FIRMS

Forensic Isotope Ratio Mass Spectrometry Network

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GOOD PRACTICE GUIDE

ISOTOPE RATIO MASS SPECTROMETRY

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Good Practice Guide for Isotope Ratio Mass Spectrometry

Second Edition 2018

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Preface to the second edition

While it has only been seven years since the first edition of the FIRMS Good Practice Guide for isotope ratio mass spectrometry (IRMS) was published, there have been a number of improvements to suggested methodologies for the determination of light-element isotope delta values. Most important are the changes to chemical packing of thermal conversion reactors for δ^2H determination that eliminate the formation of HCN and to suggested methods to account for extrinsic hydrogen.

These method improvements, together with a previous lack of information within this guide regarding compound-specific carbon, nitrogen and hydrogen as well as bulk sulphur isotope ratio analyses, which are all becoming more common, highlighted the need for the original FIRMS Good Practice Guide for IRMS to be updated. There has also been the recent discovery that LSVEC is unstable in terms of carbon isotope ratio.

The previous edition of this Guide contained a section regarding the interpretation of isotope ratio data in forensic contexts; however the FIRMS Network has since produced a separate guidance document regarding interpretation. We therefore refer interested readers to the separate document and include only a summary of the key points within this Guide.

While the some of the content may be new or revised, the basic tenets of this Guide remain the same, which are to help users to understand how their instrumentation works and know whether or not the data produced is of sufficient quality to be fit-for-purpose. I would also add that a basic understanding of how instrumental software operates in terms of data processing is also important.

As with the first edition, the contributors to this guide are members of the FIRMS Network and I thank them and in particular my co-editor Jim Carter for all of their time and efforts in preparing this Guide.

Dr Phil Dunn

Chair (2015-present) and Director

The FIRMS Network

Preface to the first edition

A few decades ago, mass spectrometry (by which I mean organic MS) was considered a “black art”. Its complex and highly expensive instruments were maintained and operated by a few dedicated technicians and its output understood by only a few academics. Despite, or because, of this the data produced were amongst the “gold standard” of analytical science.

In recent years a revolution occurred and MS became an affordable, easy to use and routine technique in many laboratories. Although many (rightly) applaud this popularisation, as a consequence the “black art” has been replaced by a “black box”:

SAMPLES GO IN → ■ → RESULTS COME OUT

The user often has little comprehension of what goes on “under the hood” and, when “things go wrong”, the inexperienced operator can be unaware of why (or even that) the results that come out do not reflect the sample that goes in.

Although (gas source) isotope ratio mass spectrometry (IRMS) pre-dates organic MS it is, only now, undergoing a similar expansion in availability and fields of applications. IRMS is now increasingly used in the forensic sciences which make the highest demands on the reliability of analytical results. The contributors to this Guide are all institutional members of the Forensic Isotope Ratio Mass Spectrometry (FIRMS) Network, forensic practitioners who apply IRMS to the most exacting of analytical sciences. In sharing our knowledge we aim to present the new (and not-so-new) user of IRMS with an understanding of the technique, from start to finish. Our aim is that IRMS does not become a “black box” and that, with greater understanding, you can obtain results that are both precise and consistent with other laboratories.

This Guide focuses on IRMS when coupled to an elemental analyser but the fundamental principles of IRMS operation and good analytical practice are applicable to all IRMS configurations.

I would wish the reader “good luck”, but luck has no place in generating IRMS data of an international standard.

Dr Jim Carter

Chair (2009-2015) and Director

The FIRMS Network

Disclaimer

Reference to or mention of any commercial product or process by specific trademark or manufacturer within this guide does not represent a recommendation or an endorsement by the FIRMS Network, nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose described.

Many of the materials used for stable isotope measurements are extremely dangerous (both to the individual and to the environment) and it is essential to read the Safety Data Sheet (SDS) prior to handling any chemical. It is also advisable to read the instrument manufacturers' safety recommendation regarding high voltages, elevated temperatures and pressurised gases that will be present.

Feedback

If you have any comments about this guide, suggestions for improvement or ideas for topics that should be included in future editions please let us know at:

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Thank you.

Contents

1 Introduction

1.1 Aims of the guide

- To demonstrate and encourage good laboratory practice in the acquisition of stable isotope ratio data.
- To demonstrate and encourage the use of recognised reference materials to report stable isotope ratio data traceable to the internationally agreed scales.
- To demonstrate and encourage the use and monitoring of quality control materials.
- To demonstrate and encourage the calculation and reporting of measurement uncertainty.
- To share practical knowledge of instrument set-up, sample analysis and trouble shooting
- To illustrate the interpretation of stable isotope ratio data in a forensic context.

1.2 Examples of applications of IRMS

The isotopic “profile”, “fingerprint", “footprint” or “signature” of a material is a combination of the ratios of the stable isotopes of a number of elements such as hydrogen, carbon, nitrogen, oxygen and sulphur ($^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$). The isotopic abundances of these elements were fixed when the Earth was formed and, on a global scale, have not changed since*. Subtle variations in the isotopic composition of materials are, however, introduced through biological, chemical and physical processes.

Isotopic variations are found in most natural and manufactured materials and the isotopic profile is therefore characteristic of the origin and history of the substance. Stable isotope ratio analysis has a wide range of applications. Some examples are given below:

- Forensic sciences
    - Determining whether samples of chemically similar substances such as drugs, explosives, fibres, paints, inks, tapes or adhesives may share a common source or history
    - Distinguishing counterfeit products (e.g. pharmaceuticals) from genuine materials
    - Comparing putative reactants with contraband products
    - Monitoring features of the environment
        - Identifying the source of pollutants such as oil spills
        - Monitoring atmospheric gases to distinguish between natural and anthropogenic sources
        - Modelling climate
        - Researching water cycle processes
    - Authenticating and tracing food
        - Establishing the geographic authenticity of foodstuffs
        - Identifying the adulteration of foods with cheaper ingredients
    - Investigating wildlife crime
- Archaeology/geosciences
    - Geochemistry and geology
        - Establishing the extent and temperature of post-burial alteration of rocks
        - Provenancing of clasts
        - Identifying the source of water samples

    - Palaeoclimatology
    - Palaeoecology
    - Palaeodietary studies
- Biological sciences
    - Ecology
        - Photosynthetic pathways
        - Food webs
        - Hydrology
        - Nutrient cycling
    - Human and plant physiology
    - Human provenancing
    - Metabolic studies
    - Sports medicine
    - Toxicology
        - Distinguishing endogenous versus exogenous (bio)chemicals

* So-called “radiogenic elements”, such as strontium and lead, are the products of radioactive decay and as a consequence the abundance of different isotopes of these elements has changed over geological time. These changes can be very useful to provenance materials containing radiogenic elements.

2 Isotope ratio scales

Variations in the natural abundance of stable isotopes within the scope of this Guide are expressed as a "relative difference of isotope ratios" [Coplen 2011], known as an "isotope-delta value" [Brand et al. 2014] using delta (δ) notation. Delta values are dimensionless quantities that represent the difference in isotope ratio of a sample relative to an internationally agreed zero-point (defined by the IAEA) as shown in equations (1) and (2).

$$\text{ratio}\,(R) = \frac{[\text{heavier isotope}]}{[\text{lighter isotope}]} \tag{1}$$

$$\delta = \left(\frac{R_{\text{Samp}}}{R_{\text{RM}}} - 1 \right) \tag{2}$$

Where R_{Samp} the isotope ratio of the sample; and

R_{RM} the isotope ratio of the scale zero-point

Each isotope system ($^{13}C/^{12}C$, $^{18}O/^{16}O$, etc.) may have one or more isotope delta scale(s), each with its own associated zero-point. Isotope-delta values are commonly expressed in parts per thousand (per mil or ‰) or in parts per million (ppm or per meg) differences from these zero-points.

Due to Harold Urey's significant role in the early development of stable isotope chemistry it has been proposed that the units for reporting δ values are named the "Urey" with per mil replaced by milli Urey (mUr) [Brand and Coplen 2012]. To date, this convention has not been widely adopted.

The expression for isotope delta in equation (2) may seem to imply that absolute isotope ratio measurements, determined using equation (1), are required for the sample and reference material (RM). However, since IRMS instruments measure relative variations of isotope ratios it is not necessary to know the "absolute" isotopic composition of the zero-point materials as both measured isotope ratios and isotope-delta values are relative quantities.

As with any measurement scale, isotope-delta scales have a subtle distinction between their *definition* (i.e. the position of the zero-point) and their *realisation* (i.e. how measurements can be made that are linked to the zero-point).

2.1 Isotope-delta scale definition and realisation

Isotope-delta scales are defined by the position of their zero-point by international agreement. Zero-points of isotope-delta scales are therefore consensus values with no associated uncertainty.

RMs with known isotope-delta values can be said to realise the appropriate scale. Defined points of isotope-delta scales are realised by RMs with no uncertainty associated to their assigned δ values (these are the primary RMs, section 2.2.2). Other RMs have associated uncertainty in their isotope-delta value and realise other points on the scale (these can be secondary RMs, tertiary RMs, etc., section 2.2). Even when a RM has no associated uncertainty to its assigned value, the analysis of the RM to realise the isotope-delta scale will introduce measurement uncertainty (section 6.5).

In practice, realisation of an isotope-delta scale involves the analysis of samples and RMs linked to the isotope-delta zero-point within the same sequence. Two or more RMs should be used to realise isotope-delta scales during each measurement sequence. The differences in measured isotopic composition between the samples and RMs can then be used to determine the differences in isotopic composition between the samples and the zero-point of the scale. Section 6.3 contains more information regarding the mathematical linking of measured isotope ratios to the appropriate isotope-delta scales. It is good practice to check that realisation of the scale has been correctly performed via the analysis of quality control (QC) materials of known isotope ratio (section 7).

Measurement results of isotope delta values should always be traceable to the appropriate zero-point and accredited laboratories must be able to demonstrate this [Barwick and Prichard 2011, BIPM 2012]. The highest metrological realisation of an isotope delta scale uses the shortest traceability chain to link to the zero-point and therefore results in the smallest measurement uncertainty.

New isotope-delta scales can be introduced:

- to address new measurement requirements for specific applications of an existing isotope system (e.g. there are three isotope-delta scales for $^{18}O/^{16}O$ ratios of oxygen, section 2.3, two of which are for the analysis of oxygen isotopes within particular matrices);
- to afford compatibility of data when an isotope system has become sufficiently widely measured that an international measurement scale is required (e.g. the recent introduction of a $\delta^{17}O$ scale [Schönemann et al. 2013]); or
- to replace an existing isotope-delta scale should a primary RM be found to be unsuitable, e.g. due to previously undiscovered heterogeneity (section 2.2.2).

Conversion of isotope-delta values from one scale to another for a particular isotope system is sometimes possible, but the measurement uncertainty thereby introduced must be accounted for.

2.1.1 Isotope-delta scales with two defined points

Significant scale contraction effects can manifest during realisation of some isotope-delta scales. These effects must be taken into account and the best approach is determination of the magnitude of the effect through careful measurement (for example the application of the so-called η correction [Meijer et al. 2000]); however this is not always straightforward.

The use of two or more RMs for scale realisation allows correction for scale contraction effects, but inter-laboratory compatibility of results is significantly improved by the use of a second defined point with no uncertainty on an isotope-delta scale. Therefore some isotope-delta scales have a second point defined by international agreement in addition to the zero-point. This is a practice which has been applied for many decades for the hydrogen and oxygen VSMOW-SLAP scales.

It is crucial that an isotope-delta scale that has two defined points is realised by two (or more) RMs.

2.2 Traceability and calibration

Note that in this Guide, and in IRMS-speak, the terminology “calibration” is more generally applied to calibration of measurement results to the appropriate δ scale rather than of the *m/z* scale. Calibration of the mass spectrometer magnet is typically performed following software installation and will very rarely need to be repeated.

Traceability can be defined as “*a property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations each contributing to the measurement uncertainty*.” [Barwick and Prichard 2011, BIPM 2012]. The “unbroken chain of calibrations” can also be referred to as a “traceability chain” and requires an established calibration hierarchy.

2.2.1 Isotope ratio calibration hierarchies

VIM 3 [BIPM 2012] defines a calibration hierarchy as a “*sequence of calibrations from a reference to the final measuring system, where the outcome of each calibration depends on the outcome of the previous calibration*.” For measurements of isotope delta, the calibration hierarchy takes the form of RMs with traceability chains linking their assigned isotope-delta values back to the zero-

points of the appropriate delta scale. Although nomenclature will vary, RMs for isotope ratio measurements may be broadly classified as:

(1) Primary RMs

(2) Secondary RMs

(3) Tertiary RMs

(4) In-house (laboratory) RMs

Note that in-house RMs may also sit at the secondary or tertiary levels depending on which RMs were used during their calibration.

For commonly measured isotope ratios of light elements within the scope of this Guide (H, C, N, O and S), the current internationally agreed zero-points for the most commonly used isotope-delta scales are:

- hydrogen ($^{2}H/^{1}H$) VSMOW (Vienna Standard Mean Ocean Water)
- carbon ($^{13}C/^{12}C$) VPDB (Vienna Pee Dee Belemnite)
- nitrogen ($^{15}N/^{14}N$) Air-N2 (atmospheric nitrogen)
- oxygen ($^{18}O/^{16}O$) VSMOW
- sulphur ($^{34}S/^{32}S$) VCDT (Vienna Canyon Diablo Troilite)

The zero-points of isotope delta scales have also been referred to as “measurement standards” although this terminology can be confusing and should be avoided.

2.2.2 Primary reference materials

The primary RM for an isotope-delta scale realises the position of the zero-point (e.g. IAEA-S-1 realises the zero-point of the VCDT $\delta^{34}S$ scale). In some instances, a primary RM can both realise and define the zero-point when it’s assigned isotope ratio is zero (e.g. VSMOW both defines and realises the zero-points of the VSMOW $\delta^{2}H$ and $\delta^{18}O$ scales). For isotope-delta scales with two defined points, there are two primary RMs, one for each fixed point on the scale. All primary RMs have exact isotope-delta values with no uncertainty. The absolute isotopic compositions of the primary RMs are not important for routine measurement of delta values but absolute values have been reported [e.g. Gröning 2004].

Over time some of the original zero-point RMs for isotope systems were found to be unsuitable as RMs due to previously undiscovered heterogeneity (e.g. the PDB calcite). In such cases replacement of the affected isotope-delta scales was required. This generally involved the elevation of an existing secondary RM to become a new primary RM for a new scale with a new zero-point. As an example, in 1995, the PDB scale was replaced by elevating the NBS 19 calcite (a secondary RM on the PDB scale) to be the primary RM realising the zero-point of a new Vienna PDB (VPDB) scale with no associated uncertainty. The exact values assigned to a primary RM on new delta scales for an isotope ratio system are chosen such that the numerical isotope delta values for materials expressed on the old and new scales are as close as possible given the available information.

In cases where primary RMs have become exhausted it is not necessary to define a new scale. A replacement RM can be carefully calibrated against the original primary RM with the minimum, but nevertheless non-zero, measurement uncertainty that is possible. Examples of such replacement RMs include VSMOW2, SLAP2 and IAEA-603 which are the replacement RMs for VSMOW, SLAP and NBS 19, respectively. The replacement RMs still realise the same defined points on the isotope delta scales, but with a larger associated uncertainty. They have also, confusingly, been referred to as “primary” RMs despite having longer traceability chains than their predecessors – however this use of terminology should be avoided.

The primary RMs (and their replacements when appropriate) kept and distributed by IAEA, NIST and USGS are listed in Table 1. The routine measurement of primary RMs and their

replacements for isotope-delta scale realisation should be avoided unless the small measurement uncertainty that these materials confer during scale realisation is essential. This ensures that the primary RMs are available for as long as possible, which is important for long-term reproducibility. For all but the most exacting of applications, the slightly larger measurement uncertainty afforded by the use of secondary or tertiary RMs does not impact on the usefulness of the results.

***Table 1**. Internationally agreed zero-points of the light element isotope ratio δ scales, their primary RM(s) and (currently available) highest metrological realisations*.

Ratio	Zero-point material	primary RM(s)	Highest metrological realisation(s)	
			Name	δ value (‰)[a,b]
$^{2}H/^{1}H$	VSMOW	VSMOW	VSMOW2[c]	0.00 ± 0.3
		SLAP	SLAP2[c]	−427.5 ± 0.3
$^{13}C/^{12}C$	VPDB	NBS 19	IAEA-603[d]	+2.46 ± 0.01
$^{15}N/^{14}N$	Atmospheric nitrogen	*NA*[e]	IAEA-N-1[f]	+0.43 ± 0.04
		USGS32	USGS32	+180
$^{17}O/^{16}O$	VSMOW	VSMOW	VSMOW2[b]	0.00 ± 0.03
		SLAP	SLAP2[b]	−29.697 ± 0.05
$^{18}O/^{16}O$	VSMOW	VSMOW	VSMOW2[b]	0.00 ± 0.02
		SLAP	SLAP2[b]	−55.5 ± 0.02
	VPDB	NBS 19	IAEA-603[d]	−2.37 ± 0.04
	Atmospheric oxygen	*NA*	*NA*	*NA*
$^{34}S/^{32}S$	VCDT	IAEA-S-1	IAEA-S-1	−0.3

[a] These δ values have been obtained from the IUPAC Technical Report [Brand et al. 2014].
[b] Uncertainties are standard uncertainties. Where no uncertainty is given, the δ value is exact and assigned by consensus.
[c] Replacements for the original VSMOW and SLAP primary RMs.
[d] Replacement for NBS 19, the original primary RM for the carbon and oxygen VPDB scales.
[e] While atmospheric nitrogen could be considered to be a primary RM, it is difficult to refine with a reproducible isotope ratio, so is best only considered the zero-point of the scale.
[f] Recommended scale anchor for combustion-based measurements (section 2.3.3).

2.2.3 Secondary reference materials

Secondary RMs are natural or synthetic compounds that have been carefully calibrated relative to the primary RM(s). For most commercially available secondary RMs, assigned δ values are agreed upon and adopted internationally. In contrast to the primary materials, all secondary RMs have some uncertainty associated with the δ values. Both the δ values and the associated uncertainties (often expressed as one standard deviation, SD) of the commercial secondary materials have been reviewed and revised over time and the reader is urged to check the latest certificates from the supplier. Revisions to the adopted values for secondary RMs result either from improvements to measurement techniques or from a change to normalisation procedures for the realisation of the scale.

A summary of the commercially available secondary (and primary) RMs for all isotopic δ scales can be found in the relevant IUPAC Technical Report [Brand et al. 2014]. This report includes RMs predominately distributed by IAEA, NIST and USGS. Note that this report is updated periodically rather than on the release of new secondary RMs or publication of revised certificates and can therefore be out-of-date. For this reason, when reporting isotopic compositions it is essential that the values and measurement uncertainties assigned to primary and/or secondary materials are given alongside sample results. Secondary isotopic RMs may have been produced

decades ago and requirements of estimation and reporting of uncertainties associated to their reference values may now be different. We recommend that end users carefully examine any uncertainty statement associated to a reference δ value.

2.2.4 Tertiary reference materials.

Tertiary RMs are those that have been calibrated using secondary RMs to provide traceability to the zero-point of the scale. These materials can include those available from commercial organisations and universities. The δ values of these materials have been assigned by internal calibration or are consensus values, obtained through inter-laboratory comparison (ILC). In general, these materials do not carry the international agreement ascribed to the materials distributed by IAEA, NIST and USGS listed in the IUPAC Technical Report [Brand et al. 2014], but may prove useful where no other RMs exists.

It is important for end-users of commercially available tertiary RMs to asses critically the traceability and measurement uncertainty claims from the supplier.

2.2.5 In-house reference materials

A stable isotope laboratory must hold suitable materials for calibration and normalisation purposes so that isotope ratios can be reported on an agreed international scale. Primary and secondary RMs are not recommended for daily use as they are in short supply. Instead, primary and/or secondary RMs are used to calibrate in-house RMs for everyday use in normalisation and quality assurance (QA). Control charts should be used to monitor laboratory performance and the status of in-house RMs (section 7.1). Any contamination of the RMs will be apparent as a step in the control chart whereas a slow change (evaporation, reaction with atmospheric water or CO_2, etc.) will show as drift. Control charts will also assist in determining whether a proposed in-house RM is likely to be suitable for long-term use.

Materials adopted as in-house RMs should be chosen for:

- isotopic homogeneity (to the smallest amount to be analysed),
- stability of isotopic composition over time,
- calibrated isotope ratios within the normal range of measurement,

In-house RMs should also be chemically similar to the samples as, according to the Principle of Identical Treatment (PIT), biases propagated during preparation will tend to cancel out. Other considerations for the choice of in-house RMs may include:

- ease of preparation, storage and handling,
- conversion characteristics within peripherals (i.e. complete conversion to analyte gas and therefore generally a single chemical compound),
- ease of replacement (when exhausted, contaminated etc.),
- non-hygroscopic (especially important when measuring hydrogen and oxygen isotopes)
- comprising only intrinsic hydrogen (hydrogen permanent within a materials) unless the extrinsic hydrogen is well characterised (sections 5.3.4 and 6.4.6)

2.2.5.1 Matrix-matched in-house reference materials

Developing or buying in-house RMs that are chemically and physically similar to materials analysed in your laboratory is highly recommended. There are a variety of reasons why analyte materials may differ in their behaviour in your preparatory system from commercially available RMs; these differences may affect the isotope ratio measurement results. A non-exhaustive list includes: differences in oxidation state, differences in the sorption of water, or the presence of an

element not present in the RM, e.g. the hydrochloride salt versus the free-base form. Therefore, it is strongly recommended to use a “matrix-matched” in-house material to enable application of the Principle of Identical Treatment (PIT) [Werner and Brand 2001].

2.2.5.2 Calibration of in-house reference materials

To calibrate an in-house RMs, a laboratory must conduct a series of analytical sequences using primary and/or secondary RMs alongside the proposed in-house RM(s). Through data acquisition and documentation, the isotopic values of in-house RMs can be linked to the zero-point of the reporting scale, establishing traceability. Many of the required measurements will form part of method validation (section 7.3).

It is not always possible to match chemically the primary and/or secondary materials to the in-house RMs (e.g. matrix matching), but every effort should be made to ensure that the in-house RMs δ values are unbiased using the intended analytical method. To that purpose, it may be necessary to obtain isotope ratio results using orthogonal, or different, techniques. For example, an in-house RMs intended for EA/IRMS may also be combusted off-line and the CO_2 measured by DI/IRMS. The laboratory may also wish to send in-house RMs to other analytical facilities to confirm the results and collect external data for a better estimate of reproducibility. Ensuring the quantitative conversion of the candidate in-house RM to the analyte gas in comparison to a primary or secondary RM known to exhibit favourable conversion characteristics is critical when assigning a value to a new in-house RM.

All of the data collected together with the methods for collecting data should be compiled into a calibration report for future reference. The report should include all sources of uncertainty so that a good estimate of overall measurement uncertainty for the δ value of the in-house RM can be calculated. The in-house RMs, alongside other quality control (QC) materials, should be monitored for possible instability or contamination using laboratory information management systems (LIMS). Periodically, primary and/or secondary RMs should be analysed as “unknown” samples to check in-house proficiency and the effectiveness of in-house RMs for data normalisation and correction. If deemed necessary, whether due to improvements in methods, changes in equipment, or changes in requirements, the laboratory must conduct a recalibration and adjustment to the isotope ratio values and associated measurement uncertainties of δ values of in-house RMs.

2.3 Overview of isotope-delta scales

The historical aspects of the δ scales for the light elements (within the scope of this Guide) in the following sections are described in more detail in the IUPAC Technical Report on international RMs for isotope ratio analysis [Brand et al. 2014].

2.3.1 The VSMOW δ scale

The original zero-point for hydrogen and oxygen measurements of water samples was Standard Mean Ocean Water (SMOW) [Craig 1961]. This was a defined value, which was not associated with a RM so it did not physically exist and measurements could not be calibrated against it directly.

This shortcoming was addressed by the production of Vienna SMOW (VSMOW) by blending distilled ocean water (latitude 0°longitude 180°) with small amounts of other waters to produce an isotopic composition close to the definition of SMOW. VSMOW then became the zero-point and primary RM of the new hydrogen and oxygen isotope delta scales that were also called VSMOW (i.e. VSMOW δ^2H = 0 exactly and δ^{18}O = 0 exactly).

Due to scale expansion/contraction effects, a second primary RM was established for both the δ^2H and δ^{18}O isotope delta scales. This material was Standard Light Antarctic Precipitation (SLAP), which was prepared from South Pole firn and is considerably depleted in heavy isotopes

with respect to VSMOW. The isotope delta values assigned to SLAP are δ^{2}H = −428 ‰ exactly and δ^{18}O = −55.5 ‰ exactly versus VSMOW. The δ^{2}H and δ^{18}O values of all hydrogen and oxygen bearing materials should be reported on this VSMOW-SLAP scale [IAEA 2017].

VSMOW and SLAP (primary RMs) have now been superseded by VSMOW2 and SLAP2 that both have an almost identical isotopic composition to their predecessors, but with associated uncertainties. VSMOW2 and SLAP2 are therefore secondary RMs on the VSMOW-SLAP scale but provide the highest metrological realisation of the hydrogen and oxygen VSMOW-SLAP scale.

2.3.2 The VPDB δ scale

The original anchor for carbon isotopic measurements consisted of calcium carbonate from a Cretaceous belemnite from the Pee Dee formation in South Carolina (PDB). The CO_2 evolved from PDB, by treatment with phosphoric acid, was adopted as the zero-point for carbon and oxygen isotopic measurements.

Upon its exhaustion, PDB was replaced by assigning exact δ^{13}C and δ^{18}O values to another carbonate (NBS 19 or “TS-limestone”) versus a hypothetical Vienna PDB creating a new isotope delta scale (VPDB) for carbon and oxygen.

There is an oxygen isotope fractionation between the carbonate and the evolved CO_2, the latter being about 10 ‰ enriched in ^{18}O with respect to the calcite (when the reaction takes place at 25 ^{o}C). This is irrelevant when measuring calcite samples against calcite RMs, but becomes problematic for dual-inlet measurements of non-carbonates or for non-calcite carbonates, which have different fractionation factors than calcite at temperatures above 25 ℃ [Sharp 2006]. Therefore, the oxygen isotopic compositions of the hypothetical VPDB calcite and of the CO_2 evolved from this calcite under standard conditions (VPDB-CO2) are different (and this holds true for any calcite sample), while the carbon isotopic compositions of these two virtual materials are identical.

