National Measurement System

FIRMS

$\delta^{13}C$ $\delta^{18}O$

$\delta^{15}N$ $\delta^{2}H$

$$\delta = \left(\dfrac{R_{Samp}}{R_{Std}} - 1 \right)$$

Good Practice Guide for Isotope Ratio Mass Spectrometry

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Preface

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 Although many (rightly) applaud this popularisation, as a consequence the "black art" has been replaced by a "black box":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 and Director

The FIRMS Network

Contents

1 Introduction

1.1 Aims of the Guide

- To enable those unfamiliar with isotope ratio mass spectrometry (IRMS) to obtain isotope ratio measurements that are reliable.

- To help to fill the lack of standardised protocols for the determination and reporting of stable isotope ratios.

- To help users to recognise common pitfalls in isotope ratio mass spectrometry and how to avoid them.

- To enable users to understand the scope and some of the limitations of isotope ratio mass spectrometry.

- This Guide is restricted to the use of elemental analyser (EA) and thermal conversion (TC) EA-systems. Coupled chromatographic systems, such as GC-IRMS or LC-IRMS, are not covered.

1.2 Examples of applications of IRMS

To establish an isotopic "profile" or "signature" for a material, the ratios of the stable isotopes of a number of elements such as ^{2}H/^{1}H, ^{13}C/^{12}C, ^{15}N/^{14}N and ^{18}O/^{16}O can be measured. 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 may be introduced during biological, chemical and physical processes. IRMS is a technique used to measure the relative abundance of isotopes in different materials.

Variations in the natural abundance of stable isotopes are expressed using delta (δ) notation as shown in equations (1) and (2):

$$\text{ratio}\,(R) = \frac{\text{abundance of the heavy isotope}}{\text{abundance of the light isotope}} \tag{1}$$

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

R_{Samp} ratio of the sample

R_{Std} ratio of the international standard (defined by the IAEA)

δ-values are commonly multiplied by 1000 so that they are reported in parts per thousand (‰ or per mil) or by 1000,000, to give results in parts per million (ppm).

Isotopic variations are found in a wide variety of materials and the isotopic profile is unique to the origin and history of the substance. IRMS therefore 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

  - Environmental forensics and monitoring

    - Identifying the source of pollutants such as oil spills

    - Monitoring atmospheric gases to distinguish between natural and anthropogenic sources

- – Climate studies

  – Water cycle research

  ➢ Food authenticity and traceability

  – Establishing the geographic authenticity of foodstuffs

  – Identifying the adulteration of foods with cheaper ingredients

  ➢ Wildlife forensics

- 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

- Biological sciences

  ➢ Ecology

  – Photosynthetic pathways

  – Food webs

  – Ecohydrology

  – Nutrient cycling

  ➢ Human and plant physiology

  ➢ Human provenancing

  ➢ Metabolic studies

  ➢ Sports medicine

  ➢ Toxicology

  – Distinguishing endogenous versus exogenous bio-chemicals

2 Instrumentation

2.1 Background

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

Prior to analysis by IRMS, samples are converted to simple gases such as H_2, CO_2, N_2, and CO, depending on the composition of the material and the isotopes of interest. The IRMS measures the ratio of ions that correspond to these gases. For example, in the analysis of carbon isotope ratios, the mass spectrometer 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.

Isotope ratios, at natural abundance levels, are measured relative to international standards (primary materials) which define the measurement scale for particular isotopes. For the isotope ratios for unknown test samples to be traceable to the international standards, it is a prerequisite to use well-characterised standards (working gas and/or solid material) whose isotope ratios have been determined against the primary materials.

There are two common instrument configurations used for gas source IRMS – continuous flow IRMS (CF-IRMS) and dual-inlet IRMS (DI-IRMS). Continuous flow systems employ an elemental analyser, as described in section 2.2.

2.2 EA-IRMS (Elemental analyser isotope ratio mass spectrometry)

There are two types of elemental analyser available:

- For the analysis of carbon and nitrogen, the sample undergoes combustion in an oxygen atmosphere (known simply as EA-IRMS);

- For the analysis of hydrogen and oxygen the sample undergoes high temperature thermal conversion (referred to in this Guide as TC/EA-IRMS). Other terms in common use include HTC-IRMS (high-temperature conversion-IRMS), HTP-IRMS (high-temperature pyrolysis-IRMS) and HTCR-IRMS (high-temperature carbon reduction-IRMS).

The key components of EA-IRMS and TC/EA-IRMS systems are shown in Figure 1.

The analysis can be divided into four steps:

- Combustion or thermal conversion of the sample material using the elemental analyser;

- Introduction of the evolved gases into the ion source of the mass spectrometer via the interface;

- Ionisation of the gas molecules followed by separation and detection of the ions in the mass spectrometer;
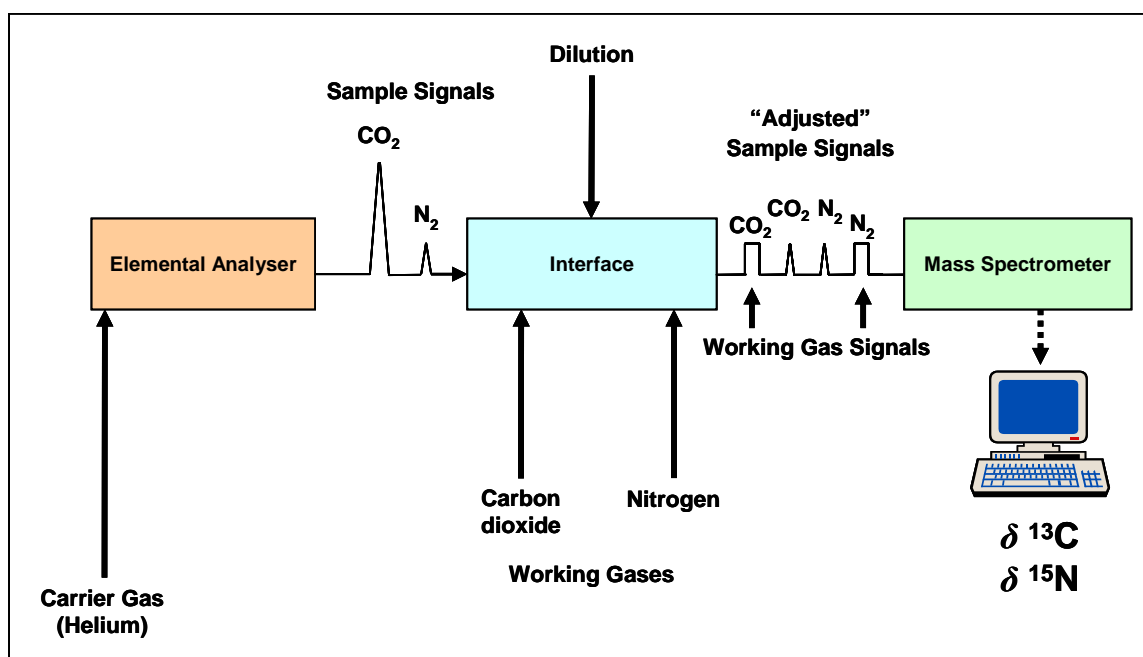
- Evaluation of the raw data.

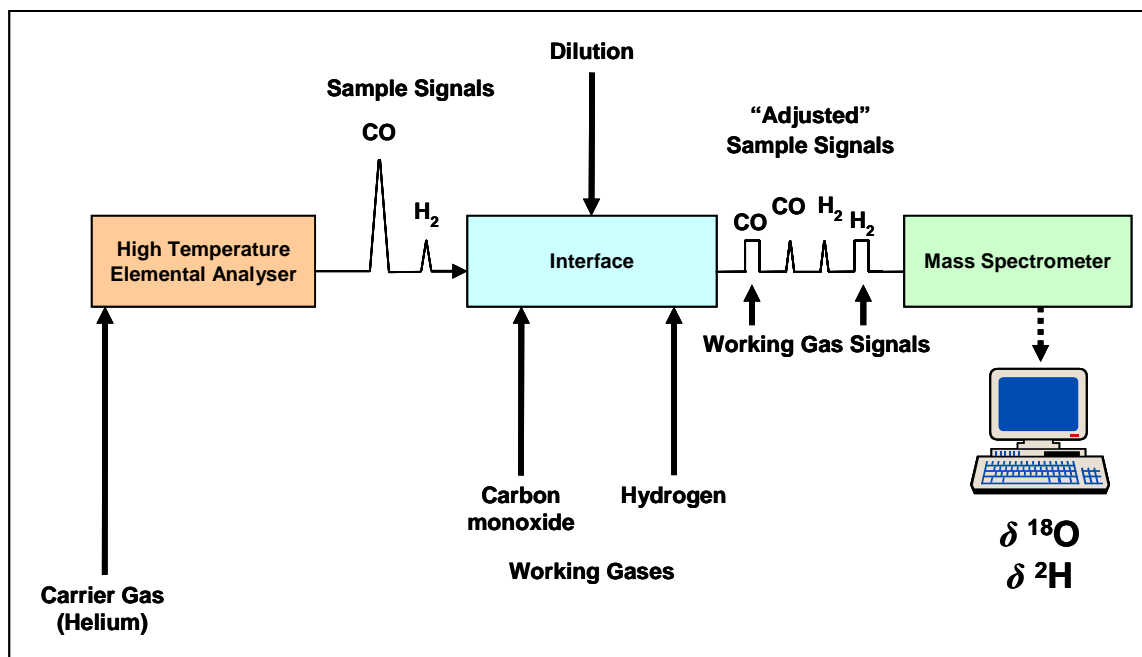*Figure 1a. Simple schematic diagram of an EA-IRMS for the determination of $\delta^{13}C$ and $\delta^{15}N$*

Figure 1b. Simple schematic diagram of a TC/EA-IRMS for the determination of δ^2H and $\delta^{18}O$

2.2.1 Elemental analyser systems

EA-IRMS is applicable to a wide range of materials. Solid substances and non-volatile liquids can be introduced into the elemental analyser system using tin (for C/N analysis) or silver (for O/H analysis) capsules, while liquids with limited viscosity can be directly injected using a liquid inlet system.

2.2.1.1 Combustion (for C and N analysis)

The analyser typically consists of a two-reactor system – 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 a packed GC column for separation of the evolved gases (CO_2 and N_2).

Combustion takes place in an O_2 atmosphere in a quartz reactor to produce CO_2, NO_x and H_2O. The reactor typically contains Cr_2O_3 and Co_3O_4+Ag (to bind sulphur and halogens), although many variations are recommended for specific applications. The reactor temperature is typically between 900-1050 °C, but the heat of combustion of tin capsules raises the temperature to about 1800 °C. It is recommended to use quartz inserts (ash crucibles or swarf crucibles) to collect the ash and residue from samples and tin capsules. Depending on the type of insert used it has to be replaced after analysing 50 to 150 samples without the need to remove the reactor.

