FIRMS

Forensic Isotope Ratio Mass Spectrometry Network

NML

UK NATIONAL MEASUREMENT LABORATORY FOR CHEMICAL AND BIOLOGICAL MEASUREMENT HOSTED AT LGC

Good Practice Guide

Isotope Ratio Mass Spectrometry

Third Edition 2025

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Good Practice Guide for Isotope Ratio Mass Spectrometry

Third Edition 2025

Editors

Dr Philip Dunn, UK

Dr Christopher Brodie, New Zealand

Mr Ethan Strak, UK

Dr Jim Carter, Australia

Contributors

Dr Christopher Brodie, New Zealand

Dr Jim Carter, Australia

Dr Philip Dunn, UK

Mr Joe Meikle, Australia

Dr Helen Salouros, Australia

Mr Ethan Strak, UK

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Dr Helen Kemp, UK, Dr Claire Lock, UK, Prof Wolfram Meier-Augenstein, UK, Dr Sabine Schneiders, Germany, Dr Gerard van der Peijl, Netherlands, Ms Lesley Chesson, US, Prof Max Coleman, USA, Mr Sean Doyle, New Zealand, Mr John Howa, USA, Dr Libby Stern, USA and Dr Federica Camin, Italy all contributed to previous editions.

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Preface to the third edition

There have been another seven years of improvements to instrumentation and analytical methods since the previous edition of the FIRMS Good Practice Guide for IRMS was published. As a result, the FIRMS network has prepared this new edition.

The major changes include a whole new chapter on sampling covering method of sampling a population, sampling for homogeneity testing of a material as well as considerations for measurement uncertainty arising from sampling. The measurement uncertainty section has been revised and expanded to touch on alternative methods to combine uncertainty components together. Methods for measurement of water isotopic composition using equilibration have also been added.

Throughout the guide, additional information has been included to expand important topics such as the difference between a reference gas and a working gas, additional tests that might be performed to check instrumental performance and initial data processing such as peak identification amongst others.

I thank my co-editors, Chris Brodie, Ehtan Strak and Jim Carter for their help as well as other members of the FIRMS network who have contributed content or suggested improvements. I hope that this guidance remains a valuable source of information for new and experienced isotope analysts alike.

Dr Phil Dunn

Chair (2025-) and Director

The FIRMS Network

Disclaimer

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

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

Feedback

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

GPG@forensic-isotopes.org.

Thank you.

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1 Terms & definitions

Term	Abbreviation	Description
absolute isotope ratio		Isotope ratio expressed as a simple ratio, e.g. $n(^{13}C)/n(^{12}C)$ rather than as a ratio relative to a standard (i.e. different to an isotope-delta value).
accuracy		Closeness of agreement between a measurement result and the true value of the property being measured.
active pharmaceutical ingredient	API	The substance within a medication that produces a pharmacological effect.
analysis of variance	ANOVA	A statistical technique for comparing groups of data by analysing variance within each group.
International Bureau of Weights and Measures	BIPM	See www.bipm.org
bulk stable isotope analysis	BSIA	The analysis of bulk material comprised of one compound or a mixture of compounds.
Canyon Diablo troilite	CDT	Canyon Diablo troilite (see *VCDT*)
cavity ring down spectroscopy	CRDS	A laser-based technique used to conduct isotope ratio measurements that is currently outside the scope of this guidance.
Cochran test		A statistical test to evaluate consistency between groups in a binary dataset. Often employed to detect outliers. May also be referred to as Cochran's statistic or Cochran's Q statistic.
combined standard uncertainty		A measure of measurement uncertainty obtained from combining all sources of uncertainty together expressed in the form of a standard deviation (σ), often denoted as "u_c."
Commission on Isotopic Abundances and Atomic Weights	CIAAW	Scientific body created to introduce uniformity in the atomic-weight values used worldwide; part of the Inorganic Chemistry Division of IUPAC. (http://www.ciaaw.org/).
compound-specific isotope analysis	CSIA	Isotopic characterisation of individual compounds.
continuous flow	CF	Automated sample preparation device and mass spectrometer in which sample analysis is conducted in a continuous stream of helium carrier gas.
definition (of an isotope-ratio delta scale)		The position of the zero-point (and any other fixed point) on an isotope-delta scale which relates absolute isotope ratios with relative measurements on that particular isotope-delta scale (such as the VPDB scale for $\delta^{13}C_{VPDB}$). This is generally done by selecting a specific reference material, such as VPDB, to serve as the reference material, although it can also be done strictly mathematically where internationally agree reference materials do not exist.

Term	Abbreviation	Description
delta (δ)		Delta notation: a measure of isotopic ratios relative to international reference materials that define the measurement scale for particular isotopes. Most commonly expressed in parts per thousand (‰).
dissolved organic carbon	DOC	The amount of organic carbon that is in solution as opposed to being suspended in water
dual inlet/isotope ratio mass spectrometry	DI/IRMS	Measurement of isotope ratios from pure gases by alternately introducing sample gas and a reference gas (of known isotopic composition) into an IRMS instrument by means of a system of valves.
electron ionisation	EI	Ionisation by electrons that are typically accelerated to energies of up to 150 eV in order to remove one or more electrons of an atom or molecule.
elemental analyser	EA	A sample preparation device in which samples are automatically converted into gases for isotope ratio analysis.
elemental analyser/isotope ratio mass spectrometry	EA/IRMS	A technique used for the measurement of nitrogen, carbon and sulphur isotope ratios that employs combustion of materials in an oxygen atmosphere followed by separation of gases evolved.
exhibit		In the context of forensics, this is any sample, artefact, or other material obtained as part of an investigation.
expanded uncertainty		The product of measurement uncertainty and a coverage factor, *k* (also referred to as a "*k-factor*"), to increase the level of confidence by expanding the range of values within which the true result lies, often denoted as *U*.
extrinsic hydrogen		Hydrogen present in a material due to interactions with external water sources. Sometimes “exchangeable hydrogen”.
Faraday collector	FC	Conducting cup or chamber that collects charged particles. The accumulated charge is subsequently measured.
flame ionisation detector	FID	An instrument which measures the concentration of analytes in a gas stream via the current produced from ions generated from the sample as it is exposed to a flame. It is often used as a detector in gas chromatography instruments.
flow injection analysis-chemical oxidation-isotope ratio mass spectrometry	FIA/CO/IRMS	A technique used to determine the bulk carbon isotopic composition using an LC/CO/IRMS instrument but bypassing the high performance liquid chromatography column.
Forensic Isotope Ratio Mass Spectrometry Network	FIRMS	See www.forensic-isotopes.org
full width at half maximum	FHMW	The width of a peak in a mass spectrum at half of its total height/intensity.
gas chromatography	GC	A separation technique in which the mobile phase is a gas.

Term	Abbreviation	Description
gas chromatography -combustion-isotope ratio mass spectrometry	GC/C/IRMS	A technique used for compound specific isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) where individual compounds are separated using gas chromatography and then combusted in an on-line reactor. An alternative acronym is *irmGC/MS* for "*isotope ratio monitoring GC/MS*."
gas chromatography -high temperature combustion-isotope ratio mass spectrometry	GC/HTC/IRMS	A technique used for compound specific isotope analysis ($\delta^{2}H$ or $\delta^{18}O$) where individual compounds are separated using gas chromatography and then converted to H_2 or CO in an on-line reactor.
Greenland Ice Sheet Precipitation	GISP	A reference material for the measurement of $\delta^{2}H$ and $\delta^{18}O$ values.
guide to the expression of uncertainty in measurement	GUM	A document that establishes general rules for evaluating and expressing uncertainty in measurement. Available freely from the International Bureau of Weights and Measures.
high performance (pressure) liquid chromatography	HPLC	A separation technique, operating with relatively high inlet pressure, in which the mobile phase is a liquid.
high temperature conversion	HTC	High temperature conversion (>1350 °C) of materials containing hydrogen and oxygen to produce H_2 and CO. Sometimes referred to as high temperature conversion (HTC), high-temperature pyrolysis (HTP, although pyrolysis is an incorrect term) or high temperature carbon reduction (HTCR) (see TC).
high temperature conversion-isotope ratio mass spectrometry	HTC/IRMS	A technique used for the measurement of hydrogen and oxygen isotope ratios, which employs high temperature thermal conversion of materials followed by gas chromatography separation of the resulting gases. Sometimes referred to as *TC/IRMS* (*thermal conversion-IRMS*) or as *TC/EA-IRMS* (*thermal conversion/elemental analyser-IRMS*).
in-house reference materials	in house RMs	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.*
Internal standard	IS	A substance, distinct from the analyte, that is added to all samples before analysis to improve accuracy by compensating for variations in sample preparation and instrument response.
International Atomic Energy Agency	IAEA	See www.iaea.org/
International Organization for Standardization	ISO	See www.iso.org

Term	Abbreviation	Description
International System of Units (Système international d'unités)	SI	The internationally agreed system of units
International Union of Pure and Applied Chemistry	IUPAC	See https://iupac.org/
intrinsic hydrogen		Hydrogen permanent within a sample. Sometimes referred to non-exchangeable hydrogen.
isobaric ions		Atomic or molecular species with the same nominal mass.
isotope amount fraction		The amount of the isotope relative to the total of all isotopes of the element in a specified material. Also written as $x(^{i}E)$ where *i* denotes the isotopic mass number and *E* denotes the chemical symbol of the element on the periodic table. Commonly found synonyms for isotope amount fraction include "*isotope abundance,*" "*isotopic abundance*," "*atom fraction*," "*mole fraction*" and "*isotope-number fraction*."
isotope number ratio	*R*	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). Sometimes also referred to as the "*absolute isotope ratio*" of a substance.
isotope ratio infra-red spectroscopy	IRIS	An optical technique using infra-red radiation to conduct isotope ratio measurements that is currently outside the scope of this guidance.
isotope ratio mass spectrometry	IRMS	The measurement of the relative quantity of the different isotopes of an element in a material using a mass spectrometer. An alternative acronym is *irm-MS* for "*isotope ratio monitoring mass spectrometry*."
isotopologue		Contraction of "isotopic analogue". These are molecules that differ only in their isotopic composition.
isotopomer		Contraction of "isotopic isomer." These are isomers with isotopic atoms, having the same number of each isotope of each element but differing in their positions.
kinetic isotope effect	KIE	The effect of isotopic substitution on the rate of a chemical reaction. Primary KIEs involve the formation or breaking of bonds containing isotopically labelled atoms in the rate determining step, while secondary KIEs typically do not.
laboratory information management systems	LIMS	The electronic records of laboratory samples and activity.
laser ablation	LA	A process in which a laser is used to vaporise the surface of a material.
light stable isotopes		Elements whose isotope delta-values are measured by gas source IRMS. Typically referring to hydrogen, carbon, nitrogen, oxygen and sulphur. Sometime referred to as bio-elements.
linearity		Changes in measured isotope-delta values as a linear function of sample size and/or peak intensity.

Term	Abbreviation	Description
liquid chromatography -chemical oxidation- isotope ratio mass spectrometry	LC/CO/IRMS	A technique whereby compounds separated by liquid chromatography are converted to carbon dioxide by chemical oxidation prior to IRMS analysis.
liquid chromatography -combustion- isotope ratio mass spectrometry	LC/C/IRMS	A technique whereby compounds separated by liquid chromatography are converted to carbon dioxide via a combustion process prior to IRMS analysis. Sometimes also referred to as *µEA/IRMS*.
liquid chromatography -isotope ratio mass spectrometry	LC/IRMS	A technique used for compound specific isotope analysis where compounds are separated using high performance liquid chromatography prior to IRMS.
	LSVEC	A reference material initially prepared for lithium isotope analysis by H Svec and later adopted as a reference material for the measurement of $\delta^{13}C$. It's use for calibration of carbon isotope-delta values is no longer recommended due to instability in its carbon isotopic composition.
mass spectrometry	MS	The study of matter through the formation of gas phase ions that are characterised using mass spectrometers (also sometimes abbreviated as "*MS*") by their mass, charge, structure and/or physio-chemical properties.
mass-to-charge ratio	*m/z*	A dimensionless quantity formed by dividing the mass of an ion in unified atomic mass units by its charge number (regardless of sign).
matrix-matched		Materials with the same chemical and physical properties that behave similarly in IRMS peripheral devices.
measurement uncertainty	MU	Parameter associated with a measurement result that characterises the dispersion of values that could reasonably be attributed to the quantity being measured. May also be abbreviated as *UoM* for "*uncertainty of measurement.*"
multicollector/ inductively coupled plasma mass spectrometry	MC/ICP-MS	A technique used for measuring isotope ratios and isotope-delta values for metallic and metalloid elements, which is currently outside the scope of this guidance.
National Institute of Standards and Technology	NIST	See www.nist.gov (formerly National Bureau of Standards, NBS).
normalisation		Aligning measured values of reference materials to their defined values (often termed "*shift*" when aligning a first RM and "*stretch*" when then aligning a second reference material) and then applying these terms to isotope ratio data measured from samples in the same analytical sequence/batch to link them to the international isotope-delta scale. This may also be referred to as "*calibration*" of measurement results to the appropriate isotope-delta scale or "*correction*" of results.

Term	Abbreviation	Description
open split		Interface between continuous flow systems such as elemental analyser, gas or liquid chromatography the isotope ratio mass spectrometer that does not affect chromatographic separation and reduces carrier flow rates from high-flow peripherals such as elemental analysers.
organic mass spectrometry	organic MS	As distinct from gas source IRMS. A range of techniques for the analysis of organic compounds involving ionisation and separation according to mass to charge ratio. The resulting mass spectrum (fragmentation pattern) provides information about the molecular mass and structure of the analyte compounds.
parts per million	ppm	A unit of concentration where the amount of substance of a component in a mixture is one unit per million units of total mixture such that 0.000001 = 1 ppm. Also found written as "*per meg.*"
Peedee belemnite	PDB	S*ee VPDB*
permille (‰)		A multiple of the unit "one" representing 10^{-3} such that 0.001 = 1 ‰. Also found written as "*per mill*," "*permill*," "*per mil*," "*permil*", or "*per mille*."
position specific isotope analysis	PSIA	A technique for the determination of isotope-delta values for specific intra-molecular sites.
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).
principle of identical treatment	PIT	The principle that all samples, reference and quality control materials, as well as the data obtained from their measurements, must be treated in the same way within a given laboratory to ensure comparability of results. Also referred to as the "identical treatment principle" or "IT principle"
proficiency test(ing)	PT	A form of inter-laboratory comparison exercise.
quality assurance	QA	The part of quality management focused on providing confidence that quality requirements will be fulfilled.
quality control	QC	The part of quality management focused on fulfilling quality requirements, i.e. planned activities designed to verify the quality of measurement results.
realisation (of an isotope-delta scale)		The method by which a measurement can be linked to a pre-defined measurement scale.
reference gas	RG	Gas evolved from a reference material (via combustion, pyrolysis, or other chemical reaction) for direct inlet measurements or introduced into the continuous flow carrier gas to facilitate isotope-delta value calculations.
reference material	RM	A material that is sufficiently homogeneous and stable with regard to specified properties, which has been demonstrated to be fit for its intended use in a measurement process. Also referred to as a certified reference material (CRM) or standard reference material (SRM).
repeatability		Measurements made by one analyst, using the same equipment over a short time period. Represents "within-batch" precision.

Term	Abbreviation	Description
resolution (of a mass spectrometer)		The ratio of the mass intended to be measured, *m*, typically taken as the heavier isotope in the context of IRMS, and the resolving power, *Δm*, of the MS.
resolving power		The minimum difference in *m/z* two ions must have to be distinguished by the mass spectrometer, often denoted as *Δm*. This can either be defined as a nominal value from the instrument's specification or by the full width of a peak in a mass spectrum at a specified height (typically either half or 10 % of the maximum intensity of the peak).
retention time	RT	The amount of time an analyte remains in a chromatography separator.
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).
simple random sampling	SRS	Selecting samples from a population at random.
sparging		Process of bubbling an inert gas (e.g. He, Ar, N_2) through a solution to remove dissolved gases and to prevent re-dissolution.
split ratio		The ratio of gas entering a gas chromatography column to the amount vented
stable isotope analysis	SIA	A technique for the analysis of isotopes of light elements.
standard		Widely adopted procedure, specification, technical recommendation, etc.
standard deviation	sd	A statistical estimate of the variation of data about the mean (average).
standard operating procedure	SOP	Step-by-step instructions that outline how to perform a specific task or process
Standard Light Antarctic Precipitation	SLAP	A reference material for the measurement of δ^2H and δ^{18}O values (now replaced by SLAP2).
Standard Mean Ocean Water	SMOW	*See VSMOW*
thermal decomposition	TD	The conversion of materials containing oxidized nitrogen using an elemental analyser without the addition of O_2 to produce N_2.
thermal conversion	TC	*See high temperature conversion (HTC)*
thermodynamic isotope effect	TIE	The effect of isotopic substitution on the equilibrium constant of a reaction.
thermal ionisation mass spectrometry	TIMS	A technique similar to high temperature conversion (HTC) where the samples are exposed to even higher temperatures capable of ionising the sample gas which is currently outside the scope of this guidance.
total organic carbon	TOC	Commonly used to denote the combined dissolved and suspended material. This may also refer simply to the total amount of carbon present

Term	Abbreviation	Description
		in a sample or refer to a detector which measures this amount by converting all carbon-containing compounds into CO_2.
traceability (of an isotope-delta measurement)		The relationship of an isotope-delta measurement to the appropriate scale through one or multiple calibration steps. Sometimes also referred to as a "*calibration chain*" or "*calibration hierarchy*."
uncertainty of measurement	UoM	S*ee measurement uncertainty*
United States Geological Survey	USGS	See www.usgs.gov
valid analytical measurement	VAM	A programme established by the UK government to improve the reliability of UK measurement results; facilitate mutual recognition of analytical data internationally; and to develop infrastructure aimed at achieving comparability and traceability of chemical measurements internationally.
Vienna Canyon Diablo troilite	VCDT	An internationally agreed zero-point for the measurement of $\delta^{34}S$ values. It is a hypothetical material.
Volatile organic compound	VOC	Organic compounds that can evaporate at room temperature and pressure.
International Vocabulary of Metrology (Vocabulaire International de Métrologie)	VIM	A glossary for metrological terms and definitions.
Vienna Peedee belemnite	VPDB	An internationally agreed zero-point for the measurement of $\delta^{13}C$ values. It is a hypothetical material.
Vienna standard mean ocean water	VSMOW	Internationally agreed zero-point for the measurement of $\delta^{2}H$ and $\delta^{18}O$ values.
working gas	WG	High purity gas introduced into the continuous flow carrier gas to facilitate isotope-delta value calculations (often referred to, although is distinct from, as the “reference gas”).
within-laboratory reproducibility		Measurements made in one laboratory over an extended time period. Other conditions such as analyst or equipment may also be varied. Represents the “between-batch” precision (sometimes referred to as “intermediate precision”).
working standard		*See In-house reference material.*

2 Introduction

2.1 Scope

This guidance includes background information pertaining to isotopic analyses by IRMS, principles of IRMS instrumentation, checking instrument performance, sampling, making measurements, processing data including estimation of measurement uncertainty, quality control and assurance and troubleshooting tips. It is focussed on obtaining and reporting isotopic composition as isotope-delta values for H, C, N, O and S.

It aims to

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

Measurements by other techniques such as optical spectroscopy or MC/ICP-MS are not within the current scope of this guidance. Nor are other element systems that may be of use in a forensic context such as Sr and Pb. Although recommendations for good practice regarding analytical methodologies for these techniques/elements are currently beyond the scope of this guide there are many sections outlining good practice in areas such as sampling, quality control and the calculation of MU that are generally applicable to isotope ratio measurements regardless of instrumentation.

2.1.1 Interpretation

Comprehensive guidance on the interpretation of stable isotope has been published as a stand-alone document by the FIRMS network (Doyle and van der Peijl 2020). Note that this interpretation guidance may be updated independently of this good practice guide and vice-versa – readers are advised to check the FIRMS website for the current version of each document.

2.1.2 Databases

The FIRMS network also provides guidance for the creation and use of databases of isotope ratio data as a stand-alone document. Again, note that this guidance on databases may be updated independently of this good practice guide and vice-versa – readers are advised to check the FIRMS website for the current version of each document.

2.2 Examples of applications of IRMS

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

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

- Forensic sciences
    - Determining whether samples of chemically similar substances such as drugs, explosives, fibres, paints, inks, polymers (films, tapes, ropes etc) or adhesives can (or cannot) be excluded as having a common source or history
    - Distinguishing counterfeit products (e.g. pharmaceuticals) from genuine materials
    - Comparing putative reactants with contraband products
    - Monitoring features of the environment
        - Identifying the source of pollutants such as oil spills
        - Monitoring atmospheric gases to distinguish between natural and anthropogenic sources
        - Modelling climate
        - Researching water cycle processes
    - Authenticating and tracing food
        - Establishing the geographic authenticity of foodstuffs
        - Identifying the adulteration of foods with cheaper ingredients
    - Investigating wildlife crime
- Archaeology/geosciences
    - Geochemistry and geology
        - Establishing the extent and temperature of post-burial alteration of rocks
        - Provenancing of clasts
        - Identifying the source of water samples
    - Palaeoclimatology
    - Palaeoecology
    - Palaeodietary studies
- Biological sciences
    - Ecology
        - Photosynthetic pathways
        - Food webs
        - Hydrology
        - Nutrient cycling
- Human and plant physiology
- Human provenancing
- Metabolic studies
- Sports medicine
- Toxicology
    - Distinguishing endogenous versus exogenous (bio)chemicals

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

3 Expressions of isotopic composition

There are a variety of different quantities that can be used to convey information regarding the isotopic composition of a material (Coplen 2011). These include isotope amount fraction, isotope ratio and isotope delta. These expressions can be inter-converted (Skrzypek and Dunn 2020b).

3.1 Isotope amount fraction, *x*

The amount fraction of isotope *i* of element E, $x(^{i}\mathrm{E})$, is the amount of the isotope relative to the total amount of all isotopes of element E in the specified material (Coplen 2011):

$$x(\,^{i}\mathrm{E}) = \frac{n(\,^{i}\mathrm{E})}{n(\mathrm{E})} \tag{1}$$

The amount of an isotope *i* of element E, $n(^{i}\mathrm{E})$, is related to the number of atoms that are of that isotope, $N(^{i}\mathrm{E})$, by the Avogadro constant, N_A, which is exactly equal to 6.022 140 76 x 10^{23} mol^{-1} (Newell et al. 2018; 'The International System of Units (SI)' 2019):

$$n(\,^{i}\mathrm{E}) = N(\,^{i}\mathrm{E})/N_A \tag{2}$$

Therefore, the numerical value of the isotope amount fraction can also be derived from the numbers of atoms of each isotope of element E. The SI units for isotope amount fraction are mol mol^{-1}. Although these units can be reduced to the unit "one," retaining the units allows the use of the SI prefixes when amount fractions are small, for example μmol mol^{-1}. Quantities with the unit one have been referred to as "dimensionless quantities" in the past.

Isotope amount fractions range from zero (absence of isotope *i* of element E in the material) to one (the material contains only isotope *i* of element E) and the sum of all isotope amount fractions for all isotopes of element E is also one.

Commonly found synonyms for isotope amount fraction include "*isotope abundance*," *"isotopic abundance*," "*atom fraction*," "*mole fraction*" and "*isotope-number fraction.*"

3.2 Isotope ratio, *R* or *r*.

The quantity "isotope ratio" generally refers to the ratio of the amounts of two isotopes or the numbers of atoms that are the two isotopes (Coplen 2011):

$$R(\,^{i}\mathrm{E}/\,^{j}\mathrm{E}) = \frac{n(\,^{i}\mathrm{E})}{n(\,^{j}\mathrm{E})} = \frac{N(\,^{i}\mathrm{E})}{N(\,^{j}\mathrm{E})} \tag{3}$$

The upper-case *R* and lower-case *r* are often used to distinguish between isotope ratios derived from numbers of atoms and amounts of isotopes, respectively (Coplen 2011); or between calibrated and uncalibrated ratios, respectively, particularly for isotope ratios of elements amenable to MC-ICP-MS measurement (Yang et al. 2018).

There is no difference in numerical value, associated uncertainty or reduced SI units between isotope ratios derived from amounts of isotopes and those derived from numbers of atoms.

The SI units for isotope amount ratio are mol mol^{-1} but these are often reduced to the SI unit "one," which is not necessary to show ('The International System of Units (SI)' 2019).

Isotope ratios range from zero (i.e., the absence of the numerator isotope ^{i}E) to infinity (i.e., the absence of the denominator isotope ^{j}E).

For the elements within the scope of this Guide, the isotope ratios have the most abundant isotope of the element (the lightest isotope in terms of mass) in the denominator and the minor isotope (the heavier isotope in terms of mass) in the numerator. As a result, equation (3) can be considered as:

$$R = \frac{[\text{heavier isotope}]}{[\text{lighter isotope}]} \tag{4}$$

3.3 Isotope delta, δ

The quantity isotope delta is the relative difference in isotope ratio between a sample and a reference (McKinney et al. 1950; Craig 1953; Coplen 2011).

$$\delta_{\mathrm{ref}}\left({}^{i}\mathrm{E}/{}^{j}\mathrm{E},\mathrm{Sample}\right)=\frac{R_{\mathrm{sample}}\left({}^{i}\mathrm{E}/{}^{j}\mathrm{E}\right)-R_{\mathrm{ref}}\left({}^{i}\mathrm{E}/{}^{j}\mathrm{E}\right)}{R_{\mathrm{ref}}\left({}^{i}\mathrm{E}/{}^{j}\mathrm{E}\right)}=\left(\frac{R_{\mathrm{sample}}\left({}^{i}\mathrm{E}/{}^{j}\mathrm{E}\right)}{R_{\mathrm{ref}}\left({}^{i}\mathrm{E}/{}^{j}\mathrm{E}\right)}-1\right) \quad (5)$$

It is common to shorten the lengthy quantity symbol for isotope delta by removing the denominator isotope and the parentheses and moving the subscript label for the reference. For example, "$\delta_{\mathrm{ref}}({}^{18}\mathrm{O}/{}^{16}\mathrm{O}$, water)" is often shortened to "$\delta^{18}\mathrm{O}_{\mathrm{ref}}$" and that water is the material in question is specified elsewhere – and this is done within this Guide for familiarity.

Equation (5) is commonly represented in the following simplified manner:

$$\delta=\frac{R_{\mathrm{sample}}-R_{\mathrm{ref}}}{R_{\mathrm{ref}}}=\left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{ref}}}-1\right) \quad (6)$$

It is vital that any shortening of quantity symbols does not introduce confusion, for example by omitting the reference for oxygen isotope delta for which there are three relatively common possibilities (section 4.2.4).

Example: Notation for isotope delta

The symbol for the hydrogen isotope delta value of a material on the VSMOW-SLAP scale (section 4.2.1.3) is commonly written as:

$\delta^{2}\mathrm{H}_{\mathrm{VSMOW\text{-}SLAP}}$

It is important to state how such an isotope delta value has been made traceable to the VSMOW-SLAP scale (sections 5.2 and 11.4).

The SI units of isotope delta can be reduced to "one," which is not necessary to show ('The International System of Units (SI)' 2019). As isotope delta values are generally small, they are commonly expressed in permille (‰) and occasionally in parts per million (per meg or ppm). Due to Harold Urey's significant role in the early development of stable isotope chemistry it has been proposed that the units for reporting isotope-delta values are named the "Urey" with permille replaced by milli Urey (mUr) (Brand and Coplen 2012). To date, this convention has not been widely adopted.

Calculations involving isotope-delta values should not use values expressed in ‰ to avoid errors in orders of magnitude. For example, –0.012 should be used rather than –12‰.

Isotope-delta values range from –1 (equivalent to –1000‰), which indicates a complete absence of the numerator isotope in the sample, i.e. $R_{\mathrm{sample}} = 0$. As R_{sample} tends towards infinity, so too does isotope delta. The reference itself has an isotope-delta value of zero by definition, because $\delta = 0$ when $R_{\mathrm{sample}} = R_{\mathrm{ref}}$ and may be referred to as the "zero-point."

To allow compatibility and comparability between isotope-delta values for the same isotope ratio, the reference used must be the same. There are internationally agreed references for isotope delta for the elements discussed in this document (chapter 4).

When expressing isotope-delta values, positive and negative values must be indicated using the appropriate symbols (i.e. "+" and "–").

The expression for isotope delta in equation (5) may seem to imply that isotope ratio measurements are required for the sample and reference. However, since IRMS instruments measure relative variations of isotope ratios it is not necessary to know the "absolute" isotopic composition of the reference as both measured isotope ratios and isotope-delta values are relative quantities.

4 Isotope delta scales

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

Each isotope system ($^{13}C/^{12}C$, $^{18}O/^{16}O$, etc.) may have one or more isotope delta scale(s), each with its own associated zero-point.

4.1 Definition of isotope-delta scales

Isotope delta scales for the elements within the scope of this guide are generally defined by selecting a specific material to serve as the reference (i.e. zero-point), rather than through strict application of equation (5) using measured isotope ratios. Originally, zero-point materials were chosen by a small number of researchers for in-house use.

Example: Peedee belemnite (PDB)

In the early 1950s, the research group in Chicago used “carbon dioxide prepared from a Cretaceous belemnite, *Belemnitella americana*, from the Peedee formation of South Carolina” as their in-house reference for both carbon and oxygen isotope-delta values of other carbonate materials (Urey et al. 1951; Craig 1953, 1957).

To allow comparability and compatibility of isotope-delta values between the small number of laboratories making measurements of isotope-delta values in the 1950s and 1960s, aliquots of these materials were exchanged to allow cross-calibration of in-house references.

As instrumental precision improved, the number of independent researchers making measurements increased and the availability of those original zero-point materials decreased, replacement zero-point materials defining new isotope-delta scales were needed. Consultants’ meetings at the IAEA in Vienna proposed the new zero-points for isotope-delta scales (Hut 1987; Gonfiantini 1984).

Presently, isotope-delta scales are defined by specific materials with exactly assigned isotope-delta values by international agreement. This involves the IAEA; the IUPAC’s CWIAA and the BIPM amongst others (Camin et al. 2025).

The materials with exactly assigned isotope-delta values are often, but not always, the zero-point of isotope-delta scales. They can be specific RMs available commercially; natural reservoirs of the element that are sufficiently large and homogeneous; or hypothetical/theoretical materials defined by other means (Skrzypek et al. 2022).

New isotope-delta scales can be introduced:

- to address new measurement requirements for specific applications of an existing isotope system (e.g. there are three isotope-delta scales for $^{18}O/^{16}O$ ratios of oxygen, section 4.2.4, two of which are for the analysis of oxygen isotopes within particular matrices),
- to afford compatibility of data when an isotope system has become sufficiently widely measured that an international measurement scale is required (e.g. the recent introduction of a $\delta^{17}O$ scale (Schönemann, Schauer, and Steig 2013)),
- to replace an existing isotope-delta scale should a primary RM be found to be unsuitable, e.g. due to previously undiscovered heterogeneity (section 5.1.1),
- inadvertently should an incorrect isotope delta value be assigned to a primary RM (e.g. section 4.2.2).

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

4.1.1 Isotope-delta scales with two defined points

The majority of isotope-delta scales across all elements are defined by a single material, often the zero-point (Brand et al. 2014). For measurements of isotope delta using IRMS, significant scale

contraction effects may occur during realisation of some isotope-delta scales (**Figure 1**). These effects must be taken into account and the best approach is determination of the magnitude of the effect through careful measurement, for example the application of the so-called η correction during dual-inlet IRMS analyses (Meijer, Neubert, and Visser 2000) as described later in section 6.2.1; however, this is not always straightforward, particularly for continuous-flow-based measurement approaches.

The use of two or more RMs for scale realisation also allows correction for scale contraction effects, and inter-laboratory compatibility of results is significantly improved by the use of a second defined point with no uncertainty on an isotope-delta scale. Therefore, some isotope-delta scales have a second point defined by international agreement in addition to the zero-point. This is a practice which has been applied for many decades for the hydrogen and oxygen VSMOW-SLAP scales (Hut 1987; Coplen 1994, 1995).

***Figure 1**. Illustration of scale contraction occurring during measurement of two RMs. Aligning the measured isotope delta value of RM1 to its defined value is often termed "shift" while then also ensuring the measured and defined isotope-delta values for RM2 are also aligned is often termed "stretch." Together these processes are known as normalisation.*

The introduction of a second point also requires a modification to the usual isotope delta equation (5). For the example of VSMOW-SLAP, this becomes (J. R. Gat and Gonfiantini 1981; Gröning 2004):

$$\delta = \left(\frac{R_{\text{sample}} - R_{\text{VSMOW}}}{R_{\text{VSMOW}}}\right) \times \left(\delta_{\text{SLAP}} / \frac{R_{\text{SLAP}} - R_{\text{VSMOW}}}{R_{\text{VSMOW}}}\right) \quad (7)$$

With the second set of parentheses containing the normalization factor based upon the defined hydrogen (or oxygen) isotope ratios of VSMOW and SLAP (R_{VSMOW} and R_{SLAP}) together with the defined isotope delta value for SLAP relative to VSMOW (δ_{SLAP}).

It is crucial that an isotope-delta scale that has two defined points is realised by two (or more) RMs (Chapter 5).

4.2 Overview of internationally agreed isotope-delta scales

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

4.2.1 Hydrogen

4.2.1.1 SMOW

The original zero-point for hydrogen isotope delta measurements of water samples was Standard Mean Ocean Water (SMOW) (Craig 1961). This was a hypothetical material with defined isotopic composition and so it did not physically exist and so it could not be used directly for calibration of instrumentally measured results. The US NBS (now NIST) RMs NBS 1 and NBS 1a were assigned hydrogen isotope-delta values relative to the hypothetical SMOW to provide a scale of practical use. This scale should no longer be used.

4.2.1.2 VSMOW

The shortcoming of a hypothetical SMOW was addressed by the production of Vienna SMOW (VSMOW) by blending distilled ocean water (latitude 0°/longitude 180°) with small amounts of other waters to produce an isotopic composition close to the definition of SMOW. VSMOW then became the zero-point and primary RM of the new hydrogen isotope delta scales that were also called VSMOW (i.e. VSMOW $\delta^2H = 0$ exactly) (J. R. Gat and Gonfiantini 1981). Single point calibration of hydrogen isotope delta for traceability to the VSMOW scale is not recommended.

4.2.1.3 VSMOW-SLAP

Due to scale contraction effects, an additional primary RM was established for the δ^2H isotope delta scales. This material was Standard Light Antarctic Precipitation (SLAP), which was prepared from South Pole firn and is considerably depleted in heavy isotopes of hydrogen with respect to VSMOW. The hydrogen isotope delta value assigned to SLAP is $\delta^2H = -428$‰ exactly versus VSMOW. The δ^2H values of all hydrogen and oxygen bearing materials should be reported on this VSMOW-SLAP scale (J. R. Gat and Gonfiantini 1981; Hut 1987; Coplen 1994, 1995).

VSMOW and SLAP (primary RMs) have now been superseded by VSMOW2 and SLAP2 that both have an almost identical isotopic composition to their predecessors, but with associated uncertainties. VSMOW2 and SLAP2 are therefore secondary RMs on the VSMOW-SLAP scale but currently provide the highest metrological realisation of the hydrogen and oxygen VSMOW-SLAP scale (Harms and Gröning 2017). The availability of VSMOW2 and SLAP2 does not prevent the continued use of the original VSMOW and SLAP should a laboratory have access to these materials.

4.2.2 Carbon

4.2.2.1 PDB

The original zero-point for carbon isotopic measurements consisted of calcium carbonate from a Cretaceous belemnite from the Peedee formation in South Carolina (PDB). The CO_2 evolved from PDB, by treatment with phosphoric acid, was adopted as the zero-point for carbon and oxygen isotopic measurements (Urey et al. 1951; Craig 1953, 1957). This scale should no longer be used.

4.2.2.2 VPDB

Upon its exhaustion, PDB was replaced by assigning an exact $\delta^{13}C$ value to another carbonate (NBS 19 or “TS-limestone”) versus a hypothetical Vienna PDB creating a new isotope delta scale (VPDB) for carbon. On this scale, NBS 19 has $\delta^{13}C_{VPDB} = +1.95$ ‰ exactly derived from an interlaboratory comparison of NBS 19 carbon isotope-delta values reported against PDB (Gonfiantini 1984).

NBS 19 is still commercially available from some suppliers. The IAEA has released IAEA-603 as their suggested replacement for NBS 19 (Fajgelj and Assonov 2016; Assonov et al. 2020), which will act as the highest metrological realisation for the carbon and oxygen VPDB scales as it has been very precisely calibrated directly against NBS 19 with a very small associated uncertainty. NBS 19 remains the primary, scale-defining RM for the VPDB scale and laboratories may continue to use it directly for calibration if they hold a stock (noting that this should only be done where the very small uncertainty imparted is of significant benefit), but IAEA-603 or other RMs traceable to NBS 19 alone such as IAEA-610, IAEA-611 and IAEA-612 should be used by laboratories that do not have access to NBS 19.

4.2.2.3 VPDB-LSVEC

NBS 19 has isotopic ratios characteristic of marine limestone and is considerably enriched in ^{13}C with respect to almost all organic carbon. It was therefore recommended that $\delta^{13}C$ values of both organic and inorganic materials were expressed relative to VPDB on a scale that was also realised by a second primary RM (LSVEC lithium carbonate) with an exact $\delta^{13}C$ value of –46.6 ‰ relative to VPDB (Coplen et al. 2006a, 2006b). This afforded better comparability of measurement results between different laboratories as scale contraction effects could be accounted for. It was not intended that this be a separate isotope delta scale.

LSVEC has been reported as suffering from incorporation of atmospheric CO_2, thereby altering its carbon isotopic composition with time, particularly for vials that are frequently opened (Qi et al. 2016; Assonov 2018). As a result, it is no longer recommended by the CIAAW to use LSVEC as a RM to realise the VPDB-LSVEC carbon isotope delta scale (Prohaska et al. 2022). Nevertheless, it is still a requirement that carbon isotope delta values be normalised to the VPDB-LSVEC scale using two or more RMs.

It has also become clear that the assigned carbon isotope delta value for LSVEC is likely too negative. As a result, the VPDB carbon isotope delta scale defined by NBS 19 alone does not provide the same isotope-delta values for materials as the VPDB-LSVEC scale defined by both NBS 19 and LSVEC (Qi et al. 2021; Hélie et al. 2021; Dunn and Camin 2024; Camin et al. 2025). For this reason, the VPDB and VPDB-LSVEC scales must be considered separate. Users must not "mix-and-match" RMs traceable to NBS 19 alone with those traceable to both NBS 19 and LSVEC within a single calibration and it must be clear which scale results are being reported on (Camin et al. 2025).

