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UNCERTAINTY CONTRIBUTIONS TO SPECIES SPECIFIC ISOTOPE DILUTION ANALYSIS

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by

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Abstract

Uncertainty Contributions to Species Specific Isotope Dilution Analysis

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Mercury speciation in solid sample matrices has been investigated using high performance liquid chromatography (HPLC) coupled with multicollector sector field (MC-SF) and quadrupole (Q) inductively coupled plasma mass spectrometry (ICP-MS) for species specific isotope dilution mass spectrometry (IDMS). ^{199}Hg enriched methylmercurychloride has been synthesised and recovered in the solid form for use as a spike material. The stability of methylmercury during the IDMS procedure was investigated using ^{199}Hg and ^{13}C labelled methylmercury isotopomers and ^{1}H Nuclear Magnetic Resonance spectroscopy. Intermolecular exchange of the methylmercury halide counter ion was observed, the halide counter ion order of preference was $I^- > Br^- > Cl^-$. No evidence was found for the decomposition, or formation, of methylmercury during equilibration with soil (NIST2710 SRM) or dogfish muscle (DORM-2 CRM), or during chromatographic separation.

The extent of equilibration between the spike and the particulate bound mercury compounds was studied by temporal monitoring of the ^{200}Hg:^{199}Hg isotope amount ratio and determining the amount of Hg species in the liquid phase. For NIST2710, complete equilibration was only achieved when concentrated HNO_3 in combination with a microwave digestion was employed. For DORM-2, complete equilibration was achieved when using 1:1 H_2O:CH_3OH $v\backslash v$ and 0.01% 2-mercaptoethanol as the solvent, even though only 47% of the analyte was extracted into the liquid phase.

The mass fraction of methylmercurychloride has been determined in DORM-2 and BCR464 lobster hepatopancreas CRM by two different procedures, single IDMS and approximate matching double IDMS. Mercury cold vapour generation of the HPLC column eluent allowed isotope amount ratios measurements by MC-SF-ICP-MS. For each CRM the mass fraction of methylmercury determined by the two IDMS methods was not statistically different, within the limits of uncertainty, from the certified values. An uncertainty budget for both IDMS procedures has been formulated to allow the performance of each method to be compared.

For single IDMS the major uncertainty contribution was derived from the within replicate uncertainty, u_{within}. The combined standard uncertainty of each replicate analysis was dominated by two components, the uncertainty associated with the natural isotopic abundance ^{200}Hg:^{199}Hg isotope amount ratio and the uncertainty associated with the ^{199}Hg enriched methylmercurychloride spike mass fraction. The between blend standard uncertainty, $u_{between}$, was the major contributor to the expanded uncertainty for approximate matching double IDMS. The combined standard uncertainty for each individual replicate was dominated by the contribution from the standard uncertainty associated with the measured ^{200}Hg:^{199}Hg isotope amount ratios in the spiked sample and the mass bias calibration blend.

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External Courses Attended

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Publications

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Poster Presentations

"Isotope Dilution for Mercury Speciation" R.Clough, E.H.Evans, S.T.Belt and B.Fairman. Poster presented at the 16th International Mass Spectrometry Conference, Edinburgh, Sept 2003.

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Jeol Annual NMR Users Day, 5th April, 2001, Oxford Instruments, Oxford, UK.

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Chapter 1 Introduction

1. Introduction

1.1. Speciation Overview

Speciation is now considered an established and mature field of analytical chemistry[1] and, depending on the method employed, has been defined variously as[2]:

- the chemical form of the analyte that is functionally important (e.g. ligands on an organometallic species or oxidation state).
- the exact structural and chemical form of the analyte (e.g. NMR derived structure).
- the nature of the chemical species as determined from an operational point of view, dependent on the method of sample preparation or analysis used (e.g. metals extractable by acetic acid or other solvent).

In order to encompass a single wider definition of speciation the definition can be framed in terms of the information which is required by the analyst, or to ask the question "What do I want to know about the analyte?". The answer poses several further questions:

- how much analyte is present in total?
- what is its chemical form/structure?
- how stable is it?
- how much is available?
- how is it distributed?
- how sure are we about all of the above?

When considered in these terms, the definition of speciation includes not only the accurate determination of the concentration of individual chemical species but also their behaviour in biological and biogeochemical systems. The International Union of Pure and Applied Chemistry (IUPAC) have clarified speciation as[3]

> "....the specific form of a chemical element defined according to its molecular, complex, electronic or nuclear structure"

leading to the identification and quantification of the different chemical and physical forms of an element existing in a sample[4]. Hence, speciation analysis can be considered to be the measurement of one or more individual chemical species in a sample. The chemical form of an element will determine its toxicity, stability and transport[5], so this measurement is a very important factor in the wider definition of speciation.

The determination of specific chemical entities is long established, especially for non-metallic species, including NO_3^-, NO_2^-, NH_4^+ and NH_3, with the characterisation of metals having a shorter history[6]. Likewise the functionality of biologically important enzymes and co-factors often depends on the speciation of individually important trace metals. Various pollution and poisoning incidents have also highlighted the need for the determination of individual chemical species.

The residents of Minamata Bay in Japan suffered acute and chronic methylmercury poisoning, with brain damage to new-born infants, as the result of a release of inorganic mercury into the bay and subsequent *in situ* biomethylation to methylmercury which, due to its lipophillic nature[7,8], bioaccumulated in fish, which formed the major part of the human diet. A spill of tetraalkyl lead in the Mediterranean raised awareness of the importance of organolead compounds[6]. A population crash, combined with changes in shell shape and meat content, decimated the oyster farming industry of Arcachon Bay in France and was traced to the use of tributyltin (TBT) as an anti-fouling agent on marine vessels[9]. Subsequently, a correlation was found between the levels of imposex observed in marine gastropods in South West England and the introduction of TBT to this region. The commercial value of the marine gastropods was also degraded due to shell shape distortion and thickening, which resulted in a lower meat content. In contrast, inorganic tin is a relatively innocuous compound.

More recently, one of the driving forces for performing speciation studies has been the introduction of legislative requirements, regulating the permissible levels of both

total elemental concentrations and, in some cases, the concentration of specific species *e.g.* tributyltin in UK water quality legislation[6], and methylmercury in fish for human consumption[4]. Speciation methods are now applied to a wide range of analytes, primarily for the determination of inorganic species and compounds of elements toxic[4] to (Al, Sb, As, Cd, Cr, Pb, Hg, Pt, Sn and the actinide series) and essential for life[10] (V, Cr, Fe, Mn, Mo, Co, Ni, Cu, Zn, B, Si, Se, I, F, S, P), and include:

- studies of biogeochemical cycles of chemical compounds
- determination of toxicity and ecotoxicity of selected elements and compounds
- quality control of food products and associated packaging
- control of medicines and pharmaceutical products
- technological process control
- research on the environmental impact of technological installations
- occupational exposure studies
- clinical analysis
- metabolism, biotransformations and excretion

1.2. Speciation in Practice

There are a number of practical difficulties associated with speciation studies, mainly associated with the requirement to preserve the speciation of the analyte in any given sample throughout the analytical procedure. The main stages in the analytical procedure are:

- sampling and storage;
- sample preparation;
- analysis;
- quality assurance and traceability of results.

1.2.1. Sampling and Storage

Sample collection, storage and preparation for analysis are factors which can have a considerable impact on the accuracy and precision of the final results. The stability of the analyte must be considered with regard to sampling and storage (what are appropriate containers?), losses of volatile species (e.g. Se), extractability from the matrix, (*e.g.* sediments), without changing the composition and/or structure of the original analyte components, solubility and stability in various aqueous and non-aqueous solvents, light sensitivity which may cause changes in molecular structure over time, pH sensitivity (will the analyte be effected by acidic or basic solutions?), temperature, and degradation over time.

The choice of sample container can be crucial to preserving sample integrity and should be carefully considered. It should:

- not contain any leachable compounds of the analyte.
- be impermeable to the analyte.
- reduce or prevent photochemical reactions or oxidation\reduction of the analyte.

Factors which determine the speciation of an analyte in the environment also need to be considered during speciation studies. For example, redox conditions govern the oxidation state of Fe and Mn species[7]. Fe(II) and Mn(II) are both soluble in anoxic natural waters, but in oxygenated waters are present as the insoluble Fe(III) and Mn(IV) forms. Furthermore, the pH of an aqueous sample may also have a controlling effect on acid-base equilibria and redox potential which may rule out the use of acidification to preserve sample integrity. The ionic strength of the solute, and one or more of the major ions contributing to ionic strength, may also affect the speciation of the desired analyte[6].

1.2.2. Sample Preparation

Once samples have been collected and suitably preserved the problem of extracting the analyte, while maintaining the integrity of the species, arises. For liquid samples, this should be relatively straightforward providing no changes occur during filtration or centrifugation and the sample can be analysed directly or after dilution. The choice of instrument for analyte detection may necessitate a preconcentration step, which can in itself give rise to experimental errors due to species transformation or loss of analyte[11].

For solid samples, an extraction step with a suitable solvent is usually required. For biological samples, toluene or methanol is regularly used, often in conjunction with ultrasonication to increase the efficiency and speed of the extraction. A soxhlet extraction may also be utilised although this is a longer process and may involve the loss of volatile analytes or speciation information due to the elevated temperatures involved[12].

Soil or sediment samples present further difficulties; drying the sample, albeit at low temperatures in air may still result in the loss of and/or speciation changes to the analyte. Sieving the dried sample into separate fractions may also give inaccurate results as some elements are associated with a particular size fraction and the use of a 'conserved' element such as aluminium as an internal standard may therefore be needed to correct for these errors[6]. The analysis of anoxic sediments and soils presents further problems as care must be taken during drying and storage to ensure anoxic conditions remain.

After a representative solid sample has been obtained various extraction protocols can be utilised. In order to determine the 'bioavailable' fraction a mild extractant or complexing agent such as ethylenediaminetetraacetic acid (EDTA)[13] can be used. One of the many sequential extraction protocols based on the method of Tessier[14] may be employed to determine metals associated with the various soil/sediment fractions. Sequential extractions, however, can be unreliable due to readsorption effects. Bermond *et*

al.[15] reported that sequential extraction protocols cannot reliably estimate trace metal speciation in soils, and the same may be true for other related solid material such as sediments and suspended particulate material (SPM).

Two further problems arise from sequential extraction protocols. First, their multi-step nature increases the risk of experimental errors, and second, the quantity of material required. For example, up to 1g can be difficult to obtain for some types of sample matrix, including suspended particulate material (SPM) as SPM concentrations for productive surface waters[16] are characteristically between 20 and 100 mg l^{-1}.

1.2.3. Analysis

There are a wide variety of separation and detection techniques available for use in speciation analysis, with the main requirements being[4]:

- sensitivity;
- selectivity;
- qualitative analysis;
- quantitative analysis;

A summary of the most commonly used techniques is presented in Table 1-1. In particular, the advent of hyphenated investigation techniques which couple powerful separation methods with sensitive detectors *e.g.* high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) and gas chromatography mass spectrometry (GC-MS) have allowed great advances in speciation studies in recent years.

Table 1-1 Analytical techniques for speciation studies (adapted from Hill[6] and Welz[12]).

Technique	Comments	Example Applications
Gas chromatography	GC has been used with various detectors which tend to be non-specific so peaks from interfering matrix constituents may give rise to confusion in peak identification.	Separations of metalloporphyrins. Determination of organometallic compounds of Sn, As, Pb and Hg.
High Performance Liquid Chromatography	The most popular detectors (UV-Vis) are rarely sensitive enough for speciation studies.	Aluminium in water samples. Cr and V with spectrophotometric detectors. Determination of organomercury compounds in wastewater and sediments.
Polarography/anodic/ cathodic stripping voltammetry	Used to differentiate between oxidation states, for kinetic experiments to determine stability constants of complexed metals. Speciation in the presence of humic/fulvic acids. Ship board monitoring applications.	Speciation of a wide variety of minor constituents of natural waters. Determination of fluorides in the presence of aluminium. Stability constants for complexes of Al, Cu, Fe, Mg, Mn, Pb, Zn in seawater.
Nuclear Magnetic Resonance	NMR can be used for a wide range of elements although the sensitivity is relatively poor (*c.a.* 1mg analyte required).	Aluminium complexes with nucleosides. Food and beverage characterisation. Site specific Isotope fractionation Compound purity.
Gas chromatography-atomic spectrometry	Heated transfer line usually required. Analytes need to be volatile or derivatised. Analytes may condense on cool spots causing sensitivity loss.	Determination of organometallic compounds in waters, biota, flue gases and petroleum. Determination of metalloporphyrins in crude oils.
High Performance Liquid Chromatography-atomic spectrometry	Ease of coupling to spectrometry. High organic content of some solvents may restrict the use of plasma based ionisation.	Determination of organotin compounds in water, molluscs and sediments. Speciation of Sb, Se, and Hg. Determination of arsenic species.
Flow Injection-atomic spectrometry	Simple coupling, FI has been used with numerous detection systems. Similar problems to HPLC-AS	Determination of inorganic Se and Cr species in waters.
Capillary zone electrophoresis-atomic spectrometry	Hard to couple as the flow rates are generally incompatible.	Speciation of organolead compounds in natural waters.
Hydride generation-atomic spectrometry	Simple coupling. Suitable only for species forming volatile derivatives during reduction.	High sensitivity determination of As, Se, Sn, Bi, Te, Sb, Pb, and Hg species.
Gas chromatography MS MS	Allows the resolution of close molecular weight compounds by two dimensional mass spectrometry.	Currently being developed for the identification of previously unresolvable compounds in oils.
HPLC Electrospray mass spectrometry	Simple coupling. Detection of molecular and fragment ions	Structural elucidation and accurrate mass measurements of organic and organo-metallic species.

1.2.4. Quality Assurance and Traceability

Having determined the amount and nature of a particular analyte in a sample the end user also requires information regarding the accuracy and precision of the results before any subsequent decisions are taken. Sulphur, for example, is present in fossil fuels and the maximum permitted mass fraction for automotive fuels sold in Europe is regulated by the European Union. The 1993 limits for sulphur, of 500 $\mu g\ g^{-1}$ and 2000 $\mu g\ g^{-1}$ for petrol and diesel respectively, are to be reduced to 50 $\mu g\ g^{-1}$, for both fuels, by 2005[17]. Suppliers of these fuels therefore have to be sure that the sulphur mass fraction is accurately determined, with a high degree of confidence, to ensure that sulphur removal costs are minimised and that regulatory limits are not breached. Quality assurance can be achieved by the inclusion of an appropriate reference material (RM) for the target analyte in the analytical procedure. With both the fuel suppliers and regulatory authorities conducting sulphur determinations in fuels it is also essential that the analytical results are traceable to the same measurement scale or reference point with a stated degree of confidence.

Traceability is formally defined by the International Organisation for Standardisation (ISO)[18] as

> " The property of the result of a measurement or the value of a standard whereby it can be related to stated references, usually national or international standards, through an unbroken chain of comparisons, all having stated uncertainties."

An example of the concept of traceability is the calibration of an analytical balance by reference masses, which are in turn calibrated, ultimately against national standard masses, the calibration of which can be traced to the primary reference kilogram. Thus, an unbroken chain of comparison is established between the laboratory measurement and a common reference point, ensuring the use of the same unit of measurement.

Measurement consistency is therefore established and the intercomparison of analytical results, both from different laboratories and within the same laboratory[19] becomes possible. Having established a traceable chain of measurements, defined by De Bièvre[20] as " a chain of successive comparisons (*i.e.* measurements) of one value to another value which ends in the value of the unit we have chosen to express the result of our measurement", to primary SI standards it is important to know how 'strong' the links in the traceability chain are. This is given by the uncertainty of each measurement[20], which has been defined by Thompson[21] as "the interval around the result of a measurement that contains the true value with high probability". Traceability and measurement uncertainty are thus intrinsically linked. As traceability relies on the comparison of the measurement result with the value of the reference material the uncertainty of the traceable measurement result has two main components, the uncertainty of the measurement result and the uncertainty of the reference value to which it has been compared[19].

The establishment of the traceability of the result of the complete measurement procedure in analytical chemistry can be achieved by a combination of the following steps[19]

- the use of traceable standards to calibrate measuring equipment
- by using, or by comparison to the results of, a primary method
- the use of a pure substance reference material (RM)
- the use of an appropriate matrix matched certified reference material (CRM)
- by using an accepted, closely defined procedure

A primary method of measurement is currently defined as follows[19]

> "A primary method of measurement is a method having the highest metrological qualities, whose operation is completely described and understood in terms of SI units and whose results are accepted without reference to a standard of the same quantity."

Hence, it follows that conventional external calibration, in which the analyte amount content is extrapolated from an instrumental response curve generated by the measurement of a series of calibration standards, does not meet the requirements of a primary method. Traceability for conventional external calibration can be achieved by direct comparison of measurement results between the primary method and the calibration method[19].

A pure standard RM can also be used to demonstrate traceability provided the response difference of the measurement system for the RM and the sample is demonstrated. However, the correction for instrumental response difference and its associated uncertainty may be unacceptably large, which is a particular problem in the case of the standard additions method or for spiking experiments[19]. A matrix matched CRM, used to compare the measurement results of the unknown with the certified results, can reduce the uncertainty compared with the use of a pure substance RM[19]. A further advantage of a matrix matched CRM is that, provided the certified value is traceable to SI units, then these measurements provide traceability to SI units for the unknown amount of the target analyte in the sample[19].

Isotope dilution mass spectrometry (IDMS) is regarded, by the Comité Consultatif Pour la Quantité de Matrière (CCQM), as having the potential to be a primary method of measurement[19]. IDMS is also regarded as a definitive analytical technique because the precision and accuracy obtainable are unsurpassed by alternative analytical methods[22]. IDMS relies on the measurement of isotope amount ratios, not interpolation from an external calibration curve, with the analyte amount content subsequently

calculated from a measurement equation which is completely described and traceable directly to SI units. Furthermore, a complete uncertainty budget, which meets the highest metrological standards[23], can be readily calculated for chemical determinations made by IDMS.

1.3. Principles of Isotope Dilution Mass Spectrometry

IDMS involves the modification of the natural isotopic composition of the target analyte, contained within a sample, by the addition of an accurately known amount of an isotopically enriched analogue, or spike, of the analyte, which acts as an internal standard. Subsequently, the resultant modified isotope amount ratio is measured by a suitable mass spectrometric technique. IDMS has a number of advantages over conventional external calibration techniques, provided that the following pre-requisites are met[24]:

- more than one, interference-free, stable isotope must be available for isotope ratio measurement;
- an isotopically enriched analogue, or spike, of the analyte must be available;
- complete equilibration between the spike and sample must be achieved;
- the mass fraction and isotopic abundances of the natural and enriched elements must be well characterised;
- the spike and sample must be chemically stable.

1.3.1. Derivation of IDMS Equations

In IDMS two isotopes are chosen, the analyte isotope, A, and the enriched, or spike, isotope, B. In a sample of natural isotopic composition the most abundant isotope is usually chosen as the analyte isotope, A, whilst the spike isotope, B, is generally of very low abundance and is usually selected to be close in mass to the analyte isotope[24]. Conversely, for the isotopically modified spike solution, the analyte isotope, A, is usually

heavily depleted and the spike isotope, B, highly enriched. The isotope amount ratio, R_B, of the analyte to spike isotopes, A:B, in the sample/spike blend is given by Equation 1-1. Throughout this work the subscript x is used to denote material of natural isotopic abundance and the subscript y used to denote isotopically modified material.

$$R_B = \frac{A_y \cdot n_y + A_x \cdot n_x}{B_y \cdot n_y + B_x \cdot n_x}$$

Equation 1-1

where

A_y = the isotope amount fraction of the analyte isotope, A, in the isotopically modified spike solution

A_x = the isotope amount fraction of the analyte isotope, A, in the natural isotopic composition sample

B_y = the isotope amount fraction of the spike isotope, B, in the isotopically modified spike solution

B_x = the isotope amount fraction of the spike isotope, B, in the natural isotopic composition sample

n_y = the number of moles of the analyte in the isotopically modified spike solution

n_x = the number of moles of the analyte in the natural isotopic composition sample

Equation 1-1 can be solved for n_x, the amount in moles of analyte originally present in the unspiked sample, (Equation 1-2).

$$n_x = n_y \cdot \frac{(A_y - R_B \cdot B_y)}{(R_B \cdot B_x - A_x)}$$

Equation 1-2

It is more usual to quote the analytical result as a mass fraction, thus Equation 1-3 is used for the IDMS calculation

$$C_x = \frac{C_y W_y M_x}{W_x M_y} \cdot \frac{A_y - R_B . B_y}{R_B . B_x - A_x}$$

Equation 1-3

where, in addition

C_x = the mass fraction of the analyte in the sample

C_y = the mass fraction of the analyte in the spike solution

W_y = mass of spike

W_x = mass of sample

M_x = molar mass of element in the sample

M_y = molar mass of element in the spike

The isotope amount fraction, *f*, can be calculated according to Equation 1-4. The ion signals for all isotopes of the analyte are measured using a suitable mass spectrometric technique. One isotope is chosen as a reference isotope, r, and the individual isotope amount ratios for all the isotopic ion signals including the reference:reference isotope amount ratio, which by definition is unity, calculated. For an element having *i* isotopes the atom fraction of isotope *q*, f_q, is given by

$$f_q = \frac{\frac{n_q}{r}}{\sum_i \frac{n_i}{r}}$$

Equation 1-4

Where

n_q = ion signal of isotope q

r = ion signal of the reference isotope

By using the terminology in Equation 1-1, where the subscript y denotes the enriched spike material and the subscript x denotes the natural isotopic abundance analyte in the sample, the isotope amount fractions can be expressed in the form of Equation 1-5. If the spike isotope amount fraction B_y is used as the reference isotope then the isotope amount fraction of the analyte isotope in the spike, A_{spike} can be expressed as

$$A_{spike} = \frac{\frac{A_y}{B_y}}{\sum_i \frac{n_{iy}}{B_y}}$$

Equation 1-5

Which can be simplified to

$$A_{spike} = \frac{R_y}{\sum_i R_{iy}}$$

Equation 1-6

Where

R_y = the isotope amount ratio of the analyte isotope to the spike isotope in the enriched spike material

$\sum_i R_{iy}$ = the sum of the ratios of all isotopes to the reference isotope, which in this case is the spike isotope.

Similarly, the isotope amount fraction of the spike isotope, B_{spike}, in the enriched spike can be expressed as

$$B_{spike} = \frac{\frac{B_y}{B_y}}{\sum_i \frac{n_{iy}}{B_y}} = \frac{1}{\sum_i R_{iy}}$$

Equation 1-7

and, with the isotope amount fraction of the spike isotope in the natural isotopic abundance sample taken as the reference isotope, B_x, the isotope amount fraction of the analyte isotope, $A_{natural}$, in the natural isotopic sample can be expressed as

$$A_{natural} = \frac{\frac{A_x}{B_x}}{\sum_i \frac{n_{ix}}{B_x}} = \frac{R_x}{\sum_i R_{ix}}$$

Equation 1-8

Where

R_x = the isotope amount ratio of the analyte isotope to the spike isotope in the natural isotopic abundance sample

$\sum_i R_{ir}$ = the sum of the ratios of the atom fraction of all isotopes to the atom fraction of the reference isotope, which in this case is the spike isotope in the natural isotopic abundance sample.

The isotope amount fraction of the spike isotope, $B_{natural}$, in the natural isotopic abundance sample can be expressed as

$$B_{natural} = \frac{\frac{B_x}{B_x}}{\sum_i \frac{n_{ix}}{B_x}} = \frac{1}{\sum_i R_{ix}}$$

Equation 1-9

Thus, the isotope amount ratio, R_B, of the reference to spike isotopes, A:B, in the sample/spike blend, B, can also be written as

$$R_B = \frac{\frac{R_y}{\sum_i R_{iy}}.n_y + \frac{R_x}{\sum_i R_{ix}}.n_x}{\frac{1}{\sum_i R_{iy}}.n_y + \frac{1}{\sum_i R_{ix}}.n_x}$$

Equation 1-10

Which can be solved for n_x, the number of moles of analyte present in the unspiked sample giving Equation 1-11.

$$n_x = n_y.\frac{R_y - R_B}{R_B - R_X}.\frac{\sum_i R_{ix}}{\sum_i R_{iy}}$$

Equation 1-11

In this form of the IDMS equation the amount of substance can be directly replaced by an amount content (mol kg^{-1}) or mass fraction *e.g.* ng g^{-1}, or any other appropriate unit provided the use of units remains constant throughout the equations[24]. Thus Equation 1-11 can be expressed in terms of a mass fraction

$$C_x = C_y \cdot \frac{m_y}{m_x} \cdot \frac{R_y - R_B}{R_B - R_x} \cdot \frac{\sum_i R_{ix}}{\sum_i R_{iy}}$$

Equation 1-12

where, in addition

C_x = is the mass fraction of the analyte in the unspiked natural isotopic abundance sample

m_x = the mass of natural isotopic abundance sample

C_y = the mass fraction of the isotopically modified spike material

m_y = the mass of the isotopically modified spike material added to the natural isotopic abundance sample

The advantage of Equation 1-12 is that the isotope amount fractions, in the sample and the spike, are not correlated[23], as they are in Equation 1-3. The latter case increases the complexity of the calculation of the measurement uncertainty of the analytical result but is not the case however for the ratio form (Equation 1-12) of the IDMS equation, thus simplifying measurement uncertainty calculations[23].

1.3.2. IDMS Advantages and Disadvantages

Incomplete analyte extraction can lead to low recovery when an external calibration standard is used to determine the analyte concentration. In IDMS, however, problems associated with incomplete extraction are negated, provided equilibration between the sample and spike isotopomers is complete[22,24]. If the spike is not fully equilibrated with the sample, a different extraction efficiency for the spike and sample will result, yielding bias in the measurement. For liquid samples, equilibration by gentle agitation should be sufficient. For solid samples complete equilibration may prove problematic because the analyte can be both adsorbed onto the surface and contained

within the lattice structure of the sample matrix, hence a destructive digestion may be required.

IDMS also has the ability to overcome a wide variety of chemical and physical interferences because the effect of these interferences would be expected to have the same effect on both of the measured isotopes, and these effects are thus cancelled in the measured isotope amount ratio[25]. However, isobaric interferences, which often apply to only one isotope in the measured isotope amount ratio, *e.g.* ^{204}Hg on ^{204}Pb, are not accounted for by IDMS. A further advantage of IDMS is that, assuming complete equilibration, losses of analyte are compensated for by losses of the spike in the same proportion because the measurand is an isotope amount ratio, rather than absolute analyte amounts[24]. This ensures an accurate determination of the target analyte, but should not be taken as an excuse for using poor experimental procedure.

IDMS does have a number of drawbacks, which are mainly cost related. Isotopically enriched spike materials, which may not be available for the analyte of interest, must be purchased and, for high accuracy and precision, expensive mass spectrometric instrumentation is required, operated by an experienced and well trained analyst[24].

1.3.3. Accuracy and Precision in IDMS

In order to achieve the best accuracy and precision a number of factors must be taken into account, Sargent *et al.*[24], in a recently published guide, summarised the critical stages as follows:

- sample preparation
- selection of the most appropriate isotopic internal standard
- characterisation of the isotopically enriched analogue

- addition of the isotopically enriched analogue
- blank correction
- instrumental analysis
- calculation of the result
- estimation of uncertainty

with the important sources of error given as:

- less than complete equilibration between the sample and spike will lead to significant systematic errors;
- isobaric and polyatomic ion interferences;
- isotopic discrimination *e.g.* isotopic fractionation, detector dead-time and mass bias during instrumental analysis.

1.3.4. Total Elemental Applications of IDMS

Isotope dilution as a quantitative technique for elemental analysis was first applied by Reynolds[26] in 1950 to determine the amounts of decay products, after neutron irradiation, of Cu^{64}, Br^{80}, Br^{82} and I^{128}. The advent of readily available user friendly instrumentation subsequently allowed elemental IDMS to be rapidly developed[24]. During the 1970's isotope dilution was introduced for the quantification of organic analytes by gas chromatographic mass spectrometry (GC-MS)[24], with spike compounds labelled with either ^{2}H, ^{13}C or ^{15}N, recent examples of which include the determination of ethanol[27] in two CRM's and cholesterol in human serum[28].

IDMS is ideally suited to analyte measurements in human serum as the complex sample matrix presents difficulties when external calibration is applied, due to factors such as different analyte ionisation efficiencies, unless the standards are closely matrix matched to the samples. There is a fundamental difference between organic and inorganic IDMS[24]. In organic IDMS the sample contains negligible amounts of the

isotopically labelled analogue, with the spike containing negligible amounts of the natural abundance analyte. For inorganic IDMS however the spike isotope is often present in significant amounts in the sample and the reference isotope present, albeit in depleted amounts in the spike material[24].

Yoshinga and Morita[29] undertook a study to compare the accuracy and precision of three analytical methods, IDMS, standard additions and external calibration, with ICP-MS as the detector, for the determination of total Hg in National Institute for Environmental Studies (NIES) CRM No. 13 human hair. IDMS, standard additions and external calibration with Pt as an internal standard gave accurate results in good agreement with the certified value and its associated uncertainty. However, external calibration with Tl as an internal standard gave results significantly lower than the certified value, which the authors attributed to the difference in the first ionisation potentials of Tl and Hg, 6.1 and 10.4 eV respectively, thus highlighting the need for the careful selection of an appropriate internal standard. The precision of the IDMS and external calibration analyses, $n = 5$, was comparable, 1% RSD and better than the CRM uncertainty of 4.5% RSD. The precision for the standard additions analysis however was poor at 10% RSD, by its nature this method is also more time consuming, requiring 3 times the number of samples.

The same authors also demonstrated the advantage of IDMS to overcome matrix effects during ICP-MS determinations of Hg in various sediment CRM's[29]. Isobutylmethylketone (IBMK), which is fairly soluble in aqueous solutions, was employed as a back extractant in the analytical procedure. The IBMK was therefore present, in varying proportions, in the samples presented for analysis, thus causing varying degrees of signal suppression, of up to 70%, of the Hg ion counts, giving rise to variable recoveries when either external calibration or standard additions was employed. In comparison, when using IDMS, the accuracy of the determinations of Hg in sediments was not affected by this varying signal suppression because the signals of the measured Hg isotopes were

affected to the same extent, and thus cancelled when the measured isotope amount ratio was calculated.

For some elements spectral interferences from polyatomic ions, *e.g.* $^{40}Ar^{16}O$ on ^{56}Fe and $^{40}Ar^{16}O^{1}H$ on ^{57}Fe, prevent accurate isotope amount ratio measurements by quadrupole ICP-MS. Sariego Muñiz *et. al.*[30] applied IDMS to the determination of Fe, Cu and Zn in human serum using a multielement spike solution enriched in ^{57}Fe, ^{65}Cu and ^{67}Zn. In order to overcome the spectral interferences, arising from polyatomic species formed from elements present in the sample matrix and ICP gases, a double focussing ICP-MS was employed, operating at a resolution of 3000. The multi-element IDMS procedure was validated by the analysis of Fe, Cu and Zn in NIST SRM 1598, Inorganic Constituents in Bovine Serum, with good agreement between the found and certified values.

In order to determine the correct isotope amount ratio by ICP-MS it is necessary to compensate for mass bias in the instrumentation. Quadrupole and sector field mass spectrometers and their associated ion optics do not transmit ions of different masses equally. This effect, known as mass bias, arises primarily from space-charge effects in the plasma interface and during ion transfer[31] which results in lighter ions being more readily deflected from the ion beam whilst heavier ions are preferentially transported[32]. Thus, if an elemental solution composed of two isotopes with an exactly 1:1 molar ratio is analysed using ICP-MS, a 1:1 isotope amount ratio will not necessarily be observed. The magnitude of the mass bias depends on isotope mass and the type of mass spectrometer used, but generally tends to be greatest at low mass and decreases with increasing mass[32]. Even very small mass-biases can have deleterious effects on the accuracy of isotope amount ratio determinations, so a correction must always be made, usually in one of two ways, bracketing or interpolation.

A reference material, certified for the isotope amount ratio of the isotopic pair under study, if available, can be used to determine the instrumental mass bias factor. The

isotope amount ratio of the CRM is measured immediately prior and post the sample analysis *i.e.* the sample is bracketed by the CRM. Subsequently the deviation of the measured isotope amount ratio from the certified value can be used to calculate the instrumental mass bias factor for the isotope pair under study. This approach is limited by the paucity of isotope amount ratio CRMs available.

It is also possible to use an alternative element, with an isotope pair of similar mass to the isotope under study, and which has a certified isotope amount ratio. The mass bias correction can be performed by interpolation using either a linear, power, or logarithmic equation. For example, the ^{205}Tl:^{203}Tl ratio can be used to correct the mass bias of ^{207}Pb:^{206}Pb. The advantage of this approach is that the mass bias correction can be measured at the same time as the isotope amount ratio of interest by spiking the sample with the isotopically certified RM.

A third approach is to prepare a calibration blend of a natural standard and the spike solution, which is matched to the spiked sample in both isotope amount ratio and ion signals to within 5% for both parameters[24]. The calibration blend is then used as a bracketing solution to measure the instrumental mass bias factor. The advantage of this approach, known as 'approximate signal matching IDMS', is that a 'double' IDMS equation can be formulated from which the mass fraction of the spike solution, which is often relatively poorly characterised and hence significantly contributes to the measurement uncertainty[57], is removed and the effects of detector linearity and detector dead time minimised.

Catterick *et al.*[57] determined the concentration of Fe, Mg and Cd in three CRM's (soft water, hard water and VDA plastic) by IDMS using the approximate matching procedure for the isotope amount ratio and the ion signal strength. The analytical results were in very close agreement with the certified values, with uncertainty estimates (coverage factor, k, of 2) at the 95% confidence level of at least 50% lower than the stated

uncertainty for the reference materials (*e.g.* Cd in VDA plastic: determined value 40.6 ± 0.6, certified value; 40.9 ± 1.2 μg/g). Turner *et al.*[33] compared electrothermal vaporisation (ETV) ICP-MS using external calibration with ETV-ID-ICP-MS, using the approximate matching approach, for the determination of Se in water and serum CRM's. The uncertainties associated with determinations by the ETV-ID-ICP-MS method were a factor of 4 less then those associated with external calibration ETV-ICP-MS. Accuracy was significantly greater by ETV-ID-ICP-MS, with the results in very close agreement with the certified values.

1.4. IDMS for Speciation Studies

Species specific IDMS, coupled with an elemental selective detector, has been undertaken for both inorganic species (*e.g.* iodine/iodate[34] by LC-ICP-MS and CrIII/CrVI by HPLC-ICP-MS[35]) and organometallic species (*e.g.* mono and dimethylmercury by GC-ICP-MS [36]). IDMS has also been used to measure species that have been previously undetectable in environmental samples. Dimethylthallium (Me_2Tl^+) had previously only been measured in laboratory experiments with detection limits at the level of ng/ml, a factor of 1000 higher than levels expected in the environment[37]. Open ocean seawater samples, collected from depths of between 10 and 4000 m from the Southern Atlantic, were spiked with the ^{203}Tl enriched dimethylthallium[37]. The ^{203}Tl:^{205}Tl isotope amount ratio was measured by positive thermal ionisation mass spectrometry, after preconcentration by an anion exchange resin, separation of inorganic Tl species by methyl isobutyl ketone, and sample clean-up stages to improve ionisation efficiency. The Me_2Tl^+ detection limit was 0.4 pg/ml for a 500ml sample and 1.4 pg/ml for total Tl for a 50 ml sample. There was a variation of between 3 and 8% for parallel sample determinations. It was found that between 3-48% of the total thallium concentration was Me_2Tl^+ and, as this compound is not known as an anthropogenic substance, that production of Me_2Tl^+ is the consequence of bioactivity.

The challenge for analysts employing IDMS for speciation studies is to achieve the same accuracy and precision inherent in the method when applied to total elemental determinations, provided the aforementioned requirements have been met (Section 1.3.3). In addition to these requirements for total elemental IDMS, further prerequisites must be carefully addressed in order to obtain accurate and precise results[38]:

- isotopically enriched analogues, or isotopomers, of the target analyte(s) must be synthesised or purchased;
- relatively mild extraction conditions are often necessary in order to maintain the integrity of the species, which may result in incomplete analyte extraction. This is not a problem, however, provided that complete equilibration of the spike and sample occurs;
- it is essential that, during analyte extraction, equilibration, separation, and measurement of the isotope amount ratio, the sample and spike isotopomers are chemically inert. Any analyte formation or decomposition, ligand exchange between the sample and spike isotopomers and/or spike decomposition will give biased results;
- the chromatographic system, and its coupling to the mass spectrometer, must be optimised for robustness, analyte stability, instrumental stability and sensitivity;
- data extraction from the resulting chromatograms.

1.4.1. Spike Isotopomer Synthesis

IDMS necessitates the purchase of isotopically enriched elements for production of the spike solution. For species-specific IDMS it is also necessary to incorporate the isotopically enriched element into the target species, which will involve synthesis and purification of the compound. Isotopically enriched elements are much more expensive than the natural isotopic abundance equivalent, ranging from dollars per mg to hundreds of dollars per mg, often with a minimum order value, which can further increase the cost of analysis. This increased expense means that the synthesis and purification procedures must be as efficient as possible. It is often not possible for large-scale synthesis and purification

procedures to be scaled down to the sub gram level without large losses of the expensive, isotopically enriched starting material. This requires novel synthetic procedures to be developed to accomplish this analytical requirement. Sutton *et al.*[39] have devised new synthetic routes for the small scale production of a suite of environmentally relevant organotin compounds (*e.g.* dibutyltin dichloride) with product yields of up to 90%. Schedlbauer and Heumann[37] synthesised Me_2Tl^+ from ^{203}Tl enriched elemental thallium using a two step synthesis which gave a yield of 25%. The stability of the Me_2Tl^+ was evaluated and under optimum storage conditions (pH 2, 0 °C) the compound was found to be stable for 30 months.

1.4.2. Analyte Stability

It is well documented in the literature[40,41,42,43,44,45,46,47,48] that methylmercury is produced from inorganic Hg, and can decompose during sample preparation and GC separation, for determinations by MS and ICP-MS. Hintelmann *et al.*[49] observed methylmercury formation during analysis by both GC and HPLC-ICP-MS. Butyl group transfer has also been observed during microwave extraction of butyltin species from two sediment CRM's, followed by separation and detection of butyl Tin species by species specific ID-GC-ICP-MS[50]. Hill *et al.*[22] observed species transformations during the determination of organotin compounds by HPLC-ID-ICP-MS. These studies have shown that the target analyte is unstable during the measurement process. Nuclear magnetic resonance (NMR) spectroscopy is a non-destructive technique which allows samples to be taken at any stage of the measurement procedure and a definitive spectrum of NMR sensitive compounds present realised. Organometallic molecules contain at least two nuclei, ^1H, and ^{13}C, which are sensitive to and easily observable by NMR. Organometallic compounds enriched in ^{13}C will give, due to coupling with ^1H atoms, a spectral signal which reflects the enrichment factor compared to their natural isotopic abundance

isotopomers. These spectral differences, can be exploited to investigate the stability of the organometallic species during species specific isotope dilution mass spectrometry.

1.4.3. Quality Control and Assurance

The final consideration for speciation studies is that of quality control and quality assurance[51] which can be achieved by the use of a certified reference material (CRM). However, they are of limited availability for speciation studies, making the selection of an appropriate CRM, which is matrix matched to the sample, difficult at times. A second method is to use spiking and recovery experiments to validate results of an analysis. Extraction efficiency can be evaluated by an acid digestion of a sub-sample followed by total elemental determination[6]. An appropriate extraction method is then chosen for use on a second sub sample followed by species specific analysis and the concentration of each species summed. If this summed concentration and the total concentration determined earlier are in close agreement the extraction efficiency can be considered to be 100%. If the extraction efficiency is less than 100%, but reproducible with an RSD of ≅ 5%, the method may be considered to be under control and valid. If the RSD is much higher *e.g.* extraction of 80% ± 20% then the method is not under control and will not produce accurate results[6].

1.4.4. Separation and Detection Techniques

1.4.4.1. Choice of Separation Technique

For speciation analysis a separation stage is usually required to isolate the target analyte from other species of the same or different elements, present either in gaseous or liquid samples, or co-extracted with the target analyte from the solid phase. Gas chromatography (GC) is ideally suited for volatile species, with a requirement to derivatise non-volatile inorganic and organometallic compounds into volatile compound, which inherently changes the speciation of the analyte during the measurement process. For example, of the three oxidation states of mercury, Hg^0, Hg^I and Hg^{II}, only Hg^0 is volatile,

therefore non-volatile mercury species must be derivatised, usually by ethylation with sodiumtetraethylborate ($NaBEt_4$), before separation by GC. Thus, the risk of undesirable reactions involving the sample and/or, in the case of species specific IDMS, the spike isotopomers is increased. The derivatisation process can also significantly add to the time required for analysis. A further problem using GC can be the coupling of a heated transfer line, which must be maintained at the column outlet temperature of up to 400 ^{0}C, to the ICP-MS sample inlet system. Any 'cool spots' will lead to analyte condensation with a subsequent loss of accuracy. In addition, a metallic transfer line may act like an aerial and couple with the radio frequency used to maintain the plasma, which may cause plasma instability and possibly instrument failure[6]. It has also been reported that methylmercurychloride is corrosive to packed and capillary GC columns[52].

In comparison with separation by GC, high performance liquid chromatography (HPLC) does not require derivatisation of the analyte, with a plethora of chromatographic conditions available in the literature for the separation of mercury species, prior to detection by a wide range of spectroscopic detectors[53]. HPLC mobile phases however, often comprise a high percentage of organic modifier *e.g.* acetonitrile or methanol, which can cause a number of problems, such as plasma instability and carbon deposition in the interface region and on the tuning lenses, if ICP-MS is coupled as the detection system. Nevertheless, these problems can be overcome by optimisation of the HPLC ICP-MS interface.

1.4.4.2 Mass Spectrometric Detection

Traditionally thermal ionisation mass spectrometry (TIMS) has been used for isotope ratio determinations[54], however, unlike inductively coupled plasma mass spectrometry (ICP-MS), it is not suited for elements such as mercury, which have a high first ionisation potential[29]. ICP-MS also offers a greater sample throughput, is tolerant of complex sample matrices, utilises smaller sample sizes, can be easily coupled with various

separation techniques and does not suffer from time dependent mass bias effects[55]. The development of multicollector sector-field (MC-SF) ICP-MS instruments, enabling the simultaneous measurement of each isotopic signal[56], has greatly improved the accuracy and precision of isotope amount ratio determinations, *c.a.* 0.005% relative standard deviation of the mean[57], compared to quadrupole ICP-MS instruments and is comparable to that obtainable with TIMS instrumentation[54].

1.5. Measurement Uncertainty

Quevauviller[58] and Quevauviller *et al.*[59,60] describe two parameters that should be considered when reviewing analytical results: (a) accuracy ("absence of systematic errors") and (b) uncertainty (coefficient of variation or confidence interval) produced by random errors and random variations in the procedure. As the 'true' value of the amount of analyte in a sample cannot be known[19] the accuracy of an analytical procedure can only be assessed by inference from the results of separate analyses for the same analyte, using the same method, on certified reference materials. Whilst it is desirable to use matrix matched CRM's, in practice it is possible to use several reference materials of different matrix types to give a greater degree of certainty for the result of the unknown samples. Quevauviller[59] refers to "statistical control" as a viable means of ensuring that a high quality of laboratory results is maintained. This can be achieved by applying simple statistical analyses, such as t-tests and analysis of variance to ensure that methods are sufficiently reproducible. Typically, the standard deviation of a number of replicate analyses is calculated to give the range within which the stated result is likely to lie.

A more holistic approach to the assessment of the accuracy and precision in chemical measurements is the use of an uncertainty budget. The estimation of the uncertainty associated with an analytical measurement allows improved intercomparison of analytical results[61], and is a requirement for ISO accredited methods[33]. The uncertainty of a

measurement is very different to the error; where error is the result of a measurement minus the 'true' value of the measurand[62] (which we cannot know[19]).

The ISO Guide to the Expression of Uncertainty in Measurement[63] has established general rules for evaluating and expressing uncertainty for a wide range of measurements and has subsequently been interpreted for analytical chemistry by Eurachem[19]. The Eurachem guide sets out procedures for the evaluation of uncertainty in analytical chemistry. The main stages in the process are identified as:

- specification - write down a clear statement of what is being measured, including the full expression used to calculate the result;
- identify uncertainty sources - produce a list of all the sources of uncertainty associated with the method;
- quantify uncertainty components - measure or estimate the magnitude of the uncertainty associated with each potential source of uncertainty identified;
- calculate total uncertainty - combine the individual uncertainty components, following the appropriate rules, to give the combined standard uncertainty for the method;
- apply the appropriate coverage factor to give the expanded uncertainty.

With the major sources of uncertainty identified as[19]

- Sampling - where sampling forms part of the procedure, effects such as random variations between different samples and any bias in the sampling procedure need to be considered.

- Instrument bias - *e.g.*, calibration of analytical balances.
- Reagent purity - *e.g.*, the purity of reagents used to prepare calibration standards will contribute to the uncertainty in the concentration of the standards.
- Measurement conditions - *e.g.*, volumetric glassware may be used at temperatures different from that at which it was calibrated.
- Sample effects - The recovery of an analyte from the sample matrix, or an instrument response, may be affected by other components in the matrix. When a spike is used to estimate recovery, the recovery of the analyte from the sample may differ from the recovery of the spike, introducing an additional source of uncertainty.
- Computational effects - *e.g.*, using an inappropriate calibration model.
- Random effects - random effects contribute to the uncertainty associated with all stages of a procedure and should be included in the list as a matter of course.

When all the uncertainties for a particular method have been identified, and combined using error propagation laws, an estimate of the overall measurement uncertainty for the method is produced, allowing the analyst to determine if the method is producing results fit for their purpose. A further advantage of the use of a full uncertainty budget is that it allows the analyst to identify the contributions of each area of the measurement procedure to the overall uncertainty, and hence attempt to minimise these uncertainties[64]. For example, if the blank correction in Ni analysis by ICP-MS contributes 83% of the overall uncertainty then major gains in reducing the measurement uncertainty can be obtained by improving the blank measurement.

1.5.1 Evaluating Uncertainty

The estimation of measurement uncertainties in ICP-MS analysis using a "cause-and-effect" approach[61, 65] is a useful and easily applied method for calculating an uncertainty budget. Figure 1-1 presents a flow chart of the steps necessary to construct an uncertainty budget. The cause and effect diagram, sometimes known as an Ishikawa or "fishbone" diagram, is constructed to enable easy identification of uncertainty sources associated with the method. A cause and effect diagram for the determination of Ni by external calibration ICP-MS is presented in Figure 1-2. The purpose is to generate an estimate of overall uncertainty without a detailed quantification of all the components. The diagram typically contains a branched hierarchical structure reducing to a single outcome, in this case an analytical result. Elements within the structure may contain uncertainties from sources such as analyte recovery (including extractability), measuring devices (*e.g.* tolerances for: balances, pipettes, volumetrics, dilution errors), repeatability, calibration, temperature and internal standards errors, which are then combined using error propagation laws.

Figure 1-1 Uncertainty budget procedure flow chart

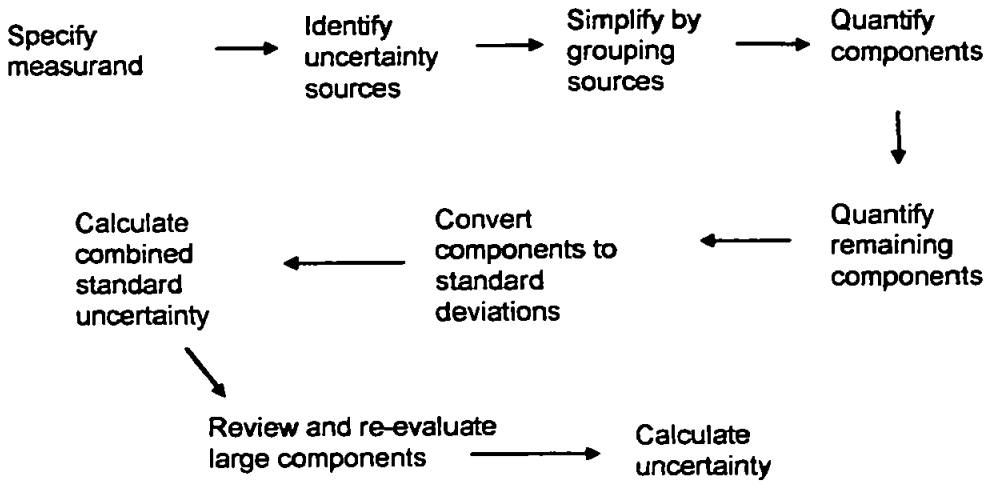
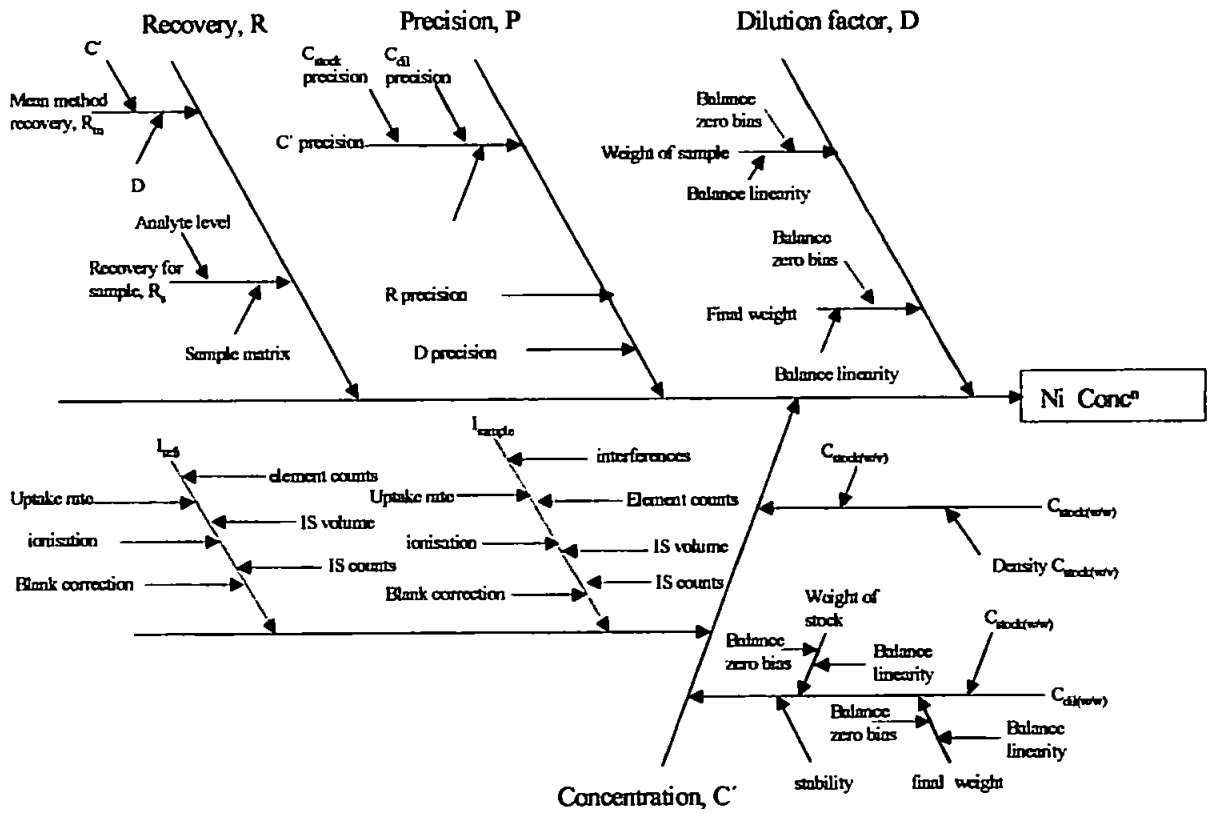


Figure 1-2 Cause and effect uncertainty diagram for the determination of Ni by ICP-MS.



Cause and Effect diagram for analysis of Ni by ICP-MS - V.J.Barwick, S.L.R.Ellison, B.Fairman, *Analytica Chimica Acta*, 1999, 394, 281.

A second approach is to use the equation used to calculate the analytical result as the model for the uncertainty budget. For example, when a standard solution is prepared, by dissolving a solid material in an appropriate solute, the final mass fraction, C, is calculated from

$$C = \frac{M_1 \times P}{M_2}$$

Equation 1-13

Where M_1 is the mass of solid, P is the purity of the solid and M_2 the diluent mass. Each of these terms will have an associated uncertainty *e.g.* the purity of the starting material may be quoted as 99.98 ± 0.02% whilst the mass of the solid and diluent have been measured to 0.0109 ± 0.0005 g and 15.2558 ± 0.0001g respectively. The uncertainty on the final mass fraction will therefore have uncertainty contributions arising from each of the components in Equation 1-13.

The individual uncertainty contributions can be combined using the spreadsheet method first described by Kragten[66], which assumes that the standard uncertainties of each measurement parameter are relatively small compared to that measurement parameter, and gives acceptable accuracy for practical purposes. The spreadsheet, which provides a simple method for the estimation and combination of uncertainties, estimates the uncertainty each parameter contributes to the overall uncertainty for the analytical result. Subsequently the individual uncertainty estimates are combined using error propagation laws to give a combined standard uncertainty for the measurand in question.

1.5.2. Uncertainties for Isotope Amount Ratio Analysis

Sanz-Medel and co-workers[67,68] investigated sources of uncertainty for lead isotope amount ratio measurements by quadrupole, double focusing and multicollector ICP-MS

instruments. Thallium was used as an internal isotope amount ratio standard and the isotope amount ratio accuracy was evaluated using NIST 981 Common Lead Isotope standard reference material and an enriched ^{204}Pb spike. Mass bias correction was performed using an exponential model for all three instruments and equations for the evaluation of the total combined uncertainty arising from the correction for dead time and mass bias were developed. They observed[67] that, in correcting for mass bias, the uncertainty in the Tl ratio contributed the main source of uncertainty for the multicollector instrument, but for the quadrupole and double focusing single collector instruments, the measured isotope amount ratio was the main source of uncertainty.

1.6. Aims and Objectives

This study has been instigated as part of the Department of Trade and Industry National Measurement System Valid Analytical Measurement (VAM) programme. The aim of this work was to investigate species specific isotope dilution mass spectrometry with regard to its accuracy and precision, thus attempting to answer the questions posed about speciation analysis at the beginning of this chapter.

Mercury was chosen as a suitable element for this investigation because:

- it is an important element from an environmental and toxicological point of view;
- it forms inorganic and organometallic species for which there exist established methods of analysis;
- it forms both volatile and non-volatile inorganic and organometallic species;
- the redox behaviour of inorganic species is well characterised;
- the ^{199}Hg nucleus, with a spin value of $I = ½$, is suitable for NMR spectroscopy;
- mercury has seven stable naturally occurring isotopes so is suitable for IDMS.

The objectives of this programme of study were to therefore develop techniques for:

- the synthesis and characterisation of isotopically enriched mercury species;
- monitoring the stability of the sample and spike isotopomers throughout the IDMS procedure;
- monitoring the extent of equilibration between the sample and spike isotopomers;
- optimisation of the coupling of the separation method to the ICP-MS system with regard to analyte stability, separation, plasma stability, instrumental sensitivity and the accuracy and precision of the measured isotope amount ratios;
- the formulation of uncertainty budget for the determination of mercury species by HPLC-ICP-MS and subsequent minimisation of the expanded uncertainty for the analytical result.

Primarily, the investigation will establish the extent to which mercury species interact with suspended particulates in equilibration solutions. These interactions of species with particulates may prevent accurate determination of the "true" concentrations of mercury species within a particular sample. Spike equilibration and species extractability will be assessed and isotope amount ratio determinations performed using Multi Collector and quadrupole ICP-MS. The effects of the different ICP-MS instrument configurations, and hence the accuracy and precision of the isotope amount ratio determinations, on the measurement uncertainty for IDMS will thus be calculated. Speciation studies will be conducted using HPLC-ICP-MS. Nuclear Magnetic Resonance spectroscopy will be employed to characterise the various species forms in order to verify their purity, stability and any functional group exchange effects.

Chapter 2 Isotopically Enriched Methylmercury Synthesis and Nuclear Magnetic Resonance Spectroscopy Studies

2. Introduction

Validation of an analytical method establishes, by systematic laboratory studies, that the method is fit-for-purpose, i.e. its performance characteristics are capable of producing results in line with the needs of the analytical problem[69]. One of the requirements of speciation analysis is that the chemical form of the analyte of interest is not altered during the measurement procedure. Species specific IDMS, which necessitates the synthesis of an isotopically enriched analogue of the target analyte, has the power, provided equilibration between the sample and spike is complete, to overcome incomplete analyte extraction and losses of the sample/spike blend. However, it is essential that the target analyte and spike material, in this case methylmercury, which is known to be unstable during conventional analytical procedures[70], are stable during the IDMS procedure otherwise biased results would be obtained. This chapter addresses the synthesis of isotopically enriched methylmercury, its characterisation using ^{1}H nuclear magnetic resonance (NMR) spectroscopy, and NMR spectroscopy studies of the stability of methylmercury during IDMS equilibration and HPLC separation.

2.1. Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic Resonance (NMR) spectroscopy is an isotope specific technique based on the principle that many nuclei have a magnetic moment, or a non-zero spin quantum number. Of these, isotopes having a ground state nuclear spin, I, of ½ are the easiest to study. In a magnetic field, such nuclei have a lower energy when aligned with the field than when opposed to the field. The energy difference between the aligned and opposed states corresponds to radio frequencies in the electromagnetic spectrum, hence the nuclei are able to absorb and re-emit radio waves. The precise resonance frequency of the

individual nucleus is dependent on the effective magnetic field at that nucleus, and is affected by electron shielding, which is in turn dependent on the chemical environment. This frequency dependence, measured as the chemical shift (δ, ppm), makes NMR spectroscopy a powerful analytical tool. Since the value of the chemical shift is proportional to the magnetic field strength the resonance frequency is ratioed to the spectrometer frequency to normalise it. As such, chemical shifts measured using different spectrometers can be compared directly.

NMR spectroscopy is primarily employed for the structural elucidation of organic molecules. The sample (typically 1 mg) is placed in a constant, homogenous magnetic field which induces individual nuclei within the molecule to align with or against the direction of the field. A pulse of radio frequency (RF) energies is applied, thereby effecting a change in the orientation of the NMR sensitive isotopes within the magnetic field. Following this 'resonance' state the energised isotopes 'relax' back to their original orientation. It is these energy changes that are measured to give an NMR spectrum, which can then be enhanced by repeated cycles of RF pulsing/relaxation to improve sensitivity. Data is acquired as a resonance frequency versus time spectrum, which is subsequently processed to a resonance frequency versus amplitude spectrum by means of a Fourier transform algorithm.

The most common isotope studied by NMR spectroscopy is the proton (^{1}H), as it the most receptive and abundant, followed by ^{13}C. However there are over 20 isotopes in the periodic table having $I = ½$ and are thus relatively straightforward to study. An important feature of NMR spectroscopy is that solvents employed should ideally not contain any of the isotope under study. Thus, deuterated solvents are employed for use in ^{1}H NMR. The use of deuterated solvents also allows the magnetic field around the sample to be built homogeneously, and, as deuterated solvents typically contain up to 0.1% ^{1}H, provide a reference point from the residual protonated solvent.

A further feature of NMR spectroscopy is that other NMR active isotopes in the molecule(s) under study will also induce changes in the spectrum due to magnetic interactions, or coupling, between these isotopes and the isotope under study. The relative intensities of these signals, or satellites, to the main peaks reflect the isotopic composition of the coupling nuclei within the molecule. Sutton *et al.*[39] took advantage of this aspect of NMR spectroscopy to characterise a suite of isotopically enriched organotin compounds, synthesised for species specific IDMS, and determine previously unreported ^{13}C chemical shifts and tin-carbon coupling constants.

In the present study, monomethylmercury, CH_3Hg^+, comprises four NMR sensitive nuclei, ^{13}C, ^{1}H,^{199}Hg and ^{201}Hg. Of the two NMR sensitive mercury isotopes ^{199}Hg has a nuclear spin quantum number of ½ and is thus readily measured whilst ^{201}Hg, with a spin quantum number of 2/3 and an electric quadrupole moment, is difficult to study by NMR spectroscopy. Therefore, ^{199}Hg was chosen as the spike isotope for both the species specific IDMS analyses and the stability studies by NMR spectroscopy. Due to the coupling of protons to ^{199}Hg and ^{13}C in the monomethylmercury molecule, enrichment in either of these isotopes will produce a spectrum that differs from the natural isotopic abundance molecule. NMR spectroscopy of organomercury compounds of natural isotopic abundance and analogues enriched in the NMR sensitive isotopes, ^{13}C and ^{199}Hg, therefore enables ligand rearrangement or formation/decomposition reactions to be observed, and the determination of which stage of the IDMS procedure *e.g.* equilibration or column separation, necessary reaction conditions, *etc*, to be elucidated. Organomercury compounds are highly toxic to human health, therefore, strict handling protocols were followed during synthetic procedures, NMR spectroscopy studies and ICP-MS analysis. A copy of the procedure for handling organomercury compounds is appended to this thesis.

2.1.1. Methylmercury Synthesis

Two possible methods were considered for the synthesis of ^{199}Hg enriched methylmercury chloride for use as a spike material during species specific IDMS. The requirements for the synthetic method were: simplicity, in order to reduce the risk of contamination from natural isotopic abundance Hg; high yield to minimise losses of isotopically enriched Hg; the recovery of the final product as a solid; safety and rapidity.

Snell *et al.*[71] prepared ^{198}Hg enriched methylmercurychloride using a two stage reaction (Equation 2-1 and Equation 2-2), to give an overall reaction efficiency of 88%.

$$^{198}HgCl_2 + 2(CH_3MgCl) \rightarrow (CH_3)_2{}^{198}Hg + 2MgCl_2$$

Equation 2-1

$$(CH_3)_2{}^{198}Hg + {}^{198}HgCl_2 \rightarrow 2CH_3{}^{198}HgCl$$

Equation 2-2

However, this synthesis has a number of drawbacks. Dimethylmercury is extremely toxic, so stringent safety procedures are required during the synthesis. The overall reaction time, including extraction and purification, was in excess of 14 hours and the final product was dissolved in a toluene/tetrahydrofuran solution requiring further time for solvent evaporation.

Rouleau and Block[72] synthesised ^{203}Hg methylmercurychloride by direct nonenzymatic methylation, using methylcobalamin ($CoC_{63}H_{91}N_{13}O_{14}P$) as the methyl group donor, (Equation 2-3) where methylcobalamin is represented by $Co\text{-}CH_3$.

$$Co - CH_3 + {}^{203}HgCl + H_2O \rightarrow (H_2O)CoIII^+ + CH_3{}^{203}HgCl$$

Equation 2-3

Methylcobalamin is a Vitamin B12 co-enzyme that can be considered to be a cobalt(III) corrinoid complex containing a co-ordinated carbanion (CH_3^-) which can be displaced under suitable reaction conditions by the electrophillic attack of Hg^{2+} ions, forming an aquocobalamin complex and the monomethylmercury anion[73], CH_3Hg^+. The synthetic procedure described by Rouleau and Block[72] is straightforward, can be completed in less than 4 hours, gives reaction efficiencies of ≥ 90% and is not known to produce dimethylmercury. The synthesised methylmercury was extracted from the aqueous phase via a benzene/hexane mixture with a back extraction into an aqueous disodium carbonate solution. However, this final extraction yields methylmercuryhydroxide as the final product. In this study methylmercurychloride was required for the species specific IDMS experiments, and a solid product was necessary for dissolution in deuterated solvents for the NMR spectroscopy stability studies. Hence the synthetic method of Rouleau and Block[72] was therefore modified to extract the ^{199}Hg enriched methylmercurychloride from the benzene/hexane extractant.