VPDB has isotopic ratios characteristic of marine limestone and is considerably enriched in ^{13}C with respect to almost all organic carbon compounds. It was therefore recommended that δ^{13}C values of both organic and inorganic materials were expressed relative to VPDB on a scale that was also realised by a second primary RM (LSVEC lithium carbonate) with an exact δ^{13}C value of −46.6 ‰ relative to VPDB [Coplen et al. 2006]. This afforded better comparability of measurement results between different laboratories as scale expansion/contraction effects could be accounted for.

NBS 19 is no longer commercially available and can be considered exhausted. The IAEA has recently released IAEA-603 as a replacement for NBS 19 [IAEA 2016], which will act as the highest metrological realisation for the carbon and oxygen VPDB scales as it has been very precisely calibrated directly against NBS 19 and has a very small associated uncertainty. NBS 19 remains the primary RM for the VPDB scale but IAEA-603 should be used by laboratories that do not have access to NBS 19 where required.

LSVEC has recently been reported as suffering from incorporation of atmospheric CO_2, thereby altering its carbon isotopic composition with time, particularly for vials that are frequently opened [Qi et al. 2016, Assonov 2018]. As a result, it is no longer recommended by the CIAAW to use LSVEC as a RM to realise the VPDB carbon isotope delta scale. Nevertheless, it is still a requirement that carbon isotope delta values be normalised to the VPDB scale using two or more RMs. It is also vital that the identities and values/uncertainties assigned to all RMs used during scale realisation are reported alongside results for samples such that data can be re-normalised when RM values and or assigned uncertainties change. This includes any intermediate RMs used during calibration of in-house RMs.

The VPDB scale is used for reporting δ^{18}O values of carbonates. When converting between the VPDB and VSMOW scales the conversion recommended by the CIAAW is [Brand et al. 2014]:

$$\delta^{18}O_{VPDB} = 0.97001 \times \delta^{18}O_{VSMOW} - 29.99 \quad (3)$$

2.3.3 Atmospheric nitrogen δ scale

The isotopic composition of atmospheric nitrogen (Air-N2) has been adopted as the zero-point for all nitrogen isotope ratio analyses as it has been shown not to vary measurably around the world or over time [Mariotti 1983, Mariotti 1984]. To be used as a practical RM, however, N_2 would need to be isolated from the atmosphere without fractionation. For convenience a number of RMs (mostly ammonium and nitrate salts) have been prepared and are distributed by the IAEA, NIST and the USGS. The Air-N2 scale now also has a second defined point which is realised by a potassium nitrate (USGS32) with an assigned $\delta^{15}N$ value of +180 ‰ exactly in addition to atmospheric nitrogen. The IUPAC's CIAAW recommends that the RM IAEA-N-1 (ammonium sulphate) be used as a scale anchor for samples that need combustion as a means of sample preparation because Air-N2 is difficult to produce free from argon, which can interfere with isotopic analysis [Brand et al. 2014].

2.3.4 Atmospheric oxygen δ scale

Oxygen isotope ratio δ values for oxygen gas have also been reported on a δ scale with the isotopic composition of atmospheric oxygen (Air-O2) as the zero-point. This scale should still be normalised such that the $\delta^{18}O$ value of SLAP is −55.5 ‰ relative to VSMOW [Gat and De Bièvre 2002; Wieser and Berglund 2009]. There are no RMs currently available that are calibrated to the Air-O2 scale.

2.3.5 The VCDT δ scale

CDT (Canyon Diablo Troilite – iron sulphide from the Canyon Diablo Barringer meteorite) was originally proposed as a scale anchor and zero-point for sulphur $\delta^{34}S$ values; however it was found to be isotopically inhomogeneous and therefore unsuitable as a primary RM [Beaudoin et al. 1994]. The zero-point of the replacement scale, Vienna CDT (VCDT) is realised by assigning the $\delta^{34}S$ value of the silver sulphide material IAEA-S-1 to be −0.3 ‰ exactly. There is currently no second primary RM for the VCDT scale, however a range of secondary RMs with known isotope-delta values is available and therefore two or more point realisation of the VCDT scale is possible and recommended. As with VPDB, VCDT is a virtual material; however, unlike VPDB, the absolute isotopic composition of VCDT, traceable to the International System of Units (SI), is known to a high degree of precision [Ding et al 2001].

3 Instrumentation

Isotope ratio mass spectrometry (IRMS) instruments are specifically designed to measure precisely, small differences in the abundances of isotopes such as $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$ and $^{34}S/^{32}S$.

Prior to analysis by IRMS, samples are converted to simple gases such as; hydrogen, carbon dioxide, nitrogen, carbon monoxide and sulphur dioxide (H_2, CO_2, N_2, CO and SO_2), depending on the composition of the material and the isotopes of interest. The IRMS instrument measures the ratio of ions that correspond to the different isotopic forms (isotopologues) of these gases. For example, for the analysis of carbon isotope ratios, the mass spectrometer simultaneously monitors ions with mass-to-charge ratios (*m/z)* of 44, 45 and 46, which correspond to the ions produced from CO_2 molecules containing ^{12}C, ^{13}C, ^{16}O, ^{17}O and ^{18}O in various combinations.

Samples are converted to these simple gases either “off-line” using classical chemical techniques or by a continuous-flow process described below. The entire element of interest within a sample can be analysed at once (bulk stable isotope analysis, BSIA) or individual compounds may be first isolated, and then converted to the analyte gas for compound-specific isotope analysis (CSIA).

3.1 Isotope ratio mass spectrometer

In the ion source of the mass spectrometer gas molecules are ionised through interaction with an electron beam (electron ionisation, EI), typically at higher energy than conventional (70 eV) organic MS.

Ions leave the source and are focussed and accelerated through a high voltage. The mass spectrometer is a sector-field instrument and ions pass through a magnetic field (and in some instruments an additional electrostatic field) before reaching the Faraday collectors. The strength of the magnetic field and the accelerating voltage determine the trajectories of the ions and, therefore, which ions will enter which Faraday collector. The use of multiple collectors allows the simultaneous measurement of ion intensity ratios, negating the smallest fluctuations in the overall intensity of the ion beam.

For nitrogen and carbon ratio measurements two suites of collectors, specifically spaced to collect *m/z* 28 and 29 and *m/z* 44, 45 and 46 are required. As an alternative a “universal” triple collector can be used in which the outer collectors are wide with respect to the dispersion of the ion beam. This “universal” collector configuration can also be used for oxygen isotope measurements (CO - *m/z* 28 and 30) and for sulphur isotope measurements (SO_2 - *m/z* 64 and 66)

For the analysis of hydrogen isotopes the magnetic field strength is greatly reduced to allow ions of *m/z* 2 and 3 ($^{1}H_2$, $^{1}H^{2}H$) to enter an additional pair of collectors. These collectors are often positioned on either side of the central collectors. Additional collectors may also be present to determine the isotopic ratios of elements such as sulphur or chlorine.

Each collector is connected to a dedicated amplifier whose gain is defined by a precise, high ohmic resistor. Each amplifier has a different gain such that ion ratios, at natural abundance levels, will produce similar signals. Typical absolute and relative amplifier gains are shown in Table 2. Some instruments provide an ability to switch the gain of certain amplifiers to facilitate the measurement of samples which that have been enriched (labelled) with stable isotopes, i.e. the relative abundance of the major and minor isotope may be close to unity.

The signals from each amplifier are recorded simultaneously typically every tenth of a second, digitised and recorded by the IRMS data system. This creates a “chromatogram” (a plot of intensity versus time) for ions of given *m/z*, the intensity being proportional to the number of ions detected.

Table 2. *Typical detector amplification factors for an IRMS instrument.*

m/z	Relative amplifier gain	Absolute amplifier gain
2	1	1×10^9
3	1,000	1×10^{12}
28, 44 or 64	1	0.3×10^9
29, 45 or 66	100	30×10^9
30 or 46	333	100×10^9

3.1.1 MS tuning

Operators often categorise an IRMS instrument as being tuned for either “sensitivity” or “linearity”. The first suite of parameters is intended to afford maximum signal intensity, the second to afford consistent ion ratios over a range of signal intensities. For continuous-flow applications the latter should be applied.

The ideal tuning parameters for an ion source are strongly dependent upon the type of instrument, cleanliness of the ion source and many other conditions. Therefore this Guide can only give very general recommendations of how to perform tuning.

To achieve good sensitivity all ion source parameters are varied to attain maximum signal intensity of the working gas.

To achieve good linearity some ion source parameters are set to “critical” values, e.g. the extraction lens voltages. All other parameters are then adjusted to maximise the signal of the working gas.

The critical values are only established through an iterative process of tuning and measuring linearity, e.g. by setting the extraction lenses to another value and adjusting all other parameters. Although very time consuming, this process will generally only need to be performed once to establish what are the “critical values”.

Most IRMS instrument software offers an “autofocus” function. This can speed up the whole process, but manual tuning is typically performed after the “autofocus” to achieve the best results. A knowledge of the “critical values” is essential to make best use of an “autotune” function.

3.2 Bulk stable isotope analysis (BSIA) techniques

Prior to analysis, the sample of interest must first be converted into simple analyte gases (H_2, CO_2, N_2, CO and SO_2 for hydrogen, carbon, nitrogen, oxygen and sulphur isotope analysis, respectively). If the entire element of interest within a sample is converted at once, then a so-called bulk isotope ratio will be the result. A bulk isotope ratio is the average isotope ratio of the material regardless of whether it is a pure, single chemical or a complex mixture of various species. There are a number of instrumental techniques that can be used for BSIA including dual-inlet (DI), elemental analyser (EA) and flow injection analysis (FIA).

3.2.1 DI/IRMS (Dual-inlet isotope ratio mass spectrometry)

Dual/inlet (DI) IRMS is generally considered to be the most precise method of measuring the isotope ratios of light elements. The technique, however, requires significant preparation and larger sample size than required for the continuous-flow methods described in later sections.

The DI technique is briefly described here because:

- It is arguably the highest precision technique available,
- it has historical significance, and
- it was the origin of the (now) ubiquitous δ notation.

Several authors have produced extensive comparisons of DI and continuous flow (CF) IRMS [Barrie and Prosser 1996; Brand 2004]. Some of these differences are summarised in Table 2. The first studies using isotope ratio mass spectrometry, using dual-inlet, were published before 1950 (e.g. Neir 1947) and the basic structure of the DI instrument has remained fundamentally unchanged although advances in electronics and vacuum technology have improved both precision and ease of measurement.

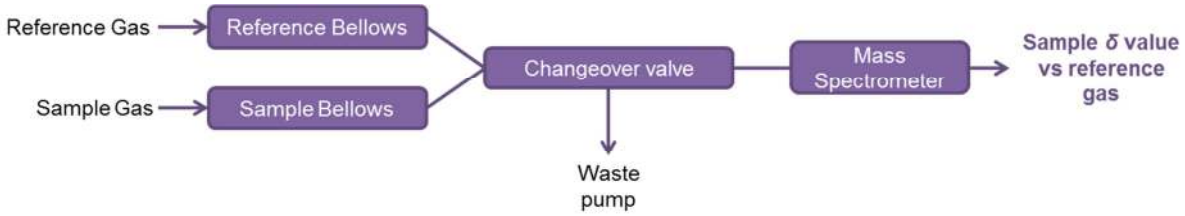

Figure 1. *Simple schematic diagram of a dual-inlet IRMS instrument for the determination of isotope ratio δ values. Note that the reference gas may be derived from a working standard rather than a primary calibration standard.*

DI/IRMS determines isotope ratios of pure gases by alternately introducing a sample gas and a reference gas of well calibrated isotopic composition into an IRMS instrument. The sample and reference gases enter the MS under nearly identical conditions, achieved by introducing the two gases into two independent variable volumes, or bellows. Both bellows are connected, via a capillary, to a crimp, which allows a small but steady flow of gas either into the mass spectrometer or to a waste line via a "change-over valve". These capillaries with crimps are designed to leak gas under viscous flow at an equal rate, for a given pressure in the bellows, preventing isotopic fractionation during flow [Halsted and Neir 1950]. The variable volume of the bellows allows the gas pressure to be adjusted such that nearly identical amounts of sample and reference gas are alternatively introduced into the ion source of the IRMS instrument.

Table 3. *Comparison between dual-inlet and continuous flow techniques.*

	Dual-Inlet	**Continuous flow**
Type of gas entering the mass spectrometer.	A pure gas (such as CO_2).	A mixed gas, e.g. CO_2 as a peak within a flow of helium.
How the sample gas and reference gas are introduced into the mass spectrometer.	The gases are repeatedly and alternately introduced into the ion source.	The sample gas peak is preceded and/or followed by introduction of working gas.
Signal intensity of sample gas.	Sample and reference gases are carefully balanced by adjustments of bellows to produce nearly identical signals for the major ion beam, avoiding linearity bias.	Sample gas varies in intensity across the peak. Ideally, the maximum intensity of the sample gas will be the same as the working gas.
Amount of sample required.	10s of µmol, or ~0.5 µmol using a cold finger volume (see below). The sample size is controlled by the need for viscous flow conditions in the capillaries.	100s of nmol, smaller if systems are optimised to 10s of nmol by GC/IRMS or LC/IRMS. Viscous flow is provided by the helium stream.

The alternating, near identical, flow of sample and reference gas allows for high precision isotope ratio measurements. The origin of delta (δ) notation comes from the observation of difference, or delta, between the sample and reference gases during a DI isotope ratio measurement [McKinney et al 1950]. Typically, 5 to 10 pairs of sample / reference gas isotope ratio measurements are made for any one sample, which are typically averaged and an outlier filter may be applied.

The reference gas in DI isotope ratio measurement may be derived from a RM (e.g. CO_2 released from the offline acid digestion of IAEA-603), or it may be a working gas (sometimes referred to a as transfer standard) for example a cylinder of high-purity CO_2 that has itself been directly calibrated to the reporting scale by DI-IRMS. Note that as sample and reference gases are introduced by identical means into a DI/IRMS instrument the term "reference gas" is used; whereas in continuous-flow techniques sample and working gases are not treated identically and hence the term "reference gas" is not appropriate. In contrast to continuous-flow techniques, an offset/shift correction (normalisation) can be applied through means other than the analysis of two or more RMs, for example by application of the so-called η correction [Meijer et al. 2000].

Some DI systems are optimised for smaller sample sizes by means of a "cold-finger" or "micro-volume" in which the sample gas is frozen into a small volume, and the reference bellows are adjusted to introduce an equivalent amount of gas in the reference-side micro-volume. The dual-inlet measurement is then conducted on these limited volumes. Because only small amounts of gas are present in these micro-volumes, there is the potential for deviation from the viscous flow regime and changes to the isotope ratios of the gases, but with care, this option can produce high quality measurements on very small amounts of gas.

DI isotope ratio measurements are commonly performed on samples of a pure gas prepared "offline". Various reaction and clean-up processes, typically conducted in vacuum lines, may be employed quantitatively to convert a sample into a pure gas for introduction to a DI-IRMS instrument. Specific procedures are used to convert solids, liquids, dissolved gases, and gas mixtures into pure gases, and will not be described further [de Groot 2009 Vol 2]. These off-line techniques are usually very time consuming, although some of the common methods have now been automated including: hydrogen and oxygen isotope ratio measurements of waters by H_2 and CO_2 equilibration; carbon and oxygen isotope ratio measurements of carbonates; and high precision carbon and oxygen isotope ratio measurements of atmospheric CO_2.

3.2.2 EA/IRMS (Elemental analyser isotope ratio mass spectrometry)

Elemental analysis(EA)/IRMS is applicable to a wide range of materials. Solid substances and non-volatile liquids can be introduced into the EA system enclosed in tin (for C/N/S analysis) capsules, while liquids with limited viscosity can be directly injected using a liquid inlet system. There are various types of EA with different reactors for different applications:

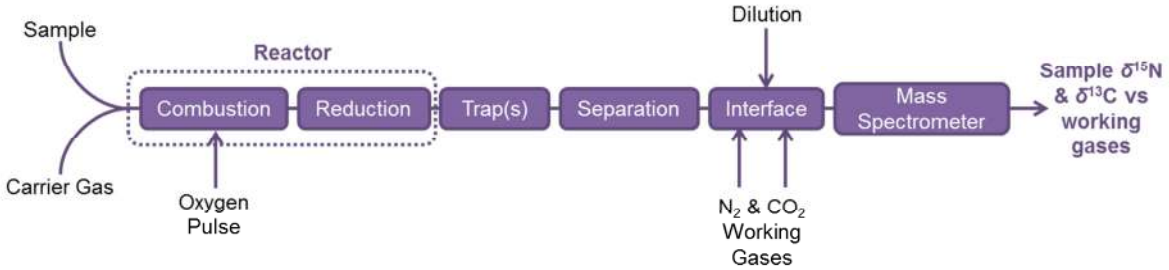

Figure 2. *Simple schematic diagram of an EA-IRMS system for the determination of $\delta^{15}N$ and $\delta^{13}C$ values. Note that materials containing elements other than H, C, N and O can yield combustion products that may need to be removed.*

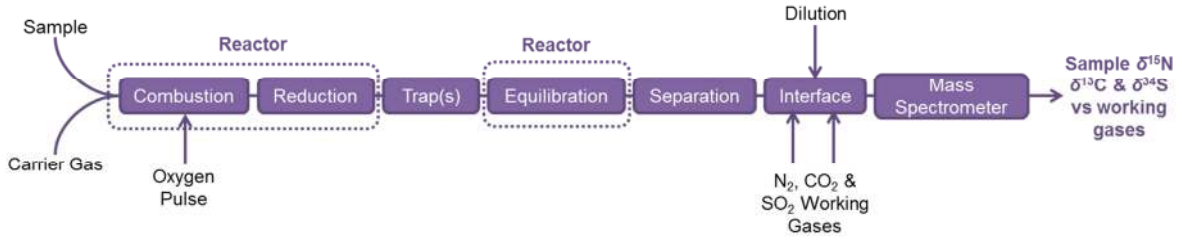

Figure 3. *Simple schematic diagram of an EA/IRMS system for the determination of $\delta^{34}S$ values in addition to $\delta^{15}N$ and $\delta^{13}C$ values. Note that the combustion, reduction and equilibration can all occur within the same reactor tube.*

3.2.2.1 Combustion (for nitrogen and carbon isotopic analysis)

The EA instrument typically contains two reactors – a "combustion" reactor, followed by a "reduction" reactor, although these can be combined in a single tube. The reactors are followed by a water-separation device and (typically) a packed GC column for separation of the evolved gases (N_2 and CO_2).

Combustion takes place in an oxygen (O_2) atmosphere in a quartz (or less frequently steel) tube to produce N_2, NO_x, CO_2 and H_2O. The reactor typically contains an oxidation catalyst [copper (II) or chromium (III) oxide] and a scavenger to bind sulphur and halogens [cobalt (II, III) oxide and/or silver], although many variations are recommended for specific applications. The reactor temperature is typically maintained between 900-1050 ℃, but the heat of combustion of the tin capsules raises the sample temperature to about 1800 ℃. It is recommended to use easily removed inserts (ash crucibles) to collect the ash, the residue from samples and tin capsules. Depending on the type of insert used this can be replaced after analysing 50 to 150 samples without the need to remove the entire reactor.

Removal of excess oxygen and reduction of the NO_x to N_2 takes place at lower temperatures, either in a cooler part of a single tube or in a separate furnace, typically maintained at 650 ℃. The reduction process typically relies on high purity elemental copper and, again, variations are recommended for specific applications.

The water formed by combustion is removed by a "water trap" typically containing magnesium perchlorate (also known as Anhydrone®) or similar desiccants. When only nitrogen isotope ratios are to be determined, CO_2 can be removed from the gas stream using a chemical trap containing soda lime or sodium hydroxide on a silica substrate, e.g. Ascarite® or Carbo-sorb®. These reagents produce water when absorbing CO_2 and should be positioned between two water traps.

Finally, the N_2 and CO_2 are separated via an isothermal gas chromatography (GC) column packed with a stationary phase such as Porapak® QS.

As an alternative to chromatographic separation, some instruments employ a "purge-and-trap" system to achieve separation [Siepers et al. 2006]. Nitrogen passes directly through the system while other evolved gases (CO_2, etc.) are collected on a number of adsorption tubes (effectively short GC columns). These traps are then sequentially heated to liberate the gases into the MS.

3.2.2.2 Thermal decomposition (for nitrogen isotopic analysis)

Traditional combustion methods, used to produce N_2 for isotopic measurements, are not quantitative for materials containing nitrogen in high oxidation states, specifically nitrates [Gentile et al 2013; Lott et al. 2015]. This can lead to bias in nitrogen isotope ratio results and conversion via thermal decomposition as opposed to combustion is recommended.

The EA configuration for thermal decomposition is the same as for combustion (section 3.2.2.1) except the oxygen "pulse" is disabled and the method timing is changed slightly such that

samples decompose at high temperature rather than combust. The net effect is a decrease in the amount of NO_x versus N_2 produced during thermal conversion of samples to gas.

3.2.2.3 Combustion (for sulphur isotopic analysis)

Elemental analysers for sulphur typically use a single combustion/reduction tube to convert sulphur within samples to sulphur dioxide (SO_2) gas, which is then passed to the MS for the determination of $\delta^{34}S$ values. The oxygen pulse may need to be larger than for N and C measurements to account for the larger sample sizes (due to the typically lower concentration of S). The addition of vanadium pentoxide to the tin capsules can also promote oxidation.

It is essential to maintain a high linear flow of carrier gas in the initial reactors to prevent SO_2 diffusing back and reacting with excess oxygen to form sulphur trioxide (SO_3) which may cause isotopic fractionation [Mambelli et al. 2016].

The copper used in the reduction stage of the reactor must be maintained at a higher temperature than for C and N analysis (830 to 910 °C) to ensure that copper sulphate does not form, which would lead to poor peak shapes and fractionation of measured sulphur isotope ratios [Duggan 1977].

An “equilibration” reactor (to ensure that the oxygen isotopic composition of the SO_2 produced from all materials is identical) can be used after the combustion/reduction reactor and water trap. This consists of a quartz tube filled with quartz chips held at 890 ℃. Alternatively, a single reacto r can be used, which consists of quartz chips, quartz wool and reduced copper wires [Fry 2007].

Sulphur can be analysed together with N and C, using tungsten (VI) oxide granules to promote combustion. Separation of the combustion products (N_2, CO_2 and SO_2) requires a shorter GC column or a ‘purge and trap’ system for the separation of the combustion gases by reversible adsorption on a series of molecular sieve traps. Such systems are capable of combusting up to 100 mg of organic samples, allowing the analysis of samples with low concentration of sulphur [Sieper et al. 2006].

Both SO_2 and SO_3 dissolve readily in water, forming acids that can damage metal components within the instrumentation, which may need to be regularly rinsed with water and occasionally with hot nitric acid to remove deposited material. PTFE or Sulfinert® treated tubing can be used in place of stainless steel for gas transfer within the elemental analyser and between the interface.

Sulphur isotope ratios have also been determined by DI/IRMS analysis of sulphur hexafluoride (SF_6) gas. Fluorine is monoisotopic and therefore there are no isobaric interferences to be corrected for during such analysis. Furthermore, SO_2^+ comprises only about half of the species formed upon ionisation of SO_2, while SF_5^+ comprises over 90 % of the ionisation products of SF_6. The chemical transformation of a material to SF_6 gas, however, is not straightforward and must be performed offline, precluding the use of CF/IRMS methods. Furthermore, the mass spectrometer needs to have a higher resolution to distinguish SF_5^+ isotopomers than is needed for SO_2^+ [Mayer and Krouse 2004].$\delta^{34}S$ measurements via SO_2 versus SF_6 have been shown to differ for sub-samples of the same material using the same MS and the contemporaneous analysis of RMs for normalisation of results is paramount.

3.2.3 HTC/IRMS (high temperature conversion isotope ratio mass spectrometry for O and H analysis)

High temperature conversion (HTC) refers to the Schuetze/Unterzaucher process in which both organic and inorganic compounds are converted to H_2, N_2 and CO gases in a strongly reducing environment at temperatures between 1350 and 1450 °C [Santrock and Hayes 1987]. The system typically comprises an outer tube made from fused alumina and an inner tube made from “glassy carbon” (a brittle form of carbon with a randomized structure). The inner tube is filled with glassy carbon particles and silver wool intended to bind sulphur and halogen atoms. Similar to combustion EA many variations are recommended for specific applications. The evolved gases

are separated via isothermal packed column GC (e.g. molecular sieve 5 Å). The products of HTC are assumed to be H_2, N_2 and CO, but reactive species can also be generated when analytes contain N, Cl, S, etc. The use of chemical traps to remove some reactive gases is sometimes recommended, placed before the GC column. Trapping materials include; activated charcoal, magnesium perchlorate, Sicapent® (phosphorous pentoxide on a binder) and Ascarite® [Hunsinger et al. 2013]. Such traps also serve to remove gases such as water and carbon dioxide that can be formed when the reactor is at a lower, "standby" temperature.

3.2.3.1 Water samples

The hydrogen and oxygen isotopic compositions of water samples are measured using a glassy carbon reactor (described above) topped with a stainless steel insert. The insert provides a hot and small volume to promote the evaporation of the water. The auto-sampler for solid samples is replaced with a liquid injection adaptor (containing a high temperature and pre-drilled septum) and a GC style liquid handling auto-sampler. The volume of water required for analysis is typically 0.15 to 0.25 μL and to facilitate this the auto-sampler must be fitted with a 0.5 or 1 μL syringe. The condition of the syringe is critical to obtaining good data and should be checked before and after an analytical sequence. When poor results are obtained the first check should always be for consistent peak height, width and shape – poor reproducibility typically points to a problem with the syringe.

3.2.3.2 Solid samples

The oxygen isotopic compositions of solid samples are measured using a glassy carbon reactor (described above) topped with a graphite adaptor which serves to funnel samples into the hot zone. For oxygen isotopic analysis of nitrogen-bearing materials the separation of N_2 and CO is essential because N_2 is isobaric with $^{12}C^{16}O$ (*m/z* 28) and also forms NO^+ which is isobaric with $^{12}C^{18}O$ (*m/z* of 30) Although CO and N_2 can be separated by the GC column, NO^+ in the ion source elevates the *m/z* 30 background long after the elution of N_2, and affects the integration of the CO peak. It is therefore necessary to eliminate or minimize the amount of N_2 entering the mass spectrometer by maximum dilution or diversion of the N_2 to improve the accuracy of $\delta^{18}O$ measurements of N-bearing materials [Werner et al. 1996, Farquar et al. 1997, Werner and Brand 2001; Bohlke et al. 2003; Gehre and Stauch 2003; Accoe et al. 2008, Brand et al. 2009; Qi et al. 2011, Hunsinger and Stern 2012]..