4.2.3 Nitrogen

Atmospheric Nitrogen (Air-N_2) has been adopted as the zero-point for all nitrogen isotope ratio analyses as it has been shown not to vary measurably around the world or over time (Mariotti 1984, 1983). To be used as a practical RM, however, N_2 would need to be isolated from the atmosphere without fractionation, which is challenging (Brand et al. 2014).

A second defined point, USGS32 potassium nitrate with an assigned $\delta^{15}N$ value of +180 ‰ exactly in addition to atmospheric nitrogen has been proposed (Coplen, Krouse, and Böhlke 1992; Böhlke, Gwinn, and Coplen 1993; Böhlke and Coplen 1995) and implemented by some studies (Dunn, Malinovsky, and Goenaga-Infante 2020) but not yet officially adopted (Camin et al. 2025).

The IUPAC CIAAW recommends that the RM IAEA-N-1 (ammonium sulphate) be used as the highest metrological realisation of the Air-N_2 scale for samples that need combustion as a means of sample preparation because Air-N_2 is difficult to produce free from argon, which can interfere with isotopic analysis (Brand et al. 2014).

4.2.4 Oxygen

For the majority of oxygen bearing materials, the VSMOW-SLAP isotope delta scale (section 4.2.1) should be used. For each of two specific matrices and for historical reasons, there are additional oxygen isotope delta scales in use.

4.2.4.1 VSMOW-SLAP

Due to scale contraction effects, a second primary RM was established for the $\delta^{18}O$ isotope delta scale. This material was SLAP, which is considerably depleted in heavy isotopes of oxygen with respect to VSMOW. The oxygen isotope delta value assigned to SLAP is $\delta^{18}O$ = –55.5‰ exactly versus VSMOW. The $\delta^{18}O$ values of all oxygen bearing materials should be reported on this VSMOW-SLAP scale (J. R. Gat and Gonfiantini 1981; Hut 1987; Coplen 1994, 1995).

VSMOW and SLAP (primary RMs) have now been superseded by VSMOW2 and SLAP2 that both have an almost identical isotopic composition to their predecessors, but with associated uncertainties. VSMOW2 and SLAP2 are therefore secondary RMs on the VSMOW-SLAP scale but currently provide the highest metrological realisation of the hydrogen and oxygen VSMOW-SLAP scale (Harms and Gröning 2017). Some suppliers may still hold stocks of the original VSMOW and SLAP RMs.

4.2.4.2 PDB

The same Cretaceous belemnite material used as the zero-point for early carbon isotope delta measurements, was also used as the equivalent oxygen isotope delta zero-point. The PDB scale was used for reporting $\delta^{18}O$ values of carbonates. This scale should no longer be used.

4.2.4.3 VPDB and VPDB-CO_2

As with carbon isotope delta, the PDB oxygen isotope delta scale was replaced by the VPDB scale with NBS 19 as the new primary RM. On this scale, NBS 19 has $\delta^{18}O_{VPDB}$ = –2.20‰ exactly.

There is an oxygen isotope fractionation between the carbonate and the evolved CO_2, the latter being about 10‰ enriched in ^{18}O with respect to the calcite (when the reaction takes place at 25 °C). This is irrelevant when measuring calcite samples against calcite RMs, but becomes problematic for DI measurements of non-carbonates or for non-calcite carbonates, which have different fractionation factors than calcite at temperatures above 25 °C (Sharp 2017). Therefore, the oxygen isotopic compositions of the hypothetical VPDB calcite and of the CO_2 evolved from this calcite under standard conditions (VPDB-CO_2) are different (and this holds true for any calcite sample), while the carbon isotopic compositions of these two virtual materials are identical.

As a result of this oxygen isotopic fractionation between carbonate and carbon dioxide, measurements of the oxygen isotope-delta values of carbon dioxide gas are sometimes made on a scale where instead of the solid NBS 19 carbonate being assigned $\delta^{18}O_{VPDB}$ = –2.20‰ exactly relative to the hypothetical VPDB carbonate, it is carbon dioxide released from acid digestion of NBS 19 under specified conditions that is assigned this isotope delta value relative to hypothetically released carbon dioxide form the acid digestion of the hypothetical VPDB carbonate under the same conditions. This hypothetical carbon dioxide released from VPDB is termed VPDB-CO_2.

4.2.4.4 Atmospheric Oxygen (Air-O_2)

Oxygen isotope delta values for oxygen gas have also been reported on an isotope delta scale with the isotopic composition of atmospheric oxygen (Air-O_2) as the zero-point. This scale should still be normalised such that the $\delta^{18}O$ value of SLAP is –55.5‰ relative to VSMOW (Joel R. Gat and DeBievre 2002; Wieser and Berglund 2009).

There are no RMs currently available that are calibrated to the Air-O_2 scale.

4.2.4.5 Scale conversions for oxygen isotope delta

There are several agreed conversion models to transform oxygen isotope-delta values from one scale to another (Camin et al. 2025; Hillaire-Marcel et al. 2021; Brand et al. 2014). These include:

$$\delta^{18}O_{\text{VSMOW-SLAP}} = 1.03092 \times \delta^{18}O_{\text{VPDB}} + 30.92‰ \quad (8)$$

$$\delta^{18}O_{\text{VSMOW-SLAP}} = 1.01419 \times \delta^{18}O_{\text{VPDB-CO2}} + 41.49‰ \quad (9)$$

$$\delta^{18}O_{\text{VSMOW-SLAP-CO2}} = 1.00029 \times \delta^{18}O_{\text{VPDB-CO2}} + 0.29‰ \quad (10)$$

4.2.5 Sulphur

4.2.5.1 CDT

CDT (Canyon Diablo troilite – iron sulphide from the Canyon Diablo Barringer meteorite) was originally proposed as a scale anchor and zero-point for sulphur $\delta^{34}S$ values (Hut 1987). This scale should no longer be used.

4.2.5.2 VCDT

The original CDT material was found to be isotopically inhomogeneous and therefore unsuitable as a primary RM (Beaudoin et al. 1994).

The zero-point of the replacement scale, Vienna CDT (VCDT) is defined by assigning the $\delta^{34}S$ value of the silver sulphide material IAEA-S-1 to be –0.3‰ exactly (Krouse and Coplen 1997).

There is currently no additional primary RM for the VCDT scale, however a range of secondary RMs with known isotope-delta values is available and therefore two-or-more point realisation of the VCDT scale is possible and recommended – for example using IAEA-S-2 and IAEA-S-3.

As with VPDB, VCDT is a virtual material; however, the absolute isotopic composition of VCDT, traceable to the International System of Units (SI), is known to a high degree of precision through measurements of IAEA-S-1 (Ding et al. 2001).

5 Traceability and calibration

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

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

Measurement results of isotope delta values should always be traceable to the appropriate scale and accredited laboratories must be able to demonstrate this (Barwick and Prichard 2011; BIPM et al. 2012). The highest metrological realisation of an isotope delta scale uses the shortest traceability chain and therefore results in the smallest MU.

RMs with known isotope-delta values can be said to realise the appropriate scale. Defined points of isotope-delta scales are realised by RMs with no uncertainty associated to their assigned isotope-delta values (these are the primary RMs, section 5.1.1). Other RMs have associated uncertainty in their isotope-delta value and realise other points on the scale (these can be secondary RMs, section 5.1). Even when a RM has no associated uncertainty to its assigned value, the analysis of the RM to realise the isotope-delta scale will introduce measurement uncertainty (section 10.7).

5.1 Reference materials and calibration hierarchies

VIM 3 (BIPM et al. 2012) defines a calibration hierarchy as a “*sequence of calibrations from a reference to the final measuring system, where the outcome of each calibration depends on the outcome of the previous calibration*.”

For measurements of isotope delta, the calibration hierarchy takes the form of RMs with traceability chains linking their assigned isotope-delta values back to the zero-point (and any other defined point) of the appropriate delta scale. Although nomenclature will vary, RMs for isotope delta measurements may be broadly classified as:

(1) Primary (or scale-defining) RMs

(2) Secondary (or scale-realising) RMs

(3) In-house (or laboratory) RMs

Given that many isotope delta RMs are value-assigned by inter-laboratory comparison studies; that within such studies laboratories may use a variety of RMs for calibration; and that not all of these RMs will have traceability chains of the same length, it can be difficult to classify reference materials for isotope delta into a hierarchy (Dunn, Malinovsky, and Goenaga-Infante 2020). As a result, use of the terms “primary,” “secondary,” etc. in relation to isotope delta RMs require clarification.

Measurement of RMs together with sample materials facilitates calibration of measurement results to the reporting isotope delta scale and thereby ensures traceability.

5.1.1 Primary (scale-defining) reference materials

The primary RM for an isotope-delta scale defines the position of the zero-point (e.g. IAEA-S-1 defines the zero-point of the VCDT δ^{34}S scale). In some instances, a primary RM can both realise and define the zero-point when it’s assigned isotope delta is zero (e.g. VSMOW both defines and realises the zero-points of the VSMOW-SLAP δ^{2}H and δ^{18}O scales). For isotope-delta scales with two defined points, there are two primary RMs, one for each fixed point on the scale. All primary RMs have exactly assigned isotope-delta values with no uncertainty.

As noted in the IUPAC Technical Report on stable isotope reference materials, the concept of primary RMs having zero uncertainty only holds for consideration of the entirety of the primary RM in question. Individual units of the primary RM, aliquots samples from a single unit, or even single

grains may exhibit a detectable degree of heterogeneity. As a result, the suppliers of some primary RMs assign a non-zero uncertainty to their isotope-delta values – for example the 2020 reference sheet for IAEA-S-1 from the IAEA provides a combined standard uncertainty in the assigned isotope delta value of 0.03‰, even though IAEA-S-1 defines the VCDT sulphur isotope delta scale (Vasileva and Assonov 2020).

The absolute isotopic compositions of the primary RMs are not important for routine measurement of delta values but absolute values have been reported (Junk and Svec 1958; Hagemann, Nief, and Roth 1970; Baertschi 1976; Ding et al. 2001; Gröning 2004; Dunn et al. 2024).

Over time some of the original zero-point RMs for isotope systems were found to be unsuitable due to previously undiscovered heterogeneity (e.g. the PDB calcite). In such cases replacement of the affected isotope-delta scales was required. This generally involved the elevation of an existing secondary RM to become a new primary RM for a new scale with a new zero-point. The exact values assigned to a primary RM on new delta scales for an isotope system are chosen such that the numerical isotope delta values for materials expressed on the old and new scales are as close as possible given the available information (Gonfiantini 1984; Hut 1987).

Example: Vienna Peedee belemnite (VPDB)

In the late 1980s, the PDB scale was replaced by elevating the NBS 19 calcite (a secondary RM on the PDB scale) to be the primary RM defining the zero-point of a new Vienna PDB (VPDB) scale with no associated uncertainty (Hut 1987).

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

The primary RMs (and their replacements when appropriate) kept and distributed by IAEA, NIST and USGS are listed in **Table 1**. The routine measurement of primary RMs and their replacements for isotope-delta scale realisation should be avoided unless the small measurement uncertainty that these materials confer during scale realisation is essential. This ensures that the primary RMs are available for as long as possible, which is important for long-term reproducibility. For all but the most exacting of applications, the slightly larger measurement uncertainty afforded by the use of secondary or tertiary RMs does not impact on the usefulness of the results.

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

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

$^{18}O/^{16}O$	VSMOW	VSMOW	VSMOW2[c]	0.00 ± 0.02
		SLAP	SLAP2[c]	−55.5 ± 0.02
	VPDB	NBS 19	IAEA-603[d]	−2.37 ± 0.04
	Atmospheric oxygen	NA	NA	NA
$^{34}S/^{32}S$	VCDT	IAEA-S-1	IAEA-S-1	−0.3

[a] These isotope-delta values have been obtained from the IUPAC Technical Report (Brand et al. 2014).

[b] Uncertainties are standard uncertainties. Where no uncertainty is given, the isotope-delta value is exact and assigned by consensus.

[c] Recommended replacements for the original VSMOW and SLAP primary RMs (Martin and Gröning 2009; Lin, Clayton, and Gröning 2010; Harms and Gröning 2017).

[d] Recommended replacement for NBS 19, the original primary RM for the carbon and oxygen VPDB scales (Fajgelj and Assonov 2016; Assonov et al. 2020).

[e] While atmospheric nitrogen could be considered to be a primary RM, it is difficult to refine with a reproducible isotope ratio, so is best only considered the zero-point of the scale.

[f] Recommended scale anchor for combustion-based measurements (Brand et al. 2014; Mariotti 1984).

[g] While there is no official IUPAC Recommendation on the use of USGS32 with exactly-assigned nitrogen isotope delta value, all currently available RMs have USGS32 with exactly assigned isotope delta within their traceability chains (Dunn, Malinovsky, and Goenaga-Infante 2020).

5.1.2 Secondary (scale realizing) reference materials

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

A summary of the commercially available secondary (and primary) RMs for all isotope-delta scales can be found in the relevant IUPAC Technical Report (Brand et al. 2014). This report includes RMs for the elements in the scope of this Guide predominately distributed by IAEA, NIST and USGS. Note that this report is updated periodically rather than on the release of new secondary RMs or publication of revised certificates. For this reason, when reporting isotopic compositions it is essential that the values and measurement uncertainties assigned to primary and/or secondary materials are given alongside sample results. Secondary isotopic RMs may have been produced decades ago and requirements of estimation and reporting of uncertainties associated to their reference values may now be different.

Other RMs may also be commercially available that have longer traceability chains rather than being directly calibrated against primary RMs. The isotope-delta values of these materials have often been assigned by a single laboratory or are consensus values, obtained through ILC. Materials distributed within PT schemes that have assigned isotope-delta values may also be available. In general, these materials do not carry the international agreement ascribed to the materials distributed by IAEA, NIST and USGS listed in the IUPAC Technical Report (Brand et al. 2014), but may prove useful where no other RMs exists.

We recommend that end users carefully examine the stated traceability and any uncertainty statement associated to the isotope-delta value of a RM.

5.1.3 In-house reference materials

A stable isotope laboratory must hold suitable materials for calibration and normalisation purposes so that isotope delta values can be reported on an agreed international scale. Primary and secondary RMs are not recommended for daily use as they are in short supply. Instead, primary and/or secondary RMs are used to calibrate in-house RMs for everyday use in normalisation and QA. Control charts should be used to monitor laboratory performance and the status of in-house RMs (section 11.1.1). Any contamination of the RMs will be apparent as a step-change in the control chart whereas a slow change (evaporation, reaction with atmospheric water or CO_2, etc.) will show as drift. Control charts will also assist in determining whether a proposed in-house RM is likely to be suitable for long-term use.

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

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

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

- ease of preparation, storage and handling,
- conversion characteristics within peripherals (i.e. complete conversion to analyte gas and therefore generally a single chemical compound),
- ease of replacement (when exhausted, contaminated etc.),
- non-hygroscopic (especially important when measuring hydrogen and oxygen isotopes),
- comprising only intrinsic hydrogen (hydrogen permanent within a materials) unless the extrinsic hydrogen is well characterised (sections 9.3.4 and 10.6.6).

5.1.3.1 Matrix-matched in-house reference materials

Developing or buying in-house RMs that are chemically and physically similar to materials analysed in your laboratory is highly recommended. There are a variety of reasons why analyte materials may differ in their behaviour in your preparatory system from commercially available RMs; these differences may affect the isotope ratio measurement results. A non-exhaustive list includes: differences in oxidation state, differences in the sorption of water, or the presence of an element not present in the RM, e.g. the hydrochloride salt versus the free-base form. Therefore, it is strongly recommended to use a “matrix-matched” in-house material to enable application of PIT (Werner and Brand 2001) described in section 5.2.1.

5.1.3.2 Calibration of in-house reference materials

A thorough description of the processes needed for characterisation of in-house RMs for stable isotope analyses is provided elsewhere (Dunn, Malinovsky, et al. 2021). Preparation and value assignment for specific matrices have also been provided (Carter and Fry 2013b; Crivellari et al. 2021; Hélie and Hillaire-Marcel 2021). A brief summary of the processes is given below:

Before beginning, a fully developed and validated method (section 11.3) must be available. Thorough planning of the process (a validation plan) is helpful before starting. There are several required assessments of any candidate material including homogeneity and stability. The aim is to provide an assigned isotope delta value with associated uncertainty for the candidate material that can then be used as a source of traceability. A laboratory should produce a validation plan for developing an in-house RM with appropriate review and sign off.

The nature of the homogeneity assessment will depend on several factors including the expected degree of heterogeneity of the candidate material, the precision of the method, the amount of candidate material available and the number of units/containers that the material is stored in. On the assumption that a small number of containers is available (e.g. <20), then testing material from each container is a possibility, otherwise a subset of containers can be tested. To allow the between-container and within-container variations to be disentangled, multiple aliquots should be taken from each container and all aliquots across all tested containers should be measured in the

same sequence/batch. Statistical methods such as ANOVA can be used to determine both within- and between-container variations and from these an estimate of the contributions to overall uncertainty from heterogeneity can be made. An example homogeneity assessment is given in section 8.4.

For stability assessment, it is the nature of the candidate material that has the most impact on the design of a study. As noted above, candidate materials are generally selected on the basis of their known stability in terms of isotopic composition over time. As a result, a long-term stability study need not be performed before use of the material – although on-going monitoring of any in-house RM during use is vital. For pure chemical compounds or similar a good starting point for stability is to refer to the manufacturer or supplier Certificate of Analysis and the "use by" or retest date therein.

A short-term or accelerated stability study is often performed by (C)RM manufacturers to check that material is stable during shipping. For an in-house RM this may seem superfluous, however such a study can highlight the integrity of the material should it not be stored under the recommended conditions for some time (e.g. left out of the fridge; power cut to freezer, etc). It can be enough simply to test some aliquots of the material left under none-ideal conditions for some time and compared to aliquots stored in ideal conditions and then provide a fitness-for-purpose statement that can be referred to later.

There needs to be a specific set of measurements made to obtain the assigned isotope delta value(s) for the in-house RM. These should be made on a small number of aliquots taken from a few of the containers and be repeated across a small number of days. These measurements provide the assigned value for the candidate in-house RM as well as a specific contribution to the associated uncertainty that also includes the contributions arising from traceability. Ideally, measurements should be made by more than one analyst and if available, more than one instrument.

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

The overall uncertainty in the assigned value for the in-house RM is a combination of the uncertainty arising from the characterisation measurements, from the homogeneity study and from any stability study that was not simply providing a fitness-for-purpose statement.

All of the data collected together with the methods for collecting data should be compiled into a calibration report for future reference. The collected data must be reviewed and deemed fit-for-purpose by an approved signatory.

In-house RMs, alongside other QC materials, should be monitored for possible instability or contamination using LIMS. Periodically, primary and/or secondary RMs should be analysed as "unknown" samples to check in-house proficiency and the effectiveness of in-house RMs for data normalisation and correction. If deemed necessary, whether due to improvements in methods, changes in equipment, or changes in requirements, the laboratory must conduct a recalibration and adjustment to the values and associated measurement uncertainties of isotope-delta of in-house RMs.

5.2 Traceability in practice

In practice, realisation of an isotope-delta scale involves the analysis of samples and RMs linked to the isotope-delta zero-point within the same sequence/batch. Two or more RMs should be used to realise isotope-delta scales during each measurement sequence/batch. The differences in measured isotopic composition between the samples and RMs can then be used to determine the differences in isotopic composition between the samples and the zero-point of the scale. Section 10.5 contains more information regarding the mathematical linking of measured isotope delta values to the appropriate isotope-delta scales. It is this process that confers traceability to measurement results.

It is good practice to check that realisation of the scale has been correctly performed via the analysis of QC materials of known isotope delta value (chapter 11).

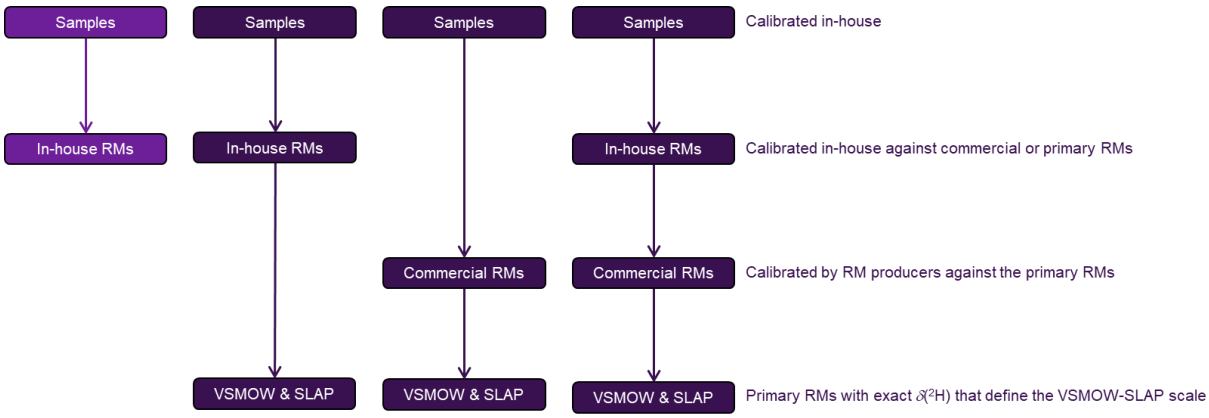

***Figure 2**. Four examples of traceability chains for isotope-delta values of samples. Three are linked by calibration chains that involve both VSMOW and SLAP to the internationally agreed VSMOW-SLAP scale. The calibration chain to the left, which uses in-house RMs as the common reference, will not provide data that is comparable with the other three.*

5.2.1 Principle of Identical Treatment (PIT)

The concept of PIT was introduced by Werner and Brand in 2001 (Werner and Brand 2001) and aimed to reduce systematic errors that can arise from differences in how samples and RMs are prepared and analysed. This is often interpreted as requiring the use of matrix matched RMs such that the RMs have chemical and physical properties similar to samples.

For a laboratory dedicated to the analysis only a few sample types (e.g. wood) this may be possible but for a laboratory with a wider remit (e.g. food) this may not be practical because (1) RMs may not exist for all matrix types and (2) they may not span the necessary range of delta-values. In addition, a laboratory testing numerous sample types would need to hold stocks of a wide range of (expensive) RMs some of which may be used only infrequently.

A more pragmatic interpretation of PIT is that the inlet device coupled to the IRMS (EA, HTC-EA etc) must quantitatively convert a given element in both RMs and samples to the analyte gas. This can be verified by analysing carefully weighed aliquots of RM and sample and comparing the yields of gas. If this degree of identical behaviour can be demonstrated, it would be acceptable to use matrix matched QC materials to ensure quality.

The PIT must also extend to any sample pretreatments, such as extraction of an analyte from a complex matrix, derivatisation etc. Such preparative steps must be proven to either not affect isotopic compositions or have equal effect on RMs and samples.

The IT Principle has subsequently been expanded (Carter and Fry 2013a) to recognise the need for identical treatment of data for all RMs, QC materials and samples, including peak integration, sample size correction, blank correction etc.

5.2.2 Working gas

Sample data from some CF/IRMS instruments are initially calculated relative to the measured isotope ratio of a pulse of gas from a high-pressure cylinder, known as a working gas (WG). This gas is regularly but incorrectly referred to as a "reference gas" (RG) The term "reference gas" should only be applied to gas that has traceability to an isotope delta scale, meaning it has a known isotopic composition and which has been treated identically as samples have been within the analytical procedure e.g. CO_2 liberated from a carbonate RM by phosphoric acid digestion and introduced by DI is a RG for measurement of carbon dioxide liberated from sample carbonates using the same procedure. This is clear in the figures showing schematic diagrams of IRMS instrumentation in Chapter 6 in which DI/IRMS uses RG introduced in the same way as the sample, while the CF/IRMS instruments use WG.

The isotopic composition of gas delivered from a high pressure cylinder should remain relatively constant over the lifetime of the cylinder provided the gas is pure (especially free from water) and maintained at a constant temperature (R. Socki et al. 2020). It is recommended that cylinders are held in a temperature-controlled environment for 24 to 48 h to equilibrate. It is also recommended that the ambient temperature in the working gas cylinder storage area and gas cylinder head pressure are checked and recorded regularly (daily to weekly). If the flow of gas from the cylinder is stopped for a period of time and restarted it may take some time (hours) for the isotopic composition of gas reaching the IRMS to stabilise. This can be especially pronounced for light gases such as hydrogen and can be compounded by additional tubing on the high-pressure side of the regulator. Filters, other than simple frits, should not be incorporated in the WG supplies as these may cause isotopic fractionation.

Cylinders that contain more than one physical phase (i.e. gas, supercritical and liquid) such as carbon dioxide can exhibit large, temperature dependent isotopic fractionation between the phases (R. A. Socki and Jacksier 2021). Use of WG that is not in equilibration between existing phases in the gas cylinders and gas plumbing lines will result in varying measured delta values of the WG and errors being introduced in calculated raw delta values for samples.

In principle, the isotopic composition of the WG need not be known and any isotope delta value could be used as an estimate of its true isotopic composition for the calculation of initial isotope-delta values of samples by instrumental software.

In practice, a good estimate of the working gas isotope delta value will lead to the isotope-delta values for samples calculated by instrumental software to be relatively close approximations of the true values. It can also be an indication as to the suitability of a particular cylinder for use as a WG. For example, hydrogen produced by electrolysis has very little deuterium with hydrogen isotope-delta values typically being very negative and outside the usual range for sample materials (δ^2H –700 to –900‰). Similarly, carbon dioxide derived from carbonates often has a carbon isotope delta value that is close to zero or even positive which is very different to organic sources of carbon.

While instrumental software might need only one WG pulse to be nominated for calculation of initial raw isotope-delta values, it is usually possible to nominate more than one WG pulse for the calculation. In this way, users can select WG pulses from the beginning and end of a run which helps mitigate drift within a single analysis.

The use of multiple WG pulses recognises that the peak areas of WG pulses (and therefore measured isotope delta values) are not all identical – indeed manufacturers often have specifications for the sd of the isotope-delta values of multiple pulses of WG (section 7.4.2); however, instrument performance can fail to meet those manufacturer specification within individual sample analysis. There is therefore some uncertainty associated with the measurement of the WG during a single analysis that is often masked by software providing only a single isotope delta value for the sample gas with no apparent uncertainty.

To include the uncertainties associated with the measurement of the WG and with its known/assigned isotope delta value(s), users will need to perform the calculations involved between integrated peak areas and initial isotope-delta values outside instrumental software (Dunn et al. 2015).

Delta-values produced by comparison to a WG must be normalised using two RMs that have been evaluated against the same working gas to be reported on the appropriate delta scale (see 10.5).

5.2.3 Selection of RMs for calibration

There are several properties of RMs of isotope delta than should be considered when choosing RMs to use for calibration purposes. These include:

***Table 2**. Considerations for selection of RMs used for calibration of isotope delta measurement results.*

Property	Considerations
Assigned isotope delta value	Calibration by interpolation is preferred. RMs used for calibration should provide a range of isotope-delta values that encompass the expected values for samples. Wider calibration ranges are generally better, particularly if calibration by extrapolation is required (Paul, Skrzypek, and Fórizs 2007; Skrzypek, Sadler, and Paul 2010; Skrzypek 2013; Meier-Augenstein and Schimmelmann 2019; Balint et al. 2024)
Uncertainty in assigned isotope delta value	Uncertainties in the assigned isotope-delta values of RMs used for calibration contribute to measurement uncertainty for sample results (section 10.7). Selecting RMs with smaller assigned uncertainty may reduce overall measurement uncertainty. The length of the traceability chain of an RM back to the scale definition affects uncertainty. Uncertainties assigned to some RMs may be unreliable (Gröning 2023).
Matrix	Ideally, RMs used for calibration would be matrix-matched to samples following PIT (Werner and Brand 2001). See section 5.2.1. Given the limited number of different matrix RMs available, less strict adherence to PIT such as using organic RMs for organic samples and inorganic RMs for inorganic samples is the pragmatic approach. Where possible, abundance of the element(s) being studied should be closely matched. Matrix-matching is critical for sample preparation approaches involving equilibria.
Nature	RMs may be shipped in units that a particular laboratory cannot make use of (e.g. NIST SRM 8562-8564 are shipped in glass ampoules that require special equipment for use) RMs may be available in several different unit “types” (for example USGS82 honey is available in 1mL vials or in batches enclosed in silver tubing each containing either 0.15 µL or 0.25 µL of honey). One unit “type” may be more easily analysed than another. Associated uncertainties for different unit types may vary.

Property	Considerations
Cost	Commercial RM produced under accreditation to ISO 17034:2016 are generally expensive but of high quality. Cheaper alternatives may be available but are likely to be of a lesser quality. Use of commercial RMs to characterise in-house (or laboratory) RMs (Hélie and Hillaire-Marcel 2021; Dunn, Malinovsky, et al. 2021; Crivellari et al. 2021) can reduce costs for routine analyses (section 5.1.3).
Quality	Commercial RMs produced under accreditation to ISO 17034:2016 will have been extensively studied using validated and accredited (to ISO/IEC 17025:2017) measurement methods. Stability and homogeneity assessments will have been carried out and assigned values are reliable within their associated expanded uncertainties. Often measurements are only performed at one laboratory (however, validation under ISO/IEC 17025:2017 requires external validation, i.e. demonstration that results obtained are comparable to other laboratories). Other commercial RMs may have less extensive characterisation studies but might involve inter-laboratory efforts to increase reliability. Some RMs are provided with limited information regarding how their values have been assigned – these RMs are of little use for calibration purposes but may be of sufficient quality for use as QA or QC materials (chapter 11). Assessment of RMs by third parties can be valuable. The IUPAC CIAAW periodically produces an IUPAC Technical Report summarising RMs for isotope delta measurements and providing recommendations (Brand et al. 2014).
Availability	RMs may be out of stock/unavailable during re-assessment exercises. RM suppliers may place limits on the amount of RM available to a customer within a particular timeframe. RM suppliers may have difficulties shipping their products to particular locations. Primary RMs that define isotope delta scales such as IAEA-S-1 should only be used for routine calibration of samples where the zero uncertainty associated to their assigned values is critical to produce results with the smallest MU possible. Typically, this will be during characterisation of new RMs (commercially or in-house).

6 Instrumentation

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

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

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

6.1 Isotope ratio mass spectrometer

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

Electrons are produced by a resistively heated metal filament. Traditionally the filament is made from tungsten that operates at temperatures close to its melting point in excess of 3,000 °C. This hot metal surface can react with water and oxygen to produce tungsten oxide.

When hydrogen gas enters the source it will react with the oxide layer to form water that increases the abundance of H_3^+ ions (see section 7.6.1). Therefore, when an instrument is swapped between $\delta^{13}C$ and $\delta^{2}H$ measurements a period of time (up to one hour) is needed for the filament to condition and the ion source to stabilise (Brand 2004).

To negate problems associated with chemical reactions some instruments employ filaments made from other metals (e.g. rhodium / tungsten) or with special coatings that can operate at lower temperatures (Brand 2004).

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

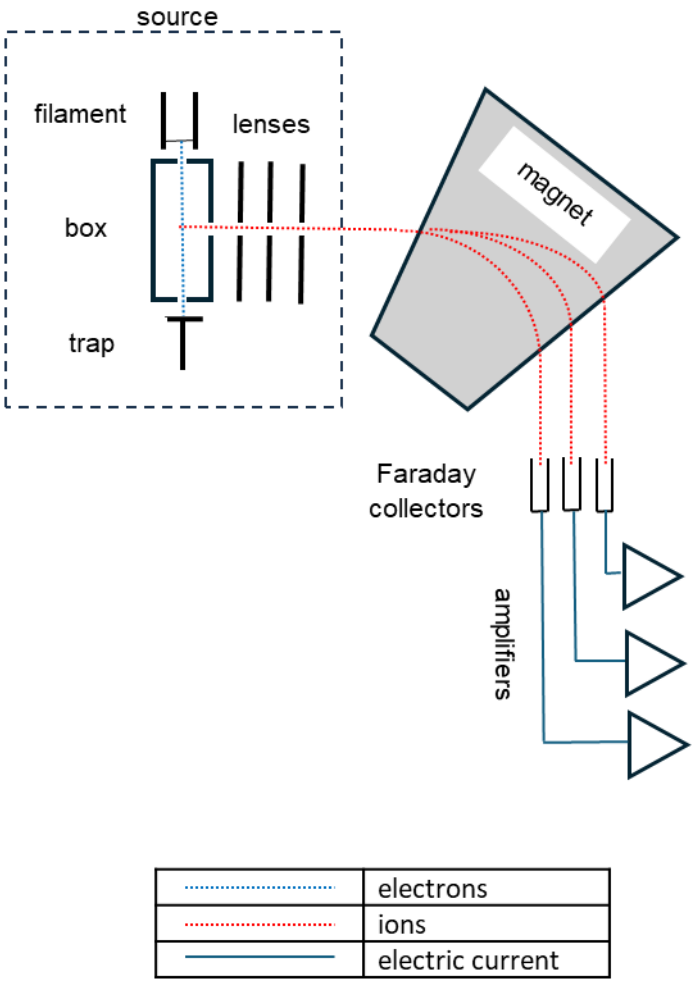

***Figure 3**. Simple schematic diagram of an isotope ratio mass spectrometer*

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

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

Each collector is connected to a dedicated amplifier whose gain is defined by a precise, high ohmic resistor. Each amplifier has a different gain such that ion ratios, at natural abundance levels, will produce similar signals. Typical absolute and relative amplifier gains are shown in **Table 4**. Some instruments provide an ability to switch the gain of certain amplifiers to facilitate the measurement of samples which that have been enriched (labelled) with stable isotopes, i.e. the relative abundance of the major and minor isotope may be close to unity.

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

***Table 3**. Mass-to-charge ratios and gas species that can be measured on low resolution (<250) isotope ratio mass spectrometers. Note: collector arrays and associated amplifiers will vary considerable between instruments and their configuration for a particular molecular gas species.*

Molecular gas species	Simultaneous mass-to-charge (*m*/*z*) ratios detected
H_2	2, 3
N_2	28, 29, 30
CO	28, 29, 30
NO	30, 31, 32
O_2	32, 33, 34
Ar	36, 38, 40
CO_2	44, 45, 46
N_2O	44, 45, 46
N_2, O_2, Ar, CO_2	28, 29, 32, 34, 36, 38, 40, 44, 45, 46
CH_3Cl	50, 52
SO_2	64, 65, 66
NO, N_2O	30, 31, 44, 45, 46
Clumped CO_2	44, 45, 46, 47, 48, 49
SO, SO_2	48, 49, 50, 52, 64, 64, 66
Kr	82, 83, 84, 86
SiF_4	85, 86, 87
Xe	124, 128, 129, 130, 132, 134
SF_6	127, 128, 129, 131

***Table 4**. Example detector amplification factors for an IRMS instrument. Not all instruments will use the same amplification.*

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

6.1.1 MS tuning

Operators often categorise an IRMS instrument as being tuned for either “sensitivity” or “linearity”. The first suite of parameters is intended to afford maximum signal intensity, the second to afford consistent ion ratios over a range of signal intensities. When tuning the mass spectrometer, operators should consider the range of sample peak intensities expected during analyses when opting to tune for sensitivity or linearity (e.g. tuning for linearity may be more important for CSIA analyses of mixtures by GC/C/IRMS where components are present at very different amounts).

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

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

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

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

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

6.2 Bulk stable isotope analysis (BSIA) techniques

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

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

DI/IRMS is generally considered to be the most precise method of measuring the isotope ratios of light elements. The technique however requires significant preparation and larger sample sizes than the CF methods described later.

The DI technique is briefly described here because:

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

Several authors have produced extensive comparisons of DI and CF/IRMS (Barrie and Prosser 1996; Brand 2004). Some of these differences are summarised in **Table 5**. The first studies using IRMS, using DI, were published before 1950 (e.g. Nier 1947) and the basic structure of the DI instrument has remained unchanged although advances in electronics and vacuum technology have improved both precision and ease of measurement.

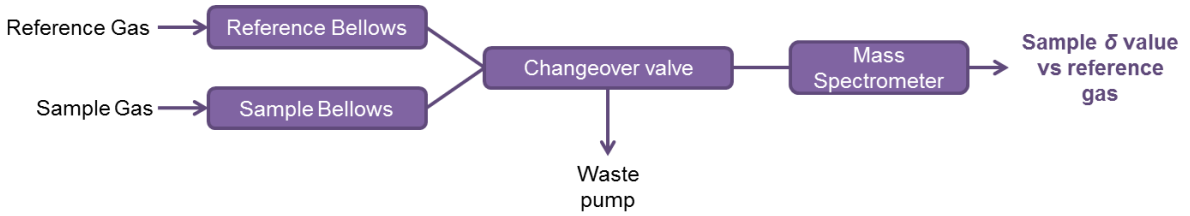

***Figure 4**. Simple schematic diagram of a DI/IRMS instrument for the determination of isotope ratio isotope-delta values. Note that the RG may be derived from a working standard rather than a primary calibration standard.*

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

***Table 5**. Comparison between dual-inlet and continuous flow techniques.*

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

The alternating, near identical, flow of sample and RG 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 RG during a DI isotope ratio measurement (McKinney et al. 1950). Typically, 5 to 10 pairs of sample and RG measurements are made for any one sample, which are typically averaged and an outlier filter may be applied.

The RG for DI isotope ratio measurement may be derived from a RM (e.g. CO_2 released from the offline acid digestion of IAEA-603), or it may be a WG (sometimes referred to a as transfer standard) for example a cylinder of high-purity CO_2 that has itself been directly calibrated to the reporting scale by DI/IRMS. Note that as sample and RG are introduced by identical means into a DI/IRMS instrument the term "reference gas" is used; whereas in continuous-flow techniques sample and WG are not treated identically and hence the term "reference gas" is not appropriate.

In contrast to continuous-flow techniques, an offset/shift correction (normalisation) can be applied through means other than the analysis of two or more RMs, for example by application of the so-

called cross-contamination or η correction (Meijer, Neubert, and Visser 2000; Srivastava 2022). This correction accounts for the possibility of a fraction of gas from the sample bellows, η, contributing to the gas from the reference bellows (and vice versa). The measured isotope delta value for the sample gas is then modified from equation (6) as follows (Meijer, Neubert, and Visser 2000):

$$\delta_{\text{meas}} = \frac{(1-\eta)R_{\text{sample}} + \eta R_{\text{ref}} - (1-\eta)R_{\text{ref}} - \eta R_{\text{sample}}}{(1-\eta)R_{\text{ref}} + \eta R_{\text{sample}}} \quad (11)$$

The isotope delta value for the sample corrected for cross-contamination can then be derived using the following expression (Meijer, Neubert, and Visser 2000):

$$\delta_{\text{true}} = \frac{\delta_{\text{meas}}}{1 - 2\eta - \eta\delta_{\text{meas}}} \quad (12)$$

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

DI isotope ratio measurements are commonly performed on samples of a pure gas prepared “offline”. Various reaction and clean-up processes, typically conducted in vacuum lines, may be employed quantitatively to convert a sample into a pure gas for introduction to a DI/IRMS instrument. Specific procedures are used to convert solids, liquids, dissolved gases, and gas mixtures into pure gases, and will not be described further (Groot 2004). These off-line techniques are usually time consuming, although some of the common methods have now been automated including: hydrogen and oxygen isotope ratio measurements of waters by H_2 and CO_2 equilibration; carbon and oxygen isotope ratio measurements of carbonates; and high precision carbon and oxygen isotope ratio measurements of atmospheric CO_2.

