Sec. 7.4.

NOTE: The procedure depends on the form of the material. For example, when $K_2Cr_2O_7$ enriched in ^{50}Cr is available, the solid can be dissolved in 1% HNO_3, followed by the addition of H_2O_2 to reduce Cr(VI) to Cr(III). The excessive H_2O_2 can be removed by boiling the solution.

7.3.5 Isotopic-abundance-certified standard solution, stock, 1 g ≈ 10 μg Cr: Weigh 31 mg of $Cr(NO_3)_3 \cdot 9H_2O$ (SRM 979) into a 500-mL polymeric container. Dissolve the solid and dilute it with 1% HNO_3 to 400 g.

7.4 The isotope-enriched spikes require characterization since a limited amount of material is usually weighed, complex treatment is involved, or the purity of the source material is limited (usually <99%). For the SIDMS method, in addition to the total concentration of the standard, the distribution of the species must be determined before it can be used (see Section 12.2.2, 2nd NOTE). Inverse IDMS and inverse SIDMS measurement is used to calibrate the isotope-enriched spike and to determine the species distribution. The characterization of $^{53}Cr(VI)$ spike solution will be illustrated as an example.

7.4.1 Calibration of total concentration of spike solution with natural material: Weigh the proper amount (W_X) of 10 μg/g ($C_{Standard}$) ^{nat}Cr standard and the proper amount (W_S) of the $^{53}Cr(VI)$ spike (nominal concentration is 10 μg/g) into a polymeric container, and dilute the mixture with 1% HNO_3 to a concentration suitable for isotope ratio

measurement. Use direct aspiration mode to determine the isotope ratio of $^{53}Cr/^{52}Cr$ ($R_{53/52}$). The concentration of the spike, C_{Spike}, can be calculated using the following equations:

$$C_{Spike} = C_S M_S$$

$$C_S = \frac{C_X W_X}{W_S}\left(\frac{^{53}A_X - R_{53/52}\,{}^{52}A_X}{R_{53/52}\,{}^{52}A_S - {}^{53}A_S}\right)$$

$$C_X = C_{Standard} / M_X$$

where C_S and C_X are the concentrations of the isotope-enriched spike and the standard with natural isotopic abundance in μmole/g, respectively. M_S and M_X are the average atomic weights of the spike and the standard in g/mole, respectively. $^{53}A_S$ and $^{53}A_X$ are the atomic fractions of ^{53}Cr for the spike and standard, respectively. $^{52}A_S$ and $^{52}A_X$ are the atomic fractions of ^{52}Cr for the spike and standard, respectively.

NOTE: The same procedure is applicable to the calibration of the isotope-enriched spike solutions in IDMS. The same procedure is also applicable to the calibration of ^{50}Cr(III) by changing isotope ^{53}Cr to ^{50}Cr.

NOTE: Average atomic weight = Σ(atomic weight of the isotope x atomic fraction)

7.4.2 Calibration of the concentration of the Cr(VI) in the ^{53}Cr(VI) spike with ^{nat}Cr(VI): Weigh the proper amount (W_X) of 10 μg/g ($C^{VI}_{standard}$) ^{nat}Cr(VI) standard and the proper amount (W_S) of the ^{53}Cr(VI) spike (nominal concentration is 10 μg/g) into a polymeric container, and dilute the mixture with reagent water to a concentration suitable for measurement. Acidify the solution to pH 1.7 - 2.0 with concentrated HNO_3. Separate the Cr(VI) with chromatography or other separation methods and measure the isotope ratio of $^{53}Cr/^{52}Cr$ in Cr(VI) species ($R^{VI}_{53/52}$). The concentration of Cr(VI) in the spike, C^{VI}_{Spike}, can be calculated using the following equations:

$$C^{VI}_{Spike} = C^{VI}_S M_S$$

$$C^{VI}_S = \frac{C^{VI}_X W_X}{W_S}\left(\frac{^{53}A_X - R^{VI}_{53/52}\,{}^{52}A_X}{R^{VI}_{53/52}\,{}^{52}A_S - {}^{53}A_S}\right)$$

$$C^{VI}_X = C^{VI}_{Standard} / M_X$$

where C^{VI}_S and C^{VI}_X are the concentrations of Cr(VI) in the isotope-enriched spike and standard with natural isotopic abundance in μmole/g, respectively. M_S and M_X are the average atomic weights of the spike and the standard in g/mole, respectively. $^{53}A_S$ and $^{53}A_X$ are the atomic fractions of ^{53}Cr for the spike and standard, respectively. $^{52}A_S$ and $^{52}A_X$ are the atomic fractions of ^{52}Cr for the spike and standard, respectively.

NOTE: This set of equations is similar to those used in the determination of total Cr in ^{53}Cr(VI) standard (Sec. 7.4.1). The general equations for inverse SIDMS are not so simple. However, for speciation of Cr(VI) and Cr(III) in standard

solutions, because the matrix is so simplified, only the reduction of Cr(VI) to Cr(III) is observed at low pH. Thus, the existence of Cr(III) species will not influence the isotope ratio of Cr(VI), and the complex equations can be simplified to the equations shown above (Ref. 3).

7.4.3 The distribution of Cr(III) and Cr(VI) in $^{53}Cr(VI)$ spike can be calculated as:

$$\text{percentage of Cr(VI)} = \frac{C_{Spike}^{VI}}{C_{Spike}} \times 100\%$$

$$\text{percentage of Cr(III)} = \left(1 - \frac{C_{Spike}^{VI}}{C_{Spike}}\right) \times 100\%$$

NOTE: No determination of the species distribution in $^{50}Cr(III)$ spike is required because only Cr(III) is present in this solution.

7.5 Blank samples -- Three types of blank samples are required for the analysis; namely, a background blank for subtracting background in isotope ratio measurement, a preparation blank for monitoring possible contamination resulting from the sample preparation procedures, and a rinse blank for flushing the system between all samples and standards.

7.5.1 The background blank consists of the same concentration(s) of the acid(s) used to prepare the final dilution of the sample solution (often 1% HNO_3 (v/v) in reagent water).

7.5.2 The preparation (or reagent) blank must be carried through the complete preparation procedure and contain the same volumes of reagents as the sample solutions.

7.5.3 The rinse blank consists of 1 to 2 % HNO_3 (v/v) in reagent water. Prepare a sufficient quantity to flush the system between standards and samples. Refer to Method 6020 regarding the interference check solution.

7.6 Refer to Method 6020 for preparing mass spectrometer tuning solution.

8.0 SAMPLE COLLECTION, PRESERVATION, AND STORAGE

Sample collection, preservation and storage requirements may vary by EPA program and may be specified in a regulation or project planning document that requires compliance monitoring for a given contaminant. Where such requirements are specified in the regulation, follow those requirements. In the absence of specific regulatory requirements, use the following information as guidance in determining the sample collection, preservation and storage requirements.

8.1 See the introductory material to SW-846 Chapter Three for inorganic analytes and Chapter Four for organic analytes.

8.2 Due to the possible degradation or interconversion of the species, samples collected for speciation analysis must be isotopically spiked as soon as possible. The measurement, however, can be carried out later provided that less than 95% degradation or

interconversion occurs with validation of specific species groups. The holding time prior to measurement depends on the preservation of the species.

8.3 Proper methods to retard the chemical activity of the species are applicable to SIDMS.

8.4 All sample containers must be prewashed with detergents, acids, and water. Polymeric containers can be used and containers are chosen for inertness and compatibility of the matrix, molecules and species to be analyzed. See SW-846 Chapters Three and Four for further information on clean chemistry procedures to reduce blank effects in these measurements.

9.0 QUALITY CONTROL

9.1 Refer to SW-846 Chapter One for guidance on quality assurance (QA) and QC protocols. When inconsistencies exist between QC guidelines, method-specific QC criteria take precedence over both technique-specific criteria and those criteria given in Chapter One, and technique-specific QC criteria take precedence over the criteria in SW-846 Chapter One. Any effort involving the collection of analytical data should include development of a structured and systematic planning document, such as a Quality Assurance Project Plan (QAPP) or a Sampling and Analysis Plan (SAP), which translates project objectives and specifications into directions for those that will implement the project and assess the results. Each laboratory should maintain a formal quality assurance program. The laboratory should also maintain records to document the quality of the data generated. All data sheets and quality control data should be maintained for reference or inspection.

9.2 Initial demonstration of proficiency

Each laboratory must demonstrate initial proficiency with the appropriate sample preparation and the isotope ratio measurement methods by generating data of acceptable bias and precision for target analytes in a clean matrix. Method 8000 provides information on how to accomplish a demonstration of proficiency for standard methods. This method, using SIDMS, has unique proficiency requirements as concentrations are determined by direct calculation based on isotopic properties of the target analyte and the enriched isotopic species spike without the use of calibration curves as described in Method 8000. During SIDMS analysis by Method 6800, analyte transformations may occur to some extent in some matrices and can be corrected by using its protocol. Proficiency is established for each species such as Cr(VI), methylmercury, tributyltin, and any polyisotopic species and each matrix such as water, soil, tissue and others. These proficiencies are to be determined using appropriate standards prepared to test proficiency of spiking, extraction, species separation, mass spectrometric operation and calculation which together constitute this method's SIDMS protocol. A suite of proficiency testing materials, as well as guidelines, for the implementation of the proficiency testing is available from Applied Isotope Technologies (info@sidms.com). Determination of total element or molecule using this method's IDMS protocol is similar to more traditional methods that are spiked with elements as the matrix spike or molecules in organic analytes. However, in this method, concentration of the species of interest is calculated directly from the isotopic element/molecule spike and not from calibration curves.

9.3 Sample quality control for preparation and analysis

The laboratory must also have procedures for documenting the effect of the matrix on method performance (precision, accuracy, method sensitivity, species conversion). At a

minimum, this should include the analysis of QC samples including background blank, preparation blank, rinse blank, matrix spike, duplicate, and laboratory control sample (LCS) and/or proficiency samples if available and were applicable for each analytical batch. A sample of known concentration, such as Standard Reference Material, an appropriate reference material or a suitable set of proficiency materials can be used as LCS. Any blank, matrix spike samples, and replicate samples should be subjected to the same analytical procedures (Sec. 11.0) as those used on actual samples.

9.3.1 Duplicate samples should be processed on a routine basis. A duplicate sample is a sample processed through the entire sample preparation and analytical procedure. A duplicate sample should be processed with each analytical batch or every 20 samples, whichever is the greater number. A duplicate sample should be prepared for each matrix type (i.e., soil, sludge, water, tissue, etc.). If statistical compilations are desired, a sufficient number of duplicate samples should be analyzed to produce the desired statistical results. Consult Method 8000 for information on developing acceptance criteria for the duplicate.

9.3.2 Spiked samples and/or standard reference materials and/or reference materials or proficiency materials (if available) should be included with each group of samples processed or every 20 samples, whichever is the greater number. A spiked sample should also be included whenever a new sample matrix is being analyzed and proficiency should be established for each sample matrix. For SIDMS, because the species may degrade or convert to other species when they are spiked into samples, low recovery may be observed. Thus, the low recovery does not immediately invalidate this method. For example, if Cr(III) is spiked into a basic solution, due to the hydrolysis of Cr(III) and the limited solubility of chromium hydroxide, low recovery of Cr(III) may be obtained. Low recovery may indicate an unfavorable matrix for preserving the corresponding species (Ref. 4). The conversion of one species to another should be calculated and recorded with the final corrected concentration. If equilibration is reached prior to species-loss, an isotopic ratio may still be measurable and will result in an acceptable species measurement. In most cases, conversion can be quantified and corrected. If a sample, isotopic spike of one species is completely converted into another species, then the matrix and/or the sample preparation procedure may be responsible for species conversion. This will indicate that this specific matrix can support the stability of only select species. The results of concentration and conversion should be recorded as an acceptable set of data. Species concentration and species conversion should be measured and recorded in the laboratory report. Speciation under this method is a relatively new field and additional equations and other QC, proficiency support, and reference materials are updated through Ref. 27.

9.3.3 Blank samples should be prepared using the same reagents and quantities used in sample preparation, placed in vessels of the same type, and processed with the samples. Also see Sec. 7.5 for more information regarding blank samples.

9.4 Lower Limit of Quantitation (LLOQ) check standard

9.4.1 The laboratory should establish the LLOQ as the lowest point of quantitation which, in most cases, is the lowest concentration in the calibration curve or direct calculation, such as Method 6800. The LLOQ is initially verified by the analysis of at least 7 replicate samples, spiked at the LLOQ and processed through all preparation and analysis steps of the method. The mean recovery and relative standard deviation of these samples provide an initial statement of precision and accuracy at the LLOQ. In most cases the mean recovery should be +/- 35% of the true value and RSD should be < 20%. In-house limits may be calculated when sufficient

data points exist. Monitoring recovery of LLOQ over time is useful for assessing precision and bias. Refer to a scientifically valid and published method such as Chapter 9 of Quality Assurance of Chemical Measurements (Taylor 1987) or the Report of the Federal Advisory Committee on Detection and Quantitation Approaches and Uses in Clean Water Act Programs (http://water.epa.gov/scitech/methods/cwa/det/index.cfm) for calculating precision and bias for LLOQ.

9.4.2 Ongoing LLOQ verification, at a minimum, is on a quarterly basis to validate quantitation capability at low analyte concentration levels. This verification may be accomplished either with clean control material (e.g., reagent water, method blanks, Ottawa sand, diatomaceous earth, etc.) or a representative sample matrix (free of target compounds). Optimally, the LLOQ should be less than or equal to the desired regulatory action levels based on the stated project-specific requirements.

10.0 CALIBRATION AND STANDARDIZATION

10.1 IDMS calibration

10.1.1 Follow the appropriate sections in Method 6020B to set up and tune the ICP-MS. Sample introduction is performed in direct aspiration mode. The following procedure is illustrated with the measurement of $^{50}Cr/^{52}Cr$ and $^{53}Cr/^{52}Cr$ isotope ratios.

10.1.2 Determine the dead time (Sec. 4.3.2). Solutions prepared from reference material SRM 979 ($Cr(NO_3)_3 \bullet 9H_2O$) are used in this determination. A range of solutions of different concentrations should be prepared, but their concentrations do not need to be accurately known. Masses 50, 52 and 53, as well as masses which could affect data quality, should be monitored. The raw count rates for each solution are measured and integrated. Assume a dead time and use the equation described in Sec. 4.3.2 to correct the integrated counts. The dead-time-corrected counts are then used for calculating the isotope ratios after background subtraction. By trial and error, the dead time is determined to bring the isotope ratios obtained from solutions of different concentrations to a constant (the relative standard deviation of the isotope ratios reaches the minimum). The isotope ratios obtained from high counts may be excluded as gain loss may occur.

NOTE: The concentration range of the solutions may be adjusted depending on the sensitivity and dynamic range of the instrument.

NOTE: For direct aspiration mode, the dead time correction can be done either before or after the integration of the raw data. However, it is simpler to do the dead time correction after the integration.

10.1.3 Determine the mass bias factor (Sec. 4.3.3). The mean of isotope ratios obtained in Sec. 10.1.2 is used for calculating the mass bias factor. The equation is provided in Sec. 4.3.3. The measurement of the mass bias factor must be done periodically between sample measurements. The interval between these measurements depends on the mass bias stability of the instrument. The relative difference between two consecutive mass bias factors should not exceed 1%.

10.2 SIDMS calibration

10.2.1 Follow the appropriate sections in Method 6020B to set up and tune the ICP-MS. Follow Sec. 10.1.2 to measure the dead time. If the calibration of the isotope-enriched spikes is required, the mass bias factors for direct aspiration mode and the altered isotope ratios for the spiked standards are measured at this step. The measured isotope ratios obtained at this step are used in the calibration of total concentrations.

10.2.2 Determine the mass bias factor (Sec. 4.3.3). Connect the outlet of the chromatographic column to the nebulizer of the ICP-MS. Background blank and an isotopic abundance certified standard are used for the measurement of the mass bias factors in time resolved analysis (TRA) mode. The raw data at each point are corrected for dead time using the equation described in Sec. 4.3.2 and then integrated by summing the data across each peak. The intervals between two consecutive injections must be long enough for the signal to return to baseline. The integrated counts are then used to calculate the isotope ratios with the equation shown in Sec. 4.3.2. Apply the equation in Sec. 4.3.3 to calculate the mass bias factors for each isotope pair by comparing the measured isotope ratios to the certified isotope ratios.

NOTE: For the TRA mode, the dead time correction must be done at each data point before the data integration.

NOTE: For other ionization and mass spectrometer types such as ESI and/or MALDI and/or MIP ionization coupled to quadrupole, TOF and/or ion-trap mass spectrometers, appropriate correction protocols should be utilized.

11.0 PROCEDURE

11.1 IDMS

11.1.1 Closed-vessel microwave digestion is used as an example method to decompose samples and to solubilize, equilibrate and stabilize the elements of interest. The following procedure is applicable to samples decomposed using Method 3052. Refer to Method 3052 for specifications of the microwave apparatus. Method 3546 is a microwave extraction method for organic analytes and is suited for IDMS and SIDMS of molecular species. Other extraction methods are available in the literature for additional species and matrices. Separation may be directly applied to molecular and elemental species that are amenable to such measurements such as water, blood, serum, plasma, drinks, and other samples.

11.1.2 Prepare or purchase the isotope-enriched spike and calibrate it with the inverse isotope dilution mass spectrometry procedure described in Secs. 7.3 and 7.4.1. Isotope enriched spikes are available as commercial products for both elemental and molecular species from Applied Isotope Technologies, Inc. (e-mail: info@sidms.com; http://www.sidms.com).

11.1.3 Weigh a representative sample to the nearest 0.001 g into an appropriate microwave digestion vessel equipped with a pressure relief mechanism. Spike the sample with the calibrated isotope-enriched spike. The concentration of the spike should be high enough so that only a small volume of the solution is used. Weigh the mass of the spike to at least three significant figures.

11.1.4 Digest the sample according to the procedure described in Method 3052 and/or extract the species from the sample matrix with an extraction method such as 3546, or separate the analytes by chromatography (see SW-846 Chapters Three and Four and the literature for additional sample preparation methods).

NOTE: For filtered and acidified aqueous samples, digestion may not be required. Sample solutions can be directly analyzed with ICP-MS, TOF, QTOF, QQQ or and/or other appropriate mass spectrometers after spiking and equilibration.

11.1.5 Measurement of the isotope ratios can be carried out using ICP-MS or ESI-TOF, QTOF, QQQ and/or other appropriate mass spectrometers.

11.1.5.1 Determine the mass bias factor periodically as described in Sec. 10.1.3.

11.1.5.2 Measure the isotope ratio of each sample. Flush the system with the rinse blank. In many cases the ideal isotope ratio is 1:1. Isotope ratios are ideally within the range from 0.1:1 to 10:1, except for blanks and samples with extremely low concentrations. Samples may be re-spiked to achieve an isotope ratio close to 1:1. If too high of a count rate is observed, samples must be diluted to avoid gain loss of the detector. Some isotope ratios can be quantified at 1:100 and 100:1 in cases of instrument sensitivity and stability. Error Propagation Factors (EPF) can be calculated to optimize the ratios for quantification.