For hydrogen isotope analysis of nitrogen- and halogen-bearing materials the use of a reactor containing metallic chromium and possibly manganese [Morrison et al. 2001; Renpenning et al. 2015; Gehre et al. 2015; Gehre et al, 2017] is recommended. In a glassy carbon reactor, existing H-C-N bonds may be partially converted to hydrogen cyanide (HCN) and existing H-Cl bonds may be partially converted to hydrogen chloride (HCl), preventing quantitative conversion of hydrogen to H_2(g). The metal based reactor captures chlorine and nitrogen, permitting quantitative, accurate and precise hydrogen isotope ratio measurements. Unfortunately, chromium based reactors cannot be used for the analysis of oxygen isotopes due to the formation of chromium oxides, which are relatively stable even at the elevated temperatures employed. High purity metallic chromium is expensive compared to glassy carbon or manganese.

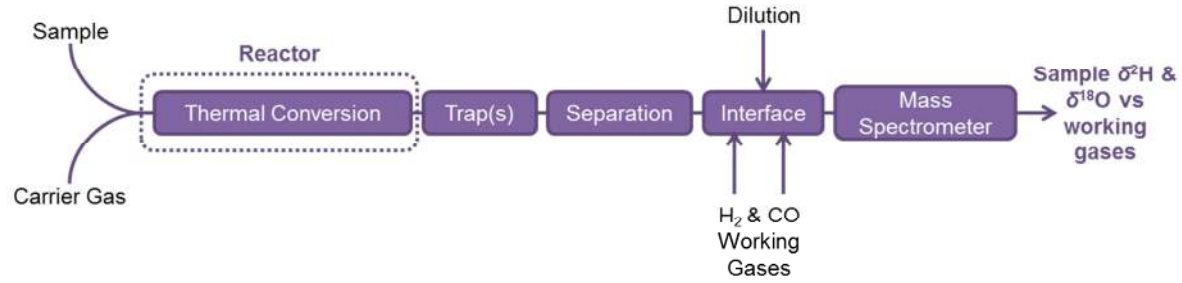

Figure 4. *Simple schematic diagram of an HTC/IRMS system for the determination of $\delta^{2}H$ and $\delta^{18}O$ values. Note that materials containing elements other than H, C and O can yield products of thermal conversion that may need to be removed.*

3.2.4 EA and HTC Interface

Some form of interface is required to connect an on-line EA or HTC system to the IRMS instrument. The interface reduces the gas volume entering the ion source and provides a means to introduce pulses of working gas and to dilute the sample gas with additional helium.

These functions of the interface make it possible to carry out measurement of $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$ isotope ratios from one sample portion. Most organic compounds contain a relatively small proportion of nitrogen and sulphur and the three gases can be diluted to give similar signal sizes. In the same manner simultaneous measurements of both $^{2}H/^{1}H$ and $^{18}O/^{16}O$ isotope ratios via HTC-IRMS are possible, but typically if samples contain no nitrogen; liquid water, cellulose etc. Distinct and mutually inconsistent analytical methods are recommended for H and O isotope ratio measurements of N-bearing material (section 3.2.3).

3.2.5 FIA/IRMS (flow injection analysis isotope ratio mass spectrometry)

LC/IRMS systems can afford the means to carry BSIA by bypassing the chromatographic separation step. This can be particularly useful for water soluble compounds, which are difficult to isolate in sufficient quantities for EA/IRMS analysis such as intact phospholipids, proteins, etc. This is known as Flow Injection Analysis/IRMS (FIA/IRMS). Further details regarding the instrumentation and principle of such analyses can be found in the LC/IRMS sections below.

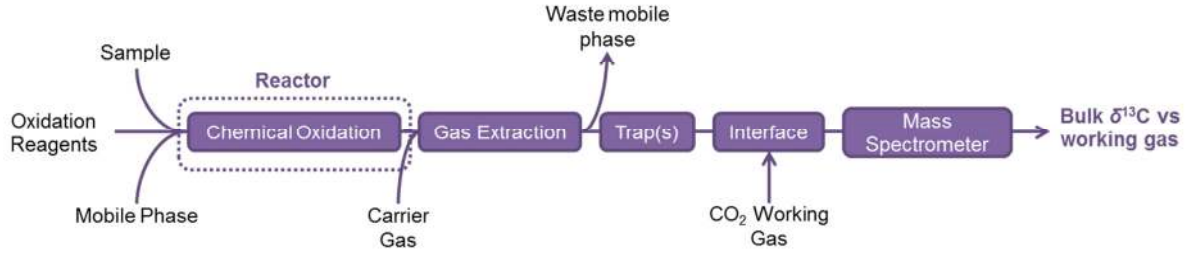

Figure 5. *Simple schematic diagram of a FIA/CO/IRMS system for the determination of $\delta^{13}C$ values*

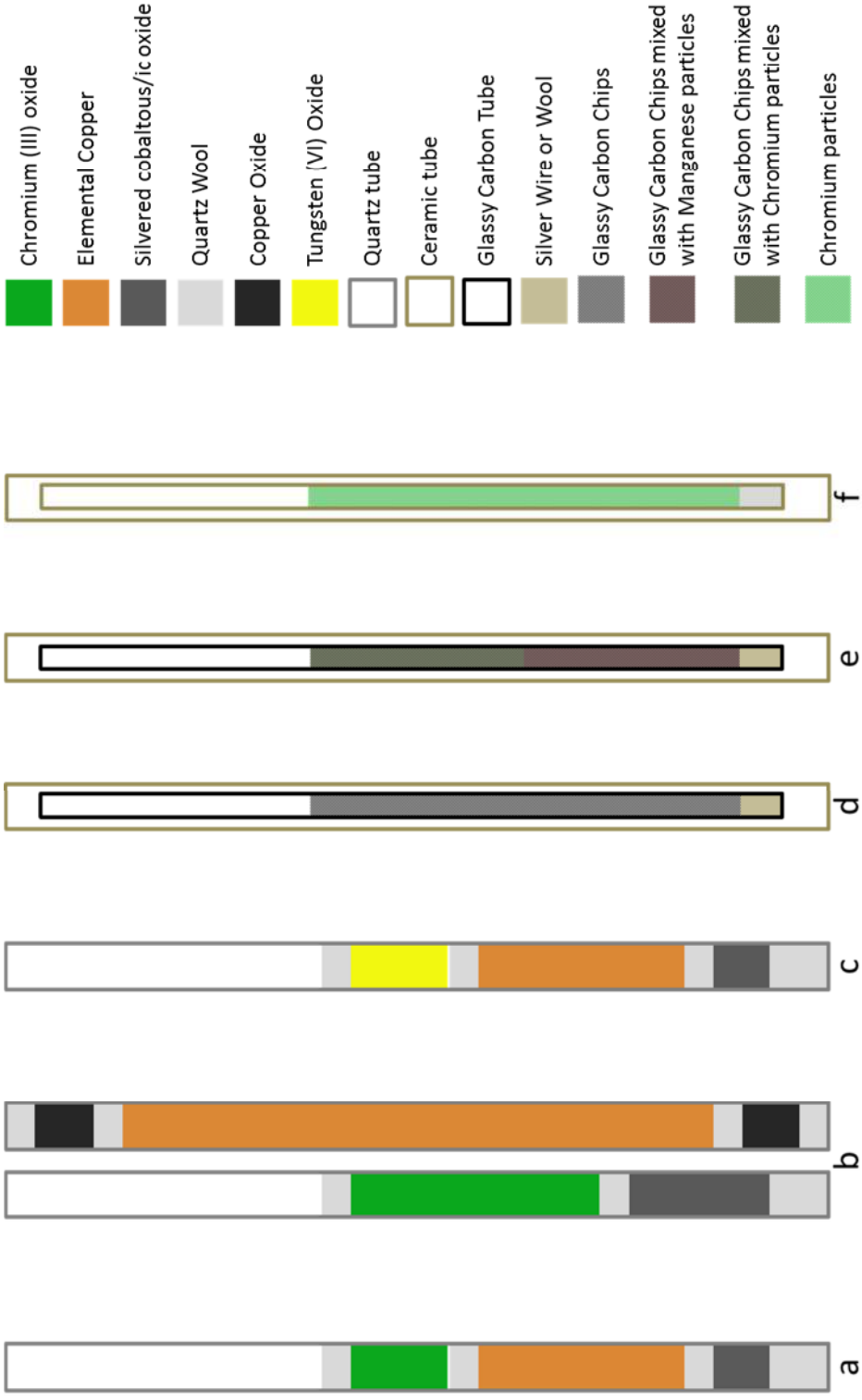

Figure 6. *Schematic representation of example EA/IRMS and HTC-IRMS combustion, reduction and thermal conversion reactors (exact amounts of chemicals required will vary depending on instruments and applications):* **a** *– single combustion/reduction reactor for N and C analysis;* **b** *– separate combustion and reduction reactors for N and C analysis;* **c** *– single combustion reduction reactor for N, C and S analysis;* **d** *– HTC reactor tube for O analysis and for H analysis of materials that do not contain N or halogens;* **e & f** *– two different reactors for the H analysis of materials containing N and/or halogens.*

3.3 Compound-specific isotope analysis (CSIA) techniques

The function of an EA or HTC system is quantitatively to convert the target element(s) present in a sample to the appropriate gas for IRMS analysis, regardless of the number of individual chemical species present. The techniques broadly described as Compound Specific Isotope Analysis (CSIA) comprise an additional stage in which some or all of the individual compounds present in a sample are separated as a function of time. Individual compounds are then converted to the appropriate gas, which is introduced to the IRMS instrument in a continuous process. A plot of the concentration of the gas evolved as a function of time appears much the same as a chromatogram produced by any number of more common detectors.

3.3.1 GC/IRMS (gas chromatography isotope ratio mass spectrometry)

Only about 15 % of organic compounds can be analysed by gas chromatography (GC), but this encompasses a wide range of forensic and environmentally important compounds from those that are gaseous at room temperature, such as methane, to relatively involatile compounds such as polychlorinated biphenyls (PCBs). Coupling the separation afforded by GC to IRMS instruments requires an interface that can convert the separated compounds into the analyte gases needed for isotopic analysis.

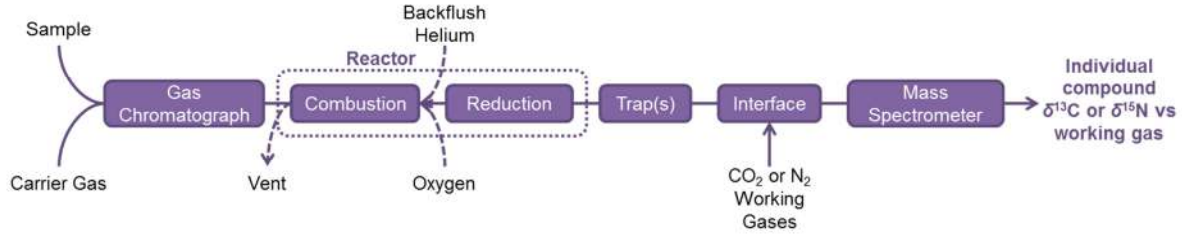

Figure 7. *Simple schematic diagram of a GC/C/IRMS system for the determination of $\delta^{13}C$ or $\delta^{15}N$ values. Note that the combustion and reduction reactors can be combined into a single tube. Dashed lines show the gas flow in back-flush mode.*

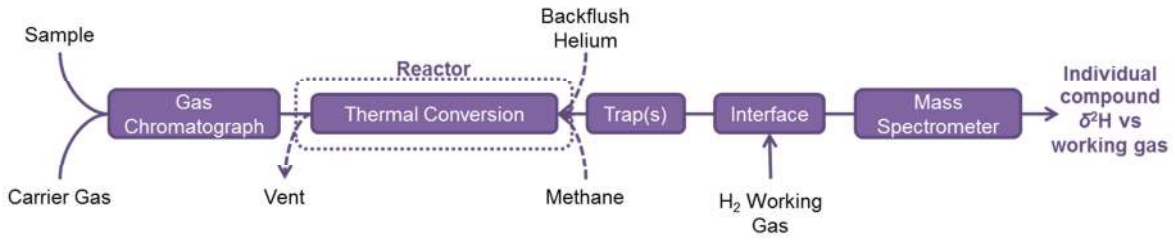

Figure 8. *Simple schematic diagram of a GC/HTC/IRMS system for the determination of $\delta^{2}H$ values. Dashed lines show the gas flow in back-flush mode.*

3.3.1.1 Gas chromatography

Whilst the gas chromatograph will require modification to accommodate the combustion/reduction reactors for GC/C/IRMS, the principles of gas chromatography for this application are no different to any other GC-based instrumentation – GC/FID, GC/MS, etc.

There are two ways in which to view GC/IRMS: either the GC is the inlet system for the IRMS or, the IRMS is the detector for the GC.

Early applications (mainly by existing IRMS users) considered the technique from the first viewpoint and employed wide-bore column (0.32 mm) and thick phases (1.0 μm and above) in order to introduce large samples to the IRMS. In practice, because the peaks from a gas chromatograph are considerably narrower than from an EA, high instantaneous concentrations are produced and acceptable results can be achieved from 10 to 100 ng of carbon (or other elements). With this understanding virtually any GC column can be coupled to an IRMS instrument regardless of diameter, length or phase thickness, with two possible exceptions. Many GC/IRMS interfaces are not well suited to high temperature applications (> 300 ℃) due to the use of polyimide ferrules, which can quickly become loose and leak with repeated temperature cycling. Also, columns that are not chemically bonded, notably Porous Layer Open Tubular (PLOT) columns, can be damaged by repeated pressure changes associated with switching between “straight” and “back-flush” modes (see details in section 3.3.1.2).

In common with many GC applications, low bleed columns (specifically manufactured for use with MS detectors) are preferred because they provide a stable (flat) baseline, which is critical to achieving repeatable peak integration and, thereby, repeatable results.

Early applications of GC/IRMS favoured split-less or on-column injection techniques in an attempt to avoid isotopic fractionation. However, in practice it is virtually impossible to introduce a sample into a GC column without some isotopic fractionation, regardless of injection technique. Therefore the injection method should be optimised for chromatographic separation provided that RMs are introduced using the same method (see PIT, section 5.1.3). Some applications, especially forensic ones, divide the GC effluent between an organic mass spectrometer and an isotope ratio mass spectrometer to obtain positive identification and stable isotopic composition from a single injection – the organic MS typically requires a very small proportion of the sample.

3.3.1.2 Combustion (C) interface

The GC/IRMS combustion interface is essentially a miniaturised version of the EA/IRMS configuration described above.

The reactors are typically made of non-porous alumina with an internal diameter of 0.5 mm. The carbon in organic compounds is converted to CO_2 using a combination of metal oxides and a platinum catalyst operating between 850 ℃ and 1100 ℃, depending on the exact nature of the packing. The reactor must be periodically regenerated by passing oxygen through the reactor to replenish the metal oxides.

Special reactor configurations may be needed for $\delta^{13}C$ measurements of certain recalcitrant compounds such as environmental pollutants [Reinnicke et al. 2012].

As depicted in Figure 7, the interface may incorporate a separate reduction reactor to remove excess oxygen and reduce nitrogen oxides or, like EA configurations these processes can be combined in a single reactor.

The water formed during combustion of the sample is typically removed using an ionic polymer membrane (Nafion®) with a counter-flow of dry helium.

For nitrogen analyses, CO_2 must be removed from the gas stream – generally by cryogenic trapping to prevent possible isobaric interferences from the production of $[^{12}C^{16}O_2]^{2+}$ [Merritt et al. 1994]. Applications measuring $^{15}N/^{14}N$ ratios by GC/C/IRMS are confounded by the relatively low abundance of nitrogen in most organic compounds and by the need for two nitrogen atoms to form one molecule of N_2 gas.

The GC interface is coupled to the IRMS instrument via an open-split. Because the flow of GC effluent is far lower than the EA carrier (typically 1-2 mL min^{-1}), the split ratio is very low and a large proportion of the sample gas is transferred to the mass spectrometer. An important function of the open-split is to reduce pressure surges as GC peaks are converted to gas.

The GC/IRMS interface operates in two modes often referred to as “straight” and “back-flush”. In straight mode the GC effluent passes through the reactor, dryer and open-split as described above. In back-flush mode the GC effluent is vented before it reaches the reactor, typically using

a simple mechanical valve inside the GC oven. Also in back-flush mode a small amount of helium flows backwards through the reactor (i.e. from the open-split to the GC oven) to ensure that no GC effluent enters the reactor.

The primary purpose of back-flush mode is to divert the GC solvent peak, which will be many orders of magnitude larger than the sample peaks. Combusting a relatively large quantity of organic material may damage the reactor (or seriously deplete its oxidation capacity) and the pressure surge from the large amount of gas evolved may damage down-stream components, including the ion source. Back-flush mode is also used when passing oxygen through a reactor (for re-generation) to avoid these gases entering the mass spectrometer. It is good practice to introduce pulses of working gas while the interface is in back-flush mode so that the baseline is as stable and reproducible as possible. After the solvent peak has been fully diverted the interface will be switched to straight mode, usually controlled as a timed function from the instrument software. Following the switch from back-flush to straight mode it will take some time for the baseline to stabilise and it is important that this is achieved before any peaks of interest elute.

3.3.1.3 High temperature conversion (HTC) interface

The GC/IRMS HTC interface is essentially a miniaturised version of the HTC/IRMS configuration described above.

The hydrogen in organic compounds is converted to H_2 by the Schuetze/Unterzaucher reaction at around 1400 ℃. The reaction requires the presence of carbon that is typically introduced by analysing a number of samples until a small deposit of carbon is formed on the lumen surface. A carbon deposit can also be formed by passing methane through the reactor in back-flush mode (3.3.1.2).

It is possible to measure $^{18}O/^{16}O$ ratios using GC/HTC/IRMS to convert organic oxygen to CO. This application employs a modified non-porous alumina tube containing an inner platinum tube with nickel wire operated at approximately 1280 ℃. An additional T-piece is also needed before the reactor, to mix a very small flow of hydrogen into the GC effluent. A recent review of this technique [Hitzfeld et al. 2016] found that both commercially available and bespoke reactors did not achieve quantitative conversion to CO with significant amounts of CO_2 being formed. The application of this technique is limited by technical complexity and by the low abundance of oxygen in most organic compounds but has been applied to the analysis of water [Wang et al. 2015].

3.3.2 LC/IRMS (liquid chromatography isotope ratio mass spectrometry)

LC/IRMS is another example of a coupled chromatographic application of IRMS for CSIA. The individual components of a mixture are separated by high pressure liquid chromatography (HPLC), then converted into the analyte gas (for example by chemical oxidation to CO_2), which can then be extracted from the mobile phase and dried before transfer to the mass spectrometer. LC/IRMS is particularly useful for small, polyfunctional compounds, which would require derivatisation for GC separation prior to IRMS analysis (amino acids from protein hydrolysates, sugars, etc.).

3.3.2.1 Liquid chromatography

Any liquid chromatographic system can be interfaced with an IRMS instrument providing the instrument control softwares are compatible. The principle by which LC-to-IRMS chemical oxidation interfaces (LC/CO/RMS) currently operate however limits the range of separations that are available. As with GC, splitting of the column eluent between the IRMS interface and a means of compound identification (mass spectrometer, PDA, etc.) allows the characterisation and determination of the isotopic composition of compounds from a single analysis.

No organic (carbon-containing) mobile phases can be used in LC/CO/IRMS and mobile phases must be limited to water and inorganic buffers or acids. If the LC system has been previously used with organic mobile phases these must be thoroughly flushed prior to LC/CO/IRMS use, which will require large volumes of carbon-free mobile phase (i.e. water). Care must also be taken with the HPLC columns used for compound separation as these can also be contaminated by previous use with organic mobile phases. The use of an LC system dedicated to LC/CO/IRMS analysis, and thereby guaranteed to be free from carbon-containing mobile phases, is recommended.

For other LC-to-IRMS interfaces such as combustion there may be other restrictions upon LC separations that can be employed.

3.3.2.2 Chemical oxidation (CO) interface

The key components of an LC/CO/IRMS system are shown in Figure 9.

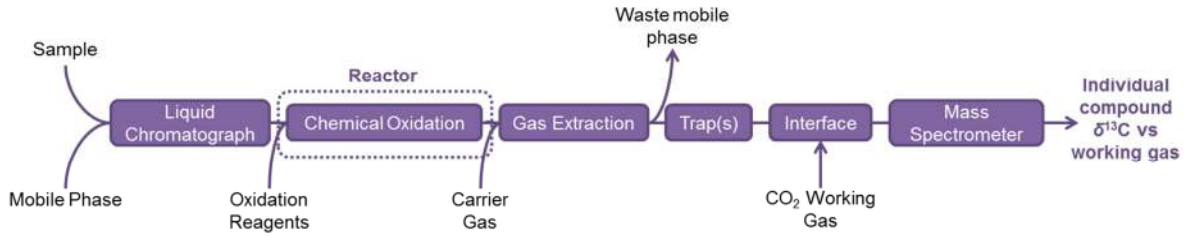

Figure 9. *Simple schematic diagram of an LC/CO/IRMS for the determination of $\delta^{13}C$ values.*

Most commercially available LC/IRMS interfaces operate via a chemical oxidation procedure akin to Total Organic Carbon (TOC) analysers in which the components of a mixture are separated by HPLC, and then oxidised to CO_2 while still in solution. Oxidation is performed by sodium persulfate and a silver nitrate catalyst can be added for difficult-to-oxidise compounds at just below the boiling point of water. A gas separator then extracts the CO_2 from solution aided by the addition of phosphoric acid. The gas stream is then dried via a Nafion® membrane and passed to the MS through an open-split.

The chemical oxidation process precludes the use of organic mobile phases during LC separation because these would also be oxidised to CO_2 and swamp the relatively tiny sample signals.

Some functional moieties are more difficult than others to oxidise under the conditions used in the chemical oxidation interfaces. For example, it has been shown that carbon atoms bonded to two or three nitrogen atoms or within aromatic N-heterocycles can be particularly difficult to oxidise completely [Diaz et al. 2013]. Halogenated compounds can also be difficult to oxidise when three halogens are bound to the same carbon atom such as trichloroacetic acid and trifluoroacetic acid [Gilevska et al. 2014].

3.3.2.3 Combustion (C) interface

A novel combustion system that allows coupling of LC-to-IRMS instruments for both N and C isotope ratio determinations is in development. This consists of a modified high-temperature combustion TOC analyser. The modifications include a three-step drying system to handle the continuous flow of water, favourable carrier and reaction gas mix and flow, and an efficient high-temperature oxidation and subsequent reduction system [Federherr et al. 2016]. This is a relatively new technique and it is not discussed in further detail within this edition.

3.4 Miscellaneous techniques

There are numerous other peripherals that may be connected to IRMS instruments for specific applications. As these are less commonly applied in forensic studies they are only briefly described below in Table 4:

Table 4. *Miscellaneous peripheral instruments that can be coupled to isotope ratio mass spectrometers*

Peripheral	Description
Carbonate analyser	Automated system for the release of carbon dioxide from carbonate samples by reaction with phosphoric acid, purification of the gas and then transfer to the bellows of a DI/IRMS system. Often able to analyse multiple samples within a sequence.
Dissolved Organic Carbon (DOC) & Total Organic Carbon (TOC) analysers	Automated systems for analysis of liquid samples. Small acidified 50-500 μL samples are injected into high temp (680-1000 ^{o}C) ovens, or 30 mL samples are oxidized at 100 ^{o}C with acidified persulfate. The resulting CO_2 is extracted for isotopic analysis.
Gas analyser	Continuous flow peripheral for the analysis of headspace or other gases when DI/IRMS is not available. Often combined with gas preparation stages such as carbonate analyser (above), or equilibrations with H_2/Pt or CO_2 for H and O isotope analysis.
Breath tester	System specifically designed to measure changes in the isotopic composition of exhaled breath CO_2 following ingestion of isotopically labelled compounds for medical diagnoses.
Preparative chromatographic systems	Preparative chromatography can be used to isolate compounds or fractions from complex mixtures prior to BSIA. This can be useful when there is insufficient material for online CSIA.
Laser sampling (ablation)	For spatially resolved and non-destructive (on a macro scale) analysis

4 Instrument set-up and preparation

4.1 Environmental control and monitoring

In order to achieve precise and reproducible measurements, an IRMS instrument must be located in an environment in which both temperature and humidity are closely controlled and monitored. The pre-installation and/or operating instructions from an instrument manufacturer will specify the acceptable range and maximum temporal variation for these parameters.

The quality of gases supplied to an instrument will also have a significant effect on the quality of data generated. Again, the instrument manufacturer should provide acceptable specifications.

It is important that the cylinders (and associated valves and regulators) supplying working gases to the IRMS instrument are also located in a temperature-controlled environment as temperature fluctuations can produce significant shifts in the isotopic composition of the working gases.

For similar reasons, the working gas cylinders should be located as close to the instrument as possible, although for safety considerations this is not always possible. Gas cylinder contents pressure should be monitored to ensure that sufficient gas is available for the sequence of analysis. The CO_2 working gas is replaced when the pressure is less than approximately 48.3 bar (700 psi), indicating that the liquid in the tank is exhausted.

The carrier gas for all CF/IRMS instrument configurations is helium, which will generally be supplied with purity better than 99.9992% (N5.2). The carrier gas supply should incorporate filters to remove trace amounts of residual oxygen and moisture. These filters are available as self-indicating (i.e. change colour when spent) cartridges that should be checked and their status recorded on a weekly basis. It is often advisable to mount a hydrocarbon filter close to the instrument to remove any traces of fluids used to manufacture the gas line tubing. These filters are typically not self-indicating and should be changed periodically.

Filters, other than simple frits, should not be incorporated in the working gas supplies as these may cause isotopic fractionation.

4.2 Safety equipment

Many of the gases used in the routine operation of IRMS are hazardous (e.g. H_2, CO and SO_2) and the laboratory should have monitoring systems to warn of dangerous gas levels. Specialised techniques (DI/IRMS and GC/C/IRMS) also require liquid nitrogen in which case the oxygen level in the laboratory should be monitored. Checking that these warning systems are functioning correctly should be an integral part of the daily instrument checks.

4.3 Testing routine

It is important to ensure that the system is working properly both at the beginning of the measurement process and throughout the sequence of samples analysed. It is recommended that laboratories develop, and follow, a specified routine of instrument checks and quality control, which is applied to every sequence of measurements. The rota of tests and their frequency should be documented in laboratory operating procedures and the accompanying records must exist, for example, in the form of an instrument logbook and/or spreadsheets.

With all instrument tests it is important to perform them regularly so there is a record of results when the instrument is working well – not just when it is broken. “Normal” operating performance for an instrument must be established during commissioning. Diagnostic tests that have specified acceptance criteria also provide a means to monitor the operability of an instrument and to ensure action can be taken where an instrument is not functioning normally.