Removal of excess oxygen and reduction of the NO_x to N_2 takes place at 650 °C in a second quartz reactor. This is typically packed with high purity Cu but, again, variations are recommended for specific applications.

The water is separated in a "water trap" containing magnesium perchlorate ($Mg(ClO_4)_2$) also known as Anhydrone®. When only nitrogen isotope ratios are to be determined it can be advantageous to remove CO_2 from the gas stream using a chemical trap containing soda lime, Ascarite® (NaOH on a silica substrate) or Carbosorb®. Depending on the nature of the reagent this may be placed before or after the water trap.

Finally, the N_2 and CO_2 are separated via an isothermal packed column GC (e.g. Porapak® QS 50/80, 3 m, 6.5 mm).

As an alternative to chromatographic separation, some instruments employ a "purge & trap" system to affect separation [1]. Nitrogen passes directly through the system while other evolved gases are collected on a number of adsorption tubes (effectively short GC columns). These traps are then electrically heated to liberate the gases.

2.2.1.2   High temperature thermal conversion (for H and O analysis)

High temperature conversion occurs at temperatures between 1350 and 1450 °C. Both organic and inorganic compounds are converted to H_2, N_2 and CO gases. The system typically comprises an outer tube made from fused alumina and an inner tube made from "glassy carbon". The inner tube is filled with glassy carbon particles and silver wool intended to bind halogen atoms. As with combustion EA many variations are recommended for specific applications. The evolved gases are separated via an isothermal packed column GC (e.g. molecular sieve 5 Å). This is important as N_2 is both isobaric with CO (both m/z 28) and known to affect the ionisation of H_2.

Although only H_2, N_2 and CO should be evolved, some workers recommend chemical traps to remove other gases, inserted before the GC column. Trapping materials include; activated charcoal, magnesium perchlorate, Sicapent® (P_2O_5 on a binder) and Ascarite®.

The use of "purge & trap" columns can result in complete baseline separation of the H_2 from the CO peak, regardless of their relative size and can prevent N_2 from interfering with the H_2 isotopic measurement [2].

2.2.2 Interface

An interface is required to connect the IRMS to the on-line elemental analyser system. The interface limits the gas volume entering the ion source and also provides a means to introduce pulses of working gas and to dilute the sample gas with additional helium.

It is therefore possible to carry out dual measurement of both $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotope ratios on one sample portion. Most organic compounds contain a relatively small proportion of nitrogen and hence the CO_2 signal is diluted to attain an appropriate signal. Similarly, in TC/EA-IRMS, if there is a high O/H-ratio in the sample the CO signal is diluted and for a low O/H-ratio the H_2 signal is diluted. This allows the simultaneous measurement of both $^2H/^1H$ and $^{18}O/^{16}O$ isotope ratios.

Due to the isobaric interference of CO and N_2, dual measurements of hydrogen and oxygen are most reliable if no nitrogen is present in the sample. Although CO and N_2 can be separated by the GC column, N_2 reacts to form NO in the ion source elevating the m/z 30 background and affecting the integration of the CO peak. It is therefore advantageous to carry out maximum dilution or divert the gas stream during elution of N_2 to improve the accuracy of $\delta^{18}O$ measurements of N-bearing materials [3].

2.2.3 Mass spectrometer

In the ion source of the mass spectrometer gas molecules are ionised through interaction with the electron beam (electron ionisation, EI).

The ions leave the source and are focussed and accelerated through a high voltage. The mass spectrometer itself is a sector-field instrument and ions pass through the magnetic field before reaching the Faraday cup detectors. The strength of the magnetic field and the accelerating voltage determine the trajectory of the ions and therefore which ions will enter the Faraday cups. The use of multiple collectors allows the simultaneous measurement of ion intensity ratios, negating fluctuations in the intensity of the ion beam.

For nitrogen and carbon ratio measurements three collectors are necessary which can be either two suites of collectors specifically spaced to collect m/z 28 and 29 or m/z 44, 45 and 46 or a universal triple collector in which the outer cups are wide with respect to the dispersion of the ion

beam. Since oxygen isotope ratio measurements are based on CO, which is isobaric with N_2, the same set of collectors are employed.

For the analysis of hydrogen the field strength is set to allow ions with m/z 2 and 3 ($^1H_2$, $^1H^2H$) to enter an additional pair of collectors. These are often positioned on either side of the central collectors. Additional cups may also be present to determine the isotopic ratio of elements such as sulphur and chlorine.

Each cup is connected to its own 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 relative amplifier gains are shown in Table 1, the absolute gain of cup 2 is typically 3×10^8 (three hundred million). Some instruments provide an ability to switch the gain of certain amplifiers to facilitate the measurement of samples which have been labelled with stable isotopes, i.e. the relative abundance of the major and minor isotope may be close to unity.

Table 1. Typical detector amplification factors for IRMS instruments

Cup	m/z	Relative amplifier gain
1	2	10
2	28 or 44	3
3	29 or 45	300
4	30 or 46	1,000
8	3	10,000

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" for ions of given m/z, the peak area being proportional to the number of ions detected.

2.3   DI-IRMS (Dual-inlet isotope ratio mass spectrometry)

Dual-inlet (DI) isotope ratio mass spectrometry is generally considered to be the most precise method of measuring the isotope ratios of light elements. The technique, however, requires significantly greater preparation time and larger sample size than is required for the continuous-flow methods described in section 2.2.

The DI technique is briefly described here because:

1.   it remains the highest precision technique available

2.   it has historical significance, and

3.   it is the basis for the ubiquitous use of delta notation.

A review of the comparison of DI and continuous flow IRMS may be found in Barrie and Prosser [4] and Brand [5]. Some of these differences are summarised in Table 2. The first studies using isotope ratio mass spectrometry, using dual-inlet, were published around 1950 (i.e. McKinney et al [6]). Since that time the basic structure of the DI instrument has remained the same although advances in mechanics and electronics have improved both precision and ease of measurements.

DI determines isotope ratios from pure gases by alternately introducing sample gas and a working gas of known isotopic composition into an IRMS. The sample and working gases enter the mass spectrometer under nearly identical conditions. This is achieved by introducing the sample gas into a variable volume, or bellows. The reference gas resides in a separate, but similar, 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 isotope fractionation during flow. The variable volume of the bellows allows the gas pressure to be adjusted such that nearly identical amounts of sample and working gas are alternatively introduced into the ion source of the IRMS.

Table 2. 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) is introduced into the ion source.	A pure gas is entrained as a chromatographic peak within a flow of helium during introduction to the ion source. Thus a mixed gas enters the ion source (e.g. CO_2 + He).
How the sample gas and working gas are introduced into the mass spectrometer	The gases are repeatedly and alternately introduced into the ion source.	The chromatographic peak of sample is preceded and/or followed by introduction of working gas.
Signal intensity of sample gas	Sample gas and working gas are carefully balanced by adjustments of bellows to produce nearly identical signals, for the major ion beam, avoiding linearity biases.	Sample gas varies in intensity across the chromatographic peak.
Amount of sample required	10s of µmol, or ~0.5 µmol using a cold finger volume. The sample size is controlled by the need for viscous flow conditions in the capillaries.	100s of nmol, smaller if systems are optimised (10s of nmol by GC-IRMS). Because viscous flow is provided by the helium stream, there is the possibility of further reduction in sample size by advancements in blank reduction, amplification and/or minimising the preparatory system.

The alternating flow of sample and working gas, at nearly identical pressures 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 working gases during a dual-inlet isotope ratio measurement. Typically, 5 to 10 pairs of sample-working gas isotope ratio measurements are made for any one sample; from 5 pairs of data, 10 comparisons of adjacent reference and sample gas are derived, which are typically averaged and an outlier filter may be applied. If the isotopic composition of the working gas is well-characterised, the offset in ratios between the working gas and sample gas may be used directly to calculate the δ-value of molecular species of the sample ($\delta^{45}CO_2$, $\delta^{46}CO_2$, or $\delta^{29}N_2$). Further corrections must be made to convert $\delta^{29}N_2$ to $\delta^{15}N$, assuming a stochastic distribution of isotopes; likewise a $\delta^{17}O$ correction must be performed (see section 6.2). As with continuous-flow measurements, a H_3^+ correction must be performed for hydrogen isotope ratio measurements (see section 3.3.4). In contrast to continuous flow techniques, an offset/shift correction is usually not needed for dual-inlet isotope ratio mass spectrometer measurements, provided the reference and sample gases are not too different in their isotopic compositions. Normalisation is seldom needed, with the exception of hydrogen isotope ratio measurements.

Some dual-inlet systems are optimised for smaller sample sizes by means of a cold-finger or micro-volume. In this configuration, 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 alteration of the isotope ratios of the gases, but with care, this option can produce high quality measurements on very small amounts of gas.

Dual-inlet isotope ratio measurements are commonly performed on samples of a pure gas prepared "offline". Various reaction and cleanup processes, typically conducted on vacuum lines, may be employed quantitatively to convert a sample into a pure gas for introduction to a dual-inlet IRMS. Specific procedures are used to convert solids, liquids, dissolved gases, and gas mixtures into pure gases, and will not be described further. These offline techniques are usually very time consuming, but are sometimes necessary to conduct particular measurements. Some of the common methods have been adapted for automated DI-IRMS. Among these automated methods are: 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 Instrument set-up and preparation

3.1 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 or operating instruction from an instrument manufacturer should specify the acceptable range and maximum daily variation for these parameters.

It is important that the cylinders (and associated valves and regulators) which supply working gases to the IRMS are also located in a temperature controlled environment. Temperature fluctuations can produce significant shifts in the isotopic composition of the working gases, especially CO_2 as the gas is in equilibrium with a fluid. It is recommended that the CO_2 working gas is replaced when the pressure is less than ~48.3 bar (700 psi), indicating that the fluid in the tank is exhausted.

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.

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.

The carrier gas for all CF-IRMS configurations is helium, which will generally be supplied with a purity better than 99.9992% (N5.2). In addition, the carrier gas supply should incorporate filters to remove residual oxygen, moisture and hydrocarbons. It is often advisable to mount a hydrocarbon filter close to the instrument to remove any traces of fluids used to manufacture the tubing. Filters should not be incorporated in the working gas supplies as these may cause isotopic fractionation.

3.2 General sequence

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.

"Normal" operating performance for an instrument must be established during commissioning. Diagnostic tests which 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.