2.2. Experimental

2.2.1. Methylmercury Recovery from Benzene/Hexane

Experiments were conducted to assess the efficiency of, and time required for, the evaporation of the benzene/hexane extractant. Three solutions of approximately 5 mg ml^{-1} methylmercury chloride were prepared in 30 ml of 50:50 *v/v* benzene:hexane (May and Baker, Dagenham, UK and BDH, Poole, UK respectively) and the solvent evaporated by passing a stream of gaseous N_2 (Air Products, Walton-on-Thames, UK) over the benzene/hexane mixture. The recovered CH_3HgCl was then dissolved in CD_3OD and ^1H NMR spectra produced to characterise the solid product.

2.2.2. Synthesis of ^{199}Hg Enriched Methylmercurychloride

^{199}Hg enriched (65.74%) HgO was obtained from AEA Technologies (Harwell, UK). Methyl cobalamine was purchased from Sigma Aldrich (Gillingham, UK), benzene from May and Baker (Dagenham, UK), hydrochloric acid (Aristar grade) and hexane from BDH (Poole, UK) and N_2 (Air Products, Walton-on-Thames, UK). All distilled, deionised water (DDW) used in the synthesis was obtained from an 18 MΩ cm^{-1} Elgastat Maxima system (Elga Ltd, High Wycombe, UK). All glassware used was first soaked for 24 hours in 10% Decon 90 (Decon Laboratories, Hove, UK) to remove organic carbon and subsequently in 10% HNO_3 for 24 hours to protonate cation exchange sites followed by washing in DDW.

Five separate syntheses were performed during this work, with the method as follows. The required mass of ^{199}Hg enriched HgO was accurately weighed into a clean sterilin container, dissolved in 400 µl of concentrated HCl, diluted with 10 ml of DDW and transferred to a 50 ml stoppered glass conical flask to be used as the reaction vessel. The sterilin container was subsequently rinsed with two further 3 ml DDW aliquots which were then added to the reaction vessel. A mass of methylcobalamin, to give at least a 1:1 molar ratio of methylcobalamin to the ^{199}Hg enriched HgO (as Hg), was accurately weighed into a separate clean sterilin container and dissolved in 10 ml of DDW. Following transfer of this solution to the reaction vessel, this container was rinsed with two further 3 ml aliquots of DDW which were added to the reaction vessel. The reaction vessel was protected from light sources because methylcobalamin is UV sensitive, and agitated by means of a magnetic stirrer for 1 hour.

For the first three syntheses performed, the methylmercurychloride was extracted using 50:50 *v/v* benzene:hexane (10 ml). Following agitation (10 minutes), the benzene:hexane layer containing the methylmercurychloride was carefully removed by pipette to a second 50 ml glass conical flask. This extraction procedure was repeated twice

more, resulting in an extractant volume of 30 ml. Aliquots of the benzene:hexane extractant were then transferred to a clean, preweighed 7 ml glass vial and the solvent evaporated under a stream of N_2, which was carefully regulated to minimise losses of methylmercurychloride by volatilisation. For the final two syntheses, dichloromethane was used as the organic extractant in the same manner. The purity and isotopic composition of the ^{199}Hg enriched methylmercurychloride was assessed by both NMR spectroscopy and HPLC-ICP-MS.

2.2.3. Synthesis of ^{13}C Enriched Methylmercuryiodide

^{13}C labelled methylmercuryiodide was synthesised by a Grignard reaction using freshly prepared $^{13}CH_3MgI$ by Dr.P.Sutton of De Montford University. All reagents were supplied by Aldrich (Gillingham, Dorset, UK). 1g of dry magnesium turnings was stirred magnetically, in a 250ml three necked round bottom flask attached to a reflux condenser, and under a nitrogen atmosphere for two hours to create a fresh reactive surface on the metal. Subsequently, 5 g of ^{13}C labelled methyliodide, dissolved in dry double distilled diethyl ether (50 ml), was added to the Mg in the round bottomed flask. The solution was gently warmed and stirred until a characteristic steely grey, indicative of the formation of the Grignard reagent, was observed. The solution was stirred at room temperature for one hour and then heated at reflux for a further 45 minutes. The reaction products were allowed to cool and settle prior to the next stage of the reaction.

Mercury (II) chloride, 4.75 g, was dissolved in 75 ml of diethyl ether in a three necked round bottomed flask attached to a reflux condenser. The solution was warmed to dissolve the $HgCl_2$ and stirred under a nitrogen atmosphere. The freshly prepared $^{13}CH_3MgI$ was added dropwise under nitrogen pressure to the $HgCl_2$ solution *via* a double ended steel needle connecting the two round bottom flasks, thus ensuring that no unreacted Mg was added to the $HgCl_2$ solution. A further 25 ml of diethyl ether was added to the

Grignard flask as a wash solution and subsequently transferred to the $HgCl_2$ solution *via* the steel needle. The resulting solution was refluxed for 12 hours to complete the reaction. Upon cooling, a crop of pale yellow crystals was precipitated in the bottom of the flask which were removed from the reaction mixture by filtration. Several large red crystals of mercury (II) iodide were also produced during the reaction and these were removed from the pale yellow crystals and discarded. The pale yellow crystals were recrystallised from boiling alcohol to yield 3.94 g of ^{13}C labelled methylmercuryiodide, with a melting point of 143°C (Lit. 143°C).

2.2.4. Nuclear Magnetic Resonance Studies

All ^{1}H spectra were acquired at 270 MHz using a Jeol EX270 Fourier Transform NMR spectrometer. The signal from the residual protio methyl (CD_2HOD) group, δ 3.3 ppm, in CD_3OD was used to reference the ^{1}H spectra, except for experiments using a solvent suppression technique when the spectra were referenced to the residual hydroxyl signal (CH_3OH and HDO) at δ 4.8 ppm. Baseline ^{1}H NMR spectra of methylmercurychloride, methylmercurybromide and methylmercuryiodide (all purchased from Sigma-Aldrich, Gillingham, UK), ^{199}Hg enriched methylmercurychloride and ^{13}C enriched methylmercury iodide were all obtained in CD_3OD. For experiments to determine the halide preference of monomethylmercury, potassium halides, (Cl, Br and I) were added to the standard solutions at twice the concentration of the mercury species present in the sample and the NMR spectra of the resulting solutions acquired.

The standard mobile phase employed for the separation of mercury species by HPLC[74], and as the equilibration solvent[75,76], comprised 50:50 methanol:water *v/v* and 0.01% 2-mercaptoethanol. However, this was not suitable for NMR studies due to the excessive proton signal from the methanol/water components. For experiments to determine the stability of mercury species during chromatographic separation a partially deuterated mobile phase comprising 50:50 D_2O:CH_3OD *v/v* and 0.01% 2-mercaptoethanol

was employed. A mixture of $^{13}CH_3HgI$ and ^{199}Hg enriched CH_3HgCl (100 and 200 μg g^{-1} respectively as Hg) was injected (100 μl) onto the HPLC column and the eluent collected in fractions of 1.5 minutes duration after UV detection at 204 nm. After preconcentration by gaseous N_2 solvent evaporation, the fractions containing the monomethylmercury species, (1.5-3 minutes elution time) were analysed by ^1H NMR spectroscopy. The partial deuteration of the HPLC mobile phase removed most of the signal arising from the H_2O in the ^1H NMR spectrum but the baseline was distorted by the very strong signal from the methyl group of the CH_3OD. To overcome this a solvent suppression technique was employed. The methyl group of the CH_3OD (δ 3.3 ppm) was pre-saturated for each transient acquisition, thus greatly reducing this signal in the spectrum.

The stability of methylmercury was investigated in the presence of two different particulate types. National Institute of Science and Technology (NIST) 2710 Montana Soil Certified Reference Material (SRM) was used to represent terrestrial material and DORM-2 dog fish muscle (National Research Council Canada (NRCC)) was employed as a biological sample. Approximately 0.5 g of the SRM was accurately weighed into a clean 7 ml glass vial to which was added 1 ml of ^{199}Hg enriched HgO (3.96 mg ml^{-1} dissolved in 1M KI/D_2O) and 1ml of $^{13}CH_3HgI$ (6.9 mg ml^{-1}, dissolved in CD_3OD, 0.02% 2-mercaptoethanol). The resulting mixture was stirred magnetically at a constant temperature, 25° C for 24 hours, followed by filtration (Autovial 0.2μm PTFE membrane syringeless filters, Whatman, Maidstone, UK) and subsequent analysis by ^1H NMR spectroscopy.

2.3. Results and Discussion

2.3.1. Recovery of Methylmercury Chloride from 50:50 *v/v* Benzene:Hexane

Methylmercurychloride was required as a solid product for this study. Experiments were conducted to assess the recovery efficiency of methylmercury chloride

from a 50:50 *v/v* benzene:hexane mixture, which was employed as an extraction solvent during the synthesis of ^{199}Hg enriched methylmercurychloride. The results of these experiments are summarised in Table 2-1. After the first experiment, the N_2 flow rate was reduced which resulted in an increased recovery of the dissolved methylmercury chloride. The chemical shift (0.91 ppm) and $^{2}J\ ^{1}H\text{-}^{199}Hg$ coupling constant (211 Hz) confirmed that CH_3HgCl was present. No benzene or hexane signals were present in the spectrum.

2.3.2. Synthesis Yields

The yields of methylmercurychloride varied from 44 to 62%, as Hg, of the starting material. The data, along with that from other workers using similar synthetic methods are presented in Table 2-2. The yields obtained for the five syntheses performed are lower than those obtained by Rouleau and Block[72] and Rodríguez Martín-Doimeadios *et al.*[77] and comparable to those obtained by Demuth and Heumann[46]. The ratio of methylcobalamin to the inorganic Hg (Table 2-2) vary with each synthesis and do not appear to correlate with the yields obtained. The recovery experiments of methylmercury chloride from 50:50 *v/v* benzene:hexane show that the low yields obtained are unlikely to be due to product loss during solvent evaporation. Rodríguez Martín-Doimeadios *et al.*[78] reported that optimal conditions for the same synthetic procedure, using 150 μg of ^{201}Hg, as 37°C for 1 hour in the dark at pH 5, buffered with 0.5 M acetic acid/sodium acetate, and an inorganic Hg:methylcobalamin ratio of 2:1. Therefore, further investigations are necessary to determine if these reaction conditions give yields greater than those already obtained when applied on a larger scale.

Table 2-1 Recoveries of methylmercurychloride after N_2 blowdown.

Initial Mass of CH_3HgCl (mg)	Mass of CH_3HgCl Recovered (mg)	Blowdown Time (Minutes)	Recovery (%)
129.6	114.7	185	88.5
140.8	137.2	255	97.4
168.5	159.1	260	94.4

Table 2-2 Methylmercurychloride yields by direct methylation with methylcobalamin.

	Inorganic Hg Mass (mg)	Me[Co]:Hg (Mole:Mole)	Yield (%)
This Work 1st synthesis	10.1	1.01	44
This Work 2nd synthesis	20.6	1.82	49
This Work 3rd synthesis	9.9	5.79	49
This Work 4th synthesis	8.0	4.88	51
This Work 5th synthesis	7.9	2.22	62
Rouleau and Block[72]	1.0	3.72	90
Rodriguez et al[77]	0.01	9.41	88
Demuth and Heumann[46]	1.9	2.02	60

2.3.3. Characterisation of Methylmercury Isotopomers

2.3.3.1. Nuclear Magnetic Resonance Studies

A comparison of the chemical shifts and the coupling constants, ^{n}J, of methylmercury halides (natural isotopic abundance, Table 2-3) confirmed the identity of the ^{199}Hg enriched isotopomer as methylmercury chloride. The different chemical shifts exhibited by the individual monomethylmercuryhalides (Table 2-3) shows the degree of electron shielding of the halide counter ion, I>Br>Cl, on the protons in the molecules. Likewise, the shielding effect on the ^{2}J ^{1}H-^{199}Hg coupling constant can also be observed for each individual methylmercuryhalide. ^{13}C labelled (>99%) methylmercuryiodide was positively characterised by ^{1}H NMR spectroscopy using the same approach. The notation used for all ^{1}H NMR spectra is shown in Table 2-4. The individual organomercury isotopomers (Table 2-4) have been numerically labelled as follows; natural abundance methylmercurychloride (*i*); ^{199}Hg enriched methylmercurychloride (*ii*); ^{199}Hg enriched methylmercuryiodide (*iii*); natural abundance methylmercuryiodide (*iv*); and ^{13}C labelled methylmercuryiodide (*v*). The ^{199}Hg enrichment in isotopomer *ii*, compared with isotopomer *i*, can be observed *via* the enhanced intensity of the ^{1}H-^{199}Hg satellite signals, which arise from spin-spin coupling between the ^{1}H and ^{199}Hg nuclei, (Figure 2-1 A and B). Similarly, a comparison of the ^{1}H NMR spectrum of isotopomer *iv* with that observed for isotopomer *v*, (Figure 2-2 A and B), demonstrates the extremely high (>99%) ^{13}C content of isotopomer *v*. Due to the almost complete ^{1}H-^{13}C coupling for isotopomer *v*, four ^{2}J ^{1}H-^{199}Hg satellites now appear in the spectrum, two for each component of the ^{1}H-^{13}C doublets.

Table 2-3 ^{1}H NMR spectroscopy chemical shifts and coupling constants for the organomercury compounds under study, all samples were dissolved in deuterated methanol, CD_3OD.

	Methylmercury Concentration (mg ml^{-1})	^{1}H Chemical Shift (δ, PPM)	^{1}J ^{1}H-^{13}C (Hz)	^{2}J ^{1}H-^{199}Hg (Hz)	Inference
CH_3HgCl	3.2	0.91	138	211	
CH_3HgBr	4.9	0.97	138	207	
CH_3HgI	3.1	1.03	138	195	
^{199}Hg enriched CH_3HgCl	3	0.91	138	211	
$^{13}CH_3HgI$	2.4	1.03	138	195	
CH_3HgCl + KBr	3	0.97	138	207	CH_3HgBr
CH_3HgCl + KI	3	1.03	138	195	CH_3HgI
CH_3HgBr + KCl	3	0.97	138	207	CH_3HgBr
CH_3HgBr + KI	4	1.03	138	195	CH_3HgI
CH_3HgI + KCl	3	1.03	138	195	CH_3HgI
CH_3HgI + KBr	3	1.03	138	195	CH_3HgI

The superscript ^{n}J denotes the number of bonds along which the coupling occurs.
Potassium halides were added at twice the methylmercury species concentration.

Table 2-4 Notation employed for the methylmercury isotopomers and ^{1}H NMR spectra.

Compound	Isotopomer Number	^{1}H NMR Signal		
		Proton	^{2}J ^{1}H-^{199}Hg	^{1}J ^{1}H-^{13}C
CH_3HgCl	*i*	α	β	
^{199}Hg enriched CH_3HgCl	*ii*	α	β	
^{199}Hg enriched CH_3HgI	*iii*	α	β	
CH_3HgI	*iv*	ε	γ	ϕ
$^{13}CH_3HgI$	*v*	ε	γ	ϕ

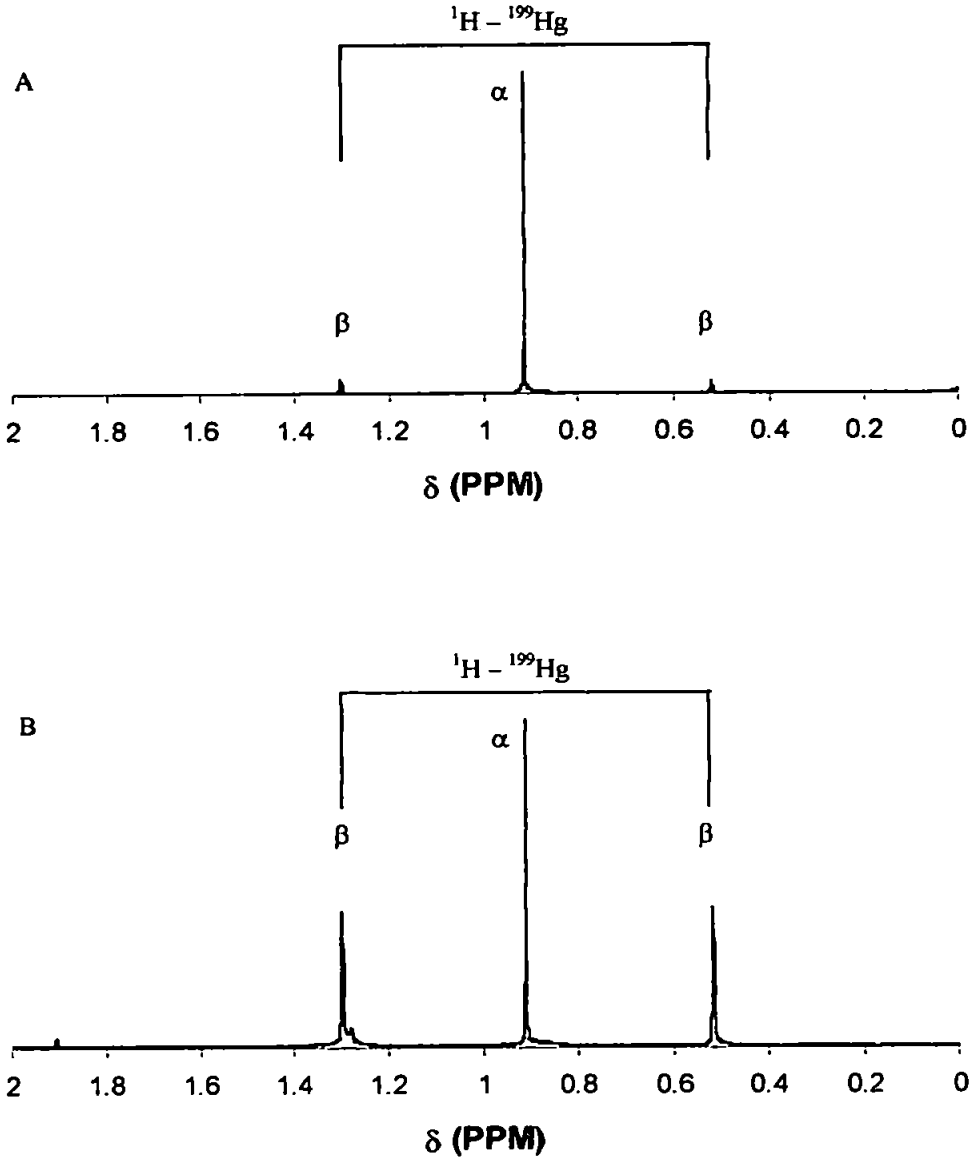


Figure 2-1 ^{1}H NMR spectra of: A, natural isotopic abundance methylmercurychloride; B, ^{199}Hg enriched (66%) methylmercurychloride, for these isotopomers the ^{1}H-^{199}Hg satellites are denoted β, The ^{199}Hg enrichment, determined by MC-SF-ICP-MS (Chapter 3 Section 3.5.3), is reflected in the increase in the relative sizes of the ^{199}Hg signals. The chemical shifts and coupling constants are given in Table 2-3.

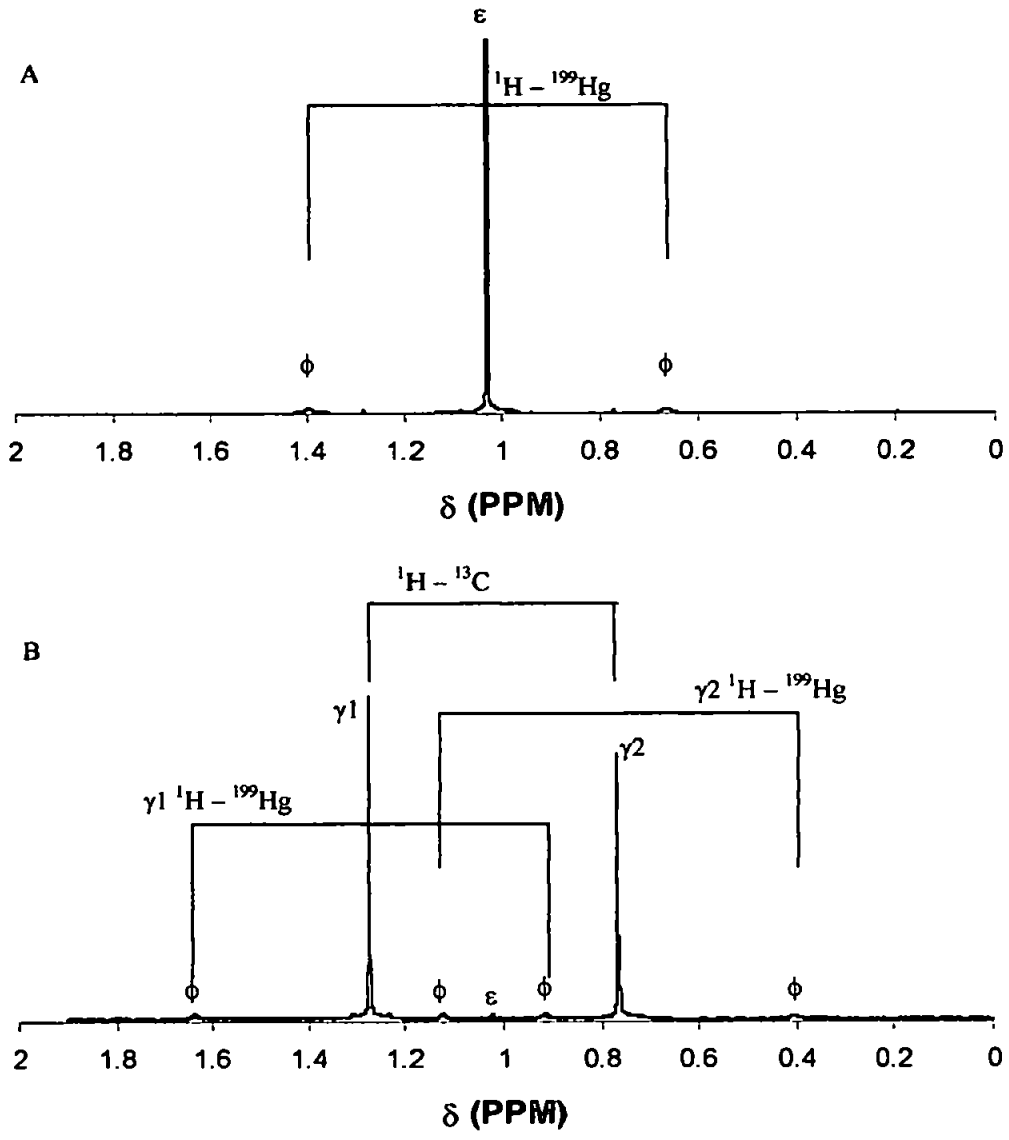


Figure 2-2 ^{1}H NMR spectra of: A, natural isotopic abundance methylmercuryiodide; and B, ^{13}C enriched (>99%) methylmercuryiodide, for these isotopomers the ^{1}H-^{13}C satellites are denoted γ with the ^{1}H-^{199}Hg satellites are denoted ϕ. The ^{13}C enrichment is reflected in the increase in the relative sizes of the ^{13}C satellite signals. The chemical shifts and coupling constants are given in Table 2-3.

2.3.3.2. Mass Spectrometry Characterisation

The synthesised product and natural isotopic abundance methylmercurychloride (Aldrich, Gillingham, UK) were analysed by HPLC-ICP-MS to determine the ^{200}Hg:^{199}Hg isotope amount ratio and check for inorganic mercury species contained in the product. A Waters 600E HPLC pump and a VG PQ3 ICP-MS instrument were coupled for the analysis. A summary of the chromatographic separation and the mass spectrometer operating conditions are shown in Table 2-5. NIST997 Thallium standard was added to the mobile phase at 50 ng/g for mass bias correction. The mass spectrometer was first tuned to ^{115}In to check sensitivity followed by direct coupling of the HPLC column eluent to the nebuliser uptake and subsequently tuned to the Tl mobile phase internal standard. The chromatograms obtained are shown in Figure 2-3. The measured and expected ^{200}Hg:^{199}Hg isotope amount ratios are presented in Table 2-6. No inorganic mercury was detected in either of the samples and the mass bias corrected measured ^{200}Hg:^{199}Hg isotope amount ratios concurred with the expected values.

Table 2-5 Operating conditions for HPLC-ICP-MS characterisation.

HPLC Conditions			
HPLC Column	HiChrom Kromasil 100 FC 18 Excel, 25 cm x 4.6 mm i.d.		
Mobile Phase	50:50 *v/v* Methanol:DDW, 0.01% 2-mercaptoethanol, 50 ng/g NIST 997 Tl SRM		
Flow Rate (ml/min)	0.9		
Injection Volume (μl)	100		
ICP-MS Operating Conditions			
RF Forward Power (W)	1450	Plasma gas (l min^{-1})	14
Reflected Power (W)	≤ 10	Auxiliary gas (l min^{-1})	0.75
Spray Chamber	Cyclonic, cooled to −5 °C	Nebuliser gas (l min^{-1})	0.52
Torch	Fassel Quartz	Dwell Time (ms)	10
Sampler and Skimmer Cones	Ni	Points per Peak	5
Nebuliser	Glass Expansion 1 ml/min Conikal	Ions Monitored	^{199}Hg, ^{200}Hg, ^{203}Tl, ^{205}Tl

Table 2-6 Measured and expected ^{200}Hg:^{199}Hg isotope amount ratios.

	Measured ^{200}Hg:^{199}Hg Isotope Amount Ratio	Standard Uncertainty	Expected ^{200}Hg:^{199}Hg Isotope Amount Ratio	Standard Uncertainty
Natural Isotopic Abundance CH_3HgCl	1.351	0.023	1.369	0.012
^{199}Hg Enriched CH_3HgCl	0.268	0.031	0.2751	2.88×10^{-5}

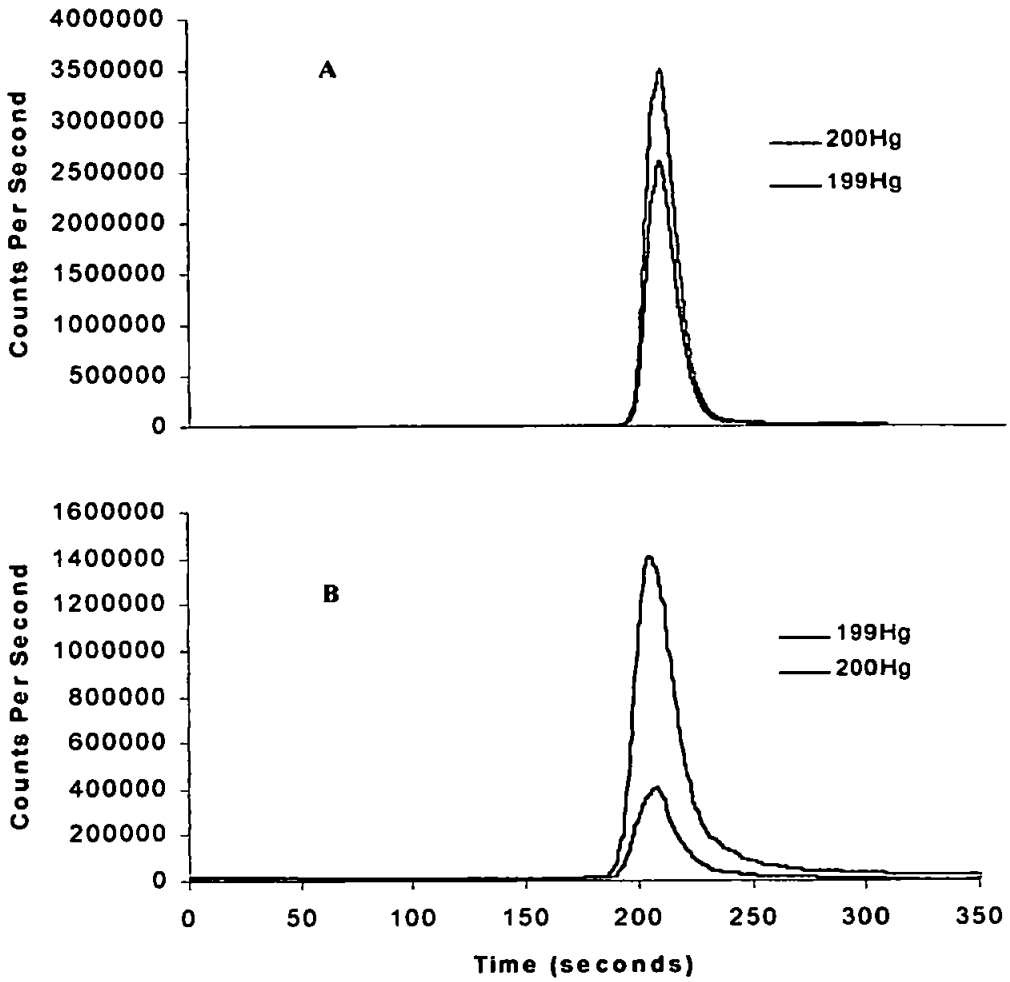


Figure 2-3 Chromatograms obtained for: A, 240 ng injection of natural isotopic abundance methylmercurychloride; B, 350 ng injection of ^{199}Hg enriched methylmercurychloride.

2.3.4. Methylmercury Halides - Halide Exchange

It is a pre-requisite for speciation studies that the chemical form of the target analyte is not altered during the analytical procedure. Therefore, experiments were designed to determine the stability of the methylmercury halide counter ion, in the presence of different inorganic halides. The ^{1}H NMR spectrum, Figure 2-4, observed for a solution containing both the natural isotopic abundance methylmercurychloride and methylmercuryiodide isotopomers, *i* and *iv* respectively, exhibited a single methyl proton signal of δ 0.97 ppm, and ^{2}J ^{1}H-^{199}Hg coupling constant of 203 Hz. In other words the ^{1}H NMR spectrum of a mixture of isotopomers *i* and *iv* was an average of the ^{1}H NMR spectra obtained for each of the individual isotopomers. From this it was deduced that the halide component of these two isotopomers, Cl^- and I^- respectively, were exchanging with each other. Since no signal was observed in either a ^{13}C or a ^{199}Hg NMR spectrum of the same solution, it was surmised that the rate of halide exchange was rapid and taking place at a rate comparable with the NMR data acquisition timescale. The rate constant, *k*, for the halide exchange was calculated as 72 s^{-1} from Equation 2-4[79].

$$k = \pi\Delta\nu/\sqrt{2}$$

Equation 2-4

where k is the rate constant and $\Delta\nu$ is the difference between the chemical shift, measured in Hertz, of the two individual organomercury halides.

The relative stability of the halide counter ion was determined by combining each compound with inorganic halides (*e.g.* KBr, KI), followed by comparison of the resulting ^{1}H NMR spectra with those obtained from authentic compounds. Using this method, and with each halide in excess, the order of preference for methylmercury was I^- >Br^- >Cl^-. Table 2-3 summarises the ^{1}H NMR spectroscopic data for various

methylmercury halides in the presence of inorganic halides. The speciation of methylmercury with regard to the halide counter ion, in the liquid phase during equilibration, chromatographic separation and subsequent detection by ICP-MS, is therefore dependent on the reagents employed.

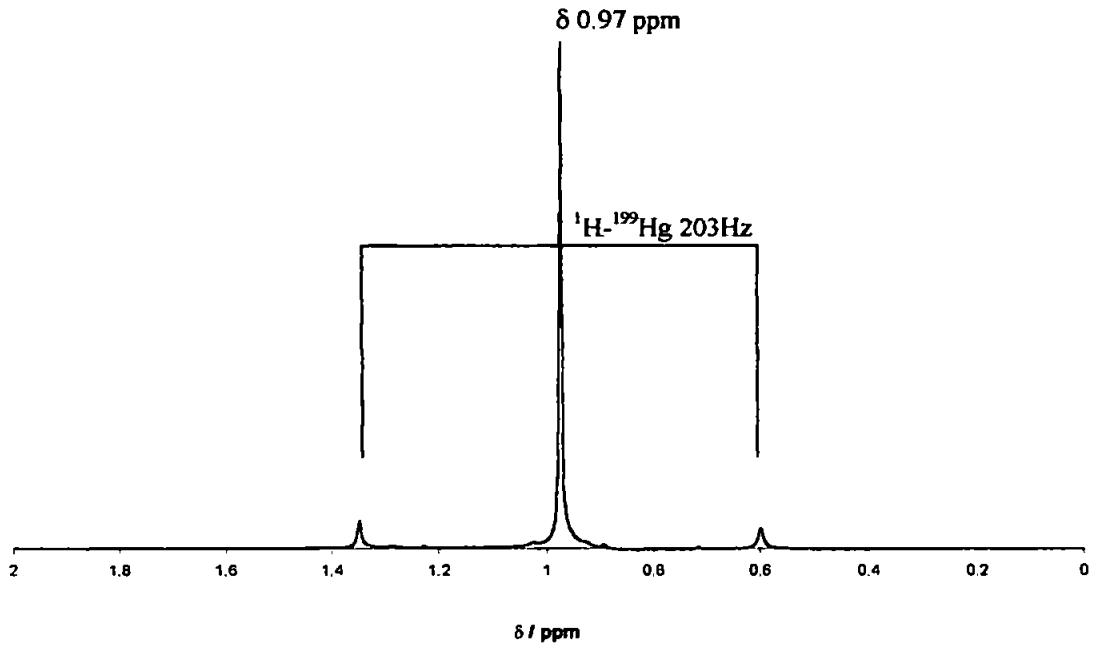


Figure 2-4 ^{1}H NMR spectrum of $^{13}CH_3HgI$ (14 mg/ml) and ^{199}Hg enriched CH_3HgCl (9 mg/ml) in CD_3OD. The signal at 0.97 ppm and the ^{2}J ^{1}H-^{199}Hg coupling constant of 203 Hz are intermediate between those of the individual compounds present.

2.3.5. Methylmercury Halides - Methyl Group Exchange

The possibility of methyl group exchange between CH_3HgI molecules, Equation 2-5, was studied by mixing together ^{13}C labelled and ^{199}Hg labelled CH_3HgCl compounds in the deuterated HPLC mobile phase. The ^{199}Hg labelled CH_3HgCl was iodinated by the addition of KI to the samples, so as both species were in the same chemical form, prior to analysis by ^1H NMR. This prevented the intermediate spectra that arise from each species having a different halide component.

$$^{13}CH_3HgI + CH_3{}^{199}HgI \rightleftharpoons {}^{13}CH_3{}^{199}HgI + CH_3HgI$$

Equation 2-5

The distinct ^1H NMR spectral patterns of the two isotopically labelled methylmercuryiodide isotopomers, *iii* and *v*, allowed the relative isotopic abundance of ^{199}Hg for each compound to be calculated when both compounds were present in the same solution. For example (Figure 2-5), the ^{199}Hg abundance in isotopomer *iii* was calculated by ratioing the peak areas of the two ^1H-^{199}Hg satellites (2β) with the total peak area (2β + α). This gave a ^{199}Hg isotopic abundance for isotopomer *iii* of 66%, as expected. Similarly, the ^{199}Hg abundance in $^{13}CH_3HgI$ could be calculated from the same ^1H NMR spectrum by ratioing the peak areas for the four ^1H-^{199}Hg satellites (2φ1 + 2φ2), with the total peak area (2φ1 + 2φ2 + γ1 + γ2). As such the ^{199}Hg abundance for isotopomer *v* was calculated as 17%.

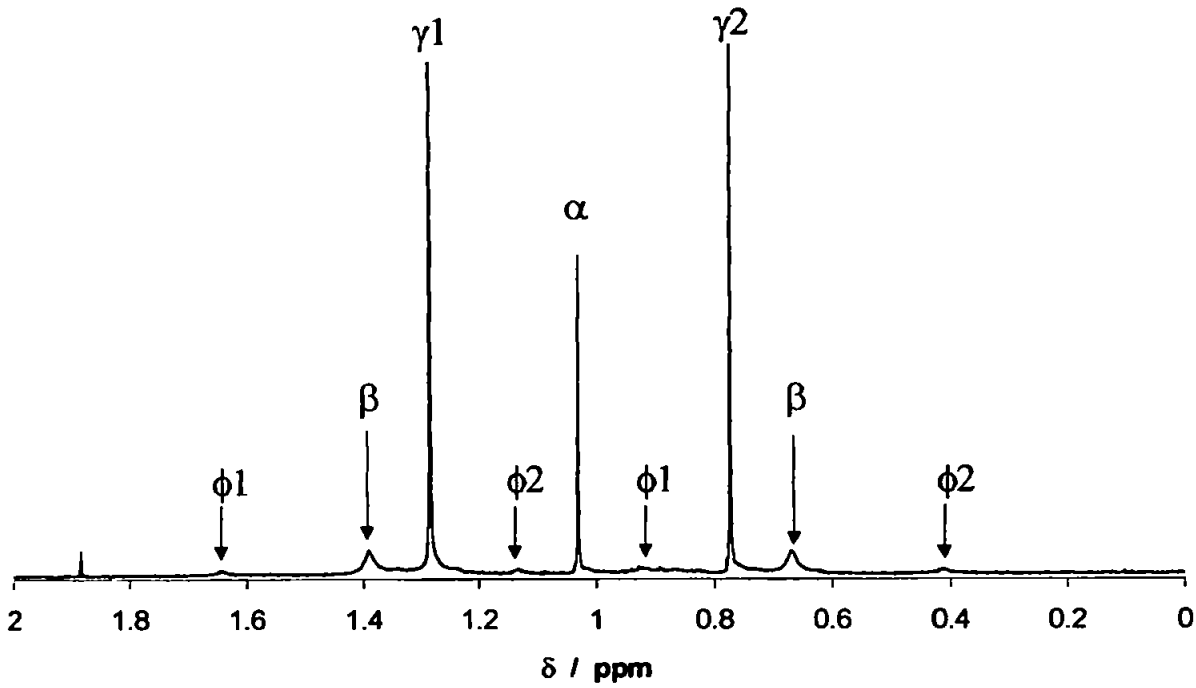


Figure 2-5 ^{1}H NMR spectrum of $^{13}CH_3HgI$ (4.8 mg/ml) and ^{199}Hg enriched CH_3HgI (2 mg/ml) in CD_3OD. The ^{1}H-^{13}C doublet, marked γ1 and γ2 arises from the $^{13}CH_3HgI$; each of γ1 and γ2 has two associated ^{1}H-^{199}Hg satellites, ϕ1 and ϕ2. The ^{199}Hg enriched CH_3HgI molecule gives three signals in the spectrum, the main proton signal, α, and two ^{1}H-^{199}Hg satellites, marked β. Note $\Delta\beta = \Delta\phi_1 = \Delta\phi_2$.

If methyl group exchange, between the methylmercuryiodide isotopomers, *iii* and *v*, as shown in Equation 2-5, had occurred, the change in the ^{199}Hg isotopic abundance in the respective molecules would alter the NMR spectral pattern obtained. For example, if $^{13}CH_3{}^{199}HgI$ had been formed, an increase in the signal from the four ^{199}Hg-^{13}C satellites, $\phi 1$ and $\phi 2$ (Figure 2-5) and a decrease in the ^{199}Hg-^{1}H satellites, γ^1 and γ^2 (Figure 2-5) would have been observed. However, since the calculated ^{199}Hg isotopic abundances, for the two labelled compounds when combined, were the same as measured for the individual species, this experiment provided no evidence for CH_3 exchange in the deuterated HPLC mobile phase.

In order to verify (or otherwise) that this absence of exchange was also the case during chromatographic separation, further solutions were analysed prior to injection onto and post elution from the HPLC column. Figure 2-6 shows the ^{1}H NMR spectrum obtained for a mixture of $^{13}CH_3HgI$ and $CH_3{}^{199}HgI$ both prior to injection onto the HPLC column (Figure 2-6A), and for the fraction collected after eluting from the column (Figure 2-6B). The chemical shift of the methyl protons for the two compounds contained in the post column fraction was 0.75 ppm, compared with 1.03 ppm for the compounds prior to HPLC separation. Similarly the ^{2}J ^{1}H-^{199}Hg coupling constant for each compound, 175 Hz, also changed from the pre-separation value of 195 Hz. This change was considered to be either due to the formation of a new monomethylmercury compound during HPLC separation, or to the effect of the change in solvent composition during evaporation by gaseous N_2, which preferentially removes the methanolic component of the HPLC mobile phase resulting in an enriched aqueous solvent.

In order to distinguish between these two possibilities, a solution containing a mixture of $^{13}CH_3HgI$ and $CH_3{}^{199}HgI$, dissolved in the partially deuterated HPLC mobile phase, was subjected to solvent evaporation by gaseous N_2 for 4 hours and subsequently analysed by ^{1}H NMR spectroscopy (Figure 2-6 C).

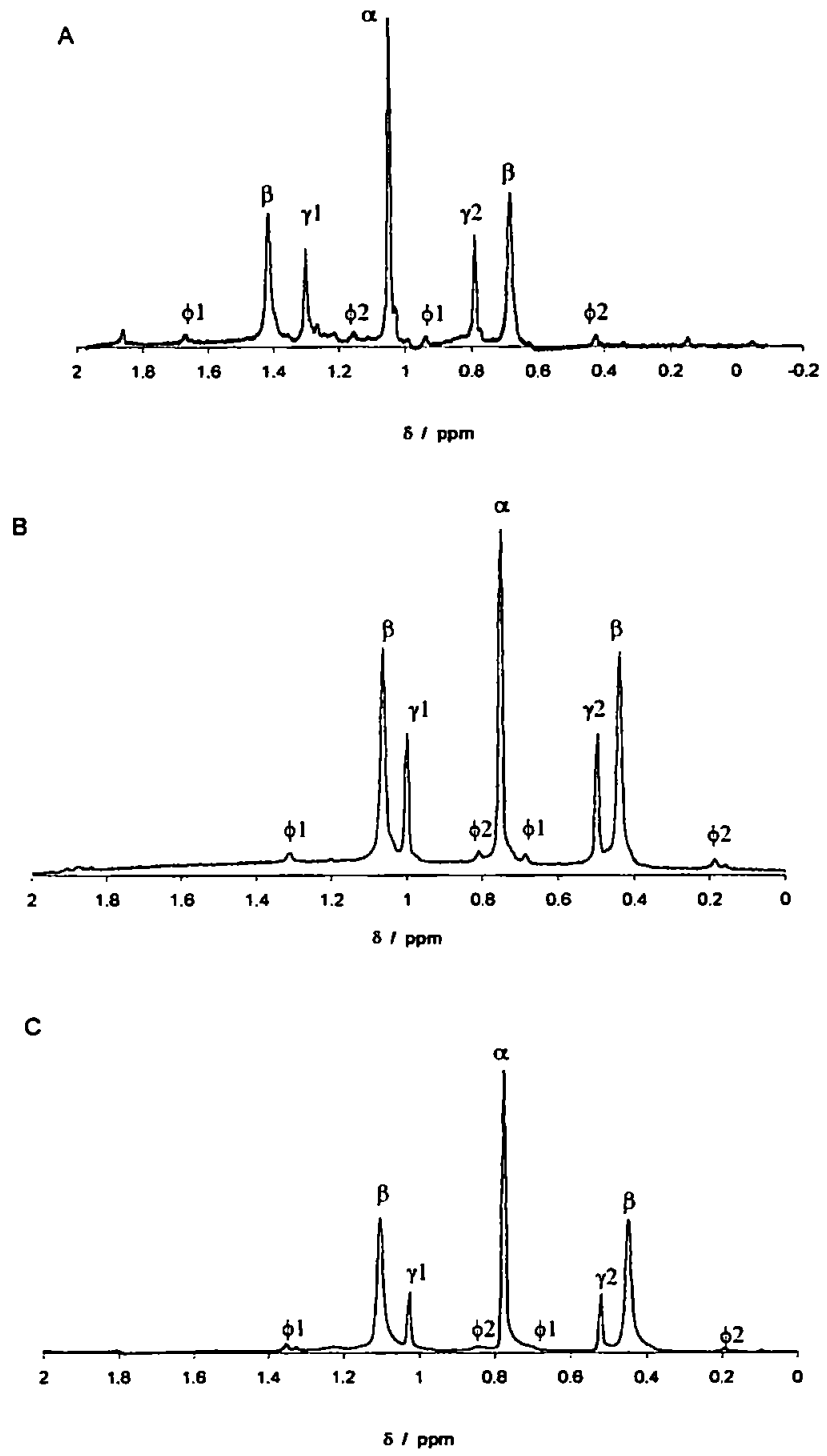


Figure 2-6 ^{1}H NMR spectrum of $^{13}CH_3HgI$ (1 mg/ml) and ^{199}Hg enriched CH_3HgCl (2 mg/ml) in 50:50 D_2O/CH_3OD *v/v*, 0.01% 2-mercaptoethanol: A, prior to injection onto the HPLC column; B, the post-column eluent fraction containing the methylmercury species; and C, the pre injection mixture after solvent evaporation by gaseous N_2. KI was added to the samples prior to analysis by ^{1}H NMR spectroscopy.

The chemical shift of the methyl protons and $^{2}J\ ^{1}H\text{-}^{199}Hg$ coupling constant for each compound was 0.77 ppm and 178 Hz respectively, which confirmed that the changes in the parameters observed for the pre- and post-HPLC separation fractions were due to the change in solvent composition. In addition, the ^{199}Hg isotopic abundance, for the two labelled isotopomers, *iii* and *v*, remained at 66% and 17% respectively after elution from the column, indicating that methyl group exchange between the two isotopomers did not occur during the HPLC procedure.

2.3.6 Formation of Methylmercury During Equilibration

Losses of the spike material before complete equilibration between the sample and spike during IDA will result in an underestimation of the amount of methylmercury present in the sample. For example, methyl group exchange from the ^{199}Hg enriched methylmercury spike to inorganic Hg, which may also be present in the sample matrix (Equation 2-6) would reduce the amount of the enriched spike which is available for equilibration with the natural analyte. Similarly, the formation of natural isotopic abundance methylmercury (Equation 2-7), *e.g.* by bacterial methylation, will cause a reduction in the value of the reference:spike isotope amount ratio, which, when measured by HPLC-ICP-MS after equilibration, again results in an underestimation of the analyte.

$$CH_3{}^{199}HgI + HgI \rightleftharpoons CH_3HgI + {}^{199}Hg^{2+}$$

Equation 2-6

$$HgI + CH_3{}^{+} \rightleftharpoons CH_3HgI$$

Equation 2-7

A solution of the ^{13}C labelled isotopomer and ^{199}Hg enriched HgI (obtained by dissolving the ^{199}Hg enriched HgO in 1M KI) was used to investigate the reactions in Equation 2-6 and Equation 2-7. The ^{1}H NMR spectra of this solution both before (Figure

2-7A) and after (Figure 2-7B), a period of equilibration with particulate matter in the form of NIST2710 SRM, a soil material, gave no evidence for the formation of methylmercury from the added ^{199}Hg enriched inorganic Hg. If any $^{13}CH_3{}^{199}HgI$ had been formed an increase in the signal intensity of the four ^{199}Hg-^{13}C satellites, Figure 2-7A and Figure 2-7B, denoted $\phi 1$ and $\phi 2$, would have been observed. Similarly, the formation of $CH_3{}^{199}HgI$, from methyl groups derived from the soil matrix and the inorganic ^{199}Hg enriched HgI, would have been reflected in an increase in the signal intensity for this compound, Figure 2-7A and Figure 2-7B, denoted α.

The ^{199}Hg isotopic abundance in the $^{13}CH_3HgI$ was 17%, *vide supra*, both prior to, and after equilibration with the SRM for 24 hours. Since the ^{199}Hg isotopic abundance of the analyte ($^{13}CH_3HgI$) remained unaltered by ^{199}Hg enriched HgCl, in the absence or presence of the particulate material, no methyl group exchange between the individual Hg species took place under these conditions. No signal for ^{199}Hg enriched methylmercuryiodide was observed in either the pre- or post-equilibration spectra, indicating that the reaction in Equation 2-7 did not occur.

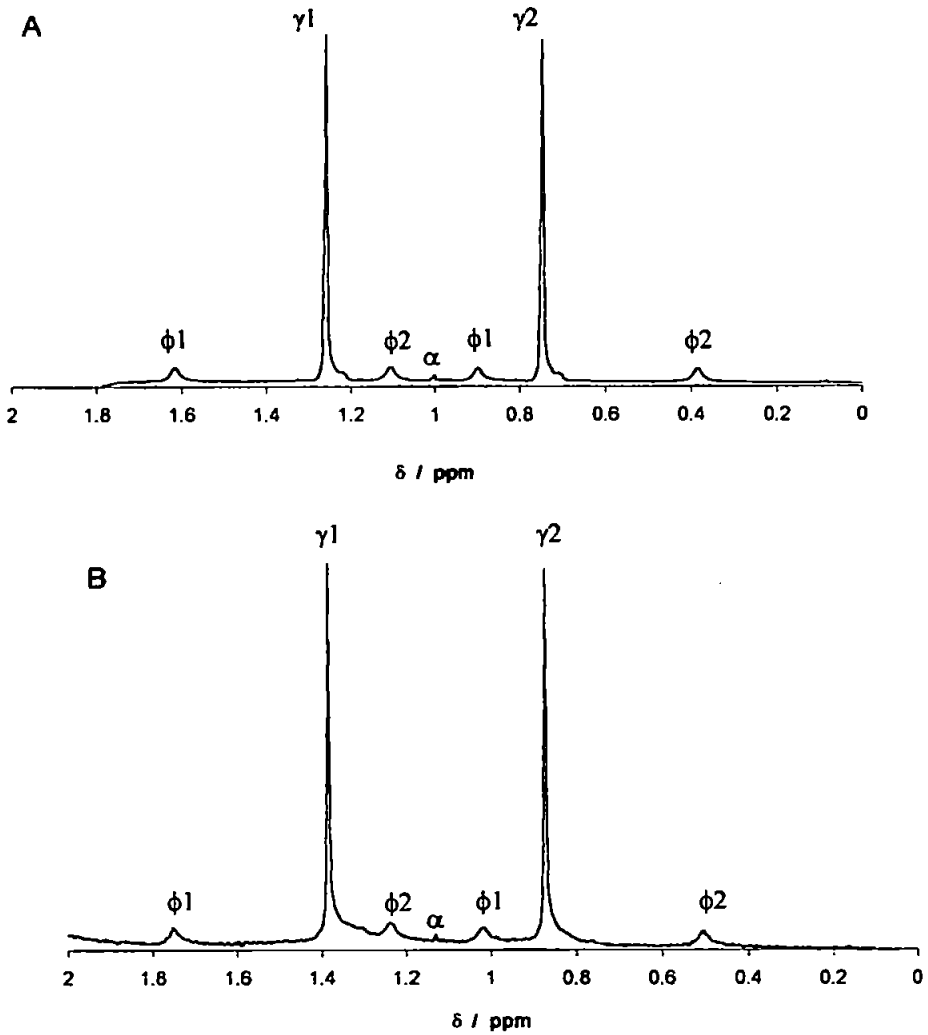


Figure 2-7 ^{1}H NMR spectrum of: A $^{13}CH_3HgI$ and ^{199}Hg enriched HgI prior to equilibration with NIST2710 SRM and B $^{13}CH_3HgI$ and ^{199}Hg enriched HgI post equilibration. The isotopic abundance of ^{199}Hg in the $^{13}CH_3HgI$, calculated from the signal peak areas remained at 17% for both samples.

A second experiment was performed using DORM-2, a dogfish muscle CRM. On this occasion, a suitable ^{1}H NMR spectrum could not be obtained because co-extracted molecules from the sample matrix obscured the spectral region of interest (0.5-1.5 ppm), Figure 2-8. Similar interferences were observed in the ^{13}C NMR spectrum, whereas the ^{199}Hg NMR spectrum was unobtainable due to the low concentration of the sample.

However, analysis of ^{199}Hg enriched CH_3HgCl using HPLC-ICP-MS has shown that this material does not decompose after equilibration with the DORM-2 CRM. A chromatogram obtained using a quadrupole ICP-MS instrument is presented in Figure 2-9. The inset shows the co-extracted inorganic Hg from the DORM-2 CRM which had a ^{200}Hg:^{199}Hg isotope amount ratio, of 1.367, compared to the IUPAC value for natural isotopic abundance Hg of 1.369[80]. From this ratio it can be deduced that the ^{199}Hg enriched CH_3HgCl spike material was stable during equilibration. If the spike had decomposed the isotope amount ratio for inorganic Hg would have changed due to a contribution from the ^{199}Hg enriched isotope. A different analytical technique is required, such as Fourier Transform Ion Cyclotron Resonance Mass Spectrometry, to establish whether any of the inorganic Hg present in the DORM-2 CRM was converted to methylmercury species during the equilibration process.

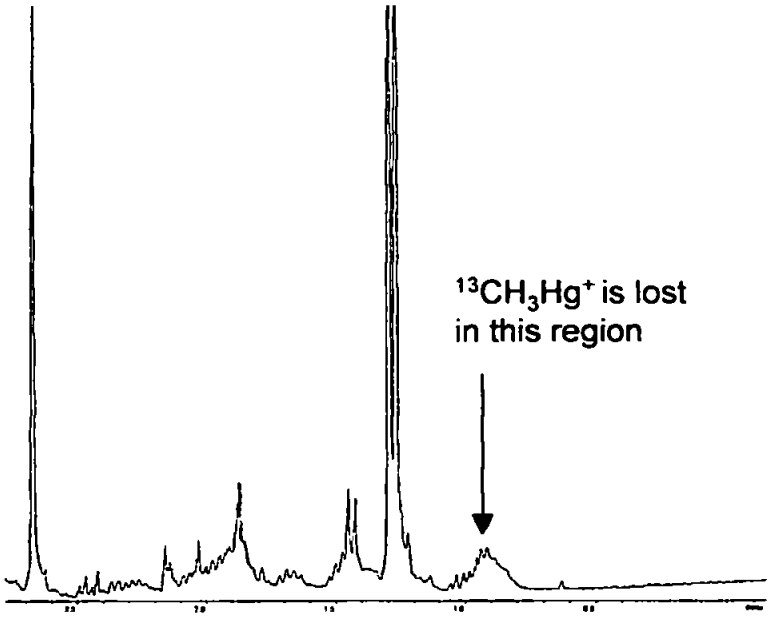


Figure 2-8 ^{1}H NMR spectrum of $^{13}CH_3HgI$ and ^{199}Hg enriched HgCl after equilibration with DORM-2 CRM. No signals were observed for the protons of $^{13}CH_3HgI$ as coextracted molecules from the dogfish muscle CRM masked the spectral region of interest.

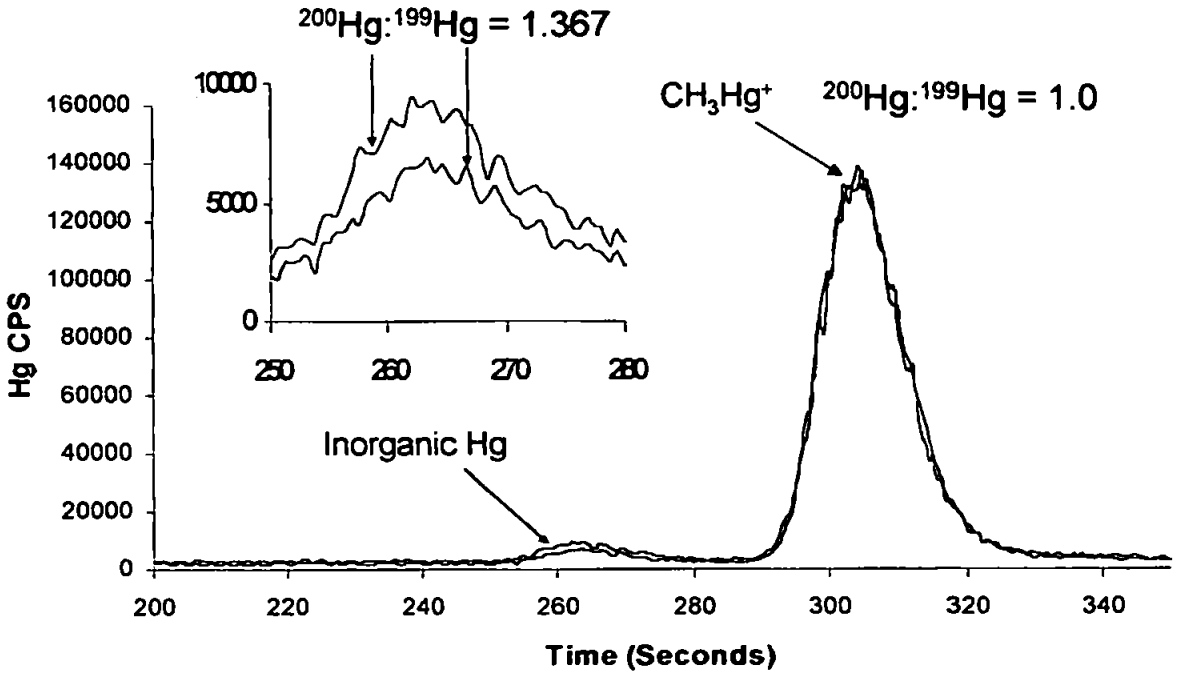


Figure 2-9 HPLC-ICP-MS chromatogram of an equilibration sample with, inset, the co-extracted inorganic Hg from DORM-2 with a natural abundance ^{200}Hg:^{199}Hg isotope amount ratio indicating that the ^{199}Hg enriched spike material was stable during the time period of the experiment.

2.4. Conclusions

^{199}Hg enriched methylmercurychloride has been successfully synthesised and extracted in the solid form using a simple and rapid procedure which does not involve the formation of dimethylmercury. No inorganic Hg has been detected in the product after analysis by NMR spectroscopy and HPLC-ICP-MS. However, synthesis yields have been lower than those reported by other workers. Recovery experiments indicate that the modification of the extraction method employed by other workers is not the cause of lower synthesis yields. Two other factors may be the cause of the lower yields obtained, either the greater starting quantity of HgO used in this work or the lower methylcobalamin to inorganic Hg ratio employed.

^{1}H NMR spectroscopy has given evidence for intermolecular halide ion exchange between methylmercurychloride and methylmercuryiodide, and that the methylmercury halide counter ion is readily exchangeable with other halides present in solution. The halide order of preference for methylmercury, as determined by ^{1}H NMR spectroscopy, has been shown to be $I^- > Br^- > Cl^-$. Thus, the speciation of methylmercuryhalides present in the original sample will be altered with respect to the halide counter ion, dependent on the halide composition of the sample matrix and the reagents employed.

The stability of the ^{199}Hg enriched methylmercurychloride spike material with $^{13}CH_3HgI$ during HPLC separation has been assessed using ^{1}H NMR spectroscopy. No evidence has been found for methyl group exchange between ^{13}C labelled and ^{199}Hg enriched methylmercury iodide, Equation 2-5, during HPLC separation. However, these experiments have shown that the chemical shift, and the ^{1}H-^{199}Hg coupling constant, of methylmercuryhalides are dependent on the solvent composition.

The stability of methylmercuryiodide in the presence of both inorganic ^{199}Hg enriched HgI and a soil material was also determined by ^{1}H NMR spectroscopy. The transfer of the methyl group from the methylmercuryiodide to inorganic Hg, Equation 2-6, was not observed by ^{1}H NMR spectroscopy. From the same experiment, no evidence was found for the formation of methylmercuryiodide, by the reaction of added inorganic mercury and methyl groups contained in the soil matrix, Equation 2-7. The stability of the spike material in the presence of a fish muscle tissue could not be determined by ^{1}H NMR spectroscopy due to co-extracted molecules giving signals which masked that from the methylmercury. However, the spike material was adjudged to be stable in the presence of DORM-2 by HPLC-ICP-MS.

Chapter 3 The Determination of Methylmercury by IDMS

3. Introduction

IDMS is capable of providing accurate and precise results providing the procedures detailed in Chapter 1 are followed, with the accuracy of the IDMS result dependent on the accuracy of the isotope ratio measurements[57]. In total analyte IDMS the required isotope amount ratio in the spiked sample solution is measured directly. For species specific IDMS a separation step is usually necessary, with a requirement to optimise the instrumental data acquisition parameters, and the subsequent isotope amount ratio calculation from the resulting chromatogram, to achieve best accuracy and precision. The aims of the work described in this Chapter were;

i. to optimise the coupling of an HPLC system to the ICP-MS instrument;

ii. to evaluate different methods of data extraction from the chromatograms obtained;

iii. to determine the mass fraction of methylmercury in a CRM by species specific IDMS.

3.1 Choice of Isotope Pair for IDMS

Conventionally, the spike isotope is of low natural abundance[24], however, ^{199}Hg enriched methylmercurychloride was synthesised for the stability studies described in Chapter 2 and this material was also used for the species specific IDMS studies. All the isotopes of mercury have a number of possible polyatomic interferences, from rare earth argides and tungsten and osmium oxides, when measured by ICP-MS. Three of the remaining six mercury isotopes available for use as the reference isotope also suffer from potential isobaric interferences, ^{196}Pt on ^{196}Hg, ^{198}Pt on ^{198}Hg and ^{204}Pb on ^{204}Hg, which are not resolvable with current ICP-MS instrumentation and were therefore discounted for use as the reference isotope. The reference isotope is usually the isotope of highest natural

abundance and chosen so it is close in mass to the spike isotope[24]. Therefore, ^{200}Hg, which is the second most abundant mercury isotope, was chosen as the reference isotope.

3.2 Equilibration Solvent

Ideally, the equilibration solvent employed should be able to extract a large proportion of the analyte from the sample matrix (*i.e.* particulate bound methylmercury), to give as high a mass fraction as possible for analysis, without compromising its speciation. A number of different solvent systems have been employed to solubilise methylmercury from particulate material, including: aqueous distillation[48]; dilute HNO_3[48]; tetramethylammonium hydroxide[81]; dilute HCl[82]; methanolic KOH[83]; toluene[84]; HNO_3/H_2O_2[85]; enzymatic hydrolysis[86]; and KBr-H_2SO_4-$CuSO_4$[43], usually in conjunction with a microwave extraction. However, because complete equilibration rather than complete extraction is required for IDMS, and the use of the above extraction methods has been shown to promote methylmercury formation (Chapter 1, Section 1.4.2), a mild extraction system was required. The HPLC mobile phase, to be used in methylmercury measurements by HPLC-ID-ICP-MS (50:50 methanol/water *v/v*, 0.01% 2-mercaptoethanol), was therefore chosen as the equilibration solvent for experiments involving a particulate CRM. The use of the HPLC mobile phase also has the advantage that no analyte would be lost with the solvent front during sample injection, thereby improving instrumental sensitivity.

3.3. The Uncertainty Budget Model

The statement of an analytical result is not considered complete without knowledge of the associated measurement uncertainty[87], which gives an estimate of the precision of the analytical method employed. Each parameter in the equation used to calculate an analytical result has an associated uncertainty which contributes to the combined uncertainty of that result. An uncertainty budget was therefore formulated for the measurement and calculation of isotope amount ratios from the HPLC-ICP-MS

chromatograms which allowed the instrumental data acquisition parameters to be optimised.