Regardless of application(s) or instrument configuration(s), daily system checks should begin with a scan of the background gases in the instrument (section 4.3.1). If these are beyond the normal range there is no point in proceeding with other tests as an investigation will be needed. If the background scan is acceptable the next step is a zero-enrichment (on-off) test of the instrument

precision with respect to the working gas (section 4.3.2). Again, if the result of this test is poorer than expected the cause must be investigated. If both background and zero-enrichment tests are successful the final test is to measure the linearity of the instrument, i.e. how the measured δ value changes with peak size (section 4.3.3).

Other tests may be needed for specific instrumental configurations and/or analyte isotope ratios (e.g. H_3^+ factor, section 4.5.1).

The user is, again, advised to consult the operating manuals of specific instruments.

4.3.1 Background gases

Instrument manufacturers will often specify acceptable levels of residual gases in the ion source.

In practice, these background levels will vary from laboratory to laboratory, depending on the instrument configuration, the grade of carrier and working gases used and many other factors. The important consideration is to monitor the background values every day the instrument is used. This will establish acceptable levels so that any changes highlight possible problems.

Figure 10 shows a typical background of residual gases for the EA/IRMS configuration. Typically the intensity of *m/z* 18, 28, 32, 40 and 44 should be recorded. Background monitoring should also include *m/z* 2 when performing hydrogen isotope measurements. Acceptable values must be determined for individual instrument configurations. Note that LC/CO/IRMS systems typically have background values much higher than those for EA/IRMS or GC/C/IRMS systems, and these may need to be recorded using a different Faraday collector.

Table 5. lists some possible causes of problems with background values (see also section 9 on troubleshooting for further information).

Table 5. *Typical problems with background values and possible causes in EA/IRMS and HTC/IRMS instruments.*

m/z	**Mol. species**	**Problem and possible cause**
2	He^{2+}	High background in $^2H/^1H$ measurements Electron energy can be adjusted to produce acceptable values
18	H_2O^+	Produces protonated species which may interfere with ions containing heavy isotope
28	N_2^+	Guide to ingress of atmospheric gases (also CO by thermolysis)
32	O_2^+	Bleed from EA oxidation catalyst.
40	Ar^+	Best guide to the ingress of atmospheric gases
44	CO_2^+	Contamination of C/N analysers or oxygen ingress into H/O analysers

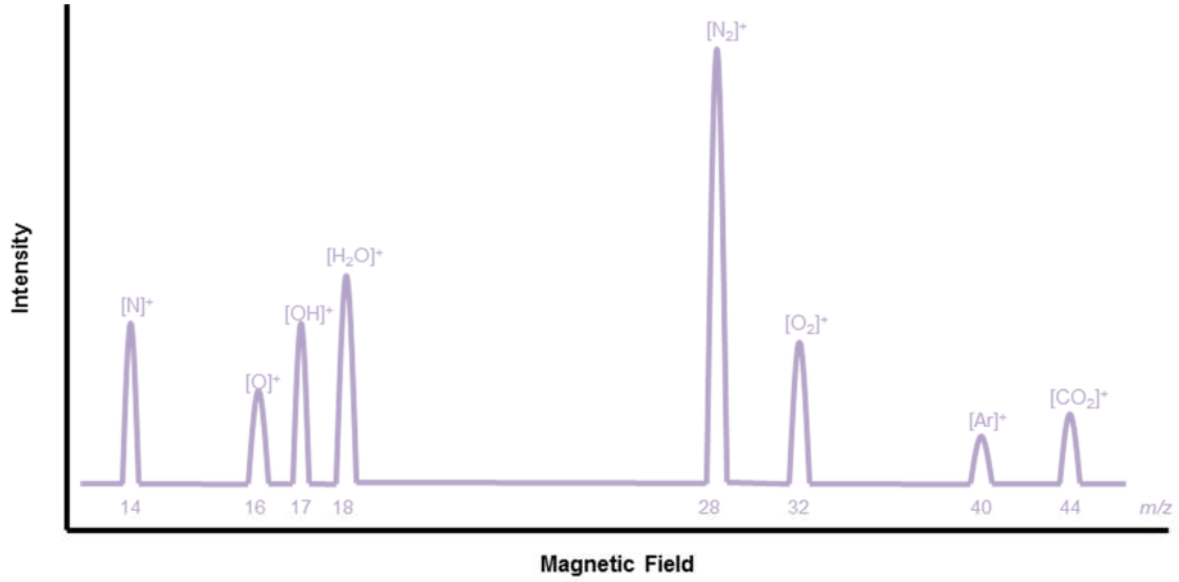

Figure 10. *Example background scan consisting of a plot of magnetic field strength and hence m/z against signal intensity.*

4.3.2 Stability (zero-enrichment or on-off)

It is important to monitor the stability of the measurement of the isotopic composition of the working gas on a daily basis. The raw data from continuous flow IRMS are (usually) initially evaluated relative to the working gas and hence the reproducibility of this measurement determines the best reproducibility that can be achieved for samples.

The measurement, known as "zero-enrichment" or "on-off" test simply involves introducing sequential pulses of working gas (typically ten) into the instrument and recording the standard deviation of the δ values, relative to one pulse defined as a reference value. This test must be performed with the intensity of the gas pulses set within the anticipated working range of sample peak heights.

As with all performance tests, acceptance criteria must be established for a specific instrument. Generally, the standard deviation for CO_2, N_2 and CO must be less than 0.1 ‰ and for H_2 less than 1.0 ‰.

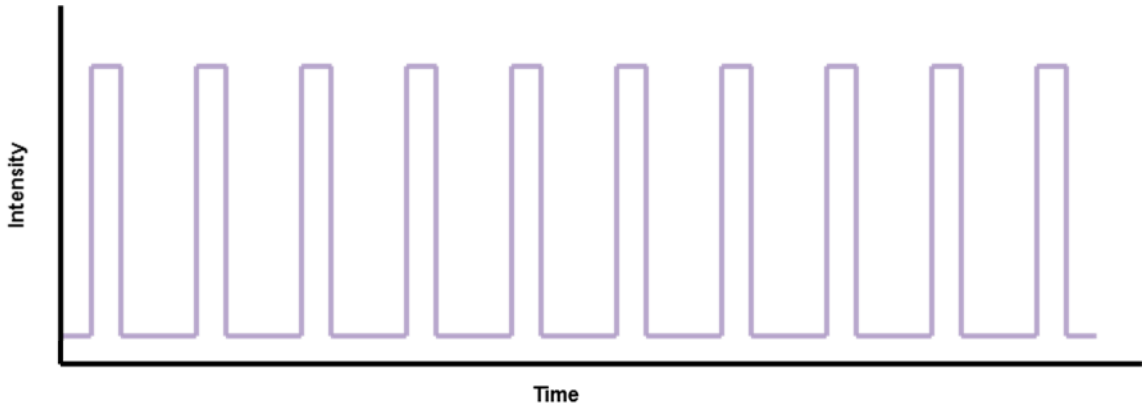

Figure 11. *Example of a zero-enrichment check or on-off test.*

4.3.3 Linearity (peak size)

Periodically (if not daily), the linearity of the instrument must be tested with respect to the working gas.

The measurement is similar to the zero-enrichment test, except that the intensity of the working gas is varied during the sequence. The intensity of the working gas pulses must encompass the intensities of the gases evolved from samples which will be established during validation.

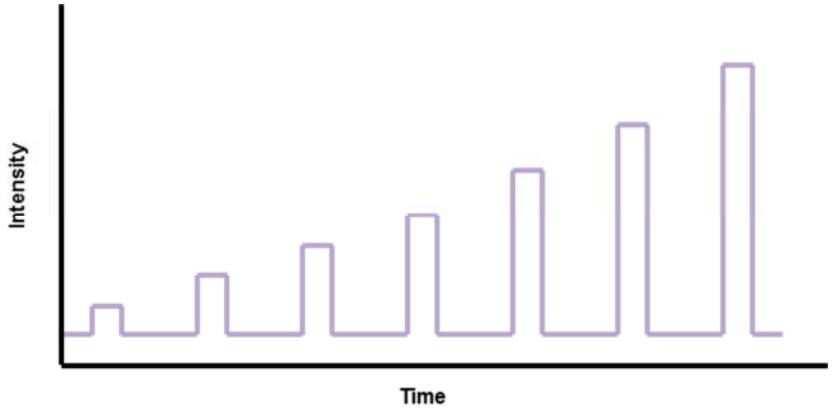

Figure 12. *Example of a linearity check using working gas.*

Ideally, the δ values for N_2, CO and CO_2 working gases, across the normal range of sample intensity, should not vary by more than the standard deviation determined for the working gas zero-enrichment test. If the variation in δ value is greater, this indicates a real effect that can be easily corrected.

The linearity test is not applicable to $^2H/^1H$ measurements, which require a daily H_3^+ factor determination (sections 4.5.1 and 6.2.2).

Linearity can also be determined from samples rather than using the working gas. This is most easily achieved on GC/ and LC/IRMS instruments where the injection volume can be varied. For EA/ and HTC/IRMS instruments, careful weighing of differing amounts of sample can be used.

4.4 EA/IRMS tests

The backgrounds (section 4.3.1) should be determined not only for the mass spectrometer alone, but also with the EA connected. The differences in the background levels and performance between these two configurations can help identify the source of any problems. Stability and linearity tests should be carried out with the EA connected to the IRMS instrument.

Components of the EA system need to be regularly renewed for proper functioning:

- Cleaning/replacing the ash collector (if used),
- Replacing the oxidation reactor reagents,
- Replacing the reduction reactor reagents,
- Replacing the trap reagents (e.g. water and CO_2), and
- Baking the GC column.

The frequency of replenishing these components will vary between instruments and with sample type and sizes. After replacing reactor or trap reagents monitoring the background gases will ensure that the system is leak-free.

4.5 HTC/IRMS tests

As with EA/IRMS configurations, the backgrounds should be determined for the mass spectrometer with the HTC connected. A stability test should be performed but a linearity test is only needed for oxygen isotope ratio measurements. In the place of a hydrogen isotope linearity test, a so-called "H_{3+} factor" determination must be performed (section 4.5.1):

Components of the HTC system need to be regularly renewed for proper functioning:

- Emptying/replacing the graphite crucible,
- Cleaning or replacing the reactor components,
- Replacing the trap reagents (if used), and
- Baking the GC column.

The frequency of replenishing these components will vary between instruments and with sample type and sizes. Always leak-check the system by monitoring the background gases after replacing reactor or trap reagents to ensure that the system is leak-free.

4.5.1 H_3^+ Factor

The term "H_3^+ factor" describes an algorithm applied to measured δ^2H data to correct for the contribution of H_3^+ species formed by ion/molecule reactions in the ion source at increasing H_2 partial gas pressures.

$$H_2^+ + H_2 \rightarrow H_3^+ + H^\bullet \qquad (4)$$

The reaction constant is proportional to both [H_2^+] and [H_2] and, for a given instrument, the number of ions formed is proportional to the number of molecules present. The ratio [H_3^+]/[H_2^+] is, therefore, a linear function of the *m/z* 2 intensity and the correction simply subtracts a portion of the *m/z* 2 intensity from the *m/z* 3 intensity.

The H_3^+ factor is determined by measuring the intensity of *m/z* 3 as a linear function of *m/z* 2, usually performed with the working gas. A sequence of gas pulses are introduced with increasing intensity of *m/z* 2. The instrument software can then calculate the H_3^+ factor. The value should be recorded in the instrument log book or spreadsheet. The H_3^+ factor should remain relatively constant but must be determined daily as any large change will be indicative of a problem.

As for other tests (background gases, zero-enrichment, etc.) it is important to monitor the H_3^+ factor to establish a range for acceptable instrument performance and an upper limit that might indicate an existing or potential problem.

4.6 GC/C/IRMS tests

Any checks that are specific to the gas chromatograph should be carried out as recommended by the instrument manufacturer. Most tests will be common to all GC analyses – e.g. cleanliness of the injector liner, etc.

4.6.1 Backgrounds

Two different background scans should be performed daily and the intensity of signals at *m/z* 18 (water), *m/z* 32 (oxygen), *m/z* 40 (argon) and *m/z* 44 (CO_2) should be recorded:

- Background scan with the interface in "back-flush" mode.
- Background scan with the interface in "straight" mode.

The differences between background gas intensities in "back-flush" and "straight" modes can identify leaks in the system (resulting in increased argon background); contamination (increase in

water/CO_2); loss of reactor efficiency/indicator of need for reactor reconditioning (reduced O_2); or loss of water trap efficiency/indicator of need to change the Nafion® membrane (increased water).

4.6.2 Argon injection test

This procedure tests for correct transfer of gas through the entire system.

Set the mass spectrometer to monitor *m/z* 40 on the middle collector (relative gain 100) and inject approximately 2 µL of laboratory air. Record the retention time (RT), height and width of the argon peak. The peak should be sharp and symmetrical with a consistent height. Changes in retention time (RT) or peak shape are indicative of leaks or blockages in the gas chromatorgraph or interface – especially peak tailing. This test can be performed with the reactor either cold or hot.

It is good practice to calculate the "dead-volume" of the GC column (based on column length and carrier flow). The difference between this time and the RT of the argon peak is the "dead-volume" of the interface which should not change.

4.6.3 Hexane vapour injection test

The GC oven should be set to a temperature at which the column will not retain hexane (typically > 100 ℃) and the mass spectrometer should be set to monitor *m/z* 44 on the middle collector (relative gain 100).

Take 1-2 µL of headspace from a vial of hexane (or similar hydrocarbon solvent) and inject into the GC. DO NOT TAKE ANY LIQUID INTO THE SYRINGE!

The peak should have a retention time very close to that of argon and individual isomers (typically but not always present) should be resolved (depending on the column stationary phase). As with the argon test above (section 4.6.2), poor peak shape indicates a problem with the system, in this case poor combustion.

To perform the same test for GC/HTC/IRMS (δ^2H analysis) simply set the mass spectrometer to monitor *m/z* 2.

4.7 LC/CO/IRMS tests

The backgrounds for an LC/CO/IRMS system should be measured daily; however the Faraday collectors on which the backgrounds are measured may be different to those used for other peripherals due to elevated background levels (consult the instrument manual). The water background will typically be significantly higher than for other peripherals connected to the IRMS instrument whereas increases in this background over the typical level indicate problems with the gas separation unit and/or water trap (i.e. the Nafion® membrane). The oxygen background provides an indication of the oxidation potential afforded by the current reagent concentrations and flow rates. The working gas stability and linearity should be monitored as described above.

The fill level of mobile phase and reagent containers should be checked to ensure sufficient solutions are available for the planned sequence of analyses (as well as any standby time at the end of the sequence). Likewise the waste container(s) should be emptied.

For LC/CO/IRMS there are two further tests that should be carried out prior to instrument use:

4.7.1 Stability of CO_2 background

The stability of the carbon dioxide background level measured on *m/z* 44 should be monitored for a period of at least 5 minutes before an analytical sequence. The Faraday collector to use will be

specified by the manufacturer. The standard deviation of the ion current should be below the manufacturer's recommended threshold or established empirically.

When using gradient elution, the stability when pumping each mobile phase background should be assessed. If different mobile phases have different levels of carbon background then any gradient between the two will result in a variable background signal and peaks eluting during the period will be difficult to integrate reliably.

4.7.2 Back-pressure

The back-pressure of the entire LC/IRMS system should be monitored frequently because in-line filters, guard columns and HPLC columns as well as components of the interface such as the reactor and gas separation unit can become clogged by particulate matter. Increases in back-pressure indicate a (partial) blockage, which should be addressed before continuing with the analytical sequence. Clear any blockages (there may be back-flushing protocols in the instrument manual supplied by the manufacturer) and ensure that the back-pressure is returned to normal before continuing.

It can be useful to determine the back-pressure for various components of the LC/IRMS system in isolation (when working normally), or before/after various components, which can be referred to when tracking down the location of a blockage.

5 Making measurements

5.1 EA-IRMS bulk nitrogen and carbon measurements

In general, better precision is obtained when measuring the isotopic composition of a single gas evolved from a sample. Due to constraints on the amount of sample and/or time available for the analysis it may be advantageous to measure two or more gases sequentially evolved from the same sample portion.

In order to measure both δ^{15}N and δ^{13}C values (or δ^{2}H and δ^{18}O values) for the same portion of a sample the IRMS instrument must "jump" between two suites of ions. To achieve this "jump" either the magnetic field strength or the accelerating voltage of the ion source is changed to focus the required ions into the collectors (historically, the magnetic field was slow to change in the timescale required).

A jump calibration must be performed daily if dual measurements are planned. This process is typically automated within the IRMS software and will determine the change in high voltage or magnetic field required.

5.1.1 Preconditioning

The EA reactor(s) should be brought to operating temperature(s), with normal helium flow, 12-24 hours before any samples are analysed. Depending on the type of elemental analyser employed, it may be necessary to carry out a "pre-conditioning" of the reactor system. This can be done by analysing a series of capsules containing homogenous material chemically similar to the samples until the δ value is stable. Note that pre-conditioning must occur before any normalisation RMs are analysed as the δ value does not need to be accurate, just consistent/stable. See section 6.

5.1.2 Blank determinations

Extraneous gases evolved from the tin capsule, which encloses a sample, will combine with those from the sample and contribute to the measured δ value. A signal from a blank analysis may also result from atmospheric gases introduced by the auto-sampler or from the oxygen pulse.

It is always advisable to carry out a blank determination prior to the analysis of samples. Empty sample capsules (folded as if loaded with sample) are introduced via the auto-sampler, using the same EA parameters as for the samples.

A high (unacceptable) blank determination is usually indicative of contamination of tweezers, capsules, work surface, etc. When high blank determinations are observed the first step should always be to try to identify and eliminate the cause.

The blank levels associated with direct injection of liquid samples into an EA system are typically very small and insignificant in comparison to the intensity of the sample gas peak.

Nitrogen measurements of low % N materials (soils, sediments, etc.) may be confounded by the inclusion of atmospheric nitrogen between particles. The magnitude of the nitrogen blank for such samples can be determined by heating a portion of the sample in a furnace at 550 ^{o}C assuming this does not significantly affect the physical form of the sample. Analysing aliquots of the pre-combusted material will reveal the magnitude of the nitrogen blank, The isotopic composition of the blank can be determined through isotope dilution i.e. using various sizes of reference materials (6.4.1).

5.1.3 Sample preparation

It is fundamentally important that samples, RMs and QC materials are prepared and analysed in an identical manner, according to the PIT [Werner and Brand 2001].

EA/IRMS determines “bulk” isotope ratios, i.e. the carbon isotope ratio is derived from all the carbon containing substances in the combusted sample. In order to obtain precise results by EA/IRMS the samples must be as homogenous as possible.

5.1.4 Sample measurement

Typically, between two and six analytical results should be acquired for each sample as more analyses provide more confidence in the experimental uncertainty of the measurement. At least two RMs for each isotope ratio of interest are analysed at the start of the sequence and (in some laboratories) again at the end. These measurements will be used to normalise the data obtained during the sequence. An in-house RM is analysed periodically throughout the sequence for quality control. A typical sequence is shown in Figure 13. To maintain sample continuity, the use of 96-well plates is recommended. The sample identification, position and the amount weighed should be recorded in a suitable template such as Figure 13. The template reflects the format of the 96-well plate in which samples will be assembled prior to analysis.

The weight of sample (using a micro balance) should be selected so that the resulting N_2 and CO_2 signal intensities (with appropriate dilution) are within the linear range of both the sample introduction device (elemental analyser) and mass spectrometer. For optimum performance the maximum intensity of the major ion from the sample peak should match the intensity of the major ion in the working gas

Materials that are water-soluble and non-volatile (sucrose, glutamic acid, etc.) may be prepared as solutions and transferred to tin capsules using a syringe or pipette [Carter and Fry 2013b]. The water can then be evaporated by gentle heating and/or vacuum and, once dry, the tin capsule can be crimped as usual. This process allows precise control over the amount of sample added to each tin capsule in a time-efficient way and has the additional benefit of ensuring homogeneity.

Further to maintain sample continuity the samples should be loaded into the auto-sampler in a prescribed sequence replicating the 96-well plate.

The information needed to identify unique samples should be recorded together with other key information such as the method of analysis, operator, date/time of analysis. Such records can take the form of sample lists within the IRMS software, external spreadsheets or written documents.

Weighed by:		Sample:				Isotope:		Typical weight:		Date weighed:			Date dessicated:	
	1	2	3	4	5	6	7	8	9	10	11	12		
A	BLANK	RM1	RM1	RM1	RM2	RM2	RM2	sample 1	sample 1	sample 1	sample 2	sample 2		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
B	sample 2	sample 3	sample 3	sample 3	sample 4	sample 4	sample 4	QC	QC	QC	sample 5	sample 5		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
C	sample 5	sample 6	sample 6	sample 6	sample 7	sample 7	sample 7	sample 8	sample 8	sample 8	QC	QC		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
D	QC	sample 9	sample 9	sample 9	sample 10	sample 10	sample 10	sample 11	sample 11	sample 11	sample 12	sample 12		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
E	sample 12	QC	QC	QC	sample 13	sample 13	sample 13	sample 14	sample 14	sample 14	sample 15	sample 15		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
F	sample 15	sample 16	sample 16	sample 16	QC	QC	QC	sample 17	sample 17	sample 17	sample 18	sample 18		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
G	sample 18	sample 19	sample 19	sample 19	sample 20	sample 20	sample 20	RM1	RM1	RM1	RM2	RM2		
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		
H	RM2	BLANK												
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg		

Figure 13. *Template for sample continuity illustrating a typical measurement sequence***.**

5.2 Bulk EA-IRMS bulk sulphur measurements

5.2.1 Sample preparation

Sample sizes for sulphur analysis are typically 10 to 20 times larger than for N and C measurements due to the low abundance of sulphur in many materials.

Some laboratories mix vanadium pentoxide (2-10 mg) with the samples in the tin capsules prior to analysis to promote oxidation. As an alternative, ammonium nitrate can be added as a solution to the sample within the tin capsules and the solvent evaporated before analysis to aid flash combustion for materials that are difficult to combust (e.g. sediments). This practice will proclude N isotope ratio data from that sample and a separate analysis will be required for determination of N isotope ratios.

5.2.2 Sample measurement

It is important to remember that measurements of sulphur isotope ratios can require different operating conditions than carbon and nitrogen isotope ratio analysis. The combustion process results in the formation of both sulphur dioxide (SO_2) and sulphur trioxide (SO_3) and the flow rate of carrier gas must be sufficient to force the gases resulting from combustion down through the reactor, thereby reducing formation of SO_3 [Mambellia et al. 2016].

The SO_3 formed is reduced to SO_2 by hot copper in the reduction stage of the reactor. The temperature of the copper is critical: if it is too cool it will react to form copper sulphate, if it is too hot it will melt.

The oxygen atoms in the SO_2 molecules are derived from the sample, from the oxygen in the elemental analyser and from any catalyst added to the sample such as vanadium pentoxide. In order to achieve consistent $\delta^{18}O$ values, the SO_2 is passed over a bed of heated quartz chips that provides a large surface area with which the SO_2 can exchange oxygen (buffering). In this way both RMs and sample pass over the same bed of quartz to ensure that the isotopic composition of SO_2 reflects variations in sulphur and not oxygen from different sources..

5.3 HTC/IRMS bulk hydrogen and oxygen measurements

Most of the information in sections 5.1.1 to 5.1.4 also applies to bulk hydrogen and oxygen isotope ratio measurements. The additional complexity of these measurements is discussed below.

A newly packed HTC reactor should be brought to maximum operating temperature over several hours and for routine operation brought to operating temperature, with normal helium flow, 12-24 hours before any samples are analysed. When changing the crucible, the reactor must be cooled to below 900 ^{o}C to prevent the glassy carbon reacting with atmospheric oxygen.

5.3.1 Blank determinations

Blank determinations for bulk hydrogen and oxygen isotope ratio measurements are performed the same as for EA/IRMS measurements, with the use of silver rather than tin capsules (section 5.1.2).

5.3.2 Sample preparation

The most important consideration when performing hydrogen and oxygen isotope ratio measurements of solid materials is appropriate drying of samples. Residual moisture will affect both $\delta^{2}H$ and $\delta^{18}O$ values so samples and reference materials must be absolutely dry. Many materials are hygroscopic or have large surface areas that will adsorb large quantities water. The

use of desiccants, vacuum, and heat can minimize the effect of water sorption and great care must be taken after desiccation to ensure the samples remain dry prior to analysis.

Special consideration must be given to the preparation of samples for hydrogen isotope ratio analysis. Hydrogen exchange in compounds with reactive functional groups may affect the total δ^2H value of a compound [Bowen et al. 2005; Qi and Coplen 2011]. Sections 5.3.4 and 6.4.6 contain more information regarding intrinsic versus extrinsic hydrogen.

5.3.3 Sample measurement

As described in section 3.2.3, the presence of elements other than H, O and C may require modification to the preparatory system. The presence of chlorine in moderately high abundance in the analyte [e.g. polyvinyl chloride (PVC) or hydrochloride salts such as cocaine hydrochloride] will generate hydrogen chloride (HCl) in a traditional, glassy carbon based HTC configuration. Likewise, when samples contain nitrogen (caffeine, proteins, explosives, etc.), a glassy carbon HTC reactor will partially convert the hydrogen present in the molecules into hydrogen cyanide (HCN). The use of a chromium reduction reactor or combinations of glassy carbon, chromium and manganese is recommended for δ^2H measurements of these compounds (section 3.2.3).

For oxygen isotope analysis HCl and HCN can be removed with an Ascarite® (or equivalent) trap with a secondary trap to remove the product water (Sicapent® or equivalent). In addition, any N_2 produced must be excluded from the mass spectrometer by diversion, dilution, or purge and trap methods.

A further consideration for HTC reactors is a possible “memory effect” associated with glassy carbon such that the first sample in a series of replicates has a composition off-set toward the composition of the previous sample [Olsen et al. 2006]. If this effect is apparent more replicate samples must be analysed and the first, or first few, replicate results excluded from subsequent data analysis.

5.3.4 Considerations for H – intrinsic and extrinsic fractions

A significant difficulty with hydrogen isotope ratio measurements is the presence of extrinsic hydrogen in the form of absorbed or adsorbed water. This must be addressed by careful and consistent drying of samples (and RMs). The method of choice can be determined for specific sample types but must be applied according to PIT; samples, QC materials and RMs must all be dried using the same procedure.