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

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

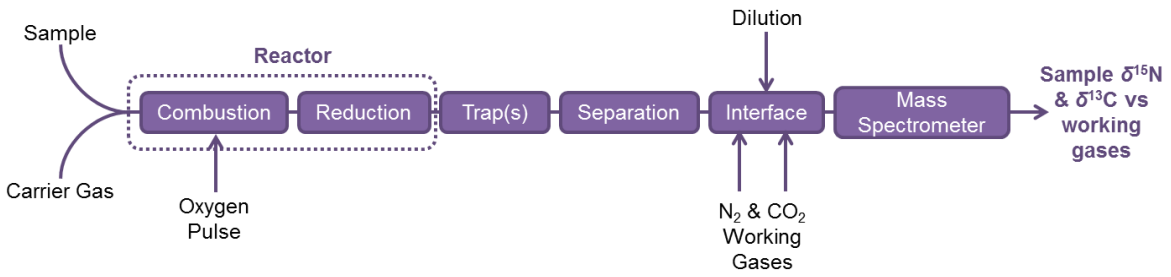

***Figure 5**. Simple schematic diagram of an EA/IRMS system for the determination of $\delta^{15}N$ and $\delta^{13}C$ values. Note that materials containing elements other than H, C, N and O can yield combustion products that may need to be removed by chemical trap(s).*

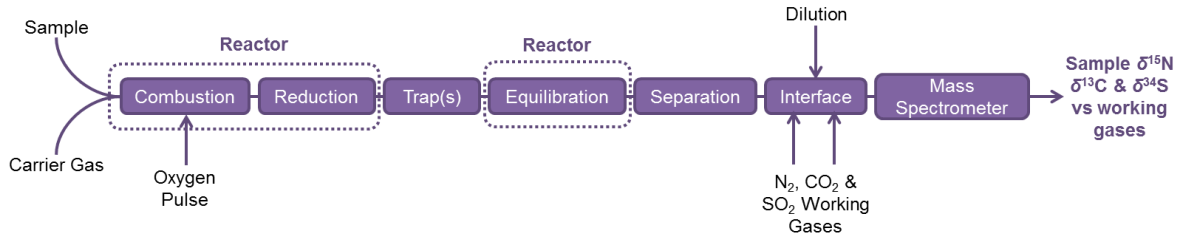

Figure 6. *Simple schematic diagram of an EA/IRMS system for the determination of $\delta^{34}S$ values in addition to $\delta^{15}N$ and $\delta^{13}C$ values. Note that the combustion, reduction and equilibration can all occur within the same reactor tube.*

6.2.2.1 Combustion (for nitrogen and carbon isotopic analysis)

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

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

Removal of excess oxygen and reduction of the NO_x to N_2 takes place at lower temperatures, either in a cooler part of a single tube or in a separate furnace, typically maintained at 600-900 °C. The reduction process typically relies on high purity elemental copper and, again, variations are recommended for specific applications.

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

Finally, N_2 and CO_2 are separated via an isothermal GC column packed with a stationary phase such as Porapak® QS. Some EAs are equipped with a GC that has multiple temperature set points, termed "ramped GC". The GC oven can be programmed as part of the method to increase the temperature to a higher set point, generally after the elution of the CO_2, to increase the speed of the elution of SO_2 gas. This results in faster sample analysis when multiple elements are measured from a single sample. This function can also be used when measuring a single isotope (N_2 or CO_2) to prevent SO_2 interfering during subsequent sample analysis.

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

6.2.2.2 Thermal decomposition (for nitrogen isotopic analysis)

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

The EA configuration for thermal decomposition is the same as for combustion (section 6.2.2.1) except the oxygen “pulse” is disabled and the method timing is changed slightly such that samples decompose at high temperature rather than combust. The net effect is a decrease in the amount of NO_x versus N_2 produced during thermal conversion of samples to gas.

6.2.2.3 Combustion (for sulphur isotopic analysis)

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

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

The copper used in the reduction stage of the reactor must be maintained at a higher temperature than for C and N analysis (830 to 910 °C) to ensure that copper sulphate does not form, which would lead to poor peak shapes and fractionation of measured sulphur isotope ratios (Dugan 1977). Packing with long copper wires is preferred as heat from the furnace is conducted along the length of the copper wires, resulting in even heat distribution. Copper particles distribute the heat in a less uniform manner. The surface area of the copper can also vary which can affect how SO_2 is transferred through the reactor, and which may result in carry-over. Reactor materials included from new can contribute a blank peak for sulphur.

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

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

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

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

Measurement methods other than gas source IRMS can also be employed to determine sulphur isotope ratios. Multicollector/ICP-MS and multicollector/TIMS have both been applied but are not within the current scope of this guide. The reader is directed to the following references as a starting point for these methods of sulphur isotope analysis (Pritzkow et al. 2005; Clough et al. 2006; Mann, Vocke Jr., and Kelly 2009).

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

High temperature conversion (HTC) refers to the Schütze/Unterzaucher process in which both organic and inorganic compounds are converted to H_2, N_2 and CO gases in a strongly reducing environment at temperatures between 1350 and 1450 °C (Schütze 1939; Unterzaucher 1952; Santrock and Hayes 1987). The system typically comprises an outer tube made from fused alumina and an inner tube made from “glassy carbon” (a brittle form of carbon with a randomized structure). The inner tube is filled with glassy carbon particles and silver wool intended to bind sulphur and halogen atoms. Similar to combustion EA, many variations are recommended for specific applications.

For oxygen measurements it is essential that the flow path is comprised only of glassy carbon or similarly inert materials. At elevated temperatures oxygen exchange will occur between CO and surfaces such as quartz and alumina. It is also possible to purchase tubes with various linings, such as molybdenum (which can also be helpful in analysis of hydrogen, see 6.2.3.2 below), for this application to provide a barrier between sample gas and the outer tube.

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

6.2.3.1 Water samples

The hydrogen and oxygen isotopic compositions of water samples can be measured using a glassy carbon reactor (described above) with a bottom-feed adaptor and topped with a stainless steel insert. The insert provides a small, hot volume to promote the evaporation of the water. The bottom-feed adaptor channels helium carrier gas upwards between the outer alumina tube and inner glassy carbon tube. The helium enters the glassy carbon tube at the top and flows down through the lumen exiting at the base. The overall operation is similar to a GC injector eliminating dead-volumes and producing sharper peaks.

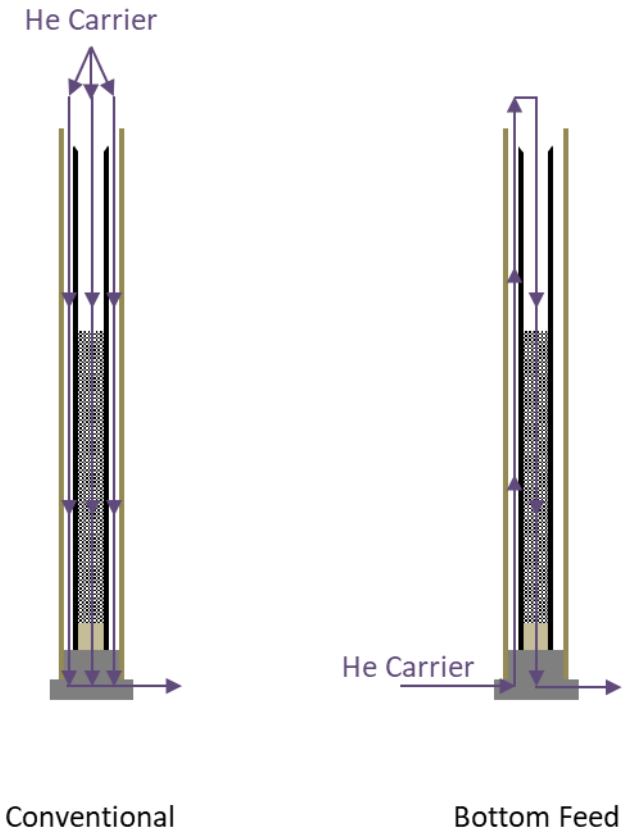

***Figure 7**. Comparison of helium gas flow paths for conventional and bottom-feed connectors in HTC instrumentation.*

The auto-sampler for solid samples is replaced with a liquid injection adaptor containing a high temperature septum. Injections can be performed manually or by a GC style liquid auto-sampler. The volume of water required for analysis is typically 0.15 to 0.25 µL which requires a 0.5 or 1 µL plunger-in-needle type syringe. The condition of the syringe is critical to obtaining good data and should be checked before and after an analytical sequence/batch. When poor results are obtained the first check should always be for consistent peak height, width and shape that will affect peak integration. Poor reproducibility typically points to a problem with the syringe.

An alternative IRMS method for hydrogen and oxygen isotopic compositions of water samples is equilibration with H_2 or CO_2 (see section 6.2.6).

Hydrogen and oxygen isotope-delta values for water can also be obtained using optical spectroscopy including cavity ring down spectroscopy (CRDS) and isotope ratio infra-red spectroscopy (IRIS). These techniques are currently outside the scope of this guidance.

6.2.3.2 Solid samples

The oxygen isotopic compositions of solid samples are measured using a glassy carbon reactor (described above) that may be topped with a graphite adaptor which serves to funnel samples into the hot zone. For oxygen isotopic analysis of nitrogen-bearing materials the baseline separation of N_2 and CO gas, which evolved from the sample in the reactor, is essential. The N_2 isotopologue $^{14}N^{14}N$ (*m/z* 28) is isobaric with the $^{12}C^{16}O$ isotopologue (*m/z* 28). Additionally, once the N_2 gas has been allowed to enter the ion source it combines with sources of oxygen, such as O_2 or H_2O, to form NO^+ ions ($^{14}N^{16}O^+$ *m/z* 30) which are isobaric with CO isotopologues that produce $^{12}C^{18}O^+$ ions (*m/z* of 30). The isobaric interferences from N_2 gas on the CO gas, especially in the form of NO^+ ions, result in a shift in the measured isotope delta value of oxygen by several permille.

Many analytical techniques in the last 40-years have included an isothermal GC column in the EA, for the baseline separation of N_2 and CO gas species. Multiple additional features have been proposed to avoid the measured oxygen isotope values being biased by N_2 and NO isotopologues, including

(1) diversion of N_2 away from the mass spectrometer (Böhlke, Mroczkowski, and Coplen 2003; Brand et al. 2009; Qi, Coplen, and Wassenaar 2011; C. Brodie 2023),

(2) applying maximum dilution to reduce the amount of N_2 gas entering the mass spectrometer (Accoe et al. 2008),

(3) the use of longer GC columns (Farquhar, Henry, and Styles 1997) with additional temperature and flow rate considerations to optimise baseline separation (C. Brodie 2023), and

(4) manual correction of raw peak area data to remove the isobaric interference on the *m/z* 30 peak area of the measured oxygen isotope value (Gehre and Strauch 2003; Accoe et al. 2008).

Additionally, purge and trap technology has proposed an N_2 diversion solution through CO adsorption and subsequent thermal desorption in a time event manner (Sieper et al. 2010).

When using techniques to divert or dilute nitrogen there is a compromise between time to remove the entire nitrogen peak and time to allow the baseline to stabilise before the CO peak elutes. These timings will need to be adjusted periodically depending on the size of the N_2 peak and the elution times of N_2 and CO. By default some software may attempt to define a background region before the CO peak. When diverting the N_2 peak this baseline region is likely to be very short and may still contain a contribution from N_2 it is, therefore, better to define a background region after the CO peak has completely eluted.

For hydrogen isotope analysis of nitrogen- and halogen-bearing materials the use of a reactor containing metallic chromium and possibly manganese (Morrison et al. 2001; S. T. Nelson and Dettman 2001; Renpenning et al. 2015; Gehre et al. 2015, 2017) is recommended. In a glassy carbon reactor, existing H-C-N bonds may be partially converted to hydrogen cyanide (HCN) and existing H-Cl bonds may be partially converted to hydrogen chloride (HCl), preventing quantitative conversion of organic hydrogen to H_2(g). Metal-based reactors capture chlorine and nitrogen, permitting quantitative, accurate and precise hydrogen isotope ratio measurements. Unfortunately, chromium-based reactors cannot be used for the analysis of oxygen isotopes due to the formation of chromium oxides, which are relatively stable even at the elevated temperatures employed. High purity metallic chromium is expensive compared to glassy carbon or manganese. Alternatively, as it is in the same chemical group as chromium but its oxides are less stable, tubes lined with molybdenum can help prevent HCN formation (particularly if also using silver capsules) making them potentially suitable for analysis of both hydrogen and oxygen isotope ratios.

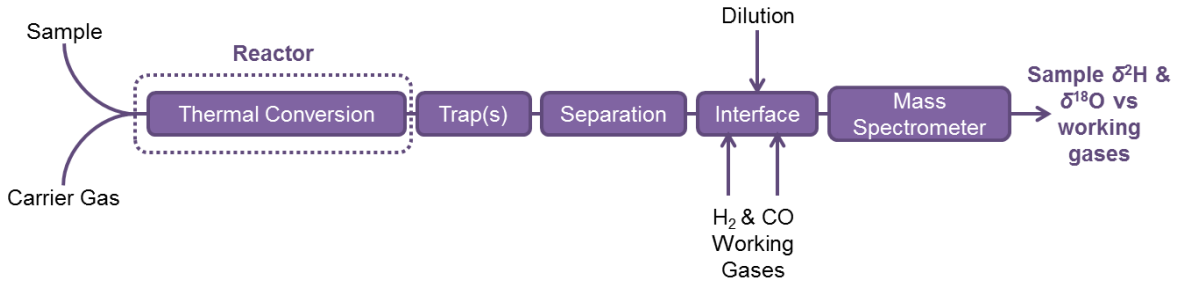

***Figure 8**. Simple schematic diagram of an HTC/IRMS system for the determination of δ^2H and $\delta^{18}O$ values. Note that materials containing elements other than H, C and O can yield products of thermal conversion that may need to be removed.*

6.2.4 EA and HTC Interface

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

These functions of the interface make it possible to carry out measurement of $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$ isotope ratios from one sample portion. Most organic compounds contain a relatively small proportion of nitrogen and sulphur and the three gases can be diluted to give similar signal sizes. In the same manner simultaneous measurements of both $^{2}H/^{1}H$ and $^{18}O/^{16}O$ isotope ratios via HTC-IRMS are possible, but typically are possible only if samples contain little or no nitrogen (e.g. liquid water, cellulose etc.). Distinct and mutually inconsistent analytical methods are recommended for H and O isotope ratio measurements of materials containing high levels of N that cannot otherwise be removed or corrected for without introducing bias (section 6.2.3).

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

LC/IRMS systems can afford the means to carry BSIA by bypassing the chromatographic separation step. This can be particularly useful for water soluble compounds, which are difficult to isolate in sufficient quantities for EA/IRMS analysis such as intact phospholipids, proteins, etc. This has also been referred to as µEA/IRMS (Krummen et al. 2004; Boschker et al. 2008; Langel and Dyckmans 2014).

Further details regarding the instrumentation and principle of such analyses can be found in the LC/IRMS section 6.3.2 below.

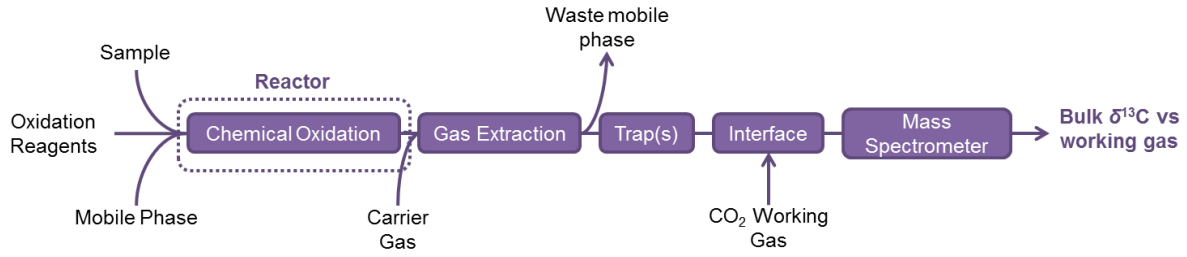

***Figure 9**. Simple schematic diagram of a FIA/CO/IRMS system for the determination of $\delta^{13}C$ values*

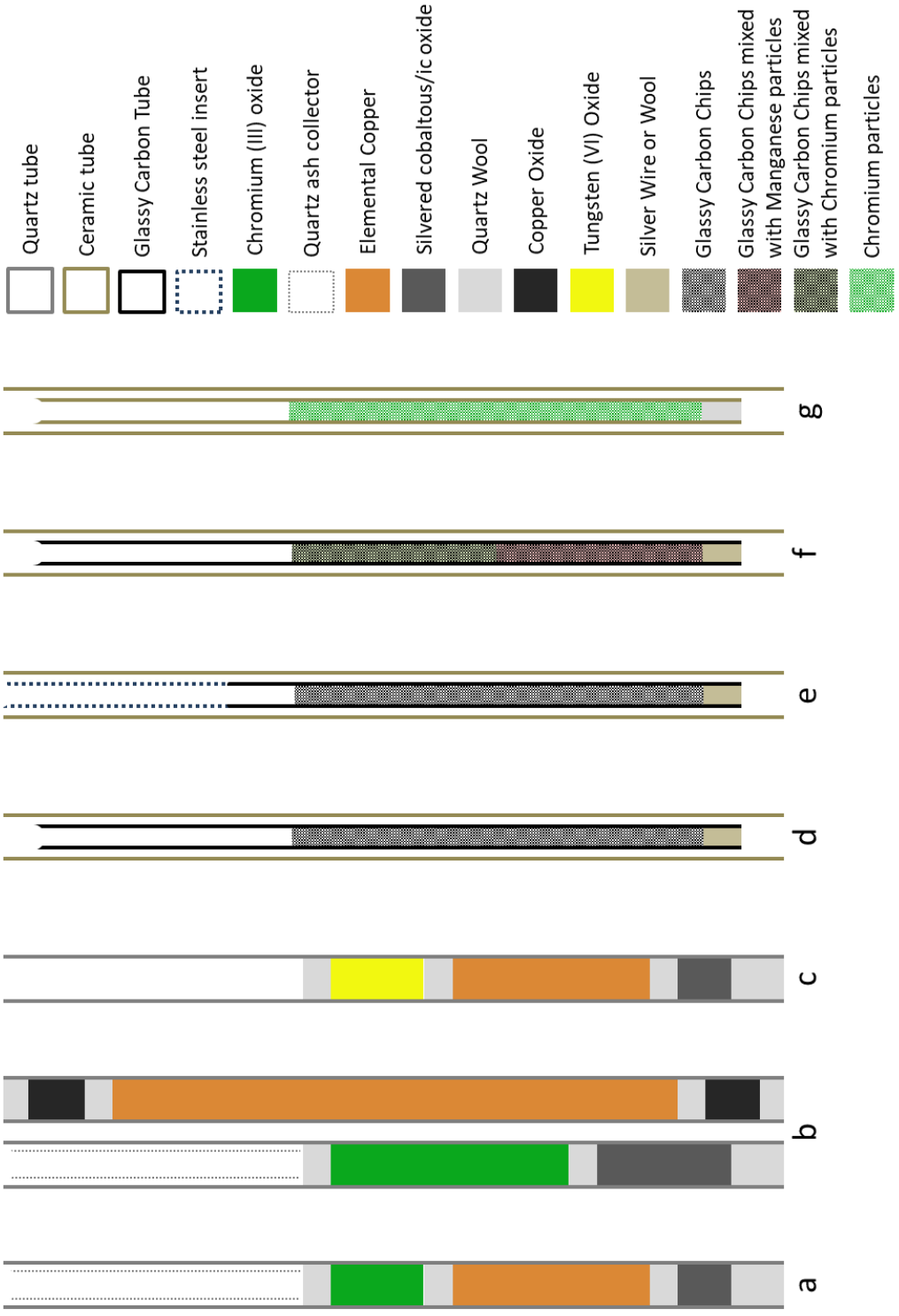

***Figure 10**. Schematic representation of example EA/IRMS and HTC/IRMS combustion, reduction and thermal conversion reactors (exact amounts of chemicals required will vary depending on instruments and applications; inserts may be present on top of inner reactor tubes; other packing schemes may be recommended by manufacturers): **a** – single combustion/reduction reactor for N and C analysis; **b** – separate combustion and reduction reactors for N and C analysis; **c** – single combustion reduction reactor for N, C and S analysis; **d** – HTC reactor tube for O analysis and for H analysis of materials that do not contain N or halogens; **e** – HTC reactor tube for analysis of liquid water introduced with a liquid autosampler; **f** & **g** – two different reactors for the H analysis of materials containing N and/or halogens.*

6.2.6 Equilibration-based methods for O and H analysis of waters

The basis for the stable isotope analysis of water was established during the Manhattan Project in the 1940s (Kirschenbaum 1951). Although IR instruments (CRDS and IRIS) can directly measure the isotopic compositions of pure waters, gas-source IRMS is not compatible with water vapour and instead measurements are made using an alternative gas as a proxy.

Historically, the stable hydrogen isotopic compositions of waters were measured via off-line conversion to hydrogen gas by reduction with chromium, uranium or zinc (Kirschenbaum 1951).

The oxygen isotopic compositions of waters can be measured by conversion to oxygen, by reaction with bromine pentafluoride and then to CO_2 by reaction with a heated graphite rod (O'Neil and Epstein 1966).

$$\mathrm{BrF_5 + H_2O \rightarrow BrF_3 + 2HF + \frac{1}{2}O_2} \quad (13)$$

Water can also be converted to hydrogen and carbon monoxide by HTC at 1450 °C (see 6.2.3.1) and, using this method both δ^2H and δ^{18}O can be measured from a single injection of water.

$$\mathrm{H_2O + C \rightarrow H_2 + CO} \quad (14)$$

Although chemically robust, this involves injecting nano-litre volumes of water into a very hot furnace (even the relatively cool upper part is hot) and any dissolved material can precipitate in the needle, quickly causing the plunger to seize.

For many decades now, measurements of δ^{18}O have been based on the principle of equilibrating water samples with CO_2 gas, the isotopic composition of which is then measured by MS.

$$\mathrm{C^{16}O_2 + H_2{}^{18}O \rightleftharpoons C^{16}O^{18}O + H_2{}^{16}O} \quad (15)$$

Initially, CO_2-H_2O equilibration was a manual, off-line process using large volumes of water (ca 25g) in large equilibration vessels (ca 250mL) (Epstein and Mayeda 1953).

A development of this method simultaneously evacuated then filled multiple vessels via capillary tubing. Water loss through the capillaries was found to be less than 0.1% and did not cause a measurable isotopic change (Roether 1970).

Most modern methodologies use variations of this methodology, operated by robotic sample handlers or re-purposed GC auto-samplers, a key feature being that gases are introduced and removed via capillary tubes. Compared to off-line methods, overall volumes are reduced with typically 200 to 300 µL of water in vessels of 4 to 12 mL capacity. Absolute volumes are not critical but must be consistent between all samples and RMs.

Modern equilibration vessels typically comprise commercially available heavy walled tubes with a screw top housing a thick chlorobutyl rubber septum; often referred to by the brand name Exetainer®.

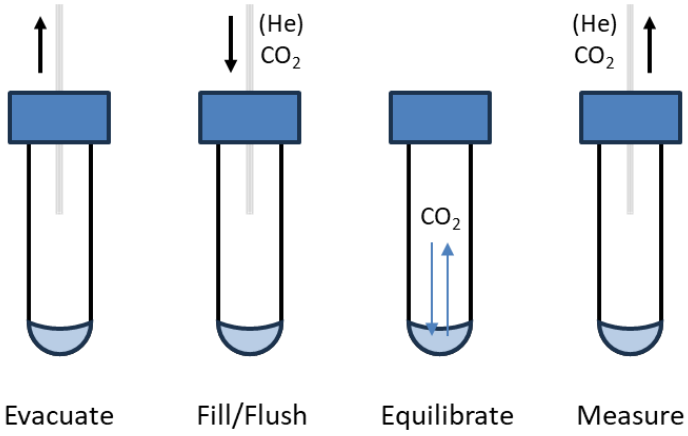

***Figure 11**. Schematic demonstrating automated operation for $\delta^{18}O$ measurements by equilibration.*

CO_2 does not isotopically exchange with oxygen bearing organic compounds such as primary, secondary or aromatic alcohols although exchange does occur with certain strong acids, aldehydes and ketones (Cohn and Urey 1938). A benefit of this is that CO_2-H_2O equilibration can be performed in the present of other, oxygen containing compounds and the technique is often used to determine the isotopic composition of water present in complex matrices such as fruit juice, wine (Hilkert and Avak 2016), plant tissue or soil (Scrimgeour 1995). This negates the need to isolate water from these matrices by vacuum or azeotropic distillation (Revesz and Woods 1990). When comparing equilibration with distillation, some authors have reported more enriched values produced by equilibration (Meyer et al. 2000)whereas others have reported a strong linear relationship with cryogenically extracted leaf water (Song and Barbour 2016).

Analogous to oxygen, the hydrogen isotopic compositions of waters can be measured from H_2 gas following H_2-H_2O equilibration. Following δ^2H measurement, the same vessels can be filled with CO_2 or He/CO_2 gas for $\delta^{18}O$ measurements (Horita et al. 1989).

An important development of this technique was the introduction of a hydrophobic Pt catalyst (0.8 to 3%) known as Hokko beads (Shoko Co Ltd), to facilitate equilibration (Ohsumi and Fujino 1986). Hokko beads are typically contained in small metal coils or bonded to glass or plastic rods that can be uniquely identified and associated with a dedicated equilibration vessel (Coplen, Wildman, and Chen 1991). Hokko rods or coils are cleaned by soaking in deionized water for 1 hr and drying overnight at 70 to 80 °C.

Despite precautions, Hokko rods may become contaminated and must be tested periodically, depending on the frequency of use and sample types. Ideally, a batch of catalyst is tested on receipt to establish suitable acceptance criteria. Testing involves using the catalysts to analyse a fixed volume of high purity water. A drift corrected, longitudinal plot of results will identify any results that deviate by more than a specified value, indicating damaged catalyst.

An alternative continuous-flow process for δ^2H measurements has been proposed in which water samples are completely vaporised and H_2-H_2O equilibration occurs at 100 °C (Huber and Leuenberger 2003). This method is reported to require only 5 µL of sample with an analysis time of approximately 5 min. Despite these apparent advantages, this method has not been widely adopted.

6.3 Compound-specific isotope analysis (CSIA) techniques

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

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

Only about 20% of known organic compounds can be directly analyzed by traditional one-dimensional GC because they need to be volatile and stable at the temperatures required for GC analysis (Ettre 2008). However, this encompasses a wide range of forensic and environmentally important compounds from those that are gaseous at room temperature, such as methane, to relatively involatile compounds such as polychlorinated biphenyls (PCBs). Coupling the separation afforded by GC to IRMS instruments requires an interface that can convert the separated compounds into the analyte gases needed for isotopic analysis.

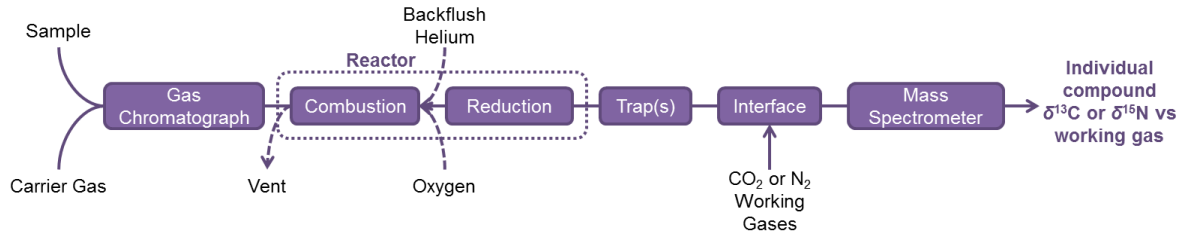

***Figure 12**. Simple schematic diagram of a GC/C/IRMS system for the determination of $\delta^{13}C$ or $\delta^{15}N$ values. Note that the combustion and reduction reactors can be combined into a single tube. Dashed lines show the gas flow in back-flush mode.*

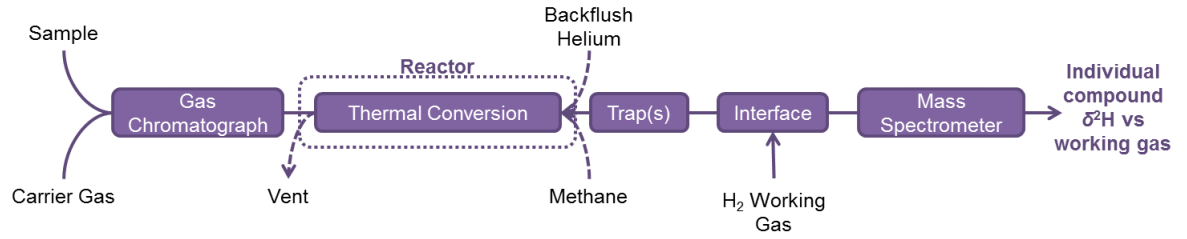

***Figure 13**. Simple schematic diagram of a GC/HTC/IRMS system for the determination of $\delta^{2}H$ values. Dashed lines show the gas flow in back-flush mode.*

6.3.1.1 Gas chromatography

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

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

Early applications (mainly by existing IRMS users) considered the technique from the first viewpoint and employed wide-bore columns (0.32 mm) with thick phases (1.0 μm and above) in order to introduce large samples to the IRMS. In practice, because the peaks from a GC are considerably narrower than from an EA, high instantaneous concentrations are produced and acceptable results can be achieved from 10 to 100 ng of carbon (or other elements). With this understanding virtually any GC column can be coupled to an IRMS instrument regardless of diameter, length or phase thickness, with two possible exceptions. Many GC/IRMS interfaces are not well suited to high temperature applications (> 300 °C) due to the use of polyimide ferrules, which can quickly become loose and leak with repeated temperature cycling. Also, columns that are not chemically bonded, notably Porous Layer Open Tubular (PLOT) columns, can be damaged by repeated pressure changes associated with switching between “straight” and “back-flush” modes (see details in section 6.3.1.2).

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

Early applications of GC/IRMS favoured split-less or on-column injection techniques in an attempt to avoid isotopic fractionation. However, in practice it is virtually impossible to introduce a sample into a GC column without some isotopic fractionation, regardless of injection technique. Therefore the injection method should be optimised for chromatographic separation provided that RMs are introduced using the same method (see PIT, section 5.2.1). Some applications, especially forensic ones, divide the GC effluent between an organic mass spectrometer and an IRMS to obtain confirmatory identification and stable isotopic composition from a single injection – the organic MS typically requires a very small proportion of the sample.

This combined technique has been limited due to technical challenges with the splitting hardware between an organic MS (under vacuum) and the combustion interface (at atmospheric pressure). A splitter must also be able to withstand the temperatures inside a GC oven and optimal performance requires all components to be inert and have low thermal mass and dead volume.

One such device is a dome splitter with one input flow (the GC effluent), two output flows and two control inputs (Boeker, Haas, and Schulze Lammers 2013). Recently new monolithic microfluidic devices (the Deans' switch) have become available in which the whole flow system is integrated into a small metal device with low thermal mass and leak-tight connections (Boeker et al. 2013).

6.3.1.2 Combustion (C) interface

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

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

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

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

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

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

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

The GC/IRMS interface operates in two modes often referred to as "straight" and "back-flush". In straight mode the GC effluent passes through the reactor, dryer and open-split as described above. In back-flush mode the effluent is vented before it reaches the reactor, typically using a mechanical valve inside the GC oven. Also in back-flush mode a small amount of helium flows backwards through the reactor (i.e. from the open-split to the GC oven) to ensure that no GC effluent enters the reactor.

The primary purpose of back-flush mode is to divert the GC solvent peak, which will be many orders of magnitude larger than the sample peaks. Combusting a relatively large quantity of organic material may damage the reactor (or seriously deplete its oxidation capacity) and the pressure surge from the gas evolved may damage down-stream components, including the ion source. Back-flush mode is also used when passing oxygen through a reactor (for re-generation) to avoid these

gases entering the mass spectrometer. It is good practice to introduce pulses of working gas while the interface is in back-flush mode so that the baseline is as stable and reproducible as possible. After the solvent peak has been fully diverted the interface will be switched to straight mode, usually controlled as a timed function from the instrument software. Following the switch from back-flush to straight mode it will take some time for the baseline to stabilise and it is important that this is achieved before any peaks of interest elute.

The additional components and connector of the GC/IRMS interface will inevitably lead to peak broadening and chromatography may need to be developed to achieve baseline resolution of CO_2 or N_2 gas peaks entering the IRMS. Note that no algorithms exist to deconvolute coeluting peaks for GC/IRMS unlike organic GC/MS in which each peak corresponds to a different compound with a unique spectrum.

6.3.1.3 High temperature conversion (HTC) interface

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

The hydrogen in organic compounds is converted to H_2 by the Schütze/Unterzaucher reaction at around 1400 °C (Schütze 1939; Unterzaucher 1952; Santrock and Hayes 1987). The reaction requires the presence of carbon that is typically introduced by analysing a number of samples until a small deposit of carbon is formed on the lumen surface. A carbon deposit can also be formed by passing methane through the reactor in back-flush mode (6.3.1.2).

It is possible to measure $^{18}O/^{16}O$ ratios using GC/HTC/IRMS to convert organic oxygen to CO. This application employs a modified non-porous alumina tube containing an inner platinum tube with nickel wire operated at approximately 1280 °C. An additional T-piece is also needed before the reactor, to mix a very small flow of hydrogen into the GC effluent. A review of this technique (Hitzfeld, Gehre, and Richnow 2017) found that both commercially available and bespoke reactors did not achieve quantitative conversion to CO with significant amounts of CO_2 being formed. The application of this technique is limited by technical complexity and by the low abundance of oxygen in most organic compounds but has been applied to the analysis of water (Wang et al. 2015).

Like the GC/C/IRMS additional components and connector will inevitably lead to peak broadening. These effects are typically less pronounced as the HTC reactor is an empty tube without packing.

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

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

6.3.2.1 Liquid chromatography

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

6.3.2.2 Chemical oxidation (CO) interface

The key components of an LC/CO/IRMS system are shown in **Figure 14**.

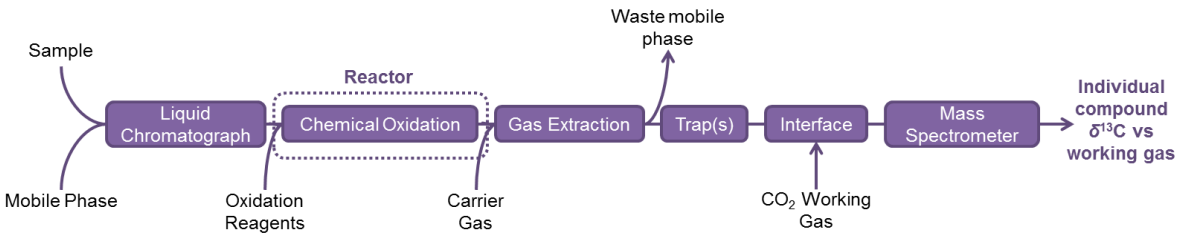

***Figure 14**. Simple schematic diagram of an LC/CO/IRMS for the determination of $\delta^{13}C$ values.*

Most commercially available LC/IRMS interfaces operate via a chemical oxidation procedure akin to TOC analysers in which the components of a mixture are separated by HPLC, and then oxidised to CO_2 while still in solution. Oxidation is performed by sodium persulfate at just below the boiling point of water. A silver nitrate catalyst can be added for difficult-to-oxidise compounds. An example for the chemical oxidation of ethanol follows:

$$6[S_2O_8]^{2-} + C_2H_5OH + 3H_2O \rightarrow BrF_3 + 12[SO_4]^{2-} + 2CO_2 + 12[H]^+ \quad (16)$$

A gas separator then extracts the CO_2 from solution aided by the addition of phosphoric acid. The gas stream is then dried via a Nafion® membrane and passed to the IRMS through an open-split.

The chemical oxidation process precludes the use of organic mobile phases during LC separation because these would also be oxidised to CO_2 and swamp the relatively tiny sample signals. mobile phases must be limited to water and inorganic buffers or acids. If the LC system has been previously used with organic mobile phases these must be thoroughly flushed prior to LC/CO/IRMS use, which will require large volumes of carbon-free mobile phase (i.e. water). Care must also be taken with the HPLC columns used for compound separation as these can also be contaminated by previous use with organic mobile phases. The use of an LC system dedicated to LC/CO/IRMS analysis and thereby guaranteed to be free from carbon-containing mobile phases is recommended.

Some functional moieties are more difficult than others to oxidise under the conditions used in the chemical oxidation interfaces. For example, incomplete oxidation has been observed even for some relatively simple chemical structures such as caffeine (Zhang et al. 2011, 2012), bentazone (Reinnicke, Bernstein, and Elsner 2010) and sulfonamides (Kujawinski et al. 2012). It has also been shown that carbon atoms bonded to two or three nitrogen atoms or within aromatic N-heterocycles can be particularly difficult to oxidise quantitatively (Cueto Díaz et al. 2013). Halogenated compounds can also be difficult to oxidise when three halogens are bound to the same carbon atom such as trichloroacetic acid and trifluoroacetic acid (Gilevska, Gehre, and Richnow 2014).

Testing for oxidation efficiency is therefore important prior to such analyses (section 7.8.3).

6.3.2.3 Other chemical reactions

The LC/CO/IRMS interface can be adapted for other chemical reactions that yield CO_2 as a product. The only published application to date uses the reaction of amino acids with ninhydrin (2,2-dihydroxyindane-1,3-dione) quantitatively to liberate CO_2 from the carbonyl group of the amino acids (LC/NR/IRMS) (Fry et al. 2018). As only the carbon atom present at the carbonyl group is converted to CO_2, the technique is also referred to as position specific isotope analysis (PSIA) although some amino acids cleave two carboxyl groups, e.g. Ala and Glu.

6.3.2.4 Combustion (C) interface

A novel combustion system that allows coupling of LC-to-IRMS instruments for both N and C isotope ratio determinations is in development. This consists of a modified high-temperature combustion TOC analyser. The modifications include a three-step drying system to handle the continuous flow of water, favourable carrier and reaction gas mix and flow, and an efficient high-

temperature oxidation and subsequent reduction system (E. Federherr et al. 2014; Kirkels et al. 2014; Eugen Federherr et al. 2015; E. Federherr, Kupka, et al. 2016; E. Federherr, Willach, et al. 2016). This is a relatively new technique and it is not discussed in further detail within this edition.