3.2.1 Mass spectrometer checks

An obvious place to begin is to check that instrument read backs indicate normal values and that these are stable and not fluctuating.

Safety equipment – Many of the gases used in the routine operation of IRMS are hazardous and the laboratory should have atmospheric monitoring systems to warn of dangerous gas levels. Checking that these warning systems are functioning correctly should be an integral part of the daily instrument checks.

3.2.2 Tuning

Instrument operators often categorise an IRMS as being tuned for either "sensitivity" or "linearity". The former suite of parameters is intended to attain maximum signal intensity, the other to attain 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 a very general idea how to perform it.

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. 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 potentially very time consuming, this process will generally only need to be performed once to establish the "critical values".

Some IRMS software programmes offer an "autofocus" function. This can speed up the whole process, but manual tuning is typically performed after the "autofocus" to achieve the best results.

3.3 Sequence of tests

A 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 log-book and/or spreadsheets. Table 3 illustrates an example of daily system checks which should be performed. Weekly, monthly, biannual and annual checks should also be scheduled; the user is advised to consult the operating manual of specific instruments.

Table 3. Example of daily system diagnostic check list

Sequence	1	2	3	4	5
Check	background	zero enrichment	H_3^+ factor	blank*	QC/QA
δ^2H of solid samples	✓	✓	✓	✓	✓
δ^2H of liquid samples	✓	✓	✓		✓
$\delta^{13}C$ of solid samples	✓	✓		✓	✓
$\delta^{13}C$ of liquid samples	✓	✓			✓
$\delta^{15}N$ of solid samples	✓	✓		✓	✓
$\delta^{18}O$ of solid samples	✓	✓		✓	✓
$\delta^{18}O$ of liquid samples	✓	✓			✓
*A blank determination is not required for liquid samples that are injected directly into the reactor. See section 5.3 for further information on blank determinations.					

3.3.1 Background

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

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

Figure 2 shows a typical background of residual gases for the EA-IRMS configuration. The intensity of at least *m/z* 18, 32, 40 and 44 should be recorded. Background monitoring should also include *m/z* 2 and 3 when performing hydrogen isotope measurements. Indicative acceptable values are shown but must be determined for individual instrument configurations.

Table 4 lists some possible causes of problems with background values (see also section 8 on troubleshooting for further information).

Table 4. Typical problems with background values and possible causes

m/z	Mol species	Problem and possible cause
2	He^{2+}	High background in D/H 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)
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
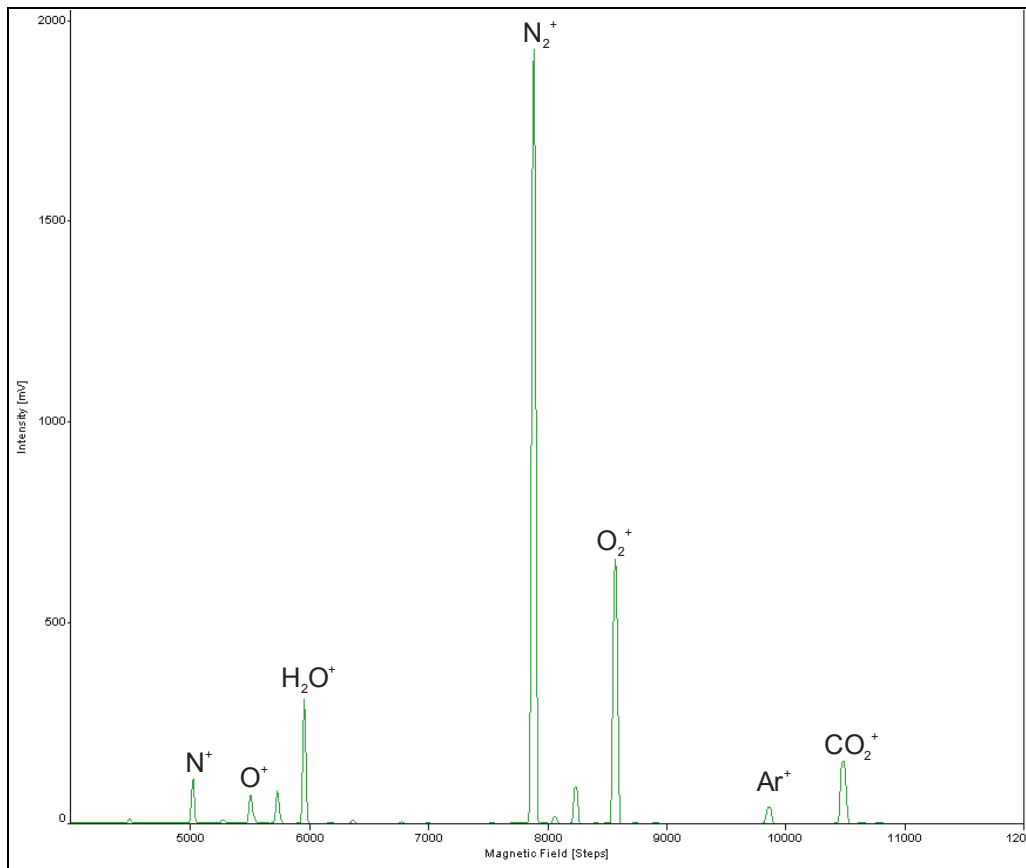
m/z	2	18	28	40	44
	maximum intensity, cup 3 (mV) (see Table 1)				
TC/EA (H&O)	220	500	30,000	50	300
EA (C&N)	n/a	500	2,500	50	150

Figure 2. Indicative signal intensities for residual gases in continuous flow IRMS configurations

3.3.2 Stability (zero enrichment)

Figure 3. Example of zero-enrichment check

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 initially evaluated relative to the working gas and hence the reproducibility of this measurement determines the best reproducibility that can be achieved.

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

As with all performance tests, acceptable 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.

3.3.3 Linearity

Periodically, the linearity of the instrument must be measured with respect to the working gas. This is not normally a daily check.

The measurement is similar to the zero-enrichment test, except that the intensity of the working gas is increased during the sequence. The intensity of the working gas pulses must encompass the intensities of the samples to be determined, i.e. if samples are measured in the range 5000 mV to 15000 mV the linearity measurement should cover the range 4000-16000 mV. The working range will be established during validation.
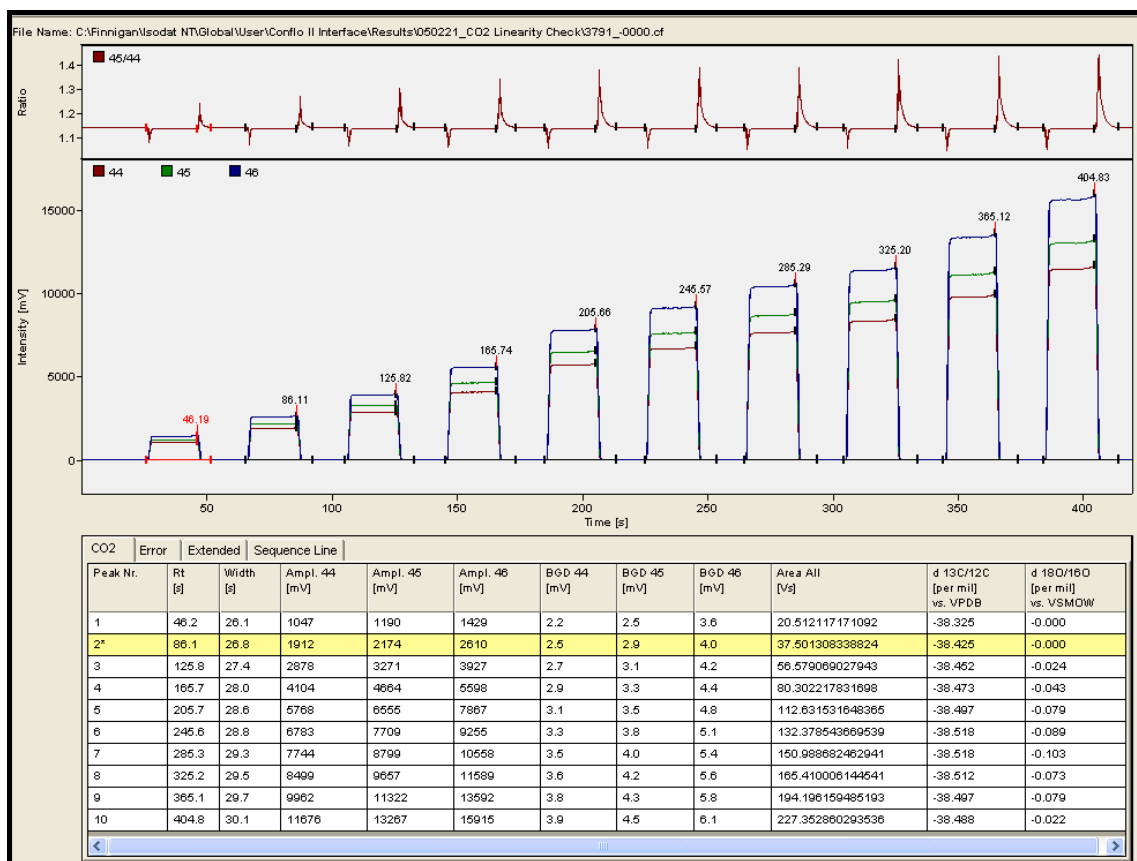
File Name: C:\Finnigan\Isodat NT\Global\User\Conflo II Interface\Results\050221_CO2 Linearity Check\3791_-0000.cf

CO2 | Error | Extended | Sequence Line

Peak Nr.	Rt [s]	Width [s]	Ampl. 44 [mV]	Ampl. 45 [mV]	Ampl. 46 [mV]	BGD 44 [mV]	BGD 45 [mV]	BGD 46 [mV]	Area All [Vs]	d 13C/12C [per mil] vs. VPDB	d 18O/16O [per mil] vs. VSMOW
1	46.2	26.1	1047	1190	1429	2.2	2.5	3.6	20.512117171092	-38.325	-0.000
2*	86.1	26.8	1912	2174	2610	2.5	2.9	4.0	37.501308338824	-38.425	-0.000
3	125.8	27.4	2878	3271	3927	2.7	3.1	4.2	56.579069027943	-38.452	-0.024
4	165.7	28.0	4104	4664	5598	2.9	3.3	4.4	80.302217831698	-38.473	-0.043
5	205.7	28.6	5768	6555	7867	3.1	3.5	4.8	112.631531648365	-38.497	-0.079
6	245.6	28.8	6783	7709	9255	3.3	3.8	5.1	132.378543669539	-38.518	-0.089
7	285.3	29.3	7744	8799	10558	3.5	4.0	5.4	150.988682462941	-38.518	-0.103
8	325.2	29.5	8499	9657	11589	3.6	4.2	5.6	165.410006144541	-38.512	-0.073
9	365.1	29.7	9962	11322	13592	3.8	4.3	5.8	194.196159485193	-38.497	-0.079
10	404.8	30.1	11676	13267	15915	3.9	4.5	6.1	227.352860293536	-38.488	-0.022

Figure 4. Example of a working gas linearity check

Typically the linearity of CO_2, N_2 and CO must be less than 0.1 ‰ per volt*. The linearity test is not applicable to $^2H/^1H$ measurements, which requires a daily H_3^+ factor determination.