Some materials contain hydrogen atoms that will undergo exchange with atmospheric water vapour although hydrogen bound to carbon is resistant to exchange under normal conditions (i.e. intrinsic). An effective method to assess if hydrogen exchange is a concern for a particular material is to weigh two portions into silver capsules in the normal way and then add a small volume of water to one of the capsules (ca. 10 μL). The isotopic composition of the water is not important but can be useful in interpreting the result. The samples are then dried, crimped and analysed in the normal way. As the liquid water evaporates it becomes enriched in ^{2}H [due to fractionation] and the final few molecules of water will have an extremely enriched δ value. If the material is genuinely exchanging hydrogen atoms with the water molecules, the sample with water added will become enriched by tens or hundreds of per mil with respect to the untreated sample. Materials with significant, readily exchangeable hydrogen may be unsuitable for δ^2H analysis with contemporary technology.

To further complicate matters almost all organic-bound hydrogen is exchangeable over geological time scales [Sessions 2016] resulting in two inputs to the isotopic signature – the hydrogen originally present (intrinsic) and the hydrogen that has been assimilated from a shifting environment over many years, decades or millennia (extrinsic). In these cases, the signal from the extrinsic hydrogen must be removed in order to reveal the original signature. An example of this conundrum is the analysis of ancient cellulose used for tree-ring dating whereas the

hydrogen isotopic composition of modern cotton is unlikely to reflect anything other than the water available to the parent plant.

If an analyte contains extrinsic hydrogen, accurate hydrogen isotope ratio measurements require one of two approaches:

- Removal of extrinsic hydrogen from the material by derivatising functional groups (for example nitration methods established for cellulose) [e.g. Boettger et al. 2007], which may not be feasible in all cases
- Duplication of measurements of the samples and RMs after equilibration with two waters of well calibrated and distinctly different hydrogen isotope composition. This is followed by a calculation of the fraction of extrinsic hydrogen and the δ^2H value of the intrinsic hydrogen [Chesson et al. 2009].

For a select group of materials (e.g. wood, keratin and hair) there are RMs available that have been characterised for the δ^2H values of the intrinsic hydrogen under defined conditions [Soto et al. 2017]. This means that samples and RMs need to be equilibrated against only one water sample to enable a calculation of the intrinsic δ^2H values of the samples.

Section 6.4.6 contains more information regarding intrinsic versus extrinsic hydrogen.

5.4 GC/ IRMS carbon and hydrogen measurements

For suitable samples, gas chromatography is capable of isolating perhaps 100 components from a very complex mixture but an important consideration for GC/IRMS is that all the components to be measured must be baseline resolved. The reason for this is that most GC stationary phases will cause a slight separation of isotopologues such that an eluting peak is not isotopically homogeneous (typically enriched in the heavier isotopes at the start and depleted at the end). As such, it is important that the entire resolved peak can be integrated. There will also be unavoidable peak broadening due to the components of the GC/IRMS interface and for this reason the initial chromatographic separation may need to be better than would be needed for quantification using a conventional GC detector.

It is generally easier to achieve better chromatographic separation during δ^2H measurements than δ^{13}C measurements as the reactor is simply an empty tube. Reactors for δ^{13}C measurements typically contain metallic wires or particles which introduce multiple pathways that inevitably cause peak broadening.

5.4.1 Preconditioning

Modern GC columns are extremely stable and typically require no pre-conditioning although columns with very thick or very polar phases may exhibit high bleed when first used. If a column does require pre-conditioning this is best done with the column disconnected from the interface – i.e. leaving the outlet end of the column loose in the oven as column bleed will contain silicon compounds that can react irreversibly with the reactor packing.

Prior to use, the oxidation reactor used for δ^{13}C measurements must be preconditioned with oxygen, a process that may take several hours at increasing temperatures. The reactor will also require periodic re-oxidizing, depending on sample size, the number of peaks in a sample and the column bleed. Re-oxidizing will typically take one hour at a reduced temperature. Once conditioned the reactor will need to be left at the operating temperature, typically for another hour, to allow excess oxygen to evolve. After this process, it is important to repeatedly inject a QC material until a consistent δ value is recorded.

As noted above, the HTC reaction requires a small amount of carbon to be present and the easiest way to achieve this is to inject a QC material until consistent δ values are recorded. This process can be accelerated by injecting a more concentrated sample than might typically be analysed.

5.4.2 Blank determinations

A blank determination for GC/IRMS is simply an injection of the solvent used to dissolve the samples; if a contaminant in the solvent co-elutes with a peak of interest it may not be obvious from the IRMS chromatogram.

Every analytical sequence should start with a blank determination to ensure that there is no contamination in the system, especially if components such as the septum or injection liner have been changed. If contaminant peaks are present, the blank determination must be repeated and if the contamination persists the cause must be investigated.

Ideally a blank determination should be performed between each sample but this would be a very inefficient use of instrument time. Practically, a blank injection at the start and end of an analytical sequence would be considered good practice.

When samples are subject to significant work-up procedures (derivatisation, etc.) it can be useful to prepare a procedural blank – i.e. progress an empty vial or pure solvent through the work-up process to ensure that no contamination is introduced. This procedure may be more appropriate to method validation than routine analysis but should be re-assessed periodically.

5.4.3 Sample preparation

Most samples that are suitable for analysis by GC will be amenable to GC/IRMS analysis and no additional sample preparation should be needed. The only caveat to this is that the concentration of the sample should be such that the peaks of interest are of a similar size to the pulses of working gas. If samples contain components at widely differing abundances, it may be necessary to prepare more than one dilution to fulfil this criterion for all the peaks of interest.

5.4.3.1 Derivatisation

Some compounds are not directly amenable to GC analysis due to their low volatility or high polarity. This can be overcome by blocking polar functional groups responsible for the low volatility with apolar moieties – i.e. derivatisation. Examples of functional groups that require derivatisation include carboxylic acids, alcohols, thiols and amines (–COOH, –OH, –SH and –NH_2). Compounds containing these groups can be derivatised via reactions including (but by no means limited to) esterification, silylation and acetylation. There are many different derivatisation options and combinations; however some are more desirable than others. Desirable qualities for a derivatising agent suitable for GC/C/IRMS include:

- The addition of as little of the analyte element as possible (e.g. methylation is generally preferable to acetylation for carbon isotope analysis),
- A reaction that goes to completion (these are typically fast reactions),
- If not complete derivatisation, a consistent fractionation between the derivatising agent and the derivative between batches,
- Lack of interference from by-products formed with compounds of interest,
- A derivatised compound that is stable (at least) for the analysis time.

Derivatives containing carbon atoms will contribute to the measured carbon isotopic composition of derivatised compounds and this contribution must be accounted for (section 6.4.5). The same applies to other elements although it is rare for derivatives to contain nitrogen atoms and therefore this is of little concern for compound-specific nitrogen isotope ratio analysis.

Derivatives containing fluorine have been reported to irreversibly poison the combustion reactor via the formation of HF, which is said to react to form metal fluorides within the reactor and can potentially also damage capillaries downstream of the combustion reactor [Meier-Augenstein

1999]. A number of other derivatives are likely to form involatile deposits upon combustion, such as trimethylsilyl derivatives.

5.4.4 Sample measurement

An analytical sequence will begin with conditioning runs, followed by an acceptable blank determination, followed by a number of RMs for δ scale normalisation. Each component to be reported should be measured by interpolation between two chemically identical RMs with widely spaced isotopic compositions that bracket the δ values expected for samples. In practice, it is usually possible to prepare a mixture of compounds such that two solutions will contain all the RMs needed. These RMs should be analysed a number of times, dictated by the length of the GC analytical sequence, using identical analytical conditions for RMs and samples – especially the split and purge flows at the GC injector.

For lengthy GC analytical runs it may not be practical to perform multiple injections of every sample. It is, however, good practice to analyse (at least) every fifth sample in duplicate and to analyse a QC sample with the same frequency.

Although it is good practice to normalise each component in a chromatogram against two identical compounds, in practice suitable compounds may not be available. In such circumstances it may be possible to normalise using RMs chemically different to the samples, providing identical GC conditions can be used and the retention times are close. If RMs with a suitable span of δ values are not available it may be necessary to normalise measurements using a single RM. This is most appropriate when the δ values of the samples do not span a wide range and are similar to the RM. The normalisation method and the caveats inherent to the method must be clearly stated when reporting such results.

No special stand-by conditions are needed during routine GC/IRMS operation. For short periods of inactivity it is advisable to reduce the reactor temperature(s) to approximately half the operating temperature(s), to conserve the reactor materials and prolong the life of the heating elements. For long term inactivity the interface can simply be switched off but it is always advisable to leave helium flow through the GC column.

5.5 LC/CO/IRMS and FIA/CO/IRMS carbon measurements

As with all CSIA applications, achieving baseline separation is essential and the development of the chromatographic separation prior to an IRMS application is crucial. The mechanism of chromatographic separation will determine the elution order of the heavy or light isotopologues – e.g. reverse phase separations tend to result in the heavier isotopologues eluting first, while ion-exchange separations have peak tails that are very enriched in ^{13}C. This fractionation of isotopologues across LC/CO/IRMS peaks is far more pronounced than with GC/IRMS and leads to > 100 ‰ variation in δ values across peaks. Baseline separation of peaks ensures that the whole tail of the peak is included in the integration and also avoids any carry-over from the tail of one peak into the baseline of the next peak.

FIA/CO/IRMS (LC/IRMS without a chromatographic column) can be used for the determination of bulk carbon isotope ratios in most water-soluble materials. This approach requires far less material than EA/IRMS and can therefore be useful when sample size is limited.

5.5.1 Preconditioning the system

Preconditioning of an LC/CO/IRMS system is also referred to as “priming.”

Mobile phases must be free from carbon (e.g. only comprising water, inorganic acids and inorganic buffers). As noted in section 3.3.2, if any component of the LC system (pump, auto-sampler, column, etc.) has been previously used with organic mobile phases these must be thoroughly flushed (a process that can take weeks).

Before analysis begins, the mobile phase(s) and reagents must be degassed to remove dissolved CO_2 and/or volatile organic compounds (e.g. ultrasonicate under vacuum for 5-15 mins). Continuous sparging of the degassed mobile phases and reagents with inert gas (e.g. He or N_2) will help prevent re-absorbtion of CO_2 from the atmosphere.

The HPLC flow rate should be set to the operating level and all reagents allowed to flow through the system for at least 10 minutes before heating the reactor to operating temperature.

5.5.2 Blank determinations

To determine the background level of dissolved CO_2 and other carbon sources in the solvent used to prepare samples, inject the solvent as though it was a sample. The abundance and isotopic composition of any signal recorded can be used to correct if necessary. As with EA measurements it is important to establish that the blank signal has a consistent isotopic composition in order for the correction to be meaningful.

For analyses involving some form of sample pre-treatment (extraction, hydrolysis, etc.) procedural blanks should also be prepared and analysed.

More information regarding blank corrections and when they should be applied can be found in section 6.4.1.

5.5.3 Sample preparation

Samples should be prepared in carbon-free solution, typically water or dilute inorganic acids/buffers. Using the same solution composition as the initial mobile phase is recommended. Sample solutions should be filtered to remove particulate matter, which can clog HPLC columns as well as other components of the interface (e.g. the gas separation unit). If the amount of sample solution is limited this can be difficult.

5.5.4 Sample measurement

Each sequence of analyses for FIA/IRMS should include blanks, RMs for normalisation and RMs to act as QC for long-term instrumental monitoring. RMs for normalisation can be primary or secondary RMs (sections 2.2.2 and 2.2.3), provided that they are soluble in the same solvent as the samples. QC materials can be calibrated in-house (section 2.2.5.2) should matrix-matched materials not be commercially available.

Like EA and GC analyses, LC/IRMS sequences should include blanks (which should be procedural blanks if appropriate) and RMs for normalisation, QC/QA and long-term instrumental monitoring together with samples. If possible, the normalisation RMs should be analysed as external standard mixtures (section 6.3.1). Each sample solution should ideally include one or more internal standard compounds of well characterised isotopic composition. These internal standards can be used to check the instrumental performance and data handling.

Repeatability of analyses should be determined not only from multiple injections from the same vial (instrument precision) but also from repeated preparations of the sample solution (which might include replicate extractions and/or hydrolyses, etc.).

As noted in section 3.3.2.2, some functional groups are difficult to oxidise by the persulfate chemistry used in most LC/CO/IRMS systems. It is therefore important to monitor the yield of CO_2 from the oxidation process to ensure complete conversion of sample compounds. When the oxidation process does not go to completion, isotopic fractionation should be expected leading to biases which can only be corrected by concurrent analysis of matrix-matched RMs.

5.5.5 After sample analysis (standby considerations)

If the LC/CO/IRMS system is to be used again within a short period of time, the flow rates of the mobile phase can simply be reduced, the oxidation reactor cooled to room temperature and reagents diverted to waste rather than passing through the oxidation reactor. There is no need to flush out the reagents from the system if only a short operational pause is expected (i.e. overnight).

For longer pauses between instrument use (e.g. greater than two days) reagents, including any buffers in the mobile phase, should be thoroughly purged from the system using water. This will ensure that the reagents do not precipitate in any part of the system, which may result in blockages. Once the reagents have been flushed, the reactor should be cooled to room temperature and the mobile phase and reagent pump flow rates reduced to minimum. Some users prefer to keep the HPLC pump and reagent pumps delivering water at low flow rates through the system while not in use.

6 Data handling

6.1 Initial data evaluation

Typically, the IRMS instrument software automatically calculates raw isotope-delta values that can be used for subsequent data handling. This process will involve the integration of the sample and working gas peak signals from the Faraday collectors; calculation of ratios of these integrated ion currents, correction for isobaric interferences where necessary and conversion of the corrected ratios to raw isotope delta values. The user may need to specify various parameters such as known/assigned isotope delta value(s) of the working gas.

Note that the raw isotope-delta values obtained for the sample are on a scale that is realised by the working gas. Sample and working gases do not follow the same continuous-flow pathway through IRMS instruments and therefore to obtain isotope-delta values on the internationally agreed reporting scales it is necessary to normalise the raw isotope-delta values using RMs (section 6.3) that are treated the same as the samples.

It is useful to understand the calculations performed by the instrument software as there can be changes to recommendations regarding some of these processes, for example to ^{17}O correction [Brand et al. 2010]. If the instrumental process can be replicated offline (e.g. in spreadsheet software) then such changes can be more easily implemented. The most important of the stages are discussed in the following sections.

6.2 Isobaric interferences

IRMS instruments typically have mass resolutions (m/Δm at 10 % valley) of 100-200 for the triple collector system and of 10-40 for the Faraday collectors used for hydrogen measurements. The low resolution does not allow for the separation of isobaric species within the mass spectrometer (e.g. $^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$ both nominally *m/z* = 45). When isobaric species interfere with the masses used to determine isotope ratios, a correction must be applied [Kaiser and Röckmann 2008] as described below:

6.2.1 ^{17}O-correction for carbon isotope ratios of CO_2

The term "^{17}O-correction" (or "oxygen correction") describes an algorithm applied to isotope ratio measurements of CO_2 for $\delta^{13}C$ and $\delta^{18}O$ determinations to correct for the contribution of ^{17}O species. This correction is often hidden from the analyst, but IRMS instrument software may provide the option to choose the algorithm. The user must be aware of this to ensure that consistent $\delta^{13}C$ and $\delta^{18}O$ values are reported.

$\delta^{13}C$ values are almost universally determined from the mass spectrum of CO_2, which contains ions spanning *m/z* 44 to 49. Of the major ions only *m/z* 44 represents a single isotopic species.

m/z 44	$^{12}C^{16}O_2$		
m/z 45	$^{13}C^{16}O_2$	$^{12}C^{17}O^{16}O$	
m/z 46	$^{12}C^{18}O^{16}O$	$^{13}C^{17}O^{16}O$	$^{12}C^{17}O_2$
m/z 47	$^{12}C^{18}O^{17}O$	$^{13}C^{18}O^{16}O$	$^{13}C^{17}O_2$
m/z 48	$^{12}C^{18}O_2$	$^{13}C^{18}O^{17}O$	
m/z 49	$^{13}C^{18}O_2$		

The contribution of the minor species (in the natural abundance range) is small except for $^{12}C^{17}O^{16}O$, which contributes approximately 7 % to the abundance of *m/z* 45. A triple collector

IRMS instrument measures simultaneously the ratios [45]/[44] and [46]/[44], which are a function of three variables ($^{13}C/^{12}C$, $^{17}O/^{16}O$ and $^{18}O/^{16}O$).

$$[45]/[44] = \left(^{13}C/^{12}C\right) + 2\left(^{17}O/^{16}O\right) \quad (5)$$

$$[46]/[44] = 2\left(^{18}O/^{16}O\right) + 2\left(^{17}O/^{16}O\right)\left(^{13}C/^{12}C\right) + \left(^{17}O/^{16}O\right)^2 \quad (6)$$

With three unknowns and two variables, a third parameter (λ) is necessary to solve these equations. λ (also referred to as "*a*" or "the exponent") describes the relationship between the three oxygen isotopes, assuming the processes that affect the abundance of ^{18}O have a corresponding effect on ^{17}O.

The ^{17}O-correction algorithm [Craig 1957] is based on ^{17}O and ^{18}O abundances determined by Alfred Nier [Neir 1950] and assumed a fractionation factor of $\lambda = 0.5$, i.e. $^{17}O/^{16}O$ variations are half the $^{18}O/^{16}O$ variations. Since that time knowledge of absolute isotope ratios and isotope relationships has improved and values of λ, measured on natural materials, have been reported between 0.5 and 0.53. A single value of λ must, however, be chosen in order to maintain comparability with published data. For this reason the "Craig" or "IAEA" algorithm is retained [Alison et al. 1995], with $\lambda = 0.5$ and defined values for all of the ratios involved.

More recently a ^{17}O-correction algorithm (the "Santrock" or "SSH" algorithm) [Santrock et al. 1985] with a fractionation factor of $\lambda = 0.516$ and an iterative correction to solve the equations for ^{13}C has been published. The SSH algorithm is often regarded as being both mathematically exact and more realistic in its approach to natural variations of isotopic composition.

IUPAC has now published a technical report on ^{17}O-corrections [Brand et al. 2010] that includes a new linear approximation for determining the ^{17}O-correction and recommends updated values for the defined isotope ratios involved [Assonov and Brenninkmeijer 2003a, Assonov and Brenninkmeijer 2003b].

Applying the Craig, SSH or IUPAC algorithm to the same raw data will produce $\delta^{13}C$ values with differences that are small but exceed the precision of modern IRMS instruments. For an average tropospheric CO_2 the bias in raw $\delta^{13}C$ value between the IAEA and SSH algorithms has been determined as 0.06 ‰. Provided that raw $\delta^{13}C$ values for sample and RMs for scale calibration are measured against the same working gas, and that the same ^{17}O correction is applied to all materials within the same sequence, then the bias in normalised $\delta^{13}C$ values introduced via the choice of ^{17}O correction approach will be <0.001 ‰.

All of these algorithms assume a mass-dependent and stochastic distribution of isotopes and ^{17}O-correction is only valid for carbon from terrestrial sources. Material from extra-terrestrial sources can have highly anomalous oxygen compositions.

6.2.2 H_3^+-correction for hydrogen isotope ratios of H_2

This correction uses an empirically determined factor (section 4.5.1) to negate the contribution of H_3^+ to the intensity of $^2H^1H$ measured at the *m/z* 3 Faraday collector. As with the ^{17}O-correction, this correction is often hidden within instrumental software and applied automatically. Equations (7) and (8) demonstrate one method to determine and apply the H_3^+ correction [Sessions et al. 2001a; Sessions et al 2001b].

$$i_{H_3} = \left[H_3^+\right]\left(i_{H_2}\right)^2 \quad (7)$$

$$R_{meas} = \frac{i_{HD} + i_{H_3}}{i_{H_2}} = \frac{i_{HD}}{i_{H_2}} + \left[H_3^+\right] \times i_{H_2} = R_{true} + \left[H_3^+\right] \times i_{H_2} \quad (8)$$

i_{H3} ion current for H_3^+

i_{H2} ion current for H_2

i_{HD}	ion current for $^{2}H^{1}H$
$[H_3^+]$	H_3^+ factor
R_{meas}	measured *m/z* 3/*m/z* 2 ion current ratio
R_{true}	true *m/z* 3/*m/z* 2 ion current ratio (i.e. corrected for H_3^+)

6.2.3 ^{13}C-correction for oxygen isotope ratios of CO and CO_2

Oxygen isotope ratio measurements based on CO and CO_2 molecules are commonly corrected for the presence of ^{13}C in an analogous manner to the ^{17}O correction (section 6.2.1) although the ^{13}C correction is typically 0.01 ‰ and contributes little to the uncertainty of measurement [Farquhar et al. 1997].

6.2.4 *m/z* 28 interferences for isotopic ratios determined on CO

The high reactor temperatures typically used in HTC systems generates a significant background of CO (*m/z* of 28 and 30) but provided this background is stable, this interference will not have adverse effects on the accuracy or precision of oxygen isotope ratio measurements. The high background does, however, mean that ^{18}O determinations are very sensitive to integration parameters.

Nitrogen gas (*m/z* of 28 and 29) has a more profound effect on oxygen isotope ratio measurements by HTC/IRMS. When N_2 enters the ion source of the mass spectrometer, NO^+ is formed at the filament, with a dominant *m/z* of 30. This interference remains long after the chromatographic N_2 peak has left the ion source has and continues to have large effects on $\delta^{18}O$ measurements and must be addressed by physically preventing N_2 from entering the ion source (section 3.2.3.2).

6.2.5 Oxygen isotope corrections for sulphur isotope ratios of SO_2

Oxygen isotope ratio correction for $\delta^{34}S$ values is possible when both SO^+ and SO_2^+ are measured for a particular material, as shown in equations (9) and (10) [Coleman 2004]:

$$\delta^{18}O = \left(24.02 \times \delta^{66}\right) - \left(23.024 \times \delta^{50}\right) \quad (9)$$

$$\delta^{34}S = \left(1.0908 \times \delta^{66}\right) - \left(0.0908 \times \delta^{18}O\right) \quad (10)$$

where $\delta^{66} = \left(\frac{^{66}\left(SO_2\right)_{samp}}{^{64}\left(SO_2\right)_{RM}} - 1 \right)$

and $\delta^{50} = \left(\frac{^{50}\left(SO^+\right)_{samp}}{^{50}\left(SO^+\right)_{RM}} - 1 \right)$

The more common approach is to avoid the need to correct the for the presence of oxygen isotope ratios by application of PIT by ensuring that the oxygen isotopic composition of the SO_2 derived from all materials within a single sequence of analyses is identical. When this is the case, only equation (10) needs to be applied. This equalisation of oxygen isotope ratios can be achieved either via an offline process (oxidation to sulphate, followed by reduction to sulphide and combustion with the addition of isotopically identical oxygen), or by the use an elemental analyser designed to “equilibrate” or “buffer” the sample SO_2 against a large pool of oxygen (e.g. quartz chips, section 3.2.2.3).

6.3 Scale calibration/normalisation

The analysis of RMs within the same sequence as samples allows the linking of the measured δ values for the samples to the zero-point of the δ scale. Where scale contraction effects occur, these also need to be corrected for using RMs of widely different isotope ratios – a process termed "normalisation." The linking of measured results to the δ scale using RMs results in realisation of the isotope-delta scale in practice within any laboratory (section 2.1).

There are a number of algorithms that convert the measured (raw) δ values of a sample to the "true" δ values reported versus an international scale. These may be performed in external spreadsheets or LIMS.

When the measured δ values of both samples and RMs are obtained relative to a working gas (typically a pure gas introduced directly into the IRMS instrument from a high pressure cylinder), equation (11) can be used to determine the sample δ values on the reporting scale:

$$\delta_{\text{true(sample)}} = \left[\frac{\left(\delta_{\text{raw(sample)}} + 1\right)\left(\delta_{\text{true(RM)}} + 1\right)}{\left(\delta_{\text{raw(RM)}} + 1\right)} \right] - 1 \qquad (11)$$

The term "normalisation error" refers to the difference between the true and normalised δ values of the sample. Inappropriate or incorrect normalisation can introduce more uncertainty to the reported value than any experimental factor. For successful normalisation, PIT must be applied to the preparation and analysis of the sample and RMs. Samples and RMs must also be of similar chemical composition so that bias introduced by differential conversion to analyte gases are minimised.

The uncertainty of normalisation can be improved by applying a normalisation factor (*n*) calculated from the measured δ value of two RMs, with δ values far apart, assuming that systematic errors are linear in the dynamic range of the overall method. The true δ value of the sample is calculated by a modified equation (11) taking the following form:

$$\delta_{\text{true(sample)}} = \left[\frac{\left(n \times \delta_{\text{raw(sample)}} + 1\right)\left(\delta_{\text{true(RM)}} + 1\right)}{\left(n \times \delta_{\text{raw(RM)}} + 1\right)} \right] - 1 \qquad (12)$$

The value of *n* remains nearly constant for a given instrument but should be determined periodically, especially if changes in sensitivity are observed. For continuous flow IRMS instruments, it is recommended that *n* is determined for each analytical sequence, termed "two-point linear normalisation", "linear shift normalisation" or "stretch-shift correction".

For isotope δ scales that are defined by two points or where it is recommended to use two or more RMs for scale realisation, equation (11) must again be slightly modified as follows:

$$\delta_{\text{true(sample)}} = \delta_{\text{true(RM1)}} + \left[\left(\delta_{\text{raw(sample)}} - \delta_{\text{raw(RM1)}}\right) \times \left(\frac{\delta_{\text{true(RM1)}} - \delta_{\text{true(RM2)}}}{\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}} \right) \right] \qquad (13)$$

The above equation can be applied using any suitable pair of RMs calibrated to the international scale. (e.g. USGS40 and USGS41a for δ^{13}C realisation). The use of primary RMs for direct calibration/normalisation of measured δ values should be avoided unless the small measurement uncertainty that these materials confer due to their position in the calibration hierarchy is appropriate for the application. This ensures that the valuable primary RMs are available to the stable isotope community for as long as possible.

Alternatively, the δ_{true} value for a sample can also be calculated using an expression of the form shown in equation (14).

$$\delta_{\text{true(sample)}} = m \times \delta_{\text{raw(sample)}} + b \qquad (14)$$

The slope of the regression line (*m*) is referred to as the "expansion factor" or "stretch factor" and the intercept (*b*) as the "additive correction factor," "shift factor" or simply "shift." Such a regression approach can also be applied when using more than two RMs for scale realisation.

This method has been used for three decades to normalise measured δ^2H and δ^{18}O values to the VSMOW scale [Sharp, 2006] and is now recommended for the normalisation of δ^{13}C measurements of both organic and inorganic materials to the VPDB scale and δ^{15}N measurements to the atmospheric nitrogen scale.