6.4 Miscellaneous techniques

There are numerous other peripherals that may be connected to IRMS instruments for specific applications. As these are less commonly applied in forensic studies they are only briefly described below in **Table 6**:

***Table 6**. Miscellaneous peripheral instruments that can be coupled to isotope ratio mass spectrometers*

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

7 Instrument set-up and preparation

7.1 Environmental control and monitoring

Environmental conditions will influence the reliable operation of IRMS instruments and their peripherals. Manufactures will specify environmental conditions within which their instrumentation has been designed and validated to operate and meet analytical performance specifications.

Analysts and laboratories should comply with instrument manufacturer's environmental requirements unless they can provide evidence to the contrary. It is generally good practice for a laboratory to maintain records of environmental conditions especially during instrument installation and or when new instruments or peripherals are added to the laboratory environment.

Laboratories are referred directly to the manufacturer and their environmental requirements that are readily available on request; however, typical requirements might include:

- temperature controlled within e.g. ±1°C/hr,
- relative humidity within a controlled range (e.g., between 40% to 60%),
- free from interfering magnet fields (e.g., from other instruments, electrical cables and water installations, local infrastructure and radio stations, elevators),
- free and protected from sources of electrostatic discharge,
- free from vibrations and/or vibrational shock across the floor / tables that instruments are installed,
- protection from power sags, surges and/or transients,
- gas lines that are leak free with the required head pressure,
- gas cylinders are stored in a temperature regulated environment with an appropriate exhaust system and detection system,
- gas supplies meet minimum purity requirements (typically 99.999% or greater). Inline filters to remove remaining impurities may be required – for example a hydrocarbon filer to remove traces of fluids used during tubing manufacture. The use of self-indicating filters is beneficial,
- peripheral preparation and inlet devices are connected to the same gas supplies and do not have separate cylinders or additional filters. This ensures that the background gases in the ion source remain stable,
- installations of instruments and laboratory fixtures and fitting comply with all local legislation.

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Operating instrumentation in sub-optimum environmental conditions can significantly undermine analytical reproducibility and repeatability and operating outside of specifications is strongly discouraged.

7.2 Safety equipment

Many of the gases used in the routine operation of IRMS are hazardous (e.g. H_2, CO, O_2 and SO_2) and the laboratory should have monitoring systems to warn of dangerous gas levels.

Specialised techniques (DI/IRMS and GC/C/IRMS) also require liquid nitrogen in which case the oxygen level in the laboratory should be monitored. Checking that these warning systems are functioning correctly should be an integral part of the routine instrument checks.

Chemical hazards are also present in the materials used for EA and HTC rector packing, FIA- and LC/CO/IRMS oxidation, moisture traps, etc. Detailed discussion of these is outside the scope of this document, however users of IRMS systems should be aware, have suitable precautionary measures in place and have completed a detailed risk assessment.

7.3 Testing routine

It is important to ensure that the system is working properly both at the beginning of the measurement process and throughout the sequence/batch 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/batch of measurements. The rota of tests and

their frequency should be documented in laboratory operating procedures and the accompanying records must exist, for example, in the form of an instrument logbook and/or spreadsheets.

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

Regardless of application(s) or instrument configuration(s), daily system checks should begin with a scan of the background gases in the instrument (section 7.4.1). If these are beyond the normal range there is no point in proceeding with other tests as an investigation will be needed. If the background scan is acceptable the next step is a zero-enrichment (on-off) test of the instrument precision with respect to a reference or working gas (section 7.4.2). Again, if the result of this test is poorer than expected the cause must be investigated. If both background and zero-enrichment tests are successful the final test is to measure the linearity of the instrument, i.e. how the measured isotope-delta value changes with peak size (section 7.4.3).

Other tests may be needed for specific instrumental configurations and/or analyte isotope ratios (e.g. H_3^+ factor, section 7.6.1).

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

7.4 Mass spectrometer tests

7.4.1 Background gases

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

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

Figure 15 shows a typical background of residual gases for the EA/IRMS configuration. Typically the intensity of *m/z* 18, 28, 32, 40 and 44 should be recorded. Background monitoring should also include *m/z* 2 when performing hydrogen isotope measurements. Acceptable values must be determined for individual instrument configurations. Note that LC/CO/IRMS systems typically have background values much higher than for EA/IRMS or GC/C/IRMS systems, and these may need to be recorded using a Faraday collector with lesser amplification.

Table 7. lists some possible causes of problems with background values (see also section 12 on troubleshooting for further information).

***Table 7**. Typical problems with background gases and possible causes in EA/IRMS and HTC/IRMS instruments.*

m/z	Mol. species	Problem and possible cause
2	He^{2+}	High background in $^2H/^1H$ measurements Electron energy can be adjusted to produce acceptable values – lower electron energies are typically used for hydrogen measurements than for applications using a triple collector
18	H_2O^+	Produces protonated species which may interfere with ions containing heavy isotope
28	N_2^+	Guide to ingress of atmospheric gases (also CO by thermolysis)
32	O_2^+	Bleed from EA oxidation catalyst.
40	Ar^+	Best guide to the ingress of atmospheric gases

m/z	Mol. species	Problem and possible cause
44	CO_2^+	Contamination of C/N analysers or oxygen ingress into H/O analysers

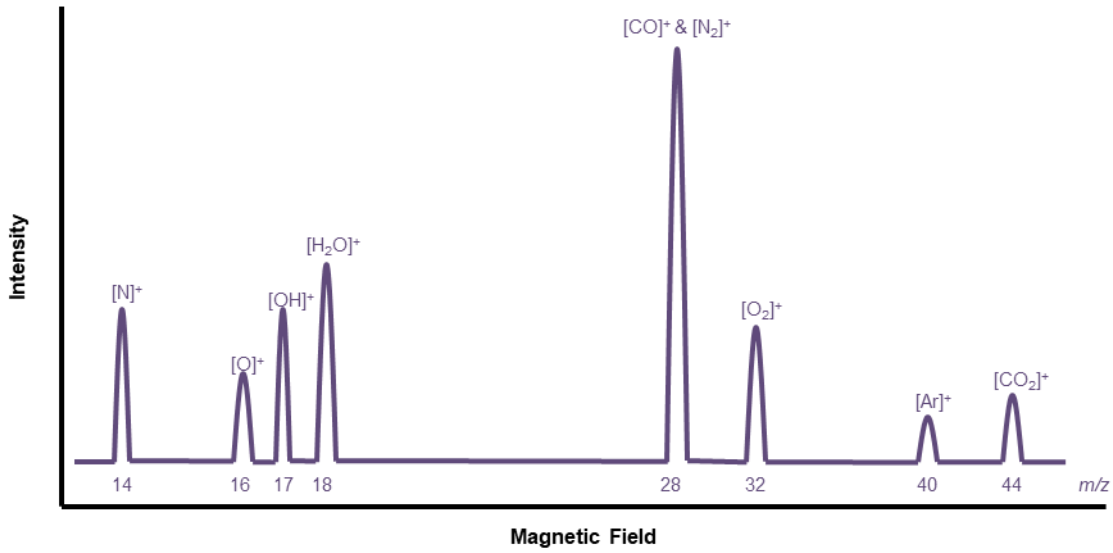


***Figure 15**. Example background scan consisting of a plot of magnetic field strength and hence m/z against signal intensity.*

7.4.2 Stability (zero-enrichment or on-off)

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

The measurement, known as “zero-enrichment” or “on-off” test simply involves introducing sequential pulses of WG (typically ten) into the instrument and recording the sd of the isotope-delta values, relative to one pulse with an assigned value (**Figure 16**). This test is best performed with the intensity of the gas pulses set within the anticipated range of sample peak heights.

Gases that might be regarded as “sticky” such as CO, CO_2 and SO_2 will require more space between peaks than gases such as H_2 and N_2. When setting up these methods it is important to examine the baseline closely and ensure that peak spacing is sufficient to negate any incremental change following each peak. Despite this the first peak of the sequence/batch may still have an anomalous delta value and may be exclude for the calculated sd.

As with all performance tests, acceptance criteria must be established for a specific instrument. In the first instance, the manufacturer’s specifications should be consulted, especially when considering the purchase of a new instrument to ensure it meets the requirements for the intended application.

In addition to calculating the sd for the delta-values of the gas pulses it is good practice to plot these values to identify any trend or drift that may indicate that the system is not fully stabilised.

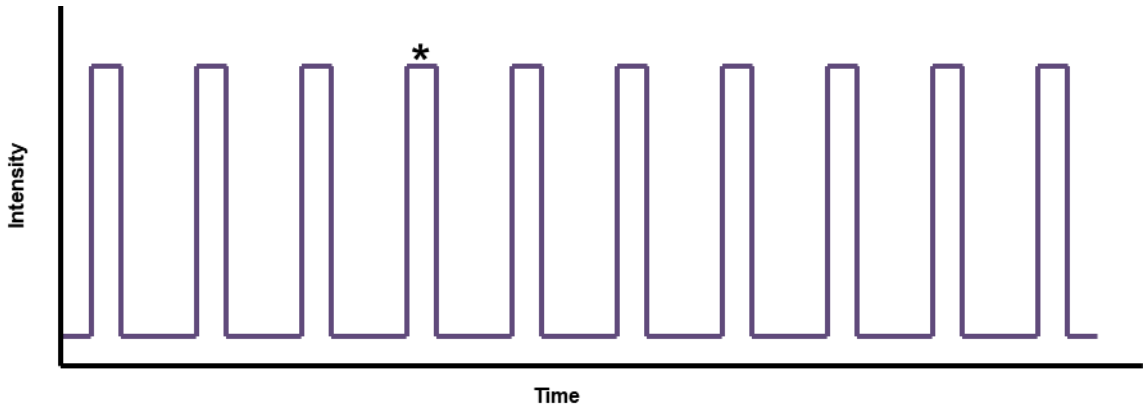


***Figure 16**. Example of a zero-enrichment check or on-off test. The fourth peak (*) is assigned a value of zero.*

7.4.3 Linearity (peak size)

Periodically (if not daily), and where analysing samples with changing concentrations of an element of interest, the linearity of the isotope ratio MS must be tested either by using (i) pulses of WG with varying intensities or (ii) analysis of a sample matrix at varying weights / injection volumes. The latter is most easily achieved on GC/ and LC/IRMS instruments where the injection volume can be varied. For EA/ and HTC/IRMS instruments, careful weighing of differing amounts of sample can be used.

The linearity measurement on the IRMS is similar to the zero-enrichment test, except that the intensity of gas peaks change (e.g. **Figure 17**).

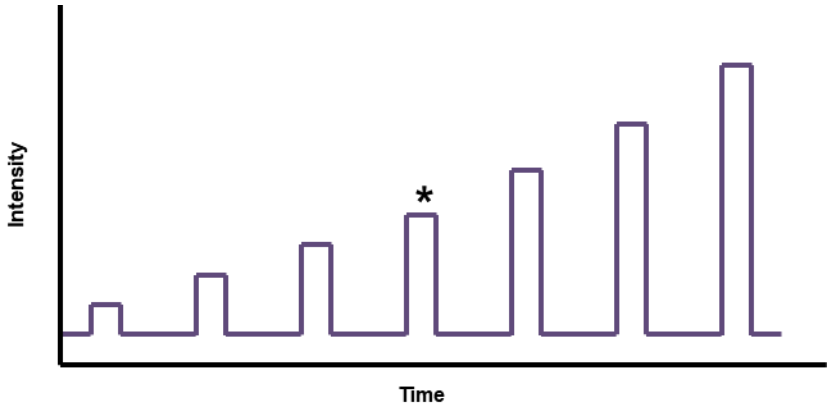


***Figure 17**. Example of a linearity check using working gas. The fourth peak (*) is assigned a value of zero.*

Where a sample matrix is introduced in varying amounts, it is possible that matrix related effect will be observed (section 10.6.3) and linearity determined using WG is not necessarily the same as linearity detected using a sample matrix injection (Balint et al. 2024). There are many confounding factors within the analysis of real samples that can appear as an effect that is proportional to amount of element or sample analysed. These include tuning, combustion dynamics, environment, sample condition, water content, capsule type, sample treatment, sample mineral content, sample weight, dilution applied, etc. As a result, WG tests can show an IRMS is operating within specifications, yet isotope delta values from sample analysis by EA/IRMS may exhibit a linearity effect (Balint et al. 2024).

Therefore, a laboratory should only use WG pulses to correct changes in isotope delta value of samples if there is evidence to support the similarity in linearity effect between samples and WG pulses for any given instrumental application.

The linearity test is not applicable to $^{2}H/^{1}H$ measurements, which require a daily H_3^+ factor determination (sections 7.6.1 and 10.4.2).

7.5 EA/IRMS tests

The backgrounds (section 7.4.1) should be determined not only for the mass spectrometer alone, but also with the EA connected. The differences in the background levels and performance between these two configurations can help identify the source of any problems. Stability and linearity tests should be carried out with the EA connected to the IRMS instrument. Any jumps between gas configurations should be tested (section 9.1).

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

- cleaning/replacing the ash collector (if used),
- replacing the oxidation reactor reagents,
- replacing the reduction reactor reagents,
- replacing the trap reagents (e.g. water and CO2), and
- baking the GC column.

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

7.6 HTC/IRMS tests

As with EA/IRMS configurations, the backgrounds should be determined for the mass spectrometer with the HTC connected. A stability test should be performed but a linearity test is only needed for oxygen isotope measurements. In the place of a hydrogen isotope linearity test, a so-called "H_3+ factor" determination must be performed (section 7.6.1):

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

- emptying/replacing the graphite crucible,
- cleaning or replacing the reactor components,
- replacing the trap reagents (if used), and
- baking the GC column.

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

7.6.1 H_3^+ Factor

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

$$H_2^+ + H_2 \rightarrow H_3^+ + H^\bullet \qquad (17)$$

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

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

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

7.7 GC/C/IRMS tests

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

7.7.1 Backgrounds

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

- background scan with the interface in “back-flush” mode and
- background scan with the interface in “straight” mode.

The differences between background gas intensities in “back-flush” and “straight” modes can identify leaks in the system (resulting in increased argon background); contamination (increase in water/CO_2); loss of reactor efficiency/indicator of need for reactor reconditioning (reduced O_2); or loss of water trap efficiency/indicator of need to change the Nafion® membrane (increased water).

7.7.2 Argon injection test

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

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

It is good practice to calculate the “hold up time” of the GC column (the time for an unretained compound such as argon to pass through) based on column length and carrier gas flow. The difference between this time and the RT of the argon peak corresponds to the volume of the interface which should not change.

7.7.3 Hexane vapour injection test

The GC oven should be set to a temperature at which the column will not retain hexane (typically > 100 °C) and the IRMS should be set to monitor *m/z* 44 on the first collector (relative gain 1).

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

The peak should have a retention time very close to that of argon and individual isomers (typically but not always present) should be resolved (depending on the column stationary phase). As with the argon test above (section 7.7.2), poor peak shape indicates a problem with the system, in this case poor combustion.

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

7.8 LC/CO/IRMS tests

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

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

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

7.8.1 Stability of CO_2 background

The stability of the carbon dioxide background level measured on *m/z* 44 should be monitored for a period of at least 5 minutes before an analytical sequence/batch. The Faraday collector to use will be specified by the manufacturer. The standard deviation of the ion current should be below the manufacturer's recommended threshold or established empirically.

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

7.8.2 Back-pressure

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

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

7.8.3 Oxidation efficiency for new matrices

As noted above (section 6.3.2.2), various classes of compounds and functional groups are not quantitatively oxidised to carbon dioxide under the usual persulfate/phosphoric acid conditions. Assessment and optimization of oxidation efficiency is therefore essential when analysing new matrices (Köster et al. 2019; Niemann et al. 2025).

A four step process for optimization of oxidation efficiency has recently been proposed (Niemann et al. 2025):

(1) Examine the chemical structure of compounds of interest for
- substituents with –I or –M effects,
- heterocycles,
- conjugated C=N bonds,
- multi-halogenations; and
- steric hinderances.

(2) Choose initial oxidation conditions,
- concentration of phosphoric acid,
- concentration of persulfate,
- mixing ratio.

(3) Test for inefficient oxidation by
- stepwise reducing reactor temperature and examining the corresponding change in obtained carbon isotope delta,
- comparison of the relationship between obtained peak area and sample concentration for the new matrix and a material known to oxidise quantitatively under the standard conditions (e.g. bicarbonate) – can be done by FIA/CO/IRMS,
- checking linearity of peak area against concentration of sample (i.e. amount of carbon injected) – can also be done by FIA/CO/IRMS,
- checking obtained carbon isotope-delta values for dependence on injected amount of carbon – can also be done by FIA/CO/IRMS,
- recognizing that deterioration of peak shape, double peaks and/or excessive tailing are all indications of severe oxidation inefficiency.

(4) Optimization of oxidation conditions

- increase concentration of oxidant,
- where chromatography permits, increase reaction time,
- add silver nitrate ($AgNO_3$) – a last resort,
- change pH,
- increase reactor temperature to beyond 100 °C.

7.9 Other instrumental condition tests

The following tests are performed less frequently than routine (daily and weekly) tests. Typically these tests will be performed when routine tests repeatedly fail and should be performed following any maintenance, especially involving the ion source. Some of these tests may be specific to certain instrument manufacturers and may only be available to service technicians.

A sequence of tests progressing from amplifier to signal stability to system stability can help to locate problems originating from the detection electronics, ion source electronics or the high voltage and magnet power supplies.

As with other tests, it is important to perform these tests when the instrument is performing satisfactorily so that unacceptable results are readily identified.

7.9.1 Amplifier test

This test provides information about the background noise of the detector electronics with no ions present. For this test the filament emission is set to zero but the high voltage must remain on. The signal intensity from each Faraday collector is measured many hundred times over several minutes and a sd calculated. The results of this test can identify instability in the ion detector electronics.

7.9.2 Signal stability test

The signal stability test measures fluctuations in the intensity of ions from a WG or RG on the apex (centre) of the ion peaks (**Figure 18**). The results of this test are indicative of the stability of the ion source electronics (lens voltages, filament current etc) and the ion detection electronics (measured by the amplifier test 7.9.1). Typically the signals from the Faraday collectors corresponding to *m/z* 44, 45 and 46 are monitored over several minutes and a sd (normalised by intensity) and a regression slope are calculated for these data. The first parameter is indicative of noise caused by the source and detector electronics and the latter is indicative of any temporal drift.

7.9.3 System stability test

The signal stability test is measured at the flank of the peak, typically at 50% of the peak height (**Figure 18**). The results of this test indicate the stability of the high voltage supply to the ion source and cumulative noise from the detection and ion source electronics (measured during the tests described in sections 7.9.1and 7.9.2) plus contributions from the magnetic power supply and gas flow.

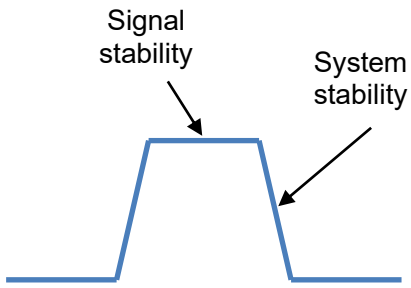


***Figure 18**. Schematic representation of the difference between "signal" and "system" stability measurements.*

7.9.4 Absolute sensitivity test

The absolute sensitivity of an IRMS instrument is usually expressed as the number of molecules required to generate one ion. For example 1000 molecules of CO_2 may yield one ion of *m/z* 44 that is detected at the Faraday collector. This test can identify whether a loss of sensitivity is due to the IRMS or to peripheral systems but can only be performed with DI/IRMS instruments. Absolute sensitivity can be express as:

$$\text{absolute sensitivity} = \frac{\Delta n_{\text{gas}}}{n_{\text{ions}}} \tag{18}$$

where n_{ion} is the number of ions produced and can be calculated from the charge accumulated in the Faraday collector over a preset time. The amount of gas consumed (Δn_{gas}) can be calculated from the pressure drop within a defined volume of a DI/IRMS bellows according to the ideal gas law that predicts the behaviour of n moles of an ideal gas at a given pressure (*P*), volume (*v*) and temperature (*T*) using the ideal gas constant (*R*).

$$P.v = n.R.T \tag{19}$$

This can be rearranged to calculate Δn_{gas}:

$$\Delta n_{\text{gas}} = \frac{\Delta P.v}{R.T} \tag{20}$$

8 Sampling

8.1 Why do we sample?

In the field of isotope forensics there are two distinct reasons to sample, (1) to obtain a representative measurement from exhibits and, (2) to put this into context by assessing the spatial and temporal variability of the background population. These could also be considered as measuring (1) within-sample homogeneity and (2) between sample variability. Sampling type (2) may also be appropriate when selecting samples to build a comparative database.

Thes two issues are addressed with different sampling strategies.

8.2 What to sample?

Before any kind of sampling strategy can be developed it is essential to decide what is to be sampled. This may sound obvious but deserves some consideration.

If our analyte is a relatively pure chemical substance such as collagen or an illicit drug the answer to the question “what to sample” can simply be “only test samples that are greater than 80% pure”. However, if our illicit drug comprises a gelatine capsule containing 10% API and 90% excipient the answer to the question of “what to sample?” is less clear. There are several possible options:

Table 8. *Possible forensic information that can be obtained from the analysis of different components of a gelatine encapsulated pharmaceutical product .*

Analyte(s)	Potential forensic value (pros/cons)
gelatine capsule	Possible information about the number of batches produced.
capsule contents	Provides an average value for all the components present. Potentially misleading. Other techniques needed to confirm chemical composition*
active ingredient	Possible information about the number of batches of API. Possible information about the synthetic routine. Time and effort needed to extract and purify the API. Extraction must not affect the isotopic composition.

*It should be noted that if a sample comprises a fixed ratio of two (or more) components, the probability of a given isotopic composition by random combinations of the components is considerably less than if the sample was a single compound. Hence, this may have greater evidential value.

Depending on the question to be addressed, a combination of approaches may be desirable.

As samples become more complex so does the questions of “what to sample?” A relatively simple manufactured product, such as a ball point pen, may contain ten or more components made from different polymers. Potentially, this provides ten points of comparison but, testing ten sub-samples from each exhibit becomes expensive and time consuming. In many cases, a small convenience sample (see below) can be tested to establish which components (if any) demonstrate variation between samples. An initial comparison can then focus on components with proven isotopic variation in their population and, if desirable, a final comparison can include a larger number of sampling sites.

For natural products the question of “what to sample” can be even more complex but can be guided by some prior knowledge of where variability exists in the population and how factors such as climate and location affect isotopic compositions (Carter and Chesson 2017).

8.3 Consider temporal variations

The natural isotopic compositions of any given material may vary in both space and time and must be considered. Temporal differences can be influenced by changes including, but not limited to

rainfall, sunlight and temperature fluctuations through seasonality and storm events, water movement across and through the terrestrial environment.

The isotopic compositions of plants, fruits and vegetables will change as a function of all these parameters but also with ripeness and will continue to change post-harvest (Carter and Chesson 2017).

For example, if the question is “is this beef from grass-fed cattle?”, a sampling strategy requires a temporal component because the carbon isotope composition of meat changes to reflect a grass-fed diet (Bahar et al. 2009).

Temporal changes are not limited to natural products as the isotopic compositions of manufactured products, such as plastic films, will depend on the feedstock used at specific manufacturing sites and may change over time. Similarly, the isotopic composition of pharmaceutical products and illicit drugs will change as a function of both the precursor chemicals and the manufacturing process.

For all these reasons, when reporting isotopic data, it is essential to state where and when samples were collected, and to note clearly relevant factors such as contemporaneous weather conditions.

8.4 Homogeneity testing

Before comparing exhibits, it is essential to assess within-sample homogeneity. For a polypropylene rope this might involve taking a sample every few metres along its entire length; for a packet of tablets this might involve testing each tablet. It is useful to note that once homogeneity has been demonstrated for a specific evidence type, without evidence to the contrary, this can be assumed for similar exhibits.

A suitable test for homogeneity, adapted from IUPAC protocols, can be easily executed in a spreadsheet (Fearn and Thompson 2001). The test is applied to replicate analyses of between 7 and 20 sub-samples. The first two columns of the spreadsheet contain replicate measurements of the samples under investigation (*result a* and *result b*). Note that, for this test, the data do not need to be normalised to the international reporting scales unless a significant stretch factor is involved.

The acceptance criteria for the test is based on a target standard deviation (*target sd*) which might be derived from MU established during method validation (see section 10.7.5) or some widely accepted performance criteria.

(1) The first step is a visual inspection of the data for obvious outliers, discordant replicates or trends.

(2) Calculate: D the difference of the two results, S the sum of the two results and D^2 the square of the difference (D).

(3) The first test is a Cochran statistical test for outliers. Calculate; n the total number of measurements, D^2_{MAX} the largest D^2 value, ΣD^2 the sum of all the D^2 values and the Cochran test statistic which is the ratio of the latter two variables (D^2_{MAX} / ΣD^2).

(4) The Cochran test statistic is compared to a table of critical values (**Table 9**). If the calculated value is less than the critical value, there is no evidence for analytical outliers at 95% confidence. If outliers are detected, these can be removed from the spreadsheet and the Cochran test statistic recalculated.

(5) Calculate further parameters: an estimate of analytical variance ($s^2_{an} = \Sigma D^2/n$), the variance of the sums (*varS* = variance of column S) and between-sample variance (s^2_{sam} = (*varS* / 2 – s^2_{an}) / 2).

(6) The allowable between-sample variance (s^2_{all}) is calculated from the target standard deviation as $(0.3 \times \textit{target sd})^2$.

(7) The critical value is then calculated using two factors (F_1 and F_2) taken from **Table 9** as ($F_1 \times s^2_{all}$) + ($F_2 \times s^2_{an}$). If the between-sample variance (s^2_{sam}) is less than the critical value then the sample is deemed to be homogeneous based on the *target sd*.

Further information regarding homogeneity testing can be found in (Thompson, Ellison, and Wood 2006).

	A	B	C	D	E
1	Result a	Result b	D (difference)	S (sum)	D^2
2	-29.49	-29.37	A2-B2	A2+B2	$(C2)^2$
3	-29.43	-29.33	A3-B3	A3+B3	$(C3)^2$
4	-29.49	-29.34	A4-B4	A4+B4	$(C4)^2$
5	-29.16	-29.18	A5-B5	A5+B5	$(C5)^2$
6	-29.34	-28.94	A6-B6	A6+B6	$(C6)^2$
7	-29.38	-29.10	A7-B7	A7+B7	$(C7)^2$
8	-29.36	-29.12	A8-B8	A8+B8	$(C8)^2$
9	-29.35	-29.24	A9-B9	A9+B9	$(C9)^2$
10	-29.15	-29.20	A10-B10	A10+B10	$(C10)^2$
11	-29.23	-29.36	A11-B11	A11+B11	$(C11)^2$
12					
13	***n***	COUNT(A2:B11)		D^2_{MAX}	MAX(E2:E11)
14	***target sd***	0.15		ΣD^2	SUM(E2:E11)
15				**Cochran statistic**	E13/E14
16					
17				s^2_{an}	E14/B13
18				**varS**	VAR(D2:D11)
19				s^2_{sam}	(E18/2-E17)/2
20				s^2_{all}	$(0.3*B14)^2$
21					
22				***critical***	(1.88*E20)+(1.01*E17)
23				***test result***	IF(E19>E22,"PASS","FAIL")

Figure 19*. Setup of a spreadsheet for testing homogeneity of multiple units of a material each analysed in duplicate.*

Table 9*. Critical values for the Cochran test statistic and factors applied during homogeneity testing.*

n	**Cochran critical value**		F_1	F_2
	95%	99%		
7	0.727	0.838	2.10	1.43
8	0.68	0.794	2.01	1.25
9	0.638	0.754	1.94	1.11
10	0.602	0.718	1.88	1.01
11	0.57	0.684	1.83	0.93
12	0.541	0.653	1.79	0.86
13	0.515	0.624	1.75	0.80
14	0.471	0.575	1.72	0.75
15	0.452	0.553	1.69	0.71
16	0.434	0.532	1.67	0.68
17	0.418	0.514	1.64	0.64
18	0.403	0.496	1.62	0.62
19	0.392	0.599	1.60	0.59
20	0.389	0.48	1.59	0.57

Example: Homogeneity testing for 10 subsamples of the same material

The data are duplicate $\delta^{13}C$ measurements of ten vials of poly(ethylene glycol) that were part of a round of the FIRMS proficiency testing scheme in 2017 (*result a* and *result b*).

The *target sd* is 0.15 ‰ as this was the standard deviation of performance assessment for the proficiency test.

The Cochran test statistic (0.432) is less than the critical value (0.602) and so there is no evidence for analytical outliers at 95% confidence.

The between-sample variance (*s2sam* = 0.001) is less than the critical value (0.023) and the sample is deemed to be homogeneous with respect to $\delta^{13}C$ based on the *target sd* of 0.15‰.

	A	B	C	D	E
1	Result a	Result b	D (difference)	S (sum)	D^2
2	-29.49	-29.37	-0.12	-58.86	0.02
3	-29.43	-29.33	-0.10	-58.76	0.01
4	-29.49	-29.34	-0.15	-58.84	0.02
5	-29.16	-29.18	0.02	-58.34	0.00
6	-29.34	-28.94	-0.40	-58.28	0.16
7	-29.38	-29.10	-0.28	-58.48	0.08
8	-29.36	-29.12	-0.23	-58.48	0.05
9	-29.35	-29.24	-0.11	-58.58	0.01
10	-29.15	-29.20	0.05	-58.35	0.00
11	-29.23	-29.36	0.13	-58.59	0.02
12					
13	***n***	20		D^2_{MAX}	0.164
14	***target sd***	0.15		ΣD^2	0.379
15				**Cochran statistic**	0.432
16					
17				s^2_{an}	0.019
18				**varS**	0.044
19				s^2_{sam}	0.001
20				s^2_{all}	0.002
21					
22				***critical***	0.023
23				***test result***	PASS

8.5 Sampling a population

Sampling theory is generally concerned with predicting parameters of a large (often assumed to be infinite) and homogeneous population based on measurements of a small sub-population. For IRMS studies, it is more likely that the samples are taken to study the variability in the population but sampling methods intended to summarise populations can be adopted.

There are many superficially appealing sampling methods that should be used with caution and assumptions must be clearly documented.

8.5.1 Convenience (or accessibility) sampling

This is probably the most widely used method in scientific experiments as samples are selected according to availability and accessibility. For example, a researcher investigating paper envelopes can simply ask staff members for examples from their desks or from their homes. The method is quick and inexpensive but the shortcomings may not always be obvious and can be serious.

8.5.2 Judgmental (or purposive) sampling

This describes a process in which the experimenter recognises that a population is heterogeneous and exercises a deliberately subjective choice in selecting a "representative sample". With this approach, there is always a risk of distortion due to personal prejudice or a lack of understanding of the population structure.

Importantly, these sampling methods have the potential to introduce bias that must be recognised when interpreting data. Generally, it is good practice to introduce some element of randomness and select samples according to a defined mechanism.

8.5.3 Simple random sampling (SRS)

This is the most straight forward method of selecting samples. Assuming that a population is ultimately homogeneous a representation of the population is provided by random sampling with equal probability assigned to each selection. For equal probability, random number tables or a random number generator can be used. Applications of SRS might include;

- selecting sacks of grain from a warehouse,
- selecting sampling locations for compacted illicit drugs (such as cocaine or heroin) by placing a grip over the surface,
- selecting manufactured products by diving batches into imaginary segments.

SRS may be inappropriate when the population has known heterogeneity, for example, if the sacks of grain in the warehouse were stacked according to different producers or the date of harvest. A different sampling strategy may then be more appropriate.

8.5.4 Stratified random sampling

This divides the population into a number of homogeneous sub-groups or strata each containing the same number of individuals. Equal size samples are then selected from each strata using SRS principles. Applications of stratified sampling might include;

- grouping illicit tablets based on similar colour and design,
- taking a proportional weight of material from exhibits seized at different locations,
- sampling a sediment core based on volume or depth.

8.5.5 Systematic (or interval) sampling

This can be applied to samples that exist in a fixed order or can be sequentially numbered. Samples are selected based on a systematic rule using a fixed interval, for example, the researcher selects the 5th sample and every 12th sample following. The effect of this type of sampling is to stratify the population but this is different to stratified sampling. With systematic sampling the samples are taken at the same relative position in the stratum whereas stratified sampling selects a position in the stratum at random. Systematic sampling is not suitable for populations with regular replication. Applications of systematic sampling might include;

- sampling a cannabis crop using a transect across a field,
- testing the homogeneity of plastic film from a production line,
- sampling tap water at the same time every day.

8.5.6 Cluster (or multistage) sampling

This is applied to a large population by dividing it into clusters based on some heterogenous property, typically geographic location. A list of clusters to be sampled is selected using SRS and samples are drawn from each cluster using either equal or unequal probability. Unequal probability

considers the size of the cluster so that larger clusters have a greater probability of being selected. Applications of cluster sampling might include;

- sampling meals from fast food outlets,
- sampling tap water from an urban distribution network,
- a commercial fish catch (itself a cluster sample).

8.6 How many samples?

It is important to identify the number of samples necessary to draw conclusions about a finite or infinite population at a given level of confidence. In forensic science, examples often involve sampling discrete containers to establish what proportion include an illicit drug. In this example, a general recommendation is that sampling should be sufficiently robust to provide 95 % confidence that greater than 90 % of the consignment contains the illicit drug.

Various methods for selecting the number of random samples have been proposed and accepted by Courts of Law. Most forensic laboratories choose a sample size based on some fraction of the consignment size, shown in **Table 10** where *n* is the sample size and *N* is the consignment size. Other laboratories take a fixed sample of one, four or 15 units regardless of consignment size.

***Table 10**. Sampling formulas by country where n is the sample size and N is the consignment size (adapted from Colón, Rodriguez, and Orlando (1993)).*

Sampling formula	Locations used
$n = \sqrt{N}$	Australia, Austria, Canada, UK, New Zealand, Hong Kong, USA
$n = \sqrt{\frac{N}{2}}$	Switzerland
$n = 10\%\ N$	Australia, Canada, USA
$n = 4\%\ N$	UK

The following formula is widely accepted for consignments greater than 20 units. It is simple to implement and can reveal heterogeneous populations (Colón, Rodriguez, and Orlando 1993).

$$n = 20 + 10\%(N - 20) \quad (21)$$

Where it is not sensible to divide a population into consignments (such as sampling a crop across a field) or the size of the population is not well defined (such as the amount of tap water in a city), it is often difficult to pre-determine how many samples are needed, In such cases, it may be necessary to conduct an initial round of sampling before reviewing the data and deciding whether more samples are needed based on the requirements of the investigation and repeating until a satisfactory set of data is obtained. Typically, at least 20 samples per sub-group are needed (Carter and Chesson 2017), so this is often a good starting amount.

8.7 Sampling uncertainty

Readers are directed to the EURACHEM / CITAC Guide to Measurement Uncertainty Arising from Sampling (Ramsey, Ellison, and Rostron 2019) and section 10.7 of this guide.

Sampling uncertainty is only one component of the overall uncertainty of measured data and, like other sources of MU, can be classed as repeatability or bias, both of which derive from random or systematic errors. Random errors, by their nature are circumstantial and cannot be captured experimentally.

Both random and systematic errors occur due to sample inhomogeneity. If a population is perfectly homogeneous it can be characterised by the analysis of any individual sample but, taking a sample from a heterogeneous population introduces uncertainty. Heterogeneity creates uncertainty either because a population is intrinsically heterogeneous, and all characteristics of the population are difficult to capture with a small sample, or due to the distribution of the heterogeneity. For example, the EURACHEM / CITAC Guide discusses errors associated with sampling powders with varying particle size and shape.

Sampling repeatability precision can be estimated by duplicating a proportion of the samples (recommended 10% and no less than 8). For example, sampling a cannabis crop can be replicated by taking multiple transects across the field with different starting points and different trajectories. As noted above, the experimenter would need to consider which parts of the plants will be sampled (leaves, flowers or whole plants) and consider possible diurnal changes in the properties of the material being collected.

Bias can be introduced by incorrect sampling or when populations are more heterogenous than the sampling strategy has assumed. Systematic bias occurs when sampling protocols are inappropriate for a given sample type or because the protocol is not closely defined. Estimating sampling bias is more difficult than estimating repeatability and may require sampling a reference material or inter-laboratory sampling trials.

Sampling and other sources of MU are combined either through a “top down” experimental approach of replication or through a “bottom up” approach in which sampling contributes one independent source to a cause-and-effect or fish-bone diagram.

9 Making measurements

9.1 EA/IRMS bulk nitrogen and carbon measurements

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

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

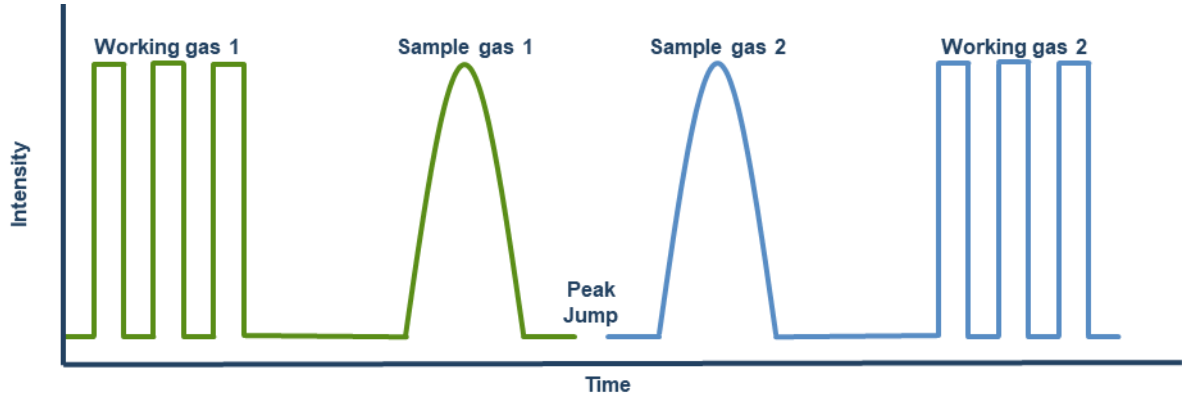


***Figure 20**. Example chromatogram for an EA/IRMS measurement including a jump between two configurations allowing measurement of two different isotope-delta values from a single sample.*

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

Generally, provided there is sufficient sample material available, it is preferable to have separate analyses for carbon and nitrogen rather than use a magnet jump. It is easier to ensure materials are prepared in matching amounts in terms of the element of interest and there is also no need for variable amounts of dilution to be applied within a single sequence/batch of analyses (this would require that the dilution system can be demonstrated to be free from fraction effects).