(* units of per mil per volt are specific to the amplifier configuration used by many Thermo Fisher Scientific® instruments.)

3.3.4 H_3^+ Factor

The term "H_3^+ correction" 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 gas pressures.

$$H_2^+ + H_2 \rightarrow H_3^+ + H$$
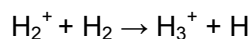
The reaction constant is proportional to both $[H_2^+]$ and $[H_2]$ and, for a given IRMS, the number of ions formed is proportional to the number of molecules present. The ratio $[H_3^+]/[H_2^+]$ is a linear function of the *m/z* 2 intensity and the correction simply subtracts a portion of the *m/z* 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 increasing the intensity by adjusting the gas pressure. The instrument software can then calculate the H_3^+. The value should be recorded in the instrument log book or spreadsheet.

The H_3^+ factor should not exceed 10 ppm/nA and should not be significantly changed from the previous value recorded, e.g. the difference should be less than 0.2. The H_3^+ factor may change significantly if the instrument has undergone maintenance or has been tuned. This should be recorded in the instrument log book.

4   Calibration

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

4.1   Overview

Variations in the isotope ratios of naturally occurring materials are reported as δ-values (e.g. $\delta^{13}C$), commonly expressed in parts per thousand (per mil or ‰) difference from the following internationally agreed zero points:

hydrogen ($^2H/^1H$)   VSMOW (Vienna Standard Mean Ocean Water)

carbon ($^{13}C/^{12}C$)   VPDB (Vienna Peedee Belemnite)

nitrogen ($^{15}N/^{14}N$)   atmospheric nitrogen (Air-N_2)

oxygen ($^{18}O/^{16}O$)   VSMOW (Vienna Standard Mean Ocean Water)

Since IRMS determines the relative variations of isotopic ratios it is not necessary to know the absolute isotopic composition of materials used for calibration and quality control. These materials have defined or agreed δ-values, for one or more element, which enable laboratories to obtain results that are both internally consistent and directly comparable with other laboratories.

All reference materials should have the desired characteristics of isotopic composition, homogeneity, chemical purity and stability.

Although nomenclature will vary, reference materials for isotope ratio measurements may be broadly classified as:

1. primary (or calibration) materials

2. secondary (or reference) materials

3. inter-laboratory comparison materials

4. in-house (or laboratory) standards

The terms 2 and 3 are used interchangeably.

4.1.1 Primary (calibration) materials

These materials define the δ-scales versus which natural variations in isotopic compositions are expressed. Of the original materials, PDB (Peedee Belemnite) is now exhausted and SMOW (Standard Mean Ocean Water) never physically existed. The International Atomic Energy Agency (IAEA) (Vienna) has now defined these scales by reference to natural or virtual materials identified by the "V" prefix.

The primary or calibration materials currently kept and distributed by the IAEA are listed in Table 5. At present, a laboratory can receive a portion of each primary material only once every three years. This control of supply is intended to ensure that each material will be available for several decades.

Again, the absolute isotopic composition of the materials is not important but values have been reported [7].

Table 5. The reference materials against which δ-scales are calibrated

Primary reference material	Nature	Isotopic ratio	δ‰	Scale
VSMOW2	water	$^2H/^1H$ $^{18}O/^{16}O$ $^{17}O/^{16}O$	0.00 ± 0.3* 0.00 ± 0.02 0.00 ± 0.03	VSMOW VSMOW VSMOW
NBS-19	calcium carbonate	$^{13}C/^{12}C$ $^{18}O/^{16}O$	+1.95* -2.20*	VPDB VPDB
*The uncertainties associated with the VSMOW2 values are given in reference 9. There are no uncertainties associated with the δ-values of NBS-19.				

4.1.1.1   The VSMOW δ-scale

VSMOW was prepared by blending distilled ocean water (latitude 0^0/longitude 180^0) with small amounts of other waters to produce an isotopic composition close to the definition of SMOW. The definition of SMOW, by Craig (1961) [8], was based on a water standard at the National Bureau of Standards (NBS-1), but SMOW itself did not physically exist and measurements could not be calibrated against it. VSMOW has now been superseded by VSMOW2.

The δ^2H and $\delta^{18}O$ values of all hydrogen and oxygen bearing materials should be calibrated versus VSMOW and normalised according to the defined differences between VSMOW and SLAP (Standard Light Antarctic Precipitation). SLAP was prepared from South Pole firn and is now superseded by SLAP2, considerably depleted in heavy isotopes with respect to VSMOW2 (δ^2H = -427.5 ‰ and $\delta^{18}O$ = -55.50 ‰ versus VSMOW2) providing an anchor for the lower end of the scale [9].

4.1.1.2   The VPDB δ-scale

PDB consisted of calcium carbonate from a Cretaceous belemnite from the Peedee formation in South Carolina. The CO_2 evolved from PDB, by treatment with phosphoric acid, was adopted as the zero point for oxygen and carbon isotopic measurements. PDB has been replaced by assigning exact values to another carbonate (NBS-19 or "TS-limestone") versus a hypothetical VPDB. There is an oxygen isotope fractionation between the carbonate and the evolved CO_2, the latter being about 10.25 ‰ higher that the calcite (when the reaction takes place at 25 ^{o}C). This is irrelevant when measuring calcite samples against calcite standards, but becomes problematic for dual inlet measurements of non-carbonates or for non-calcite carbonates, which have different fractionation factors than calcite [10].

VPDB has isotopic ratios characteristic of marine limestone and is considerably enriched in ^{13}C with respect to organic carbon compounds. It is now recommended that $\delta^{13}C$ values of both organic and inorganic materials are expressed relative to VPDB on a scale normalised by assigning a value of -46.6 ‰ to LSVEC lithium carbonate [11].

In order to maintain consistency with historical data, the VPDB scale is still used for reporting $\delta^{18}O$ values of carbonates. When converting between scales the recommended conversion is [12]:

$$\delta^{18}O_{VSMOW} = 1.03091*\delta^{18}O_{VPDB} + 30.91 \qquad\qquad (3)$$

4.1.1.3   Atmospheric nitrogen δ-scale

The isotopic composition of atmospheric nitrogen (air-N_2) has been adopted as the zero point for all nitrogen isotope ratio analyses as it does not vary measurably around the world or over time. To be used as a practical reference material, however, N_2 would need to be isolated from the atmosphere without fractionation. For convenience a number of reference materials (mostly ammonium and nitrate salts) have been prepared and are distributed by IAEA (see Table 7).

4.1.2 Secondary (reference) materials

These are natural or synthetic compounds which have been carefully calibrated versus the primary calibration materials. The δ-values of these materials are agreed and adopted internationally but, in contrast to the calibration materials, have uncertainty associated with the δ-values. Some of the materials distributed by IAEA for $\delta^{13}C$ and $\delta^{15}N$ measurements are listed in Tables 6 and 7. Both the δ-values and the associated uncertainties (expressed as a standard deviation, SD) of these materials have been reviewed and revised over time and the reader is urged to check the website:

http://nucleus.iaea.org/rpst/ReferenceProducts/ReferenceMaterials/Stable_Isotopes/index.htm

Table 8 lists some of the materials distributed by IAEA for δ^2H and $\delta^{18}O$ measurements. Some reputable researchers have published values for these materials which differ from those stated by IAEA [13].

Table 6. Secondary reference materials for $\delta^{13}C$ measurements.
The isotopic compositions are those reported by IAEA (compiled Sep 2011)

Description	NIST RM	Nature	$\delta^{13}C$ ‰	SD
USGS-41	8574	L-glutamic acid	+37.626	0.049
IAEA-CH-6	8542	sucrose	-10.449	0.033
USGS-24	8541	graphite	-16.049	0.035
IAEA-CH-3		cellulose	-24.724	0.041
USGS-40	8573	L-glutamic acid	-26.389	0.042
IAEA-600		caffeine	-27.771	0.043
NBS-22	8539	oil	-30.031	0.043
IAEA-CH-7	8540	polyethylene	-32.151	0.050
LSVEC*	8545	lithium carbonate	-46.6	0.2
* It is recommended that $\delta^{13}C$ values of both organic and inorganic materials are expressed relative to VPDB on a scale normalised by assigning a value of -46.6 ‰ to LSVEC lithium carbonate [11].				

Table 7. Secondary reference materials for $\delta^{15}N$ measurements.
The isotopic compositions are those reported by IAEA (compiled Sep 2011)

Description	NIST RM	Nature	$\delta^{15}N$ ‰	SD
USGS-32	8558	potassium nitrate	+180	1
USGS-26	8551	ammonium sulphate	+53.7	0.4
USGS-41	8574	L-glutamic acid	+47.6	0.2
IAEA-N-2	8548	ammonium sulphate	+20.3	0.2
IAEA-NO-3	8549	potassium nitrate	+4.7	0.2
USGS-35	8569	sodium nitrate	+2.7	0.2
IAEA-600		caffeine	+1.0	0.2
IAEA-N-1	8547	ammonium sulphate	+0.4	0.2
USGS-34	8568	potassium nitrate	-1.8	0.2
USGS-40	8573	L-glutamic acid	-4.5	0.1
USGS-25	8550	ammonium sulphate	-30.4	0.4

Table 8. Secondary reference materials for δ^2H and δ^{18}O measurements.
The isotopic compositions are those reported by IAEA (compiled Sep 2011)

Description	NIST RM	Nature	δ^2H ‰ (±SD)	δ^{18}O ‰ (±SD)
IAEA-602		benzoic acid		+71.4 ± 0.5
IAEA-601		benzoic acid		+23.3 ± 0.3
USGS-43		Indian hair	-50.3 ± 2.8*	+14.11 ± 0.1*
USGS-42		Tibetan hair	-78.5 ± 2.3*	+8.56 ± 0.1*
NBS-18	8543	calcite		-23.2 ± 0.1**
GISP		water	-189.5 ± 1.2	-24.76 ± 0.09
LSVEC	8545	lithium carbonate		-26.7 ± 0.2**
IAEA-CH-7	8540	polyethylene	-100.3 ± 2.0	
NBS-22	8539	oil	-120 ± 1	
SLAP2***		water	-427.5 ± 0.3	-55.50 ± 0.02
*Preliminary isotopic compositions of the non-exchangeable fractions [14]. **These values are reported against the VPDB scale for δ^{18}O. *** SLAP2 is depleted in heavy isotopes with respect to VSMOW2 and provides an anchor for the lower end of the VPDB scale.				