Linear normalisation can also be based on a best fit regression line using more than two points, termed "multiple-point linear normalisation" or simply a "calibration curve". The coefficient of determination (R^2) will indicate how closely the data obeys a linear relationship (assuming the observations are approximately evenly spaced) and the effect of random errors in the measurement of RMs (e.g. incomplete combustion) can be reduced.

A number of articles have reviewed normalisation procedures [e.g. Paul et al. 2007, Skrzypek 2013].

Regardless of the normalisation approach employed, it is essential to check that the algorithm(s) applied result in meaningful data. This is most easily achieved via the analysis of additional RMs not used for normalisation within each analytical sequence i.e. QC materials. The measured values for these RMs are then treated in the same way as the samples and the resulting, normalised δ values should agree with the known values, either obtained by certificate, inter-laboratory comparisons, or quality-controlled long-term data. This process forms part of quality control and assurance and is described further in section 7.

Example 1: Normalisation of δ^2H measurement:

	VSMOW2	**SLAP2**	**Δ**
measured (δ_{raw})	+0.3 ‰	−420.7 ‰	0.3 − −420.7 = 421.0
accepted (δ_{true})	0.0 ‰	−427.5 ‰	0.0 − −427.5 = 427.5

The "stretch factor" $m = \Delta_{true}/\Delta_{raw}$ = 427.5/421.0 = 1.01544

The "shift" or "off-set" b using VSMOW2:

$b = \delta_{true} - (\delta_{raw} \times \text{stretch}) = 0.0 - (0.3 \times 1.01544) = -0.3046$ ‰

or, using SLAP2:

$b = \delta_{true} - (\delta_{raw} \times \text{stretch}) = -427.5 - (-420.7 \times 1.01544) = -0.3046$ ‰

Adjusted δ^2H values would be calculated as:

$\delta^2H_{true} = 1.01544 \times \delta^2H_{raw} - 0.3046$

If the δ^2H_{raw} value is−189.0 ‰, the normalised δ^2H_{true} = -192.2 ‰

6.3.1 CSIA considerations

Although there are no primary RMs that can be directly analysed by CSIA using GC or LC techniques, some secondary RMs are available depending on the sample requirements (Schimmelmann et al 2016). The pedigree for CSIA-derived δ values may therefore need to be extended through the use of calibrated in-house RMs (section 2.2.5), rather than direct use of primary and/or secondary materials. In an ideal situation, each component of interest will be normalised via a pair of RMs that are the same compound but have isotopic compositions that span the expected range of the sample compound (this allows normalisation to δ scales that are defined by two anchor points). For example, for the LC/CO/IRMS analysis of glycine, two glycine RMs would be needed for normalisation that span the carbon isotopic composition of glycine expected in the samples, e.g. δ^{13}C of −46 and −10 ‰. To complicate matters further, CSIA is typically used to determine the isotope ratios of several components within a mixture, each of which would require a pair of normalisation RMs and therefore the ideal approach quickly

becomes unfeasible due to the large number of RMs that need to be sourced and/or calibrated in-house.

The use of a matrix-matched standard mixture of compounds, similar to the compounds of interest, and of known isotope ratio that span the isotopic and chromatographic ranges of the sample components, can be used as a convenient approach for data normalisation. Such a standard mixture should be analysed every 5-10 analyses and a calibration plot of measured versus "known" δ values for this standard mixture can then be used for normalisation of δ values of the sample compounds.

Ideally, each sample solution should also have one or more internal standard similar to the sample compounds (e.g. a non-naturally occurring amino acid such as norleucine can be added when analysing a protein hydrolysate) but of well characterised isotope ratio. An ideal internal standard will elute close to, but be fully resolved from, the components of interest. These internal standards can be used to check the performance of data normalisation as well as any other data correction calculations performed during processing. The internal standard should be added as early as is practical in the sample preparation process.

For FIA/IRMS normalisation can be carried out without the need for a working gas intermediate because suitable RMs can be analysed in a single sequence using multiple injections together with samples.

6.4 Other corrections

The corrections for isobaric interferences and normalisation are essential practices. There may be a need to apply other corrections to measured data but we urge users to determine whether any of the following corrections are strictly necessary before applying them because "over-correction" can introduce as many problems as it appears to solve. Furthermore, adherence to the Principle of Identical Correction is critical and therefore, if one or more of these corrections are deemed necessary, they should be applied consistently to all data within a particular analytical sequence [Carter and Fry 2013a]. The influence of any of the following corrections on the measurement uncertainty must be determined during method development and validation.

If a change in instrumental performance affects measurement data such that a correction appears necessary, it is far more advisable to identify and correct the problem then re-analyse the samples rather than apply a "correction" to bring the data back in line. It is important to note that each calculation stage from raw data to final normalised, δ values has the potential to contribute to the measurement uncertainty (section 6.5). Furthermore, the influence on the measurement uncertainty of a particular correction may be hidden if only the standard deviation of replicate analyses is considered, particularly if the standard deviation is determined using corrected data.

6.4.1 Blank correction

Blanks can arise during most IRMS measurements (both bulk and compound-specific) and the magnitude and isotopic composition of the blank should be determined. The average peak area and δ value of the blank measurement can be used to correct the data for blank contribution, as shown in equation (15).

$$\delta_{\text{blk corr}} = \frac{\delta_{\text{meas}} \times Area_{\text{meas}} - \delta_{\text{blk}} \times Area_{\text{blk}}}{Area_{\text{meas}} - Area_{\text{blk}}} \quad (15)$$

$\delta_{\text{blk corr}}$ blank corrected δ value of the sample

δ_{meas} determined (raw) δ value of the sample

δ_{blk} δ value of the blank

$Area_{\text{meas}}$ area of the sample peak

$Area_{blk}$ area of the blank peak

The blank correction may be performed by the IRMS instrument software, or in external software, using the values obtained during blank determinations.

This method of blank correction should only be applied if the reported isotopic compositions of the blank determinations are consistent. Values for small peaks can vary a great deal due to integration effects. For this reason, a laboratory may simply define a minimum acceptable sample peak size based on the intensity of the average blank peak area, i.e. a small blank peak should have minimal effect on a sample peak one hundred times larger.

A more rigorous method to determine the isotopic composition of the blank is to analyse two RMs with widely spaced δ values, at decreasing sample size. The δ values of the blank can then be determined by solving two simultaneous equations for δ value versus peak size for the two RMs [Carter and Fry 2013a].

6.4.2 Drift correction

During an analytical sequence a drift of measured δ values as a function of time may be observed as a result of changes in the isotopic composition of the working gas or changes within the ion source, such changes in the background gases (e.g. water). Traces of water, or other protonating species, can give rise to isobaric interference such as CO_2H^+ (*m/z* 45) or H_3^+ (*m/z* 3).

The presence of drift in measured δ values with time during an analytical sequence should be determined through the regular analysis of QC materials throughout the sequence (every 5-10 samples). If significant drift is detected, then a correction may be applied using the results from the QC materials. For example, if the QC materials indicate a linear drift in measured δ value with time, then equation (16) can be used to perform a drift correction for all materials analysed within the same sequence [Carter and Fry 2013a]:

$$\delta_{\text{drift corr}} = \delta_{\text{meas}} - m \times \text{position} \qquad (16)$$

$\delta_{\text{drift corr}}$ drift corrected δ value of the sample

δ_{meas} determined (raw) δ value of the sample

m slope of linear drift curve (plot of δ value versus auto-sampler position)

position auto-sampler position within the sequence (assuming this is a proxy for time)

Such an approach assumes that the δ values of the QC materials and samples drift in the same way and the effectiveness of such a drift correction should be confirmed by a second QC material also analysed throughout the sequence. An alternative approach is drift correction, combined with a linearity correction [Ohlsson and Wallmark 1999].

Drift can also be detected by comparing the calibration plot for the RMs analysed at the beginning of the sequence, with δ values measured for the same RMs analysed at the end of the sequence.

6.4.3 Linearity (peak size) correction

Instrument linearity can be determined from working gas pulses of varying amplitude or from the analysis of varying sample weights (section 4.3.3). A small linearity correction (the slope of δ value against amplitude/mass) can have a large influence on the precision and/or accuracy of measured δ value if samples are not prepared in consistent amounts. A correction for linearity can be performed using an approach analogous to drift correction (section 6.4.2):

$$\delta_{\text{linearity corr}} = \delta_{\text{meas}} - m \times \text{area} \qquad (17)$$

$\delta_{\text{linearity corr}}$ linearity corrected δ value of the sample

δ_{meas} determined (raw) δ value of the sample

m slope of linearity correction (plot of δ value versus peak area)

area peak area of sample (proxy for amount of analyte)

A more rigorous approach is to avoid any linearity effect by analysing a closely controlled weight (in terms of the element of interest) of each RM and sample in any given analytical sequence.

6.4.4 Memory correction

Some IRMS applications, particularly HTC/IRMS, exhibit observable memory effects whereby the isotope ratio of the sample gas(es) from one analysis is affected by the sample gas(es) from the previous analysis. There are two approaches to overcome this effect. The first is simply to carry out a larger number of sequential replicate analyses and discard the first few of each sample (or ensure that the first replicate consists of a very much larger amount of sample material than usual). This approach is robust but wastes sample, analysis time and may not be ideal in all situations. As an alternative, a memory correction can be applied [e.g. Gröning 2011]. This type of correction must not only include the isotopic composition of the preceding samples, but also account for any difference in intensity of the signals. A memory correction will contribute significantly to the measurement uncertainty and its effectiveness should be monitored through the use of additional QC material(s). An abnormally large memory effect, which cannot be ameliorated by one or two conditioning samples, typically indicates a problem with the system and should be investigated.

6.4.5 Correction for derivatisation

The isotope ratio measured for a derivatised compound will include contributions from the parent compound itself and from any atoms present in the derivative group. These exogenous isotopes must be accounted for and the measured δ value thereby corrected, typically via a simple mass balance equation for any given element (18) [Rieley 1994].

$$\delta_{\mathrm{c}} = \frac{n_{dc}\delta_{\mathrm{dc}} - n_{d}\delta_{\mathrm{d}}}{n_{\mathrm{c}}} \qquad (18)$$

δ_{c} δ value of the parent compound

δ_{dc} δ value of the derivatised compound

δ_{d} δ value of the derivative

n_c number of atoms in the parent compound

n_{dc} number of atoms in the derivatised compound

n_d number of atoms in the derivative

The isotopic composition of the derivatisation agent, δ_{d}, should be determined independently, e.g. by EA/IRMS. Where this is not possible or practical (e.g. for a derivatisation agent supplied in small ampoules with a different ampoule used for each batch of analyses), δ_{d} must be determined by derivatising a RM (of the same chemical formula as the sample compound) of well characterised isotope ratio (δ_{c}). Equation (18) can then be rearranged to determine δ_{d}, which can then be used for subsequent calculations.

When an isotopic fractionation is associated with the derivatisation reaction, which may occur with a low yield of derivatised compound,. It is first necessary to determine whether this difference is consistent between different derivatisation batches/reactions. If it is, then the effective stable isotope composition of the derivatising atoms(s) ($\delta_{\mathrm{d\text{-}eff}}$) can be determined using equation (19) which can then be used within equation (18) in place of δ_{d}. If it is not each sample must contain an internal standard (as discussed above) that that will form the same derivative as other components of the sample. In this way $\delta_{\mathrm{d\text{-}eff}}$ can be calculated for each sample.

$$\delta_{\text{d-eff}} = \frac{n_{dc}\delta_{\text{dc}} - n_c\delta_{\text{c}}}{n_{\text{d}}} \quad (19)$$

6.4.6 Correction for extrinsic hydrogen

Materials may contain three pools of hydrogen:

- intrinsic (permanent) hydrogen (typically H bound to C);
- extrinsic absorbed/adsorbed water, that can be removed by careful drying, and
- chemically bonded hydrogen (e.g. H bound to O, N, etc.), that interacts with environmental water

In order to determine the hydrogen isotope ratio of intrinsic hydrogen in a material, two approaches are recommended: near complete removal of the extrinsic hydrogen (e.g. nitration of cellulose) or equilibration with water(s) with well-characterised isotopic composition.

Extrinsic hydrogen (one component of which is chemically bonded hydrogen) undergoes exchange with atmospheric water and contributes to the measured hydrogen isotope ratio (section 5.3.4). As noted above the time for exchange will vary from fractions of a second to many thousands of years. To avoid unnecessary and complex sample preparation it is important to consider to what extent the presence of extrinsic hydrogen confounds the overall interpretation of the hydrogen isotopic signature.

6.4.6.1 Nitration

Protocols have been developed for carbohydrate materials (principally cellulose) that replace oxygen bonded hydrogen (O-H) with nitrate [e.g. Boettger et al. 2007]. Subsequent hydrogen isotope ratio measurements can be performed on thoroughly dried samples containing only carbon-bound intrinsic hydrogen. While useful, this nitration process is not possible for all materials.

6.4.6.2 Controlled isotope exchange

If the amount of extrinsic hydrogen in a sample is unknown and nitration (or other derivatisation) is not possible, another approach is to equilibrate two suites of RMs and samples with two waters of well characterised, but distinct, hydrogen isotope compositions. This approach requires the RMs to be chemically identical (or very similar) to the samples such as the keratin RMs developed specifically for this purpose [Soto et al. 2017].

Proposed methods include equilibration at room temperature and heated equilibration, always within sealed vessels. Regardless of the equilibration conditions, these conditions must be maintained for a sufficient duration to ensure consistent isotopic exchange between the equilibration water and the extrinsic hydrogen in the material.

It is convenient to weigh the samples into loosely-crimped silver capsules prior to equilibration to permit rapid transfer of sample to a desiccator or vacuum oven following equilibration. Samples must be thoroughly dried following equilibration and must be quickly transferred to the auto-sampler of the HTC instrument to limit further interactions with atmospheric moisture.

In addition to routine post-analysis processing the following calculations must be applied to correct for the extrinsic hydrogen [Wassenaar and Hobson 2000; Chesson et al. 2009; Wassenaar et al. 2015].

By mass balance, the measured δ^2H value of the equilibrated sample is:

$$\delta_{\text{Meas}} = f_{Ex}\delta_{Ex} + (1 - f_{Ex})\delta_{In} \quad (20)$$

f_{Ex} mole fraction of hydrogen that is susceptible to exchange

δ_{Ex} δ^2H values of the extrinsic hydrogen atoms of the material

δ_{In} δ^2H values of the intrinsic hydrogen atoms of the material.

The mole fraction of extrinsic hydrogen may be calculated by:

$$f_{Ex} = \frac{\delta_{MeasA} - \delta_{MeasB}}{\delta_{WA} - \delta_{WB}} \times \frac{1}{\alpha_{Ex-W}} \quad (21)$$

where WA and WB indicate two waters used in equilibration treatments and the measured (Meas) isotopic composition of the materials following equilibration with the two waters. Within the sealed vessels, the water vapour will not have the same isotopic composition as the equilibration water and the hydrogen isotope fractionation factor between the extrinsic hydrogen and water vapour is $\alpha_{\text{Ex-W}}$, where:

$$\alpha_{Ex-W} = \frac{\delta_{Ex} + 1}{\delta_{W} + 1} \quad (22)$$

Although the fractionation factor of water-to-vapour (α_{Ex-W}) is sometimes assumed to be 1, other values have been reported for a range of materials [Sauer et al. 2009, Chesson et al. 2009] but sensitivity analyses of this assumption have shown that it has a minimal effect on results.

From the hydrogen isotope measurements of samples equilibrated against two waters, a fractionation factor (α_{Ex-W}), and the δ^2H values of the equilibration waters, the mole fraction of extrinsic hydrogen (f_{Ex}) in the sample can be calculated. The δ^2H value of the intrinsic hydrogen (δ_{In}) can then be calculated by mass balance equation (23) [Wassenaar and Hobson 2000].

$$\delta_{In} = \frac{\delta_{MeasA} - (f_{Ex} \cdot \alpha_{Ex-W} \cdot \delta_{WA})}{1 - f_{Ex}} \quad (23)$$

6.4.6.3 Identical treatment by equilibration with reference materials

For some analytes (notably hair) suitable matrix-matched RMs exist for which f_{Ex} and δ_{In} are reported [e.g. Soto et al 2017]. For these materials, samples and RMs can be subjected to identical treatment with water vapour for a sufficient duration to allow complete isotopic equilibration. Recommended equilibration times range from 5 days at ambient temperature [Bowen et al. 2005] to 4 hours at 105 ^{o}C [Soto et al. 2017]. The samples and RMs are then subjected to identical drying and analysed for hydrogen isotope composition. By assuming that: (1) the samples and RMs have identical amounts of intrinsic hydrogen and (2) all of the extrinsic hydrogen reached equilibrium with the same water, these results can be corrected as if there were no exchange of hydrogen [e.g. Coplen 2012].

There is ongoing debate about the optimal equilibration time and temperature, as well as drying procedures to be applied to various analytes in this approach of PIT. At present good practice is, when possible, to apply identical equilibration and drying methods to samples and RMs that are a close chemical match for the samples.

6.5 Measurement uncertainty

6.5.1 What is measurement uncertainty?

The International Standard Organisation (ISO) defines measurement uncertainty (MU) as a "*non-negative parameter characterising the dispersion of the quantity values being attributed to a*

measurand, based on the information used" [Barwick 2016]. In simple terms, MU consists of a range of values that might be produced by a method within which the true value will fall with some degree of probability (which is approximately 95 % for an expanded uncertainty). Note that the true value does not need to be known to estimate the MU.

Knowledge of MU associated with a result is important because it:

- allows the reliability of a result to be assessed,
- gives confidence to any decision based upon a result,
- can help assess the fitness-for-purpose of any result, and
- allows fair comparison of measurement results

Furthermore, accreditation to ISO/IEC 17025 for calibration and testing laboratories requires assessment of measurement uncertainty.

6.5.2 How is measurement uncertainty quantified?

There is generally a four-step process to quantifying MU:

(1) Specify what is being measured, i.e. the measurand.

(2) List the sources of uncertainty for each stage of measurement, i.e. things that may cause the result to change.

(3) Quantify each of these components to uncertainty, i.e. the components should each be expressed as standard deviations in the same unit as the result.

(4) Combine the components together.

The components/sources of uncertainty can be divided into two categories: those evaluated by statistical means from experimental data/replicates ("Type A") and those evaluated by different means (e.g. from calibration certificates; "Type B"). The combination of uncertainty components requires that all are in the same mathematical format – typically a standard deviation. It also requires a measurement model to combine the different sources of uncertainty. This is ideally the equation used to calculate the result together with additional terms to account for effects, such as precision, which are not used to calculate the result.

6.5.3 Sources of uncertainty in IRMS-based analyses

The modern isotope ratio mass spectrometer is capable of measuring variations in natural isotopic ratios of most elements with an uncertainty better than 0.02 ‰. For hydrogen, the uncertainty is usually an order of magnitude greater because the natural $^{2}H/^{1}H$ isotope ratio is several orders of magnitude smaller than for other elements. Larger errors are typically introduced by sample treatments prior to IRMS analysis. Contributions to MU in IRMS-based analyses may arise from (but not limited to) the following:

- Sampling — within sample heterogeneity, background variations in isotopic composition, batch-to-batch, etc.
- Sample preparation — weighing, extraction, hydrolysis, fraction collection, derivatisation, offline conversion to analyte gas for DI/IRMS, etc.
- Instrumental analysis (preparation via peripheral) — online conversion to analyte gas, separation of gases/compounds, removal of water, open-split, etc.
- Raw data handling — integration parameters, time-shifts of signals, background correction algorithms, etc.
- Calculation of raw δ values — variations in working gas, difference in δ value and peak size/shape between working and sample gases, corrections

	for isobaric interferences, etc.
• Corrections to raw δ values	corrections applied for blank, drift, linearity or memory , etc. (including QC materials).
• Normalisation	certified and measured components of RMs used for scale calibration for entire traceability chain, etc.
• Further corrections	for derivative carbon or extrinsic hydrogen, etc.

Some of these contributions can be minimised through proper choice of analytical conditions and by applying PIT (section 5.1.3). For example, it is important that RMs have similar chemical properties to samples as combustion efficiency may vary, changing the isotopic composition of the evolved gases. Similar variations can occur in the efficiency of the reduction reactor and any chemical or physical traps that remove water, oxygen or CO_2.

6.5.4 Combining uncertainty components

To be able to combine different uncertainty contributions to give a single uncertainty estimate for the result for a particular sample, the uncertainties must be in the same mathematical form. According to internationally agreed rules for uncertainty evaluation, uncertainties should be expressed as standard deviations [ISO/IEC Guide 98-3 2008]. The basic approach for combining uncertainties is the *square root of the sum of the squares rule*. Uncertainty components $u(x_1)$……. $u(x_n)$, expressed as standard deviations, are combined as shown in equation (24) to give the uncertainty in the result *y* (assuming an additive measurement model):

$$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + ...u(x_n)^2} \quad (24)$$

This, however, requires that the uncertainty components are expressed in the same units as the measurement result (i.e. a δ value in the case of IRMS measurements) and for the uncertainty components to be independent.

For many IRMS analyses, the measurement equation contains terms that are correlated and therefore not independent. For example, section 6.3 outlines the process of normalisation using "stretch" and "shift" factors. The uncertainty in normalised results for samples [$\delta_{true(sample)}$] will have contributions from the uncertainty of the measurements of the RMs and the sample, and must also include the uncertainty in the known or certified RM δ value, as illustrated in Figure 14.

The "stretch" and "shift" factors are correlated, but the correlation term can be avoided by calculating the uncertainty directly from the input values as described in equation (13). This expression, however, also introduces difficulties when using the simple rules for combining uncertainties as the terms $\delta_{true(RM1)}$ and $\delta_{raw(RM1)}$ appear twice in the equation (13).

Estimating the MU associated with two-point scale calibration therefore requires other methods to combine uncertainty components such as the use of partial derivatives of the measurement equation, or a Monte Carlo simulation. One of the most straightforward approaches is to use a spreadsheet-based calculation [Kragten 1994].

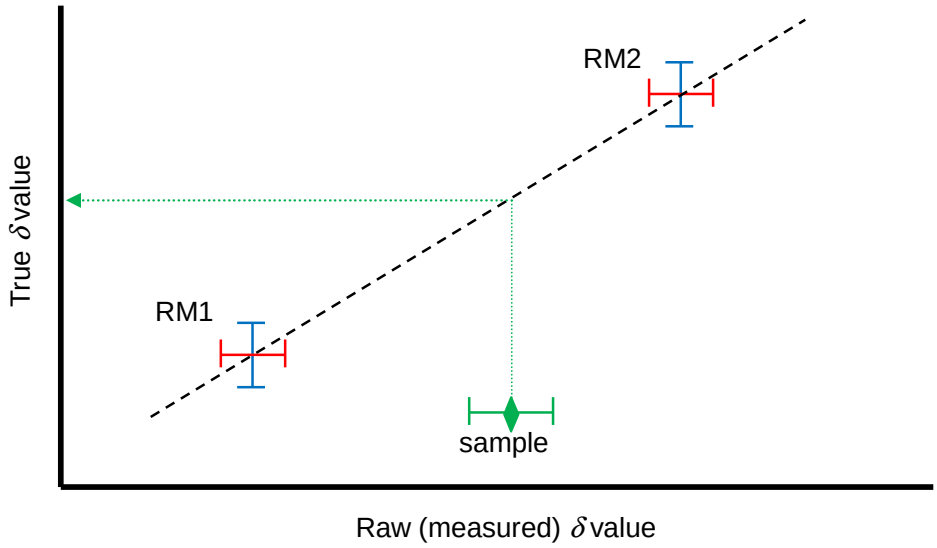

Figure 14. *Illustration of uncertainty components for scale calibration – the uncertainty in the known values of RMs (blue, vertical error bars) and the raw measured δ values for the RMs (red, horizontal error bars) and the sample (green, horizontal error bars) will contribute to the uncertainty in* δ_{true} *for the sample.*

6.5.5 Kragten spreadsheet approach to measurement uncertainty

The Kragten spreadsheet approach to the calculation of MU avoids the use of partial derivatives, does not require macros or other coding and can handle situations where the same term appears more than once in a calculation formula. This approach calculates an uncertainty budget, which will help the end-user discover if any particular contributing factors are responsible for a large proportion of the MU.

The general spreadsheet set-up is shown in Figure 15. The values of the parameters required to calculate the result, and the associated standard uncertainties, are entered into the spreadsheet in columns B and C, respectively. The formula used to calculate the result, e.g. equation (13), is entered in cell B8. Column B is then copied into columns D to H (one column for each parameter used in the calculation of $\delta_{true(sample)}$). The uncertainty given in cell C3 is added to cell D3, the uncertainty in cell C4 is added to cell E4, and so on (cells highlighted in yellow). Cells D8 to H8 show recalculated values for $\delta_{true(sample)}$, including the effect of the uncertainty in the individual parameters. Row 9 shows the differences between the recalculated values and the original calculation for $\delta_{true(sample)}$ in cell B8. The standard uncertainty in $\delta_{true(sample)}$ (cell C8) is obtained by squaring the differences in row 9, summing them and then taking the square root.

The Kragten approach can be extended to include calculations other than normalisation that may be applied during IRMS analyses such as those described in section 6.4 as well as to handle an entire sequence of analyses rather than only one sample at a time. In this way PIT can be easily adhered to as all materials analysed within a single sequence will be treated in the same way. This extension of the Kragten approach from that in Figure 15 has been described [Dunn et al. 2015], which also provides annotated, example templates, including the above example (in Excel® format) as supplementary information.

For BSIA where each replicate analysis uses a separate subsample of the material, the standard deviation of the mean can be used as described above as each replicate is fully independent. Where the instrumental replicate analyses result from multiple injections from a single vial as is typically the case in CSIA, then the replicates are not fully independent and the standard deviation must be used as the input uncertainty in a Kragten-type spreadsheet. This avoids underestimation of sampling uncertainty (pseudoreplication). Pseudoreplication can also occur

when a material has been measured several times on each of several days but where there is a significant day-to-day (or run-to-run) effect resulting in a reduction in the number of effective degrees of freedom.