9.1.1 Preconditioning

The EA reactor(s) should be brought to operating temperature(s), with normal helium flow, 12-24 hours before any samples are analysed. Depending on the type of EA employed, it may be necessary to carry out a "pre-conditioning" of the reactor system. This can be done by analysing a series of capsules containing homogenous material chemically similar to the samples until the isotope-delta value is stable. Note that pre-conditioning must occur before any normalisation RMs are analysed as the isotope-delta value does not need to be accurate, just consistent/stable. See section 10.

9.1.2 Blank determinations

A "blank" refers to gases in the analytical stream that are not directly evolved from the sample material. Depending on the size and delta-value of a blank, relative to the sample, this can result in inaccurate data being produced. In general, the blank should be ≤ 1% of the typical sample signal. Large blank signals must be investigated and eliminated rather than attempt a correction (section 10.6.1).

There are many potential sources for the blank, including:

- Sample storage containers (including during transport)

    - leachates,
    - microbial processes.
- Chemical treatment procedures:
    - chemicals and reagents,
    - glassware.
- Sampling tools and/or sample preparation surfaces.
- Contamination of capsule from
    - manufacturing process,
    - laboratory surfaces,
    - skin contact.
- Ingress of atmospheric gases:
    - oxygen pulse,
    - air captured during autosampler movement,
    - leaks in the instrumentation,
    - entrapment of air within the sample and capsule after crimping.

Good practice for the analyst is to include a blank determination at the beginning of each sample sequence/batch: the blank determination must be relative to the specific situation for sample matrix processing. Here are two examples of a blank determination:

- Example 1: a sample material is loaded directly into a capsule.
    - Blank determination: direct analysis of an empty capsule via the auto-sampler, using the same EA parameters as samples.
- Example 2: a sample undergoes a chemical treatment procedure, such as the removal of carbonates (C. R. Brodie et al. 2011).
    - Blank determination: direct analysis of an empty capsule that has undergone the same chemical treatment procedure as samples, using the same EA parameters.

For very small samples it may not be possible to completely eliminate the blanks and the isotopic composition of the blank can be determined through the analysis of two RMs (see section 10.6.1).

9.1.2.1 Materials with very low nitrogen concentrations

For samples with ≤ 1% N (such as some soils and sediments) the measurement of $\delta^{15}N$ can be confounded by the inclusion of atmospheric nitrogen between particles and, more generally, any blank, resulting in reduced data quality.

The magnitude of the nitrogen blank for such samples can be determined by heating a portion of the sample in a furnace at 550 °C assuming that this does not significantly affect the physical form of the sample. Analysing aliquots of the combusted material will reveal the magnitude of the nitrogen blank.

The magnitude of the blank for this type of sample can be reduced by placing partially crimpled sample capsules in the carousel of a zero-blank autosampler and holding them under an atmosphere of helium for several hours (Langel and Dyckmans 2017).

The isotopic composition of the blank can be determined through isotope dilution i.e. using various sizes of RMs (10.6.1).

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

9.1.3 Sample preparation

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

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

9.1.4 Sample measurement

Typically, between two and six analytical results should be acquired for each sample as more analyses provide more confidence in the experimental uncertainty of the measurement. At least two RMs for each isotope delta of interest are analysed at the start of the sequence/batch and (in some laboratories) again at the end. These measurements will be used to normalise the data obtained during the sequence/batch. A QC material is analysed periodically throughout the sequence/batch to monitor performance. A typical sequence/batch is shown in **Figure 21**. To maintain sample continuity, the use of 96-well plates is recommended. The sample identification, position and the amount weighed should be recorded in a suitable template such as **Figure 21** The template reflects the format of the 96-well plate in which samples will be assembled prior to analysis.

Blanks are not normally part of the analytical sequence/batch. The main sequence/batch should not be started until acceptable blanks have been recorded. Although some laboratories will analyse blanks at the end of the sequence/batch, this is not essential.

RMs should be analysed at the end of a long sequence/batch to ensure no drift in instrument delta-scale calibration has occurred. Alternatively, this can be affirmed by analysing a second QC material with a delta value close to the upper or lower end of the calibration range.

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

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

Further to maintain sample continuity the samples should be loaded into the auto-sampler in a prescribed sequence/batch 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.

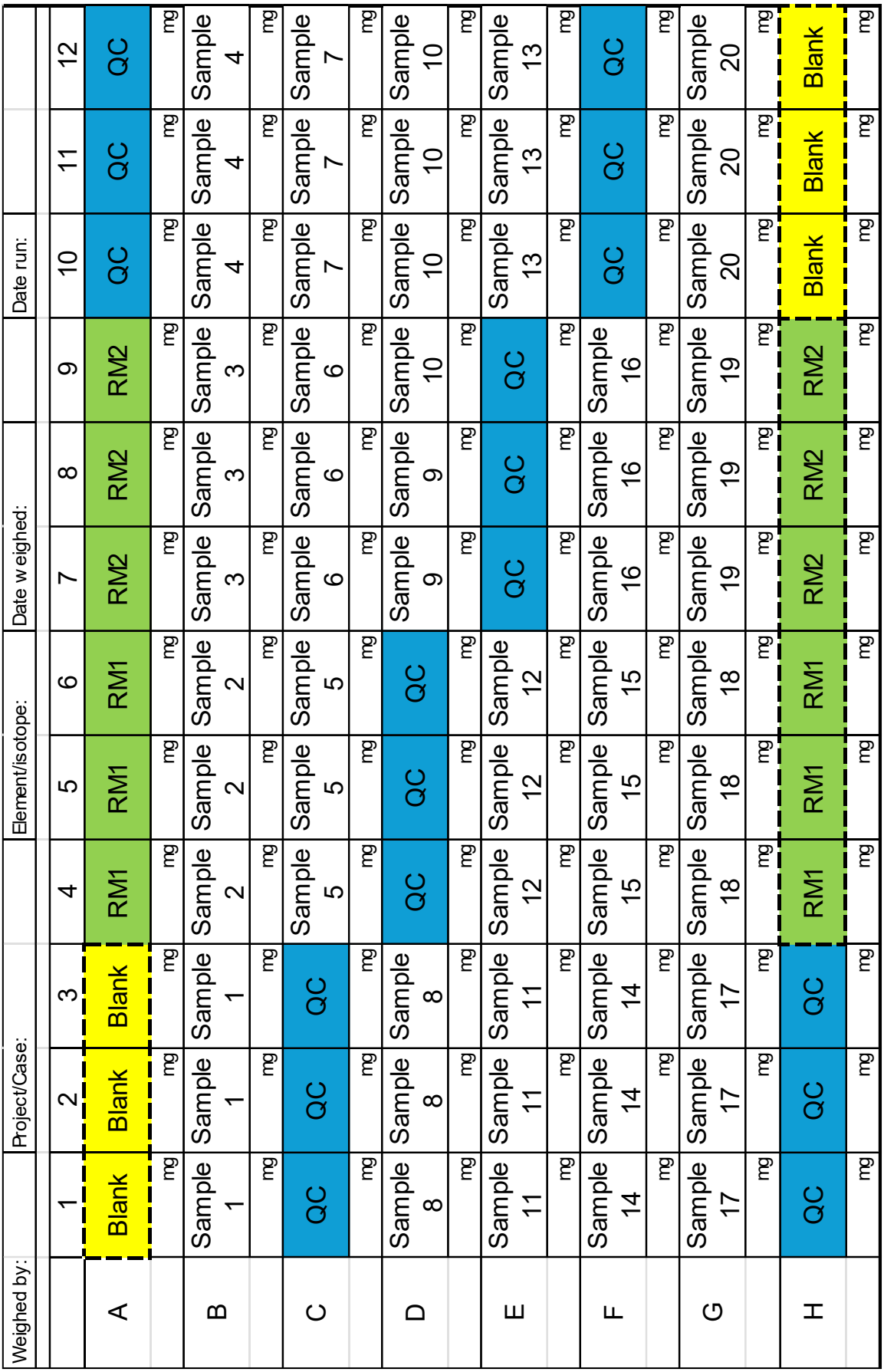


***Figure 21**. Template for sample continuity illustrating a typical measurement sequence/batch.*

9.2 EA/IRMS bulk sulphur measurements

9.2.1 Sample preparation

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

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

As water present within the instrumental system can trap the analyte SO_2, drying sample materials prior to analysis can reduce memory/carry over effects.

9.2.2 Sample measurement

Measurements of sulphur isotope delta values can require different operating conditions than carbon and nitrogen isotope analysis. The combustion process results in the formation of both sulphur dioxide (SO_2) and sulphur trioxide (SO_3) and the flow rate of carrier gas must be sufficient to force the gases resulting from combustion down through the reactor, thereby reducing formation of SO_3 (Mambelli et al. 2016).

The SO_3 formed is reduced to SO_2 by hot copper in the reduction stage of the reactor. The temperature of the copper is critical: if it is too cool it will react to form copper sulphate, if it is too hot it will melt. When using long copper wires, the heat from the furnace is conducted along the copper wires, resulting in even heat distribution. Copper particles distribute the heat in a less uniform manner. The surface area of the copper can also vary which can affect how SO_2 is transferred through the reactor, and which may result in carry-over. Reactor materials included from new can contribute a blank for sulphur.

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

9.3 HTC/IRMS bulk hydrogen and oxygen measurements

Most of the information in sections 9.1.1 to 9.1.4 also applies to bulk hydrogen and oxygen isotope ratio measurements. The additional complexity of these measurements is discussed below.

A newly packed HTC reactor should be brought to operating temperature over several hours with normal helium flow, 12-24 hours before any samples are analysed. When changing the crucible, the reactor must be cooled to below 700 ^oC to prevent the glassy carbon reacting with atmospheric oxygen.

Generally, provided there is sufficient sample material available, it is preferable to have separate analyses for oxygen and hydrogen rather than use a magnet jump. It is easier to ensure materials are prepared in matching amounts in terms of the element of interest and there is also no need for variable amounts of dilution to be applied within a single sequence/batch of analyses. Furthermore, a simple glassy carbon reactor may provide quantitative conversion of sample oxygen to CO gas but may not do the same for sample hydrogen to H_2 gas.

9.3.1 Blank determinations

Blank determinations for bulk hydrogen and oxygen isotope delta measurements are performed the same as for EA/IRMS measurements, with the use of silver rather than tin capsules (section 9.1.2).

9.3.2 Sample preparation

The most important consideration when performing hydrogen and oxygen isotope delta measurements of solid materials is appropriate drying of samples. Residual moisture will affect both δ^2H and $\delta^{18}O$ values so samples and RMs must be as dry as possible. Many materials are hygroscopic or have large surface areas that will adsorb water. The use of desiccants, vacuum, and heat can minimize the effect of water sorption and great care must be taken after desiccation to ensure the samples remain dry prior to analysis.

Special consideration must be given to the preparation of samples for hydrogen isotope delta analysis. Hydrogen exchange in compounds with reactive functional groups may affect the total δ^2H value of a compound (Bowen et al. 2005; Qi and Coplen 2011). Sections 9.3.4 and 10.6.6 contain more information regarding intrinsic versus extrinsic hydrogen.

9.3.3 Sample measurement

As described in section 6.2.3, the presence of elements other than H, O and C may require modification to the preparatory system. The presence of chlorine in moderately high abundance in the analyte [e.g. polyvinyl chloride (PVC) or hydrochloride salts such as cocaine hydrochloride] will generate hydrogen chloride (HCl) in a traditional, glassy carbon-based HTC configuration. Likewise, when samples contain nitrogen (caffeine, proteins, explosives, etc.), a glassy carbon HTC reactor will partially convert the hydrogen present in the molecules into hydrogen cyanide (HCN). The use of a chromium reduction reactor or combinations of glassy carbon, chromium and manganese is recommended for δ^2H measurements of these compounds (section 6.2.3).

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

A further consideration for HTC reactors is a possible “memory effect” associated with glassy carbon such that the first sample in a series of replicates has a composition off-set toward the composition of the previous sample (Olsen et al. 2006). If this effect is apparent more replicate samples must be analysed and the first, or first few, replicate results excluded from subsequent data analysis. Memory effects may appear less pronounced when chromium and manganese is incorporated into the reactor

9.3.4 Considerations for H – intrinsic and extrinsic fractions

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

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

To further complicate matters almost all organic-bound hydrogen is exchangeable over geological time scales (Sessions 2016) resulting in two inputs to the isotopic signature – the hydrogen originally present (intrinsic) and the hydrogen that has been assimilated from a shifting environment over many years, decades or millennia (extrinsic). In these cases, the signal from the extrinsic

hydrogen must be removed in order to reveal the original signature. An example of this conundrum is the analysis of ancient cellulose used for tree-ring dating whereas the hydrogen isotopic composition of modern cotton is unlikely to reflect anything other than the water available to the parent plant.

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

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

For a select group of materials (e.g. wood, keratin and hair) there are RMs available that have been characterised for the δ^2H values of the intrinsic hydrogen under defined conditions (Soto et al. 2017). This means that samples and RMs need to be equilibrated against only one water sample to enable a calculation of the intrinsic δ^2H values of the samples (D. B. Nelson et al. 2025).

Section 10.6.6 contains more information regarding intrinsic versus extrinsic hydrogen.

9.4 Hydrogen and oxygen isotopic measurements of waters by equilibration

9.4.1 Pre-treatment

Pre-treatments for water samples may include:

- filtering through 0.45 µm cellulose filters to remove fine particles and microorganisms,
- adding activated carbon to adsorb hydrocarbons,
- extended flushing/evacuation times to remove VOCs.

Preservation with mercuric chloride ($HgCl_2$) was once common but presents environmental risks and expense that are increasingly unacceptable. Other preservatives, such as benzalkonium chloride have been proposed (Gloël et al. 2015).

The catalyst for δ^2H measurements can become irreversibly poisoned, especially by water containing H_2S. This can be mitigated by adding copper turnings or zinc acetate to the water before introducing the catalyst.

Before performing CO_2-H_2O equilibration on biological samples (food, soil, vegetation) it is essential to eliminate any potential source of CO_2 from microbial or enzymatic activity. Once sealed in an equilibration vessel, samples can be sterilised by heating (e.g. 100 °C for 30 min) or by exposure to ionizing radiation.

H_2-H_2O equilibration is not generally suitable for measuring δ^2H in plant waters due to the presence of VOCs that may be continuously evolved even after pre-treatments (Scrimgeour 1995).

The first step of analysis is to introduce H_2 or CO_2 gas into the equilibration vessel.

9.4.2 Flush/Fill

DI measurements typically require pure H_2 or CO_2 as the equilibration gas whereas CF measurements employ a mixture of 1 to 2% H_2 in helium or 0.3 to 0.5% CO_2 in helium. The important consideration is that there is a large excess (> 10,000 times) of atoms in the water phase, compared to the gas phase, to ensure that the isotopic composition of the equilibrated gas will equal the initial composition of the water.

For oxygen measurements another consideration is to eliminate atmospheric nitrogen from the equilibration vessel. Nitrogen entering the ion source can react with residual oxygen at the hot filament to produce NO_2^+ that is isobaric with $^{12}C^{16}O^{18}O$ (*m/z* 46) and will significantly affect the measured *m/z* 46 / 44 ratio. (see section 6.2.3.2).

To introduce the equilibration gas, an instrument may use a single needle or two needles (one in, one out) often in a dual-concentric design with an outer, steel needle and an inner silica capillary.

A single needle is inserted into the equilibration vessel and then adjacent valves are opened to lower the pressure in the sample vial. Pure gas is then briefly equilibrated into this space and all valves are closed. This 'flush/fill' procedure is repeated multiple times. A double needle is used to flush the vessel without prior evacuation. This may require ten or more vial volumes of (expensive) gas mixture to displace effectively ambient nitrogen.

9.4.3 Equilibration

After H_2 or CO_2 has been introduced into the vessel it must equilibrate with the water at constant temperature. Modern instrumentation typically uses a well-insulated heating block although this precludes shaking, which earlier workers deemed important. The block temperature needs to be above room temperature for effective temperature control but cool enough to avoid excessive condensation on the underside of the septum. Temperatures in the range 22 to 40 °C are often recommended with higher temperatures promoting faster equilibration, especially with viscous samples.

With Hokko beads present, equilibrated H_2 gas is achieved after 1 hr and measurements are performed as described below. Short equilibration times are desirable because of the highly diffusive nature of both H_2 and He. Tiny leaks in the equilibration vessels may cause significant changes in the gas composition.

For consistent results it is vital that the CO_2-H_2O system establishes equilibrium. Recommended times vary significantly from 18 minutes to >18 hours and can be affected by factors such as salinity, viscosity and the physical nature of the matrix, if not pure water. Typically, equilibration times of 12 hour or more are desirable. Oxygen exchange is directly dependent on ionic concentration and longer equilibration times are required to establish equilibrium between CO_2 and saline aqueous solutions (Kim, Park, and Yun 2012).

Salinity does not affect H_2-H_2O equilibration (Lu 2016).

For equilibration of CO_2-H_2O in what are described as "pasty" matrices e.g. semi-dried fruit, it is recommended to smear the sample on the inside of the equilibration vessels to provide a high surface area and equilibrated for 6 hrs at 40 °C or overnight at 24 °C (Guyon et al. 2015; Guyon, Gaillard, and Magdas 2018). There are reports that the method is unreliable for samples containing less than 20% moisture.

For the analysis of porewater waters in soils, equilibrium can be achieved in 24 hrs at 22 °C for sandy soil, whereas loam with 2% organic content requires 96 hrs and loam with 4% organic content may not reach equilibrium after 7 days (Vadibeler et al. 2022).

Depending on the design of the heating block it is likely that water will condense on the underside of the septum during the equilibration time. When a needle pierces the septum, to sample the equilibrated gas, it is possible for liquid water to be transferred to the MS. This can cause serious problems and typically requires time and heating to remove the water. A simple solution is to gently tap the vessels to dislodge any water droplets. A more robust solution is achieved by backflushing the sample line as it enters the vessel (Seth, Schneider, and Storck 2006).

The equilibrated gas can be analysed via a DI or CF inlet system.

9.4.4 Measurements by dual inlet

Equilibrated gas is drawn from the vial into an evacuated section of sample line that is chilled to –95 °C. This section is then sealed for a few minutes while all moisture is frozen before dry, equilibrated gas is let into the bellows of a DI analyser for measurement against a WG or RG (see 6.2.1)

9.4.5 Measurements by continuous flow

Transfer to the MS is achieved via a dual-concentric needle that pushed pure helium into the vessel to displace the equilibrated gas mixture. Gas flows through a multi-port valve with a fixed volume sample loop (**Figure 22**) and, simultaneously, a flow of pure helium is directed into the IRMS.

Periodically, the valve switches to pass the gas from the sample loop into the IRMS. The valve is switched a number of times to make multiple measurements of the same sample. The timing of the valve switching is determined by the flow rate of the carrier gas and the volume of the sample loop to ensure the loop is full when the switch occurs. The concentration of H_2 or CO_2 in the equilibration gas and the size of the sample loop must be matched to present a suitable amount of gas to the MS, ideally a peak with the same intensity as the WG. As gas is withdrawn from the equilibration vessel the peak intensity will fall (**Figure 23**) but, typically, ten or more measurements can be made from a single vessel.

To reduce isobaric interference from residual atmospheric nitrogen a CF interface may incorporate a GC column for the separation of N_2 and CO_2. Systems may also contain Nafion drying membranes before and/or after the GC column to remove inevitable water vapour from the carrier gas.

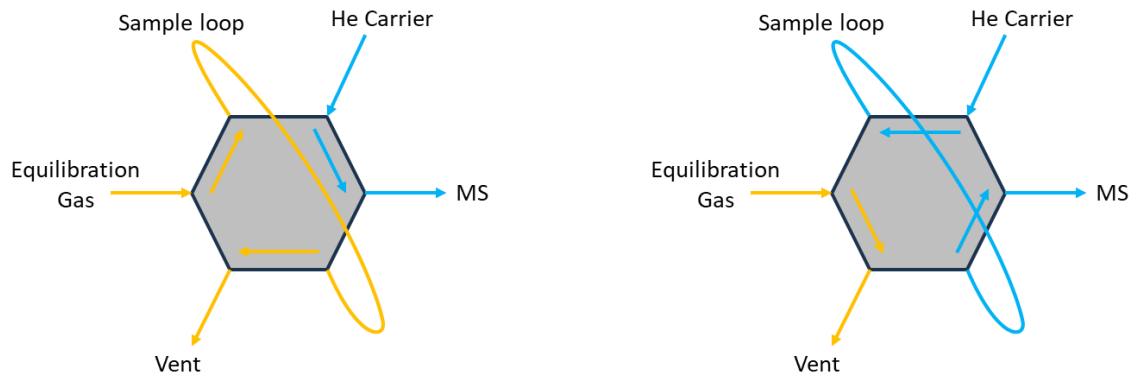


Figure 22. *Schematic for a multi-port valve with a fixed sample loop for CF measurements of equilibrated gases.*

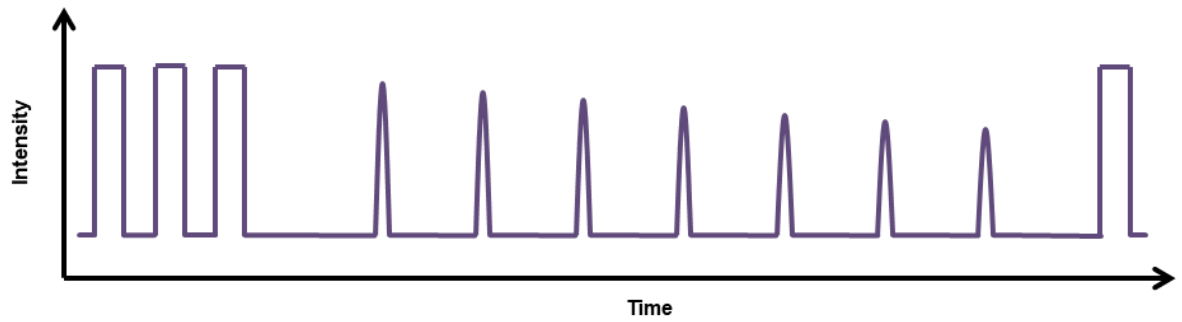


Figure 23. *Typical chromatogram obtained for measurement of gases obtained by equilibration showing the decrease in peak intensity between subsequent injections.*

9.5 GC/ IRMS carbon and hydrogen measurements

For suitable samples, GC is capable of isolating perhaps 100 components from a very complex mixture but an important consideration for GC/IRMS is that all the components to be measured must be baseline resolved. The reason for this is that most GC stationary phases will cause a slight separation of isotopologues such that an eluting peak is not isotopically homogeneous. Typically peaks are enriched in the heavier isotopes at the start and depleted at the end (Ricci et al. 1994; Brand 1996; Meier-Augenstein 1999). As such, it is important that the entire resolved peak can be integrated.

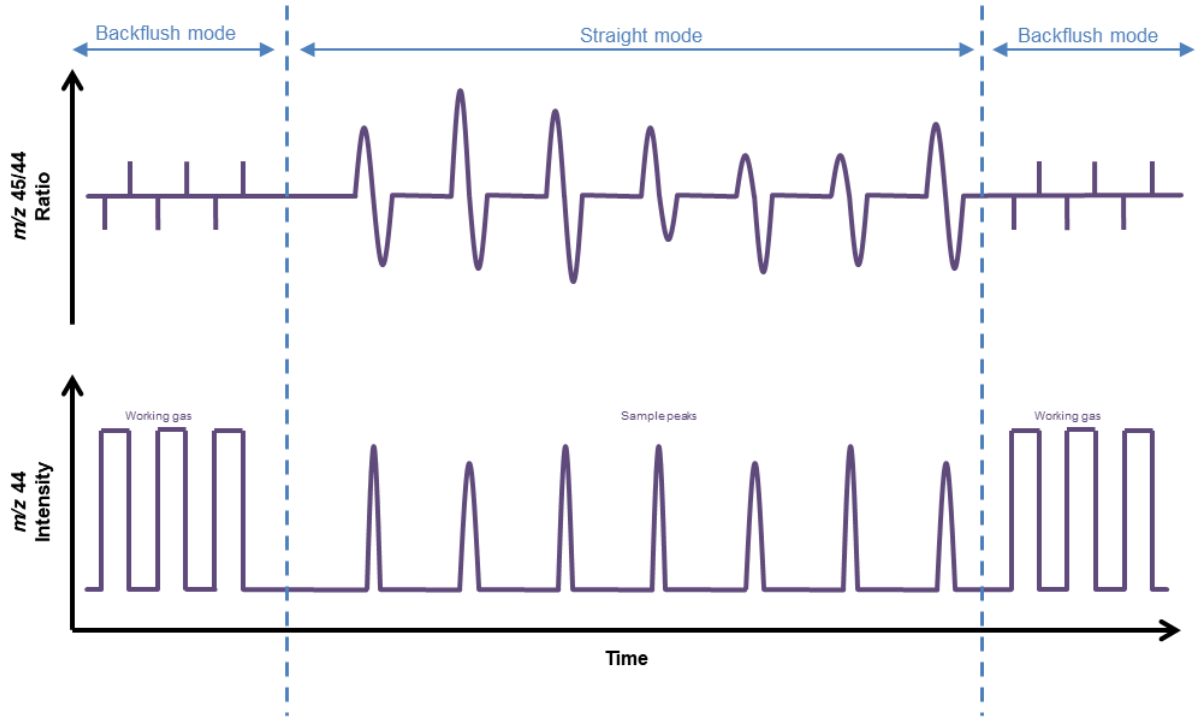


***Figure 24**. Simplified chromatogram of m/z 44 intensity and m/z 45/44 ratio for a mixture of compounds analysed by GC/IRMS for $\delta^{13}C$. The intensity ratio increases at the beginning of each peak as heavier isotopologues elute first. (Different chromatographic separation mechanisms may exhibit different partition of isotopologues within a chromatographic peak.) Working gas peaks do not generally exhibit partition of isotopologues within peaks, but rather small spikes arising from the amplifier circuit.*

To account for within run drift, working gas pulses should be introduced not only at the beginning of any analysis but also at the end. These pulses are often introduced while the instrumentation is in backflush mode to ensure a stable background.

There will also be unavoidable peak broadening due to the components of the GC/IRMS interface and for this reason the initial chromatographic separation may need to be better than would be needed for quantification using a conventional GC detector.

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

9.5.1 Preconditioning

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

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

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

9.5.2 Blank determinations

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

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

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

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

9.5.3 Sample preparation

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

9.5.3.1 Derivatisation

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

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

Derivatives containing carbon atoms will contribute to the measured carbon isotopic composition of derivatised compounds and this contribution must be accounted for (section 10.6.5). The same applies to other elements although it is rare for derivatives to contain nitrogen atoms and therefore this is of little concern for compound-specific nitrogen isotope ratio analysis.

Derivatives containing fluorine have been reported to irreversibly poison the combustion reactor via the formation of HF, which is said to react to form metal fluorides within the reactor and can potentially also damage capillaries downstream of the combustion reactor (Meier-Augenstein 1999). A number of other derivatives are likely to form involatile deposits upon combustion, such as trimethylsilyl derivatives.

9.5.4 Sample measurement

An analytical sequence/batch for GC/IRMS will be very similar to a sequence for EA/IRMS (section 9.1.4). The sequence will begin with conditioning runs, followed by an acceptable blank determination, followed by a number of RMs for isotope-delta scale normalisation. Each component to be reported should be measured by interpolation between two chemically identical RMs with widely spaced isotopic compositions that bracket the isotope-delta values expected for samples. In practice, it is usually possible to prepare a mixture of compounds such that two solutions will contain all the RMs needed. These RMs should be analysed a number of times, dictated by the length of the GC analytical sequence/batch, using identical analytical conditions for RMs and samples – especially the split and purge flows at the GC injector.

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

Although it is good practice to normalise each component in a chromatogram using two identical compounds, in practice suitable compounds may not be available. In such circumstances it may be possible to normalise using RMs chemically different to the samples, providing identical GC conditions can be used and the retention times are close. If RMs with a suitable span of isotope-delta values are not available it may be necessary to normalise measurements using a single RM. This is most appropriate when the isotope-delta values of the samples do not span a wide range and are similar to the RM. The normalisation method and the caveats inherent to the method must be clearly stated when reporting such results (Pilecky et al. 2021).

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

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

As with all CSIA applications, achieving baseline separation is essential and the development of the chromatographic separation prior to an IRMS application is crucial. The mechanism of chromatographic separation will determine the elution order of the heavy or light isotopologues – e.g. reverse phase separations tend to result in the heavier isotopologues eluting first, while ion-exchange separations have peak tails that are very enriched in ^{13}C (Piez and Eagle 1956; Bellobono 1968; van Klinken and Mook 1990; Caimi and Brenna 1997; Filer 1999; Tripp, McCullagh, and Hedges 2006). This fractionation of isotopologues across LC/CO/IRMS peaks is far more pronounced than with GC/IRMS and leads to > 100‰ variation in isotope delta value across peaks. Baseline separation of peaks ensures that the whole tail of the peak is included in the integration and avoids any carry-over from the tail of one peak into the baseline of the next peak.

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

9.6.1 Preconditioning the system

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

Mobile phases must be free from carbon (e.g. only comprising water, inorganic acids and inorganic buffers). As noted in section 6.3.2, if any component of the LC system (pump, auto-sampler, column, etc.) has been previously used with organic mobile phases these must be thoroughly flushed (a process that can take weeks).

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

The HPLC flow rate should be set to the operating level and all reagents allowed to flow through the system for at least 10 minutes before heating the reactor to operating temperature. This ensures that reagent and mobile phase flows have sufficient time to stabilise as reagent flows may be replaced by water while the instrument is in standby.

9.6.2 Blank determinations

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

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

More information regarding blank corrections and when they should be applied can be found in section 10.6.1.

9.6.3 Sample preparation

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

9.6.4 Sample measurement

Each sequence/batch of analyses for FIA/IRMS should include blanks, RMs for normalisation and RMs to act as QC for long-term instrumental monitoring. RMs for normalisation can be primary or secondary RMs (sections 5.1.1 and 5.1.2), provided that they are soluble in the same solvent as the samples. QC materials can be calibrated in-house (section 5.1.3.2) should matrix-matched materials not be commercially available.

Like EA and GC analyses, LC/IRMS sequence/batches should include blanks (which should be procedural blanks if appropriate), RMs for normalisation and QCs for long-term instrumental monitoring together with samples. If possible, the normalisation RMs should be analysed as external standard mixtures (section 10.5.1). Each sample solution should ideally include one or more IS compounds of well characterised isotopic composition to act as an internal QC material. These ISs can be used to check the instrumental performance and data handling.

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

As noted in section 6.3.2.2, some functional groups are difficult to oxidise by the persulfate chemistry used in most LC/CO/IRMS systems. It is therefore important to monitor the yield of CO_2 from the oxidation process to ensure complete conversion of sample compounds. When the oxidation process does not go to completion, isotopic fractionation should be expected leading to biases which can only be corrected by concurrent analysis of matrix-matched RMs.

9.6.5 After sample analysis (standby considerations)

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

For longer pauses between instrument use (e.g. greater than two days) reagents, including any buffers in the mobile phase, should be thoroughly purged from the system using water. This will ensure that the reagents do not precipitate in any part of the system, which may result in blockages. Once the reagents have been flushed, the reactor should be cooled to room temperature and the

mobile phase and reagent pump flow rates reduced to minimum. Some users prefer to keep the HPLC pump and reagent pumps delivering water at low flow rates through the system while not in use.

10 Data processing

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

Note that, for many CF systems, the raw isotope-delta values obtained for the sample are on a scale that is realised by the WG. Sample and WG do not follow the same pathway through IRMS instruments and therefore to obtain isotope-delta values on the internationally agreed reporting scales it is necessary to normalise the raw isotope-delta values using RMs (section 10.5) that are treated the same as the samples.

It is useful to understand the calculations performed by the instrument software as there can be changes to recommendations regarding some of these processes, for example to ^{17}O correction (Brand, Assonov, and Coplen 2010). If the instrumental process can be replicated offline (e.g. in spreadsheet software) then such changes can be more easily implemented.

Changes to settings within instrumental software relating to data processing can have significant impact in the obtained isotope-delta values. It is imperative that changes are only made when the impact is understood and when there is an objectively justifiable reason for doing so. Changes to such settings must be recorded within instrument logbooks to ensure transparency.

The most important of the stages of data processing are discussed in the following sections.

10.1 Initial data evaluation

It is always useful to inspect each datafile within instrumental software. The chromatograms of ion current versus time may show features that indicate problems within the instrumentation such as tailing or fronting, poor baseline stability, unexpected peaks interfering with peak detection or integration, overloaded signals with flat tops, peaks that are much smaller than expected based on amount of element analysed, etc.

10.2 Peak detection

Peak detection in terms of specifying the start and end time is often performed automatically by instrumental software after the user has specified some critical parameters. These may include the thresholds for the slope of the signal indicating the start and end of a peak, the minimum peak amplitude, maximum peak width, etc. As peak sizes change so will the shape and different detection parameters may be necessary. During method validation a laboratory must establish appropriate detection parameters for a given range of sample sizes, a process that may not be clearly distinct from an assessment of linearity.

In simple chromatograms such as those obtained by EA/IRMS or HTC/IRMS with consistent sample size default settings for peak detection are often suitable.

For compound-specific isotope analysis following separation by chromatographic methods, chromatograms can be significantly more complex. The number of sample peaks may be substantially greater and less well separated (but still baseline resolved), the baseline may not be stable and selection of the critical peak detection parameters becomes more important.

While instrumental software will act objectively and return the same peak areas and initial isotope-delta values when the same raw data are inputted the this does not mean that there is no uncertainty associated with this step of data processing.

The subjective choices of peak detection parameters should not be forgotten. If the peak detection parameters are changed, then so may the resulting peak areas, peak area ratios and initially calculated raw isotope delta values. As a result, two different users who each make slightly different subjective choices of peak detection parameters may obtain subtly different raw isotope-delta values from the same input data – but each of them may not be aware of this bias (section 10.7.3.1).

10.2.1 Manual peak detection

Instrumental software may also allow users to select where peaks start and end. This is a subjective process, and it is likely that there will be differences in peak start and end times selected by different users and potentially even differences for a single user.

It is recommended that manual peak detection is only employed where the complexity of a chromatogram prevents the use of automated peak detection by instrumental software and that the process is repeated, ideally by different analysts, to capture the inherent subjective uncertainty.

10.2.2 Peak "mapping"

Some instrumental software will use previous chromatograms to derive peak detection parameters for subsequent peaks detected that have the same relative retention time. While this can ensure more consistency between analyses regarding peak detection for the same compound, it is perhaps less clear as to the effect on MU.

10.3 Baseline (or background) correction

Instrumental software may have various built-in options to apply corrections to the baseline of integrated peaks. These may:

- use the signal intensity for a specified period of time directly before (or after) each peak,
- use a specific region within a chromatogram as a universal baseline, often subjectively chosen by the end user,
- apply smoothing, derive a background "function" or dynamic background (Ricci et al. 1994).

As with peak detection (section 10.2), once a subjective choice has been made regarding the baseline correction method, instrumental software will apply the method objectively and return the same peak areas from the same input data. Nevertheless, changing the baseline correction approach may substantially alter initially calculated isotope-delta values. The manner in which initial isotope-delta values change with a change in baseline correction method will depend on the nature of the chromatogram.

To adhere to PIT (section 5.2.1), the same baseline corrections should be implemented for all analyses i.e. for all compounds within a single chromatogram and all chromatograms within a sequence/batch.

10.4 Isobaric interferences

Gas source IRMS instruments typically have mass resolutions ($m/\Delta m$ at 10% peak intensity, where m is the mass of the heavier isotope and Δm is the resolving power of the instrument) of 100-200 for the triple collector system and of 10-40 for the Faraday collectors used for hydrogen measurements. The low resolution does not allow for the separation of isobaric species within the mass spectrometer (e.g. $^{13}C^{16}O_2$ and $^{12}C^{17}O^{16}O$ both nominally *m/z* 45). When isobaric species interfere with the masses used to determine isotope ratios from molecular ion ratios that cannot be resolved via chromatography, a correction must be applied (Kaiser and Röckmann 2008) as described below:

10.4.1 ^{17}O-correction for carbon isotope ratios of CO_2

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

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

m/z 44 $^{12}C^{16}O_2$

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

The contributions of the minor species (in the natural abundance range) are small except for $^{12}C^{17}O^{16}O$, which contributes approximately 7% to the abundance of *m/z* 45. A triple collector IRMS instrument measures simultaneously the ratios [45]/[44] and [46]/[44], which are a function of three variables ($^{13}C/^{12}C$, $^{17}O/^{16}O$ and $^{18}O/^{16}O$).

$$[45]/[44] = \left(^{13}\mathrm{C}/^{12}\mathrm{C}\right) + 2\left(^{17}\mathrm{O}/^{16}\mathrm{O}\right) \quad (22)$$

$$[46]/[44] = 2\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right) + 2\left(^{17}\mathrm{O}/^{16}\mathrm{O}\right)\left(^{13}\mathrm{C}/^{12}\mathrm{C}\right) + \left(^{17}\mathrm{O}/^{16}\mathrm{O}\right)^2 \quad (23)$$

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

$$R\left(^{17}\mathrm{O}/^{16}\mathrm{O}\right) = K\left(R\left(^{18}\mathrm{O}/^{16}\mathrm{O}\right)\right)^{\lambda} \quad (24)$$

The original ^{17}O-correction algorithm (Craig 1957) is based on ^{17}O and ^{18}O abundances determined by Alfred Nier (Nier 1950) and assume 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 0.50 and 0.53. A single value of λ must, however, be chosen in order to maintain comparability with published data. For this reason the "Craig" or "IAEA" algorithm is retained (Allison, Francey, and Meijer 1995), with λ = 0.5 and defined values for all of the ratios involved.

A ^{17}O-correction algorithm (the "Santrock" or "SSH" algorithm) (Santrock, Studley, and Hayes 1985) with a fractionation factor of λ = 0.516 and an iterative correction to solve the equations for ^{13}C has been published. The SSH algorithm is often regarded as being both mathematically exact and more realistic in its approach to natural variations of isotopic composition. Various approximations to this exact approach have been published (Miller, Röckmann, and Wright 2007; Kaiser 2008; Flierl and Rienitz 2024). [1]

IUPAC has published a technical report on ^{17}O-corrections (Brand, Assonov, and Coplen 2010) that includes a new linear approximation for determining the ^{17}O-correction (Assonov and Brenninkmeijer 2003b, 2003a). In 2024, the IUPAC CIAAW revised the recommended parameter set for VPDB and VPDB-CO_2 based upon a new measurement result for the absolute carbon isotope ratio of VPDB (CIAAW 2025; Dunn et al. 2024).