Other materials are available from a number of commercial organisations and universities. The δ-values of these materials have been assigned by internal calibration or are consensus values, obtained through inter-laboratory exercises. In general, these materials do not carry the international agreement ascribed to the materials distributed by IAEA but may prove useful where no other reference material exists.

When reporting isotopic compositions it is essential that the values assigned to primary and secondary materials are given alongside sample results.

4.1.3 Inter-laboratory comparison materials

Inter-laboratory comparison materials cover a broad spectrum of chemical compositions and a wide range of isotopic ratios. The δ-values of these materials, circulated in inter-laboratory comparison exercises (see section 7.2), are assigned as the consensus mean of results from participating laboratories, following appropriate statistical treatments. Subsequent to an exercise, materials may be made available. The FIRMS network organises inter-comparisons on an annual basis. Information about these exercises is available from the website:

http://forensic-isotopes.org/

4.1.4 In-house standards

An isotope laboratory must hold suitable materials for calibration and normalisation purposes so that isotopic ratios can be reported on an agreed international scale. These materials are not recommended for daily use as they are in short supply and commercial availability is restricted. Primary calibration materials or secondary reference materials must be used to verify in-house standards for everyday use in normalisation and quality assurance (QA). Control charts should be used to monitor laboratory performance and the status of in-house standards (see section 7.1). Any contamination of the standard will show as a step in the control chart whereas a slow change (e.g. evaporation) will show as drift. Control charts will also assist in determining whether a proposed in-house standard is likely to be suitable for long-term use.

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

- isotopic homogeneity (to the smallest amount to be analysed)
- consistent isotopic composition over time.

The isotopic composition of in-house standards should be within the range to be measured, since δ-values are measured more precisely when the differences between the sample and standard are small. In-house standards should also be chemically similar to the samples to ensure that errors during preparation will tend to cancel out. Other considerations for the choice of in-house standards may include:

- easy to prepare, store and handle
- a single chemical compound (preferably)
- easy to replace (when exhausted, contaminated etc)
- non-hygroscopic (especially important when measuring hydrogen and oxygen isotopes)
- comprising only non-exchangeable hydrogen (unless the non-exchangeable δ^2H value is known).

5 Making measurements

5.1 Carbon and nitrogen measurements

In general, better precision is obtained when measuring the isotopic composition of a single element although this may require modifications to the instrument set up (see section 2.2.1.1). However, due to constraints on the amount of sample and/or time available for the analysis it may be advantageous to measure two elements simultaneously in the same sample portion.

In order to measure both δ^{13}C and δ^{15}N (or δ^2H and δ^{18}O) for the same portion of a sample (as shown in Figure 1) the IRMS must "jump" between two different 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 (the magnetic field can be slow to change in the timescale required).

A jump calibration must be performed if dual measurement is planned. This process is generally automated within the IRMS software which will determine the change in high voltage or magnetic field required.

5.2 Preconditioning

Depending on the type of EA employed, it may be necessary to carry out a "pre-conditioning" of the reactor system before analysing test samples. This can be done by performing a series of EA and IRMS runs with neither samples nor empty capsules in the autosampler. If the analyser is operating correctly, both N_2 and CO_2 signals should be negligible.

5.3 Blank determinations

The gases evolved from the tin or silver capsules, which enclose samples, will combine with those from the samples and contribute to the measured δ-value. A signal from a blank analysis may also result from atmospheric gases introduced by the autosampler. 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 (4).

Depending on the type of autosampler system used, it may be necessary to carry out a blank determination prior to the analysis of samples (see Table 3 for information on when a blank may

be required). Empty sample capsules (folded as if loaded with sample) are introduced via the autosampler, using the same EA-parameters as for the samples. This can be implemented as a specific sequence, which is run every day.

$$\delta_{blk\ corr} = \frac{\delta_{meas} * Area_{meas} - \delta_{blk} * Area_{blk}}{Area_{meas} - Area_{blk}} \qquad (4)$$

$\delta_{blk\ corr}$	blank corrected δ-value of the sample
δ_{meas}	determined (raw) δ-value of the sample
δ_{blk}	δ-value of the blank
$Area_{meas}$	area of the sample peak
$Area_{blk}$	area of the blank peak

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

Usually this correction is very small and may vary unpredictably. For this reason, laboratories often define a minimum acceptable sample peak size based on the intensity of the average blank peak area, e.g. a 50 mV blank should have minimal effect on a 5000 mV sample peak.

5.4   Sample preparation

It is fundamentally important that samples, reference materials and QA materials are prepared and analysed in an identical manner, the Identical Treatment (IT) Principle [15].

Both EA-IRMS and TC/EA-IRMS are techniques for the determination of "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.

To determine the isotope ratios of single chemical species, a separation step is necessary before the combustion/conversion process, either by off-line purification processes, or by coupling techniques such as GC-C-IRMS. Special considerations are required as both approaches may introduce isotopic fractionation and the IT process is essential.

Residual moisture may affect both δ^2H and $\delta^{18}O$ values unless compounds are absolutely dry. Many compounds are hygroscopic and great care must be taken after desiccating (e.g. over phosphorus pentoxide) to ensure the samples remain dry prior to analysis.

Special consideration must be given to the preparation of samples for deuterium analysis. Hydrogen exchange in proteins and other compounds with –COOH, –NH$_2$, –OH or –SH functional groups may affect the δ^2H value of a compound (total vs. non-exchangeable δ^2H) [16, 17, 18].

5.5   Sample measurement

Typically, at least three analytical results should be acquired for each sample. For the analysis of hydrogen and oxygen more analyses may be required to obtain consistent data due to "memory effects" when the samples in a sequence have very different isotopic abundances. One reference material (RM) for each element of interest is analysed at the start of the sequence and again at the end. These values will be used to normalise the data obtained from the samples. An in-house quality control (QC) material is analysed periodically throughout the sequence for quality assurance. An important part of any validation process is to determine acceptable performance criteria for the QC materials. A typical sequence is shown in Table 9.

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

Table 9. 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) taken should be selected so that the resulting CO_2 and N_2 (or H_2 and CO) signal intensities (with or without dilution) are within the linear range of the IRMS. Ideally, the maximum intensity of the major ion from the sample peak should match the intensity of the major ion in the working gas. Very approximately, between 100 and 500 µg of the reference materials listed in Tables 6 – 8 will produce suitable signal intensities, assuming appropriate dilution.

To further maintain sample continuity the samples should be loaded into the autosampler 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.

Table 9. Template for sample continuity illustrating a typical measurement sequence

Weighed by: _____   Sample: _____   Isotope: _____   Typical weight: _____   Date weighed: _____   Date dessicated: _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLANK / mg	RM1 / mg	RM1 / mg	RM1 / mg	RM2 / mg	RM2 / mg	RM2 / mg	sample 1 / mg	sample 1 / mg	sample 1 / mg	sample 2 / mg	sample 2 / mg
B	sample 2 / mg	sample 3 / mg	sample 3 / mg	sample 3 / mg	sample 4 / mg	sample 4 / mg	sample 4 / mg	QC / mg	QC / mg	QC / mg	sample 5 / mg	sample 5 / mg
C	sample 5 / mg	sample 6 / mg	sample 6 / mg	sample 6 / mg	sample 7 / mg	sample 7 / mg	sample 7 / mg	sample 8 / mg	sample 8 / mg	sample 8 / mg	QC / mg	QC / mg
D	QC / mg	sample 9 / mg	sample 9 / mg	sample 9 / mg	sample 10 / mg	sample 10 / mg	sample 10 / mg	sample 11 / mg	sample 11 / mg	sample 11 / mg	sample 12 / mg	sample 12 / mg
E	sample 12 / mg	QC / mg	QC / mg	QC / mg	sample 13 / mg	sample 13 / mg	sample 13 / mg	sample 14 / mg	sample 14 / mg	sample 14 / mg	sample 15 / mg	sample 15 / mg
F	sample 15 / mg	sample 16 / mg	sample 16 / mg	sample 16 / mg	QC / mg	QC / mg	QC / mg	sample 17 / mg	sample 17 / mg	sample 17 / mg	sample 18 / mg	sample 18 / mg
G	sample 18 / mg	sample 19 / mg	sample 19 / mg	sample 19 / mg	sample 20 / mg	sample 20 / mg	sample 20 / mg	RM1 / mg	RM1 / mg	RM1 / mg	RM2 / mg	RM2 / mg
H	RM2 / mg	BLANK / mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg

6 Data handling

6.1 Initial data evaluation

Typically, the IRMS software automatically calculates isotope ratios relative to the working gas. Known as "single point anchoring" this requires the operator to specify the true δ-value of the working gas ($\delta_{true(wg)} = [(R_{wg}/R_{std}) - 1]*1000$). The raw δ-values of the working gas and a sample are measured and the "true" δ-value of the sample is reported as shown in equation (5):

$$\delta_{true(sample)} = \delta_{raw(sample)} + \delta_{true(wg)} + \left(\frac{\delta_{raw(sample)} * \delta_{true(wg)}}{1000} \right) \tag{5}$$

In order to minimise uncertainty the isotopic composition of the sample must be close to that of the working gas. The normalisation error becomes large as ($\delta_{true(sample)} - \delta_{true(wg)}$) increases. It is important that $\delta_{true(wg)}$ is measured frequently versus reference materials as the composition of working gas, delivered from a high pressure cylinder, may vary over time due to mass dependent processes.

Single point anchoring may also be performed relative to a reference material. The raw δ-values of both sample and reference material are measured relative to the working gas and these values used to compute the true δ-value of the sample as shown in equation (6).

$$\delta_{true(sample)} = \left[\frac{\left(\delta_{raw(sample)} + 1000 \right)\left(\delta_{true(std)} + 1000 \right)}{\left(\delta_{raw(std)} + 1000 \right)} \right] - 1000 \tag{6}$$

Like the single point anchoring to a working gas, this method will produce large normalisation errors if the isotopic composition of the sample is very different to that of the standard and if the IT principle is not strictly adhered to.

6.2 ^{17}O-correction

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

δ^{13}C values must be 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 molecular species (or isotopomer).