NOTE: Unspiked solutions are used to measure the isotopic abundance of elements and molecules such as lithium, lead, and uranium, and molecular enriched ions whose isotopic abundances can vary in nature.

11.2 SIDMS

11.2.1 SIDMS is applicable to the quantification of elemental and molecular species in various sample matrices such as water, soil, sludge, tissue, food, blood, plasma, serum, drugs, dietary supplements, etc. Solid samples require or benefit from isolation/solubilization and separation of the elemental species before analysis. Procedures for such extraction of the species from different matrices must be specifically designed to minimize and measure species conversion. Extraction procedures for tissue, hair and bacteria are found in references (Refs. 20 through 22, respectively). The following procedure is an illustration of the simultaneous determination of Cr(III) and Cr(VI) in water samples or in soil or sediment extracts. Cr(VI) is extracted from the solid using Method 3060A, mercury species by Method 3200, and organic molecules by Method 3546 and other appropriate methods (See SW-846 Chapters Three and Four).

11.2.2 There are two ways of obtaining the isotopically enriched standards used in IDMS and SIDMS. Prepare the isotope-enriched spikes of each target species and calibrate them with inverse isotope dilution mass spectrometry as described in Sec. 7.4. Species-specific isotope-enriched spikes are available as commercial products from Applied Isotope Technologies (e-mail: info@sidms.com; http://www.sidms.com).

11.2.3 Weigh an appropriate aliquot of the water, solid or semi-solid sample, or extract to the nearest 0.0001 g into a polymeric container. Spike the sample with 10 μg/g of ^{53}Cr(VI) spike to a concentration so that the isotope ratio of ^{53}Cr/^{52}Cr in Cr(VI) will be approximately 1:1. Spike the sample with ^{50}Cr(III) spike to a concentration so that the isotope ratios of ^{50}Cr/^{52}Cr in Cr(III) will also be approximately 1:1 and the species

concentrations are suitable for measurement. Thoroughly mix the spike and the sample. The isotope ratios $^{53}Cr/^{52}Cr$ for samples must be within the range of 0.1:1 to 10:1, except for blanks or samples with extremely low concentrations. If isotope ratios are found to be outside these ranges, samples should be re-spiked and re-analyzed.

NOTE: If only the Cr(VI) is of interest, the sample can be spiked only with $^{53}Cr(VI)$. However, this is based on the assumption that only unidirectional conversion can occur after spiking, i.e. reduction of Cr(VI) to Cr(III). This is usually true if the sample is acidified to a low pH after spiking, especially for matrices containing reducing agents. Molecular SIDMS uses GSH as the example of molecular species that have unidirectional species oxidation to GSSG.

11.2.4 Extract the species from the sample matrix (see Section 11.2.1)

NOTE: For aqueous samples, extraction may not be required. Sample solutions can be directly analyzed after spiking and equilibration. Highly concentrated samples may need to be adjusted in spike to sample ratio and may be adjusted to a lower spike ratio to conserve spike or sub-sampled. Chromium ore processing residues (COPR) samples are a good example of high chromium concentration where this can be applied.

11.2.5 If the solution is strongly basic, neutralize the sample with concentrated HNO_3 to avoid the hydrolysis of Cr(III). Sample preparation to preserve the molecular species is specific to the chemistry of the species and is performance based and depends on the analyses being conducted. The example of chromium species and glutathione are used to illustrate elemental and molecular species respectively. The sample preparation is dependent on the species chemistry, stoichiometry and equilibrium relationships of the species.

11.2.6 Acidify the spiked samples to pH 1.7 to 2.0; under these conditions Cr is usually retained in solution, although there might be interconversion between Cr(III) and Cr(VI). The spiked samples can be stored at ≤6 °C to retard interconversion of the Cr species. Other methods that can slow down the transformation of the species can be applied as long as no interference with the isotope ratio measurement is introduced. For example, some soil extracts contain large concentrations of reducing agents that reduce Cr(VI) rapidly after acidification. To slow down the reduction, stoichiometric amounts of $KMnO_4$ can be added to the sample; $KMnO_4$ easily oxidizes the reducing matrices.

NOTE: Studies have shown that the lower the interconversion between the species, the more precise the determination is (Ref. 3). Thus, efforts should be made to prevent or retard species interconversions.

11.2.7 The measurement of the isotope ratios in each species can be carried out using ICP-MS or other equivalent mass spectrometers following the separation of the species using chromatography or other separation methods. The use of ion-exchange chromatograph coupled with ICP-MS will be illustrated as an example in the measurement of $^{50}Cr/^{52}Cr$ and $^{53}Cr/^{52}Cr$ isotope ratios in samples containing both Cr(III) and Cr(VI) species.

11.2.7.1 Determine the mass bias factors periodically as described in Sec. 10.2.2.

11.2.7.2 Measure the isotope ratios of each sample. Flush the system with the eluent until the signal returns to the baseline. The ideal isotope ratios for $^{50}Cr/^{52}Cr$ in Cr(III) and $^{53}Cr/^{52}Cr$ in Cr(VI) are 1:1. Ratios between 0.1:1 and 10:1 are also appropriate. Samples may be re-spiked to achieve an isotope ratio close to 1:1. If excessively high count rates are observed, samples must be diluted to avoid gain loss of the detector.

NOTE: Unspiked solutions are used to measure the isotopic abundance of elements such as lithium, lead, and uranium, whose isotopic abundances can vary in nature.

NOTE: A different set of equations and examples are required for the simultaneous determination of three species with correction following the SIDMS protocol,. One example for three mercury species can be found in Ref. 24 along with a version of the equations developed for three species. Equations for the determination of two and three species are included in this reference and the formats are constructed to simplify these calculations. New explicit and determinative equations will be published in the literature and updated references and technical support will be made available through the www.SIDMS.com website (Ref. 27).

12.0 DATA ANALYSIS AND CALCULATIONS

12.1 IDMS calculations

The quantitative values must be reported in appropriate units, such as micrograms per liter (μg/L) for aqueous samples and milligrams per kilogram (mg/kg) for solid samples. If dilutions are performed, the appropriate corrections must be applied to the sample values.

12.1.1 Calculate the isotope ratios. Calculations should include appropriate interference corrections (see Sec. 4.3 for data integration, dead time correction, and mass bias correction).

12.1.2 The following equations are applied to the calculation of the concentration of the element, C_{Sample}(μg/g), in the final sample solutions:

$$C_{Sample} = C_X M_x$$

$$C_S = C_{Spike} / M_S$$

$$C_X = \frac{C_S W_S}{W_X}\left(\frac{{}^{53}A_S - R_{53/52}\,{}^{52}A_S}{R_{53/52}\,{}^{52}A_X - {}^{53}A_X}\right)$$

where, C_S and C_X are the respective concentrations of the isotope-enriched spike and the analyte in the sample, in μmole/g. M_S and M_X are the average atomic weights of the isotope-enriched spike and the sample in g/mole, respectively. $^{53}A_S$ and $^{53}A_X$ are the atomic fractions of ^{53}Cr for the isotope-enriched spike and sample, respectively. $^{52}A_S$ and $^{52}A_X$ are the atomic fractions of ^{52}Cr for the isotope-enriched spike and sample, respectively. C_{spike} is the concentration of the isotope-enriched spike in μg/g. A general guideline for IDMS calculations can be found in the documents of Section 13.5.4.

NOTE: When isotope ^{50}Cr is used, ^{53}Cr is substituted with ^{50}Cr in the above equations.

12.1.3 If appropriate or required, calculate results for solids on a dry-weight basis as follows:

(1) A separate determination of percent solids must be performed.
(2) The concentrations determined in the digest are to be reported on the basis of the dry weight of the sample as follows:

$$\text{Concentration (dry weight)(mg/kg)} = \frac{C_{Sample}}{S}$$

where, C_{Sample} = Concentration based on the wet sample (μg/g)

$$S = \frac{\%\,\text{Solids}}{100}$$

12.2 SIDMS calculations

The quantitative values must be reported in appropriate units, such as μg/L for aqueous samples and mg/kg for solid samples. If dilutions are performed, the appropriate corrections must be applied to the sample values.

12.2.1 Calculate the isotope ratios. Calculations should include appropriate interference corrections, dead time correction, and mass bias correction (Sec. 4.3).

12.2.2 The following equations are used to deconvolute the concentrations of the species at the time of spiking, as well as the conversion of the species after spiking:

$$R^{III}_{50/52} = \frac{\left({}^{50}A_X\, C_X^{III}\, W_X + {}^{50}A_S^{III}\, C_S^{III}\, W_S^{III}\right)(1-\alpha) + \left({}^{50}A_X\, C_X^{VI}\, W_X + {}^{50}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)\beta}{\left({}^{52}A_X\, C_X^{III}\, W_X + {}^{52}A_S^{III}\, C_S^{III}\, W_S^{III}\right)(1-\alpha) + \left({}^{52}A_X\, C_X^{VI}\, W_X + {}^{52}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)\beta}$$

$$R^{III}_{53/52} = \frac{\left({}^{53}A_X\, C_X^{III}\, W_X + {}^{53}A_S^{III}\, C_S^{III}\, W_S^{III}\right)(1-\alpha) + \left({}^{53}A_X\, C_X^{VI}\, W_X + {}^{53}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)\beta}{\left({}^{52}A_X\, C_X^{III}\, W_X + {}^{52}A_S^{III}\, C_S^{III}\, W_S^{III}\right)(1-\alpha) + \left({}^{52}A_X\, C_X^{VI}\, W_X + {}^{52}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)\beta}$$

$$R^{VI}_{50/52} = \frac{\left({}^{50}A_X\, C_X^{III}\, W_X + {}^{50}A_S^{III}\, C_S^{III}\, W_S^{III}\right)\alpha + \left({}^{50}A_X\, C_X^{VI}\, W_X + {}^{50}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)(1-\beta)}{\left({}^{52}A_X\, C_X^{III}\, W_X + {}^{52}A_S^{III}\, C_S^{III}\, W_S^{III}\right)\alpha + \left({}^{52}A_X\, C_X^{VI}\, W_X + {}^{52}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)(1-\beta)}$$

$$R^{VI}_{53/52} = \frac{\left({}^{53}A_X\, C_X^{III}\, W_X + {}^{53}A_S^{III}\, C_S^{III}\, W_S^{III}\right)\alpha + \left({}^{53}A_X\, C_X^{VI}\, W_X + {}^{53}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)(1-\beta)}{\left({}^{52}A_X\, C_X^{III}\, W_X + {}^{52}A_S^{III}\, C_S^{III}\, W_S^{III}\right)\alpha + \left({}^{52}A_X\, C_X^{VI}\, W_X + {}^{52}A_S^{VI}\, C_S^{VI}\, W_S^{VI}\right)(1-\beta)}$$

where,

$R^{III}_{50/52}$ is the measured isotope ratio of ^{50}Cr to ^{52}Cr of Cr(III) in the spiked sample

$^{50}A_X$ is the atomic fraction of ^{50}Cr in the sample (usually a constant in nature)

C_X^{III} is the concentration of Cr(III) in the sample (μmole/g, unknown)

W_X is the weight of the sample (g)

$^{50}A_S^{III}$ is the atomic fraction of ^{50}Cr in the ^{50}Cr(III) spike

C_S^{III} is the concentration of Cr(III) in the ^{50}Cr(III) spike (μmole/g)

W_S^{III} is the weight of the ^{50}Cr(III) spike (g)

C_X^{VI} is the concentration of Cr(VI) in the sample (μmole/g, unknown)

α is the percentage of Cr(III) oxidized to Cr(VI) after spiking (unknown)

β is the percentage of Cr(VI) reduced to Cr(III) after spiking (unknown)

NOTE: The unit of the concentrations shown above is μmole/g. The conversion factor from μmole/g to μg/g is: M, where M is the average atomic weight of the element in μg/μmole (Sec. 7.4.1). The following equation can be used to convert the unit of the concentration. Be aware that samples with different isotopic abundance have different average atomic weights.

Concentration (μmole/g) x M = Concentration (μg/g)

NOTE: Although the species distribution of the isotopic spike is determined (Sec. 7.4), the above equations assume that each isotope-enriched spike is only in one species form to simplify the equations. This has been validated for ^{50}Cr(III) and ^{53}Cr(VI) spikes prepared using the procedures described in Sec. 7.3. For other speciation analysis, this assumption must be verified experimentally, or the distribution of the species in the isotope-enriched spikes must be taken into account.

NOTE: For the quantification of the single-spiked samples, the following equations are used:

$$C_{\text{Sample}}^{\text{VI}} = C_{\text{X}}^{\text{VI}} M_{\text{X}}$$

$$C_{\text{S}}^{\text{VI}} = C_{\text{Spike}}^{\text{VI}} / M_{\text{S}}^{\text{VI}}$$

$$C_X^{VI} = \frac{C_S^{VI} W_S}{W_X} \left(\frac{^{53}A_S^{VI} - R_{53/52}^{VI} \, ^{52}A_S^{VI}}{R_{53/52}^{VI} \, ^{52}A_X - ^{53}A_X} \right)$$

where C_s^{VI} and C_x^{VI} are the respective concentrations of the isotope-enriched spike and the analyte in the sample, in μmole/g. M_s^{VI} and M_X are the average atomic weight of the isotope-enriched spike and the sample in g/mole, respectively. $^{53}A_s^{VI}$ and $^{53}A_X$ are the atomic fraction of ^{53}Cr for the isotope-enriched spike and sample, respectively. $^{52}A_s^{VI}$ and $^{52}A_X$ are the atomic fractions of ^{52}Cr for the isotope-enriched spike and sample, respectively. C_{spike}^{VI} is the concentration of the isotope-enriched spike in μg/g.

NOTE: When isotope ^{50}Cr is used, ^{53}Cr is substituted with ^{50}Cr in the above equations.

12.2.3 A computer program such as a spreadsheet can be developed to solve this set of second power, four variable equations. Solutions for the four unknowns, C_X^{III}, C_X^{VI}, α and β are required, and can be found from various mathematical approaches. An iterative approach was originally used to solve for the four unknowns (Refs. 11, 12, and 28), but an explicit, deterministic solution has since been developed. The explicit, determinative solution method eliminates the need for initialization, and recognizes the conditions under which a unique solution will be obtained.

To implement the explicit, deterministic solution method, it is helpful to first express the four equations in simplified notation:

$$R_1 = \frac{(A_1 C_x^{III} + B_1)(1-\alpha) + (A_1 C_x^{VI} + B_2)\beta}{(A_2 C_x^{III} + B_3)(1-\alpha) + (A_2 C_x^{VI} + B_4)\beta}$$

$$R_2 = \frac{(A_3 C_x^{III} + B_5)(1-\alpha) + (A_3 C_x^{VI} + B_6)\beta}{(A_2 C_x^{III} + B_3)(1-\alpha) + (A_2 C_x^{VI} + B_4)\beta}$$

$$R_3 = \frac{(A_1 C_x^{III} + B_1)\alpha + (A_1 C_x^{VI} + B_2)(1-\beta)}{(A_2 C_x^{III} + B_3)\alpha + (A_2 C_x^{VI} + B_4)(1-\beta)}$$

$$R_4 = \frac{(A_3 C_x^{III} + B_5)\alpha + (A_3 C_x^{VI} + B_6)(1-\beta)}{(A_2 C_x^{III} + B_3)\alpha + (A_2 C_x^{VI} + B_4)(1-\beta)}$$

These are the exact equations presented in 12.2.2, expressed in terms of thirteen known quantities:

- Four ion intensity ratios, corrected for dead-time and mass-bias: $\{R_1, R_2, R_3, R_4\} = \{R_{50/52}^{III}, R_{53/52}^{III}, R_{50/52}^{VI}, R_{53/52}^{VI}\}$.
- The three sample weights of each chromium isotope, using naturally occurring isotope proportions W_x: $\{A_1, A_2, A_3\} = \{^{50}A_x W_x, ^{52}A_x W_x, ^{53}A_x W_x\}$.
- The amounts of ^{50}Cr(III), ^{52}Cr(III) and ^{53}Cr(III) in the Cr(III) spike: $\{B_1, B_3, B_5\} = \{^{50}A_s^{III} N_s^{III}, ^{52}A_s^{III} N_s^{III}, ^{53}A_s^{III} N_s^{III}\}$, as well as the amounts of ^{50}Cr(VI), ^{52}Cr(VI) and ^{53}Cr(VI) in the Cr(VI) spike: $\{B_2, B_4, B_6\} = \{^{50}A_s^{VI} N_s^{VI}, ^{52}A_s^{VI} N_s^{VI}, ^{53}A_s^{VI} N_s^{VI}\}$. If W_s^{III} is the weight of the Cr(III) spike, and C_s^{III} is the concentration of Cr(III) in that spike, then $N_s^{III} = W_s^{III} C_s^{III}$.

Solving equations (1)–(4) for the unknowns α, β, C_x^{III}, and C_x^{VI}, gives the following explicit solutions:

$$\alpha = (B_2R_4A_2 - B_2A_3 + R_3B_4A_3 - B_6R_3A_2 + B_6A_1 - R_4B_4A_1)(B_1A_3 - B_1R_2A_2 - R_1B_3A_3 - B_5A_1 + B_5R_1A_2 + R_2B_3A_1 - R_1B_4A_3 + B_2A_3 - B_2R_2A_2 + R_2B_4A_1 - B_6A_1 + B_6R_1A_2)/K$$

$$\beta = (B_1R_2A_2 - B_1A_3 + R_1B_3A_3 + B_5A_1 - B_5R_1A_2 - R_2B_3A_1)(B_1A_3 - R_3B_3A_3 - B_1R_4A_2 + R_4B_3A_1 + B_5R_3A_2 - B_5A_1 - B_2R_4A_2 + B_2A_3 - R_3B_4A_3 + B_6R_3A_2 - B_6A_1 + R_4B_4A_1)/K$$

$$C_x^{III} = \frac{R_3B_5 + R_1R_4B_3 - R_1B_5 + R_2B_1 - R_4B_1 - R_3R_2B_3}{R_3R_2A_2 - R_3A_3 + R_1A_3 + A_1R_4 - R_1A_2R_4 - R_2A_1}$$

$$C_x^{VI} = \frac{R_3B_6 + R_1R_4B_4 - R_4B_2 + R_2B_2 - R_1B_6 - R_3R_2B_4}{R_3R_2A_2 - R_3A_3 + R_1A_3 + A_1R_4 - R_1A_2R_4 - R_2A_1} \quad .$$

The common value of K in the denominators of the solutions for α and β is given by:

$$K = d_1(A_1 - R_1A_2)(A_3 - R_2A_2)(A_1 - R_3A_2)(A_3 - R_4A_2),$$

where

$$d_1 = (K_{11} - K_{21})(K_{42} - K_{32}) - (K_{22} - K_{12})(K_{31} - K_{41})$$

and

$$K_{11} = \frac{B_1 - R_1B_3}{A_1 - R_1A_2} \qquad K_{12} = \frac{B_2 - R_1B_4}{A_1 - R_1A_2}$$

$$K_{21} = \frac{B_5 - R_2B_3}{A_3 - R_2A_2} \qquad K_{22} = \frac{B_6 - R_2B_4}{A_3 - R_2A_2}$$

$$K_{31} = \frac{B_1 - R_3B_3}{A_1 - R_3A_2} \qquad K_{32} = \frac{B_2 - R_3B_4}{A_1 - R_3A_2}$$

$$K_{41} = \frac{B_5 - R_4B_3}{A_3 - R_4A_2} \qquad K_{42} = \frac{B_6 - R_4B_4}{A_3 - R_4A_2} \quad .$$

If the coefficients K_{ij}, $1 \le i \le 4$ and $1 \le j \le 2$, exist and $d_1 \ne 0$, then the crossover proportions and concentrations obtained from equations (5)–(8) exist **and are unique**.