3.3.1. Mass Bias Correction

In order to determine the correct isotope amount ratio it is necessary to compensate for mass bias in the instrumentation. Quadrupole and sector field ICP-MS instruments and their associated ion optics do not transmit ions of different masses equally. The ion beam, formed after extraction from the ICP, contains ions of differing mass. Due to space charge effects arising in the plasma and the interface region[31] lighter ions are preferentially lost from and heavier ions are preferentially retained in, the ion beam[88]. In other words, if an elemental solution composed of two isotopes with an exactly 1:1 molar ratio is analysed using ICP-MS, a 1:1 isotope amount ratio will not necessarily be observed. This so-called mass bias depends on mass and the type of mass spectrometer used, but generally tends to be greatest at low mass and decreases with increasing mass[88]. Even very small mass-biases can have deleterious effects on the accuracy of isotope amount ratio determinations, so a correction must always be made, either by bracketing the sample with a reference material, certified for the isotope pair under study, or by interpolation from the mass bias observed for an isotope pair of similar mass to the isotope pair under study. No isotopically certified Hg CRM was available therefore the mass bias of $^{200}Hg:^{199}Hg$ isotope amount ratio was corrected for by interpolation of the mass bias observed for the $^{205}Tl:^{203}Tl$ isotope amount ratio using the relationship, named as the Russell correction expression[89], shown in Equation 3-1, which corrects for mass bias on the basis of the absolute mass of the isotope pairs.

$$\left(\frac{^{200}Hg}{^{199}Hg}\right)_{cor} = \frac{\left(\frac{^{200}Hg}{^{199}Hg}\right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl}\right)_{mes} \div \left(\frac{^{205}Tl}{^{203}Tl}\right)_{cer}\right)^{\left(\ln\left(\frac{RAM\ ^{200}Hg}{RAM\ ^{199}Hg}\right)+\ln\left(\frac{RAM\ ^{205}Tl}{RAM\ ^{203}Tl}\right)\right)}}$$

Equation 3-1: The Russell correction expression used for mass bias correction

Where *cor* is the corrected isotope amount ratio, *mes* is the measured isotope amount ratio, *cer* is the certified isotope amount ratio and RAM is the relative atomic mass.

The advantage of this approach is that the mass bias correction can be performed by spiking the sample with a mass bias correction standard (i.e. Tl) and measuring this isotope amount ratio at the same time as the sample. For speciation analysis by HPLC-ID-ICP-MS, it is most convenient to spike the mobile phase with the standard and monitor it continuously.

3.3.2. The IDMS Model

The ratio version of the IDMS equation, Equation 3-2, was chosen to calculate the mass fraction of methylmercury in National Research Council of Canada (NRCC) DORM-2 dogfish muscle CRM, C_X.

$$C_x = C_y \cdot \frac{m_y}{m_x . h} \cdot \frac{R_y - R_B}{R_B - R_x} \cdot \frac{\sum_i R_{ix}}{\sum_i R_{iy}}$$

Equation 3-2 The ratio version of the IDMS equation

Where,

C_x = concentration of the analyte in the sample

C_y = concentration of the analyte in the spike solution

m_s = mass of spike

m_x = mass of sample

h = moisture content correction factor

R_y = reference:spike isotope amount ratio in the spike

R_x = reference:spike isotope amount ratio in the sample

$\sum_i R_{ix}$ = sum of the isotope amount ratios of isotope *i* to the spike isotope in the sample

$\sum_i R_{iy}$ = sum of the isotope amount ratios of isotope *i* to the spike isotope in the spike

R_B = reference:spike isotope amount ratio in the sample after spiking.

For this work ^{200}Hg was chosen as the reference isotope and ^{199}Hg as the spike isotope.

It is assumed that the reference:spike isotope amount ratio in the sample after spiking, R_B (Equation 3-2), is corrected for mass bias effects. Each parameter in the Russell correction expression, used to correct for mass bias effects on the measured ^{200}Hg:^{199}Hg isotope amount ratio, has an associated uncertainty, which contributes to the overall uncertainty for the IDMS procedure. In order to include these uncertainties in the uncertainty budget for the determination of methylmercury in DORM-2 CRM Equation 3-1 and Equation 3-2 were combined, to give Equation 3-3, the notation is the same as that used for Equation 3-1 and Equation 3-2.

$$C_X = C_y \cdot \frac{m_y}{m_x . h} \cdot \frac{\left(R_y - \left(\frac{\left(\frac{^{200}Hg}{^{199}Hg} \right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl} \right)_{mes} \div \left(\frac{^{205}Tl}{^{203}Tl} \right)_{cer} \right)^{\left(\ln\left(\frac{RAM^{200}Hg}{RAM^{199}Hg} \right) + \ln\left(\frac{RAM^{205}Tl}{RAM^{203}Tl} \right) \right)}} \right) \right)}{\left(\frac{\left(\frac{^{200}Hg}{^{199}Hg} \right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl} \right)_{mes} \div \left(\frac{^{205}Tl}{^{203}Tl} \right)_{cer} \right)^{\left(\ln\left(\frac{RAM^{200}Hg}{RAM^{199}Hg} \right) + \left(\frac{RAM^{205}Tl}{RAM^{203}Tl} \right) \right)}} - R_x \right)} \cdot \frac{\sum_i R_{ix}}{\sum_i R_{iy}}$$

Equation 3-3 The ratio IDMS equation incorporating the Russell mass bias correction expression.

The standard uncertainty of each parameter in Equation 3-3 may be comprised of a number of different uncertainties. An Ishikawa diagram, Figure 3-1, was drawn up to ensure that all the individual sources of uncertainty contributing to each term in Equation 3-3 were identified and incorporated into the uncertainty budget model. The uncertainty budget was calculated following the guidelines set out by Eurachem/Citac[19].

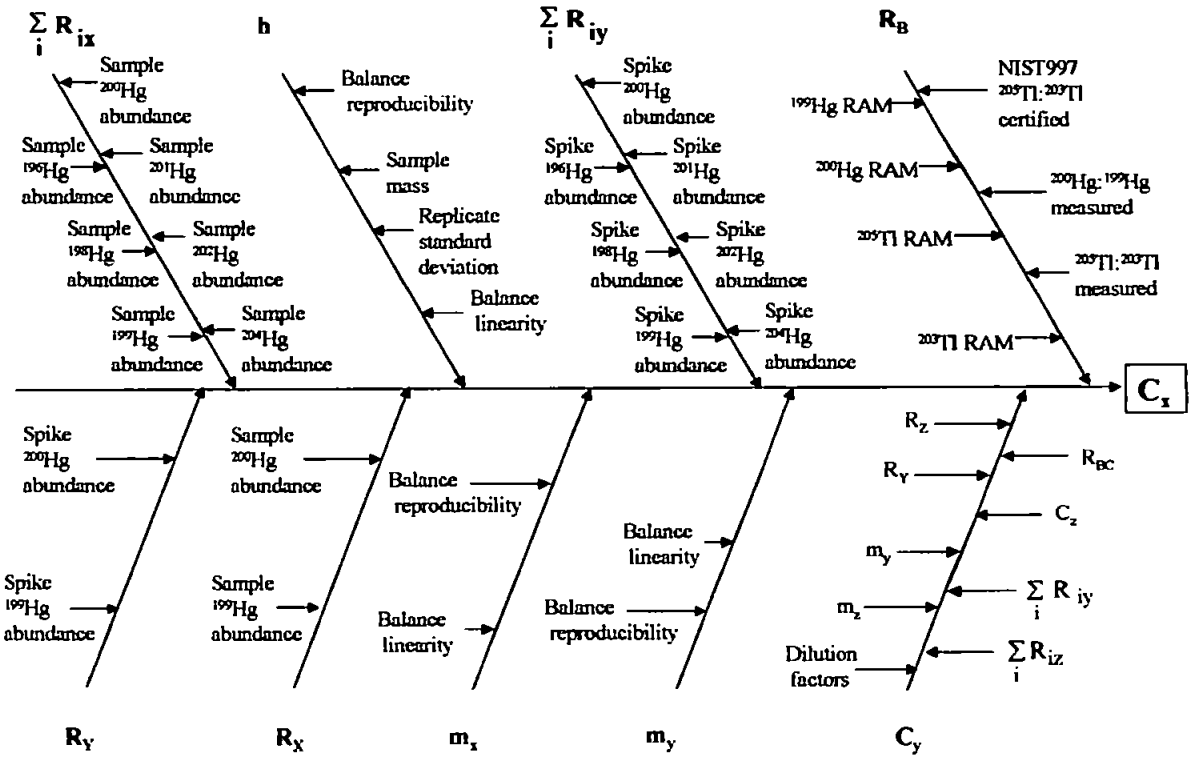


Figure 3-1 Ishikawa uncertainty diagram for the determination of mercury species by IDMS.

3.3.3. Combining Standard Uncertainties

Individual uncertainties associated with each parameter in Equation 3-3 were combined, after conversion to a standard uncertainty, using the spreadsheet method first described by Kragten[66], which assumes that the standard uncertainties of each measurement parameter are relatively small compared to that measurement parameter, and gives acceptable accuracy for practical purposes. The spreadsheet, which provides a simple method for the estimation and combination of uncertainties, estimates the contribution of each standard uncertainty to the overall uncertainty for the analytical result. The individual uncertainty estimates are subsequently combined using error propagation laws. Worked examples of the estimation of measurement uncertainty are given in the Eurachem/Citac[19] and the principles described therein were followed when constructing the spreadsheet used in this work. Each spreadsheet constructed during this work was separately validated by manual calculation. Proprietary computer software packages are also available for the estimation of measurement uncertainty *e.g.* GUM workbench™ from Metrodata GMBH, Germany, however, it was decided to avoid a 'black box' approach to the calculations and use first principles in order to fully understand the reasoning and methodologies employed.

3.3.4. Reverse IDMS of the Spike Material

The ^{199}Hg enriched CH_3HgCl spike material was not synthesised in quantities sufficient to determine its purity or to weigh with sufficient accuracy using a four figure balance. The spike material was therefore characterised by reverse isotope dilution analysis. In this procedure the spike material is isotopically diluted with a standard of natural isotopic abundance and the mass fraction of the spike solution calculated according to Equation 3-4. The measured ^{200}Hg:^{199}Hg isotope amount ratio was corrected for mass bias effects by the addition of NIST997 Tl as an internal standard and Equation 3-1 was combined with Equation 3-4 for the calculations but has been omitted here for clarity. It is possible to combine Equation 3-2 and Equation 3-4 to give a 'double' isotope dilution

equation[19,24] which eliminates the spike mass fraction, C_y, which is often relatively poorly characterised. In this study however, two separate spike solutions were prepared, each analysed using a different ICP-MS instrument. This allowed a comparison to be made of the different uncertainty contributions arising from the use of the different instruments for the spike characterisation.

$$C_y = C_z \cdot \frac{m_z}{m_y} \cdot \frac{R_z - R_{BS}}{R_{BS} - R_Y} \cdot \frac{\sum_i R_{iy}}{\sum_i R_{iz}}$$

Equation 3-4 The reverse IDMS equation

Where,

C_y = concentration of the analyte in the spike solution

C_z = concentration of the natural isotopic abundance standard

m_z = mass of natural isotopic abundance standard

m_y = mass of spike

R_z = reference:spike isotope amount ratio in the natural isotopic abundance standard

R_y = reference:spike isotope amount ratio in the spike

$\sum_i R_{iz}$ = isotope amount ratio of isotope *i* to the spike isotope in the natural isotopic abundance standard

$\sum_i R_{iy}$ = isotope amount ratio of isotope *i* to the spike isotope in the spike

R_{BS} = reference:spike isotope amount ratio in the spike and natural standard blend, corrected for mass bias effects in the manner shown in Equation 3-1.

3.4. Experimental

3.4.1 Separation and Analysis of Mercury Compounds by HPLC-ICP-MS

Two ICP-MS instruments were employed for the detection of mercury species following separation by HPLC, a VG Plasmaquad 3 quadrupole (Q) ICP-MS and a VG Axiom multicollector (MC) sector field (SF) ICP-MS (both Thermo Elemental, Winsford, UK). The coupling of the HPLC system and the data acquisition parameters for each instrument for the measurement of isotope amount ratios was optimised by analysing gravimetrically prepared solutions, of known isotopic composition, of inorganic and organomercury compounds with a range of ^{200}Hg:^{199}Hg isotope amount ratios. The HPLC conditions follow those of Harrington *et al.*[74] and are summarised in Table 3-1 along with the optimal operating conditions for each ICP-MS instrument used in this study. NIST 997 Thallium SRM was added to the mobile phase at 50 ng g^{-1} and continuously monitored during separations for the purposes of mass bias correction.

A number of problems can arise when coupling HPLC to ICP-MS, two of which are the incompatibility of the HPLC flow rate (*c.a.* 1 ml/minute) with that required for high efficiency sample nebulisation, and the deleterious effects on the plasma of a mobile phase with a high proportion of organic solvent. To overcome these potential problems the HPLC system was not directly coupled to the nebuliser sample uptake tube but a low dead volume splitter was used. This allowed the nebuliser to operate at its natural uptake rate, as determined by the nebuliser argon gas flow, and the system to be optimised for maximum sensitivity and optimum peak shape without compromising the HPLC conditions. A low flow nebuliser (0.2 ml/min Micromist, Glass Expansion, Vevey, CH) was employed in conjunction with 0.18 mm ∅ Teflon uptake tubing. The uptake rate of this system was 50 µl/minute, thereby ensuring highly efficient nebulisation and, in conjunction with a coupled spray chamber system (cyclonic and bead impact, Glass Expansion, Vevey, CH) cooled to – 5°C, reduced the organic loading in the plasma.

Table 3-1 Operating conditions for HPLC-ICP-MS characterisation.

HPLC Conditions			
HPLC Column	HiChrom Kromasil 100 FC 18 Excel, 25 cm x 4.6 mm i.d.		
Mobile Phase	50:50 *v/v* Methanol:DDW, 0.01% 2-mercaptoethanol, 50 ng/g NIST 997 Tl SRM		
Flow Rate (ml/min)	0.9		
Injection Volume (µl)	100		
VG PQ3 ICP-MS Operating Conditions			
RF Forward Power (W)	1450	Plasma gas (l min^{-1})	14
Reflected Power (W)	≤ 5	Auxiliary gas (l min^{-1})	0.75
Spray Chamber	Cyclonic, cooled to –5 °C	Nebuliser gas (l min^{-1})	0.52
Torch	Fassel Quartz	Dwell Time (ms)	10
Sampler and Skimmer Cones	Ni	Points per Peak	5
Nebuliser	Glass Expansion 0.2 ml/min Micromist	Ions Monitored	^{199}Hg, ^{200}Hg, ^{203}Tl, ^{205}Tl
VG Axiom MC-SF-ICP-MS Operating Conditions			
RF Forward Power (W)	1400	Plasma gas (l min^{-1})	14
Reflected Power (W)	≤ 10	Auxiliary gas (l min^{-1})	0.85
Spray Chamber	Coupled cyclonic and bead impact, cooled to –5 °C	Nebuliser gas (l min^{-1})	0.72
Torch	Fassel Quartz fitted with a Pt shield	Dwell Time (ms)	25
Sampler and Skimmer Cones	Ni	Points per Peak	1
Nebuliser	Glass Expansion 0.2 ml/min Micromist	Ions Monitored	^{199}Hg, ^{200}Hg, ^{203}Tl, ^{205}Tl

3.4.2. Characterisation of the ^{199}Hg Enriched CH_3HgCl

The mass fraction of the ^{199}Hg enriched CH_3HgCl was determined by reverse ID-MS with the spike isotopic composition modified by the addition of a well characterised natural standard (methylmercury chloride standard, Alfa Aesar, Ward Hill, MA, USA). Two different methylmercurychloride spike solutions were prepared, one for analysis by Q-ICP-MS (9 $\mu g\ g^{-1}$ as Hg), and the second for analysis by MC-SF-ICP-MS (11 $\mu g\ g^{-1}$ as Hg). In each case an iterative procedure was employed[24], to dilute the spike with the natural standard to give an ^{200}Hg:^{199}Hg isotope amount ratio of close to unity to minimise errors from sources such as mass bias and detector dead time[57], thus reducing the measurement uncertainty.

3.4.3. Equilibration of ^{199}Hg Enriched Methylmercury and DORM-2 CRM

Equilibration solutions of the HPLC mobile phase, 50:50 H_2O:CH_3OH *v/v* and 0.01% 2-mercaptoethanol, were prepared using HPLC grade methanol (BDH, Poole, UK), distilled deionised water (Elgastat Maxima, Elga Ltd, High Wycombe, UK) and 2-mercaptoethanol (BDH, Poole, UK). The moisture content of the DORM-2 CRM was determined, by drying separate subsamples to a constant mass at 105 $^{\circ}C$, to give the moisture correction factor, h (Equation 3-2). Approximately 3g of the DORM-2 CRM was accurately weighed and the amount of methylmercury present, after accounting for moisture content, calculated from the certified value. The mass of spike solution added was chosen so that the ^{200}Hg:^{199}Hg isotope amount ratio would be close to unity, provided complete equilibration between the sample and spike occurred. Subsequently, the spike solution was added to 25g of the equilibration solution, contained in a clean 50 ml glass conical flask. This solution was agitated throughout the timespan of the equilibration experiment by a magnetic stirrer and maintained at a temperature of 25°C by means of an electrically heated hotplate.

The DORM-2 CRM was subsequently added to the equilibration solution and 1 ml samples withdrawn at intervals up to 3000 minutes. The sampling frequency was approximately every two minutes for the first 20 minutes of the experiment followed by a reduced sampling rate of 5 minute intervals until 1 hour had elapsed, with less frequent sampling thereafter. Each sample aliquot was pipetted into a filter (Autovial 0.2μm PTFE membrane syringeless filters, Whatman, Maidstone, UK), diluted prior to filtration with 3 ml of fresh equilibration solvent, filtered, and stored in a clean 25 ml sterilin container at 4°C until analysis by HPLC-ICP-MS. Samples obtained from the equilibration experiments were analysed using Q-ICP-MS under the same instrumental conditions as those for the reverse IDMS of the spike solution.

3.5. Results and Discussion

3.5.1 Calculating Isotope Amount Ratios

In order to perform species specific ID-MS it is necessary to measure isotope amount ratios of two transient signals resulting from the chromatographic separation prior to ICP-MS detection. Two problems may arise, namely compound fractionation on the column and spectral skew resulting from too slow mass spectral scanning. It is unlikely that any on column fractionation would occur with the compounds under study because the difference in molar mass between the analyte and spike was too small (though this may not be the case if gas chromatography was used). In this case data acquisition parameters were optimised to eliminate spectral skew.

Two different approaches were assessed to determine the accuracy and precision of calculated isotope amount ratios from the multi-isotope chromatograms obtained by HPLC-ICP-MS, namely peak integration and a pseudo-steady-state approach. For peak integration the relevant peaks for the isotope pair of interest, ^{200}Hg and ^{199}Hg, of a particular species in the chromatogram were integrated to obtain the baseline-subtracted

peak integrals and the isotope amount ratio for that specie calculated using these integrals. The advantage of this approach is that the effects of spectral skew, are minimised, however, precision can be degraded because the precision inherent in rapid sampling of the isotopic pair is lost. The second approach assumed that the chromatogram was an undulating, or pseudo-steady-state, signal. The results of this approach are shown in Figure 3-2, where co-incident pairs of data points from the two isotope ion signals (*i.e.* ^{200}Hg and ^{199}Hg) were ratioed over the course of the chromatogram.

In order to achieve the best precision and accuracy, only data-points on the apices of the peaks (PQ3 n=9, Axiom n=5), for each isotope of a particular specie in the chromatogram, were chosen and baseline signal subtracted. The isotope amount ratios were calculated using each pair of corresponding data-points from the two peaks and subsequently corrected for mass bias effects. The advantage of this approach is that the inherent precision is maintained and it is possible to obtain an estimate of precision from a single peak, however, erroneous isotope amount ratios will result if spectral skew is not minimised. Both of the above methods for data extraction were evaluated and the pseudo-steady-state approach resulted in measured, mass bias corrected, isotope amount ratios which were closer to the theoretical values of the gravimetrically prepared mercury solutions, and exhibited a greater degree of precision then those obtained by peak integration (Table 3-2).

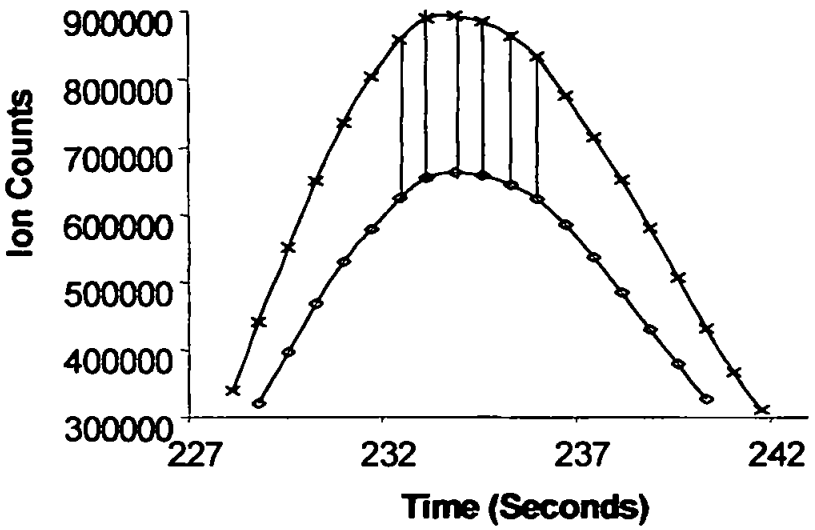


Figure 3-2 Isotope amount ratios measured using the pseudo steady state approach. The isotope amount ratio is calculated by ratioing the ion signals for coincident pairs of data points over the peak maximum.

Table 3-2 Figures of merit for HPLC-ICP-MS optimisation using the pseudo-steady-state approach for the calculation of the isotope amount ratios.

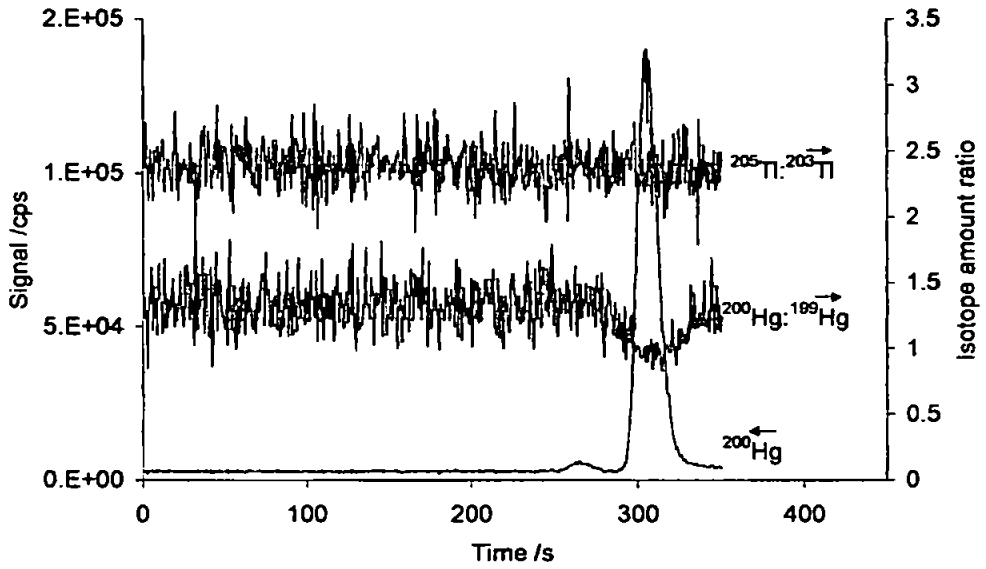
	^{200}Hg:^{199}Hg Isotope Amount Ratio	Relative Standard Uncertainty
VG PQ3		
Gravimetric Ratio	1.369 ± 0.029	0.89 %
Pseudo-steady-state measured ratio	1.371 ± 0.064	4.67 %
Peak integration measured ratio	1.355 ± 0.075	5.5 %
Gravimetric Ratio	0.2751 ± 0.00002	0.01 %
Pseudo-steady-state measured ratio	0.2758 ± 0.0057	2.1 %
Peak integration measured ratio	0.2674 ± 0.008	3.0 %
VG Axiom Multicollector		
Gravimetric Ratio	1.369 ± 0.029	0.89 %
Pseudo-steady-state measured ratio	1.369 ± 0.00051	0.037 %
Peak integration measured ratio	1.359 ± 0.013	0.92 %
Gravimetric Ratio	0.2751 ± 0.00002	0.01 %
Pseudo-steady-state measured ratio	0.2755 ± 0.00057	0.21 %
Peak integration measured ratio	0.2661 ± 0.0015	0.56 %

3.5.2. Comparison of Scanning and Simultaneous Detection

Variability of the measured isotope amount ratios during a chromatographic run are illustrated in Figure 3-3 for both the quadrupole scanning (Figure 3-3A) and the Axiom simultaneous multicollector instrument (Figure 3-3B). The Quadrupole instrument resulted in poorer precision during peak elution, due to the requirement to measure transient signals for four isotopes with a dwell time of 10 milliseconds. There is also evidence of spectral skew, (Figure 3-3A) because the measured ^{200}Hg:^{199}Hg isotope amount fell at the start of the methylmercury peak elution, and was stable only at the peak apex. When using the multicollector instrument the ^{200}Hg:^{199}Hg isotope amount ratio, calculated from background signals using Faraday detectors, was highly variable prior to the MeHg peak elution as the Hg concentration in the mobile phase was not high enough to result in a measurable signal. As the peak eluted, and the signal strength rose rapidly, the isotope amount ratio precision rapidly improved to a minimum at the peak maximum. The greater precision of the measured ^{205}Tl:^{203}Tl isotope amount ratio obtained using the multicollector, as opposed to that obtained using the quadrupole instrument, can also be clearly observed.

The precision of the mercury and thallium isotope amount ratios was comparable, 1.2% and 0.86% relative standard uncertainty respectively, when the quadrupole scanning instrument was employed. However, for the multicollector instrument the mercury isotope amount ratio was measured with improved precision compared with that obtained for the thallium isotope amount ratio, typically 0.07 and 0.24% relative standard uncertainty respectively. This was due to the poorer sensitivity of the Faraday cup detectors and the relatively low mass fraction of Tl in the mobile phase, 50 ng g^{-1}, compared to the methylmercury mass fraction, 11 $\mu g\ g^{-1}$, injected onto the column.

A



B

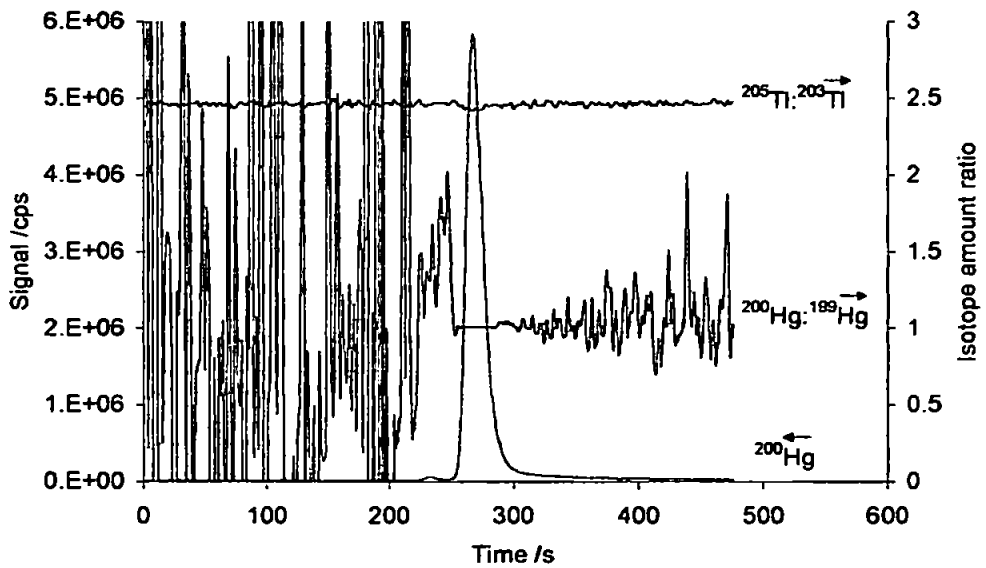


Figure 3-3 The measured ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios and ^{200}Hg signal monitored during reverse ID-MS of the spike solutions. A, PQ3 quadrupole instrument in peak jumping mode, dwell time 10ms, 100µl injection of a 900 ng/g CH_3HgCl solution; B; Axiom multicollector instrument, 25 ms dwell time, 100 µl injection of a 11µg/g CH_3HgCl solution.

The memory effect, which is a common problem in mercury analysis, is also well illustrated, Figure 3-3B, when using the multicollector instrument. As the MeHg signal strength declined after peak elution the isotope amount ratio became more variable than during, but less so than before the peak elution. However, this was not the case when using the electron multiplier detector of the quadrupole scanning instrument, Figure 3-3A, as the amount of mercury contained within the HPLC mobile phase was sufficient to provide relatively stable ion counts. To counteract this memory effect 0.6M HBr in a 50% methanol solution was aspirated for 3 minutes between each sample acquisition which reduced the blank counts to those prior to the sample run.

3.5.3 Characterisation of the Spike

Before IDMS analysis of a sample could be performed it was necessary to determine the mass fraction of CH_3HgCl in the ^{199}Hg enriched spike as accurately and precisely as possible. Reverse IDMS was performed for the spike characterisation, and full uncertainty budgets calculated, using both the simultaneous multicollector and the scanning quadrupole instruments. The spike mass fraction was calculated using Equation 3-4, the results are shown in Table 3-3. No methylmercury was detected in the blank solutions and therefore a blank correction was not included in Equation 3-4. The raw counts for each mercury isotope in the methylmercury peak were, however, baseline subtracted to account for inorganic Hg present in the ICP argon gas and the solutions used for the spike make up. The standard uncertainty of the measured ^{200}Hg:^{199}Hg isotope amount ratio entered into Equation 3-4 included the uncertainty contribution from the baseline correction which contributed less than 0.1%. The relative standard uncertainty of the spike solution characterised using the Axiom instrument was 0.70%, which was nearly an order of magnitude less than that obtained for the spike solution mass fraction using the scanning instrument, 4.1%. Individual contributions to the total standard uncertainty are shown in Figure 3-4.

Table 3-3 The mass fraction of ^{199}Hg enriched CH_3HgCl spike determined by reverse isotope dilution HPLC-ICP-MS.

Instrument	Mass Fraction ($\mu g\ g^{-1}$)	Standard Uncertainty ($\mu g\ g^{-1}$, K=1)	Relative Standard Uncertainty
VG PQ3 Q-ICP-MS	8.96 ($n = 5$)	± 0.37	4.1 %
VG Axiom MC-SF-ICP-MS	11.06 ($n = 3$)	± 0.08	0.70 %

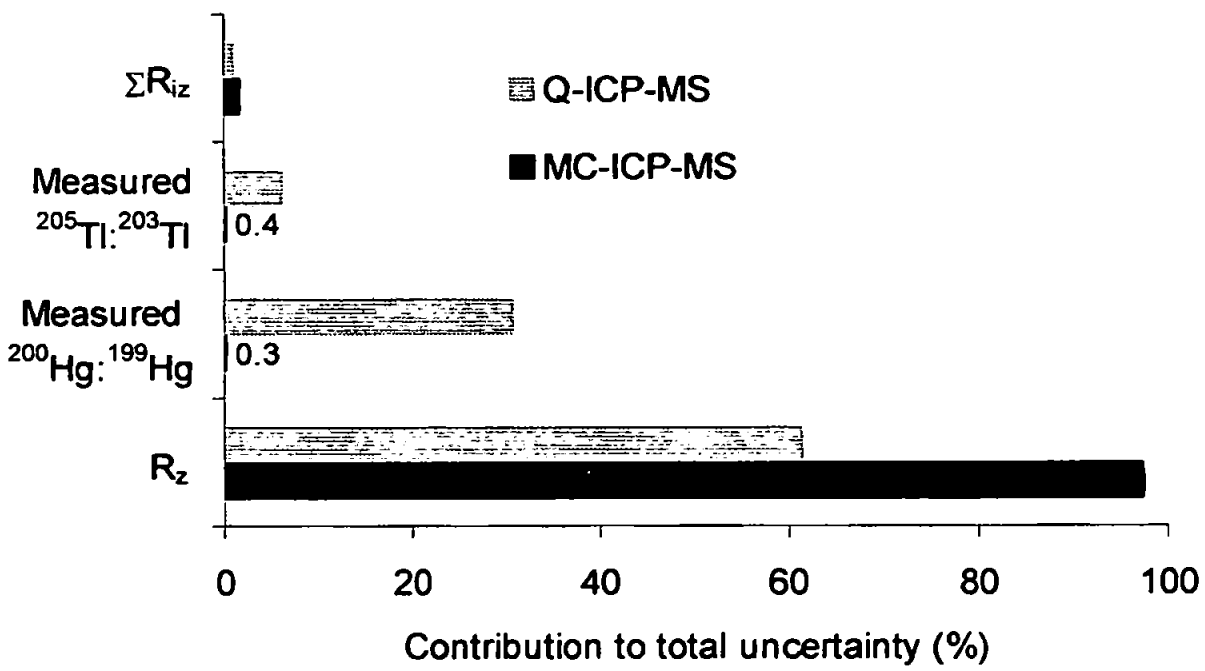


Figure 3-4 Contributions to the total standard uncertainty of the mass fraction of ^{199}Hg enriched CH_3HgCl determined by reverse IDMS.

For the PQ3 quadrupole instrument the major uncertainty contributions arose from two parameters, the natural isotopic abundance $^{200}Hg:^{199}Hg$ isotope amount ratio, R_z, and the measured $^{200}Hg:^{199}Hg$ isotope amount ratio, R_{BS}, which contributed 61 and 31 percent respectively. In comparison, when using the Axiom multicollector instrument, R_z contributed 97% of the standard uncertainty and the measured $^{200}Hg:^{199}Hg$ isotope amount ratio, R_{BS}, only contributed 0.3% of the combined standard uncertainty for the spike mass fraction. The reduction in the contribution of the measured isotope amount ratios $^{200}Hg:^{199}Hg$ and $^{205}Tl:^{203}Tl$ was due to the increased measurement precision obtainable with the Axiom multicollector instrument (Table 3-4).

The relative isotopic abundances of mercury, as given by IUPAC[80], have relatively large associated uncertainties compared to some elements, hence the large relative standard uncertainties for R_z and ΣR_{iz} (Table 3-4), which were calculated from the IUPAC data. No purity or isotopic abundance uncertainty data was supplied with the ^{199}Hg enriched HgO, hence the isotopic composition of the spike material was determined using the Axiom MC-SF-ICP-MS prior to the synthesis of the spike material using the procedures of Briche[90]. The IUPAC, AEAT and measured isotopic abundance data is shown in Table 3-5. The improved precision values obtained by this analysis resulted in the associated uncertainties of R_y and ΣR_{iy} (Table 3-4) providing a minimal contribution to the standard uncertainty for the spike mass fraction.

Table 3-4 Measured isotope amount ratios and uncertainty contributions to the mass fraction determination of the ^{199}Hg enriched CH_3HgCl spike by reverse HPLC-ID-MS.

		Relative Standard Uncertainty (%)	Contribution to spike mass fraction standard uncertainty (%)
Quadrupole HPLC-ID-ICP-MS			
R_{BC}	0.9900	0.58	31
Measured ^{205}Tl:^{203}Tl	2.4276	0.51	6
Rz	1.3693	0.89	61
ΣRiz	5.9277	0.44	1
Ry	0.2751	0.01	2.9E-06
ΣRiy	1.5155	0.009	1.4E-04
Axiom Multicollector HPLC-ID-ICP-MS			
R_{BC}	1.0029	0.045	0.3
Measured ^{205}Tl:^{203}Tl	2.4243	0.10	0.4
Rz	1.3693	0.89	97
ΣRiz	5.9277	0.44	2
Ry	0.2751	0.01	2.9E-06
ΣRiy	1.5155	0.009	1.4E-04

Table 3-5 The isotopic abundance and uncertainty data for natural abundance Hg and the stated and measured AEAT ^{199}Hg enriched HgO.

			AEAT ^{199}Hg enriched HgO		
Mercury Isotope	IUPAC Data Atom %	Standard Uncertainty	Data supplied Atom %	Axiom MC-SF-ICP-MS Atom %	Standard Uncertainty
196	0.15	0.01	0.1	0.10	0.001
198	9.97	0.12	3.08	2.93	0.0014
199	16.87	0.13	65.74	65.98	0.010
200	23.1	0.11	18.18	18.15	0.0016
201	13.18	0.05	3.95	3.96	0.0019
202	29.86	0.15	7.31	7.43	0.0047
204	6.87	0.09	1.65	1.44	0.0012

3.5.4 Analysis of Reference Material

The isotope amount ratios in the samples were determined using the PQ3 instrument preceded by a HPLC separation, Figure 3-5 shows a typical chromatogram obtained. It was not possible to measure the isotope amount ratios using the Axiom multicollector instrument as the ion signal strength was not high enough to be measured by the faraday cup multicollector array with sufficient accuracy and precision.

Two speciation analyses were performed, one using the spike material characterised by the PQ3 quadrupole ICP-MS and the second using the spike characterised with the Axiom multicollector instrument. The major contributions to the uncertainty budget for each analysis are derived from the precision values of the measured ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios. The raw counts of the mercury isotopes were baseline corrected as described in the spike characterisation. Figure 3-6 shows the major contributions to the uncertainty budget for each analysis and the data is shown in Table 3-6. For the speciation analysis conducted using the spike material characterised by the quadrupole instrument the major uncertainty component was derived from the uncertainty associated with the measured ^{200}Hg:^{199}Hg isotope amount ratio, 63%. Three other factors, the measured ^{205}Tl:^{203}Tl isotope amount ratio, (10%), the calculated natural abundance ^{200}Hg:^{199}Hg isotope amount ratio, R_x, (10%), and the spike mass fraction, C_y, (16%), also contributed significantly to the standard uncertainty. The improved spike characterisation by the multicollector instrument resulted in a smaller uncertainty contribution from C_y, (8%), to the standard uncertainty when this material was used, with the uncertainty contribution from R_x rising to 24%.

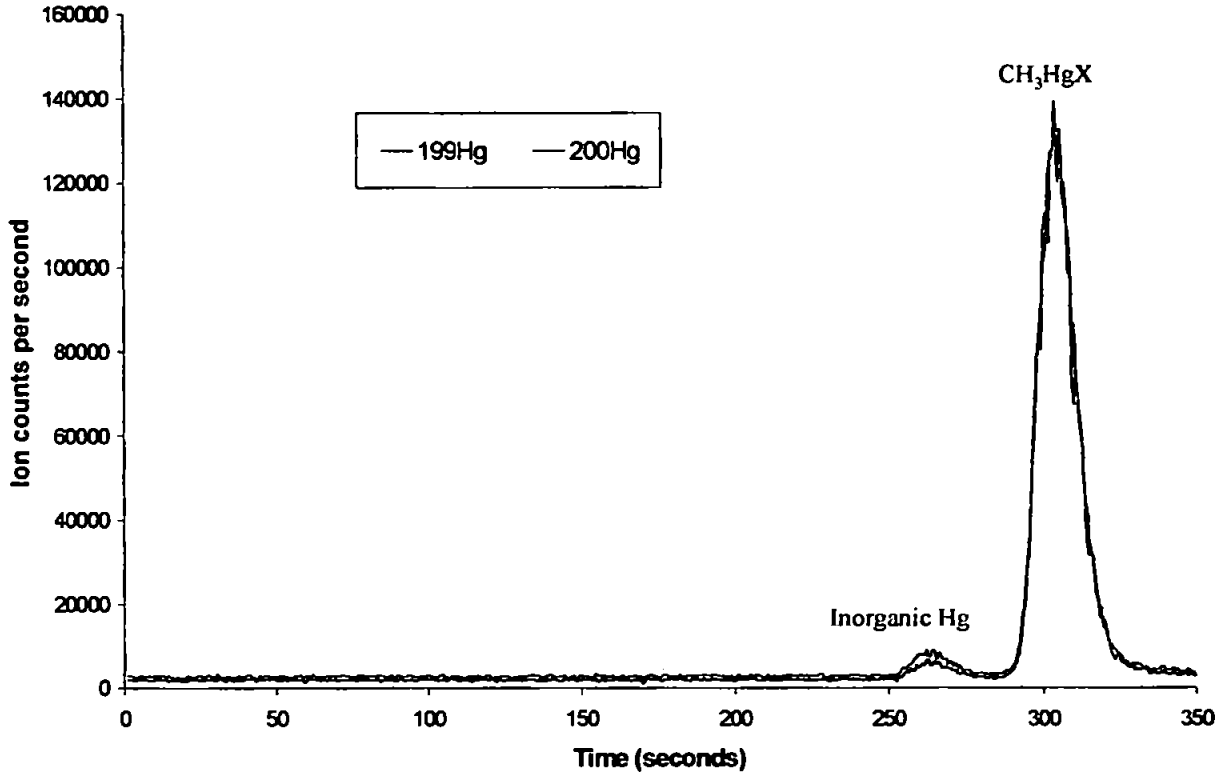


Figure 3-5 HPLC-Q-ICP-MS chromatogram of an equilibration sample.

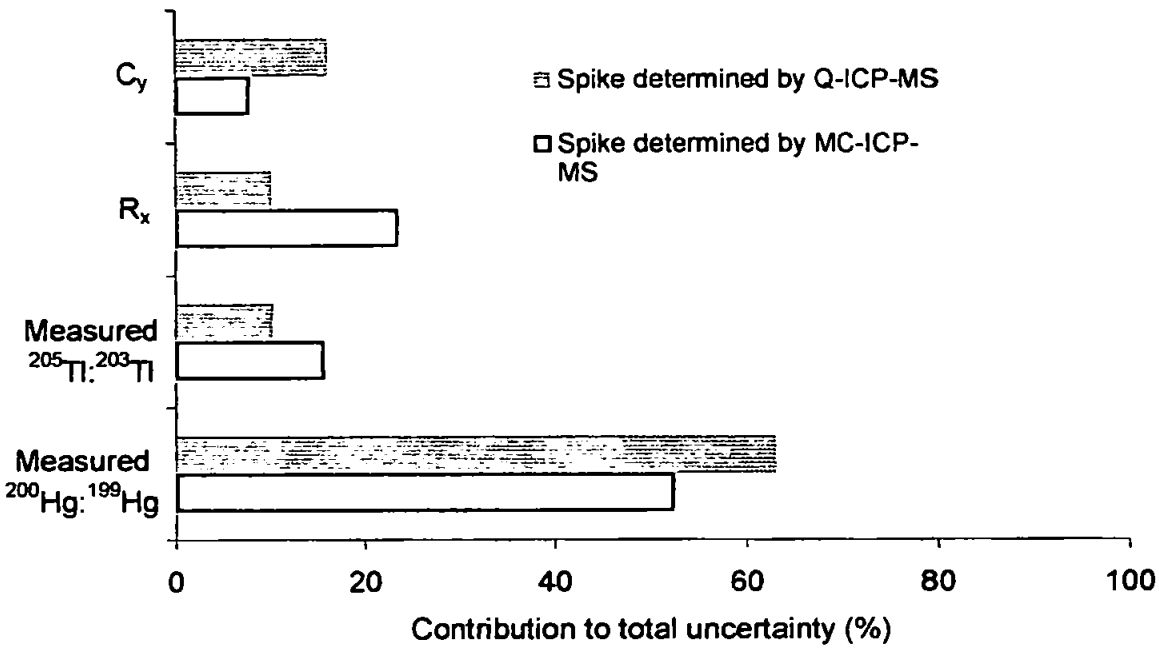


Figure 3-6 Contributions of individual standard uncertainties to the total combined expanded uncertainty for the determination of CH_3Hg^+ in DORM-2 CRM by species specific IDMS. Each uncertainty budget was dominated by the precision of the measured isotope amount ratios. The contribution from the spike uncertainty was significantly larger when it was characterised by quadrupole HPLC-ICP-MS.

Table 3-6 Contributions to uncertainty for a single sample from each equilibration experiment.

		Relative Standard Uncertainty (%)	Contribution to methylmercury mass fraction in DORM-2 standard uncertainty (%)
Spike Characterised by quadrupole HPLC-ICP-MS			
Measured ^{200}Hg:^{199}Hg	1.0021	1.9	63
Measured ^{205}Tl:^{203}Tl	2.3656	1.6	10
R_X	1.369	0.9	10
C_Y ($\mu g\ g^{-1}$)	8.96	4.0	16
Spike Characterised by multicollector HPLC-ICP-MS			
Measured ^{200}Hg:^{199}Hg	1.0115	1.1	52
Measured ^{205}Tl:^{203}Tl	2.3654	1.3	16
R_X	1.369	0.9	24
C_Y ($\mu g\ g^{-1}$)	11.1	1.9	8

The mass fraction of methylmercury in DORM-2 was calculated using Equation 3-3 and the standard uncertainty estimated, the results are shown in Table 3-7. No methylmercury was detected in the blank solutions and a blank correction was not therefore included in Equation 3-3. The mass fraction of methylmercury in DORM-2 using the spike characterised using quadrupole HPLC-ICP-MS was 4.45 $\mu g\ g^{-1}$ with a standard combined uncertainty of 0.45 $\mu g\ g^{-1}$. For the experiment conducted using the spike characterised by multicollector HPLC-ICP-MS the mass fraction of methylmercury in DORM-2 was 4.25 $\mu g\ g^{-1}$ with a standard uncertainty of 0.24 $\mu g\ g^{-1}$.

A standard uncertainty, u, corresponds to one standard deviation, which, for a normal distribution, includes approximately 68% of the values. To provide an uncertainty estimate which can be expected to cover approximately 95% of the normally distributed values u is expanded by a coverage factor, k. As the major contribution to each standard uncertainty was derived from the precision of the measured isotope amount ratios, $n = 10$ for each replicate, the degrees of freedom was considered large enough to use a coverage factor of $k = 2$, which approximates to the 95% confidence interval. Consequently the standard uncertainty for each determination of methylmercury in DORM-2 by species specific HPLC-ID-ICP-MS was expanded to give the final results in Table 3-7.

Table 3-7 The methylmercury mass fraction in DORM-2 CRM determined by species specific HPLC-ID-ICP-MS.

Determination	DORM-2 Methylmercury Mass Fraction (μg/g)	Relative Expanded Uncertainty
Spike Characterised by quadrupole ICP-MS	4.45 ± 0.90*	20%
Spike Characterised by multicollector ICP-MS	4.25 ± 0.47*	11%
DORM-2 Certified Value	4.47 ± 0.32	7%

* the reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%

3.6. Conclusions

The analytical procedure required a separation step, by HPLC, prior to the detection of the mercury species by ICP-MS. The coupling of the HPLC system to the ICP-MS instrument was optimised to ensure highly efficient sample nebulisation and minimise the effect of the HPLC mobile phase, 50% methanol, on plasma stability. Instrumental data acquisition parameters were optimised using gravimetrically prepared solutions of natural and ^{199}Hg enriched abundance methylmercurychloride. Two methods of data extraction for the calculation of isotope amount ratios from the resulting chromatograms, peak integration and a pseudo-steady-state approach, have been evaluated. The pseudo-steady-state approach gave results of greater accuracy and precision than the peak integration approach, and, as precision values can be obtained from a single sample injection onto the HPLC column, considerably reduces the sample volume and time required for analysis.

The determination of methylmercury in a fish muscle certified reference material, DORM-2, was performed by species specific IDMS and quantitative recovery in good agreement with the certified value was obtained. An uncertainty budget has been calculated for the analytical procedure, allowing the relative uncertainty contributions for each parameter in the measurement equation to be quantified and, their relative contributions to the final expanded uncertainty determined.

Two ICP-MS instruments were employed in this study for the measurement of both transient and simultaneous ion signals for the calculation of isotope amount ratios. Species specific HPLC IDMS by the Axiom multicollector instrument resulted in an lower relative expanded uncertainty due to the greater precision and accuracy obtainable with this instrument compared with a quadrupole ICP-MS. The major contributions to the expanded uncertainty using the multicollector instrument are from the uncertainties associated with the natural isotopic abundances of Hg, therefore, in order to significantly reduce the uncertainty further an improvement in the isotopic characterisation of Hg in the sample is

required prior to species specific ID. This will add considerable time to the analysis, with the possible need to resolve or account for interferences *e.g.* ^{204}Pb on ^{204}Hg. A number of elements have a relatively poor isotopic characterisation and, if ID is to become more widely used, a programme is necessary to improve the IUPAC data. The advent of modern multicollector ICP-MS instrumentation should allow this improvement. For the scanning quadrupole instrument the measured isotope amount ratios contributed >65% of the expanded uncertainty. As it is unlikely that the isotope amount ratio precision can be significantly improved the expanded uncertainty for quadrupole HPLC IDMS determinations will remain relatively large.

CHAPTER 4 Equilibration

4. Introduction

Equilibration between the natural isotopic abundance analyte contained within the sample and the isotopically modified spike solution is the key experimental stage in IDMS[24]. If this equilibration is incomplete the advantages of IDMS over analyses performed by conventional external calibration are lost and biased results occur. The determination of methylmercury in DORM-2 CRM by species specific IDMS gave results in good agreement with the certified value and equilibration could therefore have been adjudged to be complete. In order to investigate the effects of incomplete sample/spike equilibration a second sample matrix, NIST2710 Montana soil SRM was chosen to represent a terrestrial material, where the partitioning of mercury species may be different to those in DORM-2.

4.1. Monitoring Equilibration

The extent of equilibration between the target analyte in the sample and the isotopically enriched spike solution can be deduced by monitoring the reference:spike isotope amount ratio in the extractant solution with time. At the moment of addition of the particulate material to the extractant/spike solution the contribution to the ^{200}Hg: ^{199}Hg ratio, chosen as the reference and spike isotopes respectively, in the liquid phase will be solely from the spike solution. As equilibration between the ^{199}Hg enriched spike, and the natural isotopic abundance mercury proceeds this ratio will change as natural isotopic abundance Hg is released from, and enriched spike Hg is adsorbed onto the particulate material until, at equilibrium, the ^{200}Hg:^{199}Hg ratio in the liquid or solid phases is constant. If the mass fractions of analyte in the sample and spike are known the ^{200}Hg:^{199}Hg ratio at complete equilibration can be calculated, allowing a comparison to be made with the

measured ^{200}Hg:^{199}Hg ratio at equilibrium. At time $t=0$, the overall ^{200}Hg:^{199}Hg ratio can be described by Equation 4-1 where the following subscripts and superscripts apply.

e = ^{199}Hg enriched spike Hg

n = natural isotopic abundance Hg

l = liquid phase

s = solid phase

t = 0 the time of spiking before any adsorption or desorption has occurred

t_{eqb} = time t when sample/spike equilibration is complete

$$\frac{^{200}Hg_{s+l}^{t=0}}{^{199}Hg_{s+l}^{t=0}} = \frac{^{200}Hg_{el}^{t=0} + ^{200}Hg_{es}^{t=0} + ^{200}Hg_{nl}^{t=0} + ^{200}Hg_{ns}^{t=0}}{^{199}Hg_{el}^{t=0} + ^{199}Hg_{es}^{t=0} + ^{199}Hg_{nl}^{t=0} + ^{199}Hg_{ns}^{t=0}}$$

Equation 4-1

which reduces to Equation 4-2 by removing the components with a value of zero at time $t=0$ giving

$$\frac{^{200}Hg_{s+l}^{t=0}}{^{199}Hg_{s+l}^{t=0}} = \frac{^{200}Hg_{el}^{t=0} + ^{200}Hg_{ns}^{t=0}}{^{199}Hg_{el}^{t=0} + ^{199}Hg_{ns}^{t=0}}$$

Equation 4-2

At time t_{eqb}

$$\frac{^{200}Hg_{s+l}^{t=0}}{^{199}Hg_{s+l}^{t=0}} = \frac{(^{200}Hg_{el}^{t=0} - ^{200}Hg_{es}^{teqb}) + (^{200}Hg_{ns}^{t=0} - ^{200}Hg_{ns}^{teqb})}{(^{199}Hg_{ns}^{t=0} - ^{199}Hg_{ns}^{teqb}) + (^{199}Hg_{ns}^{t=0} - ^{199}Hg_{ns}^{teqb})}$$

Equation 4-3

The amount of each isotope in solution at time t can be described in terms of the amount of spike Hg adsorbed onto, or the amount of natural Hg desorbed from, the particulate material

$$^{200}Hg_{el}^{teqb} = {}^{200}Hg_{el}^{t=0} - {}^{200}Hg_{es}^{teqb}$$

Equation 4-4

$$^{200}Hg_{nl}^{teqb} = {}^{200}Hg_{ns}^{t=0} - {}^{200}Hg_{ns}^{teqb}$$

Equation 4-5

$$^{199}Hg_{el}^{teqb} = {}^{199}Hg_{el}^{t=0} - {}^{199}Hg_{es}^{teqb}$$

Equation 4-6

$$^{199}Hg_{nl}^{teqb} = {}^{199}Hg_{ns}^{t=0} - {}^{199}Hg_{ns}^{teqb}$$

Equation 4-7

substituting Equation 4-4, Equation 4-5, Equation 4-6 and Equation 4-7 into Equation 4-3 gives

$$\frac{^{200}Hg_{s+l}^{t=0}}{^{199}Hg_{s+l}^{t=0}} = \frac{^{200}Hg_{el}^{teqb} + {}^{200}Hg_{nl}^{teqb}}{^{199}Hg_{el}^{teqb} + {}^{199}Hg_{nl}^{teqb}}$$

Equation 4-8

combining the RHS to the measurand gives

$$\frac{^{200}Hg_{s+l}^{t=0}}{^{199}Hg_{s+l}^{t=0}} = \frac{^{200}Hg_{l}^{teqb}}{^{199}Hg_{l}^{teqb}}$$

Equation 4-9

and combining with Equation 4-2 gives

$$\frac{^{200}Hg_{l}^{teqb}}{^{199}Hg_{l}^{teqb}} = \frac{^{200}Hg_{el}^{t=0} + ^{200}Hg_{ns}^{t=0}}{^{199}Hg_{el}^{t=0} + ^{199}Hg_{ns}^{t=0}}$$

Equation 4-10

Allowing the calculation of the theoretical ^{200}Hg:^{199}Hg isotope amount ratio in solution, provided equilibration between the sample and spike isotopomers is complete.

4.2. Adsorption and Desorption

The isotope amount ratio in solution (R), at any time during the equilibration of enriched and natural abundance isotopomers of an analyte is given by Equation 4-11. Furthermore the total number of moles of the analyte in solution (n), at any time is the sum of the number of moles of both isotopomers in solution (Equation 4-12). Hence, combining Equation 4-11 and Equation 4-12 to give Equation 4-13, with rearrangement to Equation 4-14, and determining R and n, allows the extent of desorption and adsorption of the natural isotopic abundance particulate bound analyte and the spike analyte respectively.

$$R = \frac{A_y . n_y + A_x . n_x}{B_y . n_y + B_x . n_x}$$

Equation 4-11

where

A_y = the abundance of isotope A in the spike

A_x = the abundance of isotope A in the sample

B_y = the abundance of isotope B in the spike

B_x = the abundance of isotope A in the sample

$$n = n_y + n_x \qquad \therefore n_y = n - n_x$$

Equation 4-12

where

n = the total number of moles of analyte n_y = the total number of moles of spike analyte

n_x = the total number of moles of sample analyte

substituting Equation 4-12 in to Equation 4-11 gives

$$R = \frac{A_y(n - n_x) + A_x.n_x}{B_y(n - n_z) + B_x.n_x}$$

Equation 4-13

and rearranging gives

$$n_x = n.\frac{A_y - RB_y}{(RB_x - A_x) + (A_y - RB_y)}$$

Equation 4-14

4.3. Experimental

The extent of equilibration between the IDMS spike solution and the natural isotopic abundance analyte was determined for two certified reference materials. National Institute of Science and Technology (NIST) 2710 Montana Soil Certified Reference Material (SRM) was used to represent terrestrial material and DORM-2 dog fish muscle (NRCC) was employed as a biological sample. The experimental procedures for experiments involving DORM-2 CRM have been described in Chapter 3. NIST 2710, purchased from LGC (Teddington, UK), is certified for total Hg, 32.6 μg g^{-1}. For the total Hg IDMS determination (NIST2710 SRM) a ^{199}Hg enriched Hg^{2+} spike solution was

prepared from ^{199}Hg enriched (65.98 atom %) HgO (AEA Technology, Harwell, UK). 3.4 mg of the enriched material was dissolved in 100µg of concentrated HNO_3 (Aristar Grade, BDH, Poole, UK) and diluted with 18 MΩ cm^{-1}, distilled, deionised water (DDW, Elgastat Maxima system Elga Ltd, High Wycombe, UK) to give a nominal mass fraction of 600 µg g^{-1} in 2% HNO_3.

4.3.1. Characterisation of the ^{199}Hg enriched inorganic Hg spike

The stock ^{199}Hg enriched inorganic Hg spike solution was diluted with 2% HNO_3 to approximately 600 ng g^{-1}, and the accurate mass fraction of the diluted spike solution determined by reverse IDMS by Dr. J.Truscott. Alfa Aesar *Specpure* ICP standard solution (Johnson Matthey, Royston, UK) was diluted to 170 ng g^{-1} Hg in 2% HNO_3 and used as the natural standard for the isotopic dilution of the spike solution. When the measured isotope amount ratio is close to unity, systematic errors, from sources such as mass bias and detector dead time, are minimised, thereby reducing the measurement uncertainty[57]. An iterative procedure was therefore employed, to produce blends of the ^{199}Hg enriched inorganic Hg spike solution and the natural standard with a reference:spike isotope amount ratio (^{200}Hg:^{199}Hg) of close to unity. The procedure followed was developed during a study by the High Accuracy Analysis by Mass Spectrometry (HAAMS) sub committee of the Analytical Methods Committee of the Royal Society of Chemistry.

A blend of the spike material and the natural standard was produced and the reference:spike isotope amount ratio measured and used to calculate the mass fraction of the spike material. Subsequently the calculated spike mass fraction is used, in conjunction with the known natural standard mass fraction, to calculate the amounts of each material required to produce a new solution having a reference:spike isotope amount ratio of close to unity. The reference:spike isotope amount ratio in this second solution is subsequently

measured. Mass bias correction on this second measured reference:spike isotope amount ratio is performed by bracketing the sample with the first solution prepared and applying a correction factor from the calculated and measured reference:spike isotope amount ratio in the bracket blend. This procedure is repeated, *i.e.* natural standard/spike solution 2 becomes the mass bias correction blend for natural standard/spike solution 3, until the measured reference:spike isotope amount ratio and the ion counts of each isotope are matched to within 5%. Subsequently the mass fraction of the spike is calculated from the matched solutions using Equation 4-15. The ^{200}Hg:^{199}Hg isotope pair was chosen as the reference:spike isotope amount ratio and measured using a VG Axiom MC-SF-ICP-MS instrument (Thermo Elemental, Winsford, UK), the optimal operating conditions are shown in Table 4-1. Hydrobromic acid, 0.6M, (Aldrich, Gillingham, UK) was used as a wash solution during all ICP-MS analyses to counteract the memory effects of mercury.

$$C_y = C_z \cdot \frac{m_z}{m_y} \cdot \frac{R_z - (R_{BS} \times \frac{R_{MB}}{R_{MB}})}{(R_{BS} \times \frac{R_{MB}}{R_{MB}}) - R_Y} \cdot \frac{\sum_i R_{iy}}{\sum_i R_{iz}}$$

Equation 4-15 The reverse IDMS equation

Where,

C_y = concentration of the analyte in the spike solution

C_z = concentration of the natural isotopic abundance standard

m_z = mass of sample

m_y = mass of spike

R_z = reference:spike isotope amount ratio in the natural isotopic abundance standard

R_y = reference:spike isotope amount ratio in the spike

$\sum_i R_{iz}$ = isotope amount ratio of isotope *i* to the spike isotope in the natural isotopic abundance standard

$\sum_i R_{iy}$ = isotope amount ratio of isotope *i* to the spike isotope in the spike

R_{MB} = the calculated reference:spike isotope amount ratio in the mass bias calibration blend

R'_{MB} = the measured reference:spike isotope amount ratio in the mass bias calibration blend

R_{BC} = reference:spike isotope amount ratio in the spike and natural standard blend

^{200}Hg was chosen as the reference isotope and ^{199}Hg as the spike isotope

Table 4-1 ICP-MS and HPLC operating conditions.

HPLC Conditions	
HPLC Column	HiChrom Kromasil 100 FC 18 Excel, 25 cm x 4.6 mm i.d.
Mobile Phase	50:50 *v/v* Methanol:DDW, 0.01% 2-mercaptoethanol, 50 ng/g NIST 997 Tl SRM
Flow Rate (ml/min)	0.9
Injection Volume (μl)	100

VG Axiom MC-SF-ICP-MS Operating Conditions			
RF Forward Power (W)	1400	Plasma gas (l min^{-1})	14
Reflected Power (W)	≤ 10	Auxiliary gas (l min^{-1})	0.85
Spray Chamber	Coupled cyclonic and bead impact, cooled to –5 °C	Nebuliser gas (l min^{-1})	0.72
Torch	Fassel Quartz fitted with a Pt shield	Dwell Time (s)	10
Sampler and Skimmer Cones	Ni	Points per Peak	1
Nebuliser	Glass Expansion 0.2 ml/min Micromist, natural aspiration		
Ions Monitored	^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, ^{204}Hg, ^{205}Tl, ^{206}Pb		

4.3.2. Characterisation of the ^{199}Hg Enriched CH_3HgCl

The experimental procedure, and instrumental operating conditions, for the characterisation of the ^{199}Hg enriched CH_3HgCl spike material was described in Chapter 3 Section 3.4.2.

4.3.3. Equilibration of ^{199}Hg Enriched Inorganic Hg and NIST2710 SRM

The moisture content of the NIST2710 SRM was determined by drying separate subsamples to a constant mass at 105 ^{o}C. Approximately 2g of the NIST2710 SRM was accurately weighed and the amount of Hg present, corrected for moisture content, was calculated from the certified value. The stock ^{199}Hg enriched inorganic Hg spike solution was diluted to 55 $\mu g\ g^{-1}$ with 2% HNO_3. Approximately 1g of this diluted spike solution was added to 40 ml of the equilibration solution, 50:50 CH_3OH/DDW *v/v* 0.01% 2-mercaptoethanol, contained in a clean 50 ml glass conical flask. The NIST2710 SRM was subsequently added to the equilibration solution and 1 ml samples withdrawn at intervals up to 3000 minutes. The sampling frequency was approximately every two minutes for the first 25 minutes of the experiment then at 5-10 minute intervals until 1 hour had elapsed, with less frequent sampling thereafter. The equilibration solution was agitated throughout by a magnetic stirrer, and maintained at a temperature of 25°C by means of an electrically heated hotplate. Each sample aliquot was pipetted into a filter (Autovial 0.2μm PTFE membrane syringeless filters, Whatman, Maidstone, UK), diluted prior to filtration with 8 ml of fresh equilibration solvent, filtered, and stored in a clean 25 ml sterilin container at 4°C until analysis by MC-SF-ICP-MS (Axiom, Thermo Elemental, Winsford, UK). The instrumental operating conditions are given in Table 4-1. Hydrobromic acid, 0.6M, (Aldrich, Gillingham, UK) was used as a wash solution to counter memory effects, and Thallium (NIST911 SRM, LGC, Teddington, UK) added to the samples as an internal standard at approximately 300 ng/g to correct for mass bias and instrumental drift.