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 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] = $^{13}C/^{12}C$ + 2($^{17}O/^{16}O$)

[46]/[44] = 2($^{18}O/^{16}O$) + 2($^{17}O/^{16}O$ ^{17}. $^{13}C/^{12}C$) + ($^{17}O/^{16}O$)^2

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 three oxygen isotope

relationship, assuming that processes that affect the abundance of ^{18}O have a corresponding affect on ^{17}O.

The ^{17}O-correction algorithm introduced by Craig (1957) [19] is based on ^{17}O and ^{18}O abundances determined by Nier (1950) [20] and assumed a fractionation factor of λ = 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. Alison *et al* [21] recommended that the "Craig" or "IAEA recommended" algorithm be retained, with λ = 0.5 and defined values for all the ratios involved.

More recently Santrock, Studley and Hayes devised a ^{17}O-correction algorithm (the "Santrock" or "SSH" algorithm) (1985) [22] with a fractionation factor of λ = 0.516 and an iterative correction to solve the equations for ^{13}C. The SSH algorithm is often regarded as both mathematically exact and more realistic in its approach to natural variations of isotopic composition. Applying the Craig and SSH algorithms to the same raw data will produce $\delta^{13}C$ values with differences that exceed the precision of modern IRMS instruments. For an average tropospheric CO_2 the $\delta^{13}C$ bias between the IAEA and SSH algorithms has been determined as 0.06 ‰. IUPAC have also published a technical report on ^{17}O-corrections [23].

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

Isotope ratio measurements based on CO molecules are commonly corrected in an analogous manner although the ^{17}O correction is typically 0.01 ‰ and contributes little to the uncertainty of measurement [24].

6.3 Normalisation

The term "normalisation" describes a number of algorithms which convert raw (measured) δ-values of a sample to the "true" δ-values reported versus an international scale. Some aspects of this process are automated within the IRMS software and may be invisible to the analyst. Additional normalisation may be performed in external spreadsheets.

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, the IT principle must be applied to the preparation and analysis of the sample and reference material.

The uncertainty of normalisation can be improved by applying a normalisation factor (n) calculated from the measured δ-value of two reference materials, 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, expressed in per mil, is calculated by a modified equation (7):

$$\delta_{true(sample)} = \left[\frac{(n * \delta_{raw(sample)} + 1000)(\delta_{true(std)} + 1000)}{(n * \delta_{raw(std)} + 1000)} \right] - 1000 \qquad (7)$$

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".

The average difference between the true and measured δ-values of each standard is calculated and added to the measured δ-value of the sample to give the true δ-value of the sample. This method has significant advantage over single point anchoring as the calculation is independent of

the isotopic composition of the working gas. For best accuracy the isotopic composition of the reference standards should bracket that of the sample.

The δ_{true} value for a sample can also be calculated using an expression of the form shown in equation (8).

$$\delta_{true} = m*\delta_{raw} + b \tag{8}$$

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".

Example 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* should be calculated for either reference material bracketing the samples to verify its numerical value is the same either way.

The "shift" or "off-set" *b* for VSMOW2 = $\delta_{true} - (\delta_{raw}*stretch)$ = 0.0 – (0.3*1.01544) = -0.3046 ‰

or, using SLAP2:

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

Adjusted δ^2H values would be calculated as:

δ^2H_{true} = 1.01544*δ^2H_{raw} – 0.3046

This method has been used for three decades to convert measured δ^2H and $\delta^{18}O$ values versus the VSMOW/SLAP scale (Sharp, 2006 [10]) and is now recommended for the normalisation of $\delta^{13}C$ measurements of both organic and inorganic materials to the VPDB/LSVEC scale [11]. This approach is reported to reduce the standard deviation in results between laboratories by 39-47%.

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 reference materials (e.g. incomplete combustion) can be reduced.

Additional discussion of normalisation procedures can be found in Paul et al, 2007 [25].

6.4   Uncertainty

The modern IRMS is capable of measuring natural isotopic ratio variations with an uncertainty better than 0.02 ‰. For hydrogen, the uncertainty is usually one order of magnitude greater because the natural $^2H/^1H$ isotope ratio is several orders of magnitude smaller than for other elements. Larger errors are typically introduced by sample treatment prior to IRMS analysis.

The combined uncertainty of reported δ-values will contain contributions from:

(1) the precision of replicate measurements

(2) the bias of experimental processes

(3) the uncertainty of δ-values in reference materials used to fix and normalise the δ-scale

(4) the algorithms applied to correct and normalise the data.

Some contributions to (1) and (2) are discussed below. Both can be minimised through proper choice of analytical conditions and by applying the IT principle (see section 5.4).

Inter-laboratory comparison exercises frequently highlight reproducibility between laboratories up to 10 times the average, individual laboratory uncertainty of measurement. As with in-house measurements, between-laboratory uncertainty increases as the difference between the δ-value of the sample and reference material increases. This suggests that (3) and (4) have significant influence.

The laboratory can do nothing to address the uncertainty associated with the calibration materials and inter-laboratory comparison materials (which is limited by the homogeneity of the materials). These uncertainties have become increasingly small over time, but must still be incorporated into an uncertainty budget and into the uncertainties associated with the values of in-house standards which have been calibrated against these standards.

For true comparison of data between laboratories there must be standardisation of:

(5) the δ-scales on which results are to be reported

(6) the δ-values (and uncertainties) of reference materials for each scale

(7) the ^{17}O-correction algorithm to be applied to δ^{13}C data

(8) the normalisation algorithm to be applied.

If (5)-(8) can be agreed it is possible that the reproducibility of δ-value measurements between laboratories can approach that attainable within a single, competent laboratory.

6.4.1 Example uncertainty calculation

The combined uncertainty can be summarised by Figure 5. The blue (vertical) error bars represent the uncertainty in the δ-values of materials used for calibration/normalisation. The red (horizontal) error bars represent the uncertainty in the raw measured δ-values for each material. Green error bars represent the uncertainty in the measured δ-value for the sample.
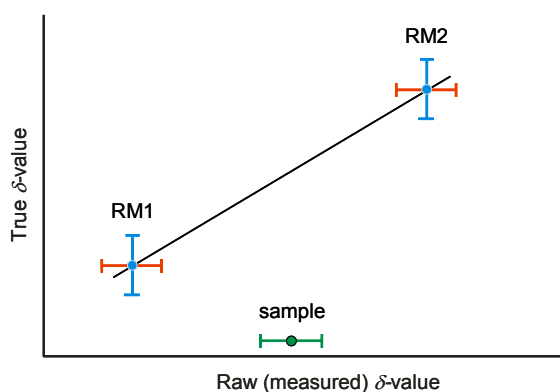

Figure 5. Illustration of uncertainty components – the uncertainty in the values of reference materials and the raw measured δ-values for the reference materials and the sample will contribute to the uncertainty in δ_{true} for the sample

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 [26]. The basic rule 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 (9) to give the uncertainty in the result y:

$$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + \ldots u(x_n)^2} \tag{9}$$

However, this requires the uncertainty components to be 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.

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 precision of the measurements of the reference materials and sample, and the uncertainty in the reference value, as illustrated in Figure 5. Calculation of the uncertainty in $\delta_{true(sample)}$ is complicated by the fact that the "stretch" and "shift" factors are correlated. Fortunately their correlation term can be avoided by calculating the uncertainty directly from the input values as described below.

If normalisation is carried out using the measured and true values for two reference materials, RM1 and RM2, the equation for calculating $\delta_{true(sample)}$ can be written as:

$$\delta_{true(sample)} = \delta_{true(RM1)} + \left[\left(\delta_{raw(sample)} - \delta_{raw(RM1)} \right) * \left(\frac{\delta_{true(RM1)} - \delta_{true(RM2)}}{\delta_{raw(RM1)} - \delta_{raw(RM2)}} \right) \right] \tag{10}$$

(a similar equation can be written using values for RM2; the result will be the same).

Since the terms $\delta_{true(RM1)}$ and $\delta_{raw(RM1)}$ appear twice in equation (10) it is not possible to use the simple rules for combining uncertainties. The most straightforward approach is to use a spreadsheet-based calculation such as that described by Kragten [27].The spreadsheet set-up is shown in Figure 6, using the calculation of the uncertainty in $\delta^2H_{true(sample)}$ as an example. Normalisation has been carried out through the analysis of VSMOW2 and SLAP2. The standard uncertainty in the true δ^2H value for the standards is given by IAEA as 0.3 ‰ in each case [9]. The standard uncertainty in $\delta_{raw(VSMOW2)}$ and $\delta_{raw(SLAP2)}$ due to random errors was 1.2 ‰ (the standard deviation of the mean of replicate measurements). The raw δ^2H value for the sample was -189.0 ‰ with a standard uncertainty of 1.5 ‰ (the standard deviation of the mean of replicate measurements).

Figure 6. Calculation of $\delta_{true(sample)}$ using a Kragten spreadsheet.

	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. (10) applied to values above	$u(\delta_{true(sample)})$ = square root of sum of squared differences	Eqn. (10) applied to values above	Eqn. (10) applied to values above	Eqn. (10) applied to values above	Eqn. (10) applied to values above	Eqn. (10) applied to values above
9			Difference	D8-B8	E8-B8	F8-B8	G8-B8	H8-B8

a) Spreadsheet set-up

	A	B	C	D	E	F	G	H
1								
2	**Parameter**	**value** $(\delta^2 H, ‰)$	**uncertainty** $(\delta^2 H, ‰)$					
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**	-192.06	-192.09	-192.89	-192.77	-190.70
9			Difference	0.1651	0.1349	-0.6687	-0.5495	1.5232

b) Calculation of uncertainty using example values

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 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.

A number of examples of the implementation of the Kragten spreadsheet are given in the Eurachem/CITAC guide [28].

6.4.2 Other sources of uncertainty

It is important that reference materials 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 which scavenge water, oxygen or CO_2.

The combustion/oxidation and reduction reactors can become contaminated with previous samples, giving rise to memory effects which can have a half life of several minutes. For this reason the first result from the analysis of a sample is often discarded as it may have a contribution from the previous sample.

During an analytical sequence a drift of results 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 as a build up of contaminants (e.g. water). Traces of water, or other protonating species, can give rise to isobaric interference of CO_2H^+ (m/z 45), H_3^+ (m/z 3) or N_2H^+ (m/z 29). It is important that the analytical sequence is designed such that drifts can be detected. Generally, a sufficient number of QA materials, throughout the sequence, can be used to identify and/or correct for drift.

7   Quality assurance

7.1   Control charts

To monitor the day-to-day performance of the IRMS system for the measurement of $\delta^{13}C$ and $\delta^{15}N$ the determined raw δ-values for the in-house standard are listed using a target value or mean value control chart with defined limits.