NOTE: This method does not require the use of calibration curves, and that the set of explicit solutions for three (or more) species is analogous to the above (albeit more lengthy).

The following mathematics is a way to solve the equations iteratively. This method was the original iterative method and can now be confirmed with the previously introduced explicit approach. To assist the analyst, additional information, technical support and

analyst training have been made available for both iterative and explicit solutions (Refs. 11, 27, and 28).

To make the expression simpler, assume

$$C_X^{III} W_X = N_X^{III},\ C_X^{VI} W_X = N_X^{VI},\ C_S^{III} W_S^{III} = N_S^{III},\ C_S^{VI} W_S^{VI} = N_S^{VI}$$

At the beginning of the iteration, arbitrary values can be assigned to N_X^{VI} and α. For example, both of them are assigned as 0s. Now we need to know the expression of N_X^{III} and β. After careful derivation, we can get the following equations:

$$\begin{cases} (1-\alpha)\left(R_{50/52}^{III}\ {}^{52}A_X - {}^{50}A_X\right)N_X^{III} + \left[R_{50/52}^{III}\left({}^{52}A_X\,N_X^{VI} + {}^{52}A_S^{VI}\,N_S^{VI}\right) - \left({}^{50}A_X\,N_X^{VI} + {}^{50}A_S^{VI}\,N_S^{VI}\right)\right]\beta \\ = \left(-R_{50/52}^{III}\ {}^{52}A_S^{III} + {}^{50}A_S^{III}\right)N_S^{III}\,(1-\alpha) \\ (1-\alpha)\left(R_{53/52}^{III}\ {}^{52}A_X - {}^{53}A_X\right)N_X^{III} + \left[R_{53/52}^{III}\left({}^{52}A_X\,N_X^{VI} + {}^{52}A_S^{VI}\,N_S^{VI}\right) - \left({}^{53}A_X\,N_X^{VI} + {}^{53}A_S^{VI}\,N_S^{VI}\right)\right]\beta \\ = \left(-R_{53/52}^{III}\ {}^{52}A_S^{III} + {}^{53}A_S^{III}\right)N_S^{III}\,(1-\alpha) \end{cases}$$

These equations can be rewritten as:

$$\begin{cases} A_1 N_X^{III} + B_1\beta = C_1 \\ A_2 N_X^{III} + B_2\beta = C_2 \end{cases}$$

The solutions are:

$$\beta = \frac{\begin{vmatrix} A_1 & C_1 \\ A_2 & C_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}} \quad \text{and} \quad N_X^{III} = \frac{\begin{vmatrix} C_1 & B_1 \\ C_2 & B_2 \end{vmatrix}}{\begin{vmatrix} A_1 & B_1 \\ A_2 & B_2 \end{vmatrix}}$$

Use these two values in the following equations to solve N_X^{VI} and α

$$\begin{cases} (1-\beta)\left(R_{50/52}^{VI}\ {}^{52}A_X - {}^{50}A_X\right)N_X^{VI} + \left[R_{50/52}^{VI}\left({}^{52}A_X\,N_X^{III} + {}^{52}A_S^{III}\,N_S^{III}\right) - \left({}^{50}A_X\,N_X^{III} + {}^{50}A_S^{III}\,N_S^{III}\right)\right]\alpha \\ = \left(-R_{50/52}^{VI}\ {}^{52}A_S^{VI} + {}^{50}A_S^{VI}\right)N_S^{VI}\,(1-\beta) \\ (1-\beta)\left(R_{53/52}^{VI}\ {}^{52}A_X - {}^{53}A_X\right)N_X^{VI} + \left[R_{53/52}^{VI}\left({}^{52}A_X\,N_X^{III} + {}^{52}A_S^{III}\,N_S^{III}\right) - \left({}^{53}A_X\,N_X^{III} + {}^{53}A_S^{III}\,N_S^{III}\right)\right]\alpha \\ = \left(-R_{53/52}^{VI}\ {}^{52}A_S^{VI} + {}^{53}A_S^{VI}\right)N_S^{VI}\,(1-\beta) \end{cases}$$

Rewrite the equation as:

$$\begin{cases} A_3 N_X^{VI} + B_3\alpha = C_3 \\ A_4 N_X^{VI} + B_4\alpha = C_4 \end{cases}$$

again:

$$\alpha = \frac{\begin{vmatrix} A_3 & C_3 \\ A_4 & C_4 \end{vmatrix}}{\begin{vmatrix} A_3 & B_3 \\ A_4 & B_4 \end{vmatrix}} \quad \text{and} \quad N_X^{VI} = \frac{\begin{vmatrix} C_3 & B_3 \\ C_4 & B_4 \end{vmatrix}}{\begin{vmatrix} A_3 & B_3 \\ A_4 & B_4 \end{vmatrix}}$$

Repeating the calculation, the variables N_X^{III} , N_X^{VI} , α and β will converge to constant values, and these values are the solution of the equations.

12.2.4 If conversion is complete from one species to another, then the starting samples may be re-spiked with the isotope-enriched spikes and analyzed, with a particular attention to retard the conversion of the species, if possible. As long as the isotope ratio can be determined, the SIDMS protocol will correct for the conversion even if extensive conversion has occurred. If 100% conversion occurs, this is the result of an active matrix. This indicates that the species converted is highly unstable in that matrix under the extraction procedure chosen.

12.2.5 If appropriate or required, calculate results for solids on a dry-weight basis as described in Sec. 12.1.3.

12.3 Examples, calculation aids, and help documents for SIDMS are available as referenced in the web (Refs. 11, 27, and 28).

12.4 Results must be reported in units commensurate with their intended use and all dilutions must be taken into account when computing final results.

13.0 METHOD PERFORMANCE

13.1 Performance data and related information are provided in SW-846 methods only as examples and guidance. The data do not represent required performance criteria for users of the methods. Instead, performance criteria should be developed on a project-specific basis, and the laboratory should establish in-house QC performance criteria for the application of this method. <u>These performance data are not intended to be and must not be used as absolute QC acceptance criteria for purposes of laboratory accreditation.</u>

13.2 Performance and use of IDMS as a definitive method in standard reference material certification has been well established in practice and in the literature. Review and discussion articles are referenced for performance criteria of this highly accurate method (Refs. 1, 9, and 10). IDMS has also been applied to analyze nutritional supplements where toxic materials were reported to contaminate zinc dietary supplements with antimony in 2010. Thirteen batches of the products and the raw material used as ingredients and the contaminated bottles were tested by Method 6800. Testing revealed that four out of the thirteen batches were not contaminated. The results were independently tested and confirmed by the manufacturer. Quantitative measurements of incoming raw material and final products tested with Method 6800 IDMS were found to be superior to less accurate results performed by other methods which were replaced by the manufacturer with Method 6800 using ICP-MS (Ref. 33). Table 16a and 16b show IDMS concentrations of tested ingredients in batches of recalled dietary supplement prescribed and distributed to hospitals.

IDMS by Method 6800 has been shown to be suitable for the analysis of highly toxic materials. Glyphosate is the active ingredient of a popular herbicide and is also being used as surrogate for VX nerve agent. I-Spike is a novel solid phase extraction (SPE) technique in which the isotopically enriched spike is preloaded onto a SPE column prior to the introduction of the sample. I-Spike solid-phase sorbent cartridges were tested to provide the placement of a molecular toxic analyte directly on the SPE column (pre-loaded enriched spikes) for safety and quality control purposes. Table 17 demonstrates the equivalent results comparing pre-equilibrating the spike with the sample prior to loading on the SPE column (liquid phase) or loading the spike and sample individually on the SPE column (solid phase) for added safety of the analyst (Ref. 34).

13.3 Accuracy, precision, and use of Method 6800 SIDMS in quantifying and correcting for species inter-transformations are shown in Tables 1 through 15, 18. Table 2(A) and Table 2(B) compare data between Method 6800 and Method 7196 for Cr(VI) in chromium ore process residues (COPR) and soil extracts. Table 1 demonstrates the ability of this method to correct for transformations of both Cr(VI) and Cr(III) in aqueous samples. Table 1 also displays the magnitude of errors that may be expected when using other methods unable to track and determine the conversion of these species. Table 2(A) indicates a sample type where double spikes were added into the same extract (spiked after extraction with Method 3060A) used for determination of Cr(VI) with Method 7196 and then analyzed with Method 6800. In this case, both the traditional 3060A/7196 methods and 3060A/6800 methods produced statistically similar data indicating that transformation had already occurred and confirmation between these two analytical methods was observed (Refs. 8 and 12). Whereas Table 2(B) indicates a situation where one set of samples were extracted with Method 3060A and analyzed with Method 7196 and another set of sub-samples of the same samples were double spiked before extraction with Method 3060A and then analyzed with Method 6800. In this case, statistically significant different results were obtained from both the traditional 3060A/7196 and the 3060A/6800 methods (Refs. 3 and 12). Method 6800 provided the ability to correct for the transformation of Cr(III) to Cr(VI) using the mathematical protocol described in Sec.12.2.3. This is also an example of how Method 6800 can be used as a diagnostic tool to isolate the origin of species-shifts in sample handling, and in analytical preparation steps of methods. Recently, NIST has released a standard reference material (SRM) where, according to the SRM's certificate of analysis, the only true value for the species of Cr(VI) was obtained by EPA Method 6800 and that the measurements made by EPA Methods 7199 and 7196A were in error by as much as 40% (Refs. 37 and 38). The European Committee for Standardization has made a similar declaration (signed by all EU member experts) accepting Method 6800 as the preferred legally defensible method for Cr(VI) measurement (Ref. 39).

The SIDMS analysis technique, applied to difficult environmental sample matrices, demonstrates the advantages of having the capability to identify and understand the transformations and method perturbations of dynamic species (Refs. 13 and 14). The study described in Table 3 demonstrates how Method 6800 can be used to field-spike reactive samples that would have degraded by the time they reached the analytical laboratory. Table 3 analytically evaluates and validates the field application of chemical spiking at the sampling site and permits evaluation of the process involving ferrous iron and chromate in the treatment or remediation of hexavalent chromium runoff by using another waste by-product, acid mine drainage (AMD) (Ref. 14). Figure 6 demonstrates the analysis of the isolated fly ash runoff and the waste stream mixed with the second waste stream of AMD. These data in Table 3 and Figure 6 as cited in the literature (Ref. 14) demonstrate the accuracy and precision of both a stable and a less stable set of samples. It was noted in Ref. 14 that applying Method 6800 at the time of sampling the small amount of residual Cr(VI) left in the sample for equilibration was enough to make quantitative measurements of Cr(VI). This can be an effective solution to

circumventing holding time issues. When a sample is highly reactive, implementing Method 6800 in the field to establish the species content at the time of sampling is an effective way of preserving the quantitative and qualitative species content.

Tables 4(A) and 4(B) demonstrate how Method 6800 can be used as a diagnostic tool to observe whether chromium species (and other species) transformation occurs during or after extraction steps. It is reported in the literature that there is a possibility of precipitation of Pb as $PbCrO_4$ during acidification. In order to determine if there is a bias introduced for Cr(VI) during acidification, the extracts were analyzed directly after acidification, without filtration and after filtration. From Table 4(A), it is observed that the results obtained for the same extract aliquots after performing filtration completely agree with those obtained from direct analysis of unfiltered samples after neutralization (Ref. 13). In Method 6800, loss of some portion of an already equilibrated sample does not cause an error, as mathematical basis of quantitation does not depend on recovery of the analyte but only on the isotopic ratio (Refs. 13 and 18). Standard calibration is adversely affected by the loss of analyte. The SIDMS, on the other hand, is unaffected by loss of analyte after isotopic species equilibration. From Table 4(B), it is observed, following extraction, that the determination of Cr(VI) is the same for three different instrumental calibrations. In most cases, similar results were obtained by analyzing the same extract using external calibration, IDMS (single spike after extraction) or SIDMS (double spike after extraction). This is because the equilibration chemistry, including species transformation, usually takes place during extraction of the sample. It can be observed from Table 4(A) and 4(B) that substantial transformation of Cr(III) to Cr(VI) can take place during extraction of the sample, and transformations generally occur nominally during post-extraction manipulation.

Table 5 demonstrates the correction necessary in some soil samples where the sample matrix causes a bias in more traditional methods. These bias corrections demonstrate the ability of Method 6800 to identify and correct for the degradation and transformation of species during the measurement process. Traditional methods such as Methods 7196, 7196A and 7199 are not able to correct for Cr(VI) transformations which may increase or decrease during Method 3060A extraction. Transformations occurring in transportation, storage, extraction, and sample preparation that have changed the species are not accounted for and all errors add to create biases that are undetectable. Method 6800 corrects for transformations occurring during extraction and other targeted steps when the enriched isotopic spike(s) are added to include those step including in the field at collection.

The SIDMS approach in Method 6800 was applied in the simultaneous determination of chromium species in food supplement, in this case biological samples, by an independent laboratory (Ref. 22). The Institute of National Measurement Standards, National Research Council of Canada has independently validated Method 6800 on brewers' yeast where they found Cr(VI) converting to Cr(III) and vise versa, and concluded that the SIDMS method enabled the accurate determination of both Cr(VI) and Cr(III) under these conditions. This work is summarized in Table 6 which was abstracted with permission from the author in Ref. 22. This study concluded that despite the presence of significant bi-directional transformation of chromium species during sample preparation, an accurate simultaneous determination of both species in yeast was made possible through the application of the SIDMS method (Table 6).

13.4 The SIDMS analysis technique has been successfully been applied for the determination of other species. For example, methylmercury and inorganic mercury have been successfully determined in biological, human and other tissues, and in soils. Table 7 demonstrates concentration measurements in soil samples (Refs. 15 and 16). Method 6800 has also been applied as a diagnostic tool to determine the concentration and to identify analytical biases caused by species transformations using the Microwave-Assisted Extraction methods such as Method 3200, used in mercury species extraction. The SIDMS protocol of

Method 6800 provides the ability to measure the concentration of species and to evaluate if species transformations have occurred. One set of samples was double-spiked before extraction and another aliquot of the same sample was double-spiked after extraction. It was observed that this extraction method did not alter the species or permit them to transform in this matrix, using this extraction protocol. The deconvoluted concentrations for each species (Table 7) obtained from both sets of extractions overlap at the 95% CI and are statistically indistinguishable (Ref. 15).

In a separate study, the SIDMS protocol was used as a diagnostic tool for the evaluation of different mercury speciation methods published in the literature and in Method 3200 (Refs. 16 and 17). From this study, it was observed that two of the five literature methods were highly prone to form inorganic mercury from methylmercury during extraction. These two published methods converted approximately ~100% of the methylmercury to inorganic mercury, including the spiked isotope enriched methylmercury. In another literature method, as much as 45% methylmercury was converted into inorganic mercury during extraction. Method 3200 and a sonication-based extraction method induced very little or no conversion of methylmercury to inorganic mercury (Table 8) (Ref. 17). Method 6800 has also been used to validate other speciation method. For example, if used in conjunction with Method 3200, Method 6800 corrects for matrix-induced and method-induced transformations of species, thus permitting the evaluation of Method 3200 and other speciation methods (Refs. 15, 17, 20, and 21).

The SIDMS protocol of Method 6800 along with Method 3200 was applied for the determination of mercury species in tissue samples (Ref. 20) (Table 9). After analyzing several fish tissue reference materials (ERM-CE464, DOLT-3, NIST-1946) with better than 20% precision, approximately 20% of the inorganic mercury was converted to methylmercury during analysis and methylmercury converted to inorganic mercury species. Here, the creation of the species of interest from inorganic mercury would be undetected without the ability of Method 6800 to evaluate and correct for the species-conversions in these speciated measurements.

The SIDMS method was applied as a diagnostic tool to evaluate eight published literature methods for mercury speciation in human hair (IAEA-085) (Ref. 21). From this study, it was observed that most of the methods induced minor or no statistically significant species transformation during extraction. However, two of the eight methods induced larger amounts of species transformation from methylmercury to inorganic mercury. One method transformed approximately 90% and the other transformed almost 20% of methylmercury to inorganic mercury during extraction (Table 10). Because an IAEA reference material was used in this study, the reference value was available for validation of all methods. The inorganic and methylmercury concentrations were obtained by the SIDMS protocol of Method 6800 as were the amount of inorganic mercury converted into methylmercury. The correction for methylmercury and inorganic mercury conversion was also accomplished. Total mercury was determined mathematically by adding inorganic and methylmercury. Without conversion correction, the accurate concentration measurement cannot be assured.

In Ref. 30, three mercury species were extracted using Method 3200 and measured individually while others were grouped as discussed in Method 3200. After the removal of the extractable mercury species, the remaining mercury species were spiked collectively and measured as a group. Ref. 30 demonstrates the ability of Method 6800 to correct for species transformations as much as 80% and obtain mass balance, after analysis. Mass balance simply requires addition of all species forms in a sample to equal to the sum of all related species. In this case, mass balance was achieved when all the mercury species that are collectively or individually present in crude oil were accounted for.

Ref. 31 and Table 14 show the application of SIDMS to human blood SRM. Here, inorganic, methyl and ethyl mercury species from air, food and water contamination are measured. Interspecies conversions of each of these species are calculated and corrected for. Through the analysis of an analytical reference material (ERMOCE464, Tuna Fish), Ref. 32 demonstrates that once the equilibration is achieved in the extraction step, incomplete extraction of the species does not affect accuracy. Ref. 30 describes the way existing methods in SW-846 Chapters Three and Four, and the literature may be used to implement 6800, along with other methods by adding the enriched species during sample preparation to overcome errors such as recovery.