Applying any of the ^{17}O-correction algorithms to the same raw data will produce $\delta^{13}C$ values with differences that are small but can exceed the precision of modern IRMS instruments. For an average tropospheric CO_2 the bias in raw $\delta^{13}C$ value between the IAEA and SSH algorithms has been determined as 0.06 ‰ (Brand, Assonov, and Coplen 2010). Provided that raw $\delta^{13}C$ values for sample and RMs for scale calibration are measured against the same RG/WG, and that the same ^{17}O correction is applied to all materials within the same sequence/batch, then the bias in normalised $\delta^{13}C$ values introduced via the choice of ^{17}O correction approach will be <0.001 ‰ (Dunn, Hill, et al. 2019; Dunn and Skrzypek 2023).

[1] The various published ^{17}O-correction algorithms differ not only in their mathematical approach or assumptions made, but also by the values assigned to isotope ratios required for the scale zero-point. These include $R(^{13}C/^{12}C)$ for VPDB as well as $R(^{17}O/^{16}O)$ and $R(^{18}O/^{16}O)$ for VPDB-CO_2. Proprietary instrumental software may have user-selectable options for implementing the ^{17}O correction. It may also have a variety of input isotope ratios for the zero point of the scale (Skrzypek and Dunn 2020a).

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

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

This correction uses an empirically determined factor (section 7.6.1) to negate the contribution of H_3^+ to the intensity of $^2H^1H$ measured at the *m/z* 3 Faraday collector. As with the ^{17}O-correction, this correction is often hidden within instrumental software and applied automatically. Equations (25) and (26) demonstrate one method to determine and apply the H_3^+ correction (Sessions, Burgoyne, and Hayes 2001a, 2001b).

$$i_{\mathrm{H_3}} = [\mathrm{H_3^+}]\left(i_{\mathrm{H_2}}\right)^2 \tag{25}$$

$$R_{\mathrm{meas}} = \frac{i_{\mathrm{HD}} + i_{\mathrm{H_3}}}{i_{\mathrm{H_2}}} = \frac{i_{\mathrm{HD}}}{i_{\mathrm{H_2}}} + [\mathrm{H_3^+}] \times i_{\mathrm{H_2}} = R_{\mathrm{true}} + [\mathrm{H_3^+}] \times i_{\mathrm{H_2}} \tag{26}$$

i_{H3} ion current for H_3^+

i_{H2} ion current for H_2

i_{HD} ion current for $^2H^1H$

$[H_3^+]$ H_3^+ factor

R_{meas} measured *m/z* 3/*m/z* 2 ion current ratio

R_{true} true *m/z* 3/*m/z* 2 ion current ratio (i.e. corrected for H_3^+)

10.4.3 ^{13}C-correction for oxygen isotope ratios of CO and CO_2

Oxygen isotope ratio measurements based on CO and CO_2 molecules are commonly corrected for the presence of ^{13}C in an analogous manner to the ^{17}O correction (section 10.4.1) although the ^{13}C correction is typically 0.01 ‰ and contributes little to MU (Farquhar, Henry, and Styles 1997; Kaiser and Röckmann 2008).

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

The high reactor temperatures typically used in HTC systems generates a significant background of CO^+ (*m/z* of 28 and 30). This is the result of oxygen from the ceramic outer tube reacting with the carbon within the inner glassy carbon reactor tube and reactor packing. Provided this background is stable, this interference will not have adverse effects on the accuracy or precision of oxygen isotope ratio measurements. The high background does, however, mean that ^{18}O determinations are very sensitive to integration parameters.

Nitrogen gas (*m/z* 28 and 29) has a more profound effect on oxygen isotope ratio measurements by HTC/IRMS. When N_2 enters the ion source of the mass spectrometer NO^+ is formed at the filament, with a dominant ion of *m/z* 30. This interference can remain long after the chromatographic N_2 peak has left the ion source and continues to have large effects on $\delta^{18}O$ measurements. This must be addressed by physically preventing or minimising N_2 entering the ion source (section 6.2.3.2).

10.4.5 Oxygen isotope corrections for sulphur isotope ratios of SO_2

Oxygen isotope ratio correction for $\delta^{34}S$ values is possible when both SO^+ and SO_2^+ are measured for a particular material, as shown in equations (27) and (28) (Coleman 2004):

$$\delta^{18}\mathrm{O} = (24.02 \times \delta^{66}) - (23.024 \times \delta^{50}) \tag{27}$$

$$\delta^{34}\mathrm{S} = (1.0908 \times \delta^{66}) - (0.0908 \times \delta^{18}\mathrm{O}) \tag{28}$$

where

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

and

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

The more common approach is to avoid the need to correct the for the presence of oxygen isotope ratios by application of PIT by ensuring that the oxygen isotopic composition of the SO_2 derived from all materials within a single sequence/batch of analyses is identical. When this is the case, only equation (28) needs to be applied. This equalisation of oxygen isotope ratios can be achieved either via an offline process (oxidation to sulphate, followed by reduction to sulphide and combustion with the addition of isotopically identical oxygen), or by the use an EA designed to "equilibrate" or "buffer" the sample SO_2 against a large pool of oxygen (e.g. quartz chips, section 6.2.2.3).

10.5 Scale calibration/normalisation

The analysis of RMs within the same sequence/batch as samples allows the linking of the isotope-delta values for the samples to the zero-point of the isotope-delta scale. Where scale contraction effects occur (**Figure 1**), these also need to be corrected for using RMs of widely different isotope ratios – a process often termed "normalisation." The linking of measured results to the isotope-delta scale using RMs results in realisation of the isotope-delta scale in practice within any laboratory (section 4.1).

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

When the measured isotope-delta values of both samples and RMs are obtained relative to a WG, equation (29) can be used to determine the sample isotope-delta values on the reporting scale:

$$\delta_{\text{true(sample)}} = \left[\frac{\left(\delta_{\text{raw(sample)}} + 1\right)\left(\delta_{\text{true(RM)}} + 1\right)}{\left(\delta_{\text{raw(RM)}} + 1\right)} \right] - 1 \qquad (29)$$

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

The uncertainty of normalisation can be improved by applying a normalisation factor (*n*) calculated from the measured isotope-delta value of two RMs, with isotope-delta values far apart, assuming that systematic errors are linear in the dynamic range of the overall method (Paul, Skrzypek, and Fórizs 2007):

$$n = \frac{\delta_{\text{true(RM1)}} - \delta_{\text{true(RM2)}}}{\delta_{\text{meas(RM1)}} - \delta_{\text{meas(RM2)}} - \left(\delta_{\text{true(RM1)}} \times \delta_{\text{meas(RM2)}}\right) + \left(\delta_{\text{meas(RM1)}} \times \delta_{\text{true(RM2)}}\right)} \qquad (30)$$

The true isotope-delta value of the sample is calculated by a modified equation (29) taking the following form:

$$\delta_{\text{true(sample)}} = \left[\frac{\left(n \times \delta_{\text{raw(sample)}} + 1\right)\left(\delta_{\text{true(RM)}} + 1\right)}{\left(n \times \delta_{\text{raw(RM)}} + 1\right)} \right] - 1 \qquad (31)$$

For a given IRMS instrument the value of *n* remains nearly constant but should be determined periodically, especially if changes in sensitivity are observed. For CF/IRMS instruments, it is

recommended that *n* is determined for each analytical sequence/batch, termed “two-point linear normalisation”, “linear shift normalisation” or “stretch/shift correction”.

For isotope isotope-delta scales that are defined by two points or where it is recommended to use two or more RMs for scale realisation, equation (29) must again be slightly modified as follows:

$$\delta_{\text{true(sample)}} = \delta_{\text{true(RM1)}} + \left[\left(\delta_{\text{raw(sample)}} - \delta_{\text{raw(RM1)}}\right) \times \left(\frac{\delta_{\text{true(RM1)}} - \delta_{\text{true(RM2)}}}{\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}}\right)\right] \quad (32)$$

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

Alternatively, the δ_{true} value for a sample can also be calculated using an expression of the form shown in equation (33).

$$\delta_{\text{true(sample)}} = m \times \delta_{\text{raw(sample)}} + b \quad (33)$$

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

This method has been used for three decades to normalise measured $\delta^{2}H$ and $\delta^{18}O$ values to the VSMOW scale (Hut 1987; Coplen 1994, 1995) and is now recommended for the normalisation of $\delta^{13}C$ measurements of both organic and inorganic materials to the VPDB and VPDB-LSVEC scales and $\delta^{15}N$ measurements to the Air-N_2 scale.

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

The use of regression to determine the slope and intercept of the calibration plot should include consideration of the uncertainties associated with both the measured delta values for calibration RMs as well as the uncertainty in their assigned values. A variety of regression approaches that do this are available including those described by Deming (Deming 1943), Williamson (Williamson 1968), York (York 1968), MacTaggart and Farwell (Mactaggart and Farwell 1992), Günther & Possolo (Günther and Possolo 2011), and Meija & Chartrand (Meija and Chartrand 2018). The differences in obtained calibration slopes and intercepts amongst the various errors-in-variables regression approaches or even in comparison to simple least squares regression may not be significant in all cases.

A number of articles have reviewed normalisation procedures (Paul, Skrzypek, and Fórizs 2007; Skrzypek, Sadler, and Paul 2010; Skrzypek 2013).

Regardless of the normalisation approach employed, it is essential to check that the algorithm(s) applied result in meaningful data. This is most easily achieved via the analysis of additional RMs not used for normalisation within each analytical sequence/batch i.e. QC materials. The measured values for these QC materials are then treated in the same way as the samples and the resulting, normalised isotope-delta values should agree with the known values, either obtained by certificate, inter-laboratory comparisons, or quality-controlled long-term data. This process forms part of quality control and assurance described further in section 11.

Example: Normalisation of δ^2H measurement:

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

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

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

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

or, using SLAP2:

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

Adjusted δ^2H values would be calculated as:

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

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

10.5.1 CSIA considerations

Although there are currently no primary RMs that can be directly analysed by CSIA using GC or LC techniques, some secondary RMs are available depending on the sample requirements (Schimmelmann et al 2016). The traceability chain for CSIA-derived isotope delta values may therefore need to be extended through the use of calibrated in-house RMs (section 0), rather than direct use of primary and/or secondary materials.

In an ideal situation, each component of interest will be calibrated via a pair of RMs that are the same compound and have isotopic compositions that span the expected range of the sample compound (this allows calibration to isotope delta scales that are defined by two points). For example, for the LC/CO/IRMS analysis of glycine, two glycine RMs would be needed for calibration that span the carbon isotopic composition of glycine expected in the samples, e.g. δ^{13}C of −46 and −10‰. To complicate matters further, CSIA is typically used to determine the isotope delta values of several components within a mixture, each of which would require a pair of normalisation RMs and therefore the ideal approach quickly becomes unfeasible due to the large number of RMs that need to be sourced and/or calibrated in-house (Pilecky et al. 2021).

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

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

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

10.5.2 Considerations for water measurements by equilibration

For each analytical sequence/batch, sample results must be normalised to the VSMOW-SLAP scale using internationally recognised or traceable laboratory RMs. Delta values for these RMs, as analyzed, are plotted against the calibrated values and a linear regression is performed (S. T. Nelson 2000) (see 10.5). Because samples are normalised directly against contemporaneous RMs, this largely negates fluctuations in equilibration temperatures and times and removes the need to calibrate carefully the WG or equilibration gases.

A major disadvantage of H_2-H_2O equilibration is the fractionation factor that gives rise to a significant contraction of the VSMOW-SLAP scale e.g. –720 to –845‰ (a range of 125‰) compared to the defined range of –428‰ to zero. This has the effect of "magnifying" errors in the measured data and increasing the overall measurement uncertainty. The fractionation is also temperature dependent and temperature fluctuations during equilibration should be limited to ± 0.1 °C, which corresponds to a 1‰ change in the δ^2H value of the hydrogen gas.

Despite this drawback, a study of day-to-day and week-to-week measurements of water and urine samples with natural abundance and enriched levels of ^{2}H concluded that the method was accurate to within 2.8‰ and reproducible to within 4.0‰ (Wong and Clarke 2012).

10.6 Other corrections

The corrections for isobaric interferences and calibration/normalisation are essential practices. There may be a need to apply other corrections to measured data but we urge users to determine whether any of the following corrections are strictly necessary before applying them because "over-correction" can introduce as many problems as it appears to solve. This is because introducing too many corrections to measured data can introduce bias to the results which can lead to false conclusions. Therefore, before covering the details of the possible corrections that can be applied to data obtained from isotope ratio measurements, it is important first to address when to apply these techniques. If the data from a particular analytical sequence/batch is of poor quality, it is important not to force it into the established QC criteria by to using large correction factors as this can introduce significant MU.

Whilst it is often not possible to completely exclude confounding variables, it is essential to establish fit-for-purpose QC criteria during method validation, including control variables such as maximum permitted blank size and effects of chemical treatment, and adhere to these limits during subsequent analysis so that correction for these variables can be kept minimal. Measured isotope ratio data which fall outside of these limits must be rejected and the root cause identified.

Thresholds indicating when particular corrections become necessary may be established during method development and validation. These remove the subjective decision of whether particular corrections should be applied or not.

If a change in instrumental performance affects measurement data such that a correction appears necessary, it is far more advisable to identify and rectify the problem then re-analyse the samples rather than apply a "correction" that makes data appear acceptable. Additionally, where investigations into deviations from QC criteria identify that a change in methodology is required, these changes must first be validated and recorded (section 11.3). Where corrections are deemed necessary correction factors must be proven to be reproducible. It is important to note that each calculation stage, from raw data to final normalisation will contribute to overall MU (section 10.7). Furthermore, the influence on the MU of a particular correction may be hidden if only the sd of replicate analyses is considered, particularly if the sd is determined using corrected data.

When applying data corrections, adherence to PIT is critical and therefore, if one or more of these corrections are deemed necessary, they should be applied consistently to all data within a particular analytical sequence/batch (Carter and Fry 2013a). Applying corrections which with varying positive or negative directional shift can add significant interpretive uncertainty which is difficult to quantify.

10.6.1 Blank correction

Blanks arise during bulk and compound specific stable isotope analysis and varies in magnitude and isotopic composition depending upon its source (See 9.1.2). Good practice includes the determination of the magnitude and isotopic composition of the blank for each sample

sequence/batch. The peak area and isotope-delta value of a blank measurement (or the average if multiple blank measurements are made) can be used to correct the data for blank contribution, as shown in equation (34).

$$\delta_{\text{blk corr}} = \frac{\delta_{\text{meas}} \times Area_{\text{meas}} - \delta_{\text{blk}} \times Area_{\text{blk}}}{Area_{\text{meas}} - Area_{\text{blk}}} \quad (34)$$

$\delta_{\text{blk corr}}$ blank corrected isotope-delta value of the sample

δ_{meas} determined (raw) isotope-delta value of the sample

δ_{blk} isotope-delta value of the blank

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

$Area_{\text{blk}}$ area of the blank peak

The blank correction may be performed by the IRMS instrument software automatically, or in external software after data export, using the peak area and isotope-delta values obtained from blank determinations in the sequence/batch. However, the instrument software may produce isotope delta values for the blank that are incorrect, particularly where the signal-to-noise ratio is low. It is therefore important for analysts to define a minimum acceptable peak size for the blank based on the average blank peak area that captures all the potential sources outlined in section 9.1.2 that is applicable to a particular sample sequence/batch. Simply stating that blanks are "negligible", is not good practice and a data driven approach must be taken and monitored over time. For example, a small blank peak (e.g., 0.05 Vs) (assuming relatively close isotope delta-values of sample and blank) is likely to have minimal effect on a sample peak one hundred times larger (e.g., 5 Vs) because the blank accounts for 1% of the total peak area.

Analysts are recommended to determine the isotopic composition of the blank by analysis of two RMs with widely spaced isotope-delta value using decreasing sample sizes (**Figure 25**). The isotope-delta values of the blank can then be determined by solving two simultaneous equations for isotope-delta value versus peak size for the two RMs (Carter and Fry 2013a; Langel and Dyckmans 2017). Alternatively, the blank may be directly quantified (peak area and isotope delta-value) using high sensitivity analytical methods (Polissar et al. 2009; Ogawa, Nagata, and Ohkouchi 2010; Baczynski et al. 2023).

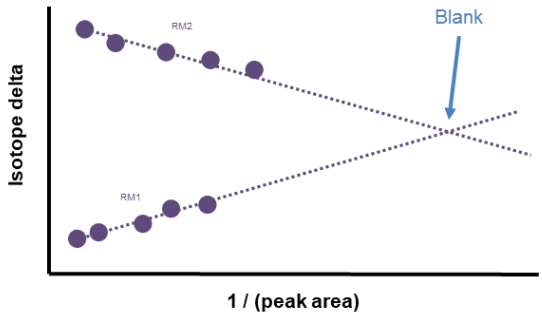


***Figure 25**. Estimation of isotopic composition and magnitude of the blank using two RMs analysed at decreasing amounts.*

An action threshold can implemented automatically within spreadsheet software to determine if a blank correction should be applied or not to any measurement sequence/batch. The magnitude of blank measurements must always be deemed acceptable before any measurement sequence/batch.

10.6.2 Drift correction

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

The buildup of ash within the reactor may also contribute to drift within a measurement sequence/batch as subsequent capsules do not reach the hottest part of the reactor.

The presence of drift in measured isotope-delta values with time during an analytical sequence/batch should be determined through the regular analysis of QC materials (every 5-10 samples). If significant drift is detected, then a correction may be applied using the results from a second QC materials. For example, if the QC materials indicate a linear drift in measured isotope-delta value with time, then equation (35) can be used to perform a drift correction for all materials analysed within the same sequence/batch (Carter and Fry 2013a):

$$\delta_{\text{drift corr}} = \delta_{\text{meas}} - m \times \text{position} \quad (35)$$

$\delta_{\text{drift corr}}$	drift corrected isotope-delta value of the sample
δ_{meas}	determined (raw) isotope-delta value of the sample
m	slope of linear drift curve (plot of isotope-delta value versus auto-sampler position)
position	auto-sampler position within the sequence/batch (assuming this is a proxy for time)

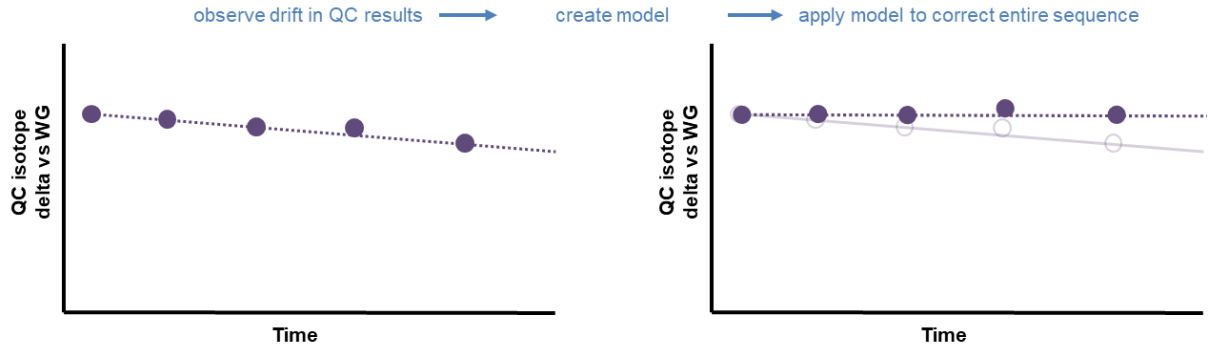


***Figure 26**. Procedure for drift correction. Note that drift may be corrected to the beginning of the sequence/batch as shown, or to the midpoint of the QC materials used to detect drift (the latter causes the mean QC value to be identical before and after application of the drift correction).*

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

Drift can also be detected by comparing calibration plots for RMs analysed at the beginning and end of the sequence/batch.

As with the blank correction thresholds may be established during method validation to determine when drift correction is appropriated. If the isotope-delta values of QC materials change in a sudden or non-linear pattern drift correction is not appropriate and the root cause of the change should be investigated.

10.6.3 Linearity (peak size) correction

Instrument linearity can be determined from WG pulses of varying amplitude or from the analysis of varying sample weights (section 7.4.3). Note that using the WG only determined linearity for the interface and IRMS – peripheral instruments may also affect linearity as a function of the amount of element analysed.

A small linearity correction (the slope of isotope-delta value against peak amplitude or areas or mass of the element within the samples) can have a large influence on the precision and/or accuracy of measured isotope-delta value if samples are not prepared in consistent amounts. A correction for linearity can be performed using an approach analogous to drift correction (section 10.6.2):

$$\delta_{\text{linearity corr}} = \delta_{\text{meas}} - m \times area \quad (36)$$

$\delta_{\text{linearity corr}}$	linearity corrected isotope-delta value of the sample
δ_{meas}	determined (raw) isotope-delta value of the sample
m	slope of linearity correction (plot of isotope-delta value versus peak area)
area	peak area of sample (proxy for amount of analyte)

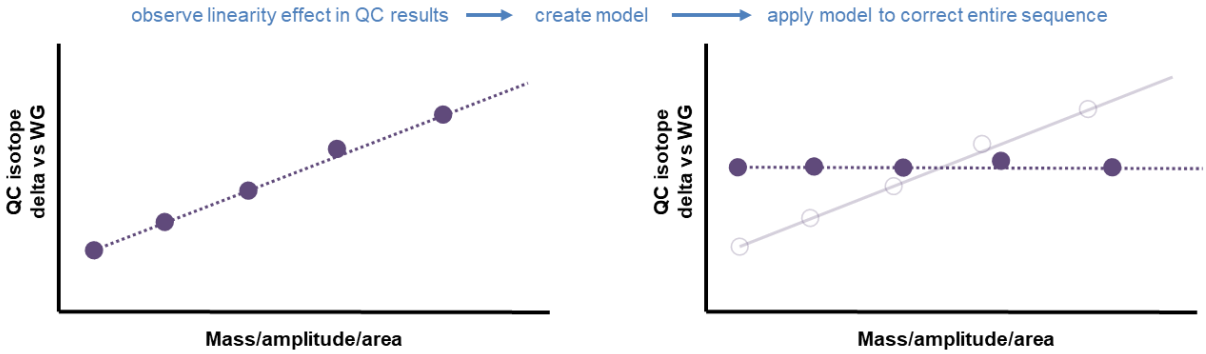


***Figure 27**. Procedure for linearity (peak size) correction.*

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

10.6.4 Memory correction

Some IRMS applications, particularly HTC/IRMS, exhibit observable memory effects whereby the isotope delta value of the sample gas(es) from one analysis is affected by the sample gas(es) from the previous analysis.

The root cause of memory effect can be elusive and there can be many contributing factors each of which can have varying proportions. For example, contamination of the autosampler and/or injection device (e.g., needle for waters), incomplete sample conversion in the reactor, chemical reactions on surfaces that are not inert (capillaries, metal connections, GC columns, and water trap chemicals), changing volumes and temperatures as gas move through the system, especially in the presence of water vapour. These possible memory sources are related to sample matrix composition, sample injection and conversion processes, cleanliness and configuration of the analytical pathway. Memory effect is a well-known challenge in the analysis of hydrogen and sulphur isotope ratios. For example, the analysis of SO_2 gas is particularly difficult and can be heavily affected by water vapour.

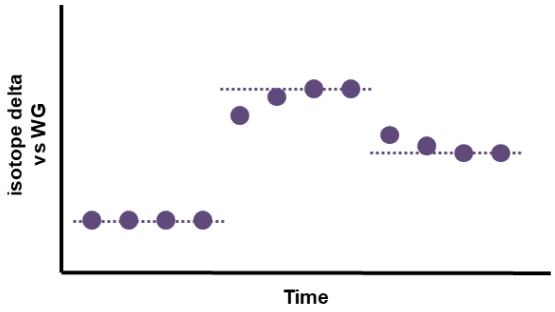


***Figure 28**. Example of a memory effect.*

There are two approaches to overcome this effect. The first is simply to carry out a larger number of sequential replicate analyses and discard the first few of each sample (or ensure that the first replicate consists of a very much larger amount of sample material than usual). This approach is robust but wastes sample, analysis time and may not be ideal in all situations.

As an alternative, a memory correction can be applied (Gröning 2011). This type of correction must include the isotopic composition of the preceding samples and also account for any difference in intensity of the signals. A memory correction will contribute significantly to the MU and its effectiveness should be monitored through the use of additional QC material(s).

An abnormally large memory effect, which cannot be ameliorated by one or two conditioning samples, typically indicates a problem with the system that should be investigated.

10.6.5 Correction for derivatisation

The isotope delta value measured for a derivatised compound will include contributions from the parent compound and from any atoms present in the derivative group. These exogenous isotopes must be accounted for and the measured isotope-delta value corrected, typically via a simple mass balance equation for a given element (37) (Rieley 1994).

$$\delta_{\mathrm{c}} = \frac{n_{\mathrm{dc}}\delta_{\mathrm{dc}} - n_{\mathrm{d}}\delta_{\mathrm{d}}}{n_{\mathrm{c}}} \qquad (37)$$

δ_{c} isotope-delta value of the parent compound

δ_{dc} isotope-delta value of the derivatised compound

δ_{d} isotope-delta value of the derivative

n_c number of atoms in the parent compound

n_{dc} number of atoms in the derivatised compound

n_d number of atoms in the derivative

The isotopic composition of the derivatisation agent, δ_{d}, should be determined independently, e.g. by EA/IRMS. Where this is not possible or practical (e.g. for a derivatisation agent supplied in small ampoules with a different ampoule used for each batch of analyses), δ_{d} must be determined by derivatising a RM (of the same chemical formula as the sample compound) of well characterised isotope delta value (δ_{c}). Equation (37) can then be rearranged to determine δ_{d}, which can then be used for subsequent calculations.

When an isotopic fractionation is associated with the derivatisation reaction, which may occur with a low yield of derivatised compound it is first necessary to determine whether this difference is consistent between different reactions. If it is, the effective stable isotope composition of the derivatising atoms(s) ($\delta_{\mathrm{d\text{-}eff}}$) can be determined using equation (38) which can then be used within equation (37) in place of δ_{d}. If it is not each sample must contain an internal standard (as discussed in section 10.5.1) that that will form the same derivative as other components of the sample. In this way $\delta_{\mathrm{d\text{-}eff}}$ can be calculated for each sample.

$$\delta_{\mathrm{d\text{-}eff}} = \frac{n_{\mathrm{dc}}\delta_{\mathrm{dc}} - n_{\mathrm{c}}\delta_{\mathrm{c}}}{n_{\mathrm{d}}} \qquad (38)$$

10.6.6 Correction for extrinsic hydrogen

Most materials contain two pool of hydrogen, intrinsic and extrinsic. The former is essentially permanent over long time periods, typically in the form of C-H bonds. The latter takes the form of absorbed/adsorbed water and hydrogen bound to polar functional groups.

Extrinsic hydrogen undergoes exchange with atmospheric moisture and contributes to the measured hydrogen isotope delta value (section 9.3.4). As noted above the time for exchange will vary from fractions of a second to many thousands of years. To avoid unnecessary and complex sample preparation it is important to consider to what extent the presence of extrinsic hydrogen confounds the overall interpretation of the hydrogen isotopic information.

It is generally difficult to obtain reliable hydrogen isotopic measurements from compounds that readily undergo substantial hydrogen exchange as discussed above (section 9.3.4). This includes compounds with highly polar function groups such as carboxylic acids and amines and also compounds such as glucose that exchange hydrogen in aqueous media due to reversible mutarotation. Larger molecules and polymeric materials may have proportionally fewer active functional groups that contribute less to the overall hydrogen isotopic composition relative to low-molecular weight compounds. Therefore, whilst it might be difficult to obtain reliable hydrogen isotopic measurements for glucose, it is possible to do so for glucose polymers, such as cellulose, which can be very important in forensic investigations.

10.6.6.1 Drying

Although many drying procedures are recommended, samples are best dried in a fan assisted oven at temperatures greater than 100 °C to constant weight. The only exceptions are samples that may decompose with liberation of significant amounts of VOCs with a concomitant change in isotopic composition.

Dried samples can be very hygroscopic and must be cooled in a desiccator with a suitable drying agent. Subsequent handling, such as measuring into capsules, should be rapid to minimise re-exposed to atmospheric moisture.

Alternatively, samples can be measuring into capsules and dried in a 96 well plate. Commonplace polystyrene 96 well plates are not suitable for drying at temperatures above 60 °C but alternative materials such as polypropylene, cycloolefin copolymer or aluminium are available.

10.6.6.2 Nitration

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

10.6.6.3 Controlled isotope exchange

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

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

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

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

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

$$\delta_{\text{Meas}} = f_{\text{Ex}}\delta_{\text{Ex}} + (1 - f_{\text{Ex}})\delta_{\text{In}} \quad (39)$$

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

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

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

The mole fraction of extrinsic hydrogen may be calculated by:

$$f_{\mathrm{Ex}} = \frac{\delta_{\mathrm{MeasA}} - \delta_{\mathrm{MeasB}}}{\delta_{\mathrm{WA}} - \delta_{\mathrm{WB}}} \times \frac{1}{\alpha_{\mathrm{Ex-W}}} \quad (40)$$

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

$$\alpha_{\mathrm{Ex-W}} = \frac{\delta_{\mathrm{Ex}} + 1}{\delta_{\mathrm{W}} + 1} \quad (41)$$

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

From the hydrogen isotope measurements of samples equilibrated against two waters, a fractionation factor ($\alpha_{Ex\text{-}W}$), and the δ^2H values of the equilibration waters, the mole fraction of extrinsic hydrogen (f_{Ex}) in the sample can be calculated. The δ^2H value of the intrinsic hydrogen (δ_{In}) can then be calculated by mass balance equation (42) (Wassenaar and Hobson 2000).

$$\delta_{In} = \frac{\delta_{MeasA} - (f_{Ex} \cdot \alpha_{Ex-W} \cdot \delta_{WA})}{1 - f_{Ex}} \quad (42)$$

Subtle differences in equilibration method such as temperature, duration, etc., can and will have an impact on the results obtained (D. B. Nelson et al. 2025).

10.6.6.4 Identical treatment by equilibration with reference materials

For some analytes (notably hair) suitable matrix-matched RMs exist for which f_{Ex} and δ_{In} have been reported (Coplen and Qi 2012, 2016; Soto et al. 2017). For these materials, samples and RMs can be subjected to identical treatment with water vapour for a sufficient duration to allow complete isotopic equilibration. Recommended equilibration times range from 5 days at ambient temperature (Bowen et al. 2005) to 4 hours at 105 °C (Soto et al. 2017). The samples and RMs are then subjected to identical drying and analysed for hydrogen isotope composition. By assuming that: (1) the samples and RMs have identical amounts of intrinsic hydrogen and (2) all of the extrinsic hydrogen reached equilibrium with the same water, these results can be corrected as if there were no exchange of hydrogen (Wassenaar and Hobson 2003; Coplen and Qi 2012).

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

10.7 Measurement uncertainty

10.7.1 What is measurement uncertainty?

The ISO defines MU as a "*non-negative parameter characterising the dispersion of the quantity values being attributed to a measurand, based on the information used*" (Barwick 2016). In simple terms, MU consists of a range of values that might be produced by a method within which the true value will fall with some degree of probability (which is usually approximately 95% for an expanded uncertainty when the data follows a normal distribution). Note that the true value does not need to be known to estimate the MU.

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

- allows the reliability of a result to be assessed,
- gives confidence to any decision based upon a result, and
- allows fair comparison of measurement results.

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

Several papers have been published that discuss MU for isotope-delta values, either focussing on specific calculations/corrections, or providing a more comprehensive overview including (Ohlsson and Wallmark 1999; Coplen 2000; Jasper 2001; Jardine and Cunjak 2005; Jardine, Kidd, and Fisk 2006; Sherwood Lollar et al. 2007; Polissar et al. 2009; Skrzypek, Sadler, and Paul 2010; Gröning 2011; Skrzypek 2013; Ohlsson 2013; Dunn et al. 2015; Szpak, Metcalfe, and Macdonald 2017; Dunn et al. 2017; Meija and Chartrand 2018; Dunn, Bilsel, et al. 2019; Gröning 2023).

Published papers and/or reports concerning RMs for isotope delta may also contain useful information regarding MU.

10.7.2 How is measurement uncertainty quantified?

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

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

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

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

(4) Combine the components together.

The components/sources of uncertainty can be divided into two categories: those evaluated by statistical means from experimental data/replicates (“Type A”) and those evaluated by different means (e.g. from calibration certificates; “Type B”).

The combination of uncertainty components requires that all are in the same mathematical format – typically a standard deviation. It also requires a measurement model to combine the different sources of uncertainty. Ideally this is the equation used to calculate the result together with additional terms to account for effects, such as precision, which are not used to calculate the result. Note that the measurement equation for isotope-delta values of the elements H, C, N, O and S is rarely equation (5) (this can be the case for determination of isotope delta of other elements by MC-ICP-MS) but more often in the form of equations (29), (31), (32) or (33) with any additional corrections as described in sections 10.6 also considered.

10.7.3 Sources of uncertainty in IRMS-based analyses

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

Contributions to MU in IRMS-based analyses may arise from (but not limited to) the following:

- Sampling
    - within sample heterogeneity,
    - between-sample heterogeneity.
- Sample preparation
    - milling,
    - drying,
    - weighing,
    - extraction,
    - hydrolysis,
    - fraction collection,
    - equilibration,
    - derivatisation,
    - offline conversion to analyte gas for DI/IRMS.
- Instrumental analysis (preparation via peripheral)
    - online conversion to analyte gas,

        - carrier flow rate,
        - reactor packing,
        - reactor temperature,
        - selection of combustion additives,
    - separation of gases/compounds,
    - removal of water,
    - open-split,
    - mass spectrometer tune parameters
- Initial data processing
    - integration parameters including peak detection,
    - baseline/background correction algorithms,
- Calculation of raw isotope-delta values
    - Isotopic variations in WG, number of WG pulses used,
    - difference in isotope-delta value and peak size/shape between WG and sample gases,
    - corrections for isobaric interferences,
        - selection of algorithm
        - selection of and uncertainty in input absolute isotope ratios.
- Corrections to raw isotope-delta value for
    - blank,
    - drift,
    - linearity,
    - memory.
- Normalisation/calibration
    - certified and measured RMs used for scale calibration
- Further corrections
    - for derivative carbon or extrinsic hydrogen, etc.

Some of these contributions can be minimised through careful choice of analytical conditions and by applying PIT (section 9.1.3). For example, it is important that RMs have similar chemical properties to samples as combustion efficiency may vary, changing the isotopic composition of the evolved gases. Similar variations can occur in the efficiency of the reduction reactor and any chemical or physical traps that remove water, oxygen or CO_2.

Identifying the contributing factors to MU is essential. This can be achieved through use of a measurement equation to identify input parameters, or a cause-and-effect diagram that similarly highlights factors contributing to measurement results (e.g. **Figure 29**). Note again that the measurement equation for isotope-delta values of the elements H, C, N, O and S is rarely equation (5) but more often in the form of equations (29), (31), (32) or (33).

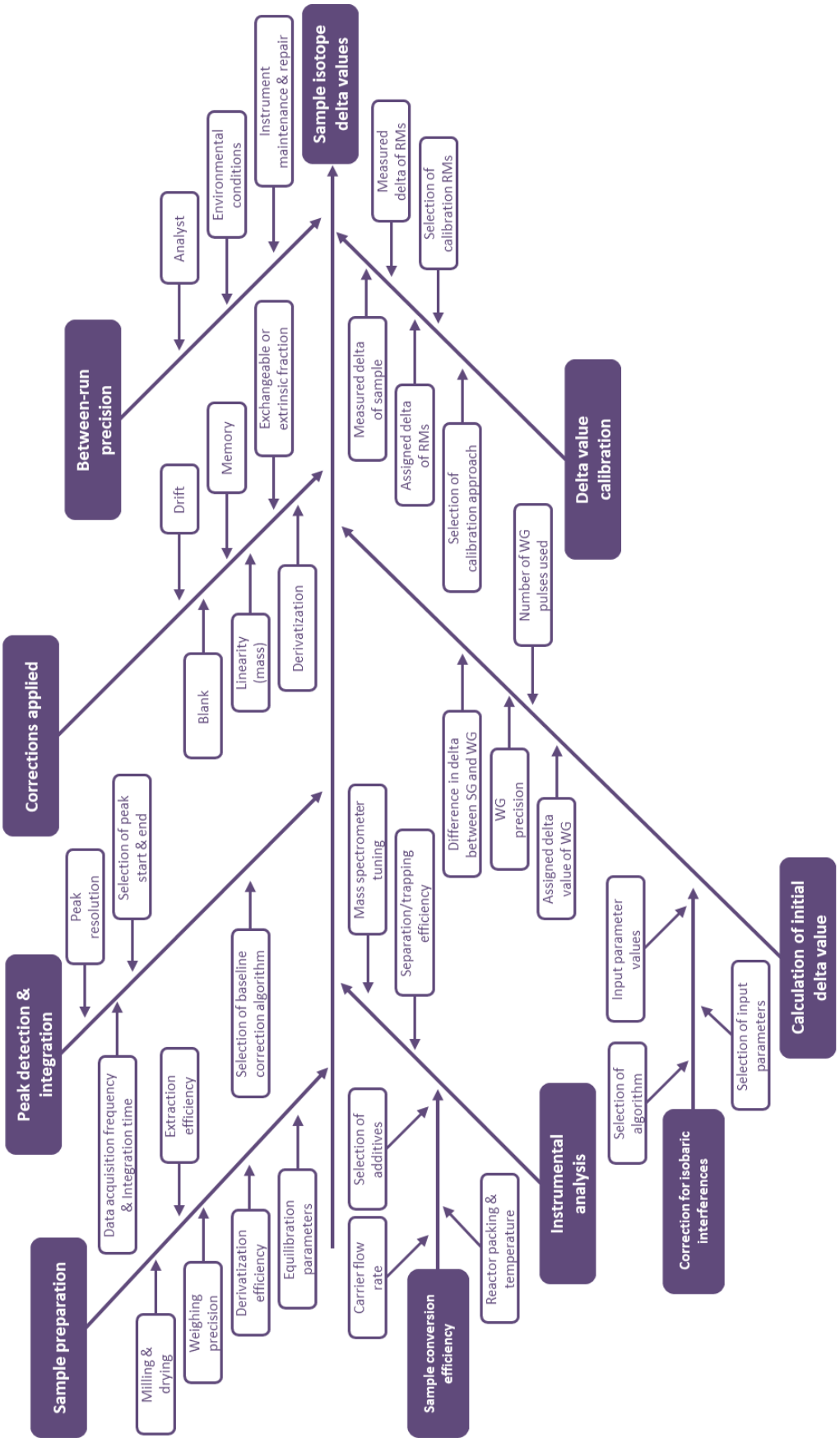


***Figure 29**. An example cause-and-effect (or fishbone) diagram to aid identification of contributions to measurement uncertainty. Not all factors identified in this figure will be relevant for all analyses described within this guide; some analyses may require additional factors beyond those noted in this figure.*

10.7.3.1 Uncertainty from subjective choices

Some aspects of IRMS instrumentation are controlled by parameters or options selected by the user. Once those choices have been made, the software will act objectively and return the same result for the same input data. Alternatively, a change to the parameter may result in a change in the result returned by the software. The subjective choices therefore introduce uncertainty.