Mean value control charts usually have a warning limit (mean ±2 σ) and an action limit (mean ±3 σ), which are determined using results from a so called prior-period with approximately 20 results determined on at least 6 different days. This should form part of the validation process. Figure 8 shows a flow chart for the interpretation of in-house quality control charts based on the "Westgard rules", adapted from the IUPAC Harmonized guidelines for internal quality control in analytical chemistry laboratories [29].

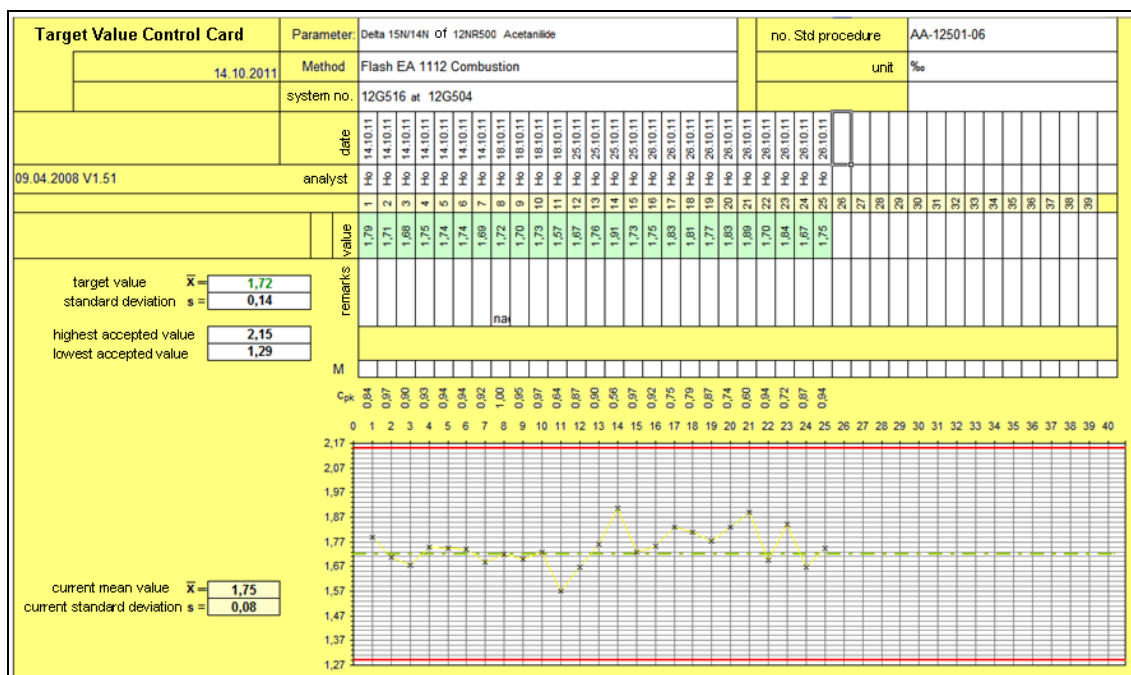Figure 7 shows an Excel®-based example for $\delta^{15}N$ using acetanilide as the in-house standard.

Figure 7. Example of a quality control chart for the determination of $\delta^{15}N$ using an in-house standard

A similar process is applied for the determination of oxygen and hydrogen but the normalisation (stretch and shift-correction) values should be recorded together with the normalised values obtained for in-house standards.
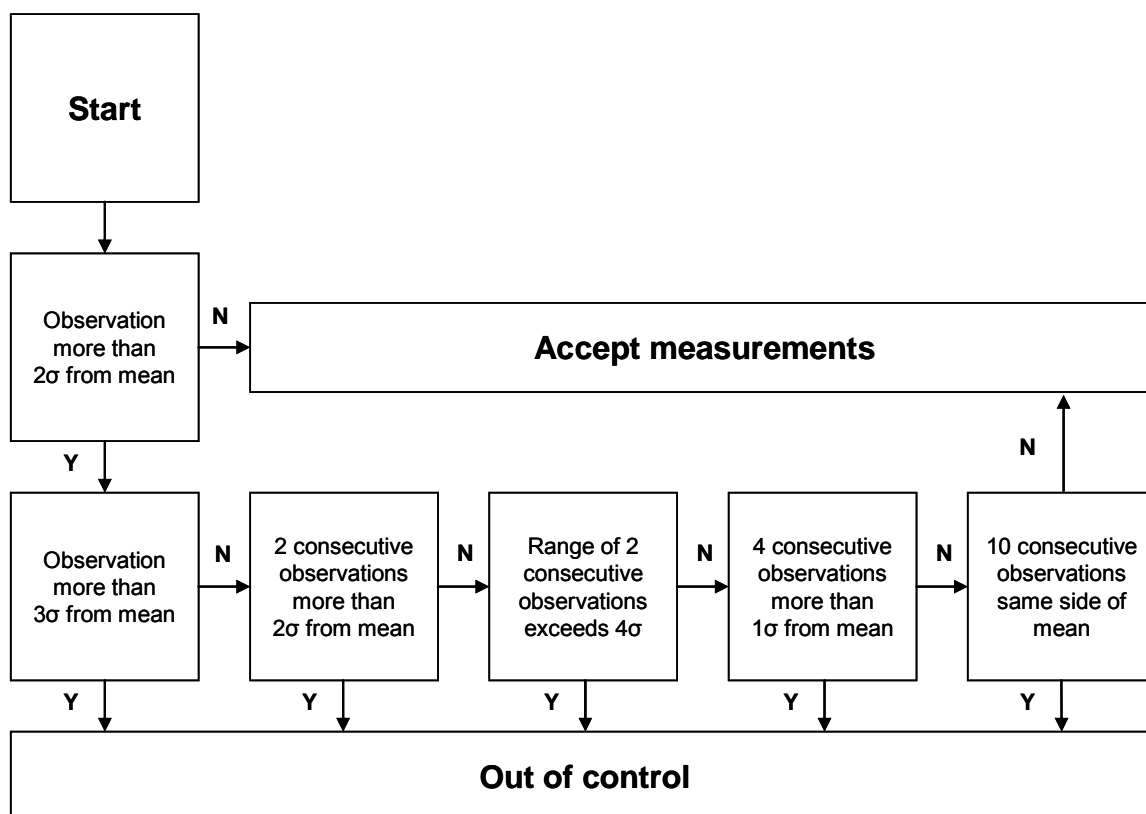
Figure 8. Schematic representation of the Westgard rules (reproduced from Pure Appl. Chem., 1995, 67, 649-666)

7.2   Inter-laboratory exercises

Inter-laboratory comparison exercises or proficiency testing schemes provide a means 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 [30].

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 inter-comparisons on an annual basis. Information about these exercises is available from the website http://www.forensic-isotopes.org.

7.3   Validation parameters

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 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.

Parameters which should be assessed include:

- Linearity – this should be determined using real samples in addition to the working gas. Note that in the context of IRMS, linearity refers to the ability of the measurement procedure to produce δ-values that are independent of the amount of material analysed;

- Stability – the standard deviation determined over 10 pulses of working gas should be determined over a significant period of time;

- Repeatability (one day, one system, one analyst, several measurements);

- Within-laboratory reproducibility (several days, different persons, one system, several measurements);

- Reproducibility (several days, different persons, several systems/laboratories) – target values should be a standard deviation equal to or less than 3.0 ‰ for δ^2H, 0.5 ‰ for δ^{18}O, and equal to or less than 0.3 ‰ for δ^{13}C and δ^{15}N;

- Selectivity – for example, can the same δ^{15}N be obtained from a drug in its free base form and as a HCl salt?

- Robustness – the effects of varying instrument parameters (temperatures, flow rates etc).

The validation document must include an estimate of the combined measurement uncertainty in results, incorporating all of the parameters addressed in the validation process. The final statement in a validation document should be a statement that the method is fit for the required purpose.

7.4   Interpretation of IRMS data in forensic science

The above activities are important in ensuring the validity of IRMS data however, to interpret IRMS data more information is generally required. An example, below, describes how IRMS is applied in a forensic science context.

Many forensic science investigations using IRMS will focus on determining if there is a link 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 realised that in general not only IRMS but a whole suite of methods should be used in such a comparative investigation, starting with a visual comparison (colour, dimensions, morphology, texture, …), often using FT-IR as well as other analytical techniques depending on whether the material is organic ((Py-)GC-MS, LC-MS) or inorganic (XRF, XRD, SEM-EDX, LA-ICPMS) in character.

First, the within sample variation in IRMS characteristics will need to be determined. This will depend on the method parameters (e.g. repeatability) and will also include sample heterogeneity. To determine the strength of potential evidence and to interpret the meaning of such a link, it is necessary to know the variation in IRMS characteristics for other similar but unrelated materials. Normally other materials of the same class will also be considered (e.g. grey duct tape). 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). For some forensically investigated materials, for example natural materials such as wood or minerals such as alumn, 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). The acquisition of appropriate data base information is of significant importance for correct evaluation of case related data. Deficiencies in background variation data must be articulated within the resultant reports and that information provided by the expert to the end user of the report.

In forensic science the conclusion may be 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"). More often, a likelihood ratio will be reported given a specific scenario (such as the scenario of the prosecution) as well as an alternative scenario (such as the scenario of the defence). (See the Bayesian approach, reference 31.)

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. In most candles the

composition of the core differs from the outer material, which has a higher melting point to promote good burning characteristics. This feature provides two points of comparison.

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. 128 packages of candles were bought in shops throughout the Netherlands and analysed. Figure 9 shows results for the isotopic analysis of one candle from each box, although for a number of boxes multiple candles were tested.

A number of candle materials recovered from crime scenes and searches were compared to each other and to the background samples. (The inner and outer components of the candles are coded i and o respectively).

- Candles from two crime scenes were found to be indistinguishable (2.001).

- One candle from the final crime scene was indistinguishable from a candle found in the suspect's pocket and a candle found during a house search (2.007). These three candles were found to be different from all other investigated background candle samples.

- A candle from a crime scene was indistinguishable from those found during a subsequent search of the suspect's house (2.003). These candles were of a "rustic" style and were comprised of a single material.