The SIDMS protocol can also be used successfully to determine the concentration of one or more analytes in the sample where species conversion, degradation and/or incomplete separation may have taken place before or during analysis (Refs. 2, 3, 5-8, 11-32, and 34-40). Additional equations and mathematical manipulations required for the determination of three simultaneous species have been developed and published. The first mathematical solutions for SIDMS were iterative. In addition to iterative equations, new explicit and deterministic SIMDS solution for three species analysis has been developed, published and is available in 6800 and Ref. 28. The explicit and determinative equations were developed for mission-critical applications, such as homeland defense and homeland security, where the detection method is required to perform predictable precision and accuracy to obtain actionable data in the field under safety scenarios. For mathematical solutions using three simultaneous species equations, tutorials and calculation aids, you are referred to the references, web sites and related publications cited in Refs. 24, 27, 32, and 35-40.

SIDMS is a general application for the detection of active species that may transform during sample collection, storage, shipping, preparation, analysis and other manipulations. In Figures 8 and 9, and Table 18 (Refs. 21, 34, 35, 36, 39, 40, and 41), glutathione, one of the most ubiquitous anti-toxin species in the human body is quantified in the oxidized and reduced form, together with the conjugated toxicant species such as inorganic and methyl mercury using ESI-QTOF-MS. This type of combined biological and inorganic quantitation is now being applied to autism and other medical research. In Ref. 35, the application of Method 6800 with MALDI-QTOF-MS demonstrates that glutathione and conjugated mercury species are quantifiable by applying the SIDMS equations to data generated with either of the commonly used ionization sources (ESI and MALDI)(Ref. 35, 42). Ref. 41 demonstrates that ESI-ion trap instruments can make the same qualitative measurements of glutathione with toxicants. However, quantitation is not possible without direct isotopic ratios as a calibration curve of glutathione with toxicants is not feasible. Figure 10 and Refs. 40 and 42 demonstrate the quantitative SIDMS analysis of sodium azide which is a toxicant and an explosive that is included with the air bags in automobiles. Lead azide is a primary component of primers in munitions and a toxicant in water. It is difficult to analyze sodium azide by GC-MS as it is not readily ionized. Method 6800 demonstrates the quantitative measurement on a TOF-MS directly without calibration curves using a mathematical determination. False positives do not occur in Method 6800 since these quantitative isotopic ratios are not achievable unless both the natural and isotopic forms of the analyte are present. Here, incomplete extraction did not affect accuracy with Method 6800. Also adduct formation due to sodium or other compounds in the sample matrix do not require matrix matching as all adduct ratios provided the same numerical concentration of the solution indicating that equilibration with the sample was achieved. Sodium azide (Figure 10 and Refs. 42 and 40), potassium cyanide, toxicants and other difficult analytes in molecular and ionic forms were tested for homeland security and environmental health assessment purposes. All requested priority toxins (including those that could not be assessed by conventional field methods) and explosives were successfully quantified in a similar manner. Analytical results of these analytes (Ref. 42) were selected from a group of analytes generally classified as Chemical, Biological, Radioactive, Nuclear, and Explosive (CBRNE) agents. Details of these

tests included in this document exist as “task reports” provided to US Air Force. Portions of these reports may be obtained from info@sidms.com (www.sidms.com), with prior request and approval.

The IDMS protocol can be used to determine simultaneously elemental concentrations as total elemental concentrations using sample decomposition methods such as Method 3052. An example of such IDMS analysis is provided for grounded and pulverized electronic components and circuit boards. The European environmental regulations referred to as Reduction of Hazardous Substances (RoHS), Waste, Electrical and Electronic Equipment (WEEE) and Registration; Evaluation, Authorization and Restriction of Chemicals (REACH) require total and speciated analyses. Table 11 demonstrates total elemental analysis by IDMS using Method 3052 microwave-enhanced sample decomposition where Cd-112, Cr-50, Pb-206 and Hg-199 isotopes were added into the sample. IDMS analysis was accomplished using a commercially available ICP-MS and total elemental content is reported in Table 11 (Ref. 26). Chromium species can be determined as described in Sec. 13.3 to complete the analysis requirements under the European RoHS regulations. The concentration of Cr(VI) was negligible in these specific samples as measured by Method 6800.

Molecular SIDMS protocols are demonstrated as examples by Refs. 34, 35, and 36 where molecular species such as reduced and oxidized glutathione are the molecular examples used to illustrate the SIDMS deconvolution of transformations of molecular species such as peptides and proteins in the immune system. These molecular species measure the red blood cell concentration of reduced and oxidized glutathione (GSH and GSSG, respectively) and the ratio of these species that is an indication of the function of the immune system and their ability to detoxify toxicants. Like Cr(VI) the GSH and GSSG must be spiked during the sampling of the blood and before shipment to the laboratory to make possible the accurate quantification of GSH and GSSG species in the blood sample. These SIDMS molecular measurements use specific sample preparation and separation specific to these species. The above equations have an almost exact adaptation of the algorithms for the Cr(III) and Cr(VI) calculated examples in sections 7.3.1 and in example calculations section 12.2.

13.5 The following documents may provide additional guidance and insight on the elemental and molecular isotope ratio measurement using ICP-MS, ESI-QTOF and ESI-QQQ, respectively:

13.5.1 K. E. Jarvis, A. L. Gray and R. S. Houk, *Handbook of Inductively Coupled Plasma Mass Spectrometry*; Blackie Academic & Professional: London, U.K. 1992, p-315.

13.5.2 G. P. Russ III, J. M. Bazan, *Spectrochim. Acta, Part B* 1987, 42B, 49- 62.

13.5.3 H. E. Taylor, *Inductively Coupled Plasma Mass Spectrometry: Practice and Techniques*, Academic Press: San Diego, CA, USA, 2001, p-48.

13.5.4 M. Sargent, R. Harte and C. Harrington, *Guidelines for Achieving High Accuracy in Isotope Dilution Mass Spectrometry*, The Royal Society of Chemistry: Cambridge, UK, 2002.

13.5.5 “Characterization of waste – State of the art document – Chromium VI specification in solid matrices” PD CEN/TR 14589:2003, British Standards, EU publication “International Standards Correspondence Index, Technical Report CEN Technical Committee CEN/TC 292, European Committee for Standardization, 2003.

13.5.6 The web sites: http://www.epa.gov/epaoswer/hazwaste/test/new-meth.htm contains Method 6800 and www.sampleprep.duq.edu and www.sidms.com, contain SIDMS/Method 6800 analysis aids, tutorials, update information and applications (Refs. 27, 28, 24, 35, 36, and 42).

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14.0 POLLUTION PREVENTION

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity and/or toxicity of waste at the point of generation. Numerous opportunities for pollution prevention exist in laboratory operations. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, a free publication available from the American Chemical Society (ACS), Committee on Chemical Safety, http://portal.acs.org/portal/fileFetch/C/WPCP_012290/pdf/WPCP_012290.pdf.

15.0 WASTE MANAGEMENT

The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* available from the American Chemical Society at the address listed in Sec. 14.2.

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17.0 TABLES, DIAGRAMS, FLOW CHARTS AND VALIDATION DATA

The following pages contain the tables and figures referenced by this method. The pages also contain two work-flow diagrams (one each for IDMS and SIDMS). A glossary follows these materials.

The data below are provided for guidance purposes only.

TABLE 1

ANALYSIS OF AN ARTIFICIALLY SYNTHESIZED WATER SAMPLE
(Refs. 6 and 7)

		Concentration (ng/g)		Conversion (%)	
Aliquot	Days after spiking	Cr(III)	Cr(VI)	Cr(III) to Cr(VI)	Cr(VI) to Cr(III)
1	1	69.8 ± 0.3	68.8 ± 0.3	4.87 ± 0.22	3.57 ± 0.03
	4	69.2 ± 0.6	69.4 ± 0.3	3.47 ± 0.11	11.9 ± 0.5
	13	70.5 ± 0.9	68.5 ± 0.4	2.80 ± 0.13	22.4 ± 0.2
2	1	69.6 ± 0.2	68.8 ± 0.4	17.6 ± 0.1	2.95 ± 0.02
	4	69.3 ± 0.7	69.6 ± 0.6	14.6 ± 1.3	11.4 ± 0.7
	13	70.7 ± 0.4	68.8 ± 0.3	12.8 ± 0.1	22.1 ± 0.3
3	1	69.8 ± 0.6	69.0 ± 0.2	23.8 ± 0.3	2.76 ± 0.08
	4	69.0 ± 0.8	69.6 ± 0.3	21.6 ± 0.2	10.2 ± 0.1
	13	70.4 ± 0.5	68.9 ± 0.8	17.6 ± 0.3	22.1 ± 0.1
True		69.67	68.63		

mean ± 95% confidence interval

Aliquots 1, 2 and 3 were from the same isotopically-spiked synthesized sample. These aliquots were treated in different ways to permit different degrees of interconversion between Cr(III) and Cr(VI). Measurements were done on different days to check the stability of the species during storage. Despite the different degrees of interconversion, the deconvoluted concentrations for both Cr(III) and Cr(VI) were always corrected to the true concentrations within experimental error.

TABLE 2 (A)

CONCENTRATIONS OF Cr(VI) IN COPR SAMPLES DETERMINED WITH METHOD 7196 AND SIDMS (SPIKED AFTER EXTRACTION)
(Refs. 8 and 12)

	Method 7196		SIDMS	
Sample	Conc. of Cr(VI) (μg/g)	Average (mean ±std)	Conc. of Cr(VI) (μg/g)	Average (mean ±std)
COPR1	1330	1410 ± 85	1373	1445 ± 70
	1410		1449	
	1500		1512	
COPR3	91.2	85.3 ± 5.2	93.9	88.8 ± 6.1
	81.5		82.1	
	83.1		90.4	
COPR4	408.9	407.8 ± 7.2	419.8	418.0 ± 9.2
	414.4		426.1	
	400.2		408.0	

COPR: chromite ore processing residue.
Method 3060A was used for Cr(VI) extraction.
Results obtained from SIDMS and Method 7196 are comparable for COPR samples.

TABLE 2(B)

OXIDATION OF Cr(III) TO Cr(VI) DURING EXTRACTION WITH METHOD 3060A FROM THREE COPR SAMPLES AND DETERMINED WITH METHOD 7196 AND SIDMS (SPIKED BEFORE EXTRACTION)
(Refs. 3 and 12)

	Total Cr	Cr(VI) ($\mu g\ g^{-1}$)		*RE*[e]
	($mg\ g^{-1}$)	SIDMS Corrected for Cr(III) to Cr(VI) conversion	Method 7196 Uncorrected for Cr(III) to Cr(VI) conversion	(%)
COPR 1	10.4[a]	2573 ± 35[d]	2671 ± 17	3.8
COPR 3	1.97[a]	161 ± 6	351 ± 8	118
COPR 4	4.60[a]	614 ± 13	877 ± 21	43
Fresh Fly Ash	0.0475[b]	8.3 ± 0.3	10.0 ± 0.4	21
Fly Ash (at 41 ft.)	0.0582[b]	3.3 ± 0.3	4.1 ± 0.1	24
SRM 1645 (River sediment)	29.6[c]	1045 ± 46	2753 ± 31	163

[a] provided by Environmental Standards, Inc.

[b] determined with EPA method 3052

[c] certified values

[d] mean ± 95% confidence interval

[e] Relative error

TABLE 3

ANALYSIS OF Cr(VI) IN LEACHATE FROM COAL FLYASH AND ACID MINE DRAINAGE (AMD) COMBINATION SAMPLES
(Ref. 14)

Sample mixture	Aliquot	Cr(VI) calculated/ $ng\ g^{-1}$ [b]	Cr(VI) measured/ $ng\ g^{-1}$
1:1 F	Aliquot 1	704	46.6 ± 8.6
1:1 L	Aliquot 2	704	17.5 ± 4.2
5:1 F	Aliquot 1	1172	963 ± 53
5:1 L	Aliquot 2	1172	25.5 ± 3.5

[a]Uncertainties are at 95% confidence interval, n = 3
[b]Only dilution of the leachate is considered
L – spiked in laboratory
F – spiked in filed

TABLE 4(A)

HEXAVALENT CHROMIUM RESULTS OBTAINED FROM INDUSTRIAL MATERIALS EXTRACTED WITH METHOD 3060A AND ANALYZED WITH METHOD 6800 (DOUBLE SPIKED BEFORE EXTRACTION)
(Ref. 13)

Sample Matrix	Sample ID	EPA Method 6800 (diagnostic SIDMS)			
		Cr^{6+} before filtration	Cr^{6+} after filtration	Cr^{3+} to Cr^{6+} before filtration	Cr^{3+} to Cr^{6+} after filtration
		($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	(%)	(%)
SRM	SRM 2704	7 ± 3	8 ± 1	50 ± 2	45 ± 4
	SRM 2711	N/A	N/A	N/A	N/A
Sediments	Sediment-1	ND	N/A	N/A	N/A
	Sediment-2	1.77 ± 0.34	N/A	N/A	N/A
Aggregate Material	Aggregate-1	308 ± 44	314 ± 28	56 ± 3	57 ± 2
	Aggregate-2	341 ± 29	334 ± 36	46 ± 1	47 ± 1
	Aggregate-3	206 ± 9	205 ± 16	48 ± 6	49 ± 7
	Aggregate-4	141 ± 6	141 ± 14	33 ± 3	33 ± 2
	Aggregate-5	223 ± 28	216 ± 17	41 ± 2	41 ± 1
	Aggregate-6	289 ± 27	291 ± 22	19 ± 3	19 ± 3
	Aggregate-7	278 ± 32	306 ± 29	20 ± 1	20 ± 1

Uncertainties are at 95% CI, n = 3

ND = not detectable (lowest measurable chromium = 0.5 ng mL^{-1} and this corresponds to 12.5 ng g^{-1} in soil or sediment sample)

NA = not applicable

TABLE 4(B)
HEXAVALENT CHROMIUM RESULTS OBTAINED FROM INDUSTRIAL MATERIALS EXTRACTED WITH METHOD 3060A AND ANALYZED WITH IC-ICP-MS (EXTERNAL CALIBRATION) AND METHOD 6800 (IDMS & SIDMS), SINGLE SPIKED & DOUBLE SPIKED AFTER EXTRACTION

(Ref. 13)

Sample	External calibration	IDMS[a]	EPA method 6800[b]	
	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	($\mu g\ g^{-1}$)	Cr^{3+} converted to Cr^{6+} (%)
SRM 2704	7 ± 2	12 ± 1	14 ± 2	10 ± 1
SRM 2711	7 ± 1	1.5 ± 0.1	12 ± 4	9 ± 4
Aggregate-1	342 ± 19	367 ± 25	342 ± 26	12 ± 3
Aggregate-2	440 ± 6	448 ± 17	409 ± 11	5 ± 2
Aggregate-3	245 ± 17	259 ± 12	242 ± 16	4 ± 1
Aggregate-4	164 ± 8	171 ± 7	184 ± 11	10 ± 2
Aggregate-5	199 ± 22	215 ± 17	214 ± 23	9 ± 2
Aggregate-6	277 ± 22	295 ± 15	319 ± 23	10 ± 2
Aggregate-7	291 ± 22	298 ± 18	328 ± 24	17 ± 4

Uncertainties are at 95% CI, n = 3

[a]Extracts were spiked with $^{53}Cr^{6+}$ spike after extraction

[b]Extracts were double spiked with $^{iso\text{-}50}Cr^{3+}$ and $^{53}Cr^{6+}$ after extraction

TABLE 5

RECOVERY OF Cr(VI) SPIKED INTO SOIL EXTRACTS
(Ref. 8)

Sample	Mass of Soil (g)	Spiked $^{nat}Cr(VI)$	Recovery (%)	
		($\mu g/g$)	Method 7196	SIDMS
1	0	2.997	101 ± 0.4	100 ± 1.3
2	1.53	3.033	91.8 ± 1.7	100 ± 0.3
3	3.06	2.993	81.9 ± 1.1	101 ± 0.3
4	3.12	1.587	71.6 ± 2.5	99.3 ± 0.3

Results obtained from SIDMS and Method 7196 are incomparable for soil extracts due to the serious matrix effects resulting from the coexisting reducing agents in soil. Method 7196 is incapable of correcting conversion of Cr(VI) leading to low recoveries. Results are based on N = 3 with uncertainties expressed in standard deviation.

TABLE 6

RESULTS FOR SPECIATION OF Cr IN YEAST
(Ref. 22)

Sample	^{Nat}Cr(III) added, (mg/kg)	^{Nat}Cr(VI) added, (mg/kg)	Measured Cr(III), (mg/kg)	Measured Cr(VI), (mg/kg)	^{Nat}Cr(III) Recovery (%)	^{Nat}Cr(VI) Recovery (%)	Measured Cr(III) + Cr(VI) (mg/kg)	Measured Total Cr (mg/kg)
Yeast	0	0	1952 ± 103	76 ± 48	NA	NA	2028 ± 57	2014 ± 16
Spiked Yeast	1784	2398	3749 ± 43	2466 ± 40	101 ± 2	100 ± 2	NA	NA

NA – not applicable
Uncertainties are at 95% confidence interval, n = 3.

TABLE 7

THE DECONVOLUTED CONCENTRATION AND TRASFORMATION OF MERCURY SPECIES IN REFERENCE SOIL (MATERIAL-1) USING SIDMS CALCULATIONS.
(Ref. 15)

	Deconvoluted Concentration		Interconversion	
	Hg^{2+} (μg/g)	CH_3Hg^+ (μg/g)	Hg^{2+} to CH_3Hg^+ (%)	CH_3Hg^+ to Hg^{2+} (%)
DSBE	3.05 ± 0.12	2.69 ± 0.10	1.3 ± 1.5	0.1 ± 1.4
DSAE	2.94 ± 0.07	2.62 ± 0.09	0.8 ± 1.5	0.7 ± 0.6

DSBE = double spiked before extraction; DSAE = double spiked after extraction.

Uncertainties are expressed at 95% CL with n = 3.

Material-1: 100% processed topsoil containing both inorganic mercury and methylmercury.

TABLE 8
EVALUATION OF DIFFERENT LITERATURE METHODS FOR MERCURY SPECIATION IN SOILS AND SEDIMENTS USING METHOD 6800.
(Ref. 16)

Sample	Extraction Method	Deconvoluted Concentration (μg/g)		% Recovery		Interconversion (%)	
		Hg^{2+}	CH_3Hg^+	Hg^{2+}	CH_3Hg^+	Hg^{2+} to CH_3Hg^+	CH_3Hg^+ to Hg^{2+}
Material-1	M-3200	2.68 ± 0.34	2.20 ± 0.29	89 ± 12	73 ± 10	0 ± 3	0 ± 9
	SONI-1	2.49 ± 0.16	1.83 ± 0.13	83 ± 5	61 ± 4	5 ± 1	45 ± 4
	SONI-2	1.88 ± 0.20	1.96 ± 0.24	63 ± 7	65 ± 8	2 ± 3	10 ± 5
	MAE	1.99 ± 0.14	2.01 ± 0.16	66 ± 8	67 ± 5	0 ± 3	7 ± 3
	CAE	NA	NA	NA	NA	NA	~ 100
Material-2	M-3200	4.09 ± 0.93	2.79 ± 0.29	91 ± 21	62 ± 6	2 ± 2	6 ± 5
	SONI-1	3.67 ± 0.16	2.65 ± 0.09	81 ± 3	59 ± 2	2 ± 1	44 ± 4
	SONI-2	3.09 ± 0.23	2.29 ± 0.20	67 ± 5	51 ± 5	1 ± 1	2 ± 2
	MAE	3.09 ± 0.24	2.26 ± 0.13	69 ± 5	50 ± 3	2 ± 1	4 ± 3
	CAE	NA	NA	NA	NA	NA	~ 100

Uncertainties are expressed at 95% CL, n = 9.