Such contribution to uncertainty may be controlled within a single laboratory where SOPs ensure all analysts apply the same choices. Nonetheless, differences may become apparent between laboratories or when subjective choices are not standardised amongst analysts.

A comprehensive uncertainty budget will include the contributions arising from subjective choices, or an assessment providing evidence that the contributions are negligible in comparison to other sources of uncertainty.

Software-controlled processes where subjective choices are important to consider include:

- peak detection (section 10.2)
- baseline corrections (section 10.3)
- algorithm and factors for correction of isobaric interferences (section 10.4)
- selection of WG peak(s) to use for calculation of initial isotope-delta values (section 5.2.2)

10.7.4 Combining uncertainty components

To combine different uncertainty contributions to obtain a single uncertainty estimate for the result for a particular sample, uncertainties must be in the same mathematical form. According to internationally agreed rules for uncertainty evaluation, uncertainties should be expressed as sds (BIPM et al. 2012). The basic approach for combining uncertainties is the *square root of the sum of the squares rule*. Uncertainty components $u(x_1)$…….. $u(x_n)$, expressed as sds, are combined as shown in equation (43) to give the uncertainty in the result y (assuming an additive measurement model):

$$u_c(y) = \sqrt{u(x_1)^2 + u(x_2)^2 + \ldots u(x_n)^2} \quad (43)$$

This requires that the uncertainty components are expressed in the same units as the measurement result (i.e. a isotope-delta value in the case of IRMS measurements) and that uncertainty components are independent.

While the GUM also discusses relative uncertainties (i.e. that may be expressed in percent, %), these should not be applied to isotope-delta values expressed in ‰. Isotope delta is itself a relative quantity and it is not expected that the uncertainty in an isotope delta value within the natural abundance range for the element should have larger uncertainty the further away from zero the value is.

For many IRMS analyses, the measurement equation contains terms that are correlated and therefore not independent. For example, section 10.5 outlines the process of normalisation using “stretch” and “shift” factors. The uncertainty in normalised results for samples [$\delta_{true(sample)}$] will have contributions from the uncertainty of the measurements of the RMs and the sample and must also include the uncertainty in the known or certified RM isotope-delta value, as illustrated in **Figure 30**. The measured terms will also have contributions from any corrections applied, such as for blank or drift.

The “stretch” and “shift” factors are correlated, but the correlation term can be avoided by calculating the uncertainty directly from the input values as described in equation (32). This expression introduces difficulties when using the simple rules for combining uncertainties as the terms $\delta_{true(RM1)}$ and $\delta_{raw(RM1)}$ appear twice in the equation (32).

Estimating MU associated with two-point or multiple-point scale calibration requires other methods to combine uncertainty components such as the use of partial derivatives, Monte Carlo simulations, or the spreadsheet-based calculation proposed by Kragten (Kragten 1994).

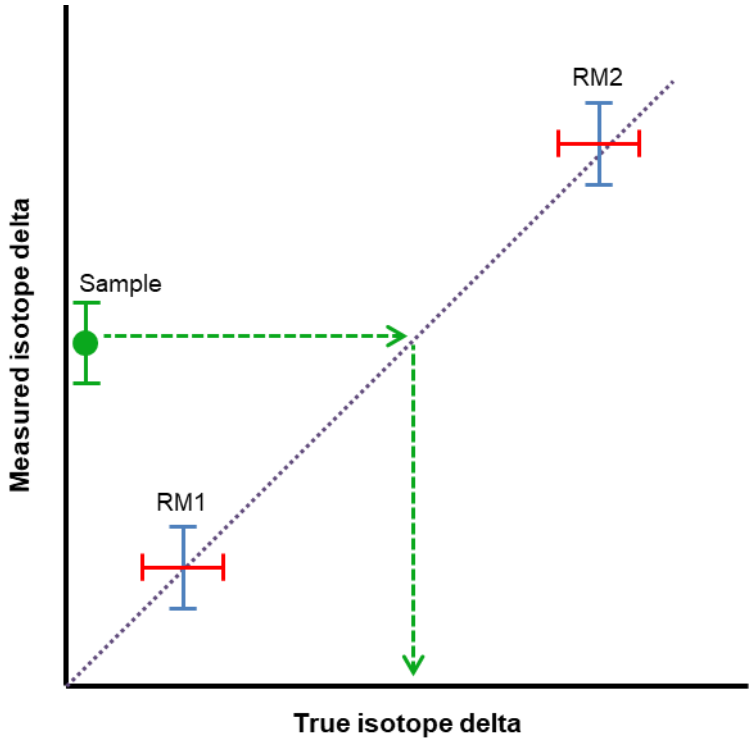


***Figure 30**. Illustration of uncertainty components for scale calibration using two RMs – the uncertainty in the known values of RMs (red, horizontal error bars) and the raw measured isotope-delta values for the RMs (blue, vertical error bars) and the sample (green, vertical error bars) will all contribute to the uncertainty in δ_{true} for the sample. Scale contraction effects will cause the slope of the calibration plot to be less than unity.*

10.7.4.1 Partial derivatives and variances from the measurement equation

This is the classical approach to combining uncertainty components found within the measurement equation (BIPM et al. 2008). For each input parameter within the measurement equation a sensitivity coefficient is required in addition to the standard uncertainty of the parameter.

The sensitivity co-efficient is the partial derivative of the measurement equation with respect to the input parameter i.e. how the measurement output changes when a specific input parameter is varied, while holding all other parameters constant. As noted above, the measurement equation will usually be in the form of one out of equations (29), (31), (32) or (33).

Using equation (32) as the example measurement equation, the sensitivity coefficient for $\delta_{\text{true(RM1)}}$ is:

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{true(RM1)}}} = \frac{\delta_{\text{raw(sample)}} - \delta_{\text{raw(RM2)}}}{\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}} \tag{44}$$

The sensitivity coefficient for $\delta_{\text{true(RM2)}}$ is:

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{true(RM2)}}} = -\frac{\delta_{\text{raw(RM1)}} - \delta_{\text{raw(sample)}}}{\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}} \tag{45}$$

The sensitivity coefficient for $\delta_{\text{raw(RM1)}}$ is:

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(RM1)}}} = -\frac{\left(\delta_{\text{true(RM1)}} - \delta_{\text{true(RM2)}}\right)\left(\delta_{\text{raw(sample)}} - \delta_{\text{raw(RM2)}}\right)}{\left(\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}\right)^2} \tag{46}$$

The sensitivity coefficient for $\delta_{\text{raw(RM2)}}$ is:

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(RM2)}}} = \frac{\left(\delta_{\text{true(RM1)}} - \delta_{\text{true(RM2)}}\right)\left(\delta_{\text{raw(sample)}} - \delta_{\text{raw(RM1)}}\right)}{\left(\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}\right)^2} \tag{47}$$

The sensitivity coefficient for $\delta_{\text{raw(sample)}}$ is:

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(sample)}}} = \frac{\delta_{\text{true(RM1)}} - \delta_{\text{true(RM2)}}}{\delta_{\text{raw(RM1)}} - \delta_{\text{raw(RM2)}}} \tag{48}$$

The square of each sensitivity coefficient is then multiplied by the variance of the relevant input parameter (i.e. the square of its standard uncertainty as this is in the form of a standard deviation). This is repeated for each input parameter and all results summed together. The result of the sum is the variance associated with the measurement result, i.e. the square of the combined standard uncertainty:

$$\begin{aligned} u^2\left(\delta_{\text{true(sample)}}\right) = &\left(\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{true(RM1)}}}\right)^2 u^2\left(\delta_{\text{true(RM1)}}\right) + \left(\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{true(RM2)}}}\right)^2 u^2\left(\delta_{\text{true(RM2)}}\right) \\ &+ \left(\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(RM1)}}}\right)^2 u^2\left(\delta_{\text{raw(RM1)}}\right) + \left(\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(RM2)}}}\right)^2 u^2\left(\delta_{\text{raw(RM2)}}\right) \\ &+ \left(\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(sample)}}}\right)^2 u^2\left(\delta_{\text{raw(sample)}}\right) \end{aligned} \tag{49}$$

The combined standard uncertainty in the measurement result is the square root of the variance:

$$u\left(\delta_{\text{true(sample)}}\right) = \sqrt{u^2\left(\delta_{\text{true(sample)}}\right)} \tag{50}$$

An uncertainty budget, i.e. the relative proportions of the combined MU arising from each contributing factor can be derived from equation (49) as the terms being summed together are proportional to the contribution to MU.

Partial derivatives of other measurement equations can be applied similarly to derive the sensitivity coefficients. Measurement equations should include any corrections applied to instrumental data including those described in Section 10.6.

Further information and description of this approach to estimation of MU can be found in the GUM and the EURACHEM Quantifying Uncertainty in Analytical Measurement (Ellison and Williams 2012; BIPM et al. 2008).

Example: Calculation of uncertainty in $\delta_{\text{true(sample)}}$ arising from two-point scale calibration using partial derivatives of the measurement equation:

RMs used for scale calibration: VSMOW2 and SLAP2.

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

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

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

Note that the values for the measurements are equal to those given in the Example on page 103.

Using equation (32) as the example measurement equation the sensitivity coefficients are:

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{true(VSMOW2)}}} = \frac{-189.0 - -420.7}{+0.3 - -420.7} = 0.550$$

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{true(SLAP2)}}} = -\frac{+0.3 - -189.0}{+0.3 - -420.7} = 0.450$$

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(VSMOW2)}}} = -\frac{(+0.3 - -427.5)(-189.0 - -420.7)}{(+0.3 - -420.7)^2} = -0.559$$

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(SLAP2)}}} = \frac{(0.0 - -427.5)(-189.0 - +0.3)}{(+0.3 - -420.7)^2} = -0.457$$

$$\frac{\partial \delta_{\text{true(sample)}}}{\partial \delta_{\text{raw(sample)}}} = \frac{0.0 - -427.5}{+0.3 - -420.7} = 1.015$$

The variance in $\delta_{\text{true(sample)}}$ is:

$$u^2\left(\delta_{\text{true(sample)}}\right) = (0.550^2 \times 0.3^2) + (0.450^2 \times 0.3^2) + (-0.559^2 \times 1.2^2) + (-0.457^2 \times 1.2^2) + (1.015^2 \times 1.5^2) = 3.116$$

The combined standard uncertainty in the in $\delta_{\text{true(sample)}}$ is:

$$u\left(\delta_{\text{true(sample)}}\right) = \sqrt{3.116} = 1.8\ ‰$$

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

The uncertainty budget can be constructed as follows:

Contribution of $\delta_{\text{true(VSMOW2)}}$	= $(0.550^2 \times 0.3^2)$/ 3.116	= 0.9%
Contribution of $\delta_{\text{true(SLAP2)}}$	= $(0.450^2 \times 0.3^2)$ / 3.116	= 0.6%
Contribution of $\delta_{\text{raw(VSMOW2)}}$	= $(-0.559^2 \times 1.2^2)$ / 3.116	= 14.5%
Contribution of $\delta_{\text{raw(SLAP2)}}$	= $(-0.457^2 \times 1.2^2)$ / 3.116	= 9.6%
Contribution of $\delta_{\text{raw(sample)}}$	= $(1.015^2 \times 1.5^2)$ / 3.116	= 74.4%

10.7.4.2 Monte Carlo simulations

The Monte Carlo approach to estimation of MU involves repeating the calculation described by the measurement equation a large number of times (usually tens of thousands). Each repetition uses a random value for each input term in the measurement equation that is selected from the underlying distribution. These distributions include uniform, triangular, normal, etc. In this way, many measurement results are simulated based upon the likely variations of the input parameters based upon their associated uncertainties.

The distribution of the large number of results obtained by simulation can be used to derive the standard combined uncertainty (this is the standard deviation across all simulated results) as well as the 95% confidence interval (this is the range covering the 2.5 to 97.5 percentile results. A histogram of the results obtained will show the distribution.

It is possible to implement Monte Carlo simulations in Microsoft Excel (Chew and Walczyk 2012). Functions such as NORMINV combined with RAND() allow random sampling from normal distributions with stated means and standard deviations. Functions providing sampling from other distributions such as uniform, triangular, etc. may also be implemented. Calculations using such input functions can simply be repeated across columns or down rows the desired number of times. Alternatively, macros can be written that copy and paste calculation results, repeat calculations using a new set of random draws and copy / paste again.

Specialised statistical software packages such as R (https://www.r-project.org/) or IBM SPSS Statistics (https://www.ibm.com/products/spss-statistics) can also be used.

10.7.4.3 Kragten spreadsheet approach to measurement uncertainty

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

The general spreadsheet set-up is shown in **Figure 31**. The values of the parameters required to calculate the result, and the associated standard uncertainties, are entered into the spreadsheet in columns B and C, respectively. The formula used to calculate the result, e.g. equation (32), is entered in cell B8. Column B is then copied into columns D to H (one column for each parameter used in the calculation of $\delta_{true(sample)}$). The uncertainty given in cell C3 is added to cell D3, the uncertainty in cell C4 is added to cell E4, and so on (cells highlighted in yellow). Cells D8 to H8 show recalculated values for $\delta_{true(sample)}$, including the effect of the uncertainty in the individual parameters. Row 9 shows the differences between the recalculated values and the original calculation for $\delta_{true(sample)}$ in cell B8. The standard uncertainty in $\delta_{true(sample)}$ (cell C8) is obtained by squaring the differences in row 9, summing them and then taking the square root.

The Kragten approach can be extended to include calculations other than normalisation that may be applied during IRMS analyses such as those described in section 10.6 as well as to handle an entire sequence/batch of analyses rather than only one sample at a time. In this way PIT can be easily adhered to as all materials analysed within a single sequence/batch will be treated in the same way. This extension of the Kragten approach from that in **Figure 31** has been described (Dunn et al. 2015), which also provides annotated, example templates, including the above example (in Excel® format) as supplementary information.

For BSIA where each replicate analysis uses a separate subsample of the material, the sd of the mean can be used as described above as each replicate is fully independent. Where the instrumental replicate analyses result from multiple injections from a single vial as is typically the case in CSIA, then the replicates are not fully independent and the sd must be used as the input uncertainty in a Kragten-type spreadsheet. This avoids underestimation of sampling uncertainty (pseudo-replication). Pseudo-replication can also occur when a material has been measured several times on each of several days but where there is a significant day-to-day (or run-to-run) effect resulting in a reduction in the number of effective degrees of freedom.

	A	B	C	D	E	F	G	H
1								
2	Parameter	value (δ^2H, ‰)	uncertainty (δ^2H, ‰)					
3	$\delta_{true(VSMOW2)}$	0.0	0.3	B3+C3	B3	B3	B3	B3
4	$\delta_{true(SLAP2)}$	−427.5	0.3	B4	B4+C4	B4	B4	B4
5	$\delta_{raw(VSMOW2)}$	0.3	1.2	B5	B5	B5+C5	B5	B5
6	$\delta_{raw(SLAP2)}$	−420.7	1.2	B6	B6	B6	B6+C6	B6
7	$\delta_{raw(sample)}$	−189.0	1.5	B7	B7	B7	B7	B7+C7
8								
9	$\delta_{true(sample)}$	Eqn. (32) applied to values above	$u(\delta_{true(sample)})$ = square root of sum of squared differences	Eqn. (32) applied to values above	Eqn. (32) applied to values above	Eqn. (32) applied to values above	Eqn. (32) applied to values above	Eqn. (32) applied to values above
10			Difference	D9-B9	E9-B9	F9-B9	G9-B9	H9-B9
11			Squared differences	$(D10)^2$	$(E10)^2$	$(F10)^2$	$(G10)^2$	$(H10)^2$

***Figure 31**. Setup of a Kragten spreadsheet for the estimation of MU arising from scale calibration of a sample δ^2H value with two RMs using equation (32).*

10.7.4.4 Combining calibrated data within or between laboratories

There are two important considerations for evaluating uncertainties associated to isotope delta values obtained by combining measurement results across measurement sequence/batches either within or between laboratories: (1) covariances in the data must be accounted for. These may arise from use of common RMs for calibration; and (2) random and systematic components of uncertainty must be considered separately – for example the systematic component of uncertainty relating to the assigned isotope delta value of a RM used for calibration cannot be reduced if more laboratories use that RM. The random component of uncertainty relating to the measurement of that RM in different laboratories can be reduced by larger numbers of measurements.

10.7.4.5 Online uncertainty tools

Several online resources relating to MU for isotope-delta values are available.

The US NIST have an “Uncertainty Machine” (https://uncertainty.nist.gov/). This allows users to specify a measurement equation, provide input parameter values and standard deviations and derive combined standard uncertainties. NIST also have a “Consensus Builder” that can be used to combine measurement results obtained by different laboratories or by application of different measurement methods, into a consensus estimate (https://consensus.nist.gov/app/nicob).

National Research Council Canada have provided an online tool (https://metrology.shinyapps.io/isotope-delta-calibration/) that can perform multiple point calibrations and derive associated MUs and also combine data from different laboratories. This is an evolution of the approach described in (Meija and Chartrand 2018).

Use of these online tools is at the risk of the user.

10.7.5 Method uncertainties from validation studies

This method for determination of MU is a top-down approach rather than the bottom-up measurement equation approach described in sections 10.7.4 and 10.7.4.3.

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

Such an estimate of MU can be easily obtained from method validation data, provided that the validation experiments are carefully planned with this in mind (Barwick et al. 2000, 2003). As

described, the precision and bias studies should be carefully planned to account for as many sources of uncertainty as possible. The sources of uncertainty that remain can either be evaluated directly from existing data or from ruggedness studies.

An example of such a MU can be found in the supplementary information to the minimum requirements for method validation of stable isotope-delta values published by the FIRMS Network (Dunn et al. 2017).

When adopting this method it is necessary periodically to update these estimations by recalculating using more recent data to account for long-term changes such as different aliquots/batches of RMs or changes in personnel.

10.7.6 Expanded measurement uncertainty

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

$$U(y) = k \times u_c(y) \tag{51}$$

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

If there are few independent replicate measurements, the effective number of degrees of freedom becomes small and the *k*-factor will need to be derived from the Student's *t* distribution. For example, when $n = 2$, 3 or 4, *k*-factors of 12.7, 4.3 and 3.2 are needed, respectively, for 95% confidence. The effective number of degrees of freedom can be increased either by performing more independent replicate measurements of the sample in question, or by using long term data for a matrix-matched in-house RM (e.g. from a control chart, section 11.1.1) as a proxy for additional measurements.

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

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

RMs used for scale calibration: VSMOW2 and SLAP2.

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

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

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

Note that the values for the measurements are equal to those given in the Example on page 99.

Kragten spreadsheet:

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

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

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

11 Quality control/assurance

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

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

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

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

One of the major contributors to the realisation of the importance of quality was the VAM program and the six principles to assure the reliability of analytical results.

(1) Analytical measurements should be made to satisfy an agreed requirement.

(2) Analytical measurements should be made using methods and equipment which have been tested to ensure they are fit for purpose.

(3) Staff making analytical measurements should be both qualified and competent to undertake the task.

(4) There should be a regular independent assessment of the technical performance of a laboratory.

(5) Analytical measurements made in one location should be consistent with those elsewhere between laboratories following methods of similar or greater quality, and

(6) Organisations making analytical measurements should have well defined quality control and quality assurance procedures.

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

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

QC is about ensuring systems are under control and performing as expected such that results may be relied upon. Meeting set performance criteria usually involves making measurements as part of the control process. Running control samples, both positive and negative, is a simple example of QC. More broadly, QC might be considered as *detection* which, in addition to measurement, might involve testing, inspection and seeking stakeholder’s views. Monitoring performance using control charts (11.1.1) is a QC activity.

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

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

Whether or not a laboratory is accredited, method validation must conform to the requirements specified in the FIRMS ten-point plan (11.3) (Dunn et al. 2017). This is essential to ensure that the the quality of results is sufficient to assure their reliability, i.e. conforming to the requirements for MU and traceability specified in ISO/IEC 17025.

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

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

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

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

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

11.1 Instrument logbook and other records

The role of logbooks is to record the performance of an instrument and to record changes to instrument hardware and software, environmental conditions and analytical procedures. From logbook records gradual changes can be observed that may not be apparent day-to-day and, importantly, step changes or failures in performance can be more easily linked to changes in hardware, procedures or environment. Instrument logbooks must be subject to periodic peer review to identify long term trends and to investigate sudden changes. Ideally, the review process will document findings and outcomes of any investigation undertaken, including root-cause-analysis.

An instrument logbook can take the form of a physical book kept close to the instrument or electronic records held in spreadsheets or databases. Often a combination of the two is most effective.

A physical logbook will allow contemporaneous, free-form records of events that may affect instrument performance. This can be especially useful for non-routine or unexpected events (e.g. power outages) that there may be no other means to record. A physical book also affords an operator the possibility to make sketches and notes of hardware such as plumbing and electrical connection for future reference.

Information that might be usefully recorded in a physical logbook would include (but not limited to):

- non-routine maintenance such as cleaning the ion source or autosampler, replacement parts, pump oil change, switching to different peripheral device,
- routine maintenance such as changing gas cylinders, reactors, crucibles, reactor packing, traps for water or other interferences/byproducts, baking out GC columns/traps,
- batch numbers for consignments of chemicals or consumables e.g. tin capsules or reactor packing materials,
- maintenance or changes to peripheral inlet systems such as EA, GC or LC instruments,
- fluctuations in environmental condition e.g. extremes of temperature or humidity or power fluctuations.

Electronic records are not as flexible as simple hand-written notes but provide a better means to monitor performance over time. An instrument operator must decide what parameters are to be

recorded and ensure these are populated at an appropriate frequency, typically every time samples are analysed. New fields can be added over time, but the real value of an electronic record is to study historic performance and visualise trends. Electronic records can usefully contain free-form fields to summarize information recorded in a physical logbook.

Information that should be record in electronic format would include (but not limited to):

- contents (outlet pressure) of working gas cylinders and record of cylinder changes,
- condition of gas filters,
- vacuum pressure,
- source tuning parameters, at least filament current (box versus trap) and H_3^+ factor but may also include accelerating voltage, electron energy, source pressure, analyser pressure, ion repeller, heater status (on/off), peak shape of analytes being measured[2]
- intensity of background gases, especially water and argon where directly measurable (see 7.4.1),
- results of daily stability and linearity tests (see 7.4.2 and 7.4.3),
- calibration data for each element e.g. slope and intercept of linear regression of the calibration plot.

Other instrument parameters and settings should be recorded when they do not meet the tolerances stated in an SOP as deviations. A long-term record of the actual instrument parameters/settings can be a useful means to identify instrumental issues requiring non-routine maintenance even if these are within specification.

Regardless of format, all logbook entries for maintenance must contain the operator's name, the date the activity was performed, a unique identifier for the instrument, as well as details of the maintenance itself.

For each analytical sequence/batch, records should be kept showing the location of samples in 96 well plates (see **Figure 21**) prior to EA analysis or autosampler sequence/batches for GC or LC inlet systems. These records can help when investigating problems of carry-over or cross-contamination in a specific batch or between batches.

Details of analytical batches must include the operator's name, start date, elements/isotope-delta values being measured, which instrument was used to make the measurement(s), a unique batch identifier, who conducted any post-run data processing and whether the batch passes QC requirements.

Physical or electronic records may be used to capture information about RMs held and used by the laboratory for calibration or QC if not within a separate controlled document. These records can include batch numbers, quantities and storage locations but importantly, should also include the certified delta-value and MU. Like all records these must be subject to periodic peer review to ensure that the delta value and MU assigned are those currently certified. Possible actions when RM delta-values are reassigned or reevaluated are discussed in section 11.4.1.

Records of the analysis of QC materials are regarded as control chard and discussed below.

11.1.1 Control charts

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

Mean value control charts usually have warning limits (mean ± 2 σ) and control limits (mean ± 3 σ). These limits will be determined using results from a *prior-period* with at least 20 results determined

[2] Analysts should note that each specific instrument and instrument software presents parameters in their own way. Not all parameters are equal across instrumentation and therefore the analyst should record the parameters appropriate to their configuration / instrumentation.

on at least 6 different days, preferably by a number of analysts. Typically, these data will have been acquired as part of the validation process.

Figure 32 shows a flow chart for the interpretation of an in-house QC material (phenacetin) based on the "Westgard rules", adapted from the IUPAC Harmonized guidelines for internal quality control in analytical chemistry laboratories (Thompson and Wood 1995). **Figure 33** shows the data from **Figure 32** modified to illustrate various breaches of the Westgard rules. Note that other rules can be adopted and applied to QC charts, such as Shewhart rules (Carroll, Pinnick, and Carroll 2003). Indeed, it is not necessary to adopt an external set of rules and use in-house rules instead, so long as they can be shown to be fit-for-purpose

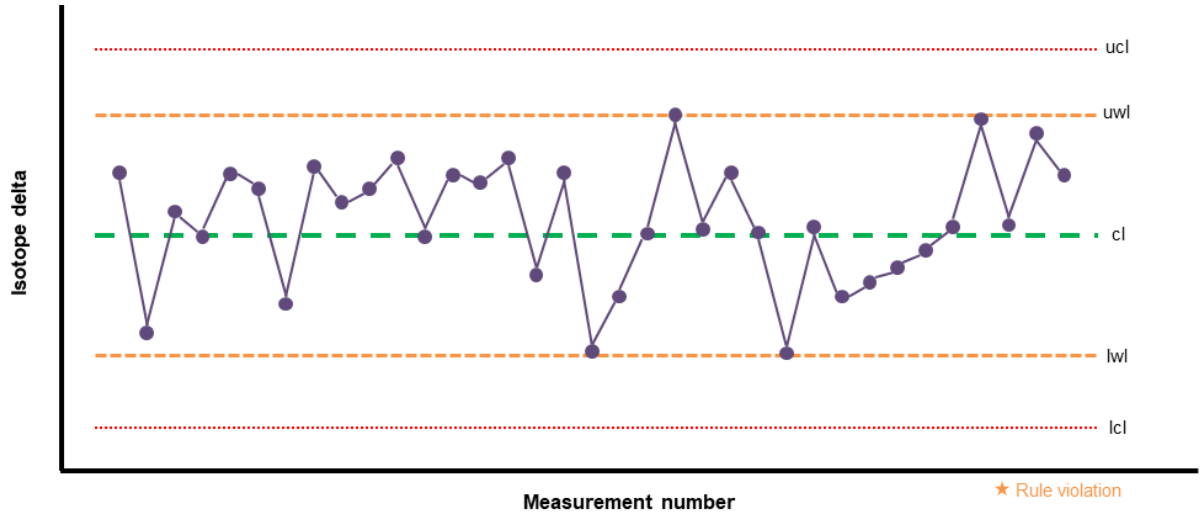


***Figure 32**. An example of a control chart for $\delta^{13}C$ measurements of a QC material; cl = centre line (mean), ucl = upper control limit (3 σ), uwl = upper warning limit (2 σ), lwl = lower warning limit (2 σ), lcl = lower control limit (3 σ). In this example all of the measurements fall within the control limits and comply with the Westgard rules.*

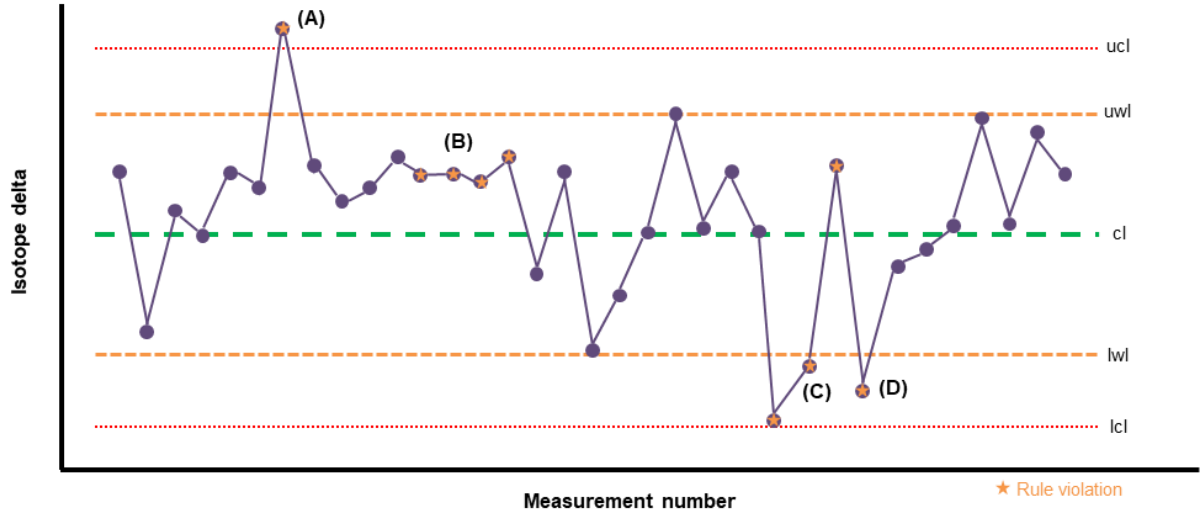


***Figure 33**. An example of a control chart showing violations of the Westgard rules; (A) result greater than 3 σ from mean, (B) 10 results on the same side of mean (also four results greater than 1 σ from mean), (C) two consecutive results greater than 2 σ from mean and, (D) two consecutive results differ by more than 4 σ.*

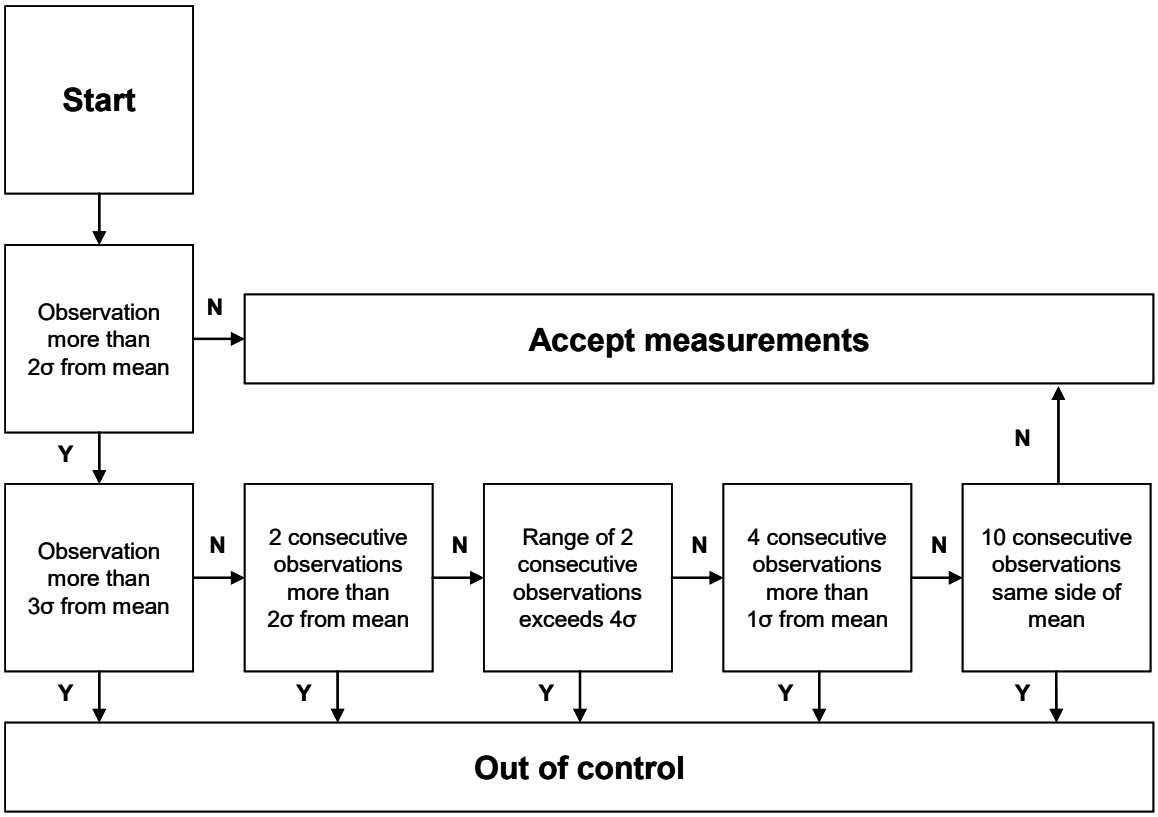


Figure 34. Schematic representation of the Westgard rules (reproduced from Thompson and Wood 1995).

11.2 Inter-laboratory exercises

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

The FIRMS Network organises an isotope ratio PT scheme in collaboration with LGC that is accredited to ISO/IEC 17043:2010. Retained materials from previous rounds are also available for use as in-house RMs. Information about this PT scheme is available from (Dunn, Carter, et al. 2021):

http://forensic-isotopes.org/

and

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

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

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

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

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

11.3 Method validation

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

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

(1) The stakeholder requirements (agreed in advance with the analytical service provider) as well as the acceptance criteria must be stated.

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

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

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

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

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

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

(8) MU for the method must be established (section 10.7). This can take into account the results from the bias, precision and ruggedness studies.

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

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

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

Stakeholder needs and acceptance criteria may not always require the use of a measurement procedure that confers the smallest possible uncertainty on measurement results. Stakeholder requirements will likely involve a balance between achievable uncertainty, cost of analysis, turnaround time, etc.

The use of validated methods for samples that fall outside the scope of the method is not good practice, unless the aim is a simple feasibility study providing an initial understanding of the isotopic composition of a new sample matrix.

To extend the scope of validation to cover a new sample matrix, a smaller verification exercise should be planned and implemented to test the performance of the method during analysis of samples of the new matrix. This results of such an exercise provides evidence that the method performance parameters are valid for the new sample matrix, and, if they are, then the scope of the validated method can be extended to cover the additional matrix type.

Ongoing use of a validated method outside of the specified scope is not acceptable practice and strongly discouraged.

11.4 Reporting isotope delta values

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

Simply providing a statement such as "carbon isotope-delta values are reported relative to VPDB where $R(^{13}C/^{12}C)$ for VPDB = 0.011180" should be avoided as insufficient information regarding traceability has been provided. In this example, should a different publication rely on a different recommended value for $R(^{13}C/^{12}C)$ for VPDB as many values have been measured or recommended over time (Dunn et al. 2024), it would not be clear if the delta values were compatible and comparable or not.

Given the evolution of the international isotope delta scales (section 4.2), the longevity of RMs produced for isotope delta calibration (some materials still currently available were initially prepared in the 1960s – see the example of NBS 22 on a following page) and periodic re-assessments by RM producers or third parties, the recommended or assigned isotope-delta values for some RMs have been changed over time.

Inconsistencies in measurement results arising from using different assigned values for the same RM has been a recognised problem for decades (Schoell, Faber, and Coleman 1983; Carter and Fry 2013a; Dunn and Skrzypek 2023).

It is therefore essential not only that the identities of RMs being used to provide traceability through calibration are reported, but also the assigned isotope delta value and associated uncertainty. This facilitates re-calculation of data should there be subsequent changes to reporting guidelines, or a need to convert data into a common reference frame (Magozzi et al. 2021). Using values recommended by e.g. the IUPAC CIAAW or IAEA Consultant's Meetings or arising from inter-laboratory characterisation studies in preference to values reported by a single laboratory is preferred.

Without this information, it is difficult to be sure that two isotope delta values purporting to be reported on the same scale are indeed comparable and compatible. This hampers the compilation of data from different sources without a rigorous assessment of data quality (Salouros et al. 2023; Dunn and Skrzypek 2023).

There is an IUPAC Technical Report detailing the minimum requirements for reporting isotope-delta values for hydrogen, carbon, nitrogen, oxygen and sulphur (Skrzypek et al. 2022). The critical aspects are:

- Isotope ratios reported as isotope-delta values must be reported on the currently agreed international scales (described in section 3 of this Guide) and the method employed must be able to produce isotope-delta values fully traceable to that scale.[3]
- At least two RMs of well characterised isotope-delta value must be used to normalise results.
- The identity and assigned isotope-delta values and uncertainties (e.g. from the certificate) of RMs used for normalisation and quality control must be reported.
- Where appropriate, the analytical protocol must be provided in sufficient detail for the reader to understand how measurements have been performed and reveal potential bias(es) (Dunn and Skrzypek 2023).
- Terminology should be used consistently (Coplen 2011).

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

- Isotope-delta values should be reported with their associated expanded uncertainties using a *k*-factor that provides the confidence required by the stakeholder(s). Generally, the approximate 95% confidence level is sufficient.

The guidance in that IUPAC Technical Report must be followed.

11.4.1 What to do if an RM supplier changes the value and/or uncertainty on a RM certificate?

Reputable RM suppliers will regularly monitor their products for ongoing stability. They may also perform re-assessments should improved measurement methods become available. As a result, assigned values and/or the associated uncertainties may change with time. If this occurs, particularly if the assigned value changes to something outside of the previous uncertainty range, then users of the RM will face the decision between three options:

- Continue to use the old value for the RM when using it for calibration of new measurement data. This affords the greatest consistency between old and new measurement results but limits comparability of new data to other laboratories due to the bias introduced by assigning different values to the same RM during calibration. The magnitude of the step change or bias to other laboratories will depend on the magnitude of the change in the assigned isotope delta value of the RM. Changes within the old, reported uncertainty may be less significant.
- Adopt the new value for calibration of new measurement results and accept that there may be a disconnect or step-change in long-term trends in e.g. QC data.
- Adopt the new value for calibration of new measurement results and also re-calculate all old data using the updated value. This may not be practical to implement if many years' worth of data from many instruments are affected. As an alternative to re-calculation, it may be possible to establish a correction for the older data, but the uncertainty introduced by such a correction will require careful assessment and propagation.

Laboratories should check the impact on measurement results of implementing each of these options in terms of changes to isotope delta values obtained and/or the achievable uncertainty.

Laboratories must also state the source of any values and uncertainties associated with calibration RMs so that others can detect the possible presence of bias. It may be helpful to collate the values and uncertainties for all RMs held by a laboratory centrally and review this list as part of the laboratory quality system.

Example: NBS 22 mineral oil for $\delta^{13}C$.

NBS 22 mineral oil was prepared in the 1960s and assigned a carbon isotope delta value relative to the original PDB of $\delta^{13}C_{PDB}$ = –29.4‰ (Hut 1987; Schoell, Faber, and Coleman 1983; Eckelmann et al. 1962).

An interlaboratory comparison in 1983 resulted in a proposed value of $\delta^{13}C_{PDB}$ = –29.81 ± 0.06‰ which was the mean and associated uncertainty of four independent direct calibrations of NBS 22 against the NBS 20 carbonate RM (Schoell, Faber, and Coleman 1983).

The USGS Reston Stable Isotope Laboratory re-assessed NBS 22 also in 1983 and reported $\delta^{13}C_{PDB}$ = –29.61 ± 0.03‰ (standard deviation of 7 independent preparations for DI/IRMS analysis) again using NBS 20 for calibration (Coplen, Kendall, and Hopple 1983).