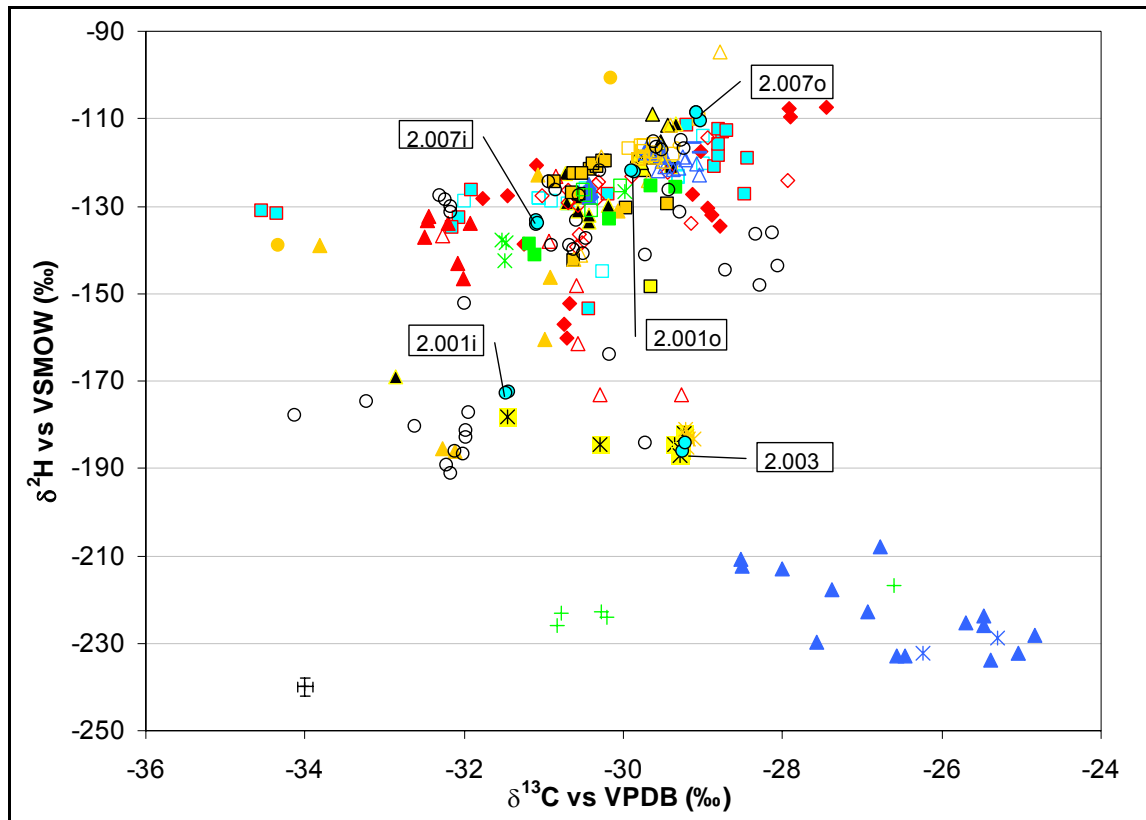This work was presented as a poster at the FIRMS2010 conference. Abstracts are available from http://www.forensic-isotopes.org.

Figure 9. Example of IRMS-results (δ^2H versus $\delta^{13}C$) for candle wax samples. *The symbols o and i refer to, respectively, the outside layer and the core of a casework candle. In this figure are also plotted results for background candle variation within the Netherlands where results denoted by one symbol refer to results for either the outside layer or core of a single candle type product from one producer (but different boxes). (Reproduced with permission from Netherlands Forensic Institute)*

8 Troubleshooting

Routine maintenance – To ensure the continued running of analytical instruments and minimise downtime, routine maintenance should be scheduled and undertaken. The frequency of routine maintenance tasks should be given in the instrument operating procedures and recorded in an instrument log book when performed. Typical routine maintenance activities include:

- Cleaning autosampler trays
- Removal of ash
- Replacement of reactors
- Replacement of H_2O traps
- Baking out GC columns
- Maintenance of turbo pumps
- Cleaning ion source.

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

8.1 Visual Inspection

A visual inspection of the equipment may reveal problems.

Indicator lights – Many analytical instruments incorporate indicator panels with lights which indicate correct or incorrect operation, e.g. loss of vacuum. Check indicator panels for system problems.

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

Settings – Each peripheral device may require specific setting for a given analysis, e.g. furnace temperatures and gas pressures. 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.

8.1.1 Elemental analyser

Symptoms	Possible causes	Resolution
High nitrogen blank	Oxygen may be contaminated with nitrogen.	Ensure suitable grade oxygen is attached for combustion.
	Auto-sampler may be leaking.	Test for leaks in the system (gas supply and elemental analyser).
Nitrogen intensity increase in subsequent samples	Reduction reactor is exhausted and NO_x is eluting from GC column. May appear as shoulder on the nitrogen peak.	Replace reduction reactor.
Poor nitrogen and/or carbon isotope ratio measurements	Reduction reactor is exhausted.	Monitor *m/z* 30 to determine if reduction reactor needs replacing.
Long tail on carbon dioxide peak	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).
	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.
Baseline drift after CO_2 peak (broad shallow peak)	Water bleeding through the GC column and detector.	Exhausted H_2O trap, replace packing with fresh material. (Monitor H_2O levels over time to prevent this.)
Peak broadening, peak separation is poor, peak tailing	Slow/restricted carrier gas flow.	Ensure sufficient carrier gas supplied. 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.	When folding sample capsules ensure they are spherical in shape. If capsules are flat they may slip under the auto-sampler tray, or be caught on the edge of the auto-sampler tray, dropping at the same time as the following sample.
	Auto-sampler not connected to PC.	Check connections (PC communication, and gas lines) are correctly configured.
	EA not connected to MS.	
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	Gas leak.	Test for leaks.
	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 failed.	Check indicator lights to see if heater has failed. Replace heater.
Rapid consumption of reduction tube chemicals	Oxygen leaking into system.	Ensure oxygen circuit is leak free. Test alternative oxygen loops if available.

8.1.2 Mass spectrometer

Symptoms	Possible causes	Resolution
Indicator light suggests acceleration voltage is OK, but there is no emission	Ion source filament failed.	Remove ion source and replace filament.
Box and trap values fluctuating	Filament has weakened and is flexing. Likely that filament will break in short time.	Remove and replace filament.
Stability checks fail acceptance criteria	Possible leak.	Check gas lines for leaks.
	Poor purity carrier gas.	Ensure suitable gas supply. Check age of gas purifying cartridge if fitted – replace if necessary.
	Gas cylinder may be reducing in pressure if near empty.	Check sufficient gas supply (cylinders are not empty and regulators are correctly set).
	Interference from contaminants leaching off GC.	Bake out column.
	Fault with electronics.	Liaise with engineer.
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.
Poor linearity over range of working gas intensities	Filament may be flexing.	Check box and trap values are stable (see above if not).
	Poor ion source tuning parameters.	Check ion source tuning.
	Gas leak.	Test system for gas leaks.
	Electronic fault.	Liaise with engineer.
Tailing on CO_2 working gas peak	Contaminated ion source.	Clean ion source.
Poor vacuum	Vacuum pump failure.	Check pumps are functioning. Observe indicator lights for pump status. (Regularly check pump oil levels and ballast pumps to avoid faults.) Ensure fixings around ion source and pressure gauge is secure.
	Poor seal around ion source/pressure gauge.	Replace aluminium 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.)

9 Glossary of terms and abbreviations

A description of some of the terms which may be encountered in relation to IRMS. Guidelines on the terminology relating to the expression of stable isotope ratio measurements have been published by Coplen [32].

Term	Description
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 be it comprised of one compound or a mixture of compounds.
Calibration material	Materials that define the δ-scales versus which natural variations in isotopic compositions are expressed. [Also known as primary measurement standards.]
CF	Continuous flow: automated sample preparation device and mass spectrometer in which sample analysis is conducted in a continuous stream of helium carrier gas.
CSIA	Compound specific isotope analysis: isotopic characterisation of individual compounds.
Delta (δ)	Delta notation: a measure of isotopic ratios relative to international standards which 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 working gas of known isotopic composition into an IRMS by means of a system of valves.
EA	Elemental analyser: an automated sample preparation device in which samples are automatically converted into pure gases for isotope ratio analysis.
EA-IRMS	Elemental analyser-isotope ratio mass spectrometry: technique used for the measurement of carbon and nitrogen isotope ratios which employs combustion of materials in an oxygen atmosphere followed by GC 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.

Term	Description
FC	Faraday cup: conducting cup or chamber that collects charged particles. The accumulated charge is subsequently measured.
FIRMS	Forensic Isotope Ratio Mass Spectrometry Network
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^{13}C and δ^{15}N) 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.]
GISP	Greenland Ice Sheet Precipitation: reference material for the measurement of δ^2H 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 (*see TC*).
HTCR	High temperature carbon reduction (*see TC*).
HTP	High-temperature pyrolysis (*see TC*).
IAEA	International Atomic Energy Agency (www.iaea.org).
In-house standard	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.*
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
LC-IRMS	Liquid chromatography-isotope ratio mass spectrometry: technique used for CSIA where compounds are separated using HPLC prior to IRMS.
Linearity	Indicates that (within an acceptable range) the isotope ratio is independent of the amount of material analysed. (Note that this differs from the general use of the term in analytical chemistry which refers to the ability to obtain a method response which is directly proportional to the value of the quantity being measured.)
Measurement uncertainty	Parameter associated with a measurement result which 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).

Term	Description
NIST	National Institute of Standards and Technology (www.nist.gov) (formerly National Bureau of Standards, NBS).
PDB	Peedee 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).
Primary measurement standard	*See Calibration material.*
PSIA	Position specific isotope analysis: technique for the determination of δ^2H and δ^{13}C values for specific intramolecular sites.
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).
Reference gas	*See Working gas.*
RM	Reference material: a material that is sufficiently homogeneous and stable with reference 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).
SIA	Stable isotope analysis.
SLAP	Standard Light Antarctic Precipitation: reference material for the measurement of δ^2H and δ^{18}O values (now replaced by SLAP2).
SMOW	Standard mean ocean water (*see VSMOW*).
TC	Thermal conversion: high temperature conversion (>1350 ^{o}C) of materials containing oxygen and hydrogen to produce CO and H_2. Sometimes referred to as high temperature conversion (HTC), high-temperature pyrolysis (HTP) or high temperature carbon reduction (HTCR).
TC/EA-IRMS	Thermal conversion elemental analyser-isotope ratio mass spectrometry: technique used for the measurement of oxygen and hydrogen isotope ratios which employs high temperature thermal conversion of materials followed by GC separation of the resulting gases.
USGS	United States Geological Survey (www.usgs.gov).
VPDB	Vienna Peedee Belemnite: Internationally agreed zero point for the measurement of δ^{13}C values.
VSMOW	Vienna Standard Mean Ocean Water: internationally agreed zero point for the measurement of δ^2H and δ^{18}O values.

Term	Description
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.
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 standard.*

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