NA – analyzed but could not perform SIDMS calculations.

TABLE 9

SIDMS ANALYSIS OF FISH TISSUE (FISH REFERENCE MATERIALS) (ERM-CE464, DOLT-3, NIST-1946) AFTER OPTIMIZED MICROWAVE-ASSISTED EXTRACTION (Ref. 20)

Reference material	*ICP-MS*	*LC-ICP-MS*				
	Total Hg ($mg\ kg^{-1}$)	*Hg^{2+} as Hg ($mg\ kg^{-1}$)*	*CH_3Hg^+ as Hg ($mg\ kg^{-1}$)*	*Sum of species[a] as Hg ($mg\ kg^{-1}$)*	*Mean conversion ± 95% C.L. (%)*	
					Hg^{2+} to CH_3Hg^+	*CH_3Hg^+ to Hg^{2+}*
ERM-CE464						
Certified Values	5.24 ± 0.10		5.12 ± 0.16			
EC	5.29 ± 0.24	0.17 ± 0.01	5.14 ± 0.10	5.31 ± 0.10		
IDMS (SSAE)	5.30 ± 0.02					
SIDMS (DSAE)		0.14 ± 0.01	5.09 ± 0.07	5.23 ±0.08	3.80 ± 0.30	0.53 ± 0.26
SIDMS (DSBE)		0.14 ± 0.02	5.16 ± 0.04	5.30 ±0.04	7.05 ± 0.78	0.10 ± 0.05
DOLT-3						
Certified Values	3.37 ± 0.14		1.72[b]			
EC	3.32 ± 0.09	1.63 ± 0.06	1.59 ± 0.07	3.22 ± 0.08		
IDMS (SSAE)	3.24 ± 0.06					
SIDMS (DSAE)		1.37 ± 0.02	1.78 ± 0.05	3.15 ± 0.06	1.99 ± 0.42	0.62 ± 0.22
SIDMS (DSBE)		1.52 ± 0.03	1.68 ± 0.03	3.20 ± 0.05	4.39 ± 0.40	1.57 ± 0.36
NIST 1946						
Certified Values	0.43 ± 0.01		0.40 ± 0.01			
EC	0.43 ± 0.02	0.016 ± 0.003	0.42 ± 0.02	0.44 ± 0.01		
IDMS (SSAE)	0.42 ± 0.01					
SIDMS (DSAE)		0.012 ± 0.005	0.412 ± 0.005	0.42 ± 0.01	6.76 ± 1.82	0.41 ± 0.13
SIDMS (DSBE)		0.010 ± 0.002	0.41 ± 0.01	0.42 ± 0.01	7.91 ± 1.20	0.48 ± 0.09

[a]Sum of species = Hg^{2+} + CH_3Hg^+ (HPLC-ICP-MS). [b]Indicative value.

Reference material	ICP-MS	LC-ICP-MS			Mean conversion ± 95% C.L. (%)	
	Total Hg (mg kg^{-1})	Hg^{2+} as Hg (mg kg^{-1})	CH_3Hg^+ as Hg (mg kg^{-1})	Sum of species[a] as Hg (mg kg^{-1})	Hg^{2+} to CH_3Hg^+	CH_3Hg^+ to Hg^{2+}
ERM-CE464						
Certified Values	5.24 ± 0.10		5.12 ± 0.16			
EC	5.29 ± 0.24	0.17 ± 0.01	5.14 ± 0.10	5.31 ± 0.10		
IDMS (SSAE)	5.30 ± 0.02					
SIDMS (DSAE)		0.14 ± 0.01	5.09 ± 0.07	5.23 ±0.08	3.80 ± 0.30	0.53 ± 0.26
SIDMS (DSBE)		0.14 ± 0.02	5.16 ± 0.04	5.30 ±0.04	7.05 ± 0.78	0.10 ± 0.05
DOLT-3						
Certified Values	3.37 ± 0.14		1.72[b]			
EC	3.32 ± 0.09	1.63 ± 0.06	1.59 ± 0.07	3.22 ± 0.08		
IDMS (SSAE)	3.24 ± 0.06					
SIDMS (DSAE)		1.37 ± 0.02	1.78 ± 0.05	3.15 ± 0.06	1.99 ± 0.42	0.62 ± 0.22
SIDMS (DSBE)		1.52 ± 0.03	1.68 ± 0.03	3.20 ± 0.05	4.39 ± 0.40	1.57 ± 0.36
NIST 1946						
Certified Values	0.43 ± 0.01		0.40 ± 0.01			
EC	0.43 ± 0.02	0.016 ± 0.003	0.42 ± 0.02	0.44 ± 0.01		
IDMS (SSAE)	0.42 ± 0.01					
SIDMS (DSAE)		0.012 ± 0.005	0.412 ± 0.005	0.42 ± 0.01	6.76 ± 1.82	0.41 ± 0.13
SIDMS (DSBE)		0.010 ± 0.002	0.41 ± 0.01	0.42 ± 0.01	7.91 ± 1.20	0.48 ± 0.09

[a]Sum of species = Hg^{2+} + CH_3Hg^+ (HPLC-ICP-MS). [b]Indicative value.

TABLE 10

EVALUATION OF DIFFERENT LITERATURE METHODS FOR MERCURY SPECIATION IN HUMAN HAIR (IAEA-085) USING METHOD 6800
(Ref. 21)

Extraction Method	**Hg^{2+} (µg/g)**	**$MeHg^{+}$ (µg/g)**	**Total Mercury (µg/g)**	**Hg^{2+} to $MeHg^{+}$ (%)**	**$MeHg^{+}$ to Hg^{2+} (%)**
Certified value	0.3	22.9 ± 1.4	23.2 ± 0.8	------	------
Akagi *et. al.*	1.96 ± 0.59	23.81 ± 0.67	25.77 ± 0.89	< 0	< 0
Diez *et. al*.	1.36 ± 0.39	24.82 ± 1.59	26.18 ± 1.64	< 0	4 ± 1
Bermejo *et. al.*	1.32 ± 0.16	22.39 ± 0.49	23.71 ± 0.52	4 ± 1	2 ± 1
Feng *et. al.*	1.15 ± 0.40	23.49 ± 0.49	24.64 ± 0.63	4 ± 2	3 ± 1
Chai *et. al.*	1.00 ± 0.56	23.62 ± 0.57	24.62 ± 0.80	2 ± 2	5 ± 2
Montuori *et. al.*	1.15 ± 0.42	25.17 ± 0.96	26.32 ± 1.05	14 ± 2	22 ± 1
Morton *et. al.*	1.09 ± 0.23	23.50 ± 1.21	24.59 ± 1.23	< 0	90 ± 5
Method 3200 (MAE)	0.59 ± 0.22	23.65 ± 1.42	24.24 ± 1.44	4 ± 2	6 ± 1
Method 3200 (UAE)	1.13 ± 0.25	19.80 ± 1.25	20.93 ± 1.28	9 ± 3	0 ± 1

Uncertainties are at 95% CL, n = 12.

MAE – Microwave-assisted extraction; UAE – Ultrasound-assisted extraction.

TABLE 11

ANALYSIS OF RoHS GROUND AND PULVERIZED CIRCUIT BOARDS FOR ALL REGULATED METALS SIMULTANEOUSLY USING IDMS
(Ref. 26)

Sample ID	Cadmium (µg/g)	Chromium (µg/g)	Lead (µg/g)	Mercury (µg/g)
2811	1.916 ± 0.054	23.829 ± 0.866	19,806 ± 973	4.134 ± 1.059
Compaq #1	2.087 ± 0.107	13.462 ± 1.252	15,257 ± 917	6.316 ± 1.709
Dell Optiplex #5	2.081 ± 0.049	20.624 ± 1.010	23,496 ± 1,354	3.838 ± 0.871
Dell Optiplex #3	2.013 ± 0.086	38.512 ± 3.113	21,634 ± 972	N/A
AVVA	2.017 ± 0.115	35.399 ± 1.045	23,883 ± 1,243	N/A

Uncertainties are at 95% CI with n = 3, Cr(VI) was less than 1% by 6800.
N/A – not analyzed.

TABLE 12

SIDMS BY HPLC-ICP-MS ANALYSIS OF THREE EXTRACTABLE MERCURY SPECIES AND NON-EXTRACTABLE MERCURY FROM CRUDE OIL AFTER EPA METHOD 3200 EXTRACTION AND 6800 QUANTIFICATION WITH MASS BALANCE.
(Ref. 30)

Sample Name	Extractable Mercury Species, µg/kg			Non-Extractable Mercury Species, µg/kg	Total Mercury*, µg/kg
	Hg^{2+}	$MeHg^{+}$	$EtHg^{+}$		
Serial # 87183	317.3 ± 3.6	0.8 ± 0.1	10.7 ± 1.1	342.5 ± 46.8	370.2 ± 47.0 (414.1 ± 24.7)
Serial # 87098	723.6 ± 3.7	0.5 ± 0.1	7.4 ± 2.2	323.3 ± 30.7	354.8 ± 31.0 (437.8 ± 25.4)

Uncertainties are at 95% CL, n = 12 (3 x 4).
*values in parentheses are total mercury determined by isotope dilution mass spectrometry (IDMS) of the crude oil after Method 3052 digestion.

TABLE 13

INTERCONVERSION OF EXTRACTABLE SPECIES USING METHOD 3200 DEMONSTRATING THAT GREATER THAN 80% CONVERSIONS ARE QUANTIFIABLE BY METHOD 6800. THESE DATA ARE PART OF THE OVERALL DATA IN TABLE 12 AND ARE DETAILED IN THE REFERENCE.

(Ref. 30)

Sample Name	Deconvoluted Concentration, µg/kg			Interconversion (%)					
	Hg^{2+}	$MeHg^{+}$	$EtHg^{+}$	Hg^{2+} to $MeHg^{+}$	$MeHg^{+}$ to Hg^{2+}	$MeHg^{+}$ to $EtHg^{+}$	$EtHg^{+}$ to $MeHg^{+}$	$EtHg^{+}$ to Hg^{2+}	Hg^{2+} to $EtHg^{+}$
Serial # 871833	17.3 ± 3.6	0.8 ± 0.1	10.7 ± 1.1	9.6 ± 0.9	2.9 ± 0.8	0.8 ± 0.1	6.1 ± 0.6	55.1 ± 2.2	2.1 ± 1.0
Serial # 870987	23.6 ± 3.7	0.5 ± 0.1	7.4 ± 2.2	7.4 ± 1.0	6.2 ± 1.0	0.9 ± 0.1	7.9 ± 0.8	86.9 ± 1.5	1.3 ± 0.1

Uncertainties are at 95% CL, n = 12 (3 x 4).

TABLE 14
MERCURY SPECIES IN NIST HUMAN BLOOD SRM-966 BY SIDMS

(Refs. 31 and 42)

Sample	Total Mercury (ng/g)	Deconvoluted Concentration (ng/g)		
		Hg2+	MeHg+	EtHg+
SRM-966 (Level 2)	31.9 ± 2.1 (29.8 ± 1.6)[a]	16.7 ± 1.2 (14.1 ± 0.9)[b]	15.3 ± 1.5 (15.6 ± 1.3)[b]	NA
HB-1	-------	1.54 ± 0.12	3.72 ± 0.48	0.09 ± 0.02
HB-2	30.63 ± 3.19 (23)[b]	5.43 ± 1.52	25.2 ± 2.8	NA
HB-3	23.38 ± 2.25 (17)[b]	4.28 ± 2.29	19.1 ± 2.3	NA

Uncertainties are at 95% CL, n = 4
[a]Certified values are in parentheses.
[b]Reference values are in parentheses

TABLE 15
CONCENTRATIONS OF MERCURY SPECIES (IN mg/Kg of Hg species) DETERMINED IN TUNA FISH TISSUE SRM (ERM0CE464) BY EXTERNAL CALIBRATION AND METHOD 6800 (SIDMS) DEMONSTRATING THAT EFFICIENCY OF EXTRACTION DOES NOT AFFECT ACCURACY AFTER EQUILIBRATION HAS BEEN REACHED IN METHOD 6800

(Ref. 32)

	External calibration			***EPA Method 6800 (SIDMS)***		
Extraction procedure	***Hg^{2+}***	***CH_3Hg^+***	***sum of species***	***Hg^{2+}***	***CH_3Hg^+***	***sum of species***
Certified value	*0.12*[a]	*5.12 ± 0.16*	*5.24 ± 0.10*	*0.12*[a]	*5.12 ± 0.16*	*5.24 ± 0.10*
A	0.06 ± 0.02	5.05 ± 0.13	5.11 ± 0.13	0.07 ± 0.02	5.22 ±0.31	5.29 ± 0.31
B	0.12 ± 0.03	5.05 ± 0.18	5.17 ± 0.18	0.07 ± 0.03	5.20 ± 0.18	5.27 ±0.18
C	0.18 ± 0.05	4.88 ± 0.17	5.06 ± 0.18	0.30 ± 0.07	5.18 ± 0.13	5.48 ± 0.15
D	0.07 ± 0.02	4.29 ± 0.39	4.36 ± 0.39	0.13 ± 0.05	5.11 ± 0.38	5.24 ± 0.38
E	0.06 ± 0.04	3.94 ± 0.12	4.00 ± 0.13	0.11 ± 0.07	5.60 ± 0.33	5.71 ± 0.34
F	0.35 ± 0.08	3.29 ± 0.14	3.64 ± 0.16	0.27 ± 0.12	5.12 ± 0.19	5.39 ± 0.22
G	0.45 ± 0.10	4.87 ± 0.20	5.32 ± 0.22	1.05 ± 0.14	5.08 ± 0.25	6.13 ± 0.29
H	0.16 ± 0.07	4.42 ± 0.14	4.58 ± 0.16	0.07 ± 0.02	5.22 ±0.31	5.29 ± 0.31

[a]Inorganic mercury concentration was calculated as the difference between certified total mercury and methylmercury concentrations. The values are means ± 95% CL (n = 3).

TABLE 16a
ANTIMONY TOXICANT CONTAMINATION IN Zn DIETARY SUPPLEMENT IN ug/g BY IDMS METHOD 6800

(Ref. 33)

Samples Directly From Manufacturer		Commercially Available Samples	
Lot	Concentration of Antimony (ppb)	Lot	Concentration of Antimony (ppb)
1	17,650 ± 145.9	3-1	14,430 ± 479.8
2	15,770 ± 246.1	3-2	15,720 ± 47.07
3	17,000 ± 67.03	3-3	15,370 ± 31.41
4	27,310 ± 276.2	11-1	96.15 ± 12.79
5	16,830 ± 236.5	11-2	79.55 ± 5.289
6	18,060 ± 84.62	11-3	65.00 ± 1.128
7	15,590 ± 88.14	14-1	0.4486 ± 0.1586
8	24,450 ± 159.0	14-2	0.3350 ± 0.05088
9	16,120 ± 57.58	14-3	0.3040 ± 0.03636
10	218.4 ± 24.50	15	0.6307 ± 0.09681
11	78.84 ± 1.337		
12	454.1 ± 12.16		
13	380.9 ± 29.22		
Stevia	31,620 ± 298.5		

n = 12, at the 95% Confidence Interval.

TABLE 16b
ZINC CONCENTRATION IN Zn DIETARY SUPPLEMENT IN ug/g BY IDMS METHOD 6800.

(Ref. 33)

Samples Directly From Manufacturer		Commercially Available Samples	
Lot	Concentration of Zinc (ppm)	Lot	Concentration of Zinc (ppm)
1	4,332 ± 84	3-1	4,150 ± 178
2	4,223 ± 96	3-2	4,034 ± 207
3	4,307 ± 94	3-3	4,105 ± 77
4	4,128 ± 77	11-1	4,121 ± 55
5	4,173 ± 117	11-2	4,146 ± 83
6	4,340 ± 126	11-3	4,041 ± 129
7	3,930 ± 81	14-1	3,935 ± 110
8	4,232 ± 134	14-2	4,142 ± 72
9	4,203 ± 93	14-3	4,131 ± 62
10	4,198 ± 100	15	4,656 ± 65
11	4,115 ± 89		
12	4,174 ± 95		
13	4,170 ± 77		
Stevia	0.9347 ± 0.1085		

Concentrations are in ppm, ug/g of dietary supplement
n = 12, at the 95% Confidence Interval.

TABLE 17

ANALYSIS OF THE PESTICIDE GLYPHOSATE (ANALOGUE FOR VX NERVE GAS) BY IDMS SOLID PHASE EXTRACTION AND i-SPIKE FROM DRINKING WATER WHERE THE ENRICHED ISOTOPES ARE ADDED FIRST TO THE EXTRACTION MEDIA FOR AUTOMATION AND FOR PERSONNEL SAFETY IN EXTREMELY TOXIC ANALYSES

(Refs. 34 and 42)

Normalized Actual Concentration (ppm)	Normalized Measured Concentration (ppm)	95% Confidence Interval	% RSD
SPE-IDMS			
100	99.54	±2.17	2.10%
50	53.05	±0.90	1.55%
i-Spike			
100	99.54	±1.90	1.83%
50	53.77	±1.16	1.96%

TABLE 18

GLUTATHIONE CONGIGATE MERCURY SPECIES QUANTIFIED BY 6800 USING ESI-TOF AND MALDI-TOF (TRUE VALUE 480.3 ug/g)

(Refs. 35 and 41)

Glutathione mercury (Glutathione$_2$-Hg^{2+} in sample by nanoESI-TOF IDMS	485 ± 18 ppm
Glutathione mercury (Glutathione$_2$-Hg^{2+} in sample by MALDI-TOF IDMS	474 ±73 ppm
Actual Concentration of Glutathione mercury dimer	480.3 ppm

Quantification by EPA Method 6800 using both nanoESI-TOF-MS and MALDI-TOF-MS

n-4, uncertainty is 95% CL

FIGURE 1

THE INFLUENCE OF THE DEAD TIME CORRECTION ON THE ISOTOPE RATIOS MEASURED WITH ICP-MS EQUIPPED WITH A CONTINUOUS DYNODE MULTIPLIER
(Ref. 12)

Gain loss occurs when the count rate exceeds 5.8×10^5.