Subsequent to the initial equilibration experiments a full factorial experiment was designed, with two factors at two levels, namely 20 and 80% methanol and 0.005 and 0.02% 2-mercaptoethanol. The same ^{199}Hg enriched spike solution was used and the experimental procedure followed that described above. The ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios in the samples taken was measured by Q-ICP-MS (PQ3, Thermo Elemental, Winsford, UK).

4.3.4. Equilibration of ^{199}Hg Enriched Methylmercury and DORM-2

The experimental procedure, and instrumental operating conditions, for the equilibration of the ^{199}Hg enriched CH_3HgCl spike material methylmercury and DORM-2 was described in Chapter 3 Section 3.4.3.

4.3.5 Conventional External Calibration

For the equilibration experiments involving NIST2710, the total Hg in solution for each sample, was measured by external calibration at the same time as the isotope amount ratio determinations. In order to obtain a useful calibration it was necessary to sum the signals obtained for all Hg isotopes (*i.e.* 198, 199, 200, 201, 202, 204), because the isotope amount fractions of the samples and calibration standards were different. Hence, the total Hg instrumental response was calculated, and the Hg mass fraction in solution interpolated from the calibration curve. The isotopic abundance of ^{196}Hg is the same in both the calibration standards and the samples, however, it has an isotopic amount fraction of only 0.1%, so it could not be used as the calibration isotope, and was omitted from the experiment because there were only nine faraday detectors available on the Axiom MC-SF-ICP-MS and it was necessary to monitor ^{206}Pb in order to correct for interferences from ^{204}Pb on ^{204}Hg. ^{203}Tl was used as an internal standard to correct for instrumental drift.

The total methylmercury amount fraction in the equilibration samples taken during the experiments involving DORM-2 CRM, described in Chapter 3 Section 3.4.3.,

was calculated in a similar fashion, but with HPLC coupled to the PQ3 instrument. The methylmercurychloride peak in each chromatogram for the isotopes ^{198}Hg to ^{204}Hg was baseline subtracted, integrated, and the integrals summed to give the total methylmercurychloride instrumental response. The signal for ^{204}Hg was again corrected for by monitoring ^{206}Pb. This analysis was performed separately from the isotope amount ratio determinations as the requirement to monitor nine isotopic signals resulted in spectral skew of the resulting chromatograms. NIST 911 Thallium SRM was added to the HPLC mobile phase as an internal standard at approximately 50 ng/g to correct for instrumental drift.

4.3.6 Microwave Digests

Microwave digestions were performed on NIST2710 using two different digestants, concentrated HNO_3 and 50:50 methanol:DDW (*v:v*) with 0.01% 2-mercaptoethanol. Approximately 150 mg of NIST 2710 was accurately weighed directly into a Teflon bomb and 4 ml of digestant added. The mass of Hg added via the NIST 2710 reference material was calculated and an equivalent mass of Hg added via the ^{199}Hg enriched spike solution. The bomb lids were tightened and each digestion left to equilibrate for 24 hrs prior to microwaving at 650W for 2 minutes using a domestic microwave oven. The digests were filtered (Autovial 0.2μm PTFE membrane syringeless filters) and the filtered extract diluted to approximately 25g with either DDW or 50:50 methanol:DDW (*v:v*) 0.01% 2-mercaptoethanol solution, depending on the original digestant, and stored at 4° C until analysis by the Axiom MC-SF-ICP-MS.

4.4. Results and Discussion

4.4.1 Equilibration of Reference Materials with the ^{199}Hg Enriched Spikes

The extent of equilibration between the spike and the natural isotopic abundance particulate bound Hg species was determined by a comparison of the theoretical ^{200}Hg:^{199}Hg isotope amount ratio at complete equilibration and the measured isotope amount ratio in solution. The theoretical ^{200}Hg:^{199}Hg isotope amount ratio, and its

associated expanded uncertainty was calculated for each experiment from the masses and mercury mass fractions of the starting materials, and the isotope amount fractions shown in Table 3-5 using Equation 4-10. It is important to note that the Hg originating from the solid phase (*i.e.* the particles of the CRM in solution) would have a natural isotopic composition but the Hg added to the liquid phase was of modified isotopic composition (*i.e.* the ^{199}Hg enriched spike).

4.4.1.1. Equilibration Between ^{199}Hg Enriched Inorganic Hg and NIST2710

The extent of equilibration, between the natural abundance particulate bound inorganic Hg (NIST2710) and the ^{199}Hg enriched inorganic Hg spike, was determined in the equilibration solution, from the measured ^{200}Hg:^{199}Hg isotope amount ratio, corrected for mass bias using Equation 3-1.

The ^{200}Hg:^{199}Hg isotope amount ratio in the liquid phase over time is shown in Figure 4-1. Equilibration initially proceeded at a rapid rate up to 100 minutes, with the system reaching an equilibrium at approximately 300 minutes. Complete equilibration, between the ^{199}Hg enriched inorganic Hg spike and the particulate bound natural isotopic abundance Hg, was not achieved because the ^{200}Hg:^{199}Hg isotope amount ratio in the equilibration did not attain the theoretical ^{200}Hg:^{199}Hg isotope amount ratio (Figure 4-1). The mass fraction of Hg in NIST2710, calculated by IDMS from the ^{200}Hg:^{199}Hg isotope amount ratio in the final sample taken during this equilibration experiment (3000 minutes), was 21.5 ± 2.7μg g^{-1} (expanded uncertainty, $k = 2$). As complete equilibration between the sample and the spike did not occur the mass fraction of Hg in NIST2710, determined by IDMS, underestimated the certified value of 32.6 ± 1.8 μg g^{-1} by 34%.

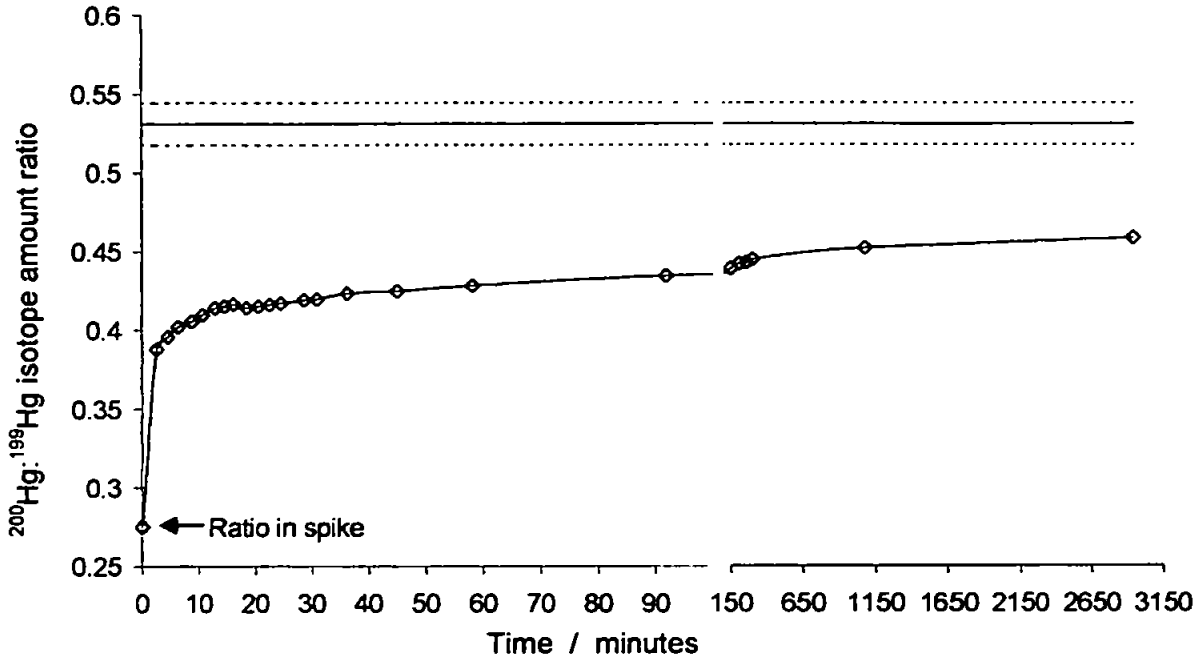


Figure 4-1 Change in ^{200}Hg:^{199}Hg isotope amount ratio in solution for the equilibration with NIST2710 over time, solid curve, with 50:50 methanol/water *v/v*, 0.01% 2-mercaptoethanol as the equilibration solvent. The solid line is the theoretical ^{200}Hg:^{199}Hg isotope amount ratio at complete equilibration, with the dashed lines representing the upper and lower limits of the expanded uncertainty, calculated using a coverage factor, *k*, of 2, which gives a level of confidence of approximately 95%.

4.4.1.2 The Effect of Solvent Composition on Equilibration

In order to study the effect of the solvent composition, on the extent of equilibration between the ^{199}Hg enriched spike and NIST2710, a full factorial experiment was designed, with the proportions of methanol and 2-mercaptoethanol employed as the equilibration solvent shown in Table 4-2. In each case equilibration between the ^{199}Hg enriched spike and NIST2710 was again incomplete, following a similar pattern to that shown in Figure 4-1, in comparison with the theoretical ^{200}Hg:^{199}Hg isotope amount ratio in solution. This incomplete equilibration again resulted in an underestimation of the mass fraction of Hg in NIST2710, with the results shown in Table 4-2. A Students t-test was used to compare the results obtained from the full factorial experiment with the mass fraction of Hg in NIST2710 obtained when 50:50 methanol/water *v/v*, 0.01% 2-mercaptoethanol was used as the equilibration solvent. In each case the results were not statistically different *i.e.* the variation in the solvent composition did not improve the extent of equilibration between the ^{199}Hg enriched spike and the particulate bound Hg.

Hg, and other metals, are co-precipitated with Fe and Mn oxyhydroxides in aquatic systems and subsequently trapped within this precipitate layer as further precipitation occurs[91]. NIST2710 SRM was prepared from soil, that was collected from land that is periodically flooded, with waters from settling ponds which contain high levels of Mn. It is thus likely that several different Fe and Mn oxyhydroxide layers had built up on the particles, rendering some of the particulate Hg unavailable for equilibration with the spike Hg dissolved in a mild solvent, *i.e.* methanol/water, 2-mercaptoethanol, causing the underestimation of Hg in NIST2710.

Table 4-2 The proportions of methanol and 2-mercaptoethanol used as equilibration solvents for the IDMS of Hg in NIST2710.

	Methanol Volume (%)	2-Mercaptoethanol Volume (%)	Mass Fraction of Hg in NIST2710 by IDMS ($\mu g\ g^{-1}$)*
1	50	0.01	21.5 ± 2.7
2	20	0.005	20.9 ± 2.6
3	20	0.02	23.4 ± 2.9
4	80	0.005	23.4 ± 2.9
5	80	0.02	26.1 ± 3.3

* The reported uncertainty is the expanded uncertainty, calculated using a coverage factor (k) of 2 which gives a level of confidence of approximately 95%.
Samples 2-5 are for full factorial experiment when the solvent composition was varied.

4.4.1.3. IDMS Microwave Digestions of NIST2710

Two IDMS microwave digestions, one with concentrated HNO_3 and the second using 50:50 methanol:DDW (*v*:*v*) 0.01% 2-mercaptoethanol were also performed on the NIST2710 SRM. The NIST2710 SRM was completely solubilised by the concentrated HNO_3 microwave digestion, and the Hg mass fraction, as determined by IDMS, of 31.7 ± 4.0 μg g^{-1} ($k = 2$) was in good agreement with the NIST2710 certified value of 32.6 ± 1.8 μg g^{-1}. In contrast, the microwave digestion using 50:50 methanol:DDW (*v*:*v*) 0.01% 2-mercaptoethanol did not completely dissolve the NIST2710SRM, and the Hg mass fraction was calculated to be 23.6 ± 3.0 μg g^{-1} ($k = 2$), comparable to that obtained, within the limits of uncertainty, by the time resolved isotope dilution equilibration analysis.

It was deduced, that when 50:50 methanol:DDW (*v*:*v*) 0.01% 2-mercaptoethanol was employed as the solvent, the ^{199}Hg enriched spike was equilibrated only with surface bound Hg, *e.g.* Hg bound to sulphur containing groups of the fulvic/humic acid layer of the particles. However, the complete solubilisation of NIST2710 *via* an HNO_3 microwave digestion, which oxidised both the soil particle coatings and the soil matrix, allowed complete equilibration between the ^{199}Hg enriched spike and the particulate bound Hg, resulting in a successful determination of Hg in NIST2710 by IDMS in comparison with the certified value.

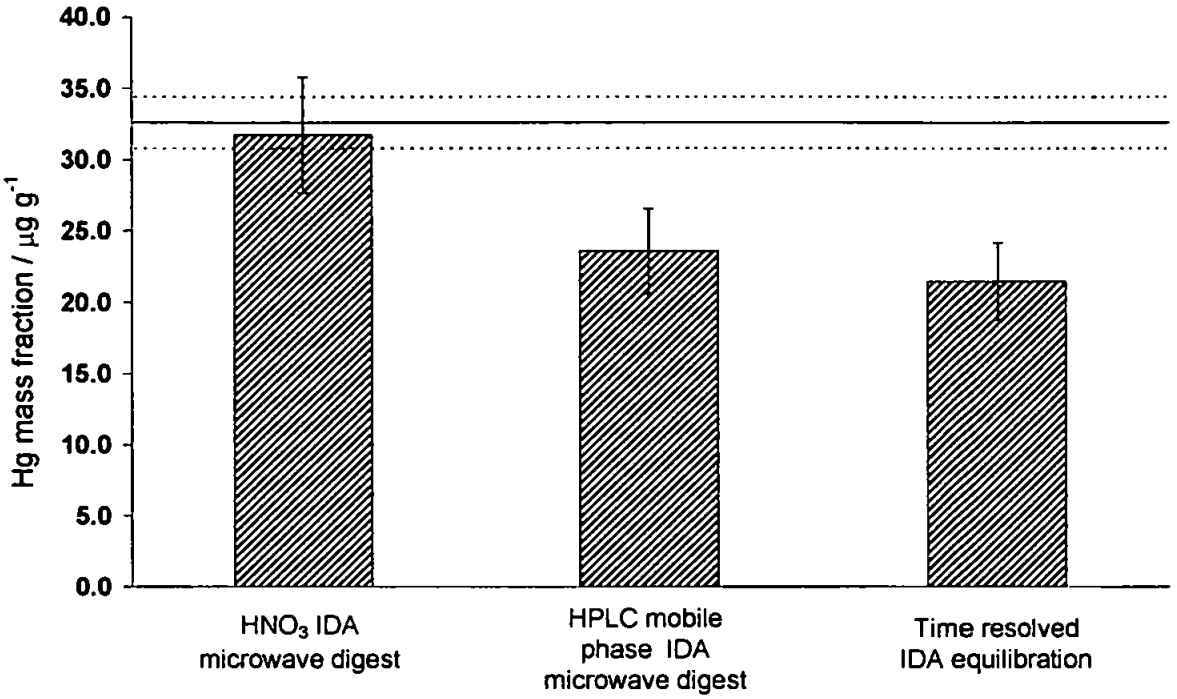


Figure 4-2 The mass fraction of Hg in NIST2710 obtained from three different isotope dilution analysis methods. The solid horizontal line is the certified mass fraction of Hg in NIST2710, with dashed lines showing the upper and lower limits (95% confidence interval). The uncertainties for the three experimental results are the expanded uncertainty ($k = 2$)

4.4.14. Equilibration Between ^{199}Hg Enriched Methylmercurychloride and DORM-2

The extent of equilibration, between the natural abundance particulate bound Methylmercury (DORM-2) and the ^{199}Hg enriched methylmercury spike, was determined in the equilibration solution, from the measured ^{200}Hg:^{199}Hg isotope amount ratio, corrected for mass bias using Equation 3-1. The rate of equilibration for DORM-2, shown in Figure 4-3, proceeded at an initial rapid rate. Complete equilibration, within the limits of uncertainty, was attained within 6 minutes from the start of the experiment. The mass fraction of methylmercury in DORM-2, determined by species specific HPLC-ID-Q-ICP-MS from the ^{200}Hg:^{199}Hg isotope amount ratio in the final sample taken at 1500 minutes, was 4.25 ± 0.47 μg g^{-1} (k = 2), in good agreement with the certified value of 4.47 ± 0.32 μg g^{-1}.

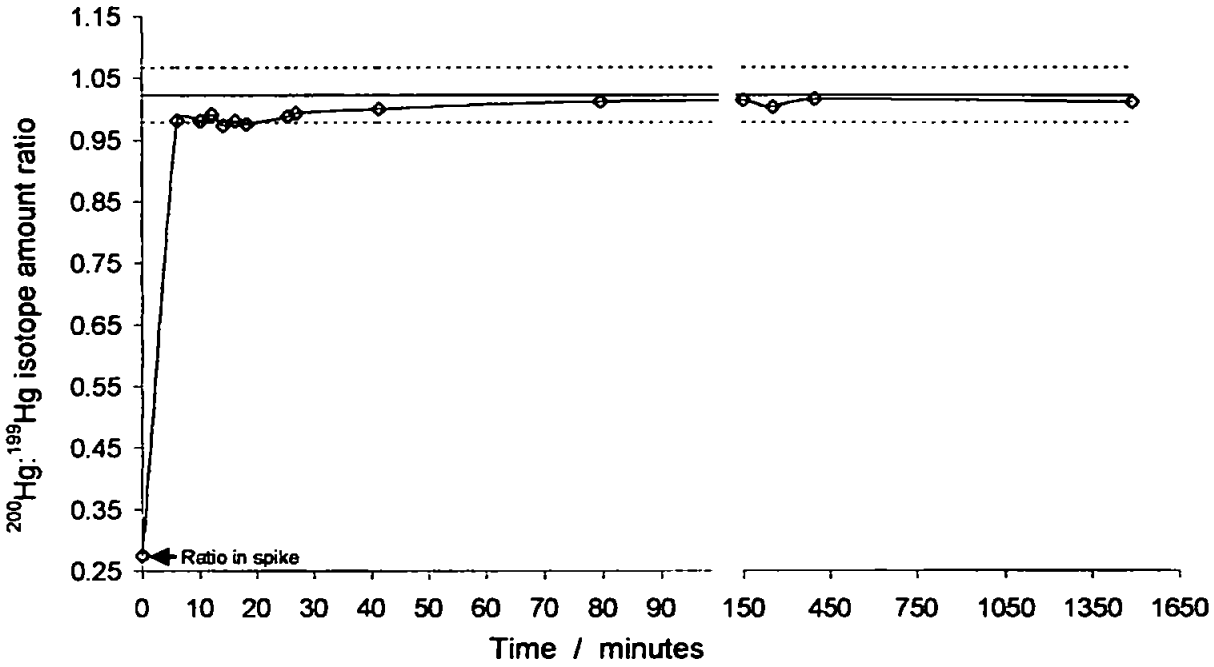


Figure 4-3 Change in $^{200}Hg:^{199}Hg$ isotope amount ratio in solution for the equilibration with DORM-2 over time, solid curve, with 50:50 methanol/water *v/v*, 0.01% 2-mercaptoethanol as the equilibration solvent. The solid horizontal line is the theoretical $^{200}Hg:^{199}Hg$ isotope amount ratio at complete equilibration, with the dashed lines representing the upper and lower limits of the expanded uncertainty ($k = 2$).

4.4.2 Adsorption and Desorption of Mercury Species from Particulates

4.4.2.1. NIST2710

The extent of desorption of Hg from the particulate phase (*i.e.* the natural abundance Hg arising from the NIST2710 SRM) and the adsorption from solution onto the particulate phase (*i.e.* adsorption of the ^{199}Hg enriched inorganic spike Hg) is shown in Figure 4-4. As can be seen 24% of the Hg from NIST2710 was desorbed from the particles after only 3 minutes (Figure 4-4B), with no significant change, within the limits of uncertainty until 1100 minutes. Thereafter, the amount of natural isotopic abundance Hg in solution increased to 37% at the end of the experiment (3000 minutes), which yielded a mass fraction of Hg in NIST2710 of 12.1 ± 2.3 μg g^{-1}, as determined by external calibration. Thus, the certified value of Hg in NIST2710 was underestimated by 63% with a relative expanded uncertainty of 19%. A coverage factor (k) of 4.3 (obtained t-tables) was used to expand the standard uncertainty, as only 2 degrees of freedom were available from the four point calibration curve[92].

The adsorption curve for the ^{199}Hg enriched Hg^{2+} spike (Figure 4-4A), showed an initial rapid adsorption, with 38% remaining in solution (*i.e.* 62% adsorbed onto the NIST2710 particles) during the first 11 minutes. Thereafter the amount of spike in solution remained constant until 1100 minutes had elapsed, whereupon a net desorption of the spike occurred, with 58% in solution at 3000 minutes. The net desorption of both the natural isotopic abundance and the spike Hg from 1100 minutes could have been due to oxidation, by the equilibration solvent, of the binding site substrate on the NIST2710 particles.

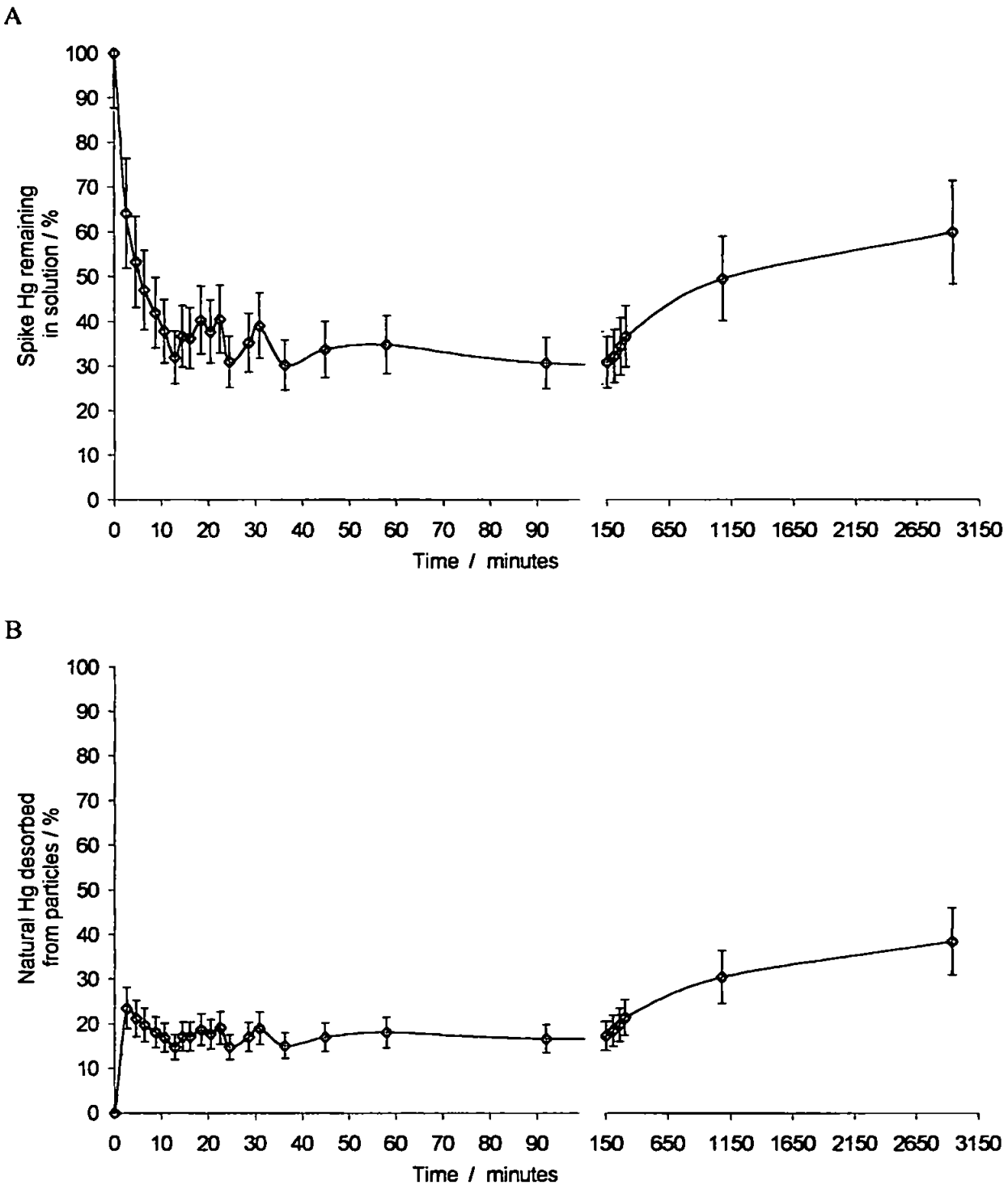


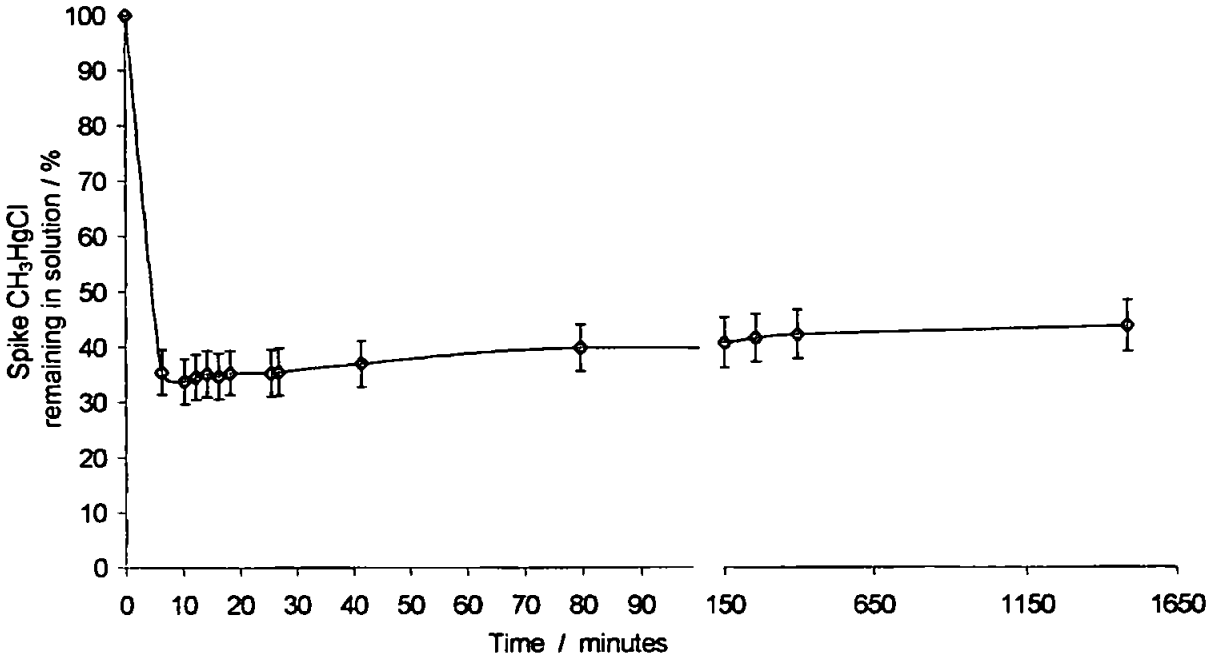
Figure 4-4 The amount of; A, ^{199}Hg enriched spike Hg remaining in solution; B, of particulate bound natural isotopic Hg desorbed during the IDMS determination of Hg in NIST2710. Uncertainty bars are the expanded uncertainty ($k = 4.3$).

4.4.2.2 DORM-2

The extent of adsorption of the spike and natural methylmercury is shown in Figure 4-5. 47% of the methylmercury in the DORM-2 CRM was desorbed within 6 minutes of the start of the experiment (Figure 4-5B), with the amount in solution remaining relatively constant for the remainder of the experiment. Likewise, 65% of the methylmercurychloride spike had been adsorbed onto the particles of DORM-2 after 6 minutes (Figure 4-5A), and this amount remained relatively constant for the duration of the experiment.

The mass fraction of methylmercury in the DORM-2 CRM, as determined by external calibration from the final sample taken at 1500 minutes, was 2.10 ± 0.5 μg g^{-1}, an underestimation of 53% compared with the certified value. By comparison the mass fraction determined by IDMS for the final sample was 4.25 ± 0.47 μg g^{-1} (k = 2), certified value of 4.47 ± 0.32 μg g^{-1} (k = 4.3). So, although only 53% of the methylmercury in DORM-2 had been brought into solution by the action of the equilibration solvent, complete equilibration had been achieved. This is an important advantage of IDMS compared with external calibration, namely that 100% extraction of the analyte from the sample matrix is not necessary, merely 100% equilibration between the solid and liquid phases is required.

A



B

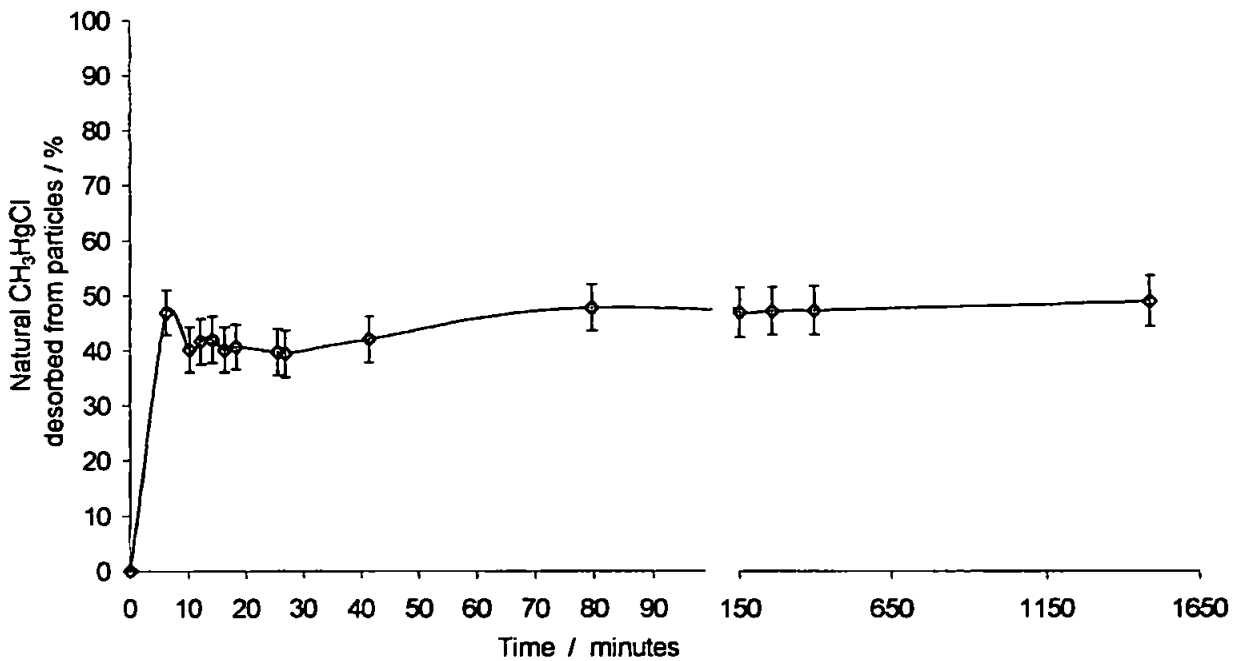


Figure 4-5 The amount of; A, ^{199}Hg enriched methylmercurychloride remaining in solution; B, of particulate bound natural isotopic methylmercurychloride desorbed during the IDMS determination of methylmercury in DORM-2. Uncertainty bars are the expanded uncertainty ($k = 4.3$).

4.4.3 Contributions to Uncertainty

4.4.3.1 Measured Isotope Amount Ratios

The standard uncertainty of the mass bias corrected $^{200}Hg:^{199}Hg$ isotope amount ratio was calculated by combining the uncertainty of each parameter in Equation 3-1. For the determination of inorganic Hg in NIST2710 by IDMS two different types of ICP-MS instrument, a multicollector and a quadrupole were used. The typical precision of the measured isotope amount ratios for both instruments and the major contributions to the measurement uncertainty is shown in Table 4-3. For the Axiom multicollector instrument, which measures the ion signal of each isotope simultaneously, the relative standard uncertainty for the mass bias corrected $^{200}Hg:^{199}Hg$ isotope amount ratio was typically 0.03%, with the major contributions to this figure derived from the measured $^{200}Hg:^{199}Hg$ isotope amount ratio (43%), the measured $^{205}Tl:^{203}Tl$ isotope amount ratio (39%) and the NIST997 certified $^{205}Tl:^{203}Tl$ isotope amount ratio (18%).

When the PQ3 quadrupole instrument was used the standard uncertainty of the mass bias corrected $^{200}Hg:^{199}Hg$ isotope amount ratio was typically 0.13%, a factor of four to five poorer than for the multicollector instrument. In this case the major contributions were derived from the measured $^{200}Hg:^{199}Hg$ isotope amount ratio (72%), the measured $^{205}Tl:^{203}Tl$ isotope amount ratio (27%), with a relatively smaller contribution from the NIST997 certified $^{205}Tl:^{203}Tl$ isotope amount ratio (1%). The sequential nature of the ion current detection of the quadrupole mass spectrometer is susceptible to small fluctuations in the ion signal, caused by variations in the nebulisation efficiency, analyte transport and plasma stability of the sample introduction and ionisation system, thereby reducing the measurement precision.

Table 4-3 Relative standard uncertainties, and the relative uncertainty contributions, of isotope amount ratio measurements by HPLC-Q-ICP-MS, MC-ICP-MS and Q-ICP-MS.

	Relative Standard Uncertainty (%)	Contribution to mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio standard uncertainty (%)
Axiom MC-ICP-MS		
Measured ^{200}Hg:^{199}Hg	0.02	43
Measured ^{205}Tl:^{203}Tl	0.03	39
NIST997 certified ^{205}Tl:^{203}Tl	0.02	18
Mass Bias Corrected ^{200}Hg:^{199}Hg	0.03	
Quadrupole ICP-MS		
Measured ^{200}Hg:^{199}Hg	0.11	72
Measured ^{205}Tl:^{203}Tl	0.13	27
NIST997 certified ^{205}Tl:^{203}Tl	0.02	1
Mass Bias Corrected ^{200}Hg:^{199}Hg	0.13	
HPLC Quadrupole ICP-MS		
Measured ^{200}Hg:^{199}Hg	1.0	80
Measured ^{205}Tl:^{203}Tl	1.0	20
NIST997 certified ^{205}Tl:^{203}Tl	0.02	0
Mass Bias Corrected ^{200}Hg:^{199}Hg	1.2	

For the determination of methylmercury in DORM-2 HPLC was coupled with a quadrupole ICP-MS instrument because the Faraday collectors on the MC-SF-ICP-MS were not sensitive enough. For these measurements the relative standard uncertainty of the mass bias corrected $^{200}Hg:^{199}Hg$ isotope amount ratio was typically 1.2%. In this case the measured isotope amount ratios, $^{200}Hg:^{199}Hg$ and $^{205}Tl:^{203}Tl$, contributed 80 and 20% respectively to the combined standard uncertainty, as shown in Table 4-3. The further decrease in precision was due not only to the sequential nature of the instrument, but also the fact that transient signals, resulting from HPLC sample introduction, were being monitored. The best accuracy and precision is obtained when continuous ion signals are measured for several minutes[24], however, the use of the pseudo steady state approach for the calculation of isotope amount ratios minimised the effects of transient signal measurements.

4.4.3.2 Isotope Dilution Analysis

The combined standard uncertainty (u) of the final analytical result is comprised of contributions from the individual standard uncertainties of the parameters in Equation 3-3, combined using the spreadsheet method of Kragten[66]. The combined standard uncertainty is then multiplied by a coverage factor (k), the value of which depends upon the available degrees of freedom, to obtain the expanded uncertainty (U), which approximates to the 95% confidence interval. The contribution of the individual parameters in Equation 3-3 can also be expressed in terms of their relative contribution to the expanded uncertainty as shown in Table 4-4 and Table 4-5, for NIST2710 and DORM-2 respectively.

Table 4-4 The mass fraction and expanded uncertainty for the determination of Hg in NIST2710 by IDMS and external calibration.

NIST2710 Total Hg Analytical method	Hg mass fraction ($\mu g\ g^{-1}$)	Expanded Uncertainty		Relative contributions to the expanded uncertainty (%)		
		absolute	relative (%)	C_y	R_x	x_{pred}
Time resolved IDMS	21.5	± 2.7 [a]	13	95	4	
HNO_3 IDMS microwave digest	31.7	± 4.0 [a]	13	95	5	
HPLC mobile phase IDMS microwave digest	23.6	± 3.0 [a]	13	95	5	
External calibration	12.1	± 2.3 [b]	19			100
Certified value	32.6	± 1.8	6			

The reported uncertainty is the expanded uncertainty, calculated using a coverage factor (k) of either; *a*, $k = 2$, or *b*, $k = 4.3$, which gives a level of confidence of approximately 95%.

Table 4-5 The mass fraction and expanded uncertainty for the determination of methylmercury in DORM-2 by IDMS and external calibration.

DORM-2 methylmercury analytical method	Methylmercury mass fraction ($\mu g\ g^{-1}$)	Expanded Uncertainty		Relative contributions to the expanded uncertainty (%)				
		absolute	relative (%)	C_y	R_x	$^{200}Hg/^{199}Hg$	$^{205}Tl/^{203}Tl$	x_{pred}
Time resolved IDMS	4.25	± 0.47 [a]	11	12	35	43	9	
External calibration	2.10	± 0.5 [b]	24					100
Certified value	4.47	± 0.32	7.1					

The reported uncertainty is the expanded uncertainty, calculated using a coverage factor (k) of either; *a*, $k = 2$, or *b*, $k = 4.3$, which gives a level of confidence of approximately 95%.

For the analysis of NIST2710 the uncertainty budget was dominated, in all cases, by the uncertainty associated with the mass fraction of the ^{199}Hg enriched inorganic Hg spike solution (C_y) which contributed 95% of the combined uncertainty. The ^{200}Hg:^{199}Hg isotope amount ratio of natural abundance mercury (R_x) contributed 4%, with the other variables in Equation 3-3 contributing less than 1%. The relative contributions to the expanded uncertainty of the mass fraction of methylmercury in DORM-2, determined by IDMS, are shown in Table 4-5. The major contributions arose from the measured ^{200}Hg:^{199}Hg isotope amount ratio (43%) and the theoretical ^{200}Hg:^{199}Hg isotope amount ratio (R_x, 35%), derived from the uncertainty associated with the natural isotopic abundance of mercury[80]. Lesser contributions arose from the spike mass fraction (C_y, 12%), the measured ^{205}Tl:^{203}Tl isotope amount ratio used for mass bias correction (9%), and ΣR_{ix} (1%) with the other parameters in Equation 3-3 contributing less than 1%. Mercury is a relatively poorly characterised element, with relatively large uncertainties associated with its isotopic composition, hence, if the precision and accuracy of the measurement of isotope amount ratios is further improved these uncertainties will start to dominate the expanded uncertainty for the measurement of mercury species by IDMS.

It is interesting to compare the uncertainty contribution of the spike (C_y) to the expanded uncertainty of the analyte mass fraction, $U(C_x)$, determined in DORM-2 and NIST2710. In the latter case the relative contribution was much greater than the former (95% *c.f.* 12%) even though the standard uncertainty of the mass fraction of each spike solution was similar (55.3 ± 3.4 μg g^{-1} *c.f.* 11.1 ± 0.21 μg g^{-1}). The reason for this is that the ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios for the IDMS analysis of Hg in NIST2710 were measured more precisely than those for the analysis of CH_3HgCl in DORM-2, thereby resulting in smaller standard uncertainties, so their relative contribution to the expanded uncertainty fell to less than 0.03%, with a consequent increase in the relative contribution of the spike.

4.4.3.3 External Calibration

An uncertainty budget was also formulated for the determination of Hg in NIST2710 and methylmercury in DORM-2 by external calibration, and the expanded uncertainty for each analysis calculated (Table 4-4 and Table 4-5 respectively). For both of these analyses the uncertainty budget was dominated (100% after rounding) by the standard deviation of the mercury mass fraction predicted from the weighted regression calibration curve (x_{pred}). In other words, the standard uncertainty of the regression fit dominated the final expanded uncertainty. For the total Hg mass fraction in NIST2710 the relative expanded uncertainty was lower for the IDMS determinations (13%) than that for the external calibration (19%). Similarly, the IDMS determination of methylmercury in DORM-2 again resulted in a lower relative expanded uncertainty (11%), compared with that obtained by external calibration (24%).

4.5 Conclusions

The mass fraction of Hg and methylmercury has been determined, by both total and species specific isotope dilution analysis, in two certified reference materials, NIST2710 and DORM-2 respectively. For the analysis of total Hg in NIST2710, complete equilibration between the sample Hg and the added spike was only achieved when a microwave digestion was performed, with concentrated HNO_3 as the solvent. For this determination the found value of 31.7 $\mu g\ g^{-1}$ was in good agreement with the certified value of 32.6 $\mu g\ g^{-1}$. When 50:50 methanol:DDW (*v*:*v*) 0.01% 2-mercaptoethanol was used as the extraction solvent incomplete equilibration, and hence an underestimate of the certified value, resulted. Only 37% of the available Hg was extracted from NIST2710 using this solvent. No significant difference, in the extent of equilibration between the sample Hg and the added spike, was observed when the methanol and 2-mercaptoethaol proportions in the equilibration solvent were varied.

In the case of species specific IDMS for methylmercury in DORM-2, when 50:50 methanol:DDW (*v*:*v*) 0.01% 2-mercaptoethanol was used as the extraction solvent complete equilibration was achieved, even though only 47% of the available methylmercury was extracted into solution, and the mass fraction of methylmercury (4.25 $\mu g\ g^{-1}$) was in good agreement with the certified value (4.47 $\mu g\ g^{-1}$), illustrating that complete equilibration, rather than complete extraction, is required to yield accurate results using IDMS. In comparison, analysis by external calibration yielded analyte recoveries of approximately half that achieved by IDMS, reflecting the poor extraction of the analytes into solution. The expanded uncertainty was calculated for each analytical method, and improved precision was obtained using IDMS compared to external calibration.

Two separate methods of mass bias correction, bracketing and interpolation, were also employed for the characterisation of the spike materials. The bracketing method gave a relative standard uncertainty for the spike mass fraction of 6.2% as opposed to 1.9% when an internal standard was employed. Despite only a small difference in these standard uncertainties the uncertainty budget for the total Hg mass fraction determinations was dominated by the spike mass fraction uncertainty whilst for species specific IDMS other parameters in the IDMS equation contributed significantly with a marked reduction in the spike contribution.

Chapter 5 Comparison of Single and Double IDMS Using Cold Vapour Sample Introduction

5. Introduction

Complete equilibration between the particulate bound methylmercury and the ^{199}Hg enriched methylmercurychloride spike was achieved for DORM-2 CRM (Chapter 3). However, insufficient methylmercury mercury was present to be measured using the Axiom multicollector ICP-MS. Therefore a scanning ICP-MS was employed, which resulted in the uncertainty budget for a single sample being dominated by the standard uncertainty of the measured isotope amount ratios. In order improve the precision of the isotope amount ratios by utilising the multicollector ICP-MS, and hence reduce the measurement uncertainty, an improvement in either sample introduction efficiency or instrumental sensitivity was required.

Cold vapour (CV) generation, in which inorganic Hg^{2+} is reduced to elemental Hg^0 vapour, has been coupled with atomic absorption spectrometry to determine total mercury[93,94,95] and, when preceded by HPLC separation, mercury species[82,96,97]. Two reducing agents have commonly been employed, $SnCl_2$ and $NaBH_4$, and the elemental Hg vapour is subsequently purged from the liquid phase by the use of a carrier gas, such as argon, *via* a gas/liquid separator. The advantage of CV generation over conventional pneumatic nebulisation is a significant increase in the mercury signal due to an improvement of the analyte transport efficiency to the ionisation source[98].

CV generation has also been coupled with ICP-MS for the determination of total mercury[99,100,101]. $SnCl_2$ was used as the reductant because the use of $NaBH_4$ produces H_2 gas, which can have deleterious effects on the plasma. Organomercury compounds are not reduced to Hg^0 by $SnCl_2$ without an oxidation step to produce Hg^{2+}, prior to reduction to Hg^0. Two common oxidants are acidified potassium permanganate or acidified bromide/bromate, however, acidified potassium permanganate suffers from two main

drawbacks. Firstly, when mixed with $SnCl_2$, the permanganate is reduced to MnO_2, which has a tendency to block the tubing. In addition, there are normally very high blank values associated with this reagent, which leads to poor limits of detection.

A gas/liquid separator can lead to band broadening of chromatographic peaks due to a dilution effect. Zhang and Combs[102] used a Scott type, double pass spray chamber as the gas liquid separator for the determination of Ge, As, Sn, Sb, Te, and Bi by hydride generation ICP-MS. To achieve this, a Meinhard nebuliser was modified so that the hydride generation outlet tube replaced the inner concentric tube, this allowed the volatile hydrides to be transported to the plasma by the argon nebuliser gas and the liquid reagents to be removed *via* the waste port. Wan *et al.*[98] used a similar approach for the speciation of mercury compounds by ion chromatography CV-ICP-MS.

Tyler *et al.*[103] used the spray chamber as both the reaction vessel and the gas/liquid separator. The sample was conventionally nebulised while the hydride reagents entered *via* a separate tube through the waste port bung. The nebuliser created an aerosol with 98% going to the drain where the hydride generation took place. Pergantis and Anderson[104] also used the spray chamber as the gas liquid separator, and in this case the sample and the hydride generation reagents were mixed *via* a t-piece prior to entry into the spray chamber, again through a separate hole in the waste port bung. The volatile hydrides were transported to the plasma by the action of the nebuliser gas, which entered conventionally along with an internal standard, with the other reagents removed *via* the drain tubing.

The best isotope amount ratio measurement precision is normally achieved by using long dwell times and continuous sample monitoring. For the acquisition of data from scanning ICP-MS instruments coupled with HPLC the dwell time is a compromise between the shortest time possible, to minimise spectral skew and compensate for short-term fluctuations in the ion signal, and the longest time possible, to allow sufficient counts

to be measured to achieve the best precision. The advantage of a multicollector instrument is that the ion signals of each isotope are measured simultaneously, thereby negating the effects of fluctuations in the ion beam caused by sample introduction and the ionisation system. Thus, the analyst has greater freedom to choose the optimal dwell time, for best accuracy and precision, of the ion amount ratio.

The spike material used in IDMS is often relatively poorly characterised, particularly for species specific IDMS, and can be a major contributor to the measurement uncertainty during conventional IDMS[57]. Henrion[105] has proposed an exact matching procedure to remove the spike solution mass fraction from the final IDMS measurement equation. In this procedure the mass bias correction factor is calculated by bracketing the sample/spike blend with a solution of a standard/spike blend having a known isotope amount ratio. The analyte mass fraction for single IDMS, with bracketing mass bias correction can be calculated according to Equation 5-1.

$$C_x = C_y \cdot \frac{m_y}{m_x} \cdot \frac{R_y - \left(\frac{R_T}{R_{BC}} \times R_B\right)}{\left(\frac{R_T}{R_{BC}} \times R_B\right) - R_x} \cdot \frac{\sum_i R_{ix}}{\sum_i R_{iy}} \cdot h$$

Equation 5-1

where

C_x = is the mass fraction of the analyte in the unspiked natural isotopic abundance sample

m_x = the mass of natural isotopic abundance sample

C_y = the mass fraction of the isotopically modified spike material

m_y = the mass of the isotopically modified spike material added to the natural isotopic abundance sample

R_y = the isotope amount ratio of the analyte isotope to the spike isotope in the enriched spike material

R_x = the isotope amount ratio of the analyte isotope to the spike isotope in the natural isotopic abundance sample

R_T = is the gravimetric reference:spike isotope amount ratio in the natural standard/spike mass bias calibration blend

R_{BC} = is the measured reference:spike isotope amount ratio in the natural standard/spike mass bias calibration blend

R_B = is the reference:spike isotope amount ratio in the sample/spike blend

$\sum_i R_{iy}$ = the sum of the ratios of all isotopes to the reference isotope, which in this case is the spike isotope.

$\sum_i R_{ix}$ = the sum of the ratios of the atom fraction of all isotopes to the atom fraction of the reference isotope, which in this case is the spike isotope in the natural isotopic abundance sample.

h = moisture content correction factor

The mass fraction of the spike material used to prepare the standard/spike blend used for mass bias correction is described by Equation 5-2

$$C_Y = C_Z \cdot \frac{m_Z}{m_{YC}} \cdot \frac{\left(\frac{R_T}{R_{BC}} \times R_{BC}\right) - R_Z}{R_Y - \left(\frac{R_T}{R_{BC}} \times R_{BC}\right)} \cdot \frac{\sum_i R_{iY}}{\sum_i R_{iZ}}$$

Equation 5-2

where

C_Y = the mass fraction of the isotopically modified spike material used to prepare the natural standard/spike mass bias calibration blend

C_Z = the mass fraction of the natural standard used to prepare the natural standard/spike mass bias calibration blend

m_Z = the mass of the natural standard used to prepare the natural standard/spike mass bias calibration blend

m_{YC} = the mass of the isotopically modified spike material used to prepare the natural standard/spike mass bias calibration blend

R_y = the isotope amount ratio of the analyte isotope to the spike isotope in the enriched spike material

R_Z = the isotope amount ratio of the analyte isotope to the spike isotope in the natural isotopic abundance sample

R_T = is the gravimetric reference:spike isotope amount ratio in the natural standard/spike mass bias bracketing blend

R_{BC} = is the measured reference:spike isotope amount ratio in the natural standard/spike mass bias bracketing blend

$\sum_i R_{iY}$ = the sum of the ratios of all isotopes to the reference isotope, which in this case is the spike isotope.

$\sum_i R_{iZ}$ = the sum of the ratios of the atom fraction of all isotopes to the atom fraction of the reference isotope, which in this case is the spike isotope in the natural isotopic abundance sample.

Combining Equation 5-1 and Equation 5-2 gives Equation 5-3

$$C_x = C_Z \cdot \frac{m_Z}{m_{YC}} \cdot \frac{\left(\frac{R_T}{R_{BC}} \times R_{BC}\right) - R_Z}{R_Y - \left(\frac{R_T}{R_{BC}} \times R_{BC}\right)} \cdot \frac{\sum_i R_{iY}}{\sum_i R_{iZ}} \cdot \frac{m_y}{m_x} \cdot \frac{R_y - \left(\frac{R_T}{R_{BC}} \times R_B\right)}{\left(\frac{R_T}{R_{BC}} \times R_B\right) - R_x} \cdot \frac{\sum_i R_{ix}}{\sum_i R_{iy}} \cdot h$$

Equation 5-3

For elements which do not vary in isotopic composition R_X is equal to R_Z and $\sum_i R_{iX}$ is equal to $\sum_i R_{iZ}$. Equation 5-3 can be simplified to Equation 5-4, the final equation from which the spike mass fraction, C_Y, has been eliminated.

$$C_x = C_Z \cdot \frac{m_Y}{m_X} \cdot \frac{m_Z}{m_{YC}} \cdot \frac{R_y - \left(\frac{R_T}{R_{BC}} \times R_B\right)}{\left(\frac{R_T}{R_{BC}} \times R_B\right) - R_x} \cdot \frac{R_T - R_Z}{R_Y - R_T} \cdot h$$

Equation 5-4

Henrion used an iterative procedure to exactly match the spike/sample and natural standard/spike blends in terms of both isotope amount ratio and ion counts, in order to minimise uncertainty contributions from systematic errors, such as mass bias, detector linearity and dead time, in the final IDMS run. However, the exact matching procedure can be time consuming depending on the number of iterations required to produce exactly matched solutions. Catterick *et al.*[57] demonstrated that matching the solutions to within 5%, in terms of isotope amount ratio and ion counts retained many of the benefits of Henrion's procedure but significantly reduced the time for the analysis.

Single IDMS, which includes the spike mass fraction in the measurement equation, has been employed so far in this work, with mass bias correction by an internal standard. The aims of the work described in this Chapter were;

- to determine the optimal dwell time for the multicollector instrument.
- to improve sensitivity by the introduction of a cold vapour generation system between the HPLC separation stage and the mass spectrometer.
- to compare single IDMS, with mass bias correction by an internal standard, with the approximate matching double IDMS technique.

5.1 Experimental

5.1.1 multicollector Optimisation

In order to determine the optimal dwell time for the Faraday cup multicollector array of the VG Axiom ICP-MS during transient signal acquisition a 30 ng g^{-1} (as Hg) solution of natural isotopic abundance methylmercury (Alfa Aesar Standard) was prepared

in the HPLC mobile phase (50:50 MeOH:DDW, 0.01% 2-mercaptoethanol). Four different dwell times were evaluated, 50, 100, 250 and 500 ms. Three different injection loop volumes, 100, 200 and 500 µl, were used to determine the minimum absolute amount of MeHg that could be injected to give accurate and precise isotope amount ratio determinations. The chromatographic set up is described in Chapter 3 Section 3.4.1., with the HPLC and typical ICP-MS conditions are shown in Table 5-1. NIST997 Tl SRM was added to the mobile phase, 50 ng g^{-1}, and continuously monitored for the purposes of mass bias correction. To determine the repeatability of isotope ratio measurements by HPLC-MC-ICP-MS a solution of methylmercurychloride, 30 ng g^{-1} as Hg, was injected onto the column, using a 500 µl sample loop volume. Eight injections were made for two different dwell times, 250 and 500 ms.

Table 5-1 Typical HPLC-ICP-MS operating conditions.

HPLC Conditions			
HPLC Column	HiChrom Kromasil 100 FC 18 Excel, 25 cm x 4.6 mm i.d.		
Mobile Phase	50:50 *v/v* Methanol:DDW, 0.01% 2-mercaptoethanol, 50 ng/g NIST 997 Tl SRM		
Flow Rate (ml/min)	1.0	Injection Volume (μl)	100, 200, 500
VG Axiom MC-SF-ICP-MS Operating Conditions			
RF Forward Power (W)	1450	Plasma gas (l min^{-1})	14
Reflected Power (W)	≤ 10	Auxiliary gas (l min^{-1})	0.85
Spray Chamber	Coupled cyclonic and bead impact, cooled to –5 °C	Nebuliser gas (l min^{-1})	0.72
Torch	Fassel Quartz fitted with a Pt shield	Dwell Time (ms)	50, 100, 250, 500
Nebuliser	Glass Expansion 0.2 ml/min Micromist	Sampler and Skimmer Cones	Ni
Ions Monitored	^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, ^{204}Hg, ^{205}Tl, ^{206}Pb		

5.1.2 Cold Vapour Optimisation

To improve the amount of mercury transported to the plasma, and hence instrumental sensitivity a cold vapour generation system was coupled between the HPLC column eluent and the VG Axiom MC-ICP-MS. A solution of acidified bromide/bromate (Convol, BDH, Poole, UK) 0.0167 M with respect to bromide, 5% HCl (BDH, Poole, UK), was introduced into the HPLC eluent post-column *via* a low dead-volume splitter. Subsequently, the combined reagents passed through a 3 m Teflon reaction coil of 0.3 mm Ø, to allow sufficient time for the oxidisation of organomercury species to Hg^{2+}. Due to the high back-pressure resulting from the reaction coil an HPLC pump (Dionex Analytical Pump, APM-1), with non-metallic pump heads, was used to introduce the acidified bromide/bromate solution.

Tin II chloride (Sigma Aldrich, Gillingham, UK), 2% m/v $SnCl_2$ in 5% *v/v* HCl, was combined with the reaction coil eluent, *via* a second low dead-volume splitter and a peristaltic pump, to reduce the Hg^{2+} to elemental Hg^0. The $SnCl_2$ solution was sparged with He gas for 30 minutes prior to use, to remove dissolved elemental mercury. A further 1 m length of 0.3 mm Ø Teflon tubing was used to introduce the resulting mixture into the ICP-MS spray chamber *via* a hole drilled into the waste port bung. Figure 5-1 shows a schematic diagram of the HPLC-CV system. The HPLC and typical ICP-MS conditions are shown in Table 5-1.

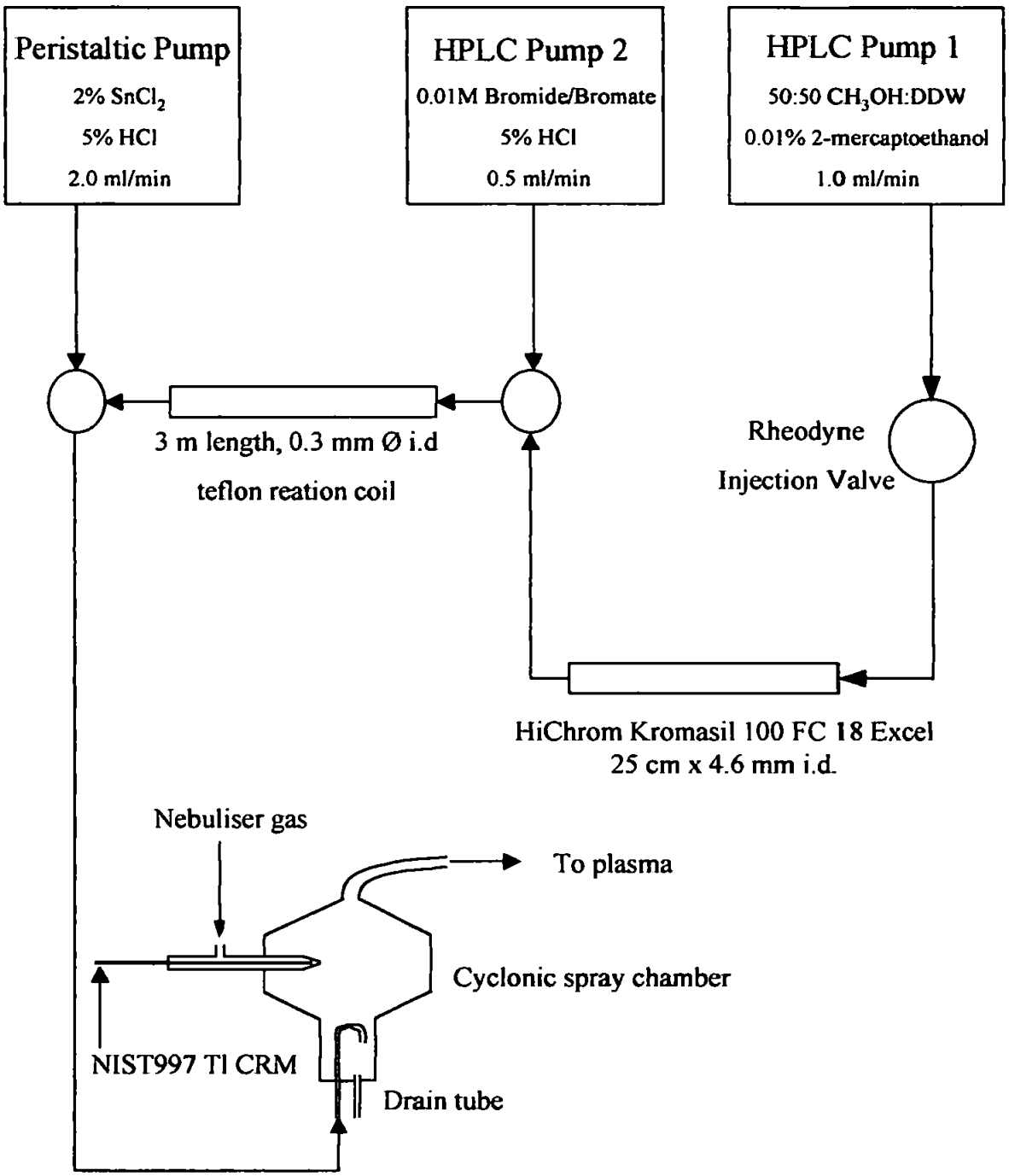


Figure 5-1 Schematic diagram of the HPLC cold vapour generation MC-ICP-MS set up.

In order to determine the optimal flow rates of the oxidant and reductant solutions natural isotopic abundance methylmercurychloride was blended with ^{199}Hg enriched methylmercurychloride to give solution having a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity. The mass fraction of this blend was approximately 1 $\mu g\ g^{-1}$, and 100µl injections were made. The HPLC flow rate was kept constant at 1.0 ml min^{-1} while the flow rates of the Br^-/BrO_3^- oxidant and $SnCl_2$ reductant were iteratively varied until an optimum combination was found which gave the maximum ^{200}Hg ion signal. The starting point for the optimisation of the oxidant and reductant flow rates was 2.5 ml min^{-1} for each reagent[106]. Subsequently, using the optimal oxidant and reductant flow rates, the reproducibility of the HPLC-CV-MC-ICP-MS was determined by 10 separate injections of a methylmercurychloride solution, 30 ng g^{-1} as Hg, with a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity.

5.1.3 Equilibration of Spike and CRM's

5.1.3.1 Reverse IDMS of Spike

The mass fraction of the ^{199}Hg enriched CH_3HgCl was determined by reverse ID-MS with the spike isotopic composition modified by the addition of a well characterised natural standard (methylmercury chloride standard, Alfa Aesar, Ward Hill, MA, USA). For this characterisation HPLC was coupled to the VG Axiom MC-ICP-MS as described in Chapter Three Section 3.41. The HPLC and typical ICP-MS operating conditions are shown in Table 5-1. The Faraday cup dwell time was set to 500 ms. The natural standard, 0.93 $\mu g\ g^{-1}$ as Hg, and the spike solution, nominally 4 $\mu g\ g^{-1}$ as Hg, were blended to give a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity to minimise errors from sources such as mass bias[57], thus reducing the measurement uncertainty. The ^{200}Hg:^{199}Hg isotope amount ratio was measured in four separate blends. The total

methylmercury mass fraction of the spike/natural standard blend was approximately 1 μg g^{-1}, as Hg, with an injection volume of 100 μl.

5.1.3.2 Equilibration Experiments

Equilibration solutions of the HPLC mobile phase, 50:50 H_2O:CH_3OH *v/v* and 0.01% 2-mercaptoethanol, were prepared using HPLC grade methanol (BDH, Poole, UK), distilled deionised water (Elgastat Maxima, Elga Ltd, High Wycombe, UK) and 2-mercaptoethanol (BDH, Poole, UK). Four different CRM's were employed in this study, DORM-2 Dogfish muscle, TORT-2 Lobster hepatopancreas (both NRC, Canada), BCR 464 Tuna fish and BCR 580 estuarine sediment (both Community Bureau of Reference (BCR, Belgium). The moisture content of each CRM was determined by drying separate subsamples to a constant mass at 105 ^{o}C for 24 hours.

The partitioning between the liquid and solid phases, for the equilibration experiments involving DORM-2 and NIST2710 CRM's, of the ^{199}Hg enriched methylmercury spike solution and the particulate bound natural abundance methylmercury, was calculated in Chapter 4, Section 4.4.2. An assumption was made that this partitioning would be similar for these experiments. The approximate amount of each CRM, corrected for moisture content, the equilibration solution volume to be used, and the spike mass fraction to be added, was calculated using the previously measured solid/liquid partition coefficients. Hence, at the end of the equilibration period enough methylmercury would be in the liquid phase to allow the measurement of isotope amount ratios by HPLC-MC-ICP-MS. The actual mass of CRM and ^{199}Hg enriched methylmercury spike solution, and equilibration solution volumes are shown in (Table 5-2), each equilibration experiment was carried out in triplicate.

Table 5-2 The CRM and ^{199}Hg enriched methylmercury spike masses.