	A	B	C	D	E	F	G	H
1								
2	**Parameter**	**value (δ^2H, ‰)**	**uncertainty (δ^2H, ‰)**					
3	$\delta_{true(VSMOW2)}$	0.0	0.3	B3+C3	B3	B3	B3	B3
4	$\delta_{true(SLAP2)}$	−427.5	0.3	B4	B4+C4	B4	B4	B4
5	$\delta_{raw(VSMOW2)}$	0.3	1.2	B5	B5	B5+C5	B5	B5
6	$\delta_{raw(SLAP2)}$	−420.7	1.2	B6	B6	B6	B6+C6	B6
7	$\delta_{raw(sample)}$	−189.0	1.5	B7	B7	B7	B7	B7+C7
8	$\delta_{true(sample)}$	Eqn. (13) applied to values above	$u(\delta_{true(sample)})$ = square root of sum of squared differences	Eqn. (13) applied to values above	Eqn. (13) applied to values above	Eqn. (13) applied to values above	Eqn. (13) applied to values above	Eqn. (13) applied to values above
9			Difference	D8-B8	E8-B8	F8-B8	G8-B8	H8-B8
			Squared differences	$(D9)^2$	$(E9)^2$	$(F9)^2$	$(G9)^2$	$(H9)^2$

Figure 15. *Setup of a Kragten spreadsheet for the estimation of measurement uncertainty arising from scale calibration of a sample δ^2H value with two RMs using equation (13).*

6.5.6 Method uncertainties from validation studies

This method for determination of MU is a top-down approach rather than the bottom-up measurement equation approach described in sections 6.5.4 and 6.5.5.

Determination of the MU for a single result or sequence of results using a Kragten-type spreadsheet is sufficient for many purposes. There are, however, situations where the long-term uncertainty of a method (rather than uncertainty in any particular result) is required, such as populating a database of isotope ratios. In such cases estimating the typical MU afforded by a particular method that can then be associated to each result obtained by that method is more useful.

Such an estimate of MU can be easily obtained from method validation data, provided that the validation experiments are carefully planned with this in mind [Barwick and Ellison 2000]. As described, the precision and bias studies should be carefully planned to account for as many sources of uncertainty as possible. The sources of uncertainty that remain can either be evaluated directly from existing data or from ruggedness studies. An example of such a MU can be found in the supplementary information to the recently published minimum requirements for method validation of stable isotope δ values [Dunn et al. 2017].

6.5.7 Expanded measurement uncertainty

The combined standard uncertainty (u_c) that arises from a Kragten-type spreadsheet, or other means to combine individual standard uncertainty components, is generally in the form of a standard deviation. In situations where the numbers of truly independent replicates and consequently of effective degrees of freedom are large, the confidence level associated with this standard uncertainty will be approximately 68 %. For many applications, particularly in forensic sciences, a higher level of confidence is required and this is achieved by multiplying the standard uncertainty by a coverage factor (*k*-factor) which results in a so-called expanded uncertainty (U):

$$U(y) = k \times u_c(y) \qquad (25)$$

Where instrumentally measured input terms that contribute to measurement uncertainty are derived from a large number of truly independent replicates and the effective number of degrees of freedom is large; the *k*-factor can be derived from a normal distribution and hence $k = 2$ can be applied to provide 95 % confidence of including the true value of the measurand. This will generally be the case for a measurement uncertainty derived from validation data (section 6.5.6). Should greater confidence be required by stakeholders (e.g. 99 %) the *k*-factor should be increased.

If there are few independent replicate measurements, the effective number of degrees of freedom becomes small and the *k*-factor will need to be derived from the Student's *t* distribution. The effective number of degrees of freedom can be increased either by performing more independent replicate measurements of the sample in question, or by using long term data for a matrix-matched in-house RM (e.g. from a control chart, section 7.1) as a proxy for additional measurements.

It is always necessary to plan measurements carefully and to check the obtained results to ensure that the selected *k*-factor is appropriate for the level of confidence required. Further detail can be found within the Eurachem/CITAC guide [Barwick et al. 2016] and the ISO Guide to the Expression of Uncertainty in Measurement [ISO/IEC Guide 98-3 2008].

Example 2: Calculation of uncertainty in $\delta_{true(sample)}$ arising from two-point scale calibration using a Kragten spreadsheet:

RMs used for scale calibration: VSMOW2 and SLAP2.

Reference δ^2H values for RMs: VSMOW2 = 0.0 ± 0.3 ‰, SLAP2 = −427.5 ± 0.3 ‰ (from the most recent IAEA reference sheets).

Measured δ^2H values for RMs: VSMOW2 = +0.3 ± 1.2 ‰, SLAP2 = −420.7 ± 1.2 ‰ (the uncertainties here are the standard deviation of the mean of independent replicate analyses).

Measured δ^2H value for sample: −189.0 ± 1.5 ‰ (again the uncertainty is the standard deviation of the mean of independent replicate measurements).

Note that the values for the measurements are equal to those given in Example 1 on page 46

Kragten spreadsheet:

	A	B	C	D	E	F	G	H
1								
2	**Parameter**	**δ^2H value (‰)**	**uncertainty (‰)**					
3	$\delta_{true(VSMOW2)}$	0.0	0.3	0.3	0.0	0.0	0.0	0.0
4	$\delta_{true(SLAP2)}$	−427.5	0.3	−427.5	−427.2	−427.5	−427.5	−427.5
5	$\delta_{raw(VSMOW2)}$	+0.3	1.2	+0.3	+0.3	+1.5	+0.3	+0.3
6	$\delta_{raw(SLAP2)}$	−420.7	1.2	−420.7	−420.7	−420.7	−419.5	−420.7
7	$\delta_{raw(sample)}$	−189.0	1.5	−189.0	−189.0	−189.0	−189.0	−187.5
8	$\delta_{true(sample)}$	**−192.2**	**1.8**	−192.1	−192.1	−192.9	−192.8	−190.7
9			Difference	+0.2	+0.1	−0.7	−0.5	+1.5
10			Squared differences	0.03	0.02	0.45	0.30	2.32

Scale calibrated δ^2H value for sample = −192.2 ± 1.8 ‰ (standard uncertainty). The expanded uncertainty assuming a large number of independent replicate analyses with $k = 2$ is ± 3.5 ‰.

A number of other examples of the implementation of the Kragten spreadsheet to other analytical systems are given in the Eurachem/CITAC guide [Barwick et al. 2016].

7 Quality control/assurance

For several decades the importance of quality has been increasingly recognised in ensuring laboratories; operate a suitable management system, are technically competent and are able to produce technically valid analytical results.

Quality is defined by ISO as “the degree to which a set of inherent characteristics of an object fulfils requirements”. ISO also recognises that the term *quality* can be used with adjectives such as *poor*, *good* or *excellent*. In the context of forensic science, however, *quality* must be *sufficient* i.e. of sufficient quality to be relied upon.

In the context of this Guide, *sufficient quality* includes the performance parameters of the analytical method: the method being the object. Quality also has a wider scope and includes characteristics which demonstrate the competence of the organisation and individuals within that organisation.

Therefore, the ISO technical definition might be restated as, “the degree to which stakeholder requirements are met by the organisation, its personnel and the analytical methods used”. The broader term *stakeholder* is used rather than the (paying) customer because, in forensic science, the requirements of a tribunal-of-fact are often paramount.

One of the major contributors to the realisation of the importance of quality was the VAM (Valid Analytical Measurement) initiative and it is worth reproducing the six VAM principles as a general guide to the measures required to assure the reliability of analytical results.

- Analytical measurements should be made to satisfy an agreed requirement
- Analytical measurements should be made using methods and equipment which have been tested to ensure they are fit for purpose
- Staff making analytical measurements should be both qualified and competent to undertake the task
- There should be a regular independent assessment of the technical performance of a laboratory
- Analytical measurements made in one location should be consistent with those elsewhere between laboratories following methods of similar or greater quality, and
- Organisations making analytical measurements should have well defined quality control and quality assurance procedures.

The VAM principles introduce two further quality terms; *quality control* and *quality assurance* which ISO defines as:

- Quality control (QC) is part of quality management focused on fulfilling quality requirements
- Quality assurance (QA) is part of quality management focused on providing confidence that quality requirements will be fulfilled

QC is about ensuring systems are under control and performing as expected such that results may be relied upon. Meeting set performance criteria usually involves making measurements as part of the control process. Running control samples, both positive and negative, is a simple example of QC. More broadly, QC might be considered as *detection* which, in addition to measurement, might involve testing, inspection and seeking stakeholder’s views. Monitoring performance using control charts (7.1) is a QC activity.

QA is about prevention i.e. having systems in place that prevent non-conformance with stakeholder requirements. Among other things this includes resources such as; competent personnel, a suitable working environment, validated methods, and traceable measurements.

Preferably, laboratories should be accredited to the standard ISO/IEC 17025 ‘General requirements for the competence of testing and calibration laboratories’ which independently assures competence and the validity of the methods. Less costly alternatives include; certifying the management system to ISO9001 or using the FIRMS Approved Practitioner scheme.

Whether or not a laboratory is accredited, method validation conforming to the requirements specified in the FIRMS ten-point plan (7.3) [Dunn et al 2017] is essential to ensure that that the quality of results is sufficient to assure their reliability, i.e. conforming to the requirements for MU and traceability specified in ISO/IEC 17025.

Conformance to international standards is important in meeting the 5th VAM principle and laboratories must consider the requirement for mutual recognition of analytical results and comparability and traceability across international borders.

Monitoring and improving performance contribute to the quality of analytical results. These activities rely on the availability of suitable RMs and participation by the laboratory in proficiency tests. Preferably, organisations providing proficiency testing should be accredited to ISO/IEC 17043 “General requirements for proficiency testing” and RM producers be accredited to ISO/IEC 17034 “General requirements for the competence of RM producers.”

Guidance on the application of and conformance to ISO/IEC 17025 are listed in the Bibliography and include:

- ILAC G19:2002 Guidance for forensic science laboratories (no charge)
- ISO Guide 98:2008 Uncertainty of measurement (charge)
- JCGM 100:2008 Joint Committee for Guides in Metrology (no charge)
- UKAS M3003 The Expression of Uncertainty and Confidence in Measurement (no charge)

A number of guidance documents are also available for laboratories that have decided, for whatever reason, not to seek accreditation to ISO17025 but, nevertheless, wish to identify and implement good practice.

7.1 Control charts

To monitor the day-to-day performance of IRMS measurements the δ values for in-house RMs (after applying all necessary corrections and normalisation) should be compared to a target value or mean value control chart with defined limits. The normalisation (stretch and shift-correction) values (for each element) should also be recorded together with the normalised values obtained for in-house RMs.

Mean value control charts usually have warning limits (mean ± 2 σ) and control limits (mean ± 3 σ). These limits will be determined using results from a *prior-period* with approximately 20 results determined on at least 6 different days, preferably by a number of analysts. Typically, these data will have been acquired as part of the validation process.

Figure 16 shows a flow chart for the interpretation of an in-house QC material (phenacetin) based on the “Westgard rules”, adapted from the IUPAC Harmonized guidelines for internal quality control in analytical chemistry laboratories [Thompson and Wood 1995]. Figure 17 shows the data from Figure 16 modified to illustrate various breaches of the Westgard rules.

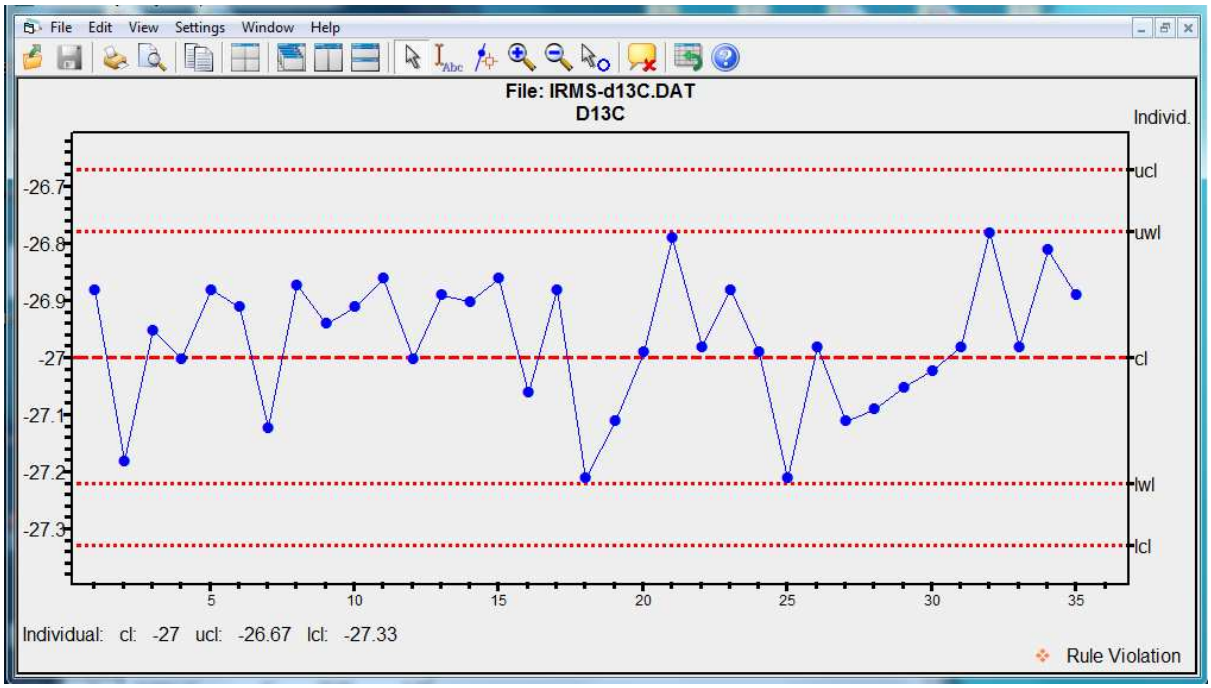

Figure 16. *An example of a control chart for $\delta^{13}C$ measurements of a QC material (phenacetin); cl = centre line (mean), ucl = upper control limit (3 σ), uwl = upper warning limit (2 σ), lwl = lower warning limit (2 σ), lcl = lower control limit (3 σ). In this example all of the measurements fall within the control limits and comply with the Westgard rules.*

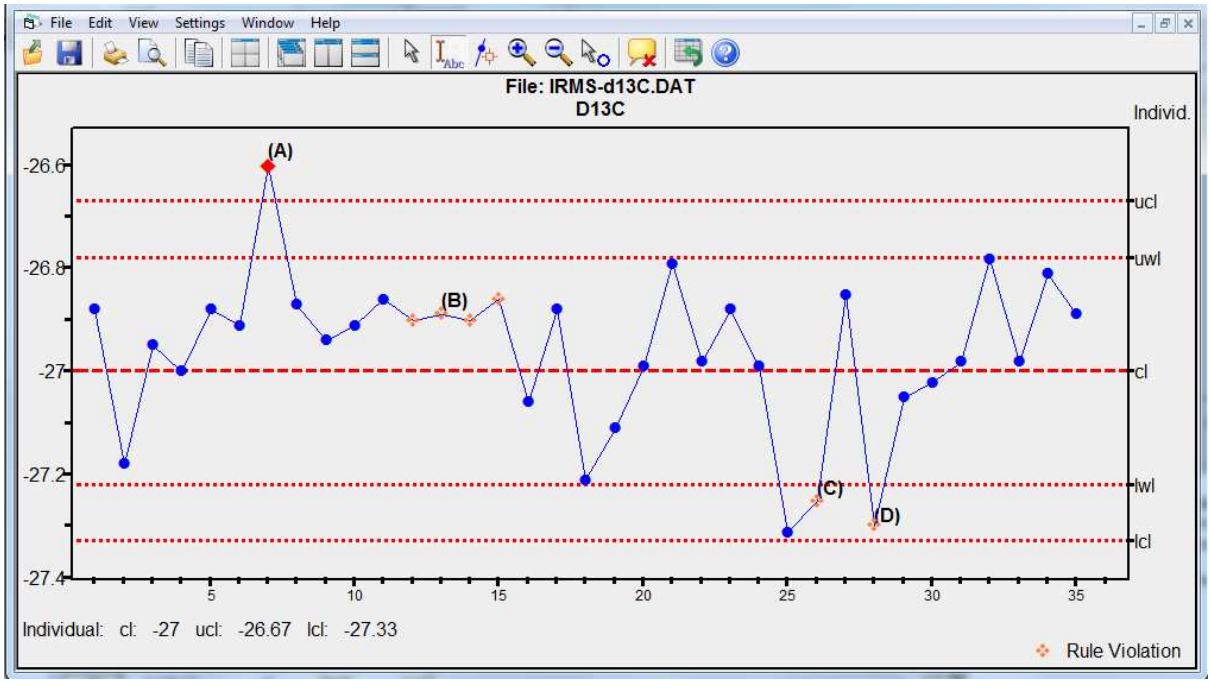

Figure 17. *An example of a control chart showing violations of the Westgard rules;* ***(A)*** *result greater than 3 σ from mean,* ***(B)*** *10 results on the same side of mean (also four results greater than 1 σ from mean),* ***(C)*** *two consecutive results greater than 2 σ from mean and,* ***(D)*** *two consecutive results differ by more than 4 σ.*

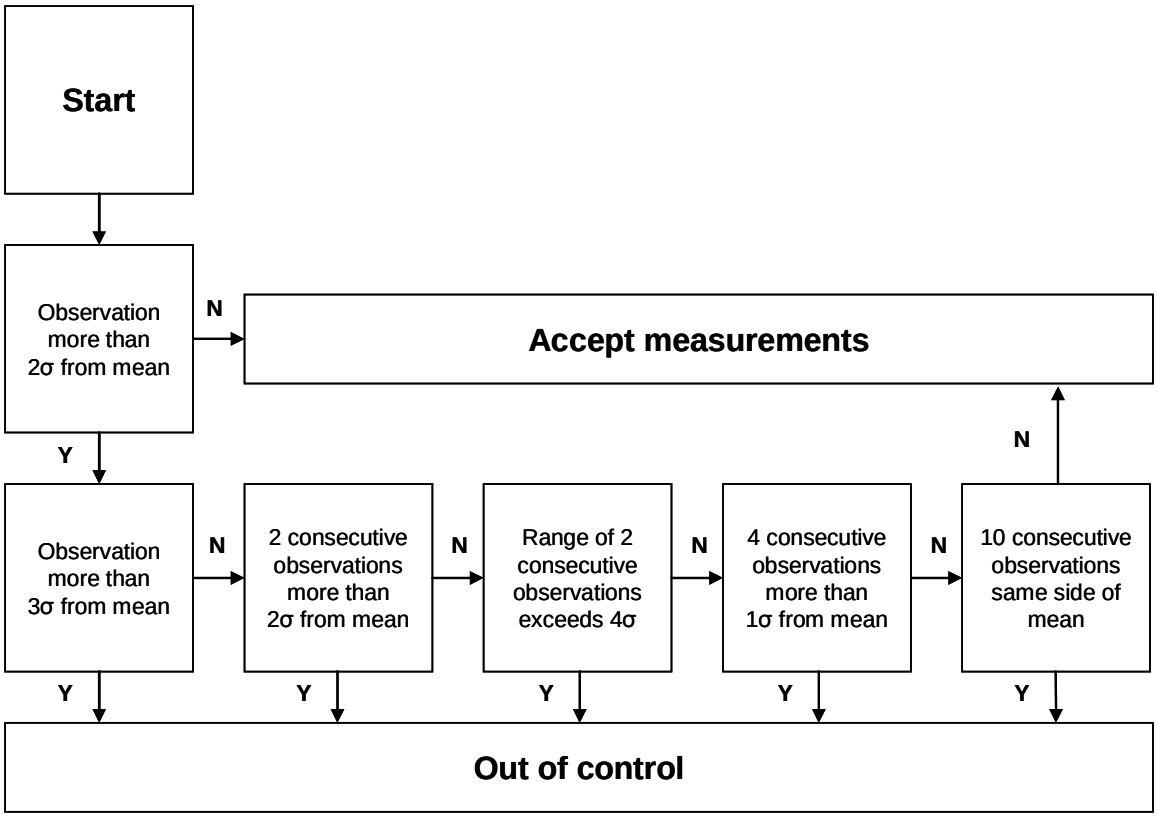

Figure 18. *Schematic representation of the Westgard rules (reproduced from Thompson and Wood 1995).*

7.2 Inter-laboratory exercises

Proficiency testing (PT) by means of ILC exercises provide a way for laboratories to check the quality of measurements and monitor the long term reproducibility of sample preparation in comparison to those obtained by other laboratories. Participation in such schemes is a fundamental requirement of any laboratory seeking or maintaining accreditation to ISO/IEC 17025:2017. It is recommended that laboratories participate in an inter-laboratory ring test at least every two years to check for reliability and accuracy of the determined results.

The FIRMS Network organises an isotope ratio PT scheme in collaboration with LGC and this scheme is accredited to ISO/IEC 17043:2010 under LGC's UKAS scope. Retained materials from previous rounds are also available for use as in-house RMs. Information about this PT scheme is available from:

http://forensic-isotopes.org/

and

https://www.lgcpt.com/productviewnarrow.aspx?SchemeID=185

Eurofins Scientific organises the Food analysis using Isotopic Techniques – PT Scheme (FIT-PTS), which is focused on isotopic analysis of foodstuffs. Samples are circulated three times a year and have included; wine, vinegar, must, honey, fruit juice, cheese, vanillin, and protein.

http://www.eurofins.com/food-and-feed-testing/food-testing-services/authenticity/fit-pts/

The IAEA organise an international Water Isotope Inter-Comparison Test (WICO) approximately every four years for laboratories engaged in routine analysis of hydrogen and oxygen stable isotopes in water:

http://www-naweb.iaea.org/napc/ih/IHS_programme_wico2016.html

7.3 Method validation

To achieve accreditation to ISO/IEC17025 or other internationally recognised quality standards, analytical techniques must be validated. A laboratory must prepare a validation plan and a validation report presenting and interpreting the data obtained. The validation process will depend on stakeholder requirements, the nature of samples to be analysed, the equipment to be used and the parameters to be measured, all of which must be defined in the validation plan.

A recent publication from members of the FIRMS Network [Dunn et al. 2017] proposed a ten-point plan as the minimum requirement for the validation of IRMS-based methods for the determination of light element isotope ratio δvalues of bulk materials. The ten points are summarised below; for more detail and an example validation report refer to the publication.

(1) The stakeholder requirements as well as the acceptance criteria must be stated.

(2) The source, scope and protocol of the method must be stated.

(3) The working range of the method, both in terms of sample mass and isotopic composition, must be clearly stated following investigation.

(4) The precision of the method, accounting for both within-run and between-run variations (ideally the within-laboratory reproducibility or intermediate precision) must be determined and reported.

(5) The bias of the method (i.e. determining the degree to which the obtained results differ from the true value) must be determined and reported.

(6) The ruggedness/robustness of the method must be determined by investigating parameters known to affect the results that have been identified during method development.

(7) The matrix variations in the performance of the method must be assessed if the scope of the method covers wide-ranging matrices. The key requirement is to demonstrate quantitative conversion of the sample to the analyte gas for each new matrix.

(8) Measurement uncertainty for the method must be established (section 6.5). This can take into account the results from the bias, precision and ruggedness studies.

(9) The external validation of the method must be demonstrated by comparison with the results obtained by other laboratories for the analysis of same materials within some form of ILC exercise.

(10) The fitness-for-purpose of the method must be determined by a dialogue between the stakeholder(s) and provider while comparing the performance of the method during the validation studies outlined above to the analytical/customer requirements of the method.

Points 4-6 should be carefully planned to allow easy estimation of the MU [Barwick and Ellison 2000]. For CSIA re it is recommend that the chromatographic separation method also be validated in-house by using conventional organic MS (or other means of compound identification) to identify the compounds being studied and to check that the gas peaks detected by the IRMS system are associated with a single compound [Meier-Augenstein 1999].

7.4 Reporting of delta values

Once an IRMS-based method has been validated, the results (and uncertainties) for sample materials obtained and the data are ready to report (e.g. to the stakeholders), the critical aspects are:

- Isotope ratios must be reported as δvalues on the currently agreed international scales (described in section 2) and the method employed must be able to produce δ values fully traceable to that scale.*
- At least two RMs of known isotope ratio must be used to normalise results.

- The identity and assigned δ values and uncertainties (e.g. from the certificate) of RMs used for normalisation and quality control must be reported.
- Where appropriate, the analytical protocol must be provided in sufficient detail for the reader to understand how measurements have been performed.
- Terminology should be used consistently [Coplen 2011].
- δ values should be reported with their associated expanded uncertainties using a *k*-factor that provides the confidence required by the stakeholder(s). Generally the 95 % confidence level is sufficient.

* In situations where a database of δ values for a particular material has been developed over many years, reporting guidelines/scales may have changed since the database was first populated. It may be possible to correct existing data to the new scale; and if it is, the contribution to MU introduced by such a correction must be propagated through to the corrected δ values. In the very rare cases where such a correction is not possible, but where compatibility of new and existing data is also a critical consideration for the stakeholders, then reporting of data on scales other than the current internationally agreed scales is permitted provided the traceability and reporting scale of the data provided to the stakeholder(s) is clearly stated [Dunn et al. 2017].

8 Interpretation of IRMS data in forensic science

A comprehensive guideline to the interpretation of stable isotope (and allied data) has been published as a stand-alone document by the FIRMS Network and this section provides only a summary of the key points from that document. Note that the interpretation guide may be updated independently of this Guide and vice-versa – readers are advised to check the FIRMS website for the current version of each document.

Forensic practitioners assist Courts by explaining the results of their examinations and analyses within the context of a specific case. In addition to stating results, each report may include opinions and conclusions based on those results - an interpretation of the significance of the results as well as the arguments in support of the opined significance. The arguments will include factors that limit or increase the significance of an opinion; e.g. the presence of unusual characteristics, two way transfers, or multiple transfers may increase the significance of findings.

The guidance in the earlier chapters of this Guide is important in ensuring the validity of IRMS data; however, to interpret IRMS data for forensic purposes more information is generally required. The following shows how the interpretation of IRMS results is applied in a forensic science context.

Many forensic science investigations using IRMS will focus on determining if there is an association between a material found at a crime scene and a similar material found at another location such as from or in association with a suspect, e.g. duct tape, paper, ecstasy tablets and many other materials. It should be noted that in general not only IRMS but a suite of methods will be used in such a comparative investigation, starting with a visual comparison (colour, dimensions, morphology, texture, etc.). Physical comparisons are often followed by chemical characterisation of organic materials using spectroscopic techniques such as Fourier transform infrared spectrometry (FTIR) and chromatographic techniques such as pyrolysis/GC/MS and LC/MS. Inorganic components may be profiled using techniques such as X-ray fluorescence (XRF) and inductively coupled plasma mass spectrometry (ICP/MS), sometimes coupled with laser ablation (LA/ICP/MS) to pin-point specific regions of an exhibit.

The first stage in evaluating IRMS based evidence is to assess the within sample variation in stable isotopic characteristics. This will depend on method parameters (e.g. repeatability) and will also highlight sample heterogeneity. Sometimes stable isotopic characteristics may be time dependent such as when solvents evaporate from the sample (inks, paints etc.) or when chemical/biological degradation occurs. In this way an association can be determined, i.e. within a given statistical confidence interval the two samples cannot be discriminated (also called a 'match').