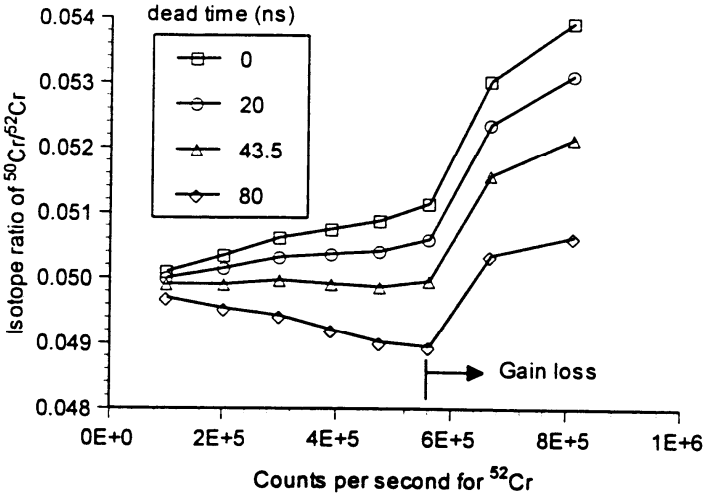


FIGURE 2

IDMS DETERMINATION OF VANADIUM IN CRUDE OIL. NUMBERS SHOWN ABOVE THE BARS ARE THE ATOMIC FRACTION
(Revised from Ref. 1)

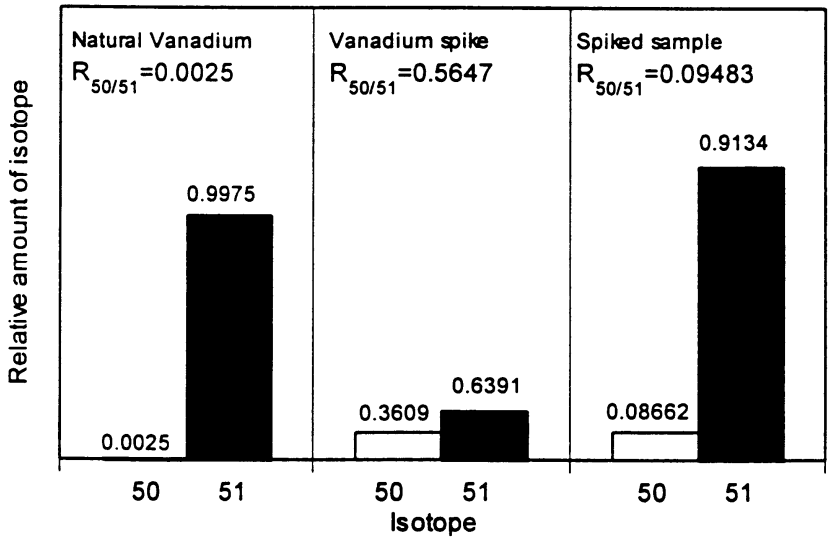


FIGURE 3

SEPARATION AND DETECTION OF Cr(III) AND Cr(VI) WITH ION-EXCHANGE CHROMATOGRAPHY COUPLED WITH AN ICP-MS
(Ref. 5)

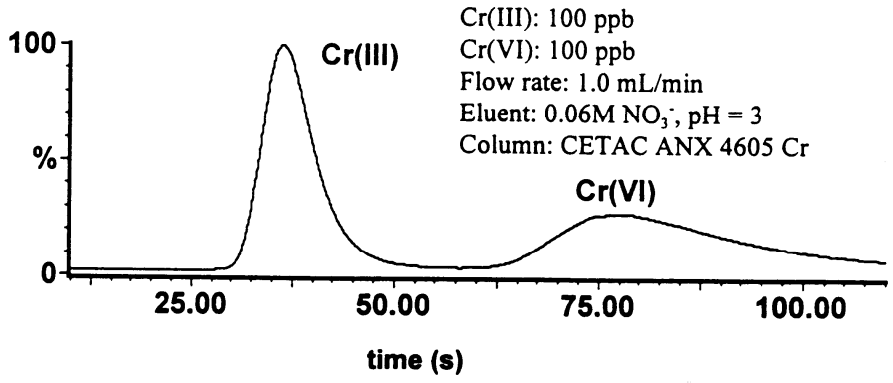


FIGURE 4

SEPARATION OF THE UNSPIKED SAMPLE AND ISOTOPICALLY SPIKED SAMPLE
(Ref. 6)

(a): Chromatograms of a solution containing Cr(III) and Cr(VI) with natural isotopic abundance.

(b): Chromatograms of the same solution spiked with isotope-enriched spikes $^{50}Cr(III)$ and $^{53}Cr(VI)$.

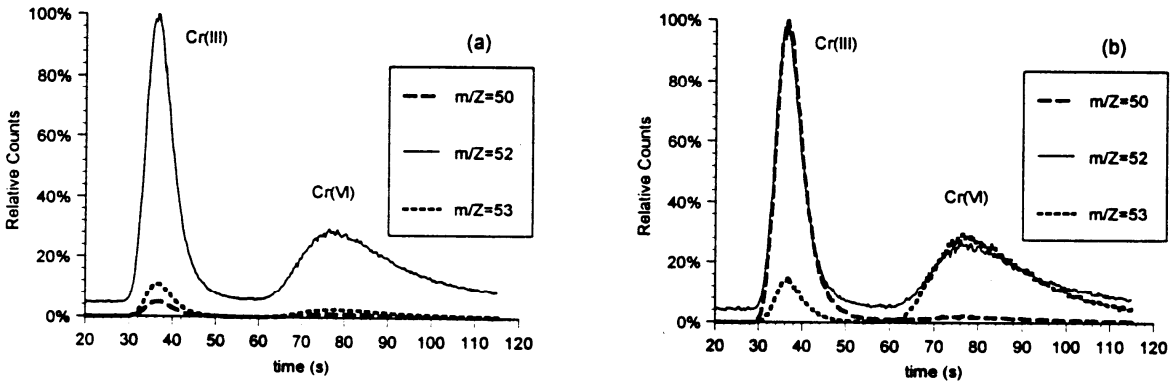
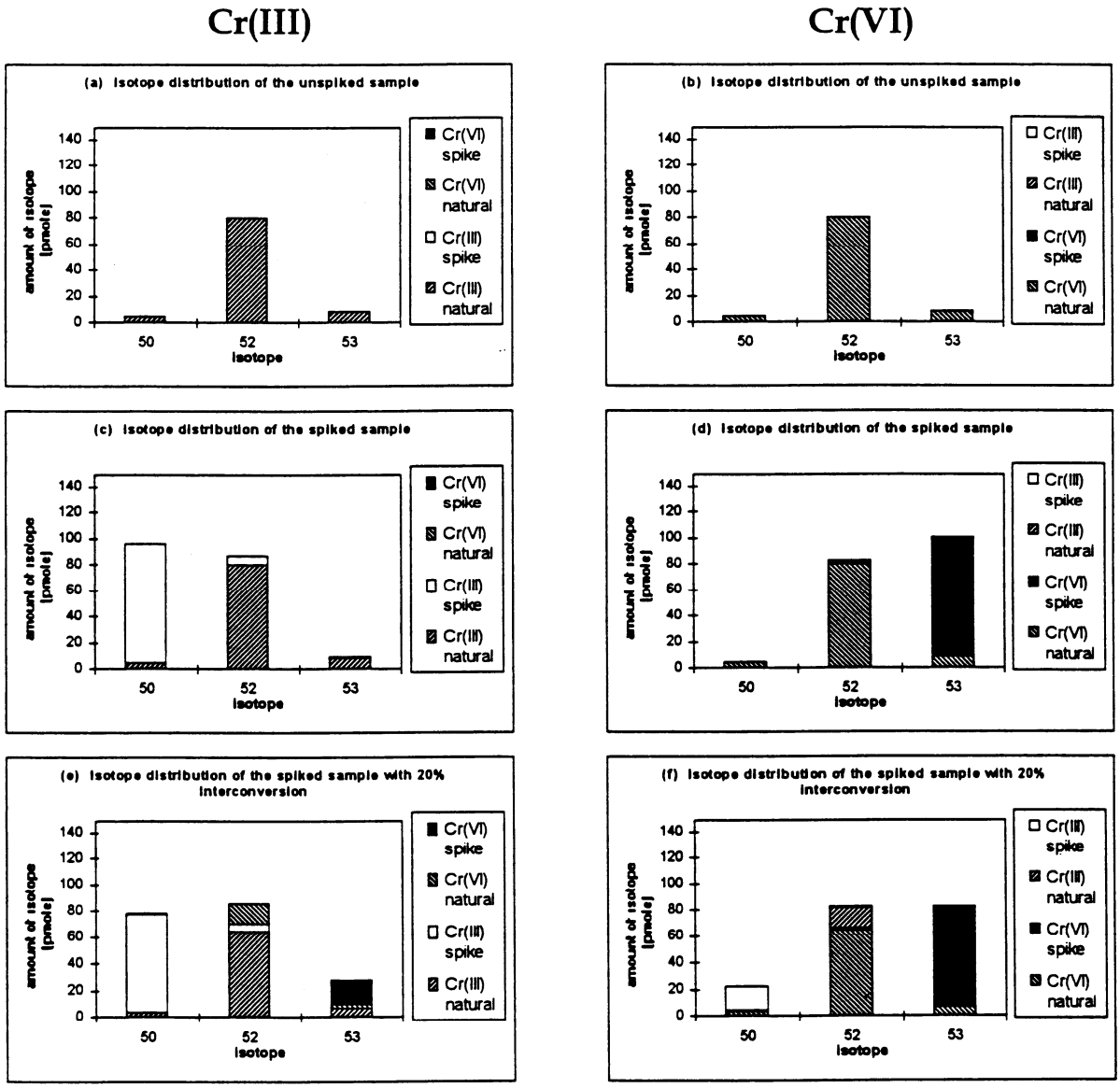


FIGURE 5

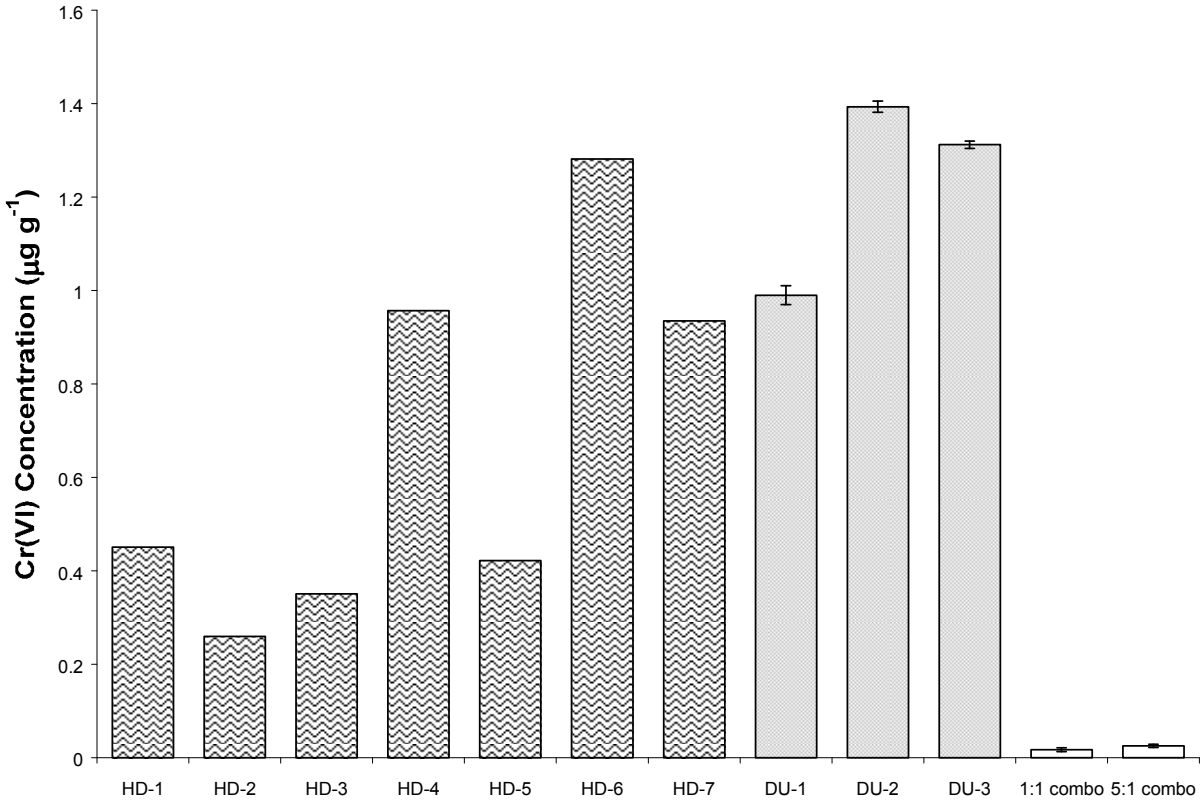
GRAPHIC CALCULATED ILLUSTRATION OF THE APPLICATION OF SIDMS TO THE SIMULTANEOUS DETERMINATION OF Cr(III) AND Cr(VI) (Ref. 6)



(a) and (b) show the initial natural isotopic abundance of species Cr(III) and Cr(VI) in a 50_l 200 ppb Cr solution in which the concentrations of both Cr(III) and Cr(VI) are 100 ppb. In (c) and (d), the sample is spiked with 100 ppb ^{50}Cr(III) (in which ^{50}Cr is enriched) and 100 ppb ^{53}Cr(VI) (in which ^{53}Cr is enriched), there is no interconversion between Cr(III) and Cr(VI). In (e) and (f), 20% of Cr(III) is converted to Cr(VI), and 20% of Cr(VI) is converted to Cr(III). Different degrees of interconversion results in different isotopic abundances, so the change of the relative isotopic abundance can be applied to the determination of the species and the degree of the interconversion.

FIGURE 6

HEXAVALENT CHROMIUM CONCENTRATIONS IN FLY ASH LEACHATE OBTAINED FROM HISTORICAL DATA (HD), STUDY WITH SIDMS (DU); AND BEFORE AND AFTER COMBINATION WITH ACID MINE DRAINAGE (AMD)
(Ref. 14)



Cr(VI) concentration in leachate before and after combination with AMD, which illustrate the dramatic and statistically significant decrease in Cr(VI) concentrations in the fly ash leachate as a result of treating with AMD. Observations from HD-1 to HD-7 (dark gray bars) were historical data obtained from the power plant. Observations from DU-1 to DU-3 (light gray bars) were obtained from the SIDMS analysis at Duquesne University during current study, and observations '1:1 combo' and '5:1 combo' were obtained by mixing leachate and AMD with the ratios of 1:1 and 5:1 (leachate to AMD), respectively. Error bars are shown at 95% confidence intervals with n = 3.

FIGURE 7

ALPHA-GLACTOSIDASE A (GLA) ENZYME MEASURED BY 6800 IDMS IN MOUSE BY NANO-ESI-QTOF MASS SPECTROMETRY AGAINST THE STANDARD FLUORESCENCE METHODS AND FOUND TO BE SUPERIOR FOR DETECTING LOWER DOSE CONCENTRATIONS IN LIVER, KIDNEY AND BRAIN (PLASMA WAS NOT DONE BY IDMS IN THIS DIAGRAM).

(Ref. 36)

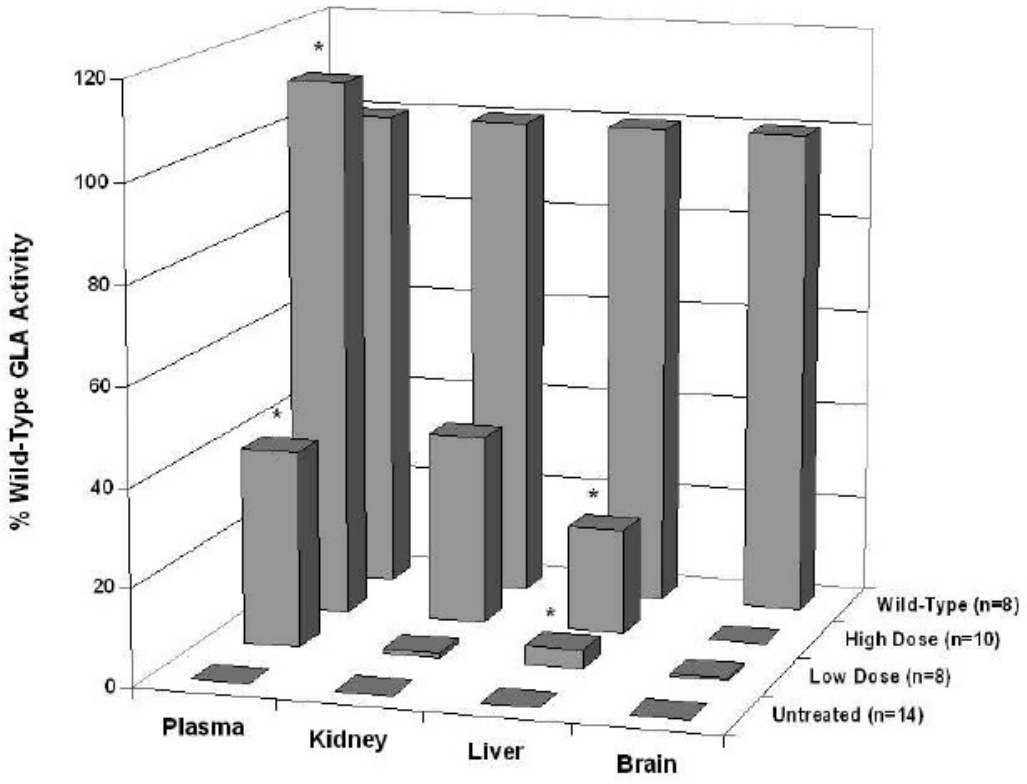


FIGURE 8

NANO-ESI-TOF-MS ANALYSIS OF GLUTATHIONE CONJUGATES WITH CH_3Hg^+ and Hg^{2+}. THE TWO NATURAL ISOTOPE SPECIES OF INORGANIC MERCURY AND METHYLMERCURY BOUND TO GLUTATHIONE IN SPECTRA 8A AND 8C. THE TWO ISOTOPICALLY ENRICHED MERCURY IN SPECIATED FORMS WITH THE ENRICHED ISOTOPE EVIDENT IN THE MERCURY GLUTATHIONE SPECTRA OF 8B AND 8D.