	Moisture Corrected Masses					
	Replicate	1	Standard Uncertainty	2	Standard Uncertainty	3	Standard Uncertainty
DORM-2							
Spike mass / g		0.9140	4.22E-05	0.7345	4.22E-05	1.0945	4.22E-05
CRM mass / g		0.5089	9.06E-05	0.4039	8.8E-05	0.4755	8.97E-05
Solution volume /ml		6.0		6.0		6.0	
TORT-2							
	Replicate	1		2		3	
Spike mass / g		0.2504	7.38E-05	0.2937	7.38E-05	0.2179	7.38E-05
CRM mass / g		2.9247	0.000223	4.5626	0.000331	3.2101	0.000241
Solution volume /ml		5.3		5.3		5.3	
BCR 464							
	Replicate	1		2		3	
Spike mass / g		1.74	6.99E-05	0.8761	4.22E-05	0.9469	4.22E-05
CRM mass / g		0.8038	6.97E-05	0.4075	5.02E-05	0.4566	5.22E-05
Solution volume /ml		6.7		6.0		6.0	
BCR580							
	Replicate	1		2		3	
Spike mass / g		1.1134	4.22E-05	1.0916	4.22E-05	1.0907	4.22E-05
CRM mass / g		3.5288	0.000262	3.4641	0.000257	3.4425	0.000256
Solution volume /ml		6.1		6.0		6.0	

The amount of each CRM and ^{199}Hg enriched methylmercury spike, to give a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity at complete equilibration, was calculated. Subsequently, the appropriate volume of equilibration solution, the required mass of ^{199}Hg enriched methylmercury spike and the preweighed CRM were added, in that order, to a clean glass conical flask of an appropriate volume. The conical flask was stoppered, protected from light, maintained at a temperature of 25°C by means of an electrically heated hotplate, and agitated by a magnetic stirrer for 24 hrs. At the end of this time period the samples were filtered (Autovial 0.2μm PTFE membrane syringeless filters, Whatman, Maidstone, UK) into clean Sterilin containers, and the supernatant stored at 4 °C until analysis by HPLC-MC-ICP-MS and HPLC-CV-MC-ICP-MS.

5.1.3.3 Measurement of Isotope Amount Ratios

Two approaches for mass bias correction of the measured ^{200}Hg:^{199}Hg isotope amount ratios were used. These were the simultaneous measurement of an internal standard, and bracketing with a methylmercury solution closely matched to the samples in terms of both ion counts and the ^{200}Hg:^{199}Hg isotope amount ratio.

5.1.3.3.1. Internal Standard Mass Bias Correction for Single IDMS

Mass bias during single IDMS runs was accounted for by the use of an internal standard. The spray chamber was used as the gas/liquid separator during analyses by HPLC-CV-MC-ICP-MS. This allowed a solution of NIST997 Tl SRM, 50 ng g^{-1} in 2% HNO_3, to be nebulised in the conventional fashion and the ^{205}Tl and ^{203}Tl isotopes to be continuously monitored.

5.1.3.3.2 Bracketing Mass Bias Correction for Double IDMS

For the bracketing method of mass bias correction blends of a natural methylmercurychloride standard (Alfa Aesar, Ward Hill, MA, USA.) and the spike

solution used for the equilibration experiments were prepared. In order to closely match the ^{200}Hg:^{199}Hg isotope amount ratio in the bracketing blend with the samples, natural standard methylmercurychloride solutions were prepared which closely matched the certified methylmercury mass fraction of each CRM. Subsequently, for each replicate of each CRM, a natural standard/spike mass bias blend was prepared. This was achieved by combining the appropriate natural standard methylmercurychloride solution with the ^{199}Hg enriched methylmercurychloride spike solution, the masses of each of these solutions closely matched those of the CRM and spike used in each replicate equilibration experiment, *i.e.* for DORM-2 replicate 1, 0.5109 g of a natural methylmercurychloride standard of 4.48 μg g^{-1} was blended with 0.9174 g of the ^{199}Hg enriched methylmercurychloride spike solution. These mass bias blends were further diluted in order to match the ion counts of the mass bias blends with those of the samples.

5.2 Results and Discussion

5.2.1 Multicollector Optimisation

For HPLC-ICP-MS the intensity of the ion signal is dependent on the amount of analyte injected onto the HPLC column, provided transport efficiency to the ICP-MS ion counting device remains constant. In order to determine the minimum amount of analyte required for accurate and precise isotope ratio measurements using HPLC coupled with the VG Axiom MC-ICP-MS, a solution of natural isotopic abundance methylmercurychloride, 30 ng/g as Hg, was prepared and injected onto the HPLC column using three different sample loop volumes of 100, 200 and 500 μl. Four injections were made using each sample loop and the dwell time varied from 50ms through 100 and 250 ms to 500 ms. Increasing the amount of methylmercurychloride injected onto the column, by increasing the sample loop volume, resulted in peak broadening. The peak area doubled as the injection volume doubled whilst the ion signal at the chromatographic peak

maximum increased by 50% for the 200 µl sample loop and 150% for the 500 µl loop compared with the peak height for a 100 µl sample injection.

The ^{200}Hg:^{199}Hg isotope amount ratio was calculated from each resulting chromatogram, using the pseudo-steady-state-approach, from a peak top width of seven seconds giving a variable number of corresponding data points dependent on the dwell time interval used (50 ms n = 141; 100 ms n = 71; 250 ms n = 29; 500 ms n = 14). The standard uncertainty of each measured ^{200}Hg:^{199}Hg isotope amount ratio was calculated as the standard deviation of the mean, *i.e.* the standard deviation divided by the square root of the number of data points of each measurement. Subsequently, the ^{200}Hg:^{199}Hg isotope amount ratio was corrected for mass bias effects using the Russell correction expression[89], Equation 3-1, and the associated combined expanded uncertainty calculated. For all measurements the expanded uncertainty for the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratios was dominated by the contribution from the measured ^{200}Hg:^{199}Hg isotope amount ratio, > 99% relative.

To determine whether the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio for each individual injection agreed with the IUPAC value a Students t test was performed using Equation 5-5, which includes the expanded uncertainties for both the measured and IUPAC values.

$$t = \frac{1 - r}{U(r)}$$

Equation 5-5

where

r is the measured ^{200}Hg:^{199}Hg isotope amount ratio divided by the IUPAC value for the ^{200}Hg:^{199}Hg isotope amount ratio and U(r) is the combined expanded uncertainty, k = 2, of r.

For each injection the measured ^{200}Hg:^{199}Hg isotope amount ratio was not statistically different from the IUPAC value. However, the expanded uncertainty of individual injections was dependent on the loop volume, hence the absolute amount of methylmercurychloride, injected onto the column. The effect of dwell time on the accuracy and precision of the measured ^{200}Hg:^{199}Hg isotope amount ratio is shown in Figure 5-2, Figure 5-3 and Figure 5-4 for 100 µl, 200 µl and 500 µl loops respectively.

The poorest precision, Figure 5-2, was obtained when a 100µl injection loop was used, with a mean relative expanded uncertainty, $U(r_m)$, of 2.5%. When the 200 µl injection volume, Figure 5-3, was used, $U(r_m)$ fell to 0.9%. The most precise results, Figure 5-4, with the least variation in the combined expanded uncertainty, were obtained when a 500 µl sample loop was used $U(r_m)$ 0.5%.

The variation in the dwell time interval, 50, 100, 250 or 500 ms, had little effect on the precision of each measurement for each sample loop volume used. This was because the number of data points acquired, from a seven second measurement acquisition period, increases as the dwell time decreases. Thus, the estimate of the standard deviation of the mean reduces because it is somewhat dependent on the number of data points.

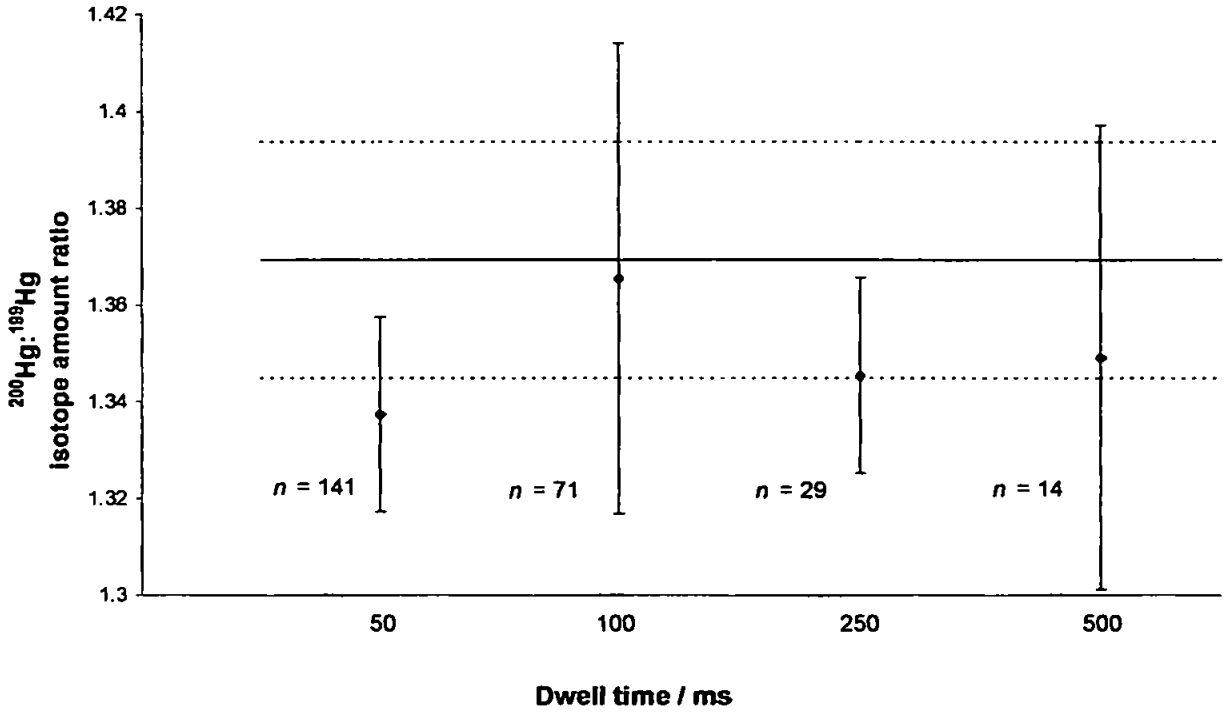


Figure 5-2 The ^{200}Hg:^{199}Hg isotope amount ratios calculated by the pseudo-steady-state approach from a 100µl injection of methylmercurychloride, 30 ng g^{-1} as Hg, the error bars represent the expanded uncertainty (k = 2). The solid horizontal line is the IUPAC value, with the dashed lines representing the upper and lower limits of the expanded uncertainty (k = 2).

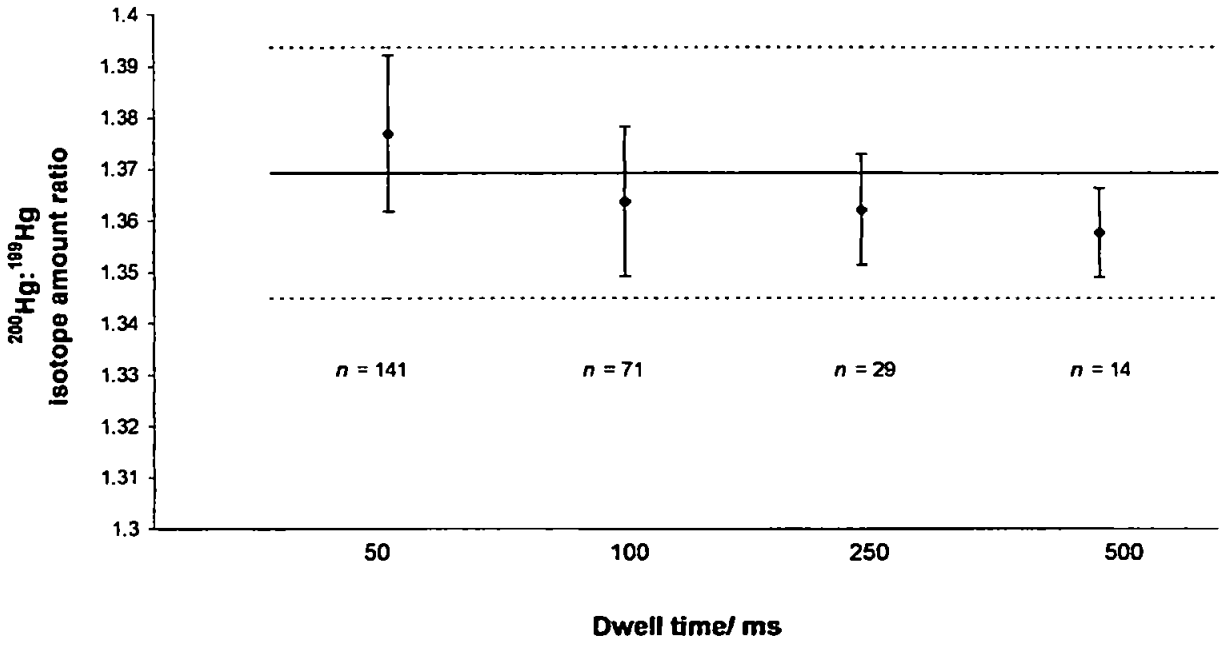


Figure 5-3 The ^{200}Hg:^{199}Hg isotope amount ratios calculated by the pseudo-steady-state approach from a 200µl injection of methylmercurychloride, 30 ng g^{-1} as Hg, the error bars represent the expanded uncertainty (k = 2). The solid horizontal line is the IUPAC value, with the dashed lines representing the upper and lower limits of the expanded uncertainty (k = 2).

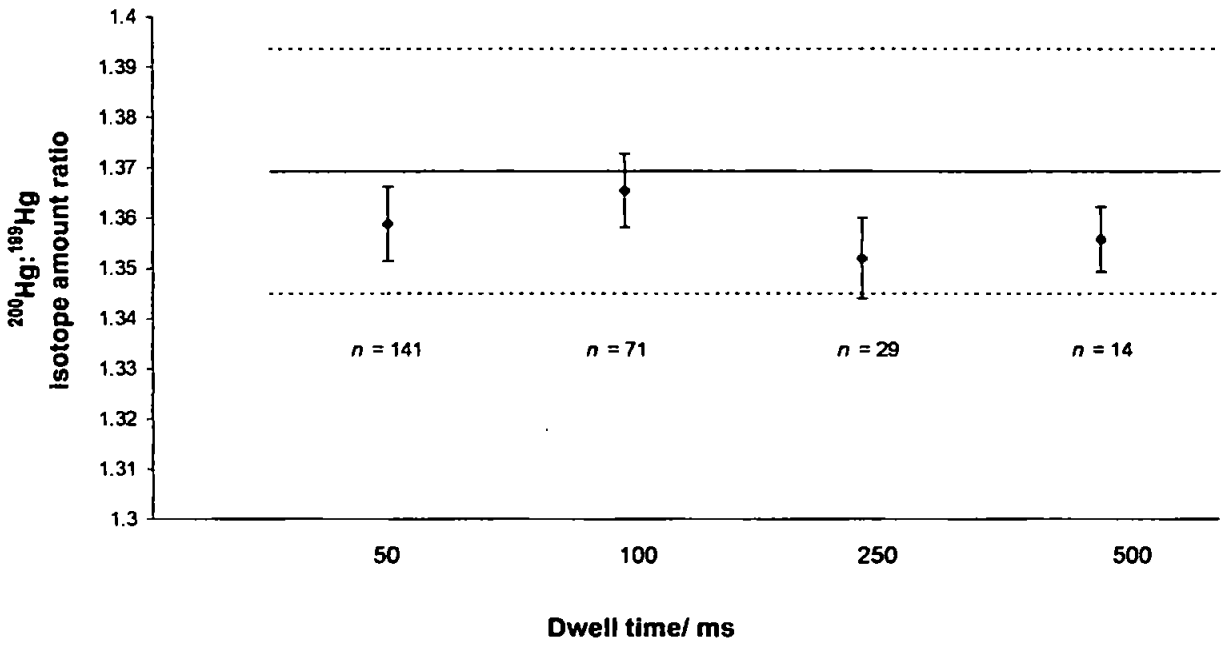


Figure 5-4 The ^{200}Hg:^{199}Hg isotope amount ratios calculated by the pseudo-steady-state approach from a 500µl injection of methylmercurychloride, 30 ng g^{-1} as Hg, the error bars represent the expanded uncertainty (k = 2). The solid horizontal line is the IUPAC value, with the dashed lines representing the upper and lower limits of the expanded uncertainty (k = 2).

Shorter dwell times presented a problem in the transfer of data from the instrument control computer to a second computer for processing. The data files were exported as comma separated value (CSV) files in two columns, time and ion counts and, as the dwell time was decreased, the number of time slices increased for a given chromatographic separation time. Nine ion signals were monitored on the Faraday multicollector array and, if the dwell time was too short, or the acquisition period too long, it was not possible to open the CSV file completely in Microsoft Excel, resulting in a loss of data. One advantage of simultaneous ion signal detection by multicollector ICP-MS is that spectral skew will only arise from isotopic fractionation during chromatographic separation and/or sample introduction. Therefore, dwell times longer than the 10 ms required for the scanning ICP-MS could be used.

To determine the repeatability of isotope ratio measurements made by HPLC-MC-ICP-MS two different dwell times, of 250 and 500 ms were selected, and eight separate analyses were made for each dwell time, using a 500 µl sample loop volume, charged with a 30 ng g^{-1} (as Hg) methylmercurychloride solution. Each mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio, obtained from the resulting chromatograms, and calculated using the pseudo-steady-state approach, was compared with the IUPAC value using Equation 5-5. No statistical difference was found for each ratio. Similarly, the repeatability of the mass bias corrected ^{200}Hg:^{199}Hg, given by the relative standard deviation, RSD (n = 8), was comparable for each dwell time, 0.41% RSD and 0.43% RSD for 250 and 500 ms respectively. These values compare favourably with that obtained by Wahlen and Wolff-Briche[107], who report a standard uncertainty range for repeat isotope ratio measurements, calculated from peak areas, by HPLC-ICP-MS of 0.3-1.4% relative. However, the individual standard uncertainty of the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio for each replicate was significantly lower for the 500 ms dwell time, typically 0.22% relative, than that obtained for the 250 ms dwell time, typically 0.43% relative. Therefore, 500 ms was selected as the optimal dwell time for best accuracy,

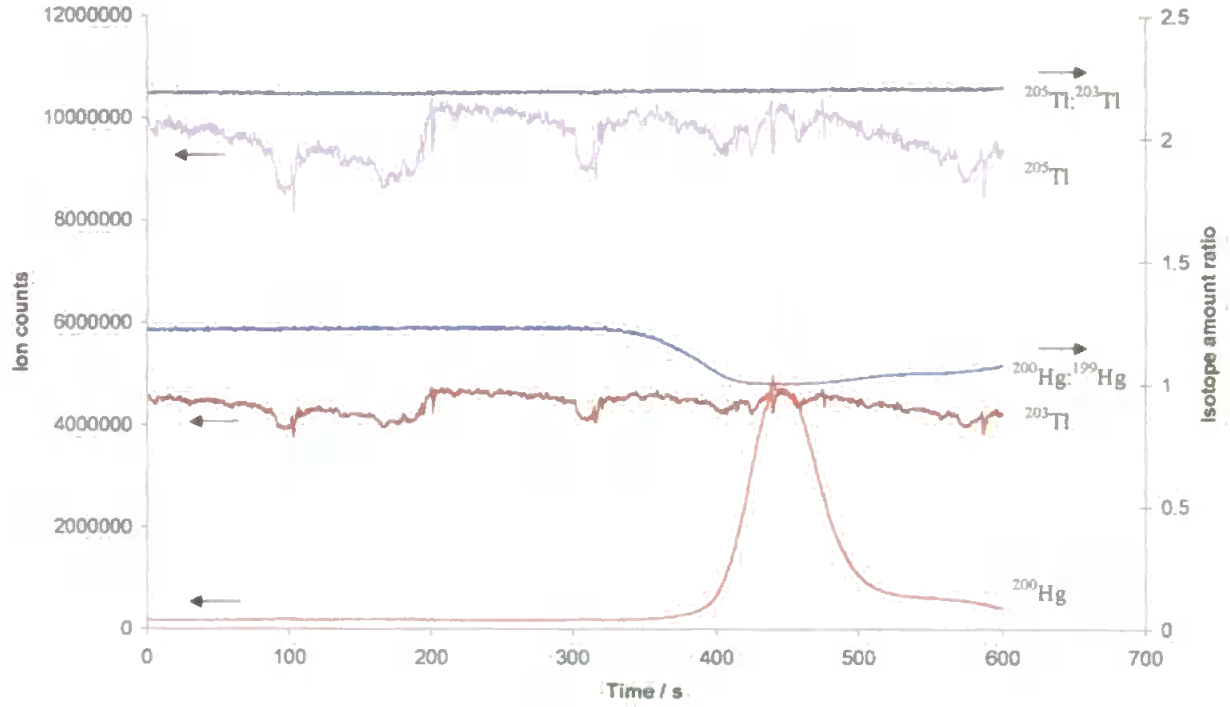
precision and data handling, for the measurement of transient HPLC ion signals by MC-ICP-MS.

5.2.2 Cold Vapour System Optimisation

In order to achieve sufficient sensitivity to allow isotope amount ratios to be measured by the Faraday cup multicollector array, a cold vapour generation system was coupled between the HPLC column eluent and the cyclonic spray chamber. The outlet tube of the CV system was directly introduced into the spray chamber to allow the mercury vapour generated to be transported to the plasma along with the nebuliser gas flow.

Initially, the CV inlet tube was placed vertically into the spray chamber so that the end of the tube rested just below the junction of the glass waste outlet and the angled side of the chamber. This arrangement resulted in a highly variable signal for the conventionally nebulised Tl solution, shown in Figure 5-5A, such that the $^{205}Tl:^{203}Tl$ isotope amount ratio was typically 2.2 as opposed to a usual measured value of 2.42. It was postulated that, due to the cyclonic action of the nebuliser gas through the spray chamber, the cold vapour generation reagents, $SnCl_2$ and Br^-/BrO_3^-, were being entrained in the nebulised Tl solution resulting in matrix effect which effected a change in the instrumental mass bias. Subsequently, the CV generation inlet tube was bent into a U shape as shown in Figure 5-1, and Figure 5-5B shows a chromatogram produced using this arrangement. In this case the measured $^{205}Tl:^{203}Tl$ isotope amount ratio was typically 2.42 and there was much less variability in the Tl signal though it was still quite noisy.

A



B

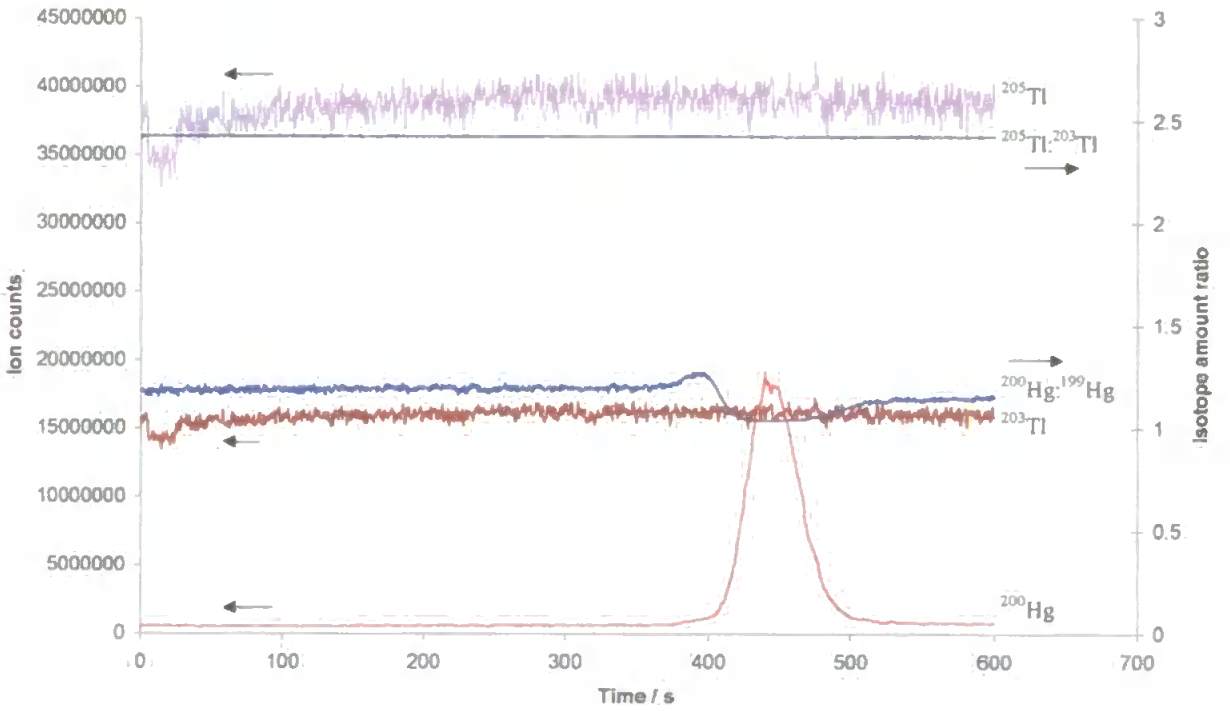


Figure 5-5 The measured, by HPLC-CV-MC-ICP-MS, ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios and ^{200}Hg signal monitored for a 100µl injection of methylmercury chloride, 1µg g^{-1} as Hg. A, with the CV generation inlet tube unbent; B, with the CV generation inlet tube formed into a 'U' shape.

The supposition that the CV generation reagents were being transported to the plasma was borne out by an increase in signal intensities. It is well known that a high matrix concentration causes space charge effects in the ion beam resulting in signal suppression and possible mass bias effects[108]. The signal intensity for the ^{205}Tl isotope rose from 10 Mcps with a straight CV inlet tube, to 40 Mcps when the CV inlet tube was bent into a U shape. Similarly, for a 100 µl injection of methylmercury chloride (1 µg g^{-1} as Hg) the signal intensity for the ^{200}Hg isotope rose from 4.6 Mcps to 18.6 Mcps at the peak maximum. The optimal flow rates for the Br^-/ BrO_3^- oxidant and the $SnCl_2$ reductant were 0.5 ml $minute^{-1}$ and 2.0 ml $minute^{-1}$ respectively.

The repeatability of the ^{200}Hg:^{199}Hg isotope amount ratio, measured by HPLC-CV-MC-ICP-MS was determined by 10 separate injections of a methylmercurychloride solution (30 ng g^{-1} as Hg) with a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity. Figure 5-6 shows the results of these injections. The repeatability, given by the standard deviation, of the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio of these injections, was 0.45% relative. The typical relative standard uncertainty of the individual mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratios was 0.22% relative.

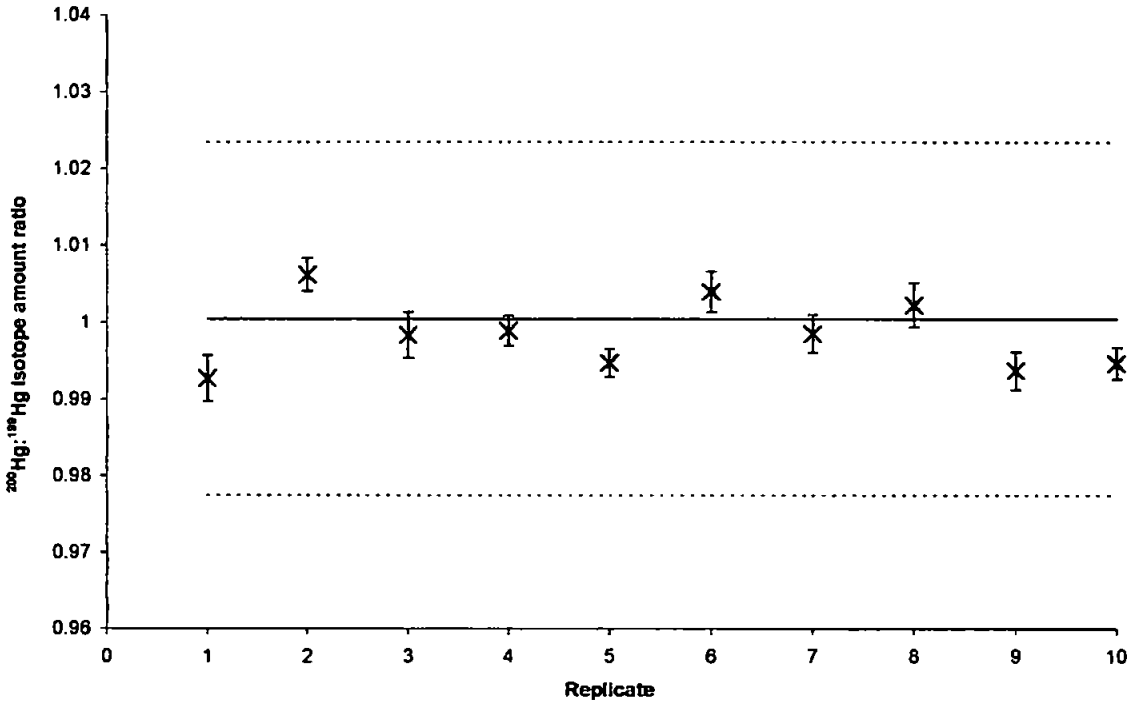


Figure 5-6 The ^{200}Hg:^{199}Hg isotope amount ratios, calculated by the pseudo-steady-state approach, from a 500µl injection of a blend of natural isotopic abundance and ^{199}Hg enriched methylmercurychloride, 30 ng g^{-1} as Hg. The error bars represent the expanded uncertainty (k = 2). The solid horizontal line is the value calculated from the masses and mass fractions of the individual solutions used to prepare the blend, with the dashed lines representing the upper and lower limits of the expanded uncertainty (k = 2).

5.2.3 Reverse IDMS of the ^{199}Hg Enriched Methylmercurychloride Spike

The mass fraction of the ^{199}Hg enriched methylmercurychloride spike solution was determined by HPLC-MC-ICP-IDMS. Four different blends of a natural isotopic abundance methylmercurychloride solution and the spike solution were produced with a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity. The spike mass fraction was calculated using Equation 3-4, and the results are shown in Table 5-3. No methylmercury was detected in the blank solutions and therefore a blank correction was not included in Equation 3-4. The raw counts for each mercury isotope in the methylmercury peak were, however, baseline subtracted to account for inorganic Hg present in the ICP argon gas and the reagents used. The standard uncertainty of the measured ^{200}Hg:^{199}Hg isotope amount ratio included the uncertainty contribution from the baseline correction, which contributed less than 0.1%.

The mean mass fraction of the ^{199}Hg enriched methylmercurychloride spike solution, was 3.74 μg g^{-1} as Hg. In order to estimate the standard uncertainty of the spike mass fraction two separate factors were combined, the between blend and the within blend standard uncertainties. The standard deviation of the mean, SD_m (0.063 μg g^{-1}) was considered to give an estimate of the 'between' blend uncertainty[23], accounting for variations due to the chromatographic separation. The mean of the standard uncertainty of each replicate (0.126 μg g^{-1}), was used as the estimate of the within blend uncertainty[23], accounting for the uncertainty of each variable in Equation 3-4 for each replicate.

Table 5-3 The mass fraction of the ^{199}Hg enriched methylmercurychloride spike solution.

Replicate	Methylmercury chloride mass fraction, as Hg ($\mu g\ g^{-1}$)	Standard Uncertainty ($\mu g\ g^{-1}$)	RSu (%)	Relative Contributions to the Standard Uncertainty (%)				
				R_Z	ΣR_{iZ}	C_Z	^{200}Hg:^{199}Hg isotope amount ratio	^{205}Tl:^{203}Tl isotope amount ratio
1	3.9244	0.128	3.25	97	1.8	0.25	0.24	0.05
2	3.6506	0.125	3.42	97	0.0	0.23	0.54	0.08
3	3.7053	0.125	3.38	98	1.7	0.23	0.12	0.11
4	3.6698	0.125	3.40	97	1.6	0.23	0.46	0.09
Mean	3.7375	0.14	3.76					

The between blend, $u_{between}$, and within blend, u_{within}, standard uncertainties were then combined, by calculating the square root of the sum of the squares of $u_{between}$ and u_{within}, giving a final standard uncertainty for the spike methylmercurychloride mass fraction of 0.14μg g^{-1} (as Hg). The major contribution to this uncertainty arose from the within blend component, 80%, with the between blend standard uncertainty contributing 20%. The within blend combined standard uncertainty for each of the four analyses was dominated by the uncertainty associated with the natural isotopic abundance ^{200}Hg:^{199}Hg isotope amount ratio, R_Z, greater than 97%. Thus, the standard uncertainties associated with the natural isotopic abundances of ^{200}Hg and ^{199}Hg, as given by IUPAC[80] from which R_Z was calculated, provides the major contribution to the standard uncertainty of the spike mass fraction as determined by HPLC-MC-ICP-IDMS. Subsequently the spike solution was diluted to 0.313 ± 0.012 μg g^{-1} (as Hg) for use in the species specific IDMS of methylmercury in the certified reference materials.

5.2.4 Analysis of Certified Reference Materials

Four certified reference materials, DORM-2, BCR464, TORT-2 and BCR580 were equilibrated for 24 hours with a ^{199}Hg enriched methylmercurychloride spike. However, the mass fraction of methylmercury in TORT-2 (0.152 μg g^{-1}) and BCR580 (0.073 μg g^{-1}) was too low to yield a measurable signal by HPLC-CV-MC-ICP-MS. For the TORT-2 Lobster hepatopancreas and BCR580 estuarine sediment CRM's the concentration of material in solution was approximately 0.6 g ml^{-1}. Methylmercury is highly lipophillic[109] and also has a high affinity for humic/fulvic coatings of sediments and soils[110], with a sediment:water partition coefficient as high as 10^5. Thus, for the experiments involving these two CRM's, not enough methylmercury was extracted into solution to be detected using the Faraday cups of the ICP-MS, due to the inability of the solvent to counteract the high affinity of methylmercury for the solid phase. Therefore, for particulate material with a low methylmercury mass fraction a destructive digestion step is

required if isotope amount ratio measurements are to be made using faraday cup multicollector ICP-MS.

For the equilibration of the ^{199}Hg enriched methylmercurychloride spike with DORM-2 and BCR464 sufficient methylmercury was extracted into the liquid phase for the ^{200}Hg:^{199}Hg isotope amount ratio to be measured by HPLC-CV-MC-ICP-MS. Two separate IDMS strategies were employed to determine the mass fraction of methylmercury in each CRM. The first was single IDMS, in which instrumental mass bias was corrected for by the use of an internal standard, NIST997 Tl SRM (50 ng g^{-1}), which was continuously nebulised in conventional fashion. The second was double IDMS, which eliminates the spike mass fraction from the final measurement equation. In the latter case a calibration blend was used to correct for mass bias using the bracketing technique. The ^{200}Hg:^{199}Hg isotope amount ratios in all samples were calculated using the pseudo-steady-state approach.

Equation 3-3 was used to calculate methylmercury mass fractions determined by single IDMS, whereas for the double IDMS calculations Equation 5-4 was used. All uncertainties were estimated using the spreadsheet method of Kragten[66], with the appropriate IDMS equation used as the model for these calculations. No methylmercury was detected in the blank solutions so a blank correction was not included in either of the IDMS equations. The raw counts for each mercury isotope in the methylmercury peak were baseline subtracted to account for inorganic Hg present in the ICP argon gas, the equilibration solution, the HPLC mobile phase and the cold vapour generation reagents. The standard uncertainty of the measured ^{200}Hg:^{199}Hg isotope amount ratio included the uncertainty contribution from the baseline correction, which contributed less than 0.1% relative.

Three replicate analyses were carried out for both DORM-2 and BCR464 CRM's by two separate procedures, single IDMS and approximate matching double

IDMS, and the mean methylmercury mass fraction calculated for each CRM with each method. The results of the individual replicates and mean values for each CRM by both IDMS procedures are shown in Table 5-4. The mean methylmercury mass fraction, as Hg, determined by single IDMS in DORM-2 was 4.24 ± 0.43 μg g^{-1}, with a found value for the double IDMS procedure of 4.29 ± 0.17 μg g^{-1}. For BCR464 the mass fraction of methylmercury was found to be 4.99 ± 0.50 μg g^{-1} by single IDMS and 5.18 ± 0.23 μg g^{-1} by double IDMS. The uncertainties quoted for the analytical results are the expanded uncertainty, U, calculated by combining the between blend ($u_{between}$) and within blend (u_{within}) standard uncertainties, as described in Section 5.2.3, and multiplying by a coverage factor, *k*, of 2, which approximates to the 95% confidence interval.

For the analysis of both reference materials the expanded uncertainty, shown in Table 5-4, of the mean found methylmercury mass fraction was significantly smaller, by approximately 60%, for double IDMS when compared with single IDMS. The estimation of the expanded uncertainty, for the mean methylmercury mass fraction found in the two CRM's, allowed the results to be compared with the certified values using Equation 5-5. There was no statistical difference at the 95% confidence level between the found values for both IDMS procedures and the certified methylmercury mass fractions (as Hg), 4.47 ± 0.32 μg g^{-1} for DORM-2 and 5.12 ± 0.16 μg g^{-1} for BCR464. For single IDMS, the contribution of u_{within} to the expanded uncertainty was much greater than that of $u_{between}$ (0.17 *c.f.* 0.044 μg g^{-1} for DORM-2 and 0.21 *c.f.* 0.045 μg g^{-1} for BCR464. In comparison, for double IDMS, the situation was reversed with $u_{between}$ contributing more than u_{within} (0.078 *c.f.* 0.04 μg g^{-1} for DORM-2 and 0.110 *c.f.* 0.04 μg g^{-1} for BCR464).

Table 5-4 The methylmercury mass fraction determined in DORM-2 and BCR464 CRM's by species specific single and double IDMS.

CRM	Methylmercury mass fraction (μg/g)	$u_{between}$ (μg g^{-1})	u_{within} (μg g^{-1})	Expanded Uncertainty (μg/g)	Relative Expanded Uncertainty (%)	Relative Contribution to the Expanded Uncertainty (%)	
						$u_{between}$	u_{within}
DORM-2							
Single IDMS	4.24	0.044	0.17	0.43	10.1	4	96
Double IDMS	4.29	0.078	0.04	0.17	4.0	83	17
Certified Value	4.47			0.32	7.2		
BCR 464							
Single IDMS	4.99	0.045	0.21	0.50	10.1	3	97
Double IDMS	5.18	0.110	0.04	0.23	4.5	89	11
Certified Value	5.12			0.16	3.1		

In addition, the combined standard uncertainty of each individual replicate analysis by double IDMS, shown in Table 5-5, was significantly lower than those obtained by the single IDMS procedure (Table 5-5), typically less than 1% relative compared with 4% relative respectively, which markedly reduced the contribution of u_{within} to the expanded uncertainty of the found methylmercury mass fractions in DORM-2 and BCR464 when compared to single IDMS.

However, the variation between replicate analyses, shown in Table 5-5 as the relative standard deviation of the mean, was greater for the double IDMS procedure, typically 2%, than for single IDMS, typically 1%, which, combined with the reduction in u_{within}, increased the relative contribution of $u_{between}$ to the expanded uncertainty. Mass bias correction for double IDMS is performed using the bracketing technique, where a mean of two measured isotope amount ratios is calculated. It has been shown (Section 5.2.2) that the repeatability of ^{200}Hg:^{199}Hg isotope amount ratio measurements in a standard solution by HPLC-CV-MC-ICP-MS was 0.45% relative standard deviation. Thus, it was conjectured that the increase in $u_{between}$ for the double IDMS method was caused by the effects of the chromatographic system.

Table 5-5 The methylmercury mass fraction determined in replicate samples of DORM-2 and BCR464 CRM's by species specific single and double IDMS.

CRM	Replicate	Methylmercury mass fraction (μg/g)	Standard Uncertainty (μg/g)	Relative Standard Uncertainty %
DORM-2				
Single IDMS	1	4.17	0.21	4.1
	2	4.32	0.22	4.3
	3	4.23	0.20	3.8
Mean		4.24		
SD_m		0.044		
Relative SD_m (%)		1.0		
Double IDMS	1	4.14	0.04	0.9
	2	4.40	0.03	0.6
	3	4.33	0.04	1.0
Mean		4.29		
SD_m		0.08		
Relative SD_m (%)		1.8		
BCR 464				
Single IDMS	1	5.08	0.25	4.1
	2	4.96	0.25	4.2
	3	4.93	0.24	4.1
Mean		4.99		
SD_m		0.045		
Relative SD_m (%)		0.9		
Double IDMS	1	5.25	0.05	0.9
	2	4.97	0.03	0.6
	3	5.33	0.04	0.7
Mean		5.18		
SD_m		0.11		
Relative SD_m (%)		2.1		

5.2.5 Contributions to Uncertainty

5.2.5.1 Single IDMS

The major relative contributions to the combined standard uncertainty for the species specific single IDMS determination of methylmercury by HPLC-CV-MC-ICP-MS, of each replicate analysis of both the DORM-2 and BCR464 CRM's, are shown in Table 5-6. In each case the uncertainty budget was dominated by the standard uncertainty associated with two variables in Equation 3-3. The natural isotopic abundance ^{200}Hg:^{199}Hg isotope amount ratio (R_X) calculated from IUPAC data[80] (1.369 ± 0.012) which typically contributed 58% relative and the standard uncertainty of the ^{199}Hg enriched methylmercurychloride spike mass fraction (C_Y, 0.313 ± 0.012 μg g^{-1} as Hg) was 40% relative. The ^{200}Hg:^{199}Hg isotope amount ratios for each replicate, measured with a precision of 0.17% or better (relative standard uncertainty), contributed a maximum of 1.7% to the combined standard uncertainty, with other parameters in Equation 3-3 contributing 1% or less to the combined standard uncertainty.

For the single IDMS determinations of methylmercury in DORM-2 described in Chapter 3, isotope amount ratio measurements were made by HPLC-Q-ICP-MS, and, with a typical standard uncertainty of 1% relative, were the major contribution to the standard uncertainty, with lesser contributions from C_Y and R_X. The improved precision obtained when isotope amount ratios were measured using the Faraday cup multicollector array of the VG Axiom instrument, markedly reduced the relative contribution of the measured isotope amount ratios to the standard uncertainty of each replicate. Hence the uncertainty contributions of R_X and C_Y increased.

Table 5-6 Relative uncertainty contributions to the standard uncertainty of the methylmercury mass fraction determined in DORM-2 and BCR464 CRM's by species specific single IDMS.

		Relative Contributions* to the Standard Uncertainty of the mass fraction of methylmercury (%)			
Sample	Replicate	Rx	Cy	Measured ^{200}Hg:^{199}Hg Isotope Amount Ratio	ΣRix
DORM-2	1	58	40	1.3	0.8
	2	54	45	0.2	0.7
	3	64	33	1.7	0.9
BCR 464	1	58	40	1.6	0.8
	2	56	43	0.4	0.7
	3	59	40	0.5	0.9

* The relative contributions to the standard uncertainty may not total 100 due to rounding

The uncertainty associated with the mass fraction of the spike solution (C_Y) was dominated by R_Z (Section 5.2.3), which was equal to R_X. The relative uncertainty contribution of C_Y was typically 40%, so the combined standard uncertainty for each replicate analysis of the CRM's is consequently dominated by R_X. Hence, when mercury isotope amount ratios are measured using a Faraday cup multicollector array, in order to significantly reduce the combined standard uncertainty of each replicate analysis the isotopic abundance of mercury in both the samples and the natural standard used for the reverse IDMS of the spike solution must be determined, with greater precision than the existing IUPAC data can provide.

5.2.5.2 Double IDMS

The major contributions to the combined standard uncertainty for the determination of methylmercury in DORM-2 and BCR 464, using the double IDMS procedure, are shown in Table 5-7. The largest contributions resulted from the uncertainty associated with the measured ^{200}Hg:^{199}Hg isotope amount ratios in the sample/spike blend, R_B, and the mass bias calibration blend, R_{BC}. However, the relative contribution which each parameter made to the combined standard uncertainty was highly variable, and dependent on the precision with which each ^{200}Hg:^{199}Hg isotope amount ratio was measured, shown in Table 5-8. For all analyses, except that for DORM-2 replicate three, the ^{200}Hg:^{199}Hg isotope amount ratio was measured with greater precision in the sample/spike blend than in the calibration blend, which resulted in the greater contributions to the measurement uncertainty from the calibration blend standard uncertainty.

Table 5-7 Relative uncertainty contributions to the standard uncertainty of the methylmercury mass fraction determined in DORM-2 and BCR464 CRM's by species specific approximate matching double IDMS.

		Relative Contributions* to the Standard Uncertainty of the mass fraction of methylmercury (%)				
Sample		Measured ^{200}Hg:^{199}Hg isotope amount ratio in spike/sample blend, R_B	Mean Measured ^{200}Hg:^{199}Hg isotope amount ratio in mass bias blend, R_{BC}	Cz	Calculated ^{200}Hg:^{199}Hg isotope amount ratio in mass bias blend, R_T	Rx
DORM-2	1	37	47	3	8	4
	2	14	74	7	3	2
	3	38	34	3	16	8
BCR464	1	43	54	3	0	0
	2	30	52	8	7	3
	3	29	47	6	12	6

* The relative contributions to the standard uncertainty may not total 100 due to rounding

Table 5-8 Isotope amount ratios measured by HPLC-CV-MC-ICP-MS for the species specific determination of methylmercury in DORM-2 and BCR464 by approximate matching double IDMS.

Sample		Measured ^{200}Hg:^{199}Hg Isotope Amount Ratio, R_B	Relative Standard Uncertainty (%)	Mean Measured ^{200}Hg:^{199}Hg in mass bias blend, R_{BC}	Relative Standard Uncertainty (%)	Degree of matching between R_{BC} and R_B (%)
DORM-2	1	0.993	0.14	1.0159	0.16	2.3
	2	1.040	0.05	1.0480	0.12	0.76
	3	0.941	0.17	0.9824	0.16	4.4
BCR 464	1	1.005	0.15	1.0090	0.18	0.40
	2	1.021	0.08	1.0328	0.10	1.1
	3	1.013	0.08	0.9941	0.11	1.9

The standard uncertainty associated with three other parameters in Equation 5-4; the natural standard mass fraction used to prepare the mass bias calibration blend, C_X, the calculated ^{200}Hg:^{199}Hg isotope amount ratio in the mass bias calibration blend, R_T, and the natural isotopic abundance ^{200}Hg:^{199}Hg isotope amount ratio, R_X, also gave significant contributions to the combined standard uncertainty for each replicate (Table 5-7). The relative uncertainty contribution of these parameters varied with each replicate, but decreased as the degree of matching between the ^{200}Hg:^{199}Hg isotope amount ratio in the mass bias calibration blend and the sample/spike blend, expressed as the absolute value of R_{BC}:R_B in percent, increased.

5.3 Conclusions

A cold vapour generation system was coupled with HPLC and ICP-MS which improved sample transport to the ICP by up to 20 fold. The resultant increase in the ion signal allowed the measurement of mercury isotope amount ratios by HPLC-MC-ICP-MS. The use of multicollector HPLC-ICP-MS improved the precision of mercury isotope amount ratio measurements by at least a factor of ten, when compared with quadrupole HPLC-ICP-MS, due to the improved signal stability and the use of longer dwell times of 500 and 10 ms respectively.

The mass fraction of methylmercurychloride has been determined in two CRM's, DORM-2 and BCR464, by two different procedures, single IDMS and approximate matching double IDMS, with the isotope amount ratios measured using HPLC-CV-MC-ICP-MS. The mass fractions determined by the two methods were not statistically different, within the limits of uncertainty, from the certified values. However, the major contributions to the expanded uncertainty for each IDMS procedure arose from different sources.

For the single IDMS procedure the major uncertainty contribution was derived from the within replicate uncertainty, u_{within}. The combined standard uncertainty of each replicate analysis was dominated by two components, the uncertainty associated with the natural isotopic abundance $^{200}Hg:^{199}Hg$ isotope amount ratio and the uncertainty associated with the ^{199}Hg enriched methylmercurychloride spike mass fraction, which was determined by reverse single IDMS. The combined standard uncertainty of the spike mass fraction was also dominated by the uncertainty of the natural isotopic abundance $^{200}Hg:^{199}Hg$ isotope amount ratio. The improvement in the precision of the measured isotope amount ratios obtained by HPLC-CV-MC-ICP-MS compared with quadrupole ICP-MS, markedly reduced this contribution to the combined standard uncertainty of each replicate. Therefore, the use of HPLC-CV-MC-ICP-MS significantly reduced the expanded uncertainty, and, in order to further reduce the measurement uncertainty associated with single IDMS the natural isotopic abundance $^{200}Hg:^{199}Hg$ isotope amount ratio in the samples and natural standard must be determined, significantly increasing the complexity of the procedure.

In the case of the analyses by approximate matching double IDMS the between blend standard uncertainty, $u_{between}$, was the major contributor to the expanded uncertainty. The combined standard uncertainty for each individual replicate was dominated by the contribution from the standard uncertainty associated with the measured $^{200}Hg:^{199}Hg$ isotope amount ratios in the spiked sample and the mass bias calibration blend. Therefore, the combined standard uncertainty of each replicate analysis can be reduced further by improving the $^{200}Hg:^{199}Hg$ isotope amount ratio measurement precision.

Three other factors, namely the mass fraction of the methylmercurychloride natural standard, the calculated $^{200}Hg:^{199}Hg$ isotope amount ratio in the mass bias calibration blend and the $^{200}Hg:^{199}Hg$ isotope amount ratio of natural isotopic abundance, also provided lesser contributions. The contributions from these parameters tended to a

minimum as the degree of matching of the $^{200}Hg:^{199}Hg$ isotope amount ratio in the sample/spike and mass bias calibration blend increased. The expanded uncertainty of the methylmercury mass fraction in each of the CRM's was reduced by approximately half for approximate matching double IDMS when compared with single IDMS, due to the lower contribution of u_{within}. Thus showing the power of the approximate matching double IDMS procedure to minimise the uncertainty for an individual analysis.

6. Conclusions and Suggestions for Future Work

6.1 Conclusions

The aim of this work was to investigate the sources of uncertainty in ID-ICP-MS for speciation analysis. An uncertainty budget was formulated for all analyses, which allowed the measurement process to be systematically studied, and different analytical procedures to be compared. ^{199}Hg enriched methylmercurychloride was synthesised, by direct non-enzymatic methylation with methylcobalamin as the methyl group donor, and extracted in the solid form for use as a spike material for species specific IDMS.

The stability of methylmercury during the IDMS procedure was investigated using ^{1}H NMR spectroscopy and solutions of ^{199}Hg enriched methylmercurychloride, ^{13}C labelled methylmercuryiodide and ^{199}Hg enriched inorganic mercury. The chemical shift, and the ^{1}H-^{199}Hg coupling constant, of methylmercuryhalides were found to be dependent on the solvent composition. Intermolecular exchange of the methylmercury halide counter ion was observed, with an order of preference of $I^- > Br^- > Cl^-$, which required samples to be iodinated prior to ^{1}H NMR spectroscopy data acquisition. Hence, the chemical form of methylmercury measured by HPLC-ICP-MS is dependent on the halogen content of the sample matrix and analytical reagents.

No evidence was found for: methyl group exchange between ^{13}C labelled and ^{199}Hg enriched methylmercuryiodide during HPLC separation; methyl group transfer between methylmercuryiodide and inorganic Hg in the presence of a soil material; or the formation of methylmercuryiodide from inorganic mercury and methyl groups contained in the soil matrix. The stability of ^{199}Hg enriched methylmercuryiodide in the presence of a fish muscle tissue could not be determined by ^{1}H NMR spectroscopy due to co-extracted

molecules giving signals which masked that from the methylmercuryiodide. However, the spike material was adjudged to be stable in the presence of DORM-2, a fish muscle CRM by HPLC-ICP-MS.

Coupling between HPLC and ICP-MS was optimised, using a low uptake naturally aspirated nebuliser, to reduce the effects of the high organic content of the mobile phase (50% methanol) on the plasma and to give the most stable ion signal. A pseudo-steady-state approach was used to calculate isotope amount ratios from the resulting chromatograms. This method of data extraction proved to be more accurate and precise than conventional peak integration, with the added advantage that an instrumental precision estimate was obtained from a single injection, thereby reducing the analytical time and the amount of sample required.

Two ICP-MS instruments were used for isotope amount ratio measurements, a quadrupole instrument capable of sequential signal monitoring, and a multicollector instrument, which monitored up to nine ion signals simultaneously. The optimal dwell time for sequential monitoring of transient ion signals was 10 ms. Spectral skew, which resulted in erroneous isotope amount ratio measurements, was observed when dwell times of a longer duration were used, whereas shorter dwell times resulted in decreasing accuracy and precision. In comparison, for the multicollector instrument the best accuracy and precision was obtained when a dwell time of 500 ms was used. Isotope amount ratios were measured with a typical standard uncertainty of 1 to 2% relative by quadrupole HPLC-ICP-MS. The use of multicollector HPLC-ICP-MS resulted in a significant improvement to the standard uncertainty of the measured ^{200}Hg:^{199}Hg isotope amount ratios, which ranged from 0.02 to 0.2% relative, dependent on the intensity of the ion signal.

The extent of equilibration between the sample and the spike was determined in a series of experiments involving a soil SRM, NIST2710. A time resolved equilibration experiment, with the HPLC mobile phase as the solvent, resulted in an underestimation of

the certified Hg mass fraction by 34% *i.e.* complete equilibration was not achieved. During this experiment 37% of the natural isotopic abundance Hg was desorbed and 42% of the ^{199}Hg enriched spike was adsorbed onto the NIST2710 particles. Complete equilibration was only achieved when the soil material was completely solubilised *via* an HNO_3 microwave digestion, rendering all of the Hg contained in the SRM available for equilibration with the ^{199}Hg enriched spike solution. The found mass fraction of mercury in NIST2710 was in good agreement, within the limits of uncertainty, with the certified value using this method. These experiments indicated that the mercury in NIST2710 was contained both within the soil matrix and adsorbed onto the particle surfaces.

A second set of time resolved equilibration experiments were carried out using DORM-2 and ^{199}Hg enriched methylmercurychloride. Complete equilibration was achieved, with the HPLC mobile phase as the solvent, despite the desorption of only 47% of the methylmercury in DORM-2 and the adsorption of 65% of the ^{199}Hg enriched methylmercurychloride spike onto the particles. The mass fraction of methylmercury in DORM-2, determined by single IDMS, was 4.25 ± 0.47 μg g^{-1} ($k = 2$), in good agreement, within the limits of uncertainty, with the certified value of 4.47 ± 0.32 μg g^{-1}. The mass fraction of methylmercury in DORM-2 as determined by external calibration from the same sample was 2.10 ± 0.5 μg g^{-1} ($k = 4.3$), an underestimation of 53% compared with the certified value.

These experiments emphasised an important advantage of IDMS over conventional external calibration, namely that complete equilibration between the sample and spike, rather than 100% extraction of the analyte into the liquid phase, was required for accurate results. However, for the determination of total Hg in a soil matrix a harsh destructive digestion was required in order to achieve complete equilibration. The extent of equilibration was determined by partitioning of the analyte and spike between the solid sample matrix and the equilibration solvent.

The formulation of an uncertainty budget for single IDMS and external calibration allowed the performance of both methods to be compared, the relative expanded uncertainty, when isotope amount ratios were measured by Q-ICP-MS, for single IDMS (11%) was less than half that for external calibration (24%). For single IDMS the major contribution arose from the measured ^{200}Hg:^{199}Hg isotope amount ratio (52%), with lesser contributions from the measured ^{205}Tl:^{203}Tl isotope amount ratio (16%), the natural isotopic abundance ^{200}Hg:^{199}Hg isotope amount ratio (24%) and the spike mass fraction, (8%). The uncertainty budget for external calibration was dominated by the standard uncertainty of the calibration curve regression fit (> 99%).

For the time resolved equilibration with DORM-2 insufficient methylmercury was brought into solution to be measured by a Faraday cup multicollector array. To allow isotope amount ratio measurements by multicollector ICP-MS a cold vapour generation system was coupled between the HPLC and ICP-MS, with the spray chamber acting as the gas/liquid separator. Sample transport to the ICP was improved by up to 20 fold using this system. Subsequently, the mass fraction of methylmercury was determined in two CRM's, DORM-2 and BCR464, by two different procedures, single IDMS and approximate matching double IDMS.

The increase in the ion signal achieved by the introduction of the cold vapour generation system allowed the measurement of mercury isotope amount ratios in the samples from these experiments by multicollector HPLC-ICP-MS. Both IDMS procedures exhibited comparable accuracy. The found mass fractions for both single and double IDMS of methylmercury in each CRM were not statistically different, within the limits of uncertainty, from the certified values. However, for double IDMS the expanded uncertainty of the found methylmercury mass fraction in each of the CRM's was reduced by approximately half when compared with single IDMS. This was due to the improved

standard uncertainty for each replicate analysis by double IDMS, 1% relative or less, compared with single IDMS, 4% relative.

For single IDMS the uncertainty budget for each individual replicate was dominated, 98% relative, by the standard uncertainty of the natural ^{200}Hg:^{199}Hg isotope amount ratio. Throughout this work the natural isotopic composition of mercury, as given by IUPAC[80], contributed significantly to the expanded uncertainty of each analysis by single and reverse IDMS, particularly when isotope amount ratios were measured with a high degree of precision using multicollector ICP-MS. To reduce this uncertainty contribution the isotopic composition of mercury in the samples and standards must be measured, which would significantly increase the complexity of the analytical procedure and may only be beneficial if multicollector instrumentation if employed.

In the case of double IDMS the major uncertainty contribution for each replicate, 72 to 97% relative, arose from the standard uncertainty of the measured ^{200}Hg:^{199}Hg isotope amount ratio in the samples and mass bias correction blend. It is unlikely, due to the optimisation of the HPLC-ICP-MS coupling, and the data extraction method used, that a further reduction in the measurement precision of isotope amount ratios resulting from transient ion signals by multicollector ICP-MS could be achieved with currently available instrumentation. Therefore, the measurement uncertainty in a single sample by species specific double IDMS, which is highly dependent on the uncertainty associated with the measured isotope amount ratios, is unlikely to be significantly improved.

6.2 Suggestions for Future Work

Isotopically enriched isotopomers of the target analyte, required for species specific IDMS, are not commercially available and not all analytical laboratories have the facilities or knowledge base to synthesise these compounds. If species specific IDMS is to become a routine method of analysis, rather than the preserve of research and National

Measurement institutions as at present, the commercial production of isotopically enriched compounds in the solid form is required. This would also have the advantage of reducing the uncertainty associated with the spike mass fraction, which is typically a factor of fifty smaller for solutions prepared from a solid of known purity compared with a mass fraction determined by reverse IDMS[23], enabling single IDMS, which is less complex than double IDMS, to be more widely used.

A wide variety of reagents and digestion procedures have previously been used to extract methylmercury from biological and sediment samples. However, a systematic study of the effect of these procedures on the stability of methylmercury has not been carried out. The stability of methylmercury during equilibration and separation by HPLC was determined by ^{1}H NMR spectroscopy using isotopically enriched isotopomers of methylmercuryhalides. Using the same approach, the stability of methylmercury and other organometallic species having NMR active isotopes, such as organotin species, which have been shown to undergo rearrangement reactions during sample preparation for GC-ID-ICP-MS[50], could be determined.

Insufficient methylmercury was brought into solution for two CRM's, TORT-2 and BCR580, certified for 152 and 70 ng g^{-1} of methylmercury respectively, to allow isotope amount ratios to be measured by faraday cup multicollector ICP-MS. For these two materials, and real environmental samples which are likely to contain even less methylmercury unless arising from polluted sites, a number of different approaches, namely preconcentration, enhanced extraction and choice of instrument, can be taken to enable ion signals to be measured simultaneously by ICP-MS.

Analyte preconcentration prior to injection onto the HPLC column has previously been used for mercury species in water samples and soil and sediment extracts. Corns[111] fitted a C_{18} guard column into the sample loop of the HPLC injection valve. As the aqueous sample was drawn through the sample loop mercury species were retained on

the guard column causing a preconcentration effect. Subsequently the valve was switched to the inject position and the retained species eluted from the guard column by the mobile phase and separated on the analytical column.

Micro-columns packed with sulphydryl cotton have also previously been used as a preconcentration media for organomercury species[112]. Water samples were passed through the micro-column where the organomercury was retained. Following this preconcentration the retained species were desorbed using an acidic solution, which was neutralised before injection onto the HPLC column. For conventional external calibration, preconcentration procedures using solid media can be unreliable due to incomplete analyte extraction[113]. However, this is negated in IDMS as analyte losses in equilibrated samples are accounted for by the action of the spike as an internal standard.

Enhanced extraction techniques *e.g.* accelerated solvent extraction or microwave digestion, have been used to increase the amount of analyte brought into solution from solid sample matrices. However, a harsh destructive digestion may alter species information in the sample matrix and so would not be appropriate. Nevertheless, as it is not necessary to bring all of the analyte into solution for accurate results by IDMS, relatively gentle reagents, *e.g.* dilute HNO_3[48], or procedures, such as very low power microwave digestion[114], may be suitable provided that it is shown that no analyte degradation occurs.

Faraday cup detectors are inherently less sensitive than secondary electron multiplier (SEM) ion counters due to a high background signal from electronic noise. A high resolution ICP-MS instrument (Neptune, Thermo Electron, Bremen, Germany) fitted with multiple SEM's has recently been introduced, enabling the simultaneous measurement of ion signals in samples at the sub ng g^{-1} level. The use of this type of instrumentation may enable the measurement of isotope amount ratios, in samples with a low analyte mass fraction, with comparable accuracy and precision to that obtained with

the VG Axiom used in this study. For samples with a low level of methylmercury a scanning ICP-MS could also be used, however, the subsequent decrease in the isotope amount ratio measurement precision available would increase the uncertainty in the analysis.

Each of the three strategies presented to allow mercury isotope amount ratios in samples of a low analyte mass fraction to be measured by multicollector ICP-MS has its merits. Preconcentration of the analyte in the sample is the simplest to perform. However, the sample volumes from the 24 hour equilibration with TORT-2 and BCR580 were 4 ml or less after filtration, giving a small preconcentration factor which may not be sufficient to give a large enough increase in the ion signal. The use of a ICP-MS equipped with multiple SEM's is not an option open to many as the Neptune instrument costs in excess of £500,000.

Increasing the amount of methylmercury in solution by an enhanced extraction procedure is the preferred option, provided analyte stability is determined, as simple chemical and/or physical digestion procedures are readily available to most analysts. For species that are trapped within the sample matrix, rather than bound to surface layers, complete equilibration, and hence accurate results by IDMS, was difficult to achieve with a mild solvent. Therefore, this approach would also have the advantage of rendering more of the analyte available for equilibration with the spike, by partially destroying the sample matrix, thus increasing the likelihood of complete equilibration.

Finally, there has recently been a debate in the literature as to whether the isotopic composition of mercury varies in nature[115,116,117,118], however, because a full uncertainty budget has not been formulated for the data sets firm conclusions cannot be drawn. There are a number of other elements (Li, B, S, Ca, Ti, Cr, Zn, Ge, Se, Zr, Mo, Ru, Pd, Cd, Sn, Te, Ba, Nd, Pt and Pb) in the IUPAC data which also have relatively large uncertainties associated with the isotopic amount fractions. Therefore, the limit on

reducing the measurement uncertainty for determinations of these elements by IDMS is likely to come from the natural isotopic abundance data. A programme, which must include full uncertainty statements, to improve the IUPAC isotopic amount fraction data and resolve the debate over possible variance of mercury is required. The advent of multicollector ICP-MS instrumentation, which are capable of the measurement of isotope amount ratios with a high degree of accuracy and precision, should enable this to be achieved.