To determine the strength of potential evidence and to interpret the meaning of such an association, it is necessary to know the variation in stable isotopic characteristics for other similar materials (e.g. grey duct tape) that are assumed not to be related to the crime. This variation is called the background variation. For reasons of transparency, a report to a Court of Law should specify what materials are considered for determining the background variation (*e.g.* grey duct tapes, from Kent, UK, collected in May to June 2017). For some forensically investigated materials, for example natural materials such as wood or minerals, some data may have been published earlier from other, non-forensic, studies. For many materials, however, this background variation will not have been determined earlier and data will normally be acquired by taking these materials from a specific market (e.g. Kent, UK in 2017). The acquisition of appropriate background data is of significant importance for correct evaluation of the value of the evidence in a case. Deficiencies in background variation data must be articulated in the resultant reports and that information must be provided by the analysts to the end user of the report.

In (UK) forensic science the conclusion is often reported in the logically correct way of reporting the likelihood of the evidence given a certain scenario or hypothesis instead of the reverse: the so-called “prosecutor’s fallacy” i.e. ignoring the prior odds of innocence. More often, a likelihood ratio will be reported given a specific scenario (such as the scenario of the prosecution) [Evett and Buckleton 1989].

This method has, for example, been used for a Dutch serial arson casework investigation where the *modus operandi* consisted of a candle together with a flammable liquid. After an initial study to determine the discriminating powers of selected techniques it was decided to use visual, GC and IRMS data for the final study. Methods were developed and within-candle and within-candle-packet variations were determined for 128 packages of candles purchased in shops throughout the Netherlands. Casework candle samples were investigated in the same way.

Using IRMS alone, the relevant casework samples could easily be discriminated from all samples from the 128 packages of candles (Figure 19 shows a typical set of results). The findings were concluded to be much more likely if the samples were from a single production batch than if the samples were from different random production batches and the correspondences were just the results of chance.

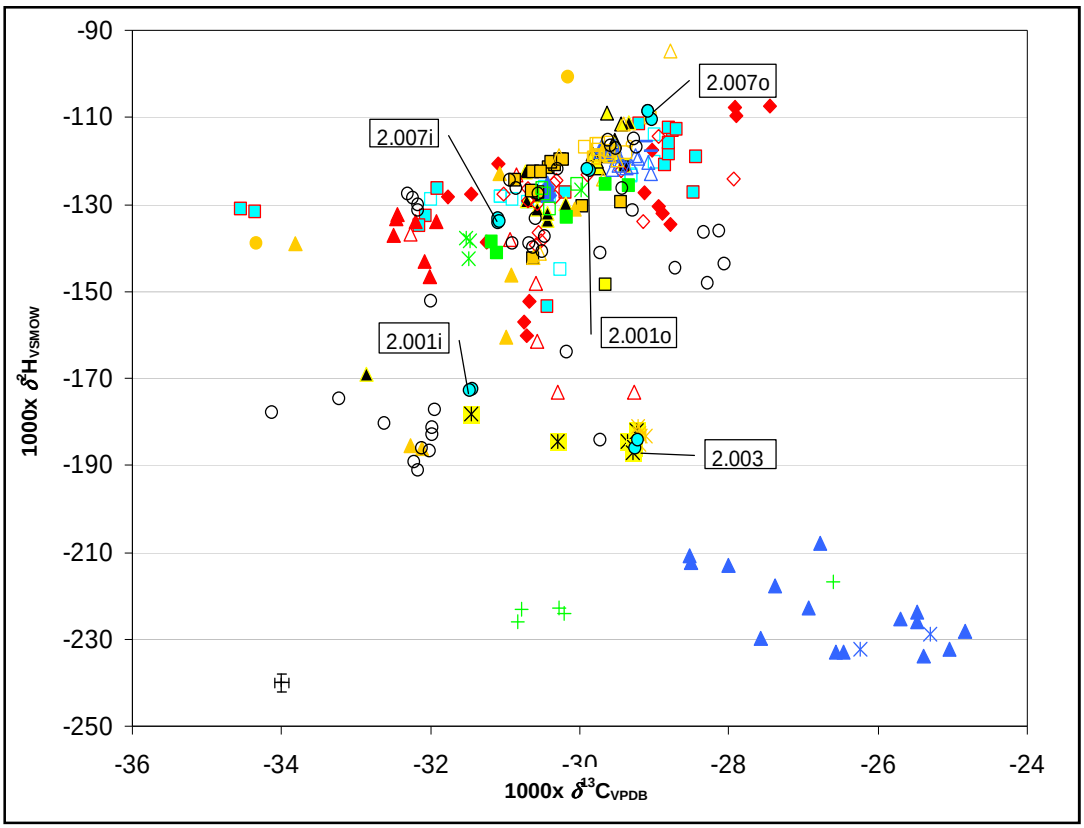

Figure 19. *Scatter-plot of δ^2H versus $\delta^{13}C$ for, (o) the outside layer and (i) the core of the candle. Different symbols identify results for a single candle type product from one producer (but different boxes acquired at different shops). (Reproduced with permission from Netherlands Forensic Institute)*

Various forensic guidance documents have been published to assist in interpretation of analytical chemistry results in a forensic investigation but a good general guidance document is the Association of Forensic Science Providers (AFSP) Standards for the formulation of evaluative forensic science expert opinion document [AFSP 2009].

More recently the European Network of Forensic Science Institutes (ENFSI) has published an ENFSI guideline for evaluative reporting in forensic science [ENFSI 2015]. This freely accessible document aligns with the AFSP Standards but is expanded. Furthermore, apart from evaluative reporting at the (sub-) source level (AFSP Standards), the ENFSI guideline focuses on evaluative reporting at the activity level which can potentially be more relevant for many Court decisions.

Other forensic guidance documents focus on other aspects of a forensic investigation such as the statistical aspects of forensic interpretation [Aitken 2011], or more general criteria for evaluating scientific and technical data, that constitute acceptable bases for forming scientific or technical expert opinions [ASTM 2013].

9 Troubleshooting

9.1 Routine maintenance

To ensure the continued performance of IRMS instruments and to minimise downtime, routine maintenance must be scheduled and undertaken according to operating procedures and recorded in an instrument log-book. Typical routine maintenance activities include:

- Cleaning auto-sampler trays
- Removal of ash
- Replacement of reactors
- Replacement of chemical traps
- Baking out GC columns
- Maintenance of vacuum pumps
- Cleaning the ion source

When troubleshooting it can be very informative to check the instrument log-book to ensure all routine maintenance has been carried out.

9.2 Visual inspection

A visual inspection of the equipment may reveal problems.

Indicator lights – Many IRMS instruments and peripheral devices incorporate indicator panels (or other forms of read-back) that indicate correct or incorrect operation, e.g. loss of vacuum. Check indicator panels for system problems.

Peripheral connections – Systems may have switches and/or valves to allow different peripheral devices and/or gases to be connected to the isotope ratio mass spectrometer. It is fundamentally important to ensure that these are correctly configured for the relevant analyses and securely connected.

Configuration – Each peripheral device may require specific settings for a given analysis, reactor packing, furnace temperatures, gas pressures etc. These parameters must be documented in operating procedures, set and checked prior to commencing instrument performance checks.

Once the operator has established that the instrumentation is in working order from the initial instrument checks further diagnostics may be required.

9.3 Elemental analyser

Symptoms	Possible causes	Resolution
High nitrogen blank	Insufficient helium purge to auto-sampler	Check purge flow. Check auto-sampler is sealed.
	Oxygen may be contaminated with nitrogen.	Ensure suitable grade oxygen is attached for combustion.
Poor nitrogen and/or carbon isotope ratio measurements	Reduction reactor is exhausted.	Monitor *m/z* 30 to determine if the reduction reactor needs replacing.
Nitrogen intensity increase in subsequent samples, or increased *m/z* 30 signal	Reduction reactor is exhausted and NO_x is eluting from the GC column. May appear as a shoulder on the nitrogen peak.	Replace reduction reactor.
Long tail on carbon dioxide peak	Sample start time too long (sample drops after O_2 has passed).	Observe combustion flash if possible. Alter sample timings and observe effect on peak shape. Only alter this after checking previous points.
	No/not enough O_2 for sample combustion.	Check O_2 flow.
	Large leak in analytical circuit.	Check for leaks.
	Blockage/restriction in oxygen gas supply.	Test gas lines for blockages/restrictions (replace any damaged or blocked sections).
Baseline drift after CO_2 peak (broad shallow peak)	Water bleeding through the GC column and detector.	Exhausted water trap, replace packing with fresh material. Monitor *m/z* 18 levels over time to prevent this.
Peak broadening, peak separation is poor, peak tailing	Slow/restricted carrier gas flow.	Clean out ash. Check carrier flow rate entering EA and MS.
	Dead volume in reactors or traps.	Check packing of reactors.
	Possible contamination or aging of GC column.	Bake out column.

Symptoms	Possible causes	Resolution
No sample peaks detected	Sample not loaded correctly.	Check samples loaded in correct order.
	Sample did not drop into reactor.	Crimp capsules so they are approximately spherical. If capsules are flat they may slip under the auto-sampler tray, or be caught on the edge of the auto-sampler tray, not dropping or dropping at the same time as the following sample.
	Samples caught between the outer reactor tube and the ash crucible.	Open the reactor and check the height of the ash crucible. Look for trapped capsules.
Unexpected, large peak with strange δ value	As above, this can also result if a capsule knocks a trapped capsule into the reactor and gas is evolved from two different samples.	Check the analytical sequence for earlier missing samples. Open the reactor and check the height of the ash crucible. Look for trapped capsules.
Furnace heater does not operate	Insufficient helium flow.	Ensure helium carrier pressure is appropriate and there are no leaks.
	Thermocouple failed (reactor may be hot, but temperature read-out differs from that expected).	Replace thermocouple.
	Furnace heater failed (temperature reading correct, but furnace will not heat).	Check/replace fuse. Replace furnace heater.
High backgrounds for N_2, H_2O, O_2, Ar	Auto-sampler seals leaking.	Test outside of auto-sampler for He leaks. Replace seals.
	GC column is contaminated.	Bake out column.
	Trap chemicals are exhausted.	Replace trap chemicals.
	Gas purity is incorrect.	Ensure correct gas supply.
	Ion source heaters or inlet valve heaters failed.	Check indicator lights to see if heater has failed. Replace heater.
Rapid consumption of reduction tube chemicals	Oxygen leaking into system.	Ensure auto-sampler is leak free. Ensure oxygen supply is leak free. Test alternative oxygen loops if available.

9.4 Mass spectrometer

Symptoms	Possible causes	Resolution
Indicator light suggests acceleration voltage is OK, but there is no emission	Ion source filament failed.	Check filament continuity on external contacts. Remove ion source and replace filament.
Box and trap values fluctuating	Filament has weakened and is flexing. Likely that filament will break soon. Wrong electrical connection to ion block or lenses	Remove and replace filament. Remove source and check connections.
False pressure reading	Dirt on electronic pressure sensor.	Remove and clean sensor.
High background with ion source needle valve closed	Air trapped in mass spectrometer.	Air may be trapped in dual-inlet valves.
Tailing on CO_2 working gas peak	Contaminated ion source. Solenoid not moving smoothly.	Clean ion source. Observe and lubricate with a tiny amount of very fine oil if movement is jerky.
Stability checks fail acceptance criteria	Poor purity carrier gas. Gas cylinder may be reduced in pressure if near empty. Interference from contaminants eluting from the GC column.	Ensure suitable gas supply. Check age of gas purifying cartridge if fitted – replace if necessary. Check sufficient gas supply (cylinders are not empty and regulators are correctly set). Bake out column.
Poor linearity over range of working gas intensities	Filament may be flexing. Poor ion source tuning parameters.	Check box and trap values are stable (see above if not). Check ion source tuning.
Poor vacuum	Vacuum pump failure.	Check pumps are functioning. Observe indicator lights for pump status. (Regularly check pump oil) Ensure fixings around ion source and pressure gauge are secure.
Poor linearity over range of working gas intensities	Poor seal around ion source/pressure gauge.	Replace O-ring seal(s).

Symptoms	Possible causes	Resolution
Poor sensitivity	Misaligned filament/source.	Following installation of a new filament, ensure the filament wire is located centrally. There may be a small amount of movement in the ion source placement; ensure this is correctly installed. (It may be useful to mark positions on the ion source and housing to assist with alignment following source removal.)
	Loose source connectors or connectors shorting.	It is not uncommon for connectors to the lenses etc. to come loose when re-installing the ion source. Adjust all the lens voltages (to their extreme values) and see that each one affects the intensity of the ion beam. If there is no effect there may be a bad connection.

9.5 GC and combustion or high temperature conversion interfaces

Symptoms	Possible causes	Resolution
High water (*m/z* 18) background	Nafion old or damaged. (The membrane can be damaged by sudden pressure changes, e.g. if solvent enters the reactor.)	Inspect with a hand lens for visible damage. Old tubing appears dark brown; new tubing is typically light in colour. Replace if necessary (soaking the end of the tubing in methanol will cause it to expand and makes it easy to fit over the fused silica).
	Excess column bleed.	Check the water background with the GC column at ambient temperature. If this is acceptable the GC column may need to be conditioned (in back-flush mode).
High O_2 (*m/z* 32) background	Reactor not conditioned.	For several hours after re-generation the reactor will bleed a significant amount of oxygen. If the O_2 background is higher than normal switch the system into “back-flush” mode for several hours. If the problem persists check that the regeneration oxygen is not bleeding into the system (i.e. isolate the oxygen cylinder).

Symptoms	Possible causes	Resolution
High Ar (*m/z* 40) background	Atmospheric leak.	This is a common problem with the GC interface with many possible causes. Before connecting a GC column to the IRMS interface it is good practice to leak test the GC injector. This will isolate any leak to the interface. Set the IRMS to monitor *m/z* 40 and apply a small flow of argon to each fitting in the interface; start at the fitting closest to the mass spectrometer. Apply the Ar only for a few seconds and wait to see any increase in signal. As you move to fittings further back in the interface remember that it will take longer for the Ar to reach the IRMS instrument.
No peaks	GC column connected to the wrong reactor.	If the interface has reactors for both ^{2}H and ^{13}C check that the correct reactor is connected.
	Reactor not at temperature or heater broken.	Check the set and read-back temperatures for the reactor.
	Broken / blocked capillary.	Perform Ar test (section 4.6.2) to ensure there is continuous He flow from the GC to the IRMS instrument.
Poor chromatography	Any number of problems with the GC injector or column.	If possible connect the GC column directly to a detector such as FID or MS. It is much easier to troubleshoot chromatographic problems without the extra complication of the IRMS interface.
Poor chromatography (not due to GC components)	Partially blocked reactor.	Perform Ar test (4.6.2) and check for changes in retention time and peak.
	Atmospheric leak	Perform Ar test and hexane tests (4.6.2 and 4.6.3) and check the peak width and height.
	Cold spots in GC.	Ensure the fittings inside the GC oven are not touching the walls.

Symptoms	Possible causes	Resolution
Sudden or gradual loss of sensitivity	Any number of problems with the GC injector or column.	If possible connect the GC column directly to a detector such as FID or MS.
Varying loss of sensitivity and peak broadening	Loose fitting in interface.	Check fittings are tight especially those subject to repeated temperature cycling or continuous elevated temperatures.
Variable δ values	Atmospheric leaks	Check the background gases and if these are high, leak check the interface as described above.
	Reactor depleted.	Re-oxidize the reactor and repeat the analyses. If these are still poor the reactor may need to be replaced.
	Reactor not conditioned.	Check the *m/z* 32 background (oxygen). If this is high, switch the system to back-flush mode and allow the system to stabilise for several hours.
Change in retention time	Leaks or blockages.	It would be uncommon to see a change in retention time that was not accompanied by a change in peak size or δ value. Typically increased retention time will be caused by blockages and reduced retention time will be caused by leaks. The Ar test (section 4.6.2) and a leak test of the GC injector are good starting points.

9.6 LC and chemical oxidation interface

Symptoms	Possible causes	Resolution
High backpressure	(Partial) blockage within system.	Examine components of system including in-line filters, HPLC column, gas separation unit and oxidation reactor for blockages and remove/flush out/replace components as required. Take care when handling narrow capillaries within the interface as these can be easily damaged.

Symptoms	Possible causes	Resolution
More negative $\delta^{13}C$ values obtained than expected for QC materials	Incomplete oxidation.	Check oxidation potential. Increase reagent concentration or flow rate or add catalyst. Reduce mobile phase flow rate.
	Non-quantitative extraction of CO_2 from mobile phase.	Increase gas flow of separation unit. Check for blockage within separation unit.
Reagent pumps not delivering solutions	(Partial) blockage within system.	Check in-line filters and replace if necessary. Disassemble pump head(s) and clean according to manufacturer's recommendations.
Elevated CO_2 background (*m/z* 44)	Change in mobile phase carbon content.	Check degassing and that sparging of mobile phase is occurring correctly. Check separate source/lot/batch of mobile phase to rule out contamination. Ensure HPLC system (including column) have been sufficiently purged of organic mobile phases.
Elevated water background (*m/z* 18)	Separation unit not operating correctly. Gas drier not operating correctly.	Check for blockages, and clear if any are found. Check purge gas flow rates. Exchange Nafion® membrane. Check gas flows.
Elevated oxygen background (*m/z* 32)	Reagents too concentrated or being delivered too quickly.	Dilute reagents (or prepare fresh solutions) and check the reagent pump flow rates. Too high an oxygen background can shorten filament lifetime.
Sequence does not run correctly, there are missing injections, etc.	Lack of communication between PC, HPLC, auto-sampler and/or interface.	Check communication cables. Test communication between parts of the system where possible.

10 Glossary of terms and abbreviations

A description of some of the terms that may be encountered in relation to IRMS. Guidelines on the terminology relating to the expression of stable isotope ratio measurements have been published [Coplen 2011] and should be followed when reporting isotope ratio data.

Term	Description
absolute isotope ratio	Isotope ratio expressed as a simple ratio (e.g. $n^{13}C/n^{12}C$) rather than as a ratio relative to a standard (i.e. a δ value).
accuracy	Closeness of agreement between a measurement result and the true value of the property being measured.
BSIA	Bulk stable isotope analysis: the analysis of bulk material comprised of one compound or a mixture of compounds.
CF	Continuous flow: automated sample preparation device and mass spectrometer in which sample analysis is conducted in a continuous stream of helium carrier gas.
CIAAW	Commission on Isotopic Abundances and Atomic Weights: scientific body created to introduce uniformity in the atomic-weight values used worldwide; part of the Inorganic Chemistry Division of IUPAC. (http://www.ciaaw.org/).
CSIA	Compound-specific isotope analysis: isotopic characterisation of individual compounds.
delta (δ)	Delta notation: a measure of isotopic ratios relative to international RMs that define the measurement scale for particular isotopes. Most commonly expressed in parts per thousand (‰).
DI/IRMS	Dual inlet/isotope ratio mass spectrometry: measurement of isotope ratios from pure gases by alternately introducing sample gas and a reference gas of known isotopic composition into an IRMS instrument by means of a system of valves.
EA	Elemental analyser: a sample preparation device in which samples are automatically converted into gases for isotope ratio analysis.
EA/IRMS	Elemental analyser/isotope ratio mass spectrometry: technique used for the measurement of nitrogen, carbon and sulphur isotope ratios that employs combustion of materials in an oxygen atmosphere followed by separation of gases evolved.
EI	Electron ionisation: ionisation of an atom or molecule by electrons that are typically accelerated to energies of up to 150 eV in order to remove one or more electrons.
extrinsic hydrogen	Hydrogen present in a material due to interactions with external water sources. Sometimes “exchangeable hydrogen”.
FC	Faraday collector: conducting cup or chamber that collects charged particles. The accumulated charge is subsequently measured.
FIA/CO/IRMS	Flow injection analysis-chemical oxidation-isotope ratio mass spectrometry: technique used to determine the bulk carbon isotopic composition using an LC/CO/IRMS instrument but bypassing the HPLC column.

Term	Description
FIRMS	Forensic Isotope Ratio Mass Spectrometry Network (www.forensic-isotopes.org)
GC	Gas chromatography: a separation technique in which the mobile phase is a gas.
GC/C/IRMS	Gas chromatography-combustion-isotope ratio mass spectrometry: technique used for CSIA (δ^{15}N and δ^{13}C) where individual compounds are separated using GC and then combusted in an on-line reactor. [Alternative acronym: irmGC/MS for isotope ratio monitoring GC/MS.]
GC/HTC/IRMS	Gas chromatography-high temperature combustion-isotope ratio mass spectrometry: technique used for CSIA (δ^{2}H or δ^{18}O) where individual compounds are separated using GC and then converted to H_2 and CO in an on-line reactor.
GISP	Greenland Ice Sheet Precipitation: reference material for the measurement of δ^{2}H and δ^{18}O values.
HPLC	High performance (pressure) liquid chromatography: separation technique, operating with relatively high inlet pressure, in which the mobile phase is a liquid.
HTC	High temperature conversion: high temperature conversion (>1350 ^{o}C) of materials containing hydrogen and oxygen to produce H_2 and CO. Sometimes referred to as high temperature conversion (HTC), high-temperature pyrolysis (HTP) or high temperature carbon reduction (HTCR) (see TC).
IAEA	International Atomic Energy Agency (www.iaea.org).
in-house reference materials	Reference material that is used routinely to normalise or verify measuring instruments or measuring systems. Sometimes referred to as “working standards”.
intermediate precision	*See Within-laboratory reproducibility.*
intrinsic hydrogen	Hydrogen permanent within a sample. Sometimes referred to non-exchangeable hydrogen.
IRMS	Isotope ratio mass spectrometry: the measurement of the relative quantity of the different isotopes of an element in a material using a mass spectrometer. [Alternative acronym: irm-MS for isotope ratio monitoring mass spectrometry.]
ISO	International Organization for Standardization (www.iso.org).
isobaric ions	Atomic or molecular species with the same nominal mass.
isotopologue	Contraction of “isotopic analogue”. These are molecules that differ only in their isotopic composition.
isotopomer	Contraction of “isotopic isomer.” These are isomers with isotopic atoms, having the same number of each isotope of each element but differing in their positions.
IUPAC	International Union of Pure and Applied Chemistry
KIE	Kinetic Isotope Effect: the effect of isotopic substitution on the rate of a chemical reaction. Primary KIEs involve the formation or breaking of bonds containing isotopically labelled atoms in the rate determining step, while secondary KIEs typically do not.

Term	Description
LC/IRMS	Liquid chromatography-isotope ratio mass spectrometry: technique used for CSIA where compounds are separated using HPLC prior to IRMS.
LC/C/IRMS	Liquid chromatography-combustion-isotope ratio mass spectrometry: technique whereby compounds separated by LC are converted to carbon dioxide via a combustion process prior to IRMS analysis.
LC/CO/IRMS	Liquid chromatography-chemical oxidation-isotope ratio mass spectrometry: technique whereby compounds separated by LC are converted to carbon dioxide by chemical oxidation prior to IRMS analysis.
Linearity	Changes in measured δ values as a function of sample size and/or peak intensity.
measurement uncertainty (MU)	Parameter associated with a measurement result that characterises the dispersion of values that could reasonably be attributed to the quantity being measured.
MS	Mass spectrometry: the study of matter through the formation of gas phase ions that are characterised using mass spectrometers by their mass, charge, structure and/or physico-chemical properties.
m/z	Mass-to-charge ratio: dimensionless quantity formed by dividing the mass of an ion in unified atomic mass units by its charge number (regardless of sign).
NIST	National Institute of Standards and Technology (www.nist.gov) (formerly National Bureau of Standards, NBS).
PDB	Pee Dee Belemnite (*see VPDB*).
precision	Measure of the degree of agreement between replicate measurement results obtained on the same sample under stipulated conditions (repeatability, intermediate precision/within-laboratory reproducibility, reproducibility).
PSIA	Position specific isotope analysis: technique for the determination of δ values for specific intra-molecular sites.
PT	Proficiency Testing: a form of inter-laboratory comparison exercise.
QA	Quality assurance: part of quality management focused on providing confidence that quality requirements will be fulfilled.
QC	Quality control: part of quality management focused on fulfilling quality requirements, i.e. planned activities designed to verify the quality of measurement results.
R	Isotope-number ratio, the amount of an isotope divided by the amount of another isotope (typically the amount of heavy isotope divided by the amount of light isotope).
RM	Reference material: a material that is sufficiently homogeneous and stable with regard to specified properties, which has been demonstrated to be fit for its intended use in a measurement process.
repeatability	Measurements made by one analyst, using the same equipment over a short time period. Represents the “within-batch” precision.
selectivity	Extent to which a measurement procedure can be used to measure a parameter without interference from other isotopic species in the mixture (often used interchangeably with specificity).

Term	Description
SI	International System of Units (Système international d'unités).
SIA	Stable isotope analysis.
SLAP	Standard Light Antarctic Precipitation: reference material for the measurement of δ^{2}H and δ^{18}O values (now replaced by SLAP2).
SMOW	Standard Mean Ocean Water (*see VSMOW*).
sparging	Process of bubbling an inert gas (e.g. He, Ar, N_2) through a solution to remove dissolved gases and to prevent re-dissolution.
standard	Widely adopted procedure, specification, technical recommendation, etc.
TC	Thermal conversion (*see HTC*).
HTC/IRMS	Thermal conversion-isotope ratio mass spectrometry: technique used for the measurement of hydrogen and oxygen isotope ratios, which employs high temperature thermal conversion of materials followed by GC separation of the resulting gases. Sometimes referred to as TC/IRMS (high temperature conversion-IRMS) or as TC/EA-IRMS (thermal conversion/elemental analyser-IRMS).
TD	Thermal decomposition: conversion of materials containing oxidized nitrogen using an elemental analyser without the addition of O_2 to produce N_2.
TIE	Thermodynamic Isotope Effect: the effect of isotopic substitution on the equilibrium constant of a reaction.
USGS	United States Geological Survey (www.usgs.gov).
VCDT	Vienna Canyon Diablo Troilite: internationally agreed zero-point for the measurement of δ^{34}S values. It is a virtual material.
VPDB	Vienna Pee Dee Belemnite: internationally agreed zero-point for the measurement of δ^{13}C values. It is a virtual material.
VSMOW	Vienna Standard Mean Ocean Water: internationally agreed zero-point for the measurement of δ^{2}H and δ^{18}O values. It was a real water material but is now replaced by VSMOW2.
within-laboratory reproducibility	Measurements made in one laboratory over an extended time period. Other conditions such as analyst or equipment may also be varied. Represents the “between-batch” precision (sometimes referred to as “intermediate precision”).
working gas	High purity gas introduced into the CF carrier gas to facilitate raw δ value calculations (often referred to as the “reference gas”).
working standard	*See In-house reference material.*

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