(Refs. 35 and 42)

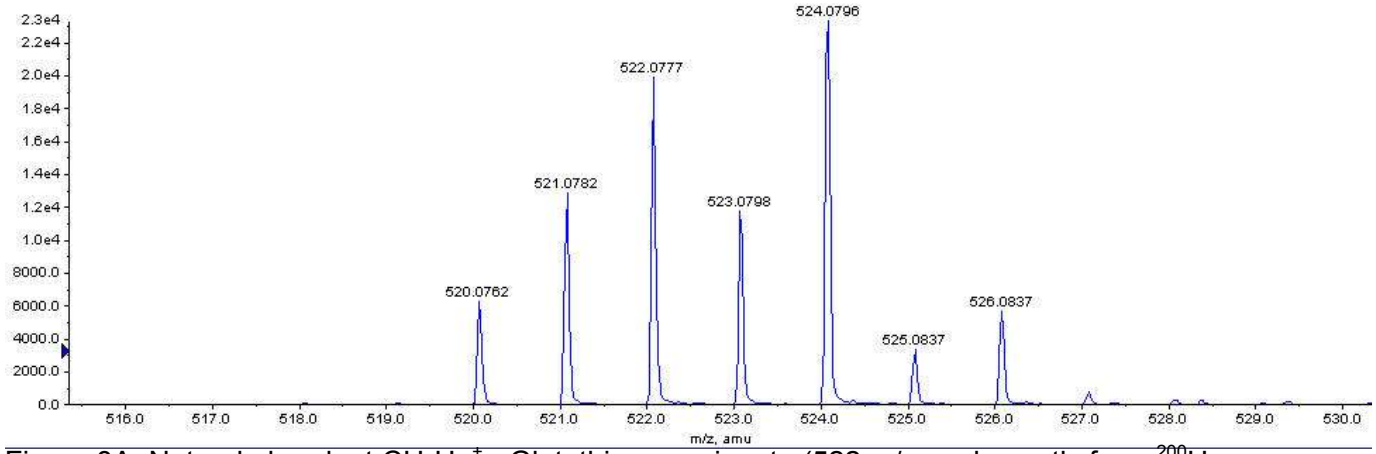


Figure 8A. Natural abundant CH_3Hg^+ - Glutathione conjugate (522 m/z peak mostly from ^{200}Hg – Glutathione conjugate, 524 m/z peak mostly from ^{202}Hg – Glutathione conjugate)

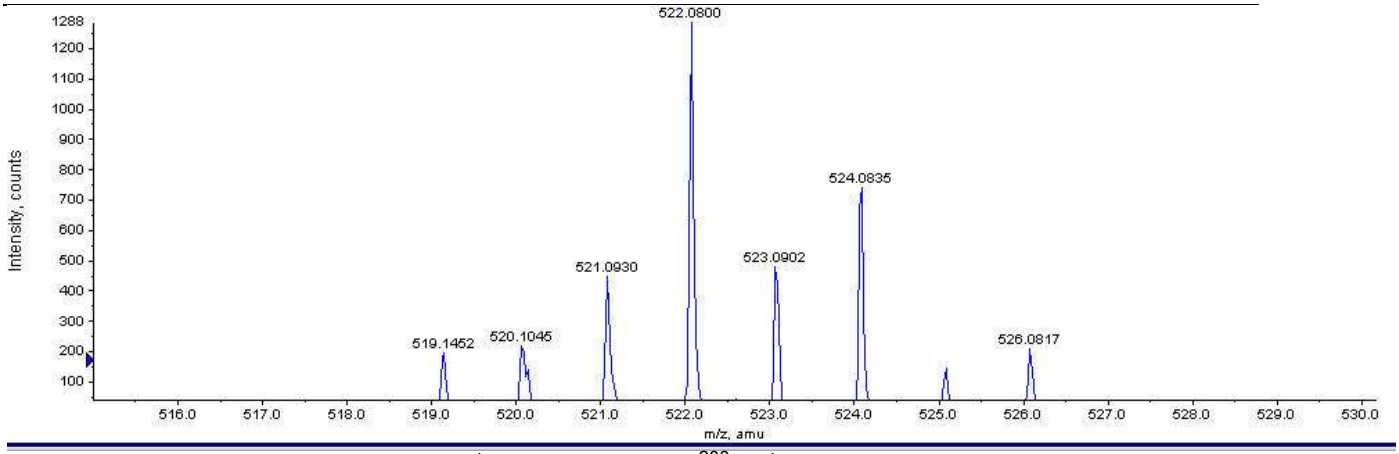


Figure 8B. Natural abundant CH_3Hg^+ spiked with $CH_3{}^{200}Hg^+$ - Glutathione conjugate (notice the 522 m/z peak in this figure compared to the one above)

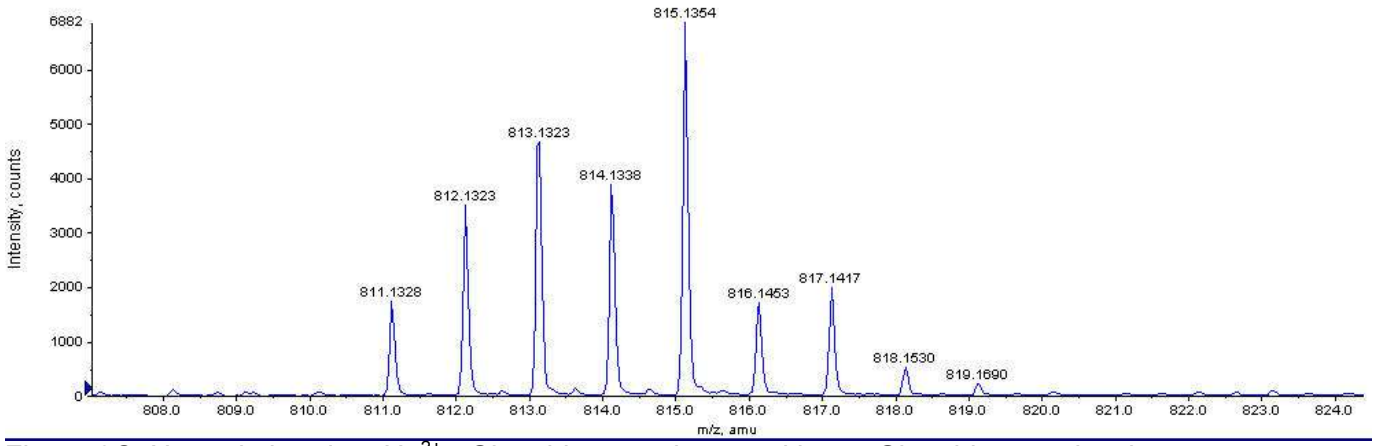


Figure 8C. Natural abundant Hg^{2+} - Glutathione conjugate with two Glutathione molecules bound to Hg^{2+} (peak at 812 m/z mostly due to $^{199}Hg^{2+}$ - Glutathione conjugate, peak at 815 m/z mostly due to ^{202}Hg – Glutathione conjugate).

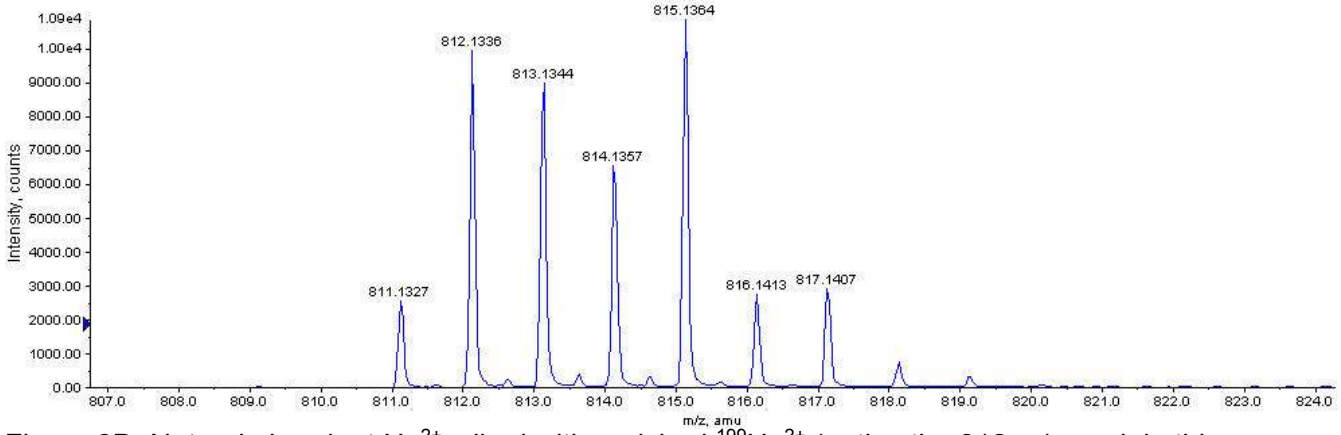


Figure 8D. Natural abundant Hg^{2+}spiked with enriched $^{199}Hg^{2+}$ (notice the 812 m/z peak in this figure compared to the one above)

FIGURE 9

NATURAL ABUNDANT REDUCED (9A) AND OXIDIZED (9B) GLUTATHIONE BIOMARKERS ANALYZED BY NANO-ESI-QTOF-MS.

(Refs. 35 and 42)

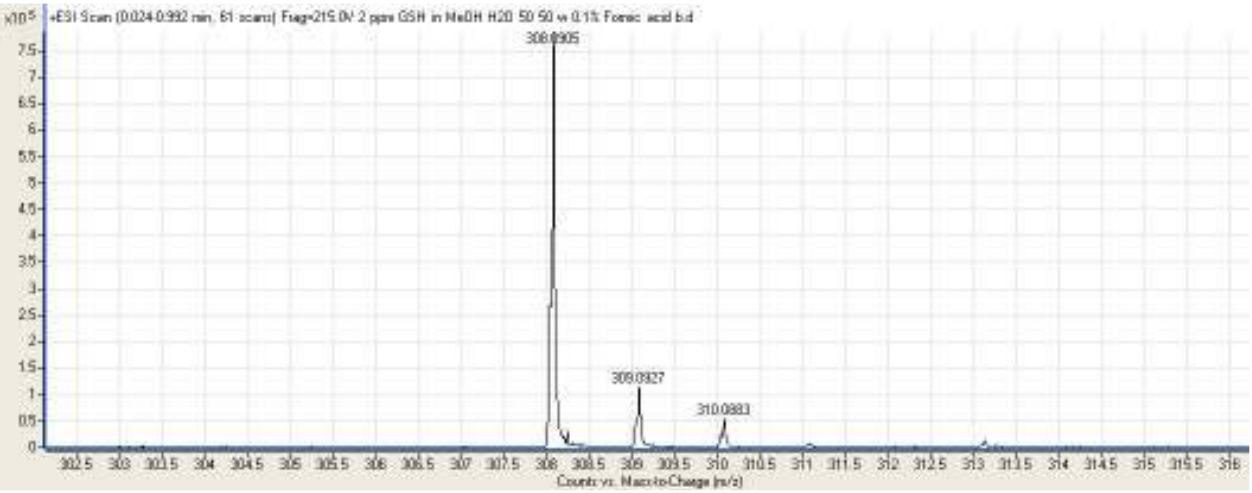


FIGURE 9A. Natural abundant Reduced Glutathione (GSH)

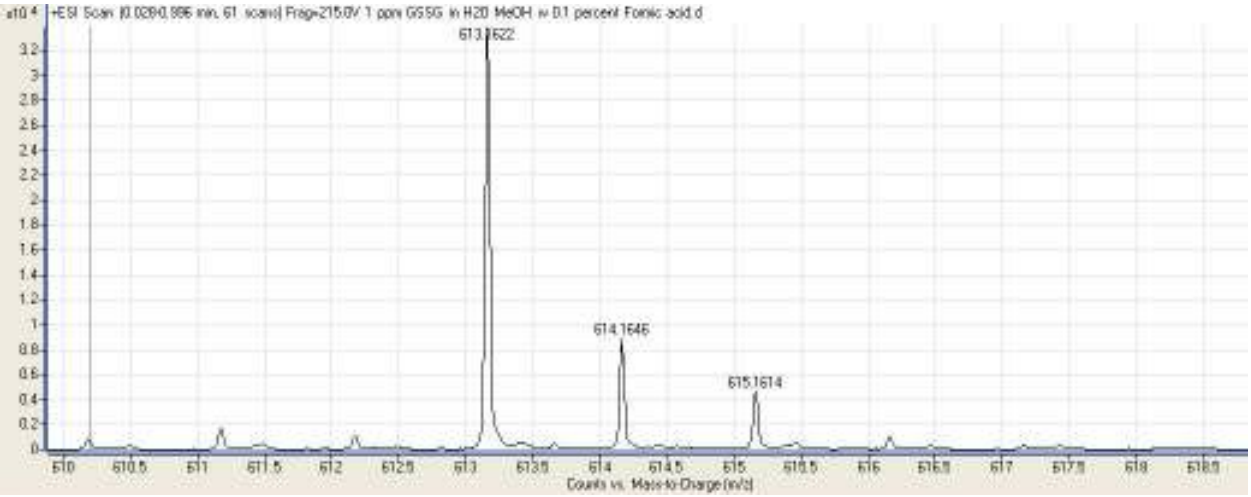


FIGURE 9B. Natural abundant oxidized Glutathione (GSSG)

FIGURE 10

SODIUM AZIDE, TOXICANT AND EXPLOSIVE, ANALYZED BY ESI-TOF-MS IN BOTH POSITIVE AND NEGATIVE MODES. EACH MODE GIVES DIFFERENT SPECIES ADDUCT FORMATION PATTERNS AND RATIOS THAT ARE STOICHIOMETRICALLY RELATED TO THE DIRECT EQUATIONS AND MATHEMATICAL SOLUTIONS. QUANTIFICATION ANALYSES WERE WITHIN 5% OF TRUE VALUE BY METHOD 6800 FOR ALL ANALYSES OF THIS TYPE.

(Refs. 40 and 42)

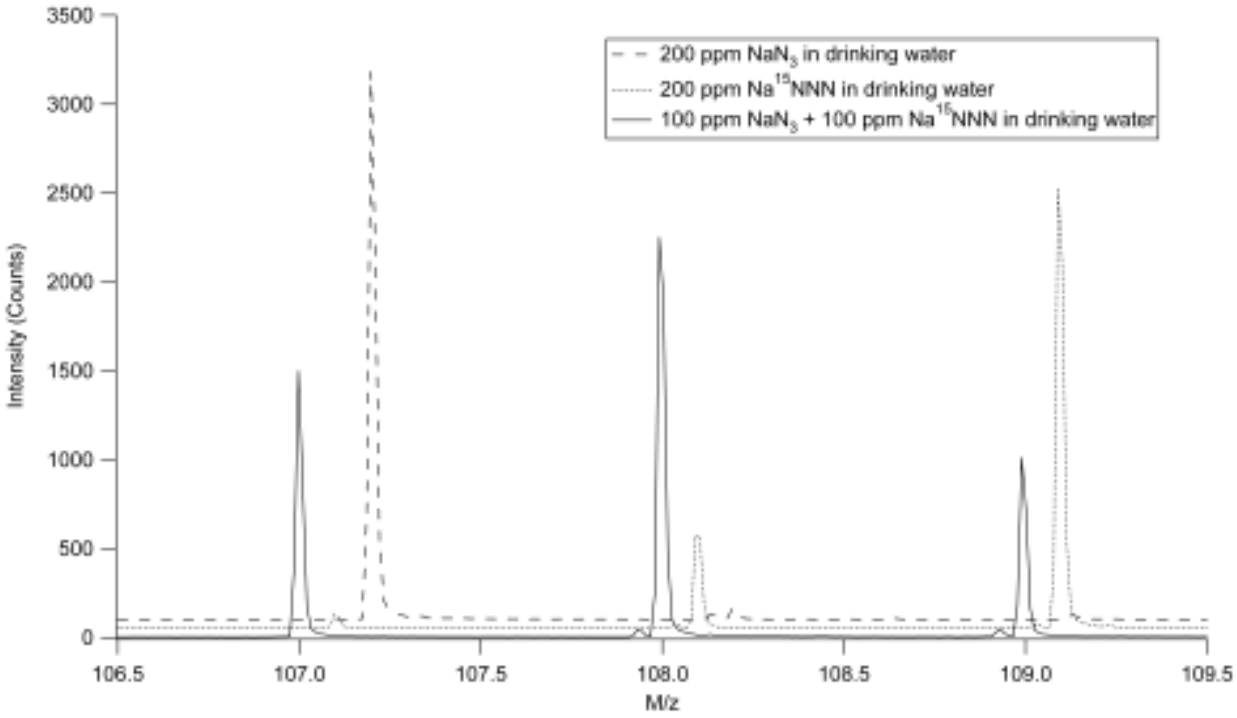
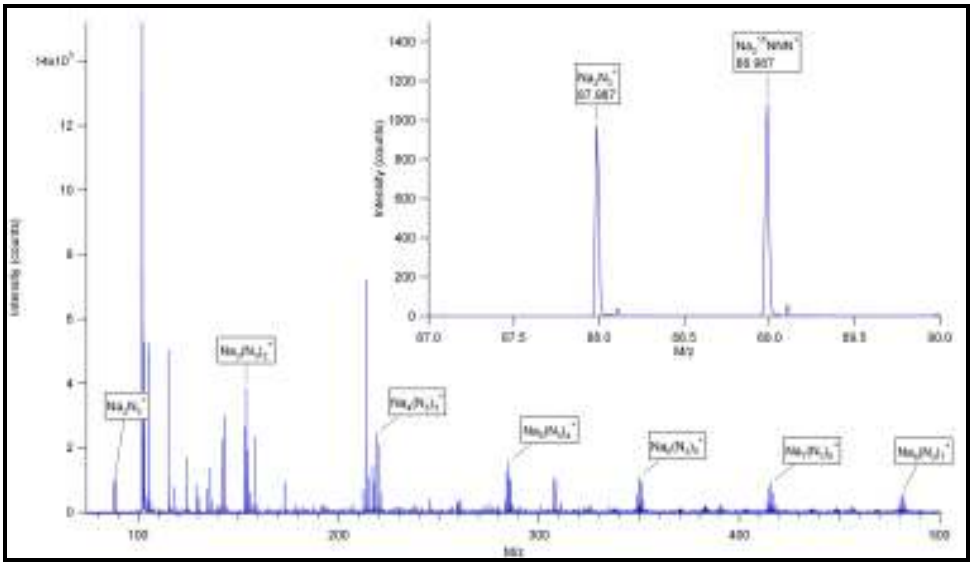


Figure 10A: An example of the unique spectra obtained from natural sodium azide, enriched sodium azide, and a mixed solution containing both natural sodium azide and enriched sodium azide in negative mode. The graphs are offset for clarity. In the mixed solution, a 1:2:1 ratio is observed as expected. Typical sample accuracy is within ± 5% of true concentration.