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Appendix

Procedure for handling organomercury compounds

Project Title: Factors affecting the accuracy and precision of isotope dilution analysis ICPMS for speciation studies.

Researcher: Robert Clough

Project Managers: Dr H.Evans

Dr S.Belt

Experimental Procedure

All work with mercury compounds *i.e.* transfer of solid and liquid compounds to sealed weighing bottles, pipetting etc, will be conducted in a dedicated fume cupboard (DB 607) which contains spill trays and a sink. When handling solids and stock solutions (>100 ppm) of organomercury compounds work must be conducted in the presence of another person.

All work is to be conducted using double hand protection (rubber and special plastic gloves available from DB 514, I. Doidge). Standard laboratory safety procedures must be rigidly adhered to. Stock compounds are to be kept in the dedicated mercury poisons locker (DB607). Dilute and stock compounds (around 10 ppm) are to be kept in sealed vials in the dedicated fume cupboard DB607, volatile dilute solutions are to be kept in the fridge in DB607.

All glassware and vessels used for stock solutions must stay in the dedicated fume cupboard (DB607) and be clearly labelled. Mercury disposal bottles must also be kept in the dedicated fume cupboard. Transport of all organomercury solids and solutions for analysis *e.g.* by NMR, ICPMS, must be carried out in labelled, sealed containers within a watertight sealed outer container.
Help from technicians or project managers must be obtained in any case of doubt.

Signed

R. Clough

Dr H.Evans

Dr M. Foulkes

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Isotope Dilution ICP-MS for Speciation Studies

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I. INTRODUCTION

Speciation is a growing feature of analytical chemistry and, depending on the method employed, has been defined variously as:

- The chemical form of the analyte that is functionally important (e.g., ligands on an organometallic species or oxidation state).
- The exact structural and chemical form of the analyte (e.g., nuclear magnetic resonance derived structure).
- The nature of the chemical species as determined from an operational point of view, dependent on the method of sample preparation or analysis used (e.g., metals extractable by acetic acid or other solvent).

In order to encompass a single wider definition of speciation the definition can be framed in terms of the information which is required by the analyst, or to ask the question "What do I want to know about

the analyte?". The answer poses several further questions:

- How much analyte is present in total?
- How much is available?
- What is its chemical form/structure?
- How is it distributed?
- How stable is it?
- How sure are we about all of the above?

When considered in these terms, the definition of speciation includes not only the accurate determination of the concentration of individual chemical species but also their behaviour in biological and biogeochemical systems. The International Union of Pure and Applied Chemistry (IUPAC) have clarified speciation as "... the specific form of a chemical element defined according to its molecular, complex, electronic or nuclear structure"[1] leading to the identification and quantification of the different chemical and physical forms of an element existing in a sample.[2] Hence, speciation analysis can be considered to be the measurement of one or more individual chemical species in a sample. The chemical form of an element will determine its toxicity, stability and transport, so this measurement is a very important factor in the wider definition of speciation.

II. OVERVIEW OF SPECIATION

The determination of distinct chemical species, as opposed to total elemental concentrations, is of growing importance in many fields. The bioavailability, toxicity and reactivity of trace metals and organometallic compounds in aquatic, sediment, soil, effluent and flue gas samples is determined by the relative proportions of the species present, rather than the total concentration of the target element.[3] The determination of specific chemical entities is long established, especially for non-metallic species including. NO_3^-, NO_2^-, NH_4^+, and NH_3, with the characterisation of metals having a shorter history.[4] Likewise the functionality of biologically important enzymes and co-factors often depends on the speciation of individually important trace metals.

The awareness of the need for speciation techniques has gradually arisen as various pollution and poisoning incidents have been investigated. The residents of Minamata Bay in Japan suffered acute and chronic mercury poisoning, with brain damage to new-born infants, as a result of a release of inorganic mercury into the bay, followed by

biomethylation to methylmercury and its subsequent bioaccumulation in fish, due to its lipophillic nature.[5,6] A spill of tetraalkyl lead in the Mediterranean raised awareness of the importance of organolead compounds.[4] A population crash, combined with changes in shell shape and meat content, decimated the oyster farming industry of Arcachon Bay in France and was traced to the use of tributyltin (TBT) as an anti-fouling agent.[7] Subsequently a correlation was found between the levels of imposex observed in gastropods in SW England and the introduction of TBT to this region. In contrast, inorganic tin is a relatively innocuous compound.

More recently, one of the driving forces for performing speciation studies has been the introduction of legislative requirements, regulating the permissible levels of both total elemental concentrations and, in some cases, the concentration of specific species e.g., tributyltin in UK water quality legislation,[4] and methylmercury in fish for human consumption.[2]

Speciation methods are now applied to a wide range of analytes, primarily for the determination of Al, Sb, As, Cd, Cr, I, Pb, P, Hg, Pt, Se, Sn and the actinide serie,[2] and include:

- Studies of biogeochemical cycles of chemical compounds.
- Determination of toxicity and ecotoxicity of selected elements.
- Quality control of food products and associated packaging.
- Control of medicines and pharmaceutical products.
- Technological process control.
- Research on the environmental impact of technological installations.
- Occupational exposure studies.
- Clinical analysis.

III. SPECIATION IN PRACTICE

There are a number of practical difficulties associated with speciation studies, mainly associated with the requirement to preserve the speciation of the analyte in any given sample throughout the analytical procedure. The main stages in the analytical procedure are:

- Sampling and storage.
- Sample preparation.
- Analysis.
- Quality assurance of results.

A. Sampling and Storage

Sample collection, storage and preparation for analysis are factors which can have a considerable impact on the accuracy and precision of the final results. The stability of the analyte must be considered with regard to sampling and storage (what are appropriate containers?), losses of volatile species (e.g., organo-mercury), extractability (from particles, e.g., sediments, without changing the composition and/or structure of the original analyte components), solubility (stability in various aqueous and non-aqueous solvents), light sensitivity (may cause changes in molecular structure over time), pH sensitivity (will the analyte be effected by acidic or basic solutions?), temperature, and degradation over time.

The choice of sample container can be crucial to preserving sample integrity and should be carefully considered. It should:

- Not contain any leachable compounds of the analyte.
- Be impermeable to the analyte.
- Reduce or prevent photochemical reactions or oxidation/reduction of the analyte.

Factors which determine the speciation of an analyte in the environment need to be considered during speciation studies. For example, redox conditions govern the oxidation state of Fe and Mn species.[5] Fe(II) and Mn(II) are both soluble in anoxic natural waters, but in oxygenated waters are present as the insoluble Fe(III) and Mn(IV) forms. Furthermore, the pH of an aqueous sample may also have a controlling effect on acid–base equilibria and redox potential which may rule out the use of acidification to preserve sample integrity. The ionic strength of the solute, and one or more of the major ions contributing to ionic strength, may also affect the speciation of the desired analyte.[4]

B. Sample Preparation

Once samples have been collected and suitably preserved the problem of extracting the analyte, while maintaining the integrity of the species, arises. For liquid samples, this should be relatively straightforward providing no changes occur during filtration or centrifugation and the sample can be analysed directly or after dilution. The choice of instrument for analyte detection may necessitate a preconcentration step, which can in itself give rise to experimental errors due to species transformation or loss of analyte.[8]

For solid samples, an extraction step with a suitable solvent is usually required. For biological samples, toluene or methanol is regularly used, often in conjunction with an ultrasonic bath to increase the efficiency and speed of the extraction. A soxhlet extraction may also be utilised although this is a longer process and may involve the loss of volatile analytes or speciation information due to the elevated temperatures involved.[40]

Soil or sediment samples present further difficulties;[4] drying the sample, albeit at low temperatures in air may still result in the loss of and/or speciation changes to the analyte. Sieving the dried sample into separate fractions may also give inaccurate results as some elements are associated with a particular size fraction and the use of a "conserved" element such as aluminium as an internal standard may therefore be needed to correct for these errors.[4] The analysis of anoxic sediments and soils presents further problems as care must be taken during drying and storage to ensure anoxic conditions remain.

After a representative solid sample has been obtained various extraction protocols can be utilised. In order to determine the bioavailable fraction a mild extractant or complexing agent such as ethylenediaminetetraacetic acid (EDTA)[9] can be used. One of the many sequential extraction protocols based on the method of Tessier[10] may be employed to determine metals associated with the various soil/sediment fractions. Sequential extractions can also be unreliable due to readsorption effects. Bermond et al.[11] reported that sequential extraction protocols cannot reliably estimate trace metal speciation in soils, and the same may be true for other related solid material such as sediments and suspended particulate material (SPM).

Two further problems arise from sequential extraction protocols. First, their multi-step nature increases the risk of experimental errors, and second, the quantity of material required. For example, up to 1 g can be difficult to obtain for some types of sample matrix, including suspended particulate material (SPM) where SPM concentrations for productive surface waters[12] are characteristically between 20 and 100 mg L^{-1}.

C. Analysis

There are a wide variety of separation and detection techniques available for use in speciation analysis, with the main requirements being[2]:

- Sensitivity.
- Selectivity.

- Quantitative analysis.
- Qualitative analysis.

A summary of the most commonly used techniques is presented in Table 1. In particular, the advent of hyphenated investigation techniques which couple powerful separation methods with sensitive detectors e.g., high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS) have allowed great advances in speciation studies in recent years.

D. Quality Assurance

The final consideration for speciation studies is that of quality control and quality assurance[13] which can be achieved using certified reference materials (CRMs). However, these are of limited availability for speciation studies, making the selection of an appropriate matrix matched CRM difficult. A second method is to use spiking and recovery experiments to validate the results.

Extraction efficiency can be evaluated by an acid digestion of a sub-sample followed by a total element determination.[4] An appropriate extraction method is then chosen for use on a second sub-sample, followed by species specific analysis, and the concentration of each species summed. If this summed concentration and the total concentration are in close agreement the extraction efficiency can be considered to be 100%. An extraction efficiency which is less than 100% but reproducible with an RSD of $\cong$5% may be considered to be under control and valid. If the RSD is much higher (e.g., extraction of $80 \pm 20\%$) then the method is not under control and will not produce accurate results.[4]

IV. ISOTOPE DILUTION ANALYSIS FOR SPECIATION STUDIES

A. Basic Theory

Isotope dilution analysis (IDA) has recently been applied to inorganic mass spectrometry after many years of use in organic analysis. It is regarded as a definitive technique because the precision and accuracy obtainable

Table 1. Analytical techniques for speciation studies (adapted from Hill[4] and Welz[40]).

Technique	Comments	Example applications
Gas chromatography	GC has been used with various detectors which tend to be non-specific so peaks from interfering matrix constituents may give rise to confusion in peak identification.	Separations of metalloporphyrins. Determination of organometallic compounds of Sn, As, Pb and Hg.
High performance liquid chromatography	The most popular detectors (UV–Vis) are rarely sensitive enough for speciation studies.	Aluminium in water samples. Cr and V with spectrophotometric detectors. Determination of organomercury compounds in wastewater and sediments.
Polarography/anodic/cathodic stripping voltammetry	Used to differentiate between oxidation states, for kinetic experiments to determine stability constants of complexed metals. Speciation in the presence of humic/fulvic acids. Ship board monitoring applications.	Speciation of a wide variety of minor constituents of natural waters. Determination of fluorides in the presence of aluminium. Stability constants for complexes of Al, Cu, Fe, Mg, Mn, Pb, Zn in seawater.

Nuclear magnetic resonance	NMR can be used for a wide range of elements although the sensitivity is relatively poor (ca. 1 mg analyte required).	Aluminium complexes with nucleosides. Food and beverage characterisation. Site specific isotope fractionation Compound purity.
Gas chromatography-atomic spectrometry	Heated transfer line usually required. Analytes need to be volatile or derivatised. Analytes may condense on cool spots causing sensitivity loss.	Determination of organometallic compounds in waters, biota, flue gases and petroleum. Determination of metalloporphyrins in crude oils.
High performance liquid chromatography-atomic spectrometry	Ease of coupling to spectrometry. High organic content of some solvents may restrict the use of plasma based ionisation.	Determination of organotin compounds in water, molluscs and sediments. Speciation of Sb, Se, and Hg.
Flow injection-atomic spectrometry	Simple coupling, FI has been used with numerous detection systems. Similar problems to HPLC-AS	Determination of arsenic species. Determination of inorganic Se and Cr species in waters.
Capillary zone electrophoresis-atomic spectrometry	Hard to couple as the flow rates are generally incompatible	Speciation of organolead compounds in natural waters
Hydride generation-atomic spectrometry	Simple coupling. Suitable only for species forming volatile derivatives during reduction.	High sensitivity determination of As, Se, Sn, Bi, Te, Sb, Pb, and Hg species.

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are unsurpassed by alternative analytical methods.[14] Isotope dilution analysis has the advantage that it can overcome problems associated with instrumental drift and matrix effects during mass spectrometric detection. Furthermore, isotope dilution analysis relies on the measurement of isotope ratios and not external calibration, so a complete uncertainty budget, which is traceable directly to S.I. units and therefore meets the highest metrological standards, can be calculated.

The IDA procedure involves the alteration of the natural isotopic abundance of an analyte in a sample by spiking with a standard of modified isotopic composition. A prerequisite for IDA is that the target analyte should have more than one stable isotope, so, because this is the case for the majority of the elements, most of them can be investigated using this method. Two stable isotopes of the target analyte are chosen, which should ideally have a large difference in natural abundance. For best practice, the isotopically enriched analogue (the spike) should have the isotope of lowest natural abundance enriched to as high an abundance as possible (the spike isotope), with the lower abundance isotope heavily depleted (the reference isotope). The isotope amount ratio in the sample is measured, after spiking and equilibration, and entered into the isotope dilution equation (Eq. 1), along with other parameters. The concentration of the analyte in the sample can then be calculated.

$$C_x = \frac{C_s W_s M_x}{W_x M_s} \times \frac{A_s - RB_s}{RB_x - A_x}$$

Equation 1. The isotope dilution analysis equation.

where,

C_x = concentration of the analyte in the sample
C_s = concentration of the analyte in the spike solution
W_s = mass of spike
W_x = mass of sample
M_x = molar mass of element in the sample
M_s = molar mass of element in the spike
A_s = abundance of reference isotope in the spike
B_s = abundance of spike isotope in the spike
A_x = abundance of reference isotope in the sample
B_x = abundance of spike isotope in the sample
R = reference and spike isotope amount ratio in the sample after spiking.

In order to achieve the best accuracy and precision and number of factors must be taken into account. Sargent et al.[15] in a recently published guide, summarise the critical stages as follows:

- Sample preparation.
- Selection of the most appropriate isotopic internal standard.
- Characterisation of the isotopically enriched analogue.
- Addition of the isotopically enriched analogue.
- Blank correction.
- Instrumental analysis.
- Calculation of the result.
- Estimation of uncertainty.

with the important sources of error given as:

- Less than complete equilibration between the sample and spike will lead to significant systematic errors.
- Isobaric and polyatomic ion interferences.
- Isotopic discrimination e.g., isotopic fractionation, detector dead-time and mass bias during instrumental analysis.

B. Spiking Procedure

1. Isotopically Enriched Standards

Isotope dilution analysis (IDA) necessitates the purchase of isotopically enriched elements for production of the spike solution. For species-specific IDA it is also necessary to incorporate the isotopically enriched element into the target species, which will involve synthesis and purification of the compound. Isotopically enriched elements are much more expensive than the natural isotopic abundance equivalent, ranging from dollars per mg to hundreds of dollars per mg, often with a minimum order value, which can further increase the cost of analysis.

This increased expense means that synthesis and purification procedures must be as efficient as possible. It is often not possible for large-scale synthesis and purification procedures to be scaled down to the sub gram level without large losses of expensive, isotopically enriched starting material. This requires novel synthetic procedures to be developed to accomplish this analytical requirement. Sutton et al.[16] devised new synthetic routes for the small scale production of a suite

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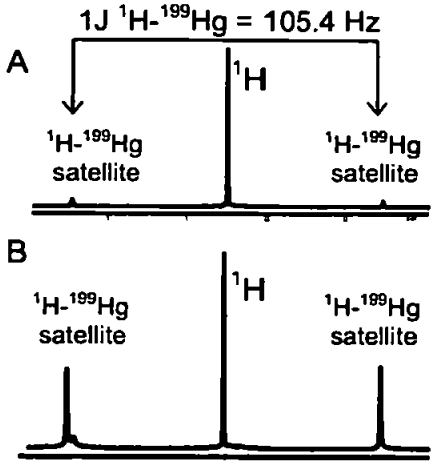

Figure 1. ^{1}H NMR spectra of A; 1 mg/ml natural isotopic abundance CH_3HgCl and B; 1 mg/mL ^{199}Hg enriched CH_3HgCl. The ^{199}Hg enrichment can be clearly seen by the increase in the peak areas of the two peaks, which arise from spin–spin coupling between the ^{1}H And ^{199}Hg nuclei. Both compounds were dissolved in deuterated methanol, CD_3OD, for spectral acquisition.

of environmentally relevant organotin compounds (e.g., dibutyltin dichloride) with product yields of up to 90%.

In our current research,^{199}Hg enriched methylmercurychloride has been produced on a milligram scale using the direct nonenzymatic methylation of $HgCl_2$ by reaction with the vitamin B_{12} co-enzyme methylcobalamine according to the procedure of Rouleau and Block[17] which gives yields of up to 90%. The product has been characterised by ^{1}H, ^{13}C and ^{199}Hg NMR spectroscopy which, as a non-destructive technique, allows for complete recovery of the isotopically labelled compound. Figure 1 shows the ^{1}H NMR spectrum of natural isotopic abundance methylmercury and its ^{199}Hg enriched analogue. The ^{199}Hg enrichment can be clearly observed by the increase in the peak areas of the two peaks which arise from the spin–spin coupling between the ^{1}H and ^{199}Hg nuclei.

The concentration of the spike solution can be determined by one of two methods. If the purity of the isotopically enriched analogue has been characterised with sufficient accuracy and minimal uncertainty, the concentration can be calculated simply from knowledge of the

masses of the compound and solvent employed. If the purity of the spike material is not certain, then reverse isotope dilution analysis is employed. In this case, the enriched spike material is treated as the sample and the isotopic abundance modified by the addition of a known quantity of a natural isotopic abundance standard which acts as the spike material. In order to simplify the calculations, concentrations are initially calculated with respect to the metallic component only and not the concentration of the organometallic species itself.

2. Reference: Spike Isotope Amount Ratio

Error propagation plots can be used to calculate the optimum reference:spike isotope amount ratio for the minimisation of errors during the measurement of the isotope amount ratio. This ratio can be calculated for a particular isotope pair using Eq. 2, examples of which are shown in fig. 2. In practice, the isotope amount ratio should lie between 1:4 and 4:1 to give reasonable counts for each isotope.

$$\sqrt{\frac{B_s}{A_s} \times \frac{B_x}{A_x}}$$

Equation 2. Calculation of the optimum isotope amount ratio for the minimisation of error propagation where A_s is the abundance of reference isotope in the spike, B_s is the abundance of spike isotope in the spike, A_x is the abundance of reference isotope in the sample and B_x is the abundance of spike isotope in the sample.

In order to further reduce systematic errors (e.g., ion counting errors due to detector dead time), it is better to fix the isotope amount ratio as close to unity as possible. In order to do this it is necessary to know the approximate concentration of the analyte in the sample prior to spiking. An exact 1:1 isotope amount ratio can be achieved by using an iterative matching procedure,[15,18] however, this can add considerable time to the method and is not always necessary. An approximate matching procedure has been developed and evaluated[15] which confers many of the benefits of the exact matching procedure but is less time consuming. To avoid matrix effects and dilution of the sample, the spiking procedure

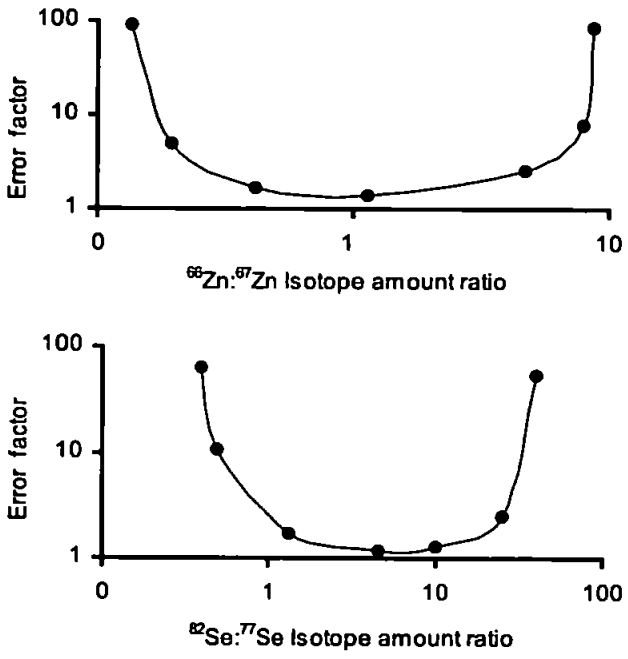

Figure 2. Error propagation plots for the ^{66}Zn:^{67}Zn and ^{82}Se:^{77}Se isotope amount ratios. These plots show the theoretical optimum spiking ratio needed to achieve the best precision for the ratio measurements.

follows the convention that the spike should be added in a small volume of a relatively high concentration.

C. Extraction and Equilibration

Incomplete analyte extraction can lead to low recovery when an external calibration standard is used to determine the analyte concentration. Isotope dilution analysis relies solely on the measurement of isotope amount ratios, so problems associated with incomplete extraction are negated, provided that the spiked sample is extracted at the same efficiency as the analyte present in the sample.[14] If the spike is not allowed to equilibrate fully with the sample, a different extraction efficiency for the spike will result, yielding errors in the measurement.

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For liquid samples, equilibration by gentle agitation should be sufficient, but for solid samples, equilibration may prove problematic because the analyte can be both adsorbed onto the surface and contained within the lattice structure of the sample matrix. A further advantage of IDA is that, assuming equilibration occurs completely, losses of analyte are compensated for by losses of the spike in the same proportion. This ensures an accurate determination of the target analyte, but should not be taken as an excuse for using poor experimental procedure.

D. Measurement of Isotope Amount Ratios

Once the sample has been spiked, equilibrated, and the target analyte has been extracted the analysis can be performed. When speciation analysis is undertaken, a separation step such as high performance liquid chromatography (HPLC) precedes isotope dilution analysis by ICP-MS. For this, so-called species specific IDA, the resulting chromatogram will show peaks corresponding to the individual species, from which the isotope abundance ratio data for each of the species must be extracted. A chromatogram showing the separation of natural isotopic abundance inorganic Hg^{2+} and methylmercurychloride is shown in Fig. 3. In order to estimate the isotope amount ratio correctly, a number of important considerations must be taken into account, as follows.

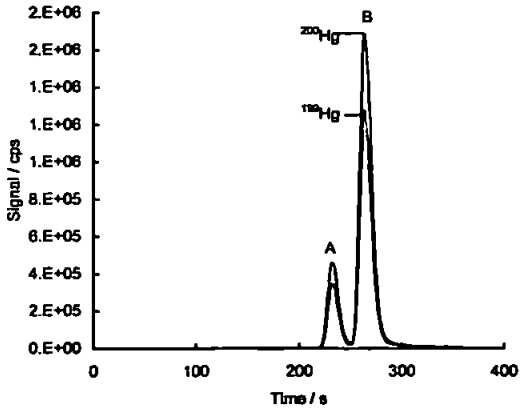

Figure 3. HPLC-ICP-MS Chromatogram of: A, inorganic mercury (210 ng) and; B, methylmercurychloride (900 ng), obtained by monitoring the ^{199}Hg and ^{200}Hg isotopes.

1. Mass Bias Correction

In order to determine the correct isotope amount ratio it is necessary to compensate for mass bias in the instrumentation. Quadrupole and sector mass spectrometers and their associated ion optics do not transmit ions of different mass equally. In other words, if an elemental solution composed of two isotopes with an exactly 1:1 molar ratio is analysed using ICP-MS, a 1:1 isotope amount ratio will not necessarily be observed. This so-called mass bias depends on mass and the type of mass spectrometer used, but generally tends to be greatest at low mass and decreases with increasing mass. Even very small mass-biases can have deleterious effects on the accuracy of isotope amount ratio determinations, so a correction must always be made, usually in one of two ways.

1.1. Bracketing

If an isotopic standard of known composition for the isotope pair under study is available, then a correction can be applied as shown in Eq. 3:

$$C = \frac{R_{cer}}{R_{mes}} \tag{3}$$

where C is the mass bias correction factor, R_{cer} is the certified isotope amount ratio for the isotope pair and R_{mes} is the measured isotope amount ratio for the isotope pair.

In practice, the isotopic standard is analysed before and after the sample (i.e., the sample is bracketed) and the mean correction factor calculated for the bracketing pair is applied to the sample.

1.2. Interpolation

It is also possible to use an alternative element, with an isotope pair of similar mass to the isotope under study, and which has a certified isotope amount ratio. The mass bias correction can be performed by interpolating (with increasing accuracy), using either a linear, power, or logarithmic equation. For example, the ^{205}Tl:^{203}Tl ratio can be used to correct the mass bias of ^{200}Hg:^{199}Hg using the relationship shown in Eq. 3.

$$\left(\frac{^{200}Hg}{^{199}Hg}\right)_{cor} = \frac{\left(\frac{^{200}Hg}{^{199}Hg}\right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl}\right)_{mes} \div \left(\frac{^{205}Tl}{^{203}Tl}\right)_{cer}\right)^{\left(\ln\left(\frac{RAM^{200}Hg}{RAM^{199}Hg}\right) \div \ln\left(\frac{RAM^{205}Tl}{RAM^{203}Tl}\right)\right)}}$$

Equation 3. The logarithmic law used for mass bias correction.

where cor is the corrected isotope amount ratio, mes is the measured isotope amount ratio, cer is the certified isotope amount ratio and RAM is the relative isotopic atomic mass.

The advantage of this approach is that the mass bias correction can be performed by spiking the sample with a mass bias correction standard (i.e., Tl) and measuring this isotope amount ratio at the same time as the sample. For speciation analysis by HPLC–ICP-MS, it is most convenient to spike the mobile phase with the standard and monitor it continuously.

2. Data Extraction

There are two ways of extracting data from the multi-isotope chromatograms obtained.

2.1. Peak Integration

Peaks for each isotope of a particular specie in the chromatogram are integrated to obtain the baseline-subtracted peak integrals and the isotope amount ratio for that specie is calculated using these integrals. The advantage of this approach is that the effects of fractionation and spectral skew, Fig. 4, are minimised, however, precision can be degraded because the precision inherent in rapid sampling of the isotopic pair is lost.

2.2. Pseudo Steady-State

The chromatogram can be treated as an undulating, or pseudo-steady-state, signal. Several data-points on the apices of the peaks (Fig. 5) for each isotope of a particular specie in the chromatogram can be chosen and baseline signal subtracted. The isotope amount ratios can then be calculated using each pair of corresponding data-points from the two peaks and subsequently corrected for mass bias effects. The advantage of this approach is that the inherent precision is maintained and it is possible to obtain an estimate of precision from a single peak, however, erroneous isotope amount ratios will result unless spectral skew is minimised. If simultaneous monitoring of the isotopes is possible (e.g., using multicollector SF-ICP-MS) then this approach should yield both the best accuracy and precision.

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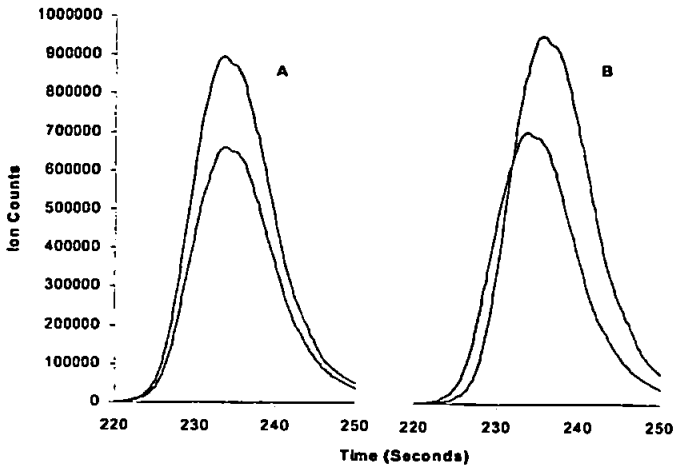

Figure 4. An illustration of spectral skew. In the case of peak A, the isotopic signals are co-incident because the two isotopes were monitored and integrated over a time interval which was rapid enough to keep pace with the rapid rise and fall of the leading and tailing edges of the peak. In the case of peak B, isotopes were monitored over a longer interval, which was too slow, and the isotopic signals are no longer co-incident (NB this is not the same as isotopic fractionation on the column). The effect has been exaggerated for illustrative purposes.

E. Quality Assurance (QA) and Uncertainty

Quevauviller et al.[19–21] describe two parameters that should be considered when reviewing analytical results: (a) accuracy ("absence of systematic errors") and (b) uncertainty (coefficient of variation or confidence interval) produced by random errors and random variations in the procedure. If the levels of uncertainty for a particular analysis were found to be too high, any results would be rendered useless. Quevauviller[19] refers to "statistical control" as a viable means of ensuring that a high quality of laboratory results is maintained. This can be achieved by applying simple statistical analyses, such as *t*-tests and analysis of variance to ensure that methods are sufficiently reproducible. The level of accuracy can be determined by the application of commercially available certified reference materials (CRM). While, it is desirable to use CRM's with matching matrix constituents (to give validity to the found values), in practice it is possible to use several reference materials of different matrix types to give a greater degree of certainty for the result of the unknown samples.

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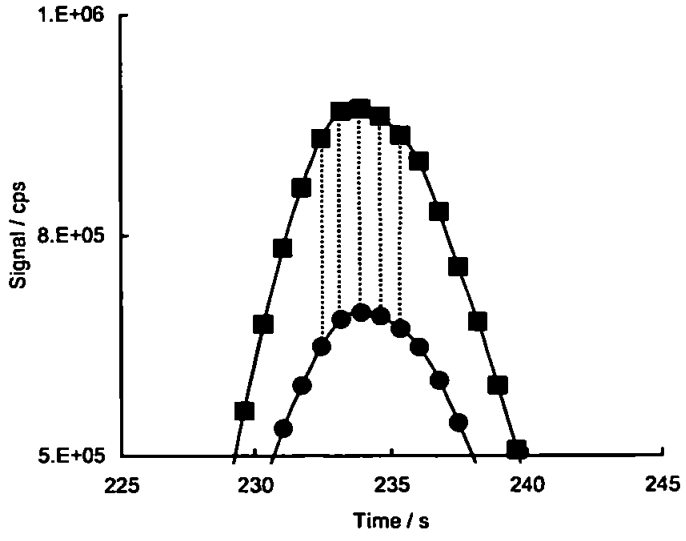

Figure 5. Isotope amount ratios measured using the pseudo steady state approach. The isotope amount ratio is calculated by ratioing the ion signals for coincident pairs of data points over the peak maximum.

The normal terms used in uncertainty measurement have been summarized[22] from the "Guide to the Expression of Uncertainty in Measurement[23]" as follows:

- The accuracy of measurement is the closeness of the agreement between the result of a measurement and a 'true' value of the measurement.
- Standard uncertainty is the uncertainty of the result of a measurement expressed as a standard deviation.
- Coverage factor (k) is a numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty.

1. Evaluating Uncertainty

The estimation of the uncertainty associated with an analytical measurement is increasingly being recognised as an essential part of the measurement process, allowing improved intercomparison of analytical results,[24] and is a requirement for ISO accredited methods.[25]

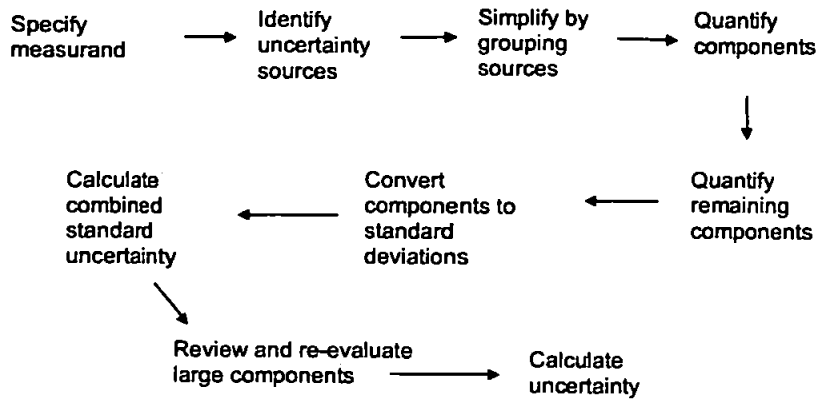

Figure 6. Uncertainty budget procedure flow chart.

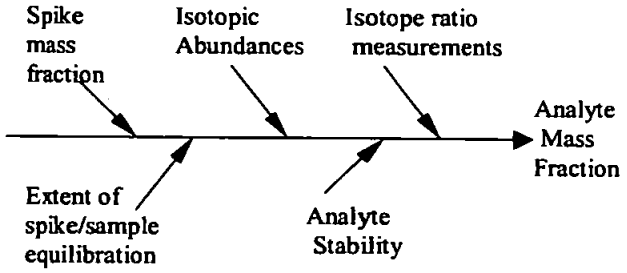

Figure 7. Simplified cause and effect diagram for the identification of sources of uncertainty in ID-MS.

The estimation of measurement uncertainties in ICP-MS analysis using a "cause-and-effect" approach[24,26] is a useful and easily applied method for calculating an uncertainty budget. Figure 6 presents a flow chart of the steps necessary to construct an uncertainty budget.

The cause and effect diagram is constructed to enable easy identification of uncertainty sources associated with the method. A simplified cause and effect diagram is presented in Fig. 7. The purpose is to generate an estimate of overall uncertainty without a detailed quantification of all the components. The diagram typically contains a branched hierarchical structure reducing to a single outcome, in this case an analytical result. Elements within the structure may contain uncertainties from sources such as analyte recovery (including

extractability), measuring devices (e.g., tolerances for: balances, pipettes, volumetrics, dilution errors), repeatability, calibration, temperature and internal standards errors.

The uncertainty of a measurement is very different to the error; where error is the result of a measurement minus the true value of the measurand[27] (which we cannot know), uncertainty has been more informally described[28] as "the interval around the result of a measurement that contains the true value with high probability".

When all uncertainties for a particular method have been combined, it is possible to determine if the level of uncertainty related to the whole procedure is within reasonable measurable levels. A further advantage of the use of a full uncertainty budget is that it allows the analyst to identify the contributions of each area of the measurement procedure to the overall uncertainty, and hence attempt to minimise these uncertainties. For example, if the precision of the measured isotope amount ratios contributes 83% of the overall uncertainty then major gains in reducing the measurement uncertainty can be obtained if the isotope amount ratios are measured with greater precision.

1.1. Uncertainties for Isotope Amount Ratio Analysis

Information on possible sources of uncertainty for isotope amount ratio analysis can be found from observations made during other studies. Sanz-Medel and co-workers[29,30] investigated sources of uncertainty for lead isotope amount ratio measurements by quadrupole, double focusing, and multicollector ICP-MS instruments. Thallium was used as an internal isotope amount ratio standard and the isotope amount ratio accuracy was evaluated using NIST 981 Common Lead Isotope standard reference material and an enriched ^{204}Pb spike. Mass bias correction was performed using an exponential model for all three instruments and equations for the evaluation of the total combined uncertainty arising from the correction for dead time and mass bias were developed. They observed[29] that, in correcting for mass bias, the uncertainty in the Tl ratio contributed the main source of uncertainty for the multicollector instrument, but for the quadrupole and double focusing single collector instruments, the measured isotope amount ratio was the main source of uncertainty.

High accuracy measurements from adapted ID-ICP-MS methodologies that utilised an iterative matching procedure have been successfully applied.[15,18] Based on a conventional analysis, an approximate match of both the isotope amount ratio (1:1) and signal intensities (each within 5% relative), was made between a spiked reference

standard and the spiked sample, providing a basis for calculations to be made by normal IDMS, but retaining the benefits of the full matching procedure. Errors from sources such as mass bias, detector dead time and characterisation of the spike material are cancelled[18] thus reducing the measurement uncertainty.

V. APPLICATIONS OF ISOTOPE DILUTION ANALYSIS

Isotope dilution analysis for speciation studies has been applied to a wide variety of sample matrices (soils, sediments, natural and artificial waters, gases and reference materials) and target analytes (inorganic, organic, organometallic and complexes). The challenge for analysts employing ID for speciation studies is to achieve the accuracy and precision inherent in the method when applied to total elemental determinations. Table 2 presents some examples retrieved from the literature. The detection limits and precision values presented in Table 2 highlight why ID analysis is the definitive and preferred method for trace/ultratrace determinations in conjunction with a variety of separation and sample introduction techniques of which there are many more than presented.

A. Total Elemental Determinations

Catterick et al.[18] determined the concentration of Fe, Mg and Cd in three CRM's (soft water, hard water and VDA plastic) by ID analysis using the approximate matching procedure for the isotope amount ratio and the ion signal strength. The analytical results were in very close agreement with the certified values, with uncertainty estimates (coverage factor, K, of 2) at the 95% confidence level of at least 50% lower than the stated uncertainty for the reference materials (e.g., Cd in VDA plastic: determined value 40.6 ± 0.6, certified value; $40.9 \pm 1.2\,\mu g/g$). Turner et al.[25] compared electrothermal vaporisation (ETV) ICP-MS using external calibration with ETV-ID-ICP-MS for the determination of Se in water and serum CRM's. The uncertainties associated with determinations by the ETV-ID-ICP-MS method were a factor of four less than those associated with external calibration ETV-ICP-MS. Accuracy was significantly greater by ETV-ID-ICP-MS, with the results again in very close agreement with the certified values. Species specific isotope dilution analysis has been undertaken for both inorganic species

(e.g., iodine/iodate[31] by LC-ICP-MS) and organometallic species (e.g., mono and dimethylmercury by GC-ICP-MS[32]). Limits of detection for the two mercury species were 2 and 8 pg (as Hg) respectively.

B. Species Specific Isotope Dilution Analysis

1. Accuracy and Precision

Interferences from matrix effects can be overcome by the use of species specific ID analysis. Ebdon et al.[33] analysed an artificial rainwater sample for trimethyllead chloride (TML, 42.75 ± 4.4 ng/mL determined by an interlaboratory comparison) by two methods; external calibration using TML calibrants and species specific ID analysis with ^{206}Pb enriched TML as the spike, separation and detection was by HPLC-ICP-MS. The external calibration HPLC-ICP-MS gave a result of 173 ± 10 ng/mL of TML as lead. No errors in the calibration procedure could be found and no spectral interferences for lead were known. The sample also contained Ca at ≈ 1500 ng/mL and Mg at ≈ 525 ng/mL, with neither of the two metals interfering with the determination of lead. The authors surmised that an "enhancing"[33] effect, observed for every analysis of the artificial rainwater by external calibration, was being caused by the sample matrix. Analysis by species specific ID-HPLC-ICP-MS gave a TML concentration of 41 ± 2 ng/mL, in good agreement with the mean interlaboratory value. Because ID relies on the measurement of isotope amount ratios, rather than species concentrations, the matrix effects observed during external calibration measurements were negated.

Isotope dilution analysis has also been used to measure species that have been previously undetectable in environmental samples. Dimethylthallium (Me_2Tl^+) had previously only been measured in laboratory experiments with detection limits at the level of ng/mL, a factor of 1000 higher than levels expected in the environment.[34] Schedlbauer and Heumann synthesised Me_2Tl^+ from ^{203}Tl enriched elementary thallium using a two step synthesis which gave a yield of 25%. The stability of the Me_2Tl^+ was evaluated and under optimum storage conditions (pH 2, 0°C) the compound was found to be stable for 30 months.

Open ocean seawater samples, collected from depths of between 10 and 4000 m from the Southern Atlantic, were spiked with the ^{203}Tl enriched dimethylthallium. The ^{203}Tl:^{205}Tl isotope amount ratio was measured by positive thermal ionisation mass spectrometry, after

Table 2. Applications of isotope dilution analysis for speciation studies.

Analyte	Comments	Detection limits/precision	Reference
Trimethyl and triethyl lead	Analysis of rainwater by reversed phase ion pairing chromatography.	Trimethyl lead:3 ng/mL Triethyl lead: 14 ng/mL	[33]
Cadmium	Environmental and biological reference materials analysed. Results were in close agreement with the certified values except for very low [Cd].	Isotope amount ratio precision $<0.1\%$. Sample precision 0.6–1.6%.	[41]
Organotin species	Reversed phase column with ion pairing agent. CRM's.	Tetra methyl lead 0.48 ng/mL	[42]
Selenium	Water and serum samples. Electro thermal vaporisation ICP-MS.	Isotope amount ratios 0.2–2.3% RSD. Sample precision 0.06–0.8.	[25]
Organomercury	GC-ICP-MS Furnace atomisation plasma ionisation MS (FAPIMS). Natural gas condensate.	2–8 pg Hg. Precision 2%	[32]

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Chromium	Anion exchange column ICP-MS. Artificial waters.	Cr(III) 0.21 ng/g. Cr(VI) 0.37 ng/g. Recovery 99.5–99.1%.	[43]
Iodine species	HPLC-ICP-MS, water samples.		[31]
Mercury	Hair and reference samples Total determination ICP-MS.	Accuracy > 99.5% Precision < 1%	[44]
Monomethyl mercury	GC-ICP-MS in CRM's. Species transformation detection.	Isotope amount ratio precision 0.4%. Species transformation LOD 2.5 pg Hg^{2+} per 1 ng/g CH_3Hg+	[45]

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preconcentration by an anion exchange resin, separation of inorganic Tl species by methyl isobutyl ketone, and sample clean-up stages to improve ionisation efficiency. The Me_2Tl^+ detection limit was 0.4 pg/mL for a 500 mL sample and 1.4 pg/mL for total Tl for a 50 mL sample. There was a variation of between 3 and 8% for parallel sample determinations. It was found that between 3–48% of the total thallium concentration was Me_2Tl^+ and, as this compound is not known as an anthropogenic substance, that production of Me_2Tl^+ is the consequence of bioactivity.

2. Species Production and Decomposition During Measurement

Isotope dilution analysis, by means of isotope amount ratio measurements of sample and spike solutions, can also be used to investigate species transformations. Such transformations will bias analytical results, and may occur at any point in the analytical procedure between sample spiking and isotope amount ratio measurement. One example of a specie transformation that may occur after spiking a sample with ^{199}Hg enriched methylmercury is given in Eq.4.

$$Hg^{2+} + CH_3{}^{199}Hg^+ \longleftrightarrow {}^{199}Hg + CH_3Hg^+$$

Equation 4. Species transformation between methylmercury and inorganic mercury.

Hill et al.[14] observed species transformations during the determination of organotin compounds by HPLC-ID-ICP-MS. The chromatogram of the standards, Fig. 8, gives a ratio for ^{120}Sn:^{116}Sn as approximately 2.25 which reflects the natural abundance ratio of these two tin isotopes.[14] Spiking with an appropriate amount of ^{116}Sn enriched TBT, calculated after conventional HPLC-ICP-MS analysis of the sample, should give TBT signals for each isotope which reflects the degree of equilibration between the sample and spike. The other tin species detected should have the natural isotope amount ratio, because only enriched TBT was spiked into the sample. The chromatogram of the ^{116}Sn enriched sample clearly indicates the presence of several unidentified peaks having a greatly enriched ^{116}Sn signal along with the reduction in size of the tributyl and triphenyl tin peaks.

Stable isotope species specific ID can also be used to investigate the analytical process with regard to analyte stability. In this case, the sample is spiked with both an isotopically enriched analogue of the target analyte and an inorganic component of the analyte enriched in a different isotope.

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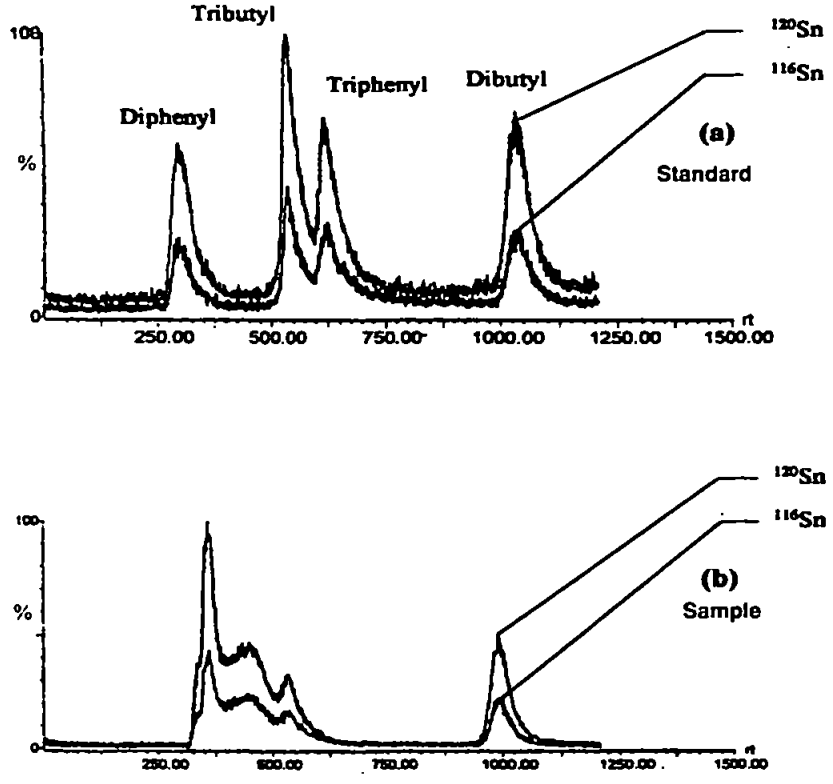

Figure 8. Organotin species transformation as indicated by HPLC-ID-ICP-MS. (From Hill et al.[14])

Artefact formation during extraction, derivatisation and detection of methylmercury by GC-ICP-MS has been observed[35–37] indicating that the methods are not under control and bias in the results will arise.

In order to investigate the processes involved, Lambertsson et al.[38] spiked sediment samples with $CH_3{}^{198}Hg^+$ and/or $^{201}Hg^+$ (^{202}Hg was used as the reference isotope) to measure mercury methylation and demethylation during the measurement process. By monitoring the change in the ^{201}Hg:^{202}Hg isotope amount ratio of the methylmercury in the sample, and by calculating the amount of $CH_3{}^{201}Hg^+$ present, the amount of inorganic mercury methylated in situ could be estimated. Similarly, the ^{198}Hg:^{200}Hg isotope amount ratio of inorganic mercury was determined. The amount of demethylated methylmercury was calculated from the difference in the concentrations between the added

$CH_3{}^{198}Hg^+$ and that determined in the sample after extraction, derivatization and detection. The detection limits for the methylation of the $^{201}Hg^+$ spike and the demethylation of the added $CH_3{}^{198}Hg^+$ were 0.1 and 0.2 ng per gram of sediment respectively.

3. Monitoring Equilibration

The use of isotopically enriched elements and/or compounds enables the extent of adsorption/desorption of the enriched spike and natural isotopic abundance analyte to be monitored. The need for complete equilibration during ID analysis was illustrated by Clough et al.[39] who spiked a sample of NIST 2710 Montana soil CRM, certified for total Hg, with an equivalent amount of ^{199}Hg enriched mercury. The equilibration solvent comprised 80:20 methanol–water v/v. The spike/particulate/solvent mixture was agitated and samples withdrawn over a timespan of 2–3000 min from the initial mixing. After filtration and dilution with fresh solvent the ^{200}Hg:^{199}Hg isotope amount ratio and the total Hg concentration of each withdrawn aliquot were determined by ICP-MS. The extent of equilibration between the spike and sample was calculated for each aliquot and compared with the theoretical equilibrium ^{200}Hg:^{199}Hg isotope amount ratio calculated from the known masses of enriched and natural abundance mercury present in the starting materials (Fig. 9).

The measured ^{200}Hg:^{199}Hg isotope amount ratio did not attain the theoretical value, indicating that the sample and spike equilibration was not complete. The system did however come to a related equilibrium point, since the ^{200}Hg:^{199}Hg isotope amount ratio was unchanged for the final 2750 min of the experiment. The final measured ^{200}Hg:^{199}Hg isotope amount ratio was corrected for mass bias using Eq. 3. The Hg mass fraction of the NIST2710 CRM of 27.6 μg/g, calculated using Eq. 1, underestimated the certified value of 32.6 μg/g. The adsorption of the spike mercury and the desorption of the particulate mercury with time was calculated from the measured ^{200}Hg:^{199}Hg isotope amount ratio and the total mercury concentration in solution, Fig. 9 shows the adsorption and desorption for the first 100 min of the experiment.

The amount of mercury desorbed from the NIST 2710 CRM at 3000 min was estimated as 8.2 μg giving the NIST 2710 CRM mercury mass fraction as 2.7 μg/g. The different mercury mass fractions for the CRM obtained IDA and the amount of Hg released into solution highlight that, in IDA, it is important to ensure complete equilibration of the enriched spike and the sample rather than necessarily requiring

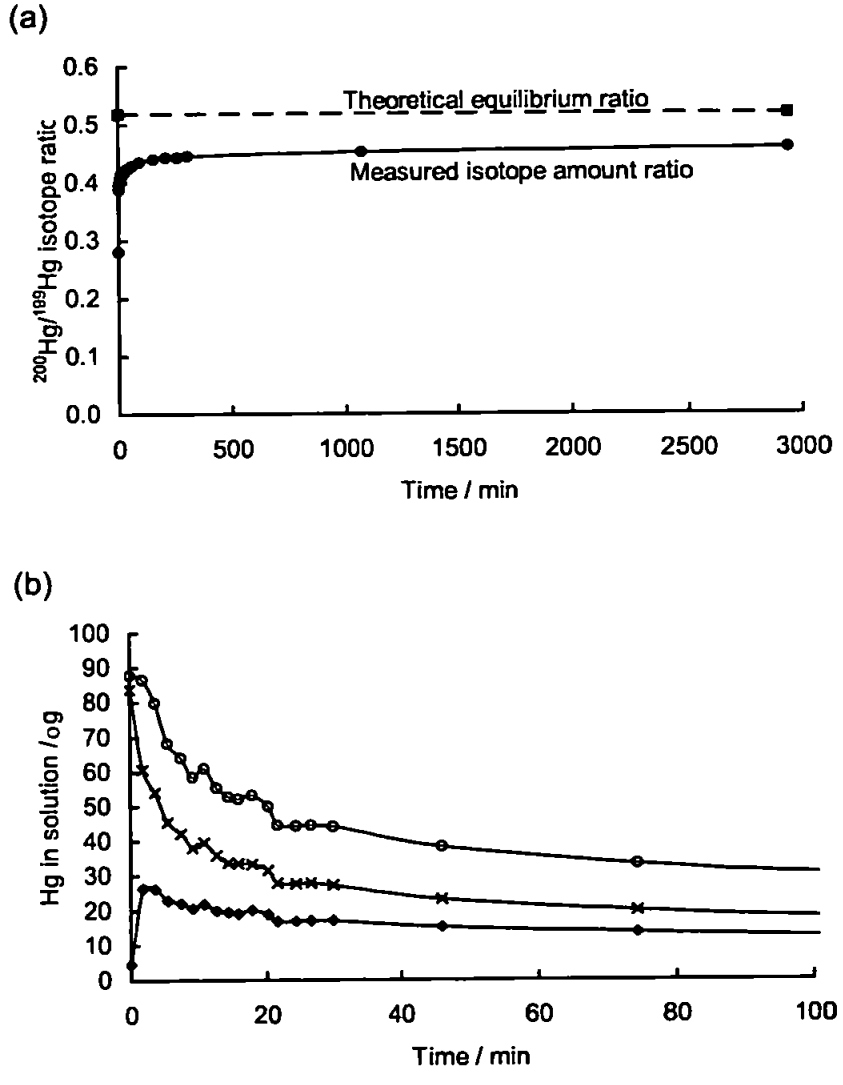

Figure 9. Extraction of inorganic Hg from a sediment certified reference material, which has been spiked with an isotopically enriched analogue. (a) Isotope amount ratio monitored in the liquid phase over the course of the extraction—note that the ratio does not reach the theoretical equilibrium value, indicating that some of the Hg is not easily exchangeable; (b) the extent of adsorption and desorption of Hg, originating from spike and sample, over the course of the extraction procedure.

complete extraction. The primary requirement for IDA with solid samples is that the analyte is sufficiently abundant in solution to be able to perform an accurate and precise isotope amount ratio measurement.

VI. CONCLUSIONS

Isotope dilution analysis can be usefully applied for speciation studies provided that:

- Appropriate isotopically enriched analogue compounds are available, or can be easily synthesised and purified.
- The spiking ratio is chosen so as to ensure the best accuracy and precision.
- The spike and sample reach equilibrium
- appropriate data acquisition and data treatment procedures are followed.

Providing the above are accounted for, the technique of species specific ID-MS is capable of highly accurate and precise analyses and correction for matrix effects. In addition, species transformation during the sample preparation stage can often be detected, and the compounds can also be used for stable isotope studies.

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Uncertainty contributions to species specific isotope dilution analysis. Part 1. Characterisation and stability of ^{199}Hg and ^{13}C isotopically enriched methylmercury by ^{1}H NMR†

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^{199}Hg-enriched methylmercurychloride has been synthesised, purified, and characterised by ^{1}H nuclear magnetic resonance spectroscopy (NMR) for use in species specific isotope dilution analysis (IDA). The stability of methylmercury during the analytical procedure was investigated using ^{199}Hg and ^{13}C isotopically labelled methylmercury analogues and ^{1}H NMR spectroscopy. The halide order of preference for methylmercury was found to be I > Br > Cl. No evidence was found for the decomposition, or formation of methylmercury during equilibration with soil (NIST 2710) or dogfish muscle (DORM-2), or during the chromatographic separations.

1. Introduction

Elemental and molecular speciation by conventional external calibration are now well established analytical techniques. The introduction of legislative requirements, regulating the concentration of specific species, *e.g.*, tributyltin in UK water quality legislation[1] and methylmercury in fish for human consumption,[2] is now one of the main driving forces for performing speciation studies, and therefore analytical results must be reported with a high degree of accuracy and precision. Isotope dilution analysis (IDA) is regarded as a definitive analytical technique because:[3]

(i) it is directly traceable to SI units;

(ii) the precision and accuracy obtainable are unsurpassed by alternative analytical methods;[4]

(iii) it can account for analyte losses or incomplete extraction.

In practice, IDA involves the alteration of the natural isotopic abundance of an analyte in a sample by spiking with a standard of modified isotopic composition and the subsequent measurement of isotope amount ratios in the spiked sample. This has a number of advantages over conventional external calibration techniques, provided that a number of prerequisites are met:

(i) more than one, interference-free, stable isotope must be available for isotope ratio measurement;

(ii) an isotopically enriched analogue of the analyte must be available;

(iii) complete equilibration between the spike and sample must be achieved;

(iv) the mass fraction concentration and isotopic abundances of the natural and enriched elements must be well characterised;

(v) isotopomers of the spike and sample must be chemically stable.

In comparison to total element analysis, species-specific IDA is more challenging, and a number of factors must be carefully addressed in order to obtain accurate and precise results:[5]

(i) isotopically enriched analogues, or isotopomers, of the target analyte(s) must be synthesised or purchased;

(ii) relatively mild extraction conditions are necessary in order to maintain the integrity of the species, which may result in incomplete analyte extraction. This is not a problem, however, provided that complete equilibration of the spike and sample occurs;

(iii) it is essential that, during analyte extraction, equilibration, separation, and measurement of the isotope amount ratio, the sample and spike isotopomers are chemically inert. Any analyte formation or decomposition, ligand exchange between the sample and spike isotopomers and/or spike decomposition will give biased results.

An example of the last point above is the formation of methylmercury from inorganic Hg during sample preparation and separation for determinations by gas chromatography inductively coupled plasma mass spectrometry (GC-ICP-MS).[6–9] For gas chromatographic separation the analyte must be presented in a volatile form. Of the three oxidation states of mercury, Hg^0, Hg^I and Hg^{II}, only Hg^0 is volatile, therefore other mercury species must be derivatised, usually by ethylation with $NaBEt_4$, before separation by GC, thus increasing the risk of undesirable reactions involving the sample and/or spike isotopomers. In comparison, high-performance liquid chromatography (HPLC) does not require derivatisation of the analyte, and can be used to separate mercury species prior to detection by a wide range of spectroscopic detectors.[10]

The aim of this work was to investigate uncertainty contributions to species-specific isotope dilution analysis. This paper addresses the synthesis of isotopically enriched methylmercury, and its characterisation using ^{1}H nuclear magnetic resonance (NMR) spectroscopy. To that end, ^{199}Hg enriched methylmercury chloride and ^{13}C enriched methylmercury iodide have been synthesised and employed to investigate the stability of methylmercury during equilibration and HPLC separation. Subsequent papers deal with uncertainty contribution during IDA[11] and the equilibration of spike and sample.[12]

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2. Experimental

2.1. Synthesis of ^{199}Hg enriched methylmercury chloride

The synthesis of methylmercury chloride by direct non-enzymatic methylation, using methylcobalamin (Me[Co]) as the methyl group donor, is based on the method of Rouleau and Block.[13] Methylcobalamine is a vitamin B_{12} co-enzyme and can be considered as a cobalt(III) corrinoid complex containing a co-ordinated carbanion (CH_3^-), which can be displaced under suitable reaction conditions by the electrophilic attack of Hg^{2+} ions, forming aquocobalamine and the monomethylmercury anion (CH_3Hg^+).[14] The overall reaction scheme for the synthesis of the methylmercury chloride isotopomer is shown in eqn. 1 where cobalamine is represented by Co. The synthetic procedure described is straightforward and can be completed within a period of six hours.

$$[Co]CH_3 + Hg^{2+} + Cl^- + H_2O \leftrightarrow H_2O[Co]^+ + CH_3HgCl \quad (1)$$

^{199}Hg enriched (65.74%) HgO was obtained from AEA Technologies (Harwell, Oxfordshire, UK). Methyl cobalamine was purchased from Sigma–Aldrich (Gillingham, Dorset, UK), benzene from May and Baker (Dagenham, Essex, UK), hydrochloric acid (Aristar grade) and hexane from Merck Ltd. (Poole, Dorset, UK) and N_2 from (Air Products, Walton-on-Thames, Surrey, UK). All distilled, deionised water (DDW) used in the synthesis was obtained from an 18 MΩ cm^{-1} Elgastat Maxima system (Elga Ltd., High Wycombe, Buckinghamshire, UK). All glassware used was first soaked for 24 h in 10% Decon 90 (Decon Laboratories, Hove, Sussex, UK), to remove organic carbon, and subsequently in 10% HNO_3 for 24 h followed by washing in DDW.

Approximately 20 mg of the ^{199}Hg enriched HgO was accurately weighed into a clean sterilin container, dissolved in 400 μl of concentrated HCl, diluted with 10 ml of DDW and transferred to a 50 ml stoppered glass conical flask as a reaction vessel. The sterilin container was subsequently rinsed with two further 3 ml DDW aliquots, which were then added to the reaction vessel. Approximately 100 mg of methylcobalamine was accurately weighed into a separate clean sterilin container and dissolved in 10 ml of DDW. Following transfer of this solution to the reaction vessel, this container was rinsed with two further 3 ml aliquots of DDW, which were added to the reaction vessel. The reaction vessel was protected from light sources since methylcobalamine is UV sensitive, and agitated by means of a magnetic stirrer for 1 h.

The methylmercury chloride was extracted using 50:50 v/v benzene–hexane (10 ml). Following agitation (10 min) the benzene–hexane layer containing the methylmercury chloride was then carefully removed by pipette to a second 50 ml glass conical flask. This extraction procedure was repeated twice more, resulting in an extractant volume of 30 ml. Aliquots of the benzene:hexane extractant were then transferred to a clean, preweighed 7 ml glass vial and the solvent evaporated under a stream of N_2, which was carefully regulated to minimise losses of methylmercury chloride by volatilisation. After evaporation solid methylmercury chloride was obtained with a yield of 60% (as Hg). The purity and isotopic composition of the ^{199}Hg enriched methylmercury chloride was assessed by both nuclear magnetic resonance (NMR) spectroscopy and HPLC-ICP-MS.

2.2. Synthesis of ^{13}C enriched methylmercury chloride

^{13}C labelled methylmercury iodide was synthesised by a Grignard reaction using freshly prepared $^{13}CH_3MgI$. All reagents were supplied by Aldrich (Gillingham, Dorset, UK). The Grignard reagent was prepared by agitating, *via* a magnetic stirrer for 2 h under a nitrogen atmosphere, 1 g of dry magnesium turnings in a 250 ml three necked round bottom flask attached to a reflux condenser, to create a fresh reactive surface on the metal. Subsequently 5 g of ^{13}C labelled methyl iodide, dissolved in dry double distilled diethyl ether (50 ml), was added to the Mg in the round bottomed flask. The solution was gently warmed and stirred until a characteristic steely grey was observed, indicating the formation of the Grignard reagent. The solution was stirred at room temperature for 1 h and then heated at reflux for a further 45 min. The reaction products were allowed to cool and settle prior to the next reaction step.

4.75 g of mercury(II) chloride was dissolved in 75 ml of diethyl ether in a three necked round bottomed flask attched to a reflux condenser. The solution was warmed to dissolve the $HgCl_2$ and stirred under a nitrogen atmosphere. The freshly prepared $^{13}CH_3MgI$ was added dropwise under nitrogen pressure to the $HgCl_2$ solution *via* a double ended steel needle connecting the two round bottomed flasks, thus ensuring that no unreacted Mg was added to the $HgCl_2$ solution. A further 25 ml of diethyl ether was added to the Grignard preparation flask as a wash solution and subsequently transferred to the $HgCl_2$ solution *via* the steel needle. The resulting solution was refluxed for 12 h to complete the reaction. Upon cooling a crop of pale yellow crystals was precipitated in the bottom of the flask which were removed from the reaction mixture by filtration. Several large red crystals of mercury(II) iodide were also produced during the reaction and these were removed from the pale yellow crystals and discarded. The pale yellow crystals were recrystallised from boiling alcohol to yield 3.94 g of ^{13}C labelled methylmercury iodide, with a melting point of 143 °C (lit. 143 °C).

2.3. Nuclear magnetic resonance studies

All ^{1}H spectra were acquired at 270 MHz using a Jeol EX270 MHz Fourier Transform NMR spectrometer. The signal from the residual proto methyl (CH_3) group, δ 3.3 ppm, in CD_3OD was used to reference the ^{1}H spectra, except for experiments using a solvent suppression technique, when the spectra were referenced to the residual hydroxyl signal (CH_3OH and HDO) at δ 4.8 ppm. Baseline ^{1}H NMR spectra of methylmercury chloride, methylmercury bromide and methylmercury iodide (all purchased from Sigma–Aldrich), ^{199}Hg enriched methylmercury chloride and ^{13}C enriched methylmercury iodide were all obtained in CD_3OD. For experiments to determine the halide preference of monomethylmercury, potassium halides (Cl, Br and I) were added to the standard solutions at twice the concentration of the mercury species present in the sample.

The standard mobile phase employed for the separation of mercury species by HPLC,[15] and as the equilibration solvent,[11,12] comprised 50:50 methanol–water v/v and 0.01% 2-mercaptoethanol. However, this was not suitable for NMR studies due to the excessive proton signal from the methanol–water components. For experiments to determine the stability of mercury species during chromatographic separation a partially deuterated mobile phase comprising 50:50 D_2O–CH_3OD v/v and 0.01% 2-mercaptoethanol was employed. A mixture of $^{13}CH_3HgI$ and ^{199}Hg enriched CH_3HgCl (100 and 200 μg g^{-1}, respectively, as Hg) was injected (100 μl) onto the HPLC column and the eluent collected in fractions of 1.5 min duration after UV detection at 204 nm. After preconcentration by gaseous N_2 solvent evaporation, the fractions containing the monomethylmercury species (1.5–3 min elution time), were analysed by ^{1}H NMR spectroscopy. The partial deuteration of the HPLC mobile phase removed most of the signal arising from the H_2O in the ^{1}H NMR spectrum but the baseline was distorted by the very strong signal from the methyl group of the CH_3OD. To overcome this a solvent suppression technique was employed. The methyl group of the CH_3OD (δ 3.3 ppm) was pre-saturated for each transient acquisition, thus greatly reducing this signal in the spectrum.

Table 1 ^{1}H NMR spectroscopy chemical shifts and coupling constants for the organomercury compounds under study: all samples were dissolved in deuterated methanol, CD_3OD

	Methylmercury concentration/mg ml^{-1}	*^{1}H chemical* shift (δ) (ppm)	^1J ^{1}H–^{13}C/Hz[a]	^2J ^{1}H–^{199}Hg/Hz[a]	Inference
CH_3HgCl	3.2	0.91	138	211	
CH_3HgBr	4.9	0.97	138	207	
CH_3HgI	3.1	1.03	138	195	
^{199}Hg enriched CH_3HgCl	3	0.91	138	211	
$^{13}CH_3HgI$	2.4	1.03	138	195	
CH_3HgCl + KBr	3	0.97	138	207	CH_3HgBr
CH_3HgCl + KI	3	1.03	138	195	CH_3HgI
CH_3HgBr + KCl	3	0.97	138	207	CH_3HgBr
CH_3HgBr + KI	4	1.03	138	195	CH_3HgI
CH_3HgI + KCl	3	1.03	138	195	CH_3HgI
CH_3HgI + KBr	3	1.03	138	195	CH_3HgI

[a]The superscript "J denotes the number of bonds along which the coupling occurs. Potassium halides were added at twice the methylmercury species concentration.

The stability of methylmercury was investigated in the presence of two different particle types. National Institute of Science and Technology (NIST) 2710 Montana Soil Certified Reference Material (CRM) was used to represent geological material and DORM-2 Dogfish Muscle (National Research Council Canada-NCR) was employed as a biological sample. Approximately 0.5 g of the CRM was accurately weighed into a clean 7 ml glass vial to which was added 1 ml of ^{199}Hg enriched HgO (3.96 mg ml^{-1} dissolved in 1 M KI–D_2O) and 1 ml of $^{13}CH_3HgI$ (6.9 mg ml^{-1}, dissolved in CD_3OD, 0.02% 2-mercaptoethanol). The resulting mixture was stirred magnetically at a constant temperature, 25 °C for 24 h, followed by filtration (Autovial 0.2 μm PTFE membrane syringeless filters, Whatman, Maidstone, UK) and subsequent analysis by ^{1}H NMR spectroscopy.

3. Results and discussion

3.1. Characterisation of methylmercury isotopomers

A comparison of the chemical shifts and the coupling constants, ^nJ, of methylmercury halides (natural isotopic abundance, Table 1) confirmed the identity of the ^{199}Hg enriched isotopomer as methylmercury chloride. ^{13}C labelled (>99%) methylmercury iodide was positively characterised by ^{1}H NMR spectroscopy using the same approach. The notation used for all ^{1}H NMR spectra is shown in Table 2. The individual organomercury isotopomers (Table 2) have been numerically labelled as follows; natural abundance methylmercury chloride (*i*), ^{199}Hg enriched methylmercury chloride (*ii*), ^{199}Hg enriched methylmercury iodide (*iii*), natural abundance methylmercury iodide (*iv*) and ^{13}C labelled methylmercury iodide (*v*). The ^{199}Hg enrichment in isotopomer *ii* compared with isotopomer *i* can be observed *via* the enhanced intensity of the ^{1}H–^{199}Hg satellite signals, which arise from spin–spin coupling between the ^{1}H and ^{199}Hg nuclei, (Fig. 1 A and B). Similarly, a comparison of the ^{1}H NMR spectrum of isotopomer *iv* with that observed for isotopomer *v*, (Fig. 1 C and D), demonstrates the extremely high (>99%) ^{13}C content of isotopomer *v*. Due to the almost complete ^{1}H–^{13}C coupling, four ^2J ^{1}H–^{199}Hg satellites now appear in the spectrum, two for each of the ^{1}H–^{13}C doublets.

Table 2 Notation employed for the methylmercury isotopomer and ^{1}H NMR spectra

Compound	Isotopomer number	^{1}H NMR signal		
		Proton	^2J ^{1}H–^{199}Hg	^1J ^{1}H–^{13}C
CH_3HgCl	*i*	α	β	
^{199}Hg enriched CH_3HgCl	*ii*	α	β	
^{199}Hg enriched CH_3HgI	*iii*	α	β	
CH_3HgI	*iv*	ε	γ	ϕ
$^{13}CH_3HgI$	*v*	ε	γ	ϕ

3.2. Methylmercury halides—halide exchange

The observed ^{1}H NMR spectrum of a solution containing both the ^{199}Hg and ^{13}C labelled methylmercury isotopomers exhibited a single methyl proton signal intermediate between that of the individual compounds. From this it was deduced that the halide component, Cl^- and I^-, respectively, was readily exchangeable with other halides. The relative stability of the halide counter ion was determined by combining each compound with inorganic halides (*e.g.*, KBr, KI), followed by comparison of the resulting ^{1}H NMR spectroscopy data with that obtained from authentic compounds. Using this method, and with each halide in excess, the order of preference for methylmercury was I > Br > Cl. Table 1 summarises the ^{1}H NMR spectroscopy data for various methylmercury halides in the presence of inorganic halides. The speciation of methylmercury, in the liquid phase during equilibration, chromatographic separation and subsequent detection by ICP-MS is therefore dependent on the reagents employed.

3.3. Methylmercury halides—methyl group exchange

Ligand exchange between CH_3HgI molecules, eqn. 2, was studied by mixing together ^{13}C labelled and ^{199}Hg labelled CH_3HgCl compounds in the deuterated HPLC mobile phase. The ^{199}Hg labelled CH_3HgCl was iodinated, so that both species were in the same chemical form, by the addition of KI to the samples prior to analysis by ^{1}H NMR. This prevented the intermediate spectra that arise from each species having a different halide component.

$$^{13}CH_3HgI + CH_3{}^{199}HgI \leftrightarrow {}^{13}CH_3{}^{199}HgI + CH_3HgI \quad (2)$$

The distinct ^{1}H NMR spectral patterns of the two isotopically labelled methylmercury iodide isotopomers, *iii* and *v*, allowed the relative isotopic abundance of ^{199}Hg for each compound to be calculated when both compounds were present in the same solution. For example (Fig. 2), the ^{199}Hg abundance in isotopomer *v* was calculated by ratioing the peak areas of the two ^{1}H–^{199}Hg satellites (Fig. 2, 2β) with the total peak area (2β + α). This gave a ^{199}Hg isotopic abundance for isotopomer *iii* of 66%, as expected. Similarly, the ^{199}Hg abundance in $^{13}CH_3HgI$ could be calculated from the same ^{1}H NMR spectrum by ratioing the peak areas for the four ^{1}H–^{199}Hg satellites (2ϕ1 + 2ϕ2), with the total peak area (2ϕ1 + 2ϕ2 + γ1 + γ2). As such the ^{199}Hg abundance for isotopomer *v* was calculated as 17%. Since the calculated ^{199}Hg

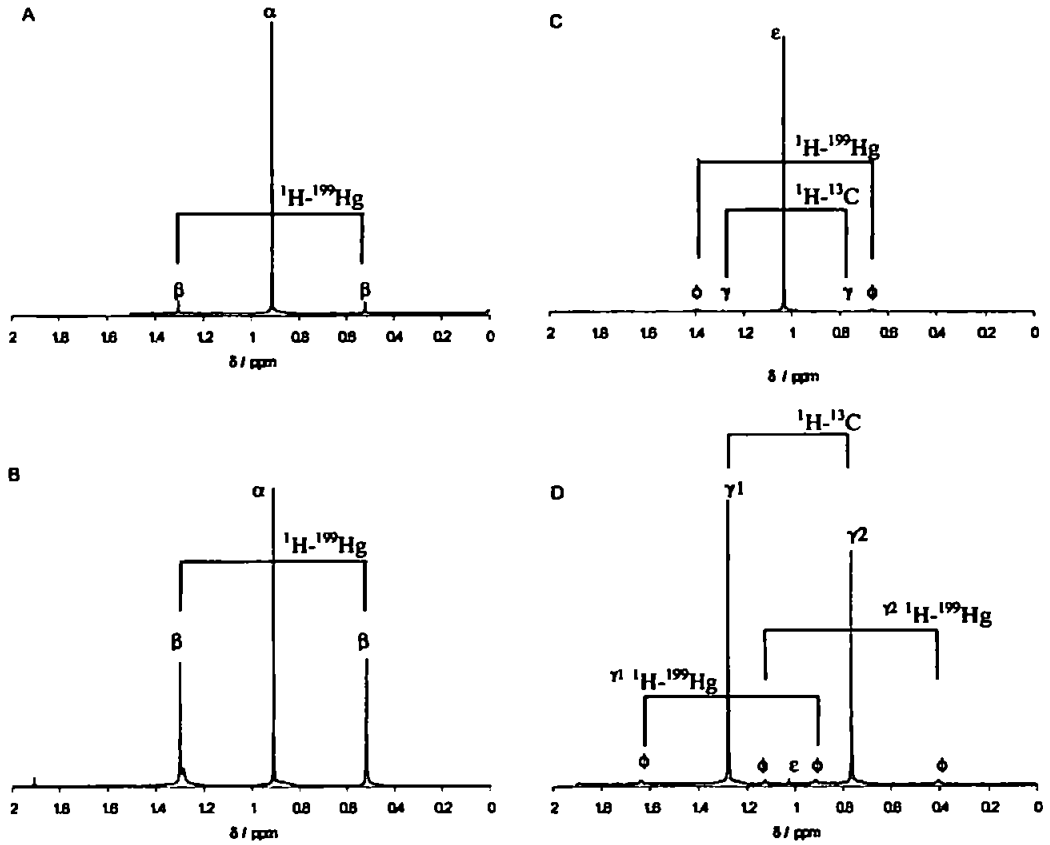

Fig. 1 ^{1}H NMR spectra of: A, natural isotopic abundance methylmercury chloride; B, ^{199}Hg enriched (66%) methylmercury chloride (for these isotopomers the ^{1}H–^{199}Hg satellites are denoted β); C, natural isotopic abundance methylmercury iodide; and D, ^{13}C enriched (>99%) methylmercury iodide (for these isotopomers the ^{1}H–^{13}C satellites are denoted γ with the ^{1}H–^{199}Hg satellites are denoted δ). The ^{199}Hg and ^{13}C enrichment is reflected in the increase in the relative sizes of the ^{199}Hg and ^{13}C satellite signals, respectively. The chemical shifts and coupling constants are given in Table 1.

isotopic abundance, for the two labelled compounds when combined, was exactly as per the individual species, this experiment provided no evidence for simple CH_3 exchange in the deuterated HPLC mobile phase.

In order to verify (or otherwise) that this absence of exchange was also the case during chromatographic separation, further solutions were analysed prior to injection onto, and post elution from, the HPLC column. Fig. 3 shows the ^{1}H NMR spectrum obtained for a mixture of $^{13}CH_3HgI$ and $CH_3{}^{199}HgI$, both prior to injection onto the HPLC column (Fig. 3A), and for the fraction collected after eluting from the column (Fig. 3B). The chemical shift of the methyl protons for the two compounds contained in the post column fraction was 0.75 ppm, compared with 1.03 ppm for the compounds prior to HPLC separation. Similarly the ^{2}J ^{1}H–^{199}Hg coupling constant for each compound, 175 Hz, also changed from the pre-separation value of 195 Hz. This change was considered to be either due to the formation of a new monomethylmercury compound during HPLC separation, or due to the effect of the change in solvent composition during evaporation by gaseous N_2, which preferentially removes the methanolic component of the HPLC mobile phase, resulting in an enriched aqueous solvent.

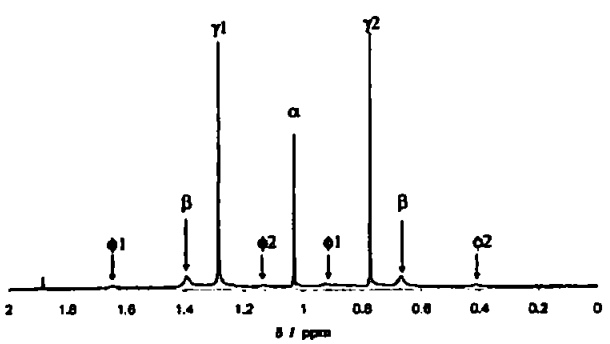

Fig. 2 ^{1}H NMR spectrum of $^{13}CH_3HgI$ (4.8 mg ml^{-1}) and ^{199}Hg enriched CH_3HgI (2 mg ml^{-1}) in CD_3OD. The ^{1}H–^{13}C doublet, marked γ1 and γ2, arises from the $^{13}CH_3HgI$: each of these doublets has two associated ^{1}H–^{199}Hg satellites marked φ1 and φ2, respectively. The ^{199}Hg enriched CH_3HgI molecule gives three signals in the spectrum, the main proton signal, α, and two ^{1}H–^{199}Hg satellites, marked β.

In order to test the second hypothesis a second solution of a mixture of $^{13}CH_3HgI$ and $CH_3{}^{199}HgI$, dissolved in the partially deuterated HPLC mobile phase, was subjected to solvent evaporation by gaseous N_2 for 4 h and subsequently analysed by ^{1}H NMR spectroscopy (Fig. 3C). The chemical shift of the methyl protons and ^{2}J ^{1}H–^{199}Hg coupling constant for each compound was 0.77 ppm and 178 Hz, respectively, which confirmed that the changes in the parameters observed for the pre- and post-HPLC separation fractions were due to the change in solvent composition. In addition, the ^{199}Hg isotopic abundance, for the two labelled isotopomers *iii* and *v*, remained at 66% and 17%, respectively, after elution from the column, indicating that methyl group exchange between the two isotopomers did not occur during the HPLC procedure.

3.4. Formation of methylmercury during equilibration

Losses of the spike material before complete equilibration between the sample and spike during IDA will result in an

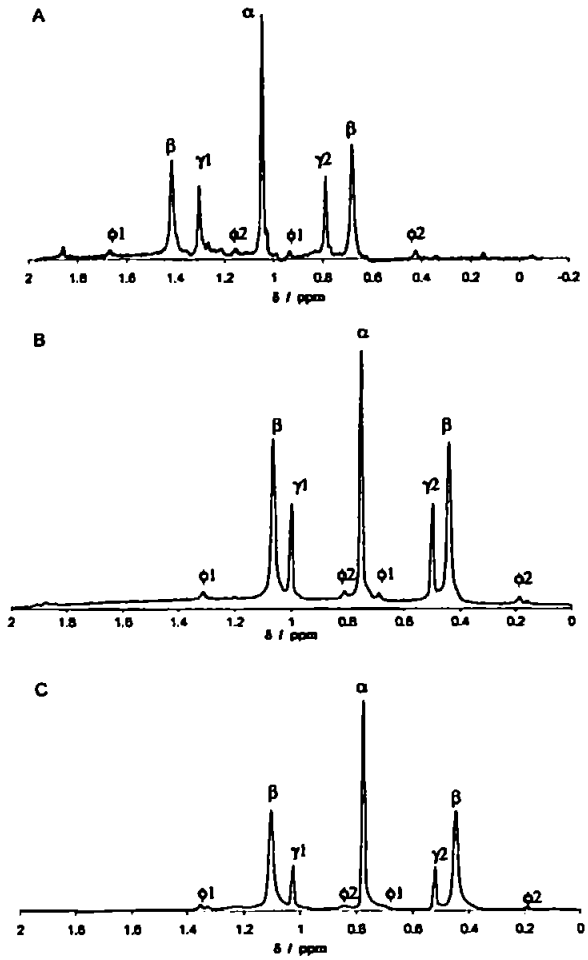

Fig. 3 ^{1}H NMR spectrum of $^{13}CH_3HgI$ (1 mg ml^{-1}) and ^{199}Hg enriched CH_3HgCl (2 mg ml^{-1}) in 50:50 D_2O–CH_3OD v/v, 0.01% 2-mercaptoethanol: A, prior to injection onto the HPLC column; B, the post-column eluent fraction containing the methylmercury species; and C, the pre-injection mixture after solvent evaporation by gaseous N_2. Spectrum B was acquired with the sample stationary in the spectrometer, hence the broadening of the signals.

underestimation of the amount of methylmercury present in the sample. Methyl group exchange from the ^{199}Hg enriched methylmercury spike to inorganic Hg, which may also be present in the sample matrix (eqn. 3) reduces the amount of the enriched spike which is available for equilibration with the natural analyte. Similarly, the formation of natural isotopic abundance methylmercury (eqn. 4) will reduce numerically the reference:spike isotope amount ratio, which, when measured by HPLC-ICP-MS after equilibration, again results in an underestimation of the analyte.

$$CH_3{}^{199}HgI + Hg^{2+} \leftrightarrow CH_3HgI + {}^{199}Hg^{2+} \quad (3)$$

$$HgI + CH_3{}^{+} \leftrightarrow + CH_3HgI \quad (4)$$

A solution of the ^{13}C labelled isotopomer and ^{199}Hg enriched HgI (obtained by dissolving the ^{199}Hg enriched HgO in 1 M KI) was used to investigate the reactions in eqn. 3 and eqn. 4. The ^{1}H NMR spectra of this solution both before (Fig. 4A) and after (Fig. 4B) a period of equilibration with particulate matter in the form of NIST2710 CRM, a soil material, gave no evidence for methyl group exchange. The ^{199}Hg isotopic abundance in the $^{13}CH_3HgI$ was 17%, see above, both prior to, and after, equilibration with the CRM for 24 h. Since the ^{199}Hg isotopic abundance of the analyte ($^{13}CH_3HgI$) remained

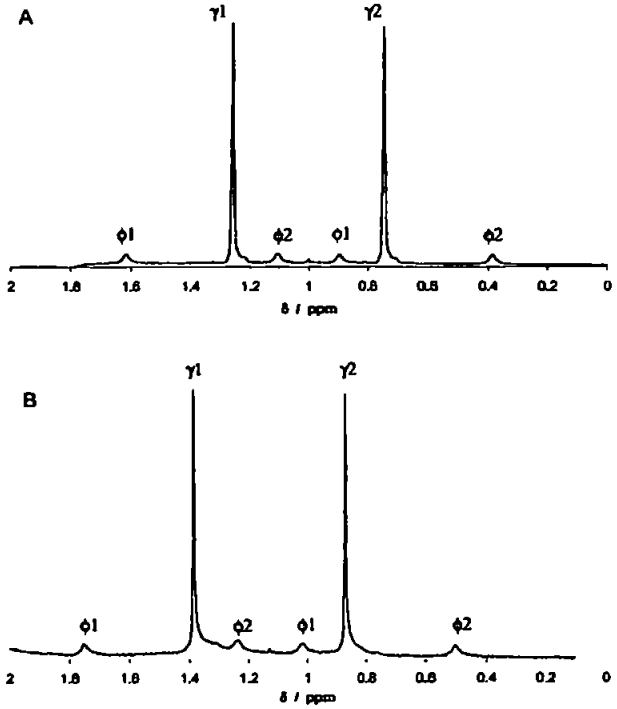

Fig. 4 ^{1}H NMR spectrum of A, $^{13}CH_3HgI$ and ^{199}Hg enriched HgCl prior to equilibration with NIST2710 CRM, and B, $^{13}CH_3HgI$ and ^{199}Hg enriched HgCl post equilibration. The isotopic abundance of ^{199}Hg in the $^{13}CH_3HgI$, calculated from the signal peak areas, remained at 17% for both samples.

unaltered by ^{199}Hg enriched HgCl, in the absence or presence of the particulate material, no methyl group exchange between the individual Hg species took place under these conditions. No signal for ^{199}Hg enriched methylmercury iodide was observed in either the pre- or post-equilibration spectra, indicating that the reaction in eqn. 4 did not occur.

A second experiment was performed using DORM-2, a dogfish muscle CRM. On this occasion a suitable ^{1}H NMR spectrum could not be obtained because co-extracted molecules from the sample matrix obscured the spectral region of interest (0.5–1.5 ppm). Similar interferences were observed in the ^{13}C NMR spectrum, whereas the ^{199}Hg NMR spectrum was unobtainable due to the low concentration of the sample. However, analysis of ^{199}Hg enriched CH_3HgCl using HPLC-ICP-MS has shown that this material does not decompose after equilibration with the DORM-2 CRM. A chromatogram obtained using a quadrupole ICP-MS instrument is presented in Fig. 5, the instrumental and chromatographic conditions having been published elsewhere.[11] The inset shows the co-extracted inorganic Hg from the DORM-2 CRM, which had a ^{200}Hg:^{199}Hg isotope amount ratio of 1.367, compared with the IUPAC value for natural isotopic abundance Hg of 1.369.[16] From this ratio it can be deduced that the ^{199}Hg enriched CH_3HgCl spike material was stable during equilibration. If the spike had decomposed the isotope amount ratio for inorganic Hg would have changed due to a contribution from the ^{199}Hg enriched isotope. A different analytical technique is required to establish whether any of the inorganic Hg present in the DORM-2 CRM was converted to methylmercury species during the equilibration process.

4. Conclusions

^{199}Hg enriched methylmercury chloride has been synthesised for use as an isotopically labelled spike material during species specific IDMS analysis for methylmercury. ^{1}H NMR spectroscopy has shown that the speciation of methylmercury will be

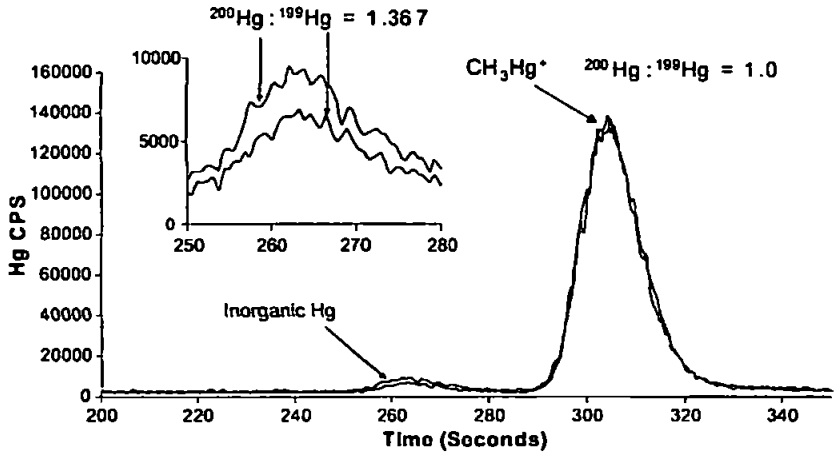

Fig. 5 HPLC-ICP-MS chromatogram of an equilibration sample with, inset, the co-extracted inorganic Hg from DORM-2 with a natural abundance ^{200}Hg:^{199}Hg isotope amount ratio, indicating that the ^{199}Hg enriched spike material was stable during the time period of the experiment.

altered with respect to the halide counter ion depending on the halide composition of the sample matrix and the reagents employed. The stability of the spike material during HPLC separation and equilibration with a soil CRM has been assessed using ^1H NMR: no evidence has been found for the breakdown or formation of methylmercury. The stability of the spike material in the presence of a fish muscle tissue could not be determined by NMR due to co-extracted molecules giving signals which masked that from the methylmercury. The spike material was adjudged to be stable in the presence of DORM-2 by HPLC-ICP-MS.

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Uncertainty contributions to species specific isotope dilution analysis. Part 2. Determination of methylmercury by HPLC coupled with quadrupole and multicollector ICP-MS†

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Species-specific isotope dilution analysis (IDA) has been used to determine the mass fraction of methylmercury in a fish muscle certified reference material, DORM-2. Two spike solutions were prepared and their mass fractions determined by coupling high-performance liquid chromatography (HPLC) with both quadrupole (Q) and multicollector (MC) inductively coupled plasma mass spectrometry (ICP-MS) and uncertainty budgets calculated. For the Q-ICP-MS determination the relative standard uncertainty of the spike was 4.1%. The major uncertainty contribution (62%) arose from the uncertainties associated with the relative isotopic abundances of Hg isotopes as given by IUPAC. The measured isotope amount ratios (^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl) contributed 37% of the combined uncertainty. For the multicollector instrument the relative standard uncertainty of the spike solution mass fraction was 0.7%. In this case the uncertainty budget was dominated by the Hg isotopic uncertainties, with a contribution of 99%. The uncertainty contributions from the measured isotope amount ratios were reduced to 0.7% of the total when using MC-ICP-MS. The two spike solutions were separately used to determine the mass fraction of methylmercury in DORM-2 CRM by species-specific IDA, using HPLC coupled with quadrupole ICP-MS. The values found were 4.45 ± 0.90 μg g^{-1} and 4.25 ± 0.47 μg g^{-1} using spikes characterised by Q- and MC-ICP-MS, respectively (the uncertainties quoted are the expanded uncertainty, $k = 2$), compared with a certified value of 4.47 ± 0.32. The major uncertainty contribution for each determination was from the measured isotope amount ratios (70%) with lesser contributions from the uncertainties associated with the natural isotopic abundance of Hg and the spike mass fraction.

1. Introduction

The estimation of the uncertainty associated with an analytical measurement is increasingly being recognised as an essential part of the measurement process, allowing improved intercomparison of analytical results,[1] and is a requirement for ISO accredited methods.[2] Isotope dilution analysis (IDA), which is regarded as a definitive analytical technique[3] because the precision and accuracy obtainable are unsurpassed by alternative analytical methods,[4] allows the estimation of a measurement uncertainty which is related directly to SI units, and therefore meets the highest metrological standards.[5] The calculation of an uncertainty budget also aids the development of an analytical method by allowing the analyst to identify which areas of the measurement process provide the major contributions to the overall measurement uncertainty and therefore focus on these areas in order to minimise the uncertainty estimate.

The measurement of isotope amount ratios during IDA, as opposed to single isotopic signals during conventional external calibration, also confers a number of advantages provided equilibration is complete:

(i) total extraction of the analyte from the sample matrix is not required;[6]

(ii) losses of the analyte are compensated for by losses of the spike in the same proportion;

(iii) as the spike material acts as an internal standard, problems associated with instrumental drift and matrix effects during mass spectrometric detection can also be overcome.[7]

Traditionally, thermal ionisation mass spectrometry (TIMS) has been used for isotope ratio determinations: however, unlike inductively coupled plasma mass spectrometry (ICP-MS), it is not suited to elements such as mercury, which have a high first ionisation potential. ICP-MS also offers greater sample throughput, is tolerant of complex sample matrices, utilises smaller sample sizes, can be easily coupled with various separation techniques, and does not suffer from time-dependent mass fractionation effects.[8] The development of multicollector sector-field (MC-SF) ICP-MS instruments, enabling the simultaneous measurement of each isotopic signal,[9] has greatly improved the accuracy and precision of isotope amount ratio determinations (*ca.* 0.005% relative standard error[10]) compared with quadrupole ICP-MS instruments and is comparable to that obtainable with TIMS instrumentation.

The aim of this work was to develop a method for species-specific IDA analysis for methylmercury and, by the use of an uncertainty budget, determine the factors which affect the accuracy and precision of the method. Species specific IDA necessitates the synthesis of isotopically labelled analogues, or isotopomers, of the target analyte. For this work, ^{199}Hg enriched methylmercury chloride has been produced and characterised by nuclear magnetic resonance spectroscopy (NMR).[11] Methylmercury formation from inorganic Hg has been observed during separation and analysis by gas chromatography.[12–14] The stability of methylmercury during equilibration and subsequent separation by high-performance liquid chromatography (HPLC)[6,11] and an account of the procedures required to produce accurate and precise results by species specific IDA-HPLC-ICP-MS[15] has been previously reported by us. In this work the mercury species were separated by high-performance liquid chromatography (HPLC) coupled to inductively coupled

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plasma mass spectrometry (ICP-MS) using both scanning quadrupole and simultaneous multicollector instruments.

2. Theory

2.1. The uncertainty budget model

The formulation and calculation of an uncertainty budget for an analytical measurement requires that all sources of uncertainty associated with the analytical procedure are identified and incorporated into the model used for the calculation of the expanded uncertainty of the analytical result. In this case the basis for the uncertainty model was the ID equation used to calculate the analyte mass fraction, eqn. 1. IDMS equations are available which use relative isotopic abundances:[10] however, the uncertainty calculation is subsequently more complex as the isotopic abundances are correlated.[5] Isotope amount ratios are used, as opposed to relative isotopic abundances, in eqn. 1, which simplified the uncertainty calculation by eliminating the relative isotopic abundance correlation.

$$C_x = C_y \times \frac{m_y}{m_x \times h} \times \frac{R_y - R_B}{R_B - R_x} \times \frac{\sum_i R_{ix}}{\sum_i R_{iy}} \quad (1)$$

where C_x = concentration of the analyte in the sample, C_y = concentration of the analyte in the spike solution, m_y = mass of spike, m_x = mass of sample, h = moisture content correction factor, R_y = reference:spike isotope amount ratio in the spike, R_x = reference:spike isotope amount ratio in the sample, $\sum_i R_{ix}$ = isotope amount ratio of isotope i to the spike isotope in the sample, $\sum_i R_{iy}$ = isotope amount ratio of isotope i to the spike isotope in the spike and R_B = reference:spike isotope amount ratio in the sample after spiking.

For this work ^{200}Hg was chosen as the reference isotope and ^{199}Hg as the spike isotope.

2.1.1. Mass bias correction. In order to determine the correct isotope amount ratio it is necessary to compensate for mass bias in the instrumentation. Quadrupole and sector field mass spectrometers and their associated ion optics do not transmit ions of different masses equally. In other words, if an elemental solution composed of two isotopes with an exactly 1:1 molar ratio is analysed using ICP-MS, a 1:1 isotope amount ratio will not necessarily be observed. This so-called mass bias depends on mass and the type of mass spectrometer used, but generally tends to be greatest at low mass and decreases with increasing mass. Even very small mass-biases can have deleterious effects on the accuracy of isotope amount ratio determinations, so a correction must always be made, either by bracketing the sample with a reference material, certified for the isotope pair under study, or by interpolation from the mass bias observed for an isotope pair of similar mass to the isotope pair under study. No isotopically certified Hg CRM was available, therefore the mass bias of ^{200}Hg:^{199}Hg isotope amount ratio was corrected for by interpolation of the mass bias observed for the ^{205}Tl:^{203}Tl isotope amount ratio using the relationship, named as the Russell correction expression,[16] shown in eqn. 2, which corrects for mass bias on the basis of the absolute mass of the isotope pairs.

$$\left(\frac{^{200}Hg}{^{199}Hg}\right)_{cor} = \frac{\left(\frac{^{200}Hg}{^{199}Hg}\right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl}\right)_{mes} \div \left(\frac{^{205}Tl}{^{203}Tl}\right)_{cer}\right)^{\left(\ln\left(\frac{RAM^{200}Hg}{RAM^{199}Hg}\right) + \ln\left(\frac{RAM^{205}Tl}{RAM^{203}Tl}\right)\right)}} \quad (2)$$

where cor is the corrected isotope amount ratio, mes is the measured isotope amount ratio, cer is the certified isotope amount ratio and RAM is the relative atomic mass.

The advantage of this approach is that the mass bias correction can be performed by spiking the sample with a mass bias correction standard (*i.e.*, Tl) and measuring this isotope amount ratio at the same time as the sample. For speciation analysis by HPLC-ICP-MS, it is most convenient to spike the mobile phase with the standard and monitor it continuously.

2.2. Uncertainty budget model incorporating mass bias correction

The ID equation, eqn. 1, assumes that the measured isotope amount ratio has been corrected for mass bias effects arising from the mass spectrometer. Each parameter in the model used for mass bias correction will have an uncertainty associated with it which contributes to the overall uncertainty for the ID procedure. The method chosen for mass bias correction of the measured isotope amount ratios must therefore be included in the model used to calculate the analytical result if the uncertainty contributions arising from mass bias correction are to be included in the final uncertainty estimate. Eqn. 1 and eqn. 2 were therefore combined to give the model for the calculation of the mass fraction of methylmercury in the DORM-2-CRM, eqn. 3: the notation is the same as that used for eqns. 1 and 2.

$$C_X = C_y \times \frac{m_y}{m_x \times h} \times \frac{R_y - \left(\frac{\left(\frac{^{200}Hg}{^{199}Hg}\right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl}\right)_{mes} + \left(\frac{^{205}Tl}{^{203}Tl}\right)_{cer}\right)^{\left(\ln\left(\frac{RAM^{200}Hg}{RAM^{199}Hg}\right) - \ln\left(\frac{RAM^{205}Tl}{RAM^{203}Tl}\right)\right)}}\right)}{\left(\frac{\left(\frac{^{200}Hg}{^{199}Hg}\right)_{mes}}{\left(\left(\frac{^{205}Tl}{^{203}Tl}\right)_{mes} + \left(\frac{^{205}Tl}{^{203}Tl}\right)_{cer}\right)^{\left(\ln\left(\frac{RAM^{200}Hg}{RAM^{199}Hg}\right) - \left(\frac{RAM^{205}Tl}{RAM^{203}Tl}\right)\right)}}\right) - R_x} \times \frac{\sum_i R_{ix}}{\sum_i R_{iy}} \quad (3)$$

2.3. Reverse IDMS of the spike material

The ^{199}Hg enriched CH_3HgCl spike material was not synthesised in quantities sufficient to determine its purity or to weigh with sufficient accuracy using a four figure balance. The spike material was therefore characterised by reverse isotope dilution analysis. In this procedure the spike material is isotopically diluted with a standard of natural isotopic abundance and the mass fraction of the spike solution calculated according to eqn. 4. The measured ^{200}Hg:^{199}Hg isotope amount ratio was corrected for mass bias effects by the addition of NIST 997 Tl as an internal standard and eqn. 2 was combined with eqn. 4 for the calculations but has been omitted here for clarity. It is possible to combine eqn. 1 and eqn. 4 to give a 'double' isotope dilution equation[3,17] which eliminates the spike mass fraction, C_y, which is often relatively poorly characterised. In this study, however, two separate spike solutions were prepared, each analysed using a different ICP-MS instrument. This allowed a comparison to be made of the different uncertainty contributions arising from the use of the different instruments for the spike characterisation.

$$C_y = C_z \times \frac{m_z}{m_y} \times \frac{R_z - R_{BC}}{R_{BC} - R_Y} \times \frac{\sum_i R_{iy}}{\sum_i R_{iz}} \quad (4)$$

where C_y = concentration of the analyte in the spike solution, C_z = concentration of the natural isotopic abundance standard, m_z = mass of natural isotopic abundance standard,

m_y = mass of spike, R_z = reference:spike isotope amount ratio in the natural isotopic abundance standard, R_y = reference: spike isotope amount ratio in the spike, $\sum_i R_{iz}$ = isotope amount ratio of isotope i to the spike isotope in the natural isotopic abundance standard, $\sum_i R_{iy}$ = isotope amount ratio of isotope i to the spike isotope in the spike and R_{BC} = reference:spike isotope amount ratio in the spike and natural standard blend, corrected for mass bias effects in the manner shown in eqn. 3.

2.4. Combining standard uncertainties

The uncertainty budget was calculated following the guidelines set out by Eurachem/CITAC.[17] Individual uncertainties associated with each parameter in eqn. 3 were combined, after conversion to a standard uncertainty, using the spreadsheet method first described by Kragten,[18] which assumes that the standard uncertainties of each measurement parameter are relatively small compared with that measurement parameter, and gives acceptable accuracy for practical purposes. The spreadsheet, which provides a simple method for the estimation and combination of uncertainties, estimates the uncertainty each parameter contributes to the overall uncertainty for the analytical result. The individual uncertainty estimates are subsequently combined using error propagation laws: worked examples of the estimation of measurement uncertainty are given in the Eurachem/CITAC Guide[17] and the principles described therein were followed when constructing the spreadsheet used in this work.

3. Experimental

3.1. Separation and analysis of mercury compounds by HPLC-ICP-MS

Two ICP-MS instruments were employed for the detection of mercury species following separation by HPLC, a VG Plasmaquad 3 quadrupole (Q) ICP-MS and a VG Axiom *multicollector (MC) sector field (SF)* ICP-MS (both Thermo Elemental, Winsford, UK). The coupling of the HPLC system and the data acquisition parameters for each instrument for the measurement of isotope amount ratios was optimised by analysing gravimetrically prepared solutions, of known isotopic composition, of inorganic and organomercury compounds with a range of ^{200}Hg:^{199}Hg isotope amount ratios. The HPLC conditions follow those of Harrington *et al.*[19] and are summarised in Table 1, along with the optimal operating conditions for each ICP-MS instrument used in this study. NIST 997 Thallium CRM was added to the mobile phase at 50 ng g^{-1} and continuously monitored during separations for the purposes of mass bias correction.

A number of problems can arise when coupling HPLC to ICP-MS, two of which are the incompatibility of the HPLC flow rate (*ca.* 1 ml min^{-1}) with that required for high efficiency sample nebulisation, and the deleterious effects on the plasma of a high organic content mobile phase. To overcome these potential problems the HPLC system was not directly coupled to the nebuliser sample uptake tube but a low dead volume splitter was used. This allowed the nebuliser to operate at its natural uptake rate, as determined by the nebuliser argon gas flow, and the system to be optimised for maximum sensitivity and optimum peak shape without compromising the HPLC conditions. A low flow nebuliser (0.2 ml min^{-1} Micromist, Glass Expansion, Vevey, Switzerland) was employed in conjunction with 0.18 mm diameter Teflon uptake tubing. The uptake rate of this system was 50 µl min^{-1}, thereby ensuring highly efficient nebulisation and, in conjunction with a coupled spray chamber system (cyclonic and bead impact, Glass Expansion, Vevey, Switzerland) cooled to −5 °C, reducing the organic loading in the plasma.

3.1.1. Characterisation of the ^{199}Hg enriched CH_3HgCl. The *mass fraction of the ^{199}Hg enriched CH_3HgCl was determined* by reverse ID-MS with the spike isotopic composition modified by the addition of a well characterised natural standard (methylmercury chloride standard, Alfa Aesar, Ward Hill, MA, USA). Two different methylmercury chloride spike solutions were prepared, one for analysis by Q-ICP-MS (9 µg g^{-1} as Hg), and the second for analysis by MC-SF-ICP-MS (11 µg g^{-1} as Hg). In each case an iterative procedure was employed[3] to dilute the spike with the natural standard to give a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity to minimise errors from sources such as mass bias and, for analysis by Q-ICP-MS, detector dead time,[10] thus reducing the measurement uncertainty.

3.2. Equilibration of ^{199}Hg enriched methylmercury and DORM-2 CRM

Equilibration solutions, 50:50 H_2O-CH_3OH v/v and 0.01% 2-mercaptoethanol, the HPLC mobile phase, were prepared using HPLC grade methanol (Merck Ltd., Poole, Dorset, UK),

Table 1 Operating conditions for HPLC-ICP-MS characterisation

HPLC conditions			
HPLC column	HiChrom Kromasil 100 FC 18 Excel, 25 cm × 4.6 mm id		
Mobile phase	50:50 v/v methanol–DDW, 0.01% 2-mercaptoethanol, 50 ng g^{-1} NIST 997 Tl CRM		
Flow rate/ml min^{-1}	0.9		
Injection volume/µl	100		
VG PQ3 ICP-MS operating conditions			
RF forward power/W	1450	Plasma gas/l min^{-1}	14
Reflected power/W	≤5	Auxiliary gas/l min^{-1}	0.75
Spray chamber	Cyclonic, cooled to −5 °C	Nebuliser gas/l min^{-1}	0.52
Torch	Fassel quartz	Dwell time/ms	10
Sampler and skimmer cones	Ni	Points per peak	5
Nebuliser	Glass Expansion 0.2 ml min^{-1} Micromist	Ions monitored	^{199}Hg, ^{200}Hg, ^{203}Tl, ^{205}Tl
VG Axiom MC-SF-ICP-MS operating conditions			
RF forward power/W	1400	Plasma gas/l min^{-1}	14
Reflected power/W	≤10	Auxiliary gas/l min^{-1}	0.85
Spray chamber	Coupled cyclonic and bead impact, cooled to −5 °C	Nebuliser gas/l min^{-1}	0.72
Torch	Fassel quartz fitted with a Pt shield	Dwell time/ms	25
Sampler and skimmer cones	Ni	Points per peak	1
Nebuliser	Glass Expansion 0.2 ml min^{-1} Micromist	Ions monitored	^{199}Hg, ^{200}Hg, ^{203}Tl, ^{205}Tl

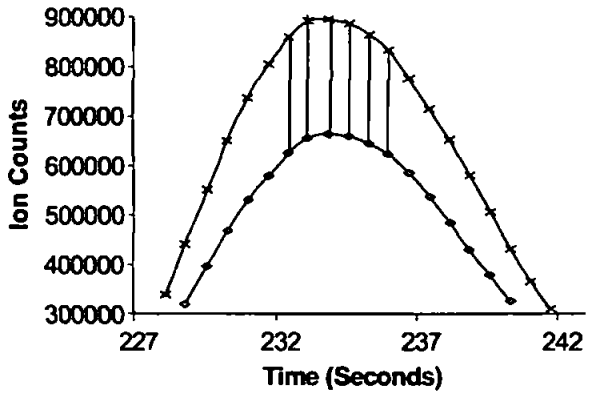

Fig. 1 Isotope amount ratios measured using the pseudo-steady-state approach. The isotope amount ratio is calculated by ratioing the ion signals for coincident pairs of data points over the peak maximum.

distilled deionised water (Elgastat Maxima, Elga Ltd., High Wycombe, UK) and 2-mercaptoethanol (Merck Ltd.). The moisture content of the DORM-2 CRM was determined, by drying separate subsamples to a constant mass at 105 °C, to give the moisture correction factor, h (eqn. 1). Approximately 3 g of the DORM-2 CRM was accurately weighed and the amount of methylmercury present, after accounting for moisture content, calculated from the certified value. The mass of spike solution added was chosen so that the ^{200}Hg:^{199}Hg isotope amount ratio would be close to unity, provided that complete equilibration between the sample and spike occurred. Subsequently, the spike solution was added to 25 g of the equilibration solution contained in a clean 50 ml glass conical flask. This solution was agitated throughout the timespan of the equilibration experiment by a magnetic stirrer and maintained at a temperature of 25 °C by means of an electrically heated hotplate. The DORM-2 CRM was subsequently added to the equilibration solution and 1 ml samples withdrawn at intervals up to 3000 min. The sampling frequency was approximately every 2 min for the first 20 min of the experiment followed by a reduced sampling rate of 5 min intervals until 1 h had elapsed, with less frequent sampling thereafter. Each sample aliquot was pipetted into a filter (Autovial 0.2 μm PTFE membrane syringeless filters, Whatman, Maidstone, Kent, UK), diluted prior to filtration with 3 ml of fresh equilibration solvent, filtered, and stored in a clean 25 ml sterilin container at 4 °C until analysis by HPLC-ICP-MS. Samples obtained from the equilibration experiments were analysed using Q-ICP-MS under the same instrumental conditions as those for the reverse ID-MS of the spike solution.

4. Results and discussion

4.1. Calculating isotope amount ratios

In order to perform species specific ID-MS it is necessary to measure isotope amount ratios of two transient signals resulting from the chromatographic separation prior to ICP-MS detection. Two problems may arise, namely compound fractionation on the column and spectral skew resulting from too slow mass spectral scanning. It is unlikely that any on column fractionation would occur with the compounds under study because the difference in molar mass between the analyte and spike was too small (though this may not be the case if gas chromatography was used). In this case data acquisition parameters were optimised to eliminate spectral skew.

Two different approaches were assessed to determine the accuracy and precision of calculated isotope amount ratios from the multi-isotope chromatograms obtained by HPLC-ICP-MS, namely peak integration and a pseudo-steady-state approach. For peak integration the relevant peaks for the isotope pair of interest, ^{200}Hg and ^{199}Hg, of a particular specie in the chromatogram were integrated to obtain the baseline-subtracted peak integrals and the isotope amount ratio for that specie calculated using these integrals. The advantage of this approach is that the effects of spectral skew are minimised: however, precision can be degraded because the precision inherent in rapid sampling of the isotopic pair is lost. The second approach assumed that the chromatogram was an undulating, or pseudo-steady-state, signal. The results of this approach are shown in Fig. 1, where co-incident pairs of data points from the two isotope ion signals (*i.e.* ^{200}Hg and ^{199}Hg) were ratioed over the course of the chromatogram.

In order to achieve the best precision and accuracy, only data-points on the apices of the peaks (PQ3 n = 9, Axiom n = 5) for each isotope of a particular specie in the chromatogram, were chosen and the baseline signal subtracted. The isotope amount ratios were calculated using each pair of corresponding data-points from the two peaks and subsequently corrected for mass bias effects. The advantage of this approach is that the inherent precision is maintained and it is possible to obtain an estimate of precision from a single peak: however, erroneous isotope amount ratios will result if spectral skew is not minimised. Both of the above methods for data extraction were evaluated and the pseudo-steady-state approach resulted in measured, mass bias corrected, isotope amount ratios which were closer to the theoretical values of the gravimetrically prepared mercury solutions, and exhibited a greater degree of precision then those obtained by peak integration (Table 2).

4.2. Comparison of scanning and simultaneous detection

Variability of the measured isotope amount ratios during a chromatographic run are illustrated in Fig. 2 for both the quadrupole scanning (Fig. 2A) and the Axiom simultaneous multicollector instrument (Fig. 2B). The quadrupole instrument resulted in poorer precision during peak elution, due to the requirement to measure transient signals for four isotopes with a dwell time of 10 ms. There is also evidence of spectral skew, (Fig. 2A) because the measured ^{200}Hg:^{199}Hg isotope amount ratio fell at the start of the methylmercury peak elution, and was stable only at the peak apex. When using the multicollector instrument the ^{200}Hg:^{199}Hg isotope amount ratio, calculated from background signals using Faraday

Table 2 Figures of merit for HPLC-ICP-MS optimisation using the pseudo-steady-state approach for the calculation of the isotope amount ratios

	Gravimetric ratio	Pseudo-steady-state measured ratio	Relative standard uncertainty
VG PQ3			
^{205}Tl:^{203}Tl (n = 9)	2.387 ± 0.001	2.407 ± 0.012	0.50%
^{200}Hg:^{199}Hg (n = 9)	1.369 ± 0.029	1.371 ± 0.064	4.67%
^{200}Hg:^{199}Hg (n = 9)	0.2751 ± 0.00002	0.2758 ± 0.0057	2.1%
VG Axiom Multicollector			
^{205}Tl:^{203}Tl (n = 5)	2.387 ± 0.001	2.395 ± 0.0036	0.15%
^{200}Hg:^{199}Hg (n = 5)	1.369 ± 0.029	1.369 ± 0.00051	0.037%
^{200}Hg:^{199}Hg (n = 5)	0.2751 ± 0.00002	0.2755 ± 0.00057	0.21%

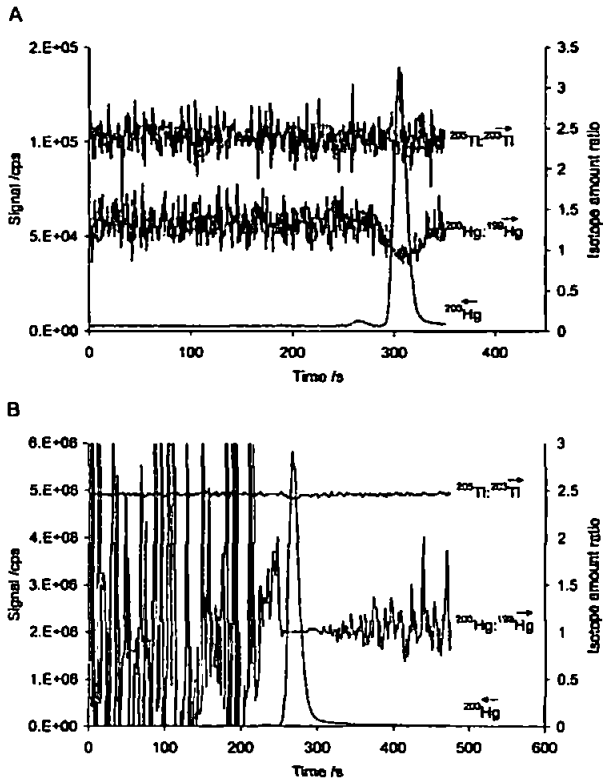

Fig. 2 The measured ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios and ^{200}Hg signal monitored during reverse ID-MS of the spike solutions. A, PQ3 quadrupole instrument in peak jumping mode, dwell time 10 ms, 100 µl injection of a 900 ng g^{-1} CH_3HgCl solution; B; Axiom multicollector instrument, 25 ms dwell time, 100 µl injection of an 11 µg g^{-1} CH_3HgCl solution.

detectors, was highly variable prior to the MeHg peak elution as the Hg concentration in the mobile phase was not high enough to result in a measurable signal. As the peak eluted, and the signal strength rose rapidly, the isotope amount ratio precision rapidly improved to a minimum at the peak maximum. The greater precision of the measured ^{205}Tl:^{203}Tl isotope amount ratio obtained using the multicollector can also be clearly observed. The memory effect, which is a common problem in mercury analysis, is also well illustrated. As the MeHg signal strength declined after peak elution the isotope amount ratio became more variable than during, but less so than before, the peak elution. To counteract this memory effect 0.6 M HBr in a 50% methanol solution was aspirated for 3 min between each sample acquisition, which reduced the blank counts to those prior to the sample run.

4.3. Characterisation of the spike

Before IDMS analysis of a sample could be performed it was necessary to determine the mass fraction of CH_3HgCl in the ^{199}Hg enriched spike as accurately and precisely as possible. Reverse IDMS was performed for the spike characterisation,

Table 3 The mass fraction of ^{199}Hg enriched CH_3HgCl spike determined by reverse isotope dilution HPLC-ICP-MS

Instrument	Mass fraction/ µg g^{-1}	Standard uncertainty/ µg g^{-1}, $K = 1$)	Relative standard uncertainty
VG PQ3 Q-ICP-MS	8.96 ($n = 5$)	±0.37	4.1%
VQ Axiom MC-SF-ICP-MS	11.06 ($n = 3$)	±0.08	0.70%

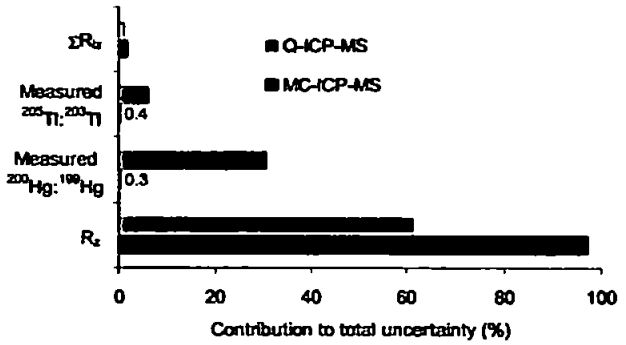

Fig. 3 Contributions to the total standard uncertainty of the mass fraction of ^{199}Hg enriched CH_3HgCl determined by reverse IDMS.

and full uncertainty budgets calculated, using both the simultaneous multicollector and the scanning quadrupole instruments. The spike mass fraction was calculated using eqn. 4, the results being shown in Table 3. No methylmercury was detected in the blank solutions and therefore a blank correction was not included in eqn. 4. The raw counts for each mercury isotope in the methylmercury peak were, however, baseline subtracted to account for inorganic Hg present in the ICP argon gas and the solutions used for the spike make up. The standard uncertainty of the measured ^{200}Hg:^{199}Hg isotope amount ratio entered into eqn. 4 included the uncertainty contribution from the baseline correction which contributed less than 0.1%. The relative standard uncertainty of the spike solution characterised using the Axiom instrument was 0.70%, which was an order of magnitude less than that obtained for the spike solution mass fraction using the scanning instrument, 4.1%. Individual contributions to the total standard uncertainty are shown in Fig. 3.

For the PQ3 quadrupole instrument the major uncertainty contributions arose from two parameters, the natural isotopic abundance ^{200}Hg:^{199}Hg isotope amount ratio, R_z, and the measured ^{200}Hg:^{199}Hg isotope amount ratio, R_{BC}, which contributed 61 and 31 per cent., respectively. By comparison, when using the Axiom multicollector instrument, R_z contributed 97% of the standard uncertainty and the measured ^{200}Hg:^{199}Hg isotope amount ratio, R_{BC}, only contributed 0.3% of the combined standard uncertainty for the spike mass fraction. The reduction in the contribution of the measured isotope amount ratios ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl was due to the increased measurement precision obtainable with the Axiom multicollector instrument (Table 4). The relative

Table 4 Measured isotope amount ratios and uncertainty contributions to the mass fraction determination of the ^{199}Hg enriched CH_3HgCl spike by reverse HPLC-ID-MS

		Relative standard uncertainty (%)	Contribution to spike mass fraction standard uncertainty (%)
Quadrupole HPLC-ICP-MS			
R_{BC}	0.9900	0.58	31
Measured ^{205}Tl:^{203}Tl	2.4276	0.51	6
R_z	1.3693	0.89	61
ΣR_{iz}	5.9277	0.44	1
R_y	*0.2751*	*0.01*	2.9 E−06
ΣR_{iy}	1.5155	0.009	1.4 E−04
Axiom multicollector HPLC-ICP-MS			
R_{BC}	1.0029	0.045	0.3
Measured ^{205}Tl:^{203}Tl	2.4243	0.10	0.4
R_z	1.3693	0.89	97
ΣR_{iz}	5.9277	0.44	2
R_y	0.2751	0.01	2.9E-06
ΣR_{iy}	1.5155	0.009	1.4E-04

Table 5 The isotopic abundance and uncertainty data for natural abundance Hg and the stated and measured AEAT ^{199}Hg enriched HgO

			AEAT ^{199}Hg enriched HgO		
Isotope	IUPAC data (atom %)	Standard uncertainty	Data supplied (atom %)	Axiom MC-SF-ICP-MS (atom %)	Standard uncertainty
196	0.15	0.01	0.1	0.10	0.001
198	9.97	0.12	3.08	2.93	0.0014
199	16.87	0.13	65.74	65.98	0.010
200	23.1	0.11	18.18	18.15	0.0016
201	13.18	0.05	3.95	3.96	0.0019
202	29.86	0.15	7.31	7.43	0.0047
204	6.87	0.09	1.65	1.44	0.0012

isotopic abundances of mercury, as given by IUPAC,[20] have relatively large associated uncertainties compared with some elements, hence the large relative standard uncertainties for R_z and ΣR_{iz} (Table 4), which were calculated from the IUPAC data. No purity or isotopic abundance uncertainty data were supplied with the ^{199}Hg enriched HgO, hence the isotopic composition of the spike material was determined using the Axiom MC-SF-ICP-MS prior to the synthesis of the spike material.[21] The IUPAC, AEAT and measured isotopic abundance data is shown in Table 5. The improved precision values obtained by this analysis resulted in the associated uncertainties of R_y and ΣR_{iy} (Table 4) providing a minimal contribution to the standard uncertainty for the spike mass fraction.

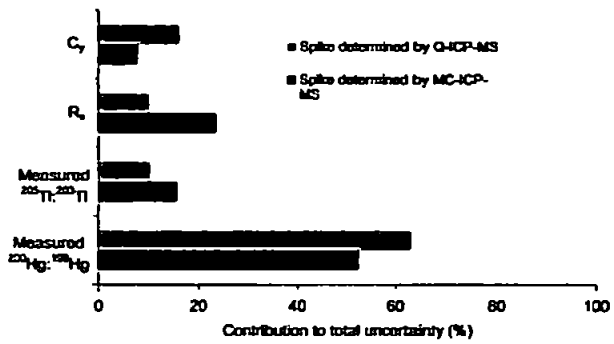

Fig. 5 Contributions of individual standard uncertainties to the total combined expanded uncertainty for the determination of CH_3Hg^+ in DORM-2 CRM by species specific IDMS. Each uncertainty budget was dominated by the precision of the measured isotope amount ratios. The contribution from the spike uncertainty was significantly larger when it was characterised by quadrupole HPLC-ICP-MS.

4.4. Analysis of reference material

The isotope amount ratios in the samples were determined using the PQ3 instrument preceded by a HPLC separation (see Fig. 4). It was not possible to measure the isotope amount ratios using the Axiom multicollector instrument as the signal strength was not high enough to be measured by the Faraday cup multicollector array with sufficient accuracy and precision.

Two speciation analyses were performed, one using the spike material characterised by the PQ3 quadrupole ICP-MS and the second using the spike characterised with the Axiom multi-collector instrument. The major contributions to the uncertainty budget for each analysis are derived from the precision values of the measured ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios. The raw counts of the mercury isotopes were baseline corrected, as described in the spike characterisation. Fig. 5 shows the major contributions to the uncertainty budget for each analysis and the data is shown in Table 6. For the speciation analysis conducted using the spike material characterised by the quadrupole instrument the major uncertainty component was derived from the uncertainty associated with the measured ^{200}Hg:^{199}Hg isotope amount ratio, 63%. Three other factors, the measured ^{205}Tl:^{203}Tl isotope amount ratio (10%), the calculated natural abundance ^{200}Hg:^{199}Hg isotope amount ratio, R_x (10%), and the spike mass fraction, C_y (16%), also contributed significantly to the standard uncertainty. The improved spike characterisation by the multicollector instrument resulted in a smaller uncertainty contribution from C_y (8%), to the standard uncertainty when

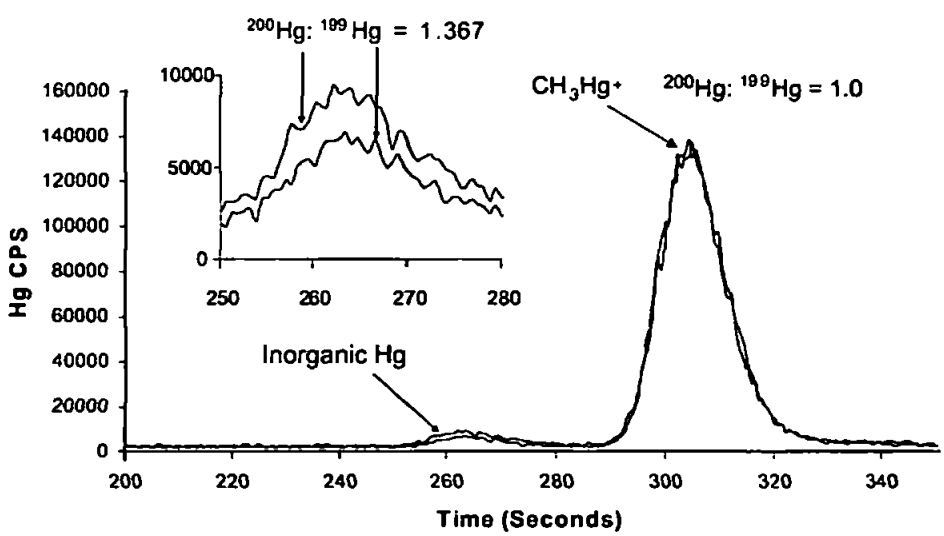

Fig. 4 HPLC-ICP-MS chromatogram of an equilibration sample with, inset, the co-extracted inorganic Hg from DORM-2 with a natural abundance ^{200}Hg:^{199}Hg isotope amount ratio, indicating that the ^{199}Hg enriched spike material was stable during the time period of the experiment.

Table 6 Contributions to uncertainty for a single sample from each equilibration experiment

		Relative standard uncertainty (%)	*Contribution to* methylmercury mass fraction in DORM-2 standard uncertainty (%)
Spike characterised by quadrupole HPLC-ICP-MS			
Measured ^{200}Hg:^{199}Hg	1.0021	1.9	63
Measured ^{205}Tl:^{203}Tl	2.3656	1.6	10
R_x	1.369	0.9	10
C_y/μg g^{-1}	8.96	4.0	16
Spike characterised by multicollector HPLC-ICP-MS			
Measured ^{200}Hg:^{199}Hg	1.0115	1.1	52
Measured ^{205}Tl:^{203}Tl	2.3654	1.3	16
R_x	1.369	0.9	24
C_y/μg g^{-1}	11.1	1.9	8

Table 7 The methylmercury mass fraction in DORM-2 CRM determined by species-specific HPLC-ID-ICP-MS

Determination	DORM-2 methylmercury mass fraction/μg g^{-1}	Relative expanded uncertainty
Spike characterised by quadrupole ICP-MS	4.45 ± 0.90[a]	20%
Spike characterised by multicollector ICP-MS	4.25 ± 0.47[a]	11%
DORM-2 Certified Value	4.47 ± 0.32	7%

[a]The reported uncertainty is an expanded uncertainty calculated using a coverage factor of 2 which gives a level of confidence of approximately 95%.

this material was used, with the uncertainty contribution from R_x rising to 24%.

The mass fraction of methylmercury in DORM-2 was calculated using eqn. 3 and the standard uncertainty estimated: the results are shown in Table 7. No methylmercury was detected in the blank solutions and a blank correction was not therefore included in eqn. 3. The mass fraction of methylmercury in DORM-2 using the spike characterised using quadrupole HPLC-ICP-MS was 4.45 μg g^{-1} with a standard combined uncertainty of 0.45 μg g^{-1}. For the experiment conducted using the spike characterised by multicollector HPLC-ICP-MS the mass fraction of methylmercury in DORM-2 was 4.25 μg g^{-1} with a standard uncertainty of 0.24 μg g^{-1}.

A *standard uncertainty, u, corresponds to one standard* deviation, which, for a normal distribution, includes approximately 68% of the values. To provide an uncertainty estimate which can be expected to cover approximately 95% of the normally distributed values u is expanded by a coverage factor, k. As the major contribution to each standard uncertainty was derived from the precision of the measured isotope amount ratios, $n = 10$ for each replicate, the degrees of freedom was considered large enough to use a coverage factor of $k = 2$, which approximates to the 95% confidence interval. Consequently the standard uncertainty for each determination of methylmercury in DORM-2 by species-specific HPLC-ID-ICP-MS was expanded to give the final results in Table 7.

5. Conclusions

The analytical procedure required a separation step, by HPLC, prior to the detection of the mercury species by ICP-MS. The coupling of the HPLC system to the ICP-MS instrument was optimised to ensure highly efficient sample nebulisation and minimise the effect of the HPLC mobile phase, 50% methanol, on plasma stability. Instrumental data acquisition parameters were optimised using gravimetrically prepared solutions of natural and ^{199}Hg enriched abundance methylmercury chloride. Two methods of data extraction for the calculation of isotope amount ratios from the resulting chromatograms, peak integration and a pseudo-steady-state approach, have been evaluated. The pseudo-steady-state approach gave results of greater accuracy and precision than the peak integration approach and, as precision values can be obtained from a single sample injection onto the HPLC column, considerably reduces the sample volume and time required for analysis.