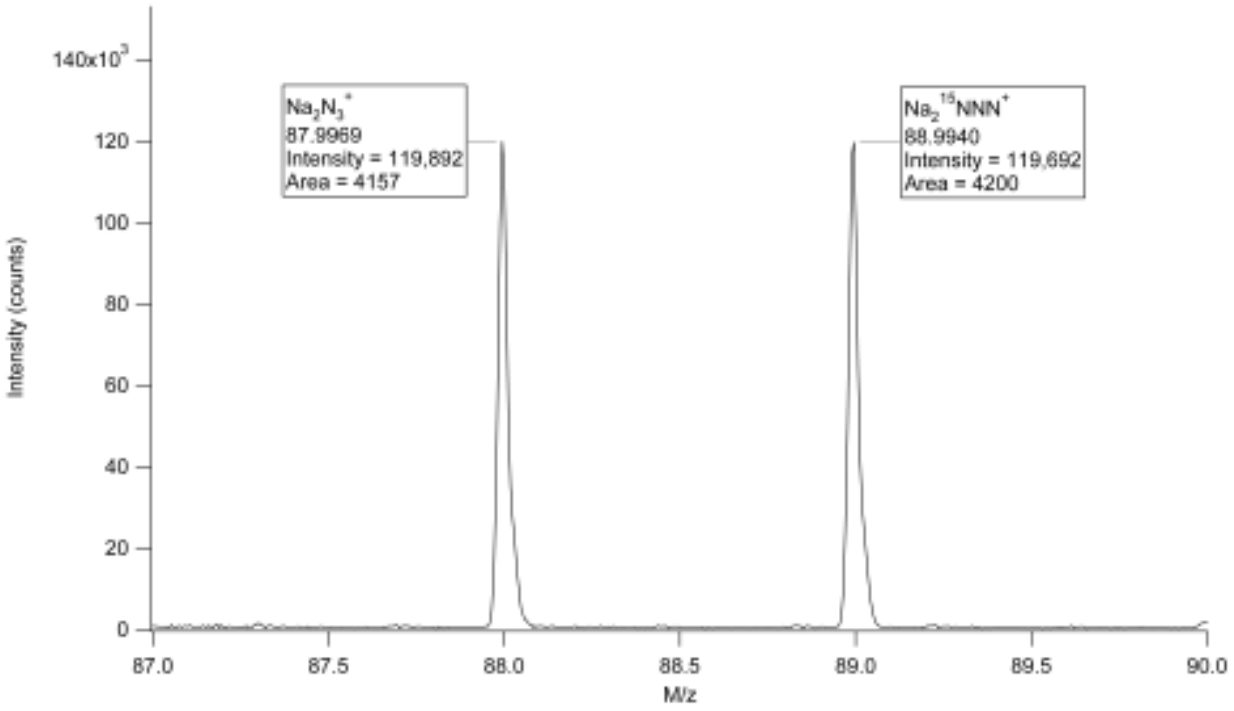


FIGURE 10B. Spectrum of 20 ppm NaN_3 and 20 ppm $Na^{15}NNN$ in water in positive mode and enlargement of one of the specific fragmentation patters. Each fragmentation pattern gives the same direct mathematical concentration. In positive mode, these two peaks form a mathematical pattern of one natural (left) and one enriched (right) providing quantitative data within 1% of the true value (in this particular case). In addition, these positive azide analyses in water are examples of solid phase extraction (SPE) elution solution spectrum for an i-Spike ESI-ID-TOF-MS of sodium azide in water using 1:1:1 Optima ACN:Optima MeOH:Optima H_2O with 5% 0.5 M NaOH in water. The i-Spike method loads the enriched isotopic molecule first and then loads the sample where the analyte is extracted and the two are equilibrated on the solid phase media and then eluted in the final isotopic and molecular form after which any portion of the sample analyte gives the same correct quantitative solution.

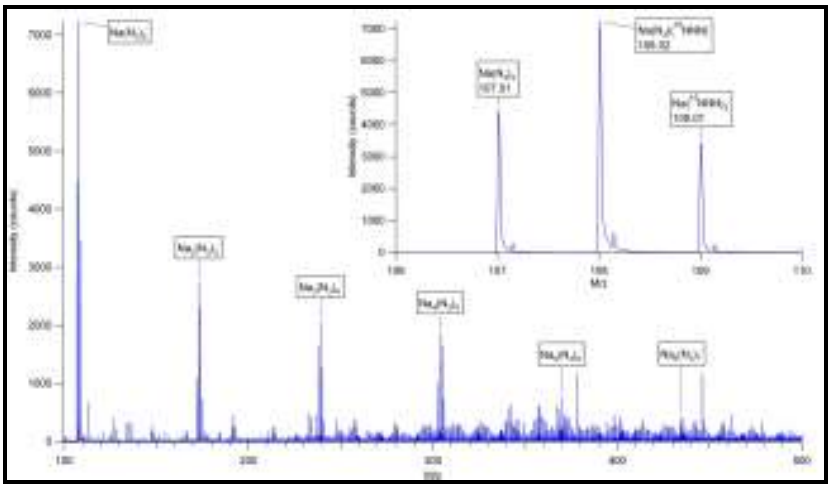
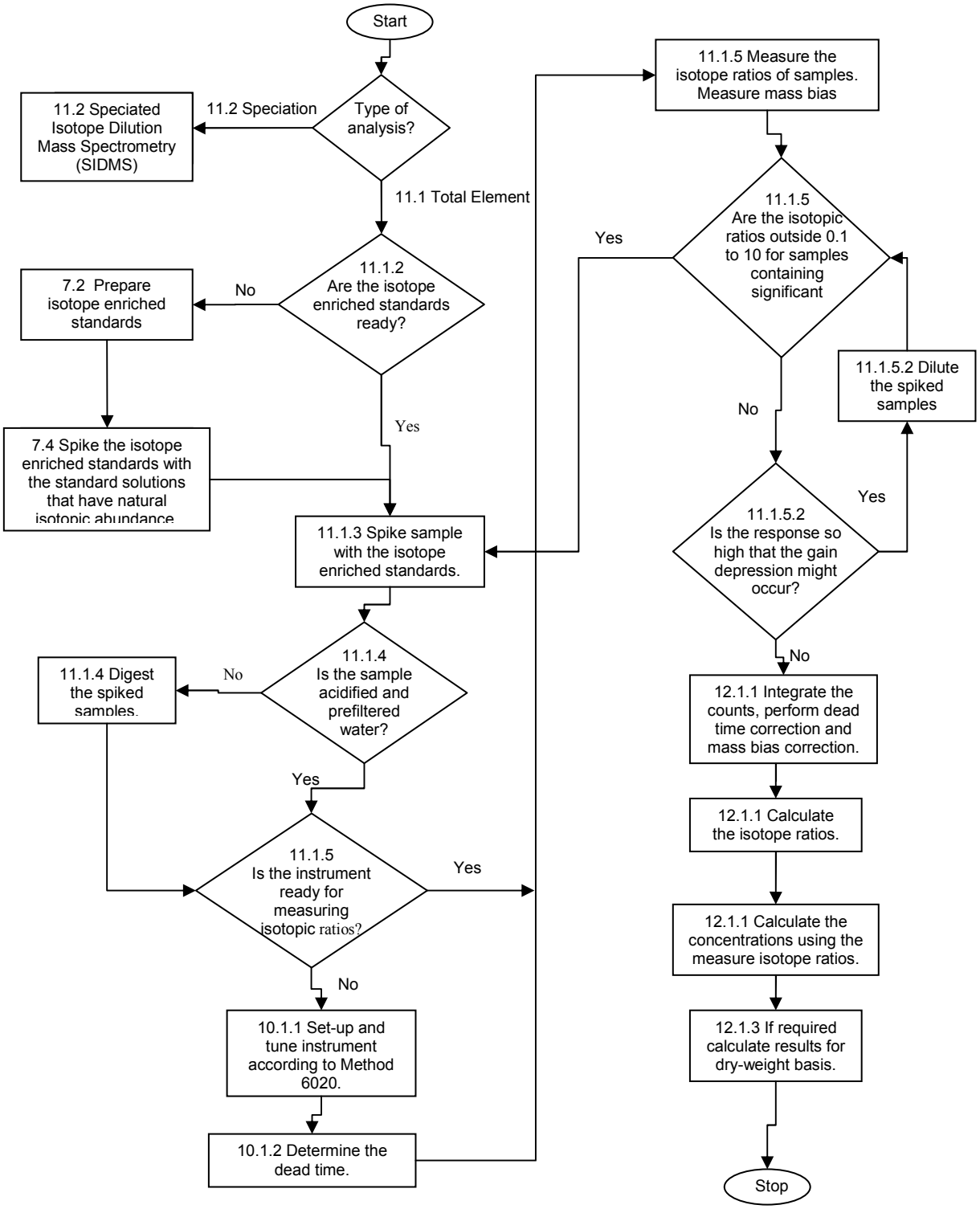
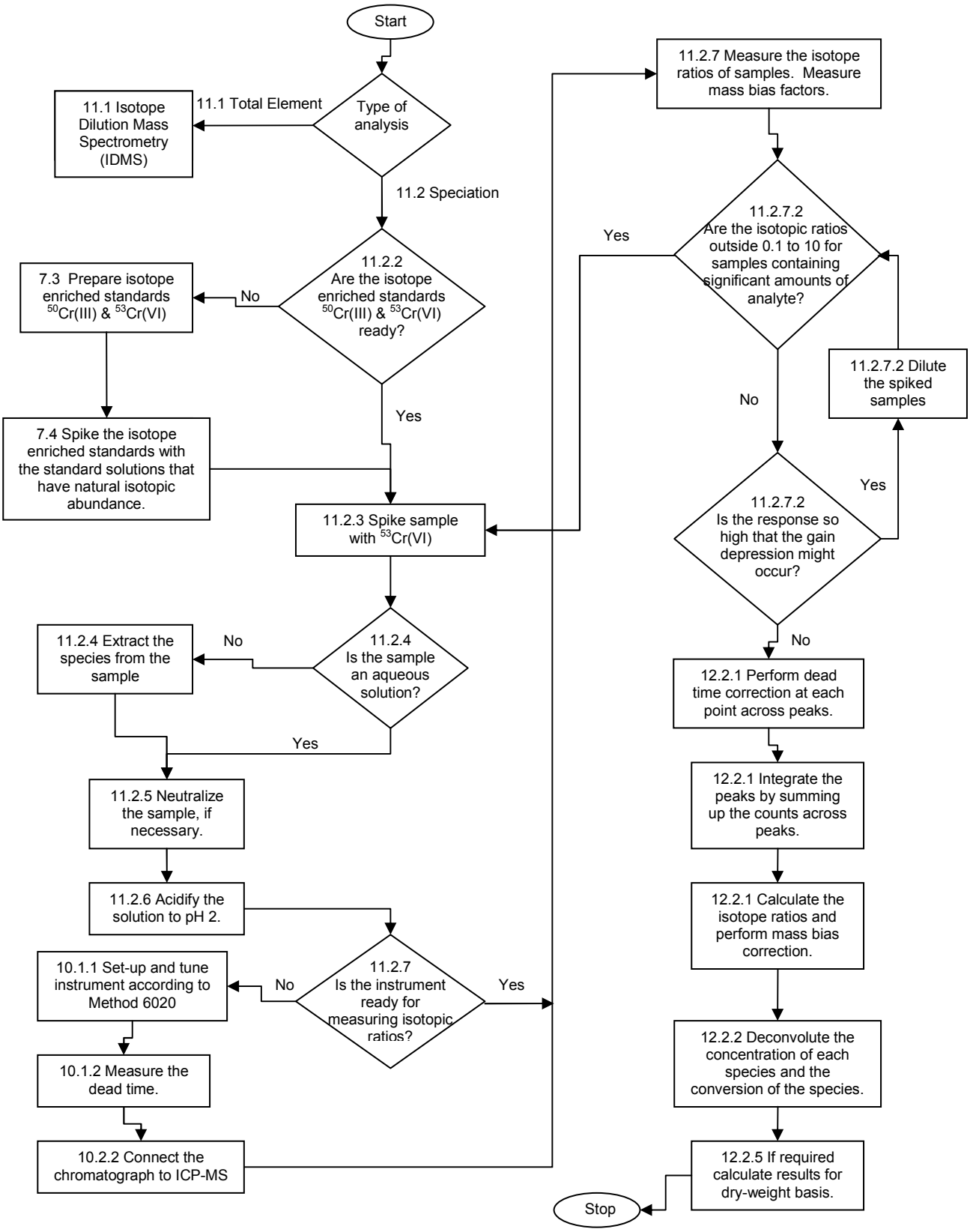


FIGURE 10C. Spectrum of 20 ppm NaN_3 and 20 ppm $Na^{15}NNN$ in water in negative mode. Here a different fractionation pattern is a 1:2:1 (similar to the example in Figure 10A) and provides quantification accuracy within 5%. Note that the negative and positive ionization patterns confirm each other using the opposite charged fragmentation pattern.

ELEMENTAL AND MOLECULAR SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY: ISOTOPE DILUTION MASS SPECTROMETRY (IDMS)




Start
Type of analysis
11.1 Total Element
11.1 Isotope Dilution Mass Spectrometry (IDMS)
11.2 Speciation
11.2.2 Are the isotope enriched standards ^{50}Cr(III) & ^{53}Cr(VI) ready?
No
7.3 Prepare isotope enriched standards ^{50}Cr(III) & ^{53}Cr(VI)
Yes
7.4 Spike the isotope enriched standards with the standard solutions that have natural isotopic abundance.
11.2.3 Spike sample with ^{53}Cr(VI)
11.2.4 Is the sample an aqueous solution?
No
11.2.4 Extract the species from the sample
Yes
11.2.5 Neutralize the sample, if necessary.
11.2.6 Acidify the solution to pH 2.
11.2.7 Is the instrument ready for measuring isotopic ratios?
No
10.1.1 Set-up and tune instrument according to Method 6020
10.1.2 Measure the dead time.
10.2.2 Connect the chromatograph to ICP-MS
Yes
11.2.7 Measure the isotope ratios of samples. Measure mass bias factors.
11.2.7.2 Are the isotopic ratios outside 0.1 to 10 for samples containing significant amounts of analyte?
Yes
11.2.7.2 Dilute the spiked samples
No
11.2.7.2 Is the response so high that the gain depression might occur?
Yes
No
12.2.1 Perform dead time correction at each point across peaks.
12.2.1 Integrate the peaks by summing up the counts across peaks.
12.2.1 Calculate the isotope ratios and perform mass bias correction.
12.2.2 Deconvolute the concentration of each species and the conversion of the species.
12.2.5 If required calculate results for dry-weight basis.
Stop

GLOSSARY

Atomic mass unit (symbol: u or amu) or dalton (symbol: Da) – the unit used to indicate mass on an atomic or molecular scale. One amu or u or Da is defined as the mass of one twelfth of the isotope of carbon-12.

Mass-to-charge ratio (m/z) – is the mass of the ion divided by the charge of the ion and is the functional unit measured in a mass spectrometer.

Isotope dilution mass spectrometry (IDMS) – A quantitative method for total concentration determination of an analyte based on the measurement of the isotope ratio of a nuclide using mass spectrometry after isotope dilution, both molecular and ionic.

Isotope dilution – Mixing of a given nuclide with one or more of its isotopes. The isotope usually has an enriched isotopic abundance different from that occurring naturally.

Speciation analysis – IUPAC defines speciation analysis as analyzing: "Chemical compounds that differ in isotopic composition, conformation, oxidation or electronic state, or in the nature of their complexed or covalently bound substituent, can be regarded as distinct chemical species"

Speciated isotope dilution mass spectrometry (SIDMS) – A quantitative method for determining molecular or ionic or elemental species and interconversions between them based on the measurement of isotope ratio(s) in each species of a nuclide using mass spectrometry after speciated isotope dilution. Samples are mixed with one or more isotopic spikes which have different isotopic abundances and are artificially converted to chemical forms corresponding to the species to be analyzed. The spiked samples are then subjected to the separation of the species, followed by the measurement of the altered isotope ratios in each species. Both species concentrations and the extent of species conversions can be mathematically deconvoluted.

Isotopic abundance – The relative number of atoms of a particular isotope in a mixture of the isotopes of an element or molecule, expressed as a fraction of all the atoms of the element.

Isotopes – Nuclides having the same atomic number but different mass numbers.

Species – Chemical forms in which molecule and or an element exists.

Natural isotopic abundance –Isotopic abundance of elements from natural sources. Most elements (except lithium, lead and uranium) found in nature have a constant isotope abundance.

Isotope ratio – Ratio of the isotopic abundances of two isotopeic forms of molecular ions.

Speciation (or speciated) analysis – Quantification of elements in specific chemical forms.

Isotope-enriched material – Material containing elements artificially enriched in minor isotopes.

Isotopic spike (Isotope-enriched spike) – Standards prepared from isotope-enriched materials.

i-Spike – Method of isotopic species equilibration that takes place rapidly by preloading isotopically enriched spike onto a sorbent-packed column prior to the introduction of the sample in both IDMS and SIDMS.

Dead time – The interval during which the detector and its associated counting electronics are unable to resolve successive pulses. The measured counts are lower than the true counts if no correction is performed.

Gain loss – The loss of gain in detector caused by the inability of the multiplier's dynode string to supply enough current to maintain constant dynode voltage drops. The measured counts are lower than the true counts, and cannot be mathematically corrected if gain loss occurs.

Mass bias – The deviation of the measured isotope ratio from the true value caused by the differential sensitivity of the instrument to mass. This effect may occur in the ionization process or from differential transmission/detection by the mass spectrometer.

Mass bias factor – A number used to correct for the mass bias of the measured isotope ratios. Mass bias factor is measured by employing an isotopically certified standard.

Isotopic-abundance-certified standard (Isotopically certified standard) – Standard material with certified isotopic abundance.

Inverse isotope dilution – Analysis method to determine the concentrations of isotopic spikes. A known quantity and isotopic abundance of an isotopic spike is mixed with a known amount and isotopic abundance (usually tabulated natural isotopic abundance or certified isotopic abundance) of standard(s) and the altered isotope ratio(s) is (are) measured and used in the calculation to find the concentration of the isotopic spike. Usually, a high purity source of the natural material is used to calibrate and determine the concentration of the separated isotopic spike solution using this method. Only in the case of such elements as uranium, lead, and lithium are the natural isotopic abundances not constant in terrestrial materials. Molecules have natural isotopes also such as C-13, O-18, O18, N-15 that can alter the purity of the natural molecule and can be mathematically corrected.

Spiking – Addition of the enriched isotopic species (spike) to the extract or appropriate sample.

Single spiking – Addition of one enriched isotopic spike to the sample.

Double spiking – Addition of two isotopic spikes to the sample. The two isotopic spikes are enriched in different isotopes, and are prepared in different chemical forms, each of which corresponds to a species form.

Unidirectional conversion – One directional transformation occurring between two species. One species can convert to the other; the reverse transformation does not occur.

Interconversion – Uni- or bi-directional transformation occurring between two species. Some species convert back and forth between two or more chemical forms.

Time resolved analysis (TRA) – A data collection mode in which the data can be acquired at designated intervals for a continuously aspirated sample, over a user-defined period of time.

APPENDIX A
SUMMARY OF REVISIONS TO METHOD 6800
(AS COMPARED TO PREVIOUS REVISION 0, FEBRUARY 2007)

1. Update for molecular SIDMS capability as well as examples of Homeland Defense and Homeland Security and Human Health examples.
2. Improved overall method formatting for consistency with new SW-846 methods style guidance.
3. Inserted text describing use and application of LLOQ (Sec. 9.4).
4. Minor editorial and technical revisions were made throughout to improve method clarity. The revision number was changed to one and the date published was changed to July 2014.
5. New explicit and determinative SIDMS equations were added to the iterative equations previously described.
6. Minor corrections to equations were made for accuracy.
7. Examples of applications and data validating these applications were included.
8. Literature examples of more molecular applications of the method were expanded.