The determination of methylmercury in a fish muscle certified reference material, DORM-2, was performed by species-specific IDMS and quantitative recovery in good agreement with the certified value was obtained. An uncertainty budget has been calculated for the analytical procedure, allowing the relative uncertainty contributions for each parameter in the measurement equation to be quantified and their relative contributions to the final expanded uncertainty determined.

Two ICP-MS instruments were employed in this study for the measurement of both transient and simultaneous ion signals for the calculation of isotope amount ratios. Species-specific HPLC-ID-MS by the Axiom multicollector instrument resulted in a lower relative expanded uncertainty due to the greater precision and accuracy obtainable with this instrument compared with a quadrupole ICP-MS. The major contributions to the expanded uncertainty using the multicollector instrument are from the uncertainties associated with the natural isotopic abundances of Hg, therefore, in order to significantly reduce the uncertainty further, an improvement in the isotopic characterisation of Hg in the sample is required prior to species-specific ID. This will add considerable time to the analysis, with the possible need to resolve or account for interferences, *e.g.*, ^{204}Pb on ^{204}Hg. A number of elements have a relatively poor isotopic characterisation and, if ID is to become more widely used, a programme is required to improve the IUPAC data. The advent of modern multicollector ICP-MS instrumentation should allow this improvement. For the scanning quadrupole instrument the measured isotope amount ratios contributed >65% of the expanded uncertainty. As it is unlikely that the isotope amount ratio precision can be significantly improved the expanded uncertainty for quadrupole HPLC IDMS determinations will remain relatively large.

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Investigation of equilibration and uncertainty contributions for the determination of inorganic mercury and methylmercury by isotope dilution inductively coupled plasma mass spectrometry

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Abstract

The mass fractions of Hg and methylmercury, in two certified reference materials, NIST2710 and DORM-2, have been determined by total and species-specific isotope dilution analysis (IDA), respectively, and uncertainty budgets for each analysis calculated. The mass fraction of Hg in NIST2710 was determined by ID using multicollector sector field inductively coupled plasma mass spectrometry (MC-SF-ICP-MS) whilst the mass fraction of methylmercury in DORM-2 was determined using HPLC coupled with quadrupole ICP-MS.

The extent of equilibration between the spike and the particulate bound mercury compounds was studied temporally by monitoring the ^{200}Hg:^{199}Hg isotope amount ratio and by determining the total amount of Hg in the liquid phase. For the NIST2710 complete equilibration was only achieved when concentrated HNO_3 in combination with a microwave digestion was employed, and good agreement between the found ($31.7 \pm 4.0\ \mu g\ g^{-1}$, expanded uncertainty $k = 2$) and certified ($32.6 \pm 1.8\ \mu g\ g^{-1}$) values was obtained. For DORM-2 complete equilibration of methylmercury between the liquid and solid phases was achieved when using 50:50 H_2O:CH_3OH (v/v) and 0.01% 2-mercaptoethanol as the solvent. Even though only 50% of the analyte was extracted into the liquid phase, complete equilibration was achieved, hence, the found methylmercury mass fraction ($4.25 \pm 0.47\ \mu g\ g^{-1}$, expanded uncertainty $k = 2$) was in good agreement with the certified value ($4.47 \pm 0.32\ \mu g\ g^{-1}$).

Keywords: Inductive coupled plasma mass spectrometry; Isotope dilution analysis; Mercury speciation; Uncertainty contributions

1. Introduction

Isotope dilution analysis (IDA), which has recently been applied to inorganic mass spectrometry after many years of use in organic analysis, is regarded as a definitive analytical technique as the precision and accuracy obtainable are unsurpassed by alternative analytical methods [1]. The challenge for analysts employing ID for speciation studies is to achieve the accuracy and precision inherent in the method when applied to total elemental determinations. IDA which has been applied to a wide variety of sample matrices (soils [2], sediments [3], natural [4,5] and artificial waters [6], gases [7] and reference materials [8]) and target analytes (inorganic, organic, organometallic and complexes), comprises the modification of the natural isotopic abundance of an analyte in a sample

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by spiking with a standard of modified isotopic composition and known mass fraction. Two isotopes are selected as the reference and spike isotopes, respectively, and the resulting isotope amount ratio of the reference:spike isotopes after spiking is measured using a suitable mass spectrometric technique [9]. Inductively coupled plasma mass spectrometry (ICP-MS), both quadrupole (Q) and multicollector (MC), which is tolerant of complex sample matrices, utilises small sample sizes, is easily coupled with various separation techniques and does not suffer from time dependent mass bias effects [10], has been previously used for both isotope amount ratio and conventional external calibration measurements.

Incomplete analyte extraction can lead to low recovery when an external calibration standard is used to determine the analyte concentration. IDA relies solely on the measurement of isotope amount ratios, so problems associated with incomplete extraction are negated, provided that the spiked sample is extracted at the same efficiency as the analyte present in the sample [1]. The key stage, therefore, in the IDA procedure is the equilibration of the isotopically modified spike and the analyte contained within the sample. Provided equilibration is complete the spike material acts as the 'ideal' internal standard, because only isotope amount ratios are measured and no external calibration is necessary, and analyte losses are compensated for by losses of the spike in the same proportion, thereby ensuring an accurate determination of the target analyte. The role of the spike material as an internal standard also negates problems associated with instrumental drift and matrix effects during mass spectrometric detection [10]. If the spike is not allowed to equilibrate fully with the sample, a different extraction efficiency for the spike will result, yielding errors in the measurement. For liquid samples, equilibration by gentle agitation should be sufficient, but for solid samples, equilibration may prove problematic because the analyte can be both adsorbed onto the surface and contained within the lattice structure of the sample matrix.

Species-specific IDA [11] requires that isotopically enriched analogues, or isotopomers, of the target analyte are synthesised. Synthesis and characterisation of ^{199}Hg enriched methylmercurychloride, using nuclear magnetic resonance spectroscopy and HPLC-ICP-MS has previously been reported by Clough et al. [12]. For organometallic species, in order to simplify the calculations, the analyte mass fraction, as determined by IDA, is calculated with respect to the metallic component of the species. In this paper we evaluated two methods of data extraction for the calculation of isotope amount ratios from the chromatograms obtained, namely peak integration and a pseudo-steady-state approach [13].

Every analytical measurement, upon which important decision are often made, has an associated uncertainty, resulting from errors arising in the various stages of sampling and analysis, which characterises the range of values within which the 'true' value is asserted to lie [14]. The estimation of the uncertainty associated with an analytical measurement is increasingly being recognised as an essential part of the measurement process [15], because it facilitates improved intercomparison of analytical results, and is a requirement for ISO accredited methods [16]. In order to estimate the uncertainty associated with an analytical measurement a clear statement of the measurand, and the quantities used to derive it, is required [17]. IDA allows the estimation of a measurement uncertainty which meets the highest metrological standards [18] because it is derived through a measurement equation which is described in terms of SI units. The formulation of an uncertainty budget can be used as a method development tool, allowing the analyst to identify which areas of the measurement process provide the major contributions to the overall measurement uncertainty, making it possible to focus on these areas in order to improve the accuracy and precision of the analytical result. It also allows an analytical method to be monitored in order to determine if the method is under control.

This paper describes the determination of total Hg and methylmercury in two certified reference materials, NIST2710 and DORM-2, respectively, by IDA. A method for monitoring the extent of equilibration between the natural isotopic abundance sample and the isotopically modified spike material has been developed, and the extent of the adsorption and desorption of the spike and analyte contained within the sample, and hence the degree of extraction of the natural isotopic abundance analyte has also been calculated. An uncertainty budget was constructed for both the IDA and conventional external calibration analyses to

allow the results of the two different methods to be compared.

2. Theory

2.1. The IDA uncertainty budget model

The formulation and calculation of an uncertainty budget for an analytical measurement requires that all sources of uncertainty associated with the analytical procedure are identified and incorporated into the model used for the calculation of the expanded uncertainty of the analytical result. In this case the basis for the uncertainty model was the IDA equation used to calculate the analyte mass fraction (Eq. (2.1)). IDA equations are available which use relative isotopic abundances [19], however, the uncertainty calculation is subsequently more complex as the isotopic abundances are correlated [18], hence isotope amount ratios are used in Eq. (2.1) (the isotope dilution equation), in preference to relative isotopic abundances:

$$C_x = C_y \frac{m_y}{m_x h} \frac{R_y - R_B}{R_B - R_x} \frac{\sum_i R_{ix}}{\sum_i R_{iy}} \quad (2.1)$$

where C_x is the concentration of the analyte in the sample, C_y the concentration of the analyte in the spike solution, m_y the mass of spike, m_x the mass of sample, h the moisture content correction factor, R_y the reference:spike isotope amount ratio in the spike, R_x the reference:spike isotope amount ratio in the sample, $\sum_i R_{ix}$ the isotope amount ratio of isotope i to the spike isotope in the sample, $\sum_i R_{iy}$ the isotope amount ratio of isotope i to the spike isotope in the spike, and R_B the reference:spike isotope amount ratio in the sample after spiking.

For this work ^{200}Hg was chosen as the reference isotope and ^{199}Hg as the spike isotope.

2.1.1. Mass bias correction

Quadrupole and sector field mass spectrometers and their associated ion optics do not transmit ions of different masses equally, therefore, if an elemental solution composed of two isotopes with an exactly 1:1 molar ratio is analysed using ICP-MS, a 1:1 isotope amount ratio will not necessarily be observed. Even very small mass biases can have deleterious effects on the accuracy of isotope amount ratio determinations, so a correction must always be made. For this work, as no mercury isotope amount ratio CRM was available, mass bias was corrected by the use of a Tl internal standard, and interpolation of the measured ^{205}Tl:^{203}Tl isotope amount ratio, using the Russell correction expression [20], shown in Eq. (2.2) (the Russell law used for mass bias correction):

$$\left(\frac{^{200}\mathrm{Hg}}{^{199}\mathrm{Hg}}\right)_{cor} = \frac{(^{200}\mathrm{Hg}/^{199}\mathrm{Hg})_{mes}}{((^{205}\mathrm{Tl}/^{203}\mathrm{Tl})_{mes}/(^{205}\mathrm{Tl}/^{203}\mathrm{Tl})_{cer})^{(\ln(\mathrm{RAM}^{200}\mathrm{Hg}/\mathrm{RAM}^{199}\mathrm{Hg})/\ln(\mathrm{RAM}^{205}\mathrm{Tl}/\mathrm{RAM}^{203}\mathrm{Tl}))}} \quad (2.2)$$

where cor is the corrected isotope amount ratio, mes the measured isotope amount ratio, cer the certified isotope amount ratio and RAM the relative isotopic atomic mass.

2.1.2. The IDA uncertainty budget model incorporating mass bias correction

The value for each parameter in the isotope dilution equation, Eq. (2.1), obtained either by an analytical measurement or a mathematical calculation, has an associated standard uncertainty. In order to ensure that all sources of uncertainty were included in the uncertainty budget model, a cause and effect approach [15] was taken. The cause and effect diagram, shown in Fig. 1, consists of a branched hierarchical structure reducing to a single outcome, in this case the mass fraction of the mercury species as determined by IDA, C_x. Each main branch of the cause and effect diagram is a parameter in Eq. (2.1), which allowed the factors which contribute to the standard uncertainty of that parameter to be identified, quantified, combined and subsequently included in the uncertainty budget model.

The IDA equation (Eq. (2.1)) assumes that the measured isotope amount ratio in the sample (R_B) has been corrected for mass bias effects arising from the mass spectrometer. For the ^{200}Hg:^{199}Hg isotope amount ratio determined in each sample, mass bias was corrected for, using the Russell law (Eq. (2.2)), by monitoring the ^{205}Tl:^{203}Tl isotope amount ratio of the added internal standard. Each parameter in the Russell law has an associated uncertainty, which contributes to the overall uncertainty for the IDA procedure. Eqs. (2.1) and (2.2) were therefore combined to give Eq. (2.3) (the ID

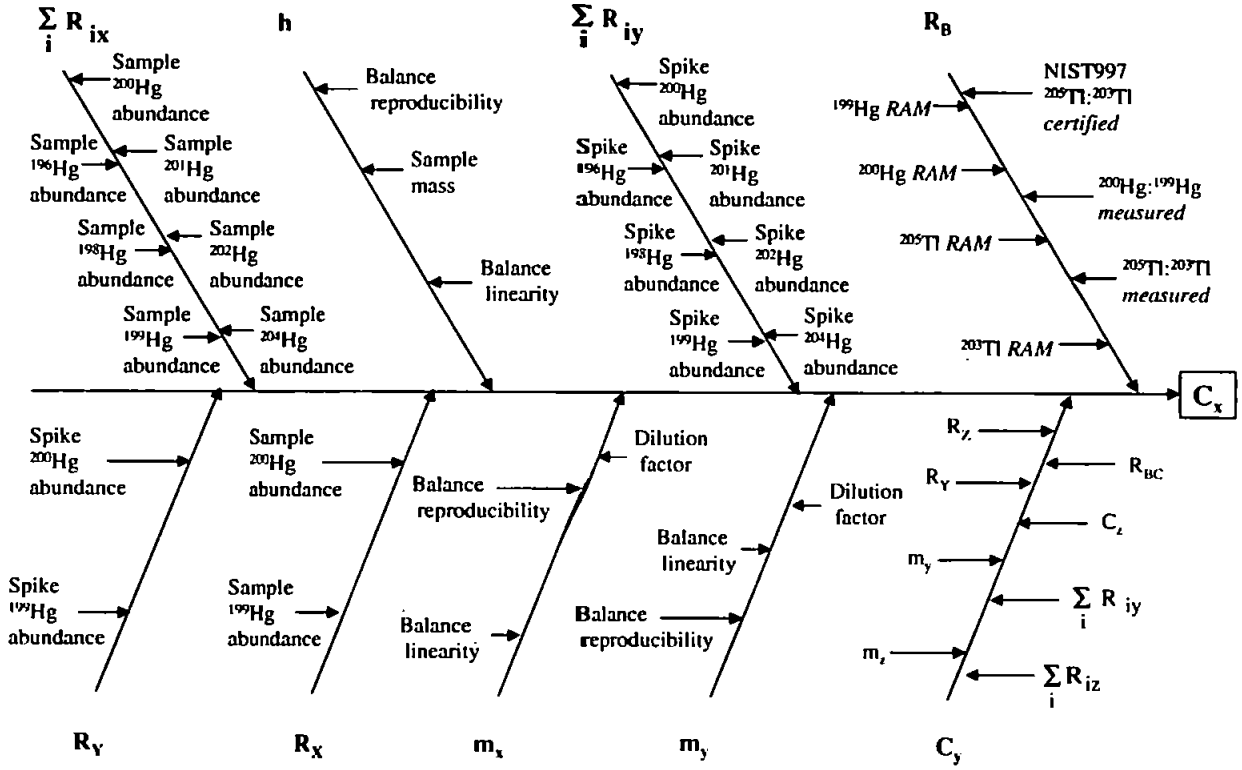

Fig. 1. Cause and effect uncertainty diagram for the determination of mercury species by IDA.

equation incorporating the mass bias correction term), the model for the calculation of the mass fraction of mercury in the CRM by IDA, the notation is the same as that used for Eqs. (2.1) and (2.2). The uncertainty budget was calculated following the guidelines set out by Eurachem/Citac [17]. Individual uncertainties associated with each parameter in Eq. (2.3) were combined, after conversion to a standard uncertainty, using the spreadsheet method first described by Kragten [21]:

$$C_x = C_y \frac{m_y}{m_x h} \frac{R_y - (((^{200}Hg/^{199}Hg)_{mes}/((^{205}Tl/^{203}Tl)_{mes}/(^{205}Tl/^{203}Tl)_{cer})^{(\ln(RAM^{200}Hg/RAM^{199}Hg)/\ln(RAM^{205}Tl/RAM^{203}Tl))}) \frac{\sum_i R_{ix}}{\sum_i R_{iy}}}{((^{200}Hg/^{199}Hg)_{mes}/((^{205}Tl/^{203}Tl)_{mes}/(^{205}Tl/^{203}Tl)_{cer})^{(\ln(RAM^{200}Hg/RAM^{199}Hg)/(RAM^{205}Tl/RAM^{203}Tl))}) - R_x} \quad (2.3)$$

2.2. External calibration uncertainty model

An uncertainty budget was also formulated for the determination of Hg species by interpolation from an external calibration curve. Most software packages use an unweighted regression to derive the equation that describes the data from a calibration series. This method does not take into account the variation in the instrumental response for each of the calibration standards [22], thus underestimating the uncertainty associated with the calculation of the mass fraction of the analyte contained in a sample. A weighted regression equation, however, includes the standard deviation of the instrumental response for the calibration standards, and was therefore employed, calculated according to Miller and Miller [22], for the calculation of mass fractions determined using an

external calibrant. In addition, the standard uncertainty associated with correction for instrumental drift, using an internal standard, was also included in the calculations. The uncertainty associated with the predicted value of the analyte (x_{pred}), from the weighted linear regression equation, was subsequently calculated and multiplied by a coverage factor to give the expanded uncertainty for the predicted mass fraction in solution.

3. Experimental

The extent of equilibration between the IDA spike solution and the natural isotopic abundance analyte was determined for two certified reference materials. National Institute of Science and Technology (NIST) 2710 Montana Soil Certified Reference Material (CRM) was used to represent terrestrial material and DORM-2 dog fish muscle (National Research Council Canada-NCR) was employed as a biological sample. NIST2710 is certified for total Hg, 32.6 $\mu g\,g^{-1}$, and DORM-2 for methylmercury, 4.47 $\mu g\,g^{-1}$, both reference materials were purchased from LGC (Teddington, UK). Two spike solutions were prepared from ^{199}Hg enriched (65.98 atom%) HgO (AEA Technology, Harwell, UK). For the total Hg IDA determination (NIST2710 CRM) a ^{199}Hg enriched Hg^{2+} spike solution was prepared, 3.4 mg of the enriched material was dissolved in 100 μg of concentrated HNO_3 (Aristar Grade, BDH, Poole, UK) and diluted with 18 $M\Omega\,cm^{-1}$, distilled, deionised water (DDW, Elgastat Maxima System Elga, High Wycombe, UK) to give a nominal mass fraction of 600 $\mu g\,g^{-1}$ in 2% HNO_3.

For the species-specific methylmercury IDA (DORM-2 CRM) ^{199}Hg enriched methylmercurychloride was synthesised from the AEA Technology ^{199}Hg enriched HgO and characterised by ^{1}H nuclear magnetic resonance spectroscopy [12]. Approximately 4 mg of the ^{199}Hg enriched methylmercurychloride spike material was dissolved in 0.9 g of HPLC grade methanol (BDH, Poole, UK) and subsequently diluted with 50:50 H_2O:CH_3OH (v/v) and 0.01% 2-mercaptoethanol (BDH, Poole, UK) to give a nominal mass fraction of 350 $\mu g\,g^{-1}$ as Hg. This solution was further diluted with 50:50 H_2O:CH_3OH (v/v) and 0.01% 2-mercaptoethanol to give a stock ^{199}Hg enriched methylmercurychloride spike solution of approximately 10 $\mu g\,g^{-1}$ as Hg. The two spike solutions were characterised by reverse IDA because no purity data was supplied with the ^{199}Hg enriched HgO. In this case the ^{199}Hg enriched spike solutions were treated as the sample and the isotopic composition modified by the addition of a well characterised natural standard and the ^{200}Hg:^{199}Hg isotope amount ratio measured by ICP-MS. Hydrobromic acid, 0.6 M (Aldrich, Gillingham, UK) was used as a wash solution during all ICP-MS analyses to counteract the memory effects of mercury.

3.1. Characterisation of the ^{199}Hg enriched inorganic Hg spike

The stock ^{199}Hg enriched inorganic Hg spike solution was diluted with 2% HNO_3 to approximately 600 $ng\,g^{-1}$, and the accurate mass fraction of the diluted spike solution determined by reverse IDA mass spectrometry. Alfa Aesar *Specpure* ICP standard solution (Johnson Matthey, Royston, UK) was diluted to 170 $ng\,g^{-1}$ Hg in 2% HNO_3 and used as the natural standard for the isotopic dilution of the spike solution. When the measured isotope amount ratio is close to unity, systematic errors, from sources such as mass bias and detector dead time, are minimised, thereby reducing the measurement uncertainty [19]. An iterative procedure [9] was therefore employed, to produce blends of the ^{199}Hg enriched inorganic Hg spike solution and the natural standard with a reference:spike isotope amount ratio (^{200}Hg:^{199}Hg) of close to unity. The ^{200}Hg:^{199}Hg isotope amount ratio (in the blend R_{BC}) was subsequently measured using an Axiom MC-SF-ICP-MS instrument (Thermo Elemental, Winsford, UK), and the Hg mass fraction of the spike solution calculated using Eq. (3.1) (the reverse IDMS equation). Operating conditions are shown in Table 1:

$$C_y = C_z \frac{m_z}{m_y} \frac{R_z - R_{BC}}{R_{BC} - R_y} \frac{\sum_i R_{iy}}{\sum_i R_{iz}} \quad (3.1)$$

where C_y is the concentration of the analyte in the spike solution, C_z the concentration of the natural isotopic abundance standard, m_z the mass of sample, m_y the mass of spike, R_z the reference:spike isotope amount ratio in the natural isotopic abundance standard, R_y the reference:spike isotope amount ratio in the spike, $\sum_i R_{iy}$ the isotope amount ratio of isotope

Table 1
ICP-MS and HPLC operating conditions

VG axiom MC-SF-ICP-MS operating conditions	
RF forward power (W)	1400
Reflected power (W)	≤10
Spray chamber	Coupled cyclonic and bead impact, cooled to −5 °C
Torch	Fassel quartz fitted with a Pt shield
Sampler and skimmer cones	Ni
Plasma gas (l min^{-1})	14
Auxiliary gas (l min^{-1})	0.85
Nebuliser gas (l min^{-1})	0.72
Dwell time (s)	10
Points per peak	1
Nebuliser	Glass expansion 0.2 ml/min micromist, natural aspiration
Ions monitored	^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, ^{204}Hg, ^{205}Tl, ^{206}Pb
VG PQ3 ICP-MS operating conditions	
RF forward power (W)	1450
Reflected power (W)	≤5
Spray chamber	Cyclonic, cooled to −5 °C
Torch	Fassel quartz
Sampler and skimmer cones	Ni
Plasma gas (l min^{-1})	14
Auxiliary gas (l min^{-1})	0.75
Nebuliser gas (l min^{-1})	0.52
Dwell time (ms)	10
Points per peak	5
Nebuliser	Glass expansion 0.2 ml/min micromist, natural aspiration
Ions monitored	
IDA	^{199}Hg, ^{200}Hg, ^{203}Tl, ^{205}Tl
External calibration	^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{203}Tl, ^{204}Hg, ^{205}Tl, ^{206}Pb
HPLC conditions	
HPLC column	HiChrom Kromasil 100 FC 18 Excel, 25 cm × 4.6 mm i.d.
Mobile phase	50:50 (v/v) methanol:DDW, 0.01% 2-mercaptoethanol, 50 ng/g NIST997 Tl CRM
Flow rate (ml/min)	0.9
Injection volume (μl)	100

i to the spike isotope in the natural isotopic abundance standard, $\sum_i R_{iz}$ the isotope amount ratio of isotope i to the spike isotope in the spike, and R_{BC} the reference:spike isotope amount ratio in the spike and natural standard blend, corrected for mass bias effects.

^{200}Hg was chosen as the reference isotope and ^{199}Hg as the spike isotope.

3.2. Characterisation of the ^{199}Hg enriched CH_3HgCl spike

The methylmercurychloride spike was characterised by reverse IDA using a natural isotopic abundance methylmercury chloride standard (Alfa Aesar, Ward Hill, MA, USA) diluted to 11 μg g^{-1} in 50:50 H_2O:CH_3OH (v/v) and 0.01% 2-mercaptoethanol. For this analysis, solutions of the spike and natural standard were prepared to give a ^{200}Hg:^{199}Hg isotope amount ratio of close to unity. In order to measure the reference:spike isotope amount ratio of the methylmercury spike/natural standard blends, HPLC was coupled with the multicollector Axiom instrument, the HPLC conditions follow those of Harrington et al. [23] and are shown in Table 1. NIST997 thallium CRM was added to the mobile phase at 50 ng g^{-1} and continuously monitored during separations for the purposes of mass bias correction (Eq. (2.2)). The HPLC system was not directly coupled to the nebuliser sample uptake tube but a low dead volume splitter was used. This allowed the nebuliser to operate at its natural uptake rate, as determined by the nebuliser argon gas flow, and the system to be optimised for maximum sensitivity and optimum peak shape without compromising the HPLC conditions. A low flow nebuliser (0.2 ml/min Micromist, Glass Expansion, Vevey, CH) was employed in conjunction with Ø0.18 mm Teflon uptake tubing. The uptake rate of this system was 50 μl/min, thereby ensuring highly efficient nebulisation and, in conjunction with a coupled spray chamber system (cyclonic and bead impact, Glass Expansion, Vevey, CH) cooled to −5 °C, reduced the organic loading in the plasma. The methylmercury mass fraction in the stock spike solution used to produce the spike/natural standard blends was calculated using Eq. (3.1).

3.3. Equilibration of ^{199}Hg enriched inorganic Hg and NIST2710 CRM

The moisture content of the NIST2710 CRM was determined, by drying separate subsamples to a constant mass at 105 °C, to give the moisture correction factor, h (Eq. (2.1)). Approximately 2 g of the NIST2710 CRM was accurately weighed and the amount of Hg present, corrected for moisture content, calculated from the certified value. The stock ^{199}Hg

enriched inorganic Hg spike solution was diluted to 55 $\mu g\,g^{-1}$ with 2% HNO_3. Approximately 1 g of this diluted spike solution was added to 40 ml of the equilibration solution, 50:50 H_2O:CH_3OH (v/v) and 0.01% 2-mercaptoethanol, contained in a clean 50 ml glass conical flask. The NIST2710 CRM was subsequently added to the equilibration solution and 1 ml samples withdrawn at intervals up to 3000 min. The sampling frequency was approximately every 2 min for the first 25 min of the experiment then at 5–10 min intervals until 1 h had elapsed, with less frequent sampling thereafter. The equilibration solution was agitated throughout by a magnetic stirrer, and maintained at a temperature of 25 °C by means of an electrically heated hotplate. Each sample aliquot was pipetted into a filter (Autovial 0.2 μm PTFE membrane syringeless filters, Whatman, Maidstone, UK), diluted prior to filtration with 8 ml of fresh equilibration solvent, filtered, and stored in a clean 25 ml sterilin container at 4 °C until analysis by MC-SF-ICP-MS. The instrumental operating conditions are given in Table 1. Hydrobromic acid (0.6 M; Aldrich, Gillingham, UK) was used as a wash solution to counter memory effects, and thallium (NIST911 CRM; LGC, Teddington, UK) added to the samples as an internal standard at approximately 300 ng/g to correct for mass bias and instrumental drift.

3.4. Equilibration of ^{199}Hg enriched methylmercury and DORM-2 CRM

This procedure followed that described for inorganic Hg, but with the following alterations to the method. Approximately 3 g of the DORM-2 CRM was accurately weighed and the amount of methylmercury present, corrected for moisture content, calculated from the certified value. The mass of spike solution added was chosen so that the ^{200}Hg:^{199}Hg isotope amount ratio would be close to unity, provided complete equilibration between the sample and spike occurred. The equilibration solution volume was reduced to 30 ml, the experimental timespan to 1500 min and samples were diluted to a volume of 4 ml prior to filtration. Samples obtained from the DORM-2 equilibration experiments were analysed using HPLC-Q-ICP-MS, with the optimum operating conditions shown in Table 1. NIST911 thallium CRM was added to the HPLC mobile phase as an internal standard at approximately 50 ng/g to correct for mass bias.

3.5. Conventional external calibration

For the equilibration experiment involving NIST-2710, the total Hg in solution for each sample, was measured by external calibration at the same time as the isotope amount ratio determination. In order to obtain a useful calibration it was necessary to sum the signals obtained for all Hg isotopes (i.e. 198, 199, 200, 201, 202 and 204), because the isotopic abundances of the samples and calibration standards were different. Hence, the total Hg instrumental response was calculated, and the Hg mass fraction in solution interpolated from the calibration curve. The isotopic abundance of ^{196}Hg is the same in both the calibration standards and the samples, however, it has an isotopic abundance of only 0.1%, so it could not be used as the calibration isotope, and was omitted from the experiment because there were only nine detectors available on the Axiom MC-SF-ICP-MS and it was necessary to monitor ^{206}Pb in order to correct for interferences from ^{204}Pb on ^{204}Hg. ^{203}Tl was used as an internal standard to correct for instrumental drift.

The methylmercury content in the equilibration samples taken during the experiment involving DORM-2 CRM was calculated in a similar fashion, but with HPLC coupled to the PQ3 instrument. The methylmercurychloride peak in each chromatogram for the isotopes ^{198}Hg to ^{204}Hg was baseline subtracted, integrated, and the integrals summed to give the total methylmercurychloride instrumental response. The signal for ^{204}Hg was again corrected for by monitoring ^{206}Pb. This analysis was performed separately from the isotope amount ratio determinations as the requirement to monitor nine isotopic signals resulted in spectral skew of the resulting chromatograms. NIST911 thallium CRM was added to the HPLC mobile phase as an internal standard at approximately 50 ng/g to correct for instrumental drift.

3.6. Microwave digests

Microwave digestions were performed on NIST2710 using two different digestants, concentrated HNO_3 and 50:50 methanol:DDW (v/v) with 0.01% 2-mercaptoethanol. Approximately 150 mg of NIST2710 was

accurately weighed directly into a Teflon bomb and 4 ml of digestant added. The mass of Hg added via the NIST2710 reference material was calculated and an equivalent mass of Hg added via the ^{199}Hg enriched spike solution. The bomb lids were tightened and each digestion left to equilibrate for 24 h prior to microwaving at 650 W for 2 min using a domestic microwave oven. The digests were filtered (Autovial 0.2 μm PTFE membrane syringeless filters) and the filtered extract diluted to approximately 25 g with either DDW or 50:50 methanol:DDW (v/v) and 0.01% 2-mercaptoethanol solution, depending on the original digestant, and stored at 4 °C until analysis by the Axiom MC-SF-ICP-MS.

4. Results and discussion

4.1. Equilibration of reference materials with the ^{199}Hg enriched spikes

The extent of equilibration between the spike and the natural isotopic abundance particulate bound Hg species was determined by a comparison of the theoretical ^{200}Hg:^{199}Hg isotope amount ratio at complete equilibration and the measured isotope amount ratio in solution. The theoretical ^{200}Hg:^{199}Hg isotope amount ratio was calculated for each experiment from the masses and mercury mass fractions of the starting materials using Eq. (4.1) where l is the liquid phase, s the solid phase, e the ^{199}Hg enriched spike, n the natural abundance mercury, eqm the equilibrium and t the time:

$$\frac{^{200}\mathrm{Hg}_{\mathrm{l}}^{t=\mathrm{eqm}}}{^{199}\mathrm{Hg}_{\mathrm{l}}^{t=\mathrm{eqm}}} = \frac{^{200}_{\mathrm{e}}\mathrm{Hg}_{\mathrm{l}}^{t=0} + {}^{200}_{\mathrm{n}}\mathrm{Hg}_{\mathrm{s}}^{t=0}}{^{199}_{\mathrm{e}}\mathrm{Hg}_{\mathrm{l}}^{t=0} + {}^{199}_{\mathrm{n}}\mathrm{Hg}_{\mathrm{s}}^{t=0}} \quad (4.1)$$

IUPAC data [24] was used for the relative isotopic abundances of natural Hg. No uncertainty data was supplied with the ^{199}Hg enriched HgO, therefore the isotopic abundances of this material were measured by MC-SF-ICP-MS, and the subsequent uncertainties associated with these measurements calculated [12]. The isotopic abundances used, for the natural and ^{199}Hg enriched mercury, are shown in Table 2. It is important to note that the Hg originating from the solid phase (i.e. the particles of the CRM in solution) would have a natural isotopic composition but the Hg added to the liquid phase was of modified isotopic composition (i.e. the ^{199}Hg enriched spike). The expanded uncertainty ($k = 2$) for the theoretical equilibrium ratio for each experiment was calculated using the standard uncertainties of the masses, concentrations, and isotopic abundances of both the reference material and ^{199}Hg enriched spike solution, Eq. (4.1) was used as the model for these calculations.

4.1.1. Equilibration between ^{199}Hg enriched inorganic Hg and NIST2710

The extent of equilibration, between the natural abundance particulate bound inorganic Hg (NIST2710) and the ^{199}Hg enriched inorganic Hg spike, was determined in the equilibration solution, from the measured ^{200}Hg:^{199}Hg isotope amount ratio, corrected for mass bias using Eq. (2.2). The ^{200}Hg:^{199}Hg isotope amount ratio in the liquid phase over time is shown in Fig. 2. Equilibration initially proceeded at a rapid rate up to 100 min, with the system reaching an equilibrium at approximately 300 min. Complete equilibration, between the ^{199}Hg enriched inorganic Hg spike and the particulate bound

Table 2
The isotopic abundance and uncertainty data for natural abundance Hg and the AEA Technology ^{199}Hg enriched HgO

Isotope	Natural abundance Hg		AEAT ^{199}Hg enriched HgO	
	IUPAC data [24] (atom%)	Standard uncertainty	Axiom MC-SF-ICP-MS (atom%)	Standard uncertainty [12]
196	0.15	0.01	0.15	0.001
198	9.97	0.12	2.93	0.0014
199	16.87	0.13	65.98	0.010
200	23.1	0.11	18.15	0.0016
201	13.18	0.05	3.96	0.0019
202	29.86	0.15	7.43	0.0047
204	6.87	0.09	1.44	0.0012

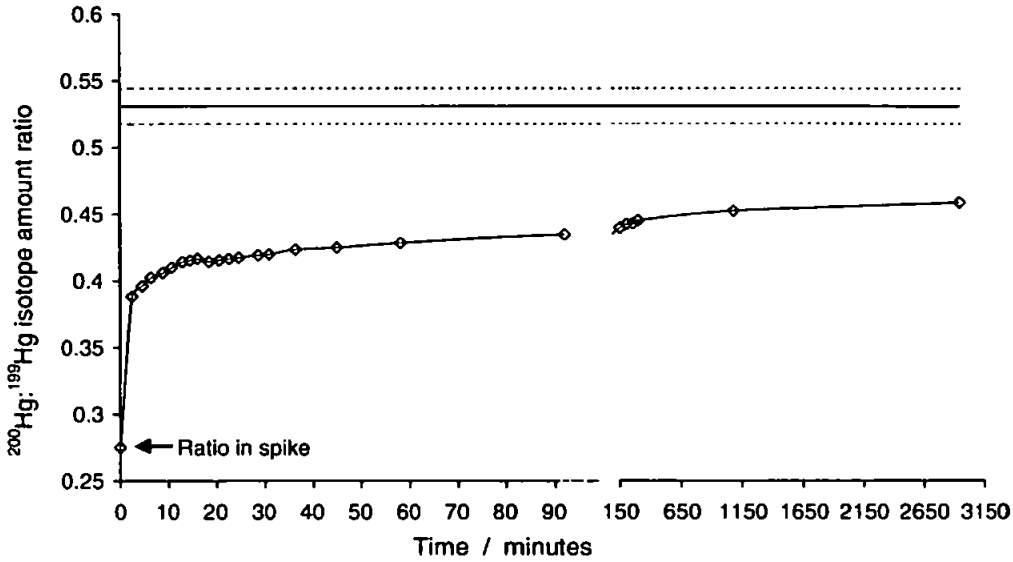

Fig. 2. Change in $^{200}Hg:^{199}Hg$ isotope amount ratio in solution for the equilibration with NIST2710 over time, solid curve. The solid line is the theoretical $^{200}Hg:^{199}Hg$ isotope amount ratio at complete equilibration, with the dashed lines representing the upper and lower limits of the expanded uncertainty, calculated using a coverage factor, k, of 2, which gives a level of confidence of approximately 95%.

natural isotopic abundance Hg, was not achieved because the $^{200}Hg:^{199}Hg$ isotope amount ratio in the equilibration did not attain the theoretical $^{200}Hg:^{199}Hg$ isotope amount ratio (Fig. 2). The mass fraction of Hg in NIST2710, calculated by IDA from the $^{200}Hg:^{199}Hg$ isotope amount ratio in the final sample taken during the equilibration experiment (3000 min), was $21.5 \pm 2.7\,\mu g\,g^{-1}$ (expanded uncertainty, $k = 2$). Complete equilibration between the sample and the spike did not occur so the mass fraction of Hg in NIST2710, determined by IDA, underestimated the certified value of $32.6 \pm 1.8\,\mu g\,g^{-1}$ by 34%.

Hg, and other metals, are coprecipitated with Fe and Mn oxyhydroxides in aquatic systems and subsequently trapped within this precipitate layer as further precipitation occurs [25]. NIST2710 CRM was prepared from soil, that was collected from land that is periodically flooded, with waters from settling ponds which contain high levels of Mn. It is thus likely that several different Fe and Mn oxyhydroxide layers had built up on the particles, rendering some of the particulate Hg unavailable for equilibration with the spike Hg dissolved in a mild solvent, causing the underestimation of Hg in NIST2710.

Two IDA microwave digestions, one with concentrated HNO_3 and the second using 50:50 methanol:DDW (v/v) and 0.01% 2-mercaptoethanol were also performed on the NIST2710 CRM. The NIST2710 CRM was completely solubilised by the concentrated HNO_3 microwave digestion, and the Hg mass fraction, as determined by IDA, of $31.7 \pm 4.0\,\mu g\,g^{-1}$ ($k = 2$) was in good agreement with the NIST2710 certified value. In contrast, the microwave digestion using 50:50 methanol:DDW (v/v) and 0.01% 2-mercaptoethanol did not completely dissolve the NIST2710CRM, and the Hg mass fraction was calculated to be $23.6 \pm 3.0\,\mu g\,g^{-1}$ ($k = 2$), comparable to that obtained, within the limits of uncertainty, by the time resolved isotope dilution equilibration analysis. It was deduced that, when 50:50 methanol:DDW (v/v) and 0.01% 2-mercaptoethanol was employed as the solvent, the ^{199}Hg enriched spike was equilibrated only with surface bound Hg, e.g. Hg bound to sulphur containing groups of the fulvic/humic acid layer of the particles. The results of the three different IDA determinations of Hg in NIST2710 CRM are shown in Fig. 3.

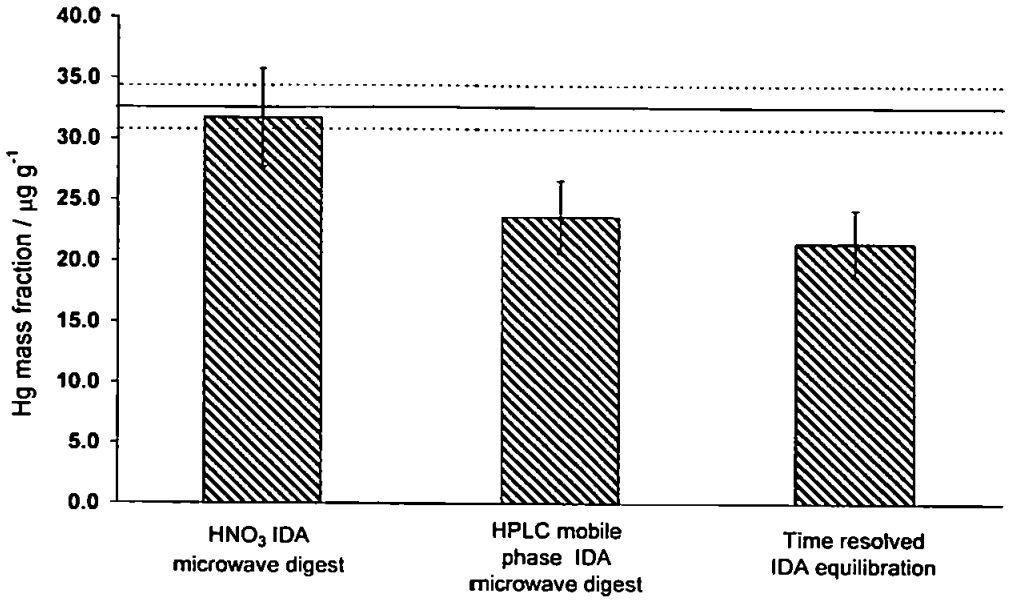

Fig. 3. The mass fraction of Hg in NIST2710 obtained from three different IDA methods. The solid horizontal line is the certified mass fraction of Hg in NIST2710, with dashed lines showing the upper and lower limits (95% confidence interval). The uncertainties for the three experimental results are the expanded uncertainty ($k = 2$).

4.1.2. *Equilibration between ^{199}Hg enriched methylmercurychloride and DORM-2*

Equilibration between the ^{199}Hg enriched methylmercurychloride spike and the natural isotopic abundance methylmercury in DORM-2 was monitored in the same fashion as that used for the soil CRM. The rate of equilibration for DORM-2, shown in Fig. 4, proceeded at an initial rapid rate. Complete equilibration,

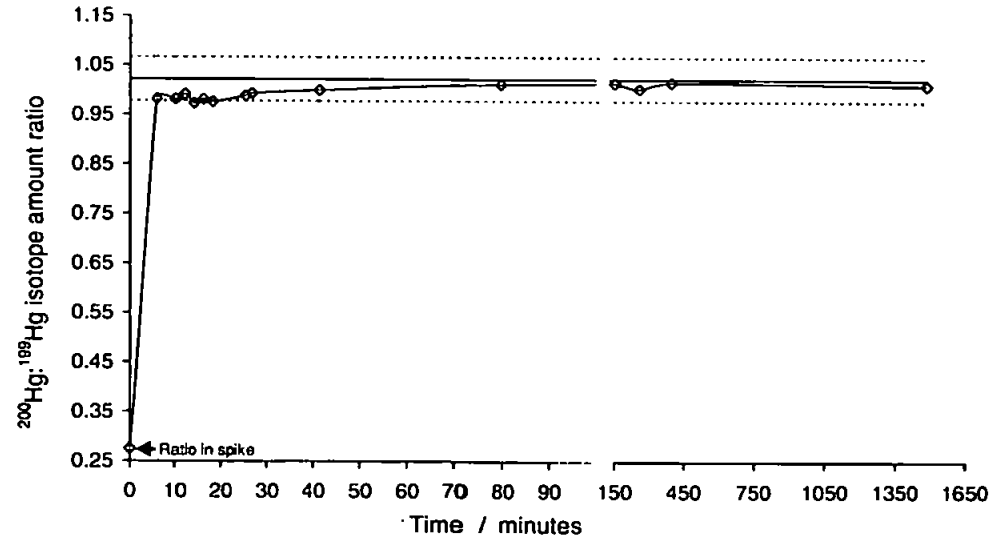

Fig. 4. Change in ^{200}Hg:^{199}Hg isotope amount ratio in solution for the equilibration with DORM-2 over time, solid curve. The solid line is the theoretical ^{200}Hg:^{199}Hg isotope amount ratio at complete equilibration, with the dashed lines representing the upper and lower limits of the expanded uncertainty ($k = 2$).

within the limits of uncertainty, was attained within 6 min from the start of the experiment. The mass fraction of methylmercury in DORM-2, determined by species-specific HPLC-IDA-Q-ICP-MS from the ^{200}Hg:^{199}Hg isotope amount ratio in the final sample taken at 1500 min, was $4.25 \pm 0.47\,\mu g\,g^{-1}$ ($k = 2$), in good agreement with the certified value of $4.47 \pm 0.32\,\mu g\,g^{-1}$.

4.2. Adsorption and desorption

The isotope amount ratio in solution (R), at any time during the equilibration of enriched and natural abundance isotopomers of an analyte is given by Eq. (4.2). Furthermore the total number of moles of the analyte in solution (n), at any time is the sum of the number of moles of both isotopomers in solution (Eq. (4.3)). Hence, combining Eqs. (4.2) and (4.3) to give Eq. (4.4), with rearrangement to Eq. (4.5), and determining R and n, allows the extent of desorption and adsorption of the natural isotopic abundance particulate bound analyte and the spike analyte, respectively:

$$R = \frac{A_y n_y + A_x n_x}{B_y n_y + B_x n_x} \tag{4.2}$$

where A_y is the abundance of isotope A in the spike, A_x the abundance of isotope A in the sample, B_y the abundance of isotope B in the spike, and B_x the abundance of isotope A in the sample

$$n = n_y + n_x, \quad \therefore n_y = n - n_x \tag{4.3}$$

where n is the total number of moles of analyte, n_y the total number of moles of spike analyte, and n_x the total number of moles of sample analyte. Substituting Eq. (4.3) into Eq. (4.2) gives

$$R = \frac{A_y(n - n_x) + A_x n_x}{B_y(n - n_x) + B_x n_x} \tag{4.4}$$

and rearranging gives

$$n_x = n\frac{A_y - RB_y}{(RB_x - A_x) + (A_y - RB_y)} \tag{4.5}$$

4.2.1. NIST2710

The extent of desorption of Hg from the particulate phase (i.e. the natural abundance Hg arising from the NIST2710 CRM) and the adsorption from solution onto the particulate phase (i.e. adsorption of the spike Hg) is shown in Fig. 5. As can be seen 24% of the Hg from NIST2710 was desorbed from the particles after only 3 min (Fig. 5B), with no significant change, within the limits of uncertainty until 1100 min. Thereafter, the amount of natural isotopic abundance Hg in solution increased to 37% at the end of the experiment (3000 min), which yielded a mass fraction of Hg in NIST2710 of $12.1 \pm 2.3\,\mu g\,g^{-1}$, as determined by external calibration. The certified value of Hg in NIST2710 was underestimated by 63% with a relative expanded uncertainty of 19%. A coverage factor (k) of 4.3 (obtained from t-tables) was used to expand the standard uncertainty, as only two degrees of freedom were available from the four point calibration curve [22]. The adsorption curve for the ^{199}Hg enriched Hg^{2+} spike (Fig. 5A), showed an initial rapid adsorption, with 38% remaining in solution (i.e. 62% adsorbed onto the NIST2710 particles) during the first 11 min. Thereafter the amount of spike in solution remained constant until 1100 min had elapsed, whereupon a net desorption of the spike occurred, with 58% in solution at 3000 min. The net desorption of both the natural isotopic abundance and the spike Hg from 1100 min was attributed to oxidation, by the equilibration solvent, of the binding site substrate on the NIST2710 particles.

4.2.2. DORM-2

The extent of adsorption of the spike and natural methylmercury is shown in Fig. 6. Forty eight percent of the methylmercury in the DORM-2 CRM was desorbed within 6 min of the start of the experiment (Fig. 6B), with the amount in solution remaining relatively constant for the remainder of the experiment. Likewise, 65% of the methylmercurychloride spike had been adsorbed onto the particles of DORM-2 after 6 min (Fig. 6B), and this amount remained relatively constant for the duration of the experiment. The mass fraction of methylmercury in the DORM-2-CRM, as determined by external calibration from the final sample taken at 1500 min, was $2.10 \pm 0.5\,\mu g\,g^{-1}$, an underestimation of 53% compared with the certified value. By comparison the mass fraction determined by IDA for the final sample was $4.25 \pm 0.47\,\mu g\,g^{-1}$ ($k = 2$), certified value of $4.47 \pm 0.32\,\mu g\,g^{-1}$ ($k = 4.3$). So, although only 53% of the methylmercury in DORM-2 had been brought into solution by the action of the equilibration solvent, complete equilibration had been achieved, demonstrating that complete extraction of

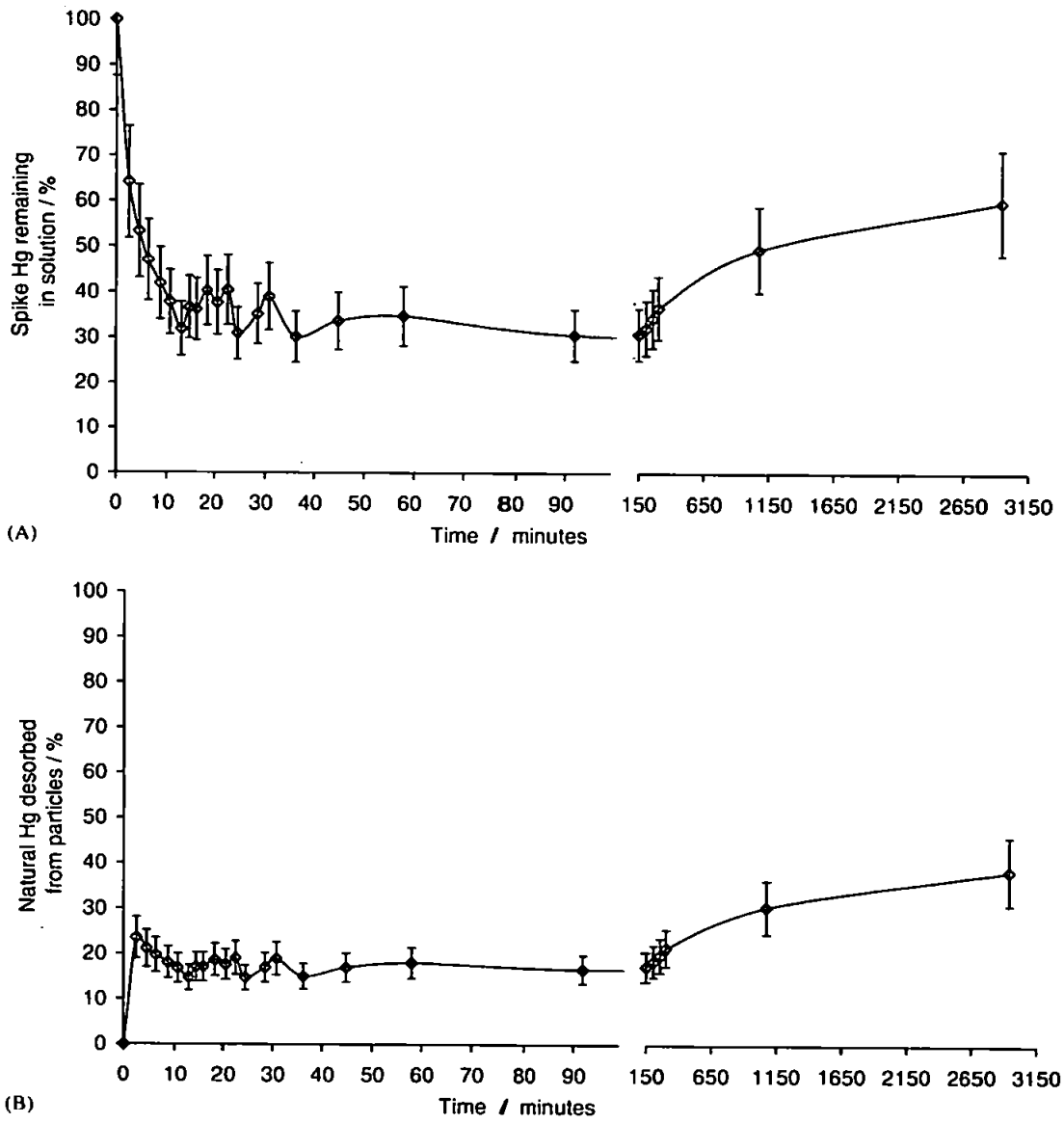

Fig. 5. The amount of (A) ^{199}Hg enriched spike Hg remaining in solution and (B) particulate bound natural isotopic Hg desorbed during the IDA determination of Hg in NIST2710. Uncertainty bars are the expanded uncertainty ($k = 4.3$).

the analyte is not necessary for accurate results by IDA.

4.3. Contributions to uncertainty

4.3.1. Isotope dilution analysis

The standard uncertainty of the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio was calculated by combining the uncertainty of each parameter in Eq. (2.2). For the determination of inorganic Hg in NIST2710 a multicollector ICP-MS instrument, which measures the ion signal of each isotope simultaneously, was used. The relative standard uncertainty for the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio was typically 0.03%, with the major contributions to this figure derived from the measured

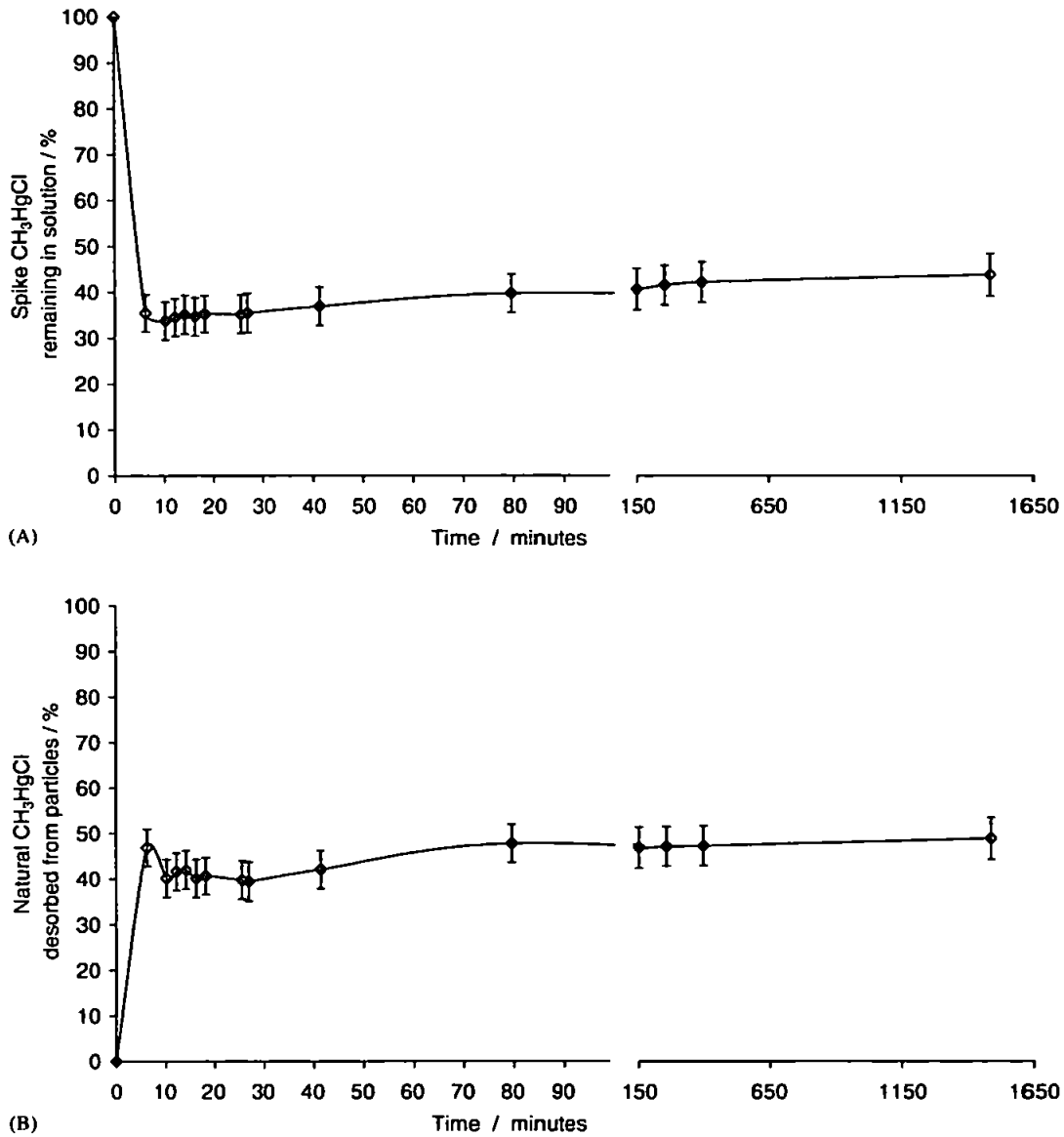

Fig. 6. The amount of (A) ^{199}Hg enriched spike Hg remaining in solution and (B) particulate bound natural isotopic Hg desorbed during the IDA determination of methylmercury in DORM-2. Uncertainty bars are the expanded uncertainty ($k = 4.3$).

^{200}Hg:^{199}Hg isotope amount ratio (43%), the measured ^{205}Tl:^{203}Tl isotope amount ratio (39%) and the NIST997 certified ^{205}Tl:^{203}Tl isotope amount ratio (18%).

For the determination of methylmercury in DORM-2, HPLC was coupled with a quadrupole ICP-MS instrument because the Faraday collectors on the MC-SF-ICP-MS were not sensitive enough. For these measurements the relative standard uncertainty of the mass bias corrected ^{200}Hg:^{199}Hg isotope amount ratio was typically 1.2%. In this case the measured isotope amount ratios, ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl, contributed 80 and 20%, respectively, to the combined standard uncertainty. The higher

Table 3
The mass fraction and expanded uncertainty for the determination of Hg in NIST2710 by IDA and external calibration[a]

NIST2710 total Hg analytical method	Hg mass fraction ($\mu g\,g^{-1}$)	Expanded uncertainty		Relative contributions to the expanded uncertainty (%)		
		Absolute	Relative (%)	C_y	R_x	x_{pred}
Time resolved IDA	21.5	±2.7 ($k = 2$)	13	95	4	
HNO_3 IDA microwave digest	31.7	±4.0 ($k = 2$)	13	95	5	
HPLC mobile phase IDA microwave digest	23.6	±3.0 ($k = 2$)	13	95	5	
External calibration	12.1	±2.3 ($k = 4.3$)	19			100
Certified value	32.6	±1.8	6			

[a] The reported uncertainty is the expanded uncertainty, calculated using a coverage factor (k) which gives a level of confidence of approximately 95%.

standard uncertainty observed using HPLC-Q-ICP-MS was due to the sequential nature of the instrument, so isotope amount ratio determinations were susceptible to fluctuations in the ion signal caused by the sample introduction system, and the fact that transient signals, resulting from HPLC sample introduction, were being monitored. The best accuracy and precision is obtained when continuous ion signals are measured for several minutes [9]; however, the use of the pseudo-steady-state approach [11–13] for the calculation of isotope amount ratios minimised the effects of transient signal measurements.

The combined standard uncertainty (u) of the final analytical result is comprised of contributions from the individual standard uncertainties of the parameters in Eq. (2.3), combined as shown in Fig. 1. The combined standard uncertainty is then multiplied by a coverage factor (k), the value of which depends upon the available degrees of freedom, to obtain the expanded uncertainty (U), which approximates to the 95% confidence interval. The contribution of the individual parameters in Eq. (2.3) can also be expressed in terms of their relative contribution to the expanded uncertainty as shown in Tables 3 and 4, for NIST2710 and DORM-2, respectively. For the analysis of NIST2710 the uncertainty budget was dominated, in all cases, by the uncertainty associated with the mass fraction of the ^{199}Hg enriched inorganic Hg spike solution (C_y) which contributed 95% of the combined uncertainty. The ^{200}Hg:^{199}Hg isotope amount ratio of natural abundance mercury (R_x) contributed 4%, with the other parameters in Eq. (2.3) contributing less than 1%.

The relative contributions to the expanded uncertainty of the mass fraction of methylmercury in DORM-2, determined by IDA, are shown in Table 4. The major contributions arose from the measured ^{200}Hg:^{199}Hg isotope amount ratio (43%) and the theoretical ^{200}Hg:^{199}Hg isotope amount ratio (R_x, 35%), derived from the uncertainty associated with the natural isotopic abundance of mercury [24]. Lesser contributions arose from the spike mass fraction (C_y, 12%), the measured ^{205}Tl:^{203}Tl isotope amount ratio used for mass bias correction (9%), and $\sum R_{ix}$ (1%). Mercury is a relatively poorly characterised

Table 4
The mass fraction and expanded uncertainty for the determination of methylmercury in DORM-2 by IDA and external calibration[a]

DORM-2 methylmercury analytical method	Methylmercury mass fraction ($\mu g\,g^{-1}$)	Expanded uncertainty		Relative contributions to the expanded uncertainty (%)				
		Absolute	Relative (%)	C_y	R_x	$^{200}Hg/^{199}Hg$	$^{205}Tl/^{203}Tl$	x_{pred}
Time resolved IDA	4.25	±0.47 ($k = 2$)	11	12	35	43	9	
External calibration	2.10	±0.5 ($k = 4.3$)	24					100
Certified value	4.47	±0.32	7.1					

[a] The reported uncertainty is the expanded uncertainty, calculated using a coverage factor (k) which gives a level of confidence of approximately 95%.

element, with relatively large uncertainties associated with its isotopic composition, hence, if the precision and accuracy of the measurement of isotope amount ratios is further improved these uncertainties will start to dominate the expanded uncertainty for the measurement of mercury species by IDA.

It is interesting to compare the relative uncertainty contribution of the spike (C_y) to the expanded uncertainty of the analyte mass fraction (C_x) determined in DORM-2 and NIST2710. In the latter case the relative contribution was much greater than the former (95%, cf. 12%) even though the standard uncertainty of the mass fraction of each spike solution was similar ($55.3 \pm 3.4\,\mu g\,g^{-1}$, cf. $11.1 \pm 0.21\,\mu g\,g^{-1}$). The reason for this is that the measured ^{200}Hg:^{199}Hg and ^{205}Tl:^{203}Tl isotope amount ratios were determined using the MC-SF-ICP-MS instrument in the final IDA of NIST2710, thereby resulting in extremely small standard uncertainties for these parameters, so their relative contribution to the expanded uncertainty fell to less than 0.03%, with a consequent increase in the relative contribution of C_y.

4.3.2. External calibration

An uncertainty budget was also formulated for the determination of Hg in NIST2710 and methylmercury in DORM-2 by external calibration, and the expanded uncertainty for each analysis calculated (Tables 3 and 4, respectively). For both these analyses the uncertainty budget was dominated (100% after rounding) by the standard deviation of the mercury mass fraction predicted from the weighted regression calibration curve (x_{pred}). In other words, the standard uncertainty of the regression fit dominated the final expanded uncertainty. For the total Hg mass fraction in NIST2710 the relative expanded uncertainty was lower for the IDA determinations (13%) than that for the external calibration (19%). Similarly, the IDA determination of methylmercury in DORM-2 again resulted in a lower relative expanded uncertainty (11%), compared with that obtained by external calibration (24%).

5. Conclusions

The mass fraction of Hg and methylmercury has been determined, by both total and species-specific IDA, in two certified reference materials, NIST2710 and DORM-2, respectively. For the analysis of total Hg in NIST2710, complete equilibration between the sample Hg and the added spike was only achieved when a microwave digestion was performed, with concentrated HNO_3 as the solvent. For this determination the found value of $31.7\,\mu g\,g^{-1}$ was in good agreement with the certified value of $32.6\,\mu g\,g^{-1}$. When 50:50 methanol:DDW (v/v) and 0.01% 2-mercaptoethanol was used as the extraction solvent incomplete equilibration and hence an underestimate of the certified value, resulted. Only 37% of the available Hg was extracted from NIST2710 using this solvent.

In the case of species-specific IDA for methylmercury in DORM-2, when 50:50 methanol:DDW (v/v) and 0.01% 2-mercaptoethanol was used as the extraction solvent complete equilibration was achieved, even though only 53% of the available methylmercury was extracted into solution, and the mass fraction of methylmercury ($4.25\,\mu g\,g^{-1}$) was in good agreement with the certified value ($4.47\,\mu g\,g^{-1}$), illustrating that complete equilibration, rather than complete extraction, is required for to yield accurate results using IDA.

In comparison analysis by external calibration yielded analyte recoveries of approximately half that achieved by IDA, reflecting the poor extraction of the analytes into solution. The expanded uncertainty was calculated for each analytical method, and improved precision was obtained using IDA compared to external calibration.

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