The 1987 IAEA Consultants' Meeting report contains results from an interlaboratory comparison for NBS 22 (among other RMs) for carbon isotope delta relative to the new VPDB scale of $\delta^{13}C_{VPDB}$ = –29.73 ± 0.09‰ which was the mean and standard deviation of the eight reported results (Hut 1987).

A subsequent IAEA Consultants' Meeting in 1993 suggested that NBS 22 should be assigned $\delta^{13}C_{VPDB}$ = –29.739 ± 0.124‰ on the basis of collated data (Gonfiantini, Stichler, and Rozanski 1995).

In 1999, during characterisation of CO_2 RMs for carbon isotope delta NIST reported a new value of $\delta^{13}C_{VPDB}$ = –29.9 ± 0.1‰ for NBS 22 (Verkouteren 1999); while Wieser & Brand reported a result using laser ablation followed by combustion and analysis by IRMS and obtained $\delta^{13}C_{VPDB}$ = –29.72 ± 0.20‰ for NBS 22 (Wieser and Brand 1999).

In 2003, the USGS and Max Planck Institute for Biogeochemistry in Jena re-assessed NBS 22 using a combination of offline DI-IRMS and online EA-IRMS analyses and suggested that $\delta^{13}C_{VPDB}$ = –29.91 ± 0.03‰ for NBS 22 be adopted having normalised their results to LSVEC having $\delta^{13}C_{VPDB}$ = –46.48‰ exactly (Qi et al. 2003). This value was later recalculated following adoption of the VPDB-LSVEC scale to $\delta^{13}C_{VPDB}$ = –29.99 ± 0.03‰ (Coplen et al. 2006b).

In 2005, the Commonwealth Scientific and Industrial Research Organisation (CSIRO) reported that NBS 22 and other organic RMs required re-assessment following adoption of the VPDB scale and recommended a value of $\delta^{13}C_{VPDB}$ = –29.95 ± 0.05‰ (Stalker, Bryce, and Andrew 2005).

During the 2006 inter-laboratory comparison where the VPDB-LSVEC scale was proposed, a new value for NBS 22 was reported of $\delta^{13}C_{VPDB\text{-}LSVEC}$ = –30.03 ± 0.09‰ (expanded uncertainty with k = 1.96) (Coplen et al. 2006b). This study also reported a number of results calibrated to NBS 19 alone with mean and standard deviation of the mean of $\delta^{13}C_{VPDB}$ = –29.84 ± 0.05‰.

In 2016, an inter-laboratory characterisation of new organic RMs also provided a new recommended value for NBS 22 of $\delta^{13}C_{VPDB\text{-}LSVEC}$ = –30.02 ± 0.04‰ (standard uncertainty) (Schimmelmann et al. 2016).

The USGS reported a value of $\delta^{13}C_{VPDB\text{-}LSVEC}$ = –29.99 ± 0.07‰ for NBS 22 in 2015 obtained during characterisation of a new L-glutamic acid RM (Qi et al. 2016).

During their 2021 characterisation of a new carbonate RM that reported results traceable to both the VPDB and VPDB-LSVEC scales, the USGS again assessed NBS 22 and found $\delta^{13}C_{VPDB}$ = –29.90 ± 0.05‰ and $\delta^{13}C_{VPDB\text{-}LSVEC}$ = –29.99 ± 0.05‰ (Qi et al. 2021).

Plots of these results have been summarised in publications from Hélie et al. 2021 and Meija 2023.

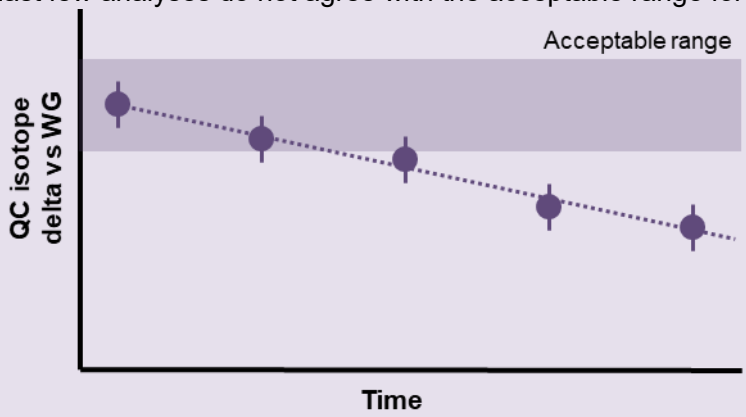
11.4.2 What to do if a sequence/batch containing "irreplaceable" samples fails QC criteria

It may be tempting to introduce additional corrections to data to bring QC results into line with the expected values this is, however, fraught with potential issues.

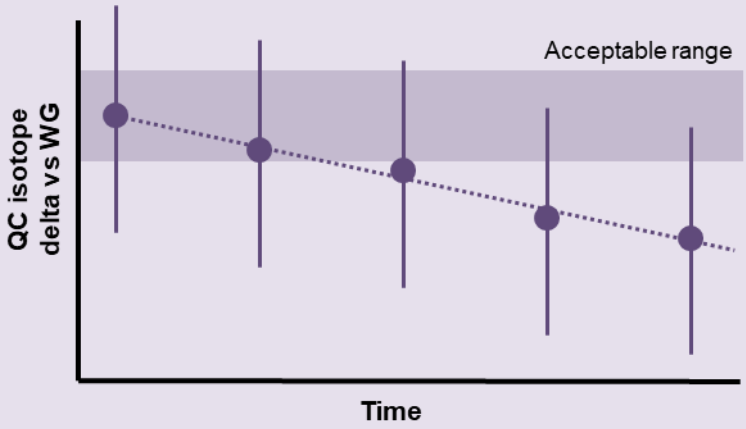
Expanding the MU obtained for data treated with additional corrections further to ensure the same level of confidence is a transparent approach. Reporting a confidence interval at specified confidence may be preferred over reporting an expanded MU with a larger *k*-factor. For example, instead of using the typical $k = 2$ to derive an expanded MU with 95% confidence from the combined MU, one could report a 95% confidence interval that has been obtained from the combined MU using a larger *k*-factor.

Example: Data failing QC criteria.

In a sequence/batch of analyses, the QC material interspersed throughout the sequence indicates a drift of isotope-delta values significantly outside the amount of drift expected for the instrument. The last few analyses do not agree with the acceptable range for the QC material.



Instead of applying a large drift correction, the uncertainty associated to each QC result is uncreased until the uncertainty overlaps with the expected value:



An equivalent expansion of uncertainty is then applied to each sample result within the sequence/batch to ensure that the reported uncertainty interval has a high enough probability of containing the true value.

12 Troubleshooting

Troubleshooting is a normal part of working with IRMS instrumentation and its associated peripherals. The troubleshooting process can be streamlined if an instrument is operating within the manufacturer specifications, environmental conditions are satisfactory, daily to weekly checks have been performed (section 11.1) and the analytical method is fit-for-purpose (section 11.3).

12.1 Routine maintenance

To ensure the continued performance of IRMS instruments and to minimise downtime, routine maintenance must be scheduled and undertaken according to operating procedures and recorded in an instrument logbook (section 11.1). Typical routine maintenance activities include:

- clean auto-sampler trays,
- remove ash from EA reactor tubes,
- replace reactors,
- replace chemical traps,
- baking EA GC columns,
- maintenance of vacuum pumps,
- clean the ion source.

When troubleshooting it can be very informative to check the instrument logbook to ensure when routine maintenance has been performed.

12.2 Visual inspection

Never overlook the obvious - a visual inspection of the equipment may reveal problems.

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

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

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

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

12.3 Planned and unplanned shutdowns

Operating manuals will contain information about the correct procedures for shutting down the IRMS and peripheral devices. Operators should read and be familiar with these processes in case it becomes necessary to shut down the instrument at short notice.

The following text provides some general advice about shutting down instrumentation. Readers are reminded to take all necessary precautions and use PPE when working with elevated pressures and temperatures, high voltages, etc.

When performing routing maintenance on the IRMS, it is important to switch off the filament emission, high voltage and source heaters before venting the instrument to atmospheric pressure. This with prevent oxidation of hot source components by atmospheric oxygen.

When performing routine maintenance of peripheral equipment and inlet systems it Is advisable to isolate the IRMS to prevent accidental ingress of atmospheric gases.

Always switch off the ion source emission and high voltage before isolating or reconnecting a peripheral that may have concomitant sudden changes in source pressure.

Following maintenance, it is important to allow sufficient time for carrier gas to flow through peripherals and displace any atmospheric gases, or gases evolved from new components, before reconnecting to the IRMS.

Following a sudden loss of carrier gas, don't panic. The biggest danger is that atmospheric gases will enter and damage the ion source or enter hot components that must be blanketed by an inert gas.

- Isolate the IRMS and/or switch off the ion source emission and high voltage.
- Bring all ovens, furnaces and reactors to room temperature as quickly as practical.
- If a GC is in use open the oven door to drop the column temperature quickly.
- Typically, do not switch off peripherals. This may cool furnaces and reactors quickly but turning off cooling fans can dissipate heat to sensitive components.

After carrier gas is restored, check the condition of self-indicating gas filters that may have been affected by an ingress of air. Replace if necessary.

Check for physical damage and allow sufficient time for the system to flush with carrier gas before bringing ovens, furnaces and reactors to working temperatures.

Following a sudden loss of electrical power, don't panic.

- If possible, physically isolate the IRMS vacuum system.
- Switch off any physical electrical switches for vacuum, heaters etc. This may prevent further damage if power returns suddenly.
- When power is lost, ovens, furnaces and reactors will cool automatically but loss of cooling fans may cause heat to dissipate to sensitive components. Consider removing panels to allow air to circulate.
- If a GC is in use open the oven door to drop the column temperature quickly.
- Turbo pumps will continue spinning, maintaining a high vacuum, for several minutes but this may have the effect of sucking oil from a staled rotary pump.

When power is restored check for physical damage. Ensure the vacuum system is working correctly and allow sufficient time for working vacuum to be established. Switch on the ion source emission and high voltage and check correct function. Allow sufficient time for the system to flush with carrier gas as failure of electronic valves may have stopped this. Once the IRMS and peripherals appear to be operational bring ovens, furnaces and reactors to working temperatures.

12.4 Mass spectrometer

Symptoms	Possible causes	Resolution
Indicator light suggests acceleration voltage is OK, but there is no emission	Ion source filament failed.	After turning off HV, check filament continuity on external contacts. Remove ion source and replace filament.
Box and trap values fluctuating	Filament has weakened and is flexing. Likely to break soon. Wrong electrical connection to ion block or lenses	Remove and replace filament. Remove source and check connections.
False pressure reading	Some pressure sensors are affected by magnetic field (e.g. Penning gauge). Dirt on pressure sensor.	Change magnet setting to see if vacuum readback changes. Remove and clean sensor.
High background with ion source needle valve closed	Air trapped in mass spectrometer.	Air may be trapped in DI valves.
Tailing on CO_2 WG peak	Solenoid not moving smoothly. Capillary moved or broken inside open-split. Contaminated ion source.	Observe and lubricate with a tiny amount of very fine oil if movement is jerky. Use a hand-lens to examine the open-split. Clean ion source.
Stability checks fail acceptance criteria	Poor purity carrier gas. Change to WG cylinder temperature Gas cylinder may be reduced in pressure is near empty. Interference from contaminants eluting from the GC column.	Ensure suitable gas supply. Check condition of gas filters and replace if necessary. WG composition can change with temperature. Monitor laboratory temperature and control as best as possible Check sufficient gas supply (cylinders are not empty and regulators are correctly set). Bake out column.
Poor linearity over range of working gas intensities	Filament may be moving. Poor ion source tuning parameters.	Check box and trap values are stable (see above if not). Check ion source tuning.
Poor vacuum	False vacuum reading Leak Vacuum pump failure.	See above. Check argon and nitrogen backgrounds levels. Ensure fixings around ion source and pressure gauge are secure. Check pumps are functioning.

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

12.5 Elemental analyser

Symptoms	Possible causes	Resolution
High nitrogen blank	Insufficient helium purge to auto-sampler	Check purge flow. Check auto-sampler is sealed.
	Oxygen may be contaminated with nitrogen.	Ensure suitable grade oxygen is attached for combustion. Run blanks with O_2 pulse disabled
Poor nitrogen and/or carbon isotope ratio measurements	Insufficient oxygen for combustion	Check O_2 supply/flow
	Reduction reactor is exhausted	Monitor *m/z* 30 to determine if the reduction reactor needs replacing.
Nitrogen intensity increase in subsequent samples, or increased *m/z* 30 signal	Reduction reactor is exhausted and NO_x is eluting from the GC column. May appear as a shoulder on the nitrogen peak.	Check amount of unoxidized copper. Replace reduction reactor if necessary.
Long tail on carbon dioxide peak	Sample start time too long (sample drops after O_2 has passed).	Observe combustion flash if possible. Alter sample timings and observe effect on peak shape. Only alter this after checking previous points.
	No/not enough O_2 for sample combustion.	Check O_2 flow.
	Large leak in analytical circuit.	Check for leaks.
	Blockage/restriction in O_2 gas supply.	Test gas lines for blockages/restrictions (replace any damaged or blocked sections).
Baseline drift after CO_2 peak (broad shallow peak)	Water bleeding through the GC column and detector.	Exhausted water trap, replace packing with fresh material. Monitor *m/z* 18 levels over time to identify when this occurs.
	GC column is saturated	Bake out the column at >100 °C
Peak broadening, peak separation is poor, peak tailing	Slow/restricted carrier gas flow.	Clean out ash. Check carrier flow rate entering EA and MS.
	Dead volume in reactors or traps.	Check packing of reactors.
	Possible contamination or aging of GC column.	Bake out column.

Symptoms	Possible causes	Resolution
No sample peaks detected	Sample not loaded correctly.	Check samples loaded in correct order.
	Sample did not drop into reactor.	Crimp capsules so they are approximately spherical. If capsules are flat they may slip under the auto-sampler tray, or be caught on the edge of the auto-sampler tray, not dropping or dropping at the same time as the following sample.
	Samples caught between the outer reactor tube and the ash crucible.	Open the reactor and check the height of the ash crucible. Look for trapped capsules.
	Check carrier gas flow from EA to IRMS	If possible, inject Ar and monitor *m/z* 40 (see 7.7.2)
	Incorrect chemical traps	e.g. check CO_2 sorbent is not installed when analysing carbon
Unusually, large peak with unexpected isotope-delta values	As above, this can also result if a capsule knocks a trapped capsule into the reactor and gas is evolved from two different samples.	Check the analytical sequence/batch for earlier missing samples. Open the reactor and check the height of the ash crucible. Look for trapped capsules.
Furnace heater does not operate	Insufficient helium flow.	Ensure helium carrier pressure is appropriate and there are no leaks.
	Thermocouple failed (reactor may be hot, but temperature read-out differs from that expected).	Check electrical continuity. Replace thermocouple.
	Furnace heater failed (temperature reading correct, but furnace will not heat).	Check/replace fuse.
		Check electrical continuity. Replace furnace heater.
High backgrounds for N_2, H_2O, O_2, Ar	Auto-sampler seals leaking.	Test outside of auto-sampler for He leaks. Replace seals.
	GC column is contaminated.	Bake out column.
	Trap chemicals are exhausted.	Replace trap chemicals.
	Gas purity is incorrect.	Ensure correct gas supply.
	Ion source heaters or inlet valve heaters failed.	Check indicator lights to see if heater has failed. Replace heater.

Symptoms	Possible causes	Resolution
Rapid consumption of reduction tube chemicals	Oxygen pulse too large	Test smaller O_2 loops or flows. The oxygen pulse should only be sufficient to combust the sample and capsule.
	Oxygen leaking into system.	Ensure auto-sampler is leak free.

12.6 GC and combustion or high temperature conversion interfaces

Symptoms	Possible causes	Resolution
High water (*m/z* 18) background	Nafion® old or damaged. (The membrane can be damaged by sudden pressure changes, e.g. if solvent enters the reactor.)	Inspect with a hand lens for visible damage. Old tubing appears dark brown; new tubing is typically light in colour. Replace if necessary (soaking the end of the tubing in methanol will cause it to expand and makes it easy to fit over the fused silica).
	Excess column bleed.	Check the water background with the GC column at ambient temperature. If this is acceptable the GC column may need to be conditioned (in back-flush mode).
High O_2 (*m/z* 32) background	Reactor not conditioned.	For several hours after re-generation the reactor will bleed a significant amount of oxygen. If the O_2 background is higher than normal switch the system into “back-flush” mode for several hours. If the problem persists check that the regeneration O_2 is not bleeding into the system (i.e. isolate the oxygen cylinder).
High Ar (*m/z* 40) background	Atmospheric leak.	This is a common problem with the GC interface with many possible causes. Before connecting a GC column to the IRMS interface it is good practice to leak test the GC injector. This will isolate any leak to the interface. Set the IRMS to monitor *m/z* 40 and apply a small flow of argon to each fitting in the interface; start at the fitting closest to the mass spectrometer. Apply the Ar only for a few seconds and wait to see any increase in signal. As you move to fittings further back in the interface remember that it will take longer for the Ar to reach the IRMS instrument.

Symptoms	Possible causes	Resolution
No peaks	GC column connected to the wrong reactor.	If the interface has reactors for both ^{2}H and ^{13}C check that the correct reactor is connected.
	Reactor not at temperature or heater broken.	Check the set and read-back temperatures for the reactor.
	Broken / blocked capillary.	Perform Ar test (section 7.7.2) to ensure there is continuous He flow from the GC to the IRMS instrument.
Poor chromatography	Any number of problems with the GC injector or column.	If possible connect the GC column directly to a detector such as FID or MS. It is much easier to troubleshoot chromatographic problems without the extra complication of the IRMS interface.
Poor chromatography (not due to GC components)	Partially blocked reactor.	Perform Ar test (7.7.2) and check for changes in retention time and peak.
	Atmospheric leak	Perform Ar test and hexane tests (7.7.2 and 7.7.3) and check the peak width and height.
	Cold spots in GC.	Ensure the fittings inside the GC oven are not touching the walls.
Sudden or gradual loss of sensitivity	Any number of problems with the GC injector or column.	If possible connect the GC column directly to a detector such as FID or MS.
Varying loss of sensitivity and peak broadening	Loose fitting in interface.	Check fittings are tight especially those subject to repeated temperature cycling or continuous elevated temperatures.
Variable isotope-delta values	Atmospheric leaks	Check the background gases and if these are high, leak check the interface as described above.
	Reactor depleted.	Re-oxidize the reactor and repeat the analyses. If these are still poor the reactor may need to be replaced.

Symptoms	Possible causes	Resolution
	Reactor not conditioned.	Check the *m/z* 32 background (O_2). If this is high, switch the system to back-flush mode and allow the system to stabilise for several hours.
Change in retention time	Leaks or blockages.	It would be uncommon to see a change in retention time that was not accompanied by a change in peak size or isotope-delta value. Simplistically increased retention time will be caused by blockages and reduced retention time will be caused by leaks. The Ar test (section 7.7.2) and a leak test of the GC injector are good starting points.

12.7 LC and chemical oxidation interface

Symptoms	Possible causes	Resolution
High backpressure	(Partial) blockage within system.	Examine components of system including in-line filters, HPLC column, gas separation unit and oxidation reactor for blockages and remove/flush out/replace components as required. Take care when handling narrow capillaries within the interface as these can be easily damaged.
More negative $\delta^{13}C$ values obtained than expected for QC materials	Incomplete oxidation.	Check oxidation potential. Increase reagent concentration or flow rate or add catalyst. Reduce mobile phase flow rate.
	Non-quantitative extraction of CO_2 from mobile phase.	Increase gas flow of separation unit. Check for blockage within separation unit.
Reagent pumps not delivering solutions	(Partial) blockage within system.	Check in-line filters and replace if necessary. Disassemble pump head(s) and clean according to manufacturer's recommendations.
Elevated CO_2 background (*m/z* 44)	Change in mobile phase carbon content.	Check degassing and that sparging of mobile phase is occurring correctly. Check separate source/lot/batch of mobile phase to rule out contamination. Ensure HPLC system (including column) have been sufficiently purged of organic mobile phases.
Elevated water background (*m/z* 18)	Separation unit not operating correctly. Gas drier not operating correctly.	Check for blockages, and clear if any are found. Check purge gas flow rates. Exchange Nafion® membrane. Check gas flows.

Symptoms	Possible causes	Resolution
Elevated oxygen background (*m/z* 32)	Reagents too concentrated or being delivered too quickly.	Dilute reagents (or prepare fresh solutions) and check the reagent pump flow rates. Too high an oxygen background can shorten filament lifetime.
Sequence/batch does not run correctly, there are missing injections, etc.	Lack of communication between PC, HPLC, auto-sampler and/or interface.	Check communication cables. Test communication between parts of the system where possible.

13 Bibliography (further reading)

Aitkin C., and F. Taroni 2004. *Statistics and the Evaluation of Evidence for Forensic Scientists* 2nd ed. John Wiley and Sons Chichester, UK ISBN 0-470-84367-5.

Barnett V. 1974. *Elements of Sampling Theory*. The English Universities Press Ltd, London 1974 ISBN 0-340-17386-6.

Carter, J. F., and L. A. Chesson, eds. 2017. *Food Forensics: Stable Isotopes as a Guide to Authenticity and Origin*. Boca Raton Fl: CRC Press.

Cook, C. S., B. R. Erkkila, S. Chakraborty, B. J. Tipple, T. E. Cerling, J. R. Ehleringer. 2017. *Stable Isotope Biogeochemistry and Ecology: Laboratory Manual*. Seattle WA: Kindle Direct Publishing.

Coplen, T. B., J. A. Hopple, J. K. Böhlke, H. S. Peiser, S. E. Rieder, H. R. Krouse, K. J. R. Rosman, T. Ding, R.D. Vocke, K.M. Revesz. A. Lamberty, P.D.P. Taylor, and P. De Bièvre. 2002. *Compilation of Minimum and Maximum Isotope Ratios of Selected Elements in Naturally Occurring Terrestrial Materials and Reagents.* Reston Va: U.S. Geological Survey Water-Resources Investigations Report 01-4222.

Coplen, T. B., J. K. Böhlke, P. De Bièvre, T. Ding, N. E. Holden, J. A. Hopple, H. R. Krouse, A. Lamberty , H.S. Peiser , K. Revesz , S.E. Rieder , K.J.R. Rosman , E. Roth , P.D.P. Taylor , R.D. Vocke Jr., and Y.K. Xiao. 2002. Isotope-abundance Variations of Selected Elements (IUPAC Technical Report), *Pure and Applied Chemistry* 74, no. 10: 1987-2017.

Coplen, T. B., Y. Shrestha. 2016. Isotope-abundance variations and atomic weights of selected elements: 2016 (IUPAC Technical Report), *Pure and Applied Chemistry*, 88, no. 12:1203-1224.

de Groot, P. A., ed. 2004. *Handbook of Stable Isotope Analytical Techniques Volume I*. Amsterdam: Elsevier Science.

de Groot, P. A. 2008. Handbook of Stable Isotope Analytical Techniques Volume 2. Amsterdam: Elsevier Science.

Doyle, S. 2018. *Quality Management in Forensic Science*. Cambridge Ma: Academic Press. ISBN 9780128054161.

Ehleringer, J. R., and S. M. Matheson. 2010. Stable Isotopes and Courts. *Utah Law Review* 2010, no. 2: 385-442.

ISO/IEC 17025:2017 General requirements for the competence of testing and calibration laboratories - https://www.iso.org/standard/66912.html.

ISO/IEC Guide 98-3:2008. Uncertainty of Measurement -- Part 3: Guide to the Expression of Uncertainty in Measurement (GUM:1995). This document is also available from the BIPM.

Meier-Augenstein, W. 2018. Stable Isotope Forensics – Methods and Forensic Applications of Stable Isotope Analysis. 2nd ed. Oxford: Wiley-Blackwell .

Ramsey, M. H., S. L. R. Ellison, and P. Rostron (eds) Eurachem / EUROLAB / CITAC / Nordtest / AMC Guide: Measurement uncertainty arising from sampling, 2019, ISBN 978-0-948926-35-8.

Thompson M., S. L. R. Ellison, and R. Wood. 2006. The International Harmonized Protocol for the Proficiency Testing of Analytical Chemical Laboratories, *Pure and Applied Chemistry*, 78, 145-196.

14 References

Accoe, Frederik, Michael Berglund, Benny Geypens, and Philip Taylor. 2008. 'Methods to Reduce Interference Effects in Thermal Conversion Elemental Analyzer/Continuous Flow Isotope Ratio Mass Spectrometry $\delta^{18}O$ Measurements of Nitrogen-Containing Compounds'. *Rapid Communications in Mass Spectrometry* 22 (14): 2280–86. doi:10.1002/rcm.3609.

Allison, C. E., R. J. Francey, and H. A. J Meijer. 1995. 'Recommendations for the Reporting of Stable Isotope Measurements of Carbon and Oxygen in CO_2 Gas'. In *Reference and Intercomparison Materials for Stable Isotopes of Light Elements*, TECDOC 825:155–62. Vienna: IAEA.

Assonov, Sergey S. 2018. 'Summary and Recommendations from the International Atomic Energy Agency Technical Meeting on the Development of Stable Isotope Reference Products (21–25 November 2016)'. *Rapid Communications in Mass Spectrometry* 32 (10). Wiley Online Library: 827–30. doi:10.1002/rcm.8102.

Assonov, Sergey S., and Carl A. M. Brenninkmeijer. 2003a. 'On the ^{17}O Correction for CO_2 Mass Spectrometric Isotopic Analysis'. *Rapid Communications in Mass Spectrometry* 17 (10): 1007–16. doi:10.1002/rcm.1012.

———. 2003b. 'A Redetermination of Absolute Values for $^{17}R_{VPDB\text{-}CO2}$ and $^{17}R_{VSMOW}$'. *Rapid Communications in Mass Spectrometry* 17 (10): 1017–29. doi:10.1002/rcm.1011.

Assonov, Sergey S., M. Groening, A. Fajgelj, J.-F. Hélie, and C. Hillaire-Marcel. 2020. 'Preparation and Characterization of IAEA-603, a New Primary Reference Material Aimed at the VPDB Scale Realisation for $\delta^{13}C$ and $\delta^{18}O$ Determination'. *Rapid Communications in Mass Spectrometry* 34 (20): e8867. doi:10.1002/rcm.8867.

Baczynski, Allison A., Christopher R. Brodie, Oliver Kracht, and Katherine H. Freeman. 2023. 'Sequential Measurement of ^{13}C, ^{15}N, and ^{34}S Isotopic Composition on Nanomolar Quantities of Carbon, Nitrogen, and Sulfur Using Nano-Elemental Analysis/Isotope Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 37 (5): e9444. doi:10.1002/rcm.9444.

Baertschi, P. 1976. 'Absolute ^{18}O Content of Standard Mean Ocean Water'. *Earth and Planetary Science Letters* 31 (3): 341–44. doi:10.1016/0012-821X(76)90115-1.

Bahar, B., A. P. Moloney, F. J. Monahan, S. M. Harrison, A. Zazzo, C. M. Scrimgeour, I. S. Begley, and O. Schmidt. 2009. 'Turnover of Carbon, Nitrogen, and Sulfur in Bovine Longissimus Dorsi and Psoas Major Muscles: Implications for Isotopic Authentication of Meat1'. *Journal of Animal Science* 87 (3): 905–13. doi:10.2527/jas.2008-1360.

Balint, Sawyer, Morgan Schwartz, Drew N. Fowler, Stella Linnekogel, Sáde Cromratie Clemons, and Laura K. Burkemper. 2024. 'Experimental Assessment of Elemental Analyzer Isotope Ratio Mass Spectrometry Normalization Methodologies for Environmental Stable Isotopes'. *Rapid Communications in Mass Spectrometry* 38 (17): e9837. doi:10.1002/rcm.9837.

Barrie, A., and S. J. Prosser. 1996. 'Automated Analysis of Light-Element Stable Isotopes by Isotope Ratio Mass-Spectrometry'. In *Mass Spectrometry of Soils, Edited by T. W. Boutton*, 1–46. New York: Marcel Dekker Inc.

Barwick, Vicki J. 2016. *EURACHEM/CITAC Guide: Guide to Quality in Analytical Chemistry - an Aid to Accreditation*. 3rd ed. www.eurachem.org.

Barwick, Vicki J., S. L. R. Ellison, M. J. Q. Rafferty, and R. S. Gill. 2000. 'The Evaluation of Measurement Uncertainty from Method Validation Studies'. *Accreditation and Quality Assurance* 5 (3): 104–13. doi:10.1007/s007690050022.

Barwick, Vicki J., and Stephen L. R. Ellison. 2000. 'The Evaluation of Measurement Uncertainty from Method Validation Studies'. *Accreditation and Quality Assurance* 5 (2): 47–53. doi:10.1007/s007690050010.

Barwick, Vicki J., Stephen L. R. Ellison, Mark J. Q. Rafferty, and Rattanjit S. Gill. 2003. 'The Evaluation of Measurement Uncertainty from Method Validation Studies'. In *Measurement Uncertainty in Chemical Analysis*, edited by Paul De Bièvre and Helmut Günzler, 187–96. Berlin, Heidelberg: Springer. doi:10.1007/978-3-662-05173-3_34.

Barwick, Vicki J., and E. Prichard. 2011. *EURACHEM Guide: Terminology in Analytical Measurement: Introduction to VIM 3*. 1st ed. www.eurachem.org.

Beaudoin, Georges, B. E. Taylor, D. Rumble, and M. Thiemens. 1994. 'Variations in the Sulfur Isotope Composition of Troilite from the Cañon Diablo Iron Meteorite'. *Geochimica et Cosmochimica Acta* 58 (19): 4253–55. doi:10.1016/0016-7037(94)90277-1.

Bellobono, I. R. 1968. 'Isotope Separation Factors of ^{14}C-Amino Acids in Ion-Exchange on Resins with Carboxylic Groups'. *Journal of Chromatography A* 34 (January): 515–19. doi:10.1016/0021-9673(68)80096-2.

BIPM, IEC, IFCC, ILAC, IUPAC, IUPAP, ISO, and OIML. 2008. 'Evaluation of Measurement Data — Guide to the Expression of Uncertainty in Measurement (GUM)'. JCGM 100.

———. 2012. 'The International Vocabulary of Metrology—Basic and General Concepts and Associated Terms (VIM)'. JCGM 200.

Boeker, Peter, Torsten Haas, and Peter Schulze Lammers. 2013. 'Theory and Practice of a Variable Dome Splitter for Gas Chromatography-Olfactometry'. *Journal of Chromatography A* 1286 (April): 200–207. doi:10.1016/j.chroma.2013.02.057.

Boeker, Peter, Jan Leppert, Bodo Mysliwietz, and Peter Schulze Lammers. 2013. 'Comprehensive Theory of the Deans' Switch As a Variable Flow Splitter: Fluid Mechanics, Mass Balance, and System Behavior'. *Analytical Chemistry* 85 (19). American Chemical Society: 9021–30. doi:10.1021/ac401419j.

Boettger, Tatjana, Marika Haupt, Kay Knöller, Stephan M. Weise, John S. Waterhouse, Katja T. Rinne, Neil J. Loader, et al. 2007. 'Wood Cellulose Preparation Methods and Mass Spectrometric Analyses of δ^{13}C, δ^{18}O, and Nonexchangeable δ^{2}H Values in Cellulose, Sugar, and Starch: An Interlaboratory Comparison'. *Analytical Chemistry* 79 (12). American Chemical Society: 4603–12. doi:10.1021/ac0700023.

Böhlke, J. K., and Tyler B. Coplen. 1995. 'Interlaboratory Comparison of Reference Materials for Nitrogen-Isotope-Ratio Measurements'. In *Reference and Intercomparison Materials for Stable Isotopes of Light Elements*, TECDOC 825:51–66. Vienna: IAEA.

Böhlke, J. K., C. J. Gwinn, and Tyler B. Coplen. 1993. 'New Reference Materials for Nitrogen-Isotope-Ratio Measurements'. *Geostandards Newsletter* 17 (1): 159–64. doi:https://doi.org/10.1111/j.1751-908X.1993.tb00131.x.

Böhlke, J. K., S. J. Mroczkowski, and Tyler B. Coplen. 2003. 'Oxygen Isotopes in Nitrate: New Reference Materials for ^{18}O:^{17}O:^{16}O Measurements and Observations on Nitrate-Water Equilibration'. *Rapid Communications in Mass Spectrometry* 17 (16): 1835–46. doi:10.1002/rcm.1123.

Boschker, H. T. S., T. C. W. Moerdijk-Poortvliet, P. van Breugel, M. Houtekamer, and J. J. Middelburg. 2008. 'A Versatile Method for Stable Carbon Isotope Analysis of Carbohydrates by High-Performance Liquid Chromatography/Isotope Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 22 (23): 3902–8. doi:10.1002/rcm.3804.

Bowen, Gabriel J., Lesley Chesson, Kristine Nielson, Thure E. Cerling, and James R. Ehleringer. 2005. 'Treatment Methods for the Determination of δ^{2}H and δ^{18}O of Hair Keratin by Continuous-Flow Isotope-Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 19 (17): 2371–78. doi:10.1002/rcm.2069.

Brand, Willi A. 1996. 'High Precision Isotope Ratio Monitoring Techniques in Mass Spectrometry'. *Journal of Mass Spectrometry* 31 (3): 225–35. doi:10.1002/(SICI)1096-9888(199603)31:3<225::AID-JMS319>3.0.CO;2-L.

———. 2004. 'Mass Spectrometer Hardware for Analyzing Stable Isotope Ratios'. In *Handbook of Stable Isotope Analytical Techniques*, edited by P.A. de Groot, 1:835–56. Amsterdam: Elsevier.

Brand, Willi A., Sergey S. Assonov, and Tyler B. Coplen. 2010. 'Correction for the ^{17}O Interference in $\delta(^{13}C)$ Measurements When Analyzing CO_2 with Stable Isotope Mass Spectrometry (IUPAC Technical Report)'. *Pure and Applied Chemistry* 82 (8): 1719–33. doi:10.1351/PAC-REP-09-01-05.

Brand, Willi A., and Tyler B. Coplen. 2012. 'Stable Isotope Deltas: Tiny, yet Robust Signatures in Nature'. *Isotopes in Environmental and Health Studies* 48 (3). Taylor & Francis: 393–409. doi:10.1080/10256016.2012.666977.

Brand, Willi A., Tyler B. Coplen, Anita T. Aerts-Bijma, J. K. Böhlke, Matthias Gehre, Heike Geilmann, Manfred Gröning, et al. 2009. 'Comprehensive Inter-Laboratory Calibration of Reference Materials for δ^{18}O versus VSMOW Using Various on-Line High-Temperature Conversion Techniques'. *Rapid Communications in Mass Spectrometry* 23 (7): 999–1019. doi:10.1002/rcm.3958.

Brand, Willi A., Tyler B. Coplen, Jochen Vogl, Martin Rosner, and Thomas Prohaska. 2014. 'Assessment of International Reference Materials for Isotope-Ratio Analysis (IUPAC Technical Report)'. *Pure and Applied Chemistry* 86 (3). De Gruyter: 425–67. doi:10.1515/pac-2013-1023.

Brodie, Chris R., Melanie J. Leng, James S. L. Casford, Christopher P. Kendrick, Jeremy M. Lloyd, Zong Yongqiang, and Michael I. Bird. 2011. 'Evidence for Bias in C and N Concentrations and $\delta^{13}C$ Composition of Terrestrial and Aquatic Organic Materials Due to Pre-Analysis Acid Preparation Methods'. *Chemical Geology* 282 (3): 67–83. doi:10.1016/j.chemgeo.2011.01.007.

Brodie, Christopher. 2023. 'Simple Software Solution for N_{18} Diversion When Measuring $\delta^{18}O$ Values of Nitrogen-Rich Samples Materials Using a Thermo Scientific EA-IRMS System'. *MethodsX* 11 (December): 102268. doi:10.1016/j.mex.2023.102268.

Caimi, Richard J., and J. Thomas Brenna. 1997. 'Quantitative Evaluation of Carbon Isotopic Fractionation during Reversed-Phase High-Performance Liquid Chromatography'. *Journal of Chromatography A* 757 (1): 307–10. doi:10.1016/S0021-9673(96)00694-2.

Camin, Federica, Dinka Besic, Paul J. Brewer, Colin E. Allison, Tyler B. Coplen, Philip J. H. Dunn, Matthias Gehre, et al. 2025. 'Stable Isotope Reference Materials and Scale Definitions—Outcomes of the 2024 IAEA Experts Meeting'. *Rapid Communications in Mass Spectrometry* 39 (14): e10018. doi:10.1002/rcm.10018.

Carroll, Tomás A., Heather A. Pinnick, and Wallace E. Carroll. 2003. 'Probability and the Westgard Rules'. *Annals of Clinical & Laboratory Science* 33 (1). Association of Clinical Scientists: 113–14.

Carter, James F., and Lesley Ann Chesson, eds. 2017. *Food Forensics: Stable Isotopes as a Guide to Authenticity and Origin*. Boca Raton: CRC Press. doi:10.1201/9781315151649.

Carter, James F., and B. Fry. 2013a. 'Ensuring the Reliability of Stable Isotope Ratio Data—beyond the Principle of Identical Treatment'. *Analytical and Bioanalytical Chemistry* 405 (9): 2799–2814. doi:10.1007/s00216-012-6551-0.

———. 2013b. '"Do It Yourself" Reference Materials for $\delta^{13}C$ Determinations by Isotope Ratio Mass Spectrometry'. *Analytical and Bioanalytical Chemistry* 405 (14): 4959–62. doi:10.1007/s00216-013-6851-z.

Chesson, Lesley A., David W. Podlesak, Thure E. Cerling, and James R. Ehleringer. 2009. 'Evaluating Uncertainty in the Calculation of Non-Exchangeable Hydrogen Fractions within Organic Materials'. *Rapid Communications in Mass Spectrometry* 23 (9): 1275–80. doi:10.1002/rcm.4000.

Chew, Gina, and Thomas Walczyk. 2012. 'A Monte Carlo Approach for Estimating Measurement Uncertainty Using Standard Spreadsheet Software'. *Analytical and Bioanalytical Chemistry* 402 (7): 2463–69. doi:10.1007/s00216-011-5698-4.

CIAAW. 2025. 'The Isotopic Composition of VPDB'. *Chemistry International* 47 (2). De Gruyter: 34–35. doi:10.1515/ci-2025-0216.

Clough, Robert, Peter Evans, Tim Catterick, and E. Hywel Evans. 2006. '$\delta^{34}S$ Measurements of Sulfur by Multicollector Inductively Coupled Plasma Mass Spectrometry'. *Analytical Chemistry* 78 (17). American Chemical Society: 6126–32. doi:10.1021/ac060875h.

Cohn, Mildred, and Harold C. Urey. 1938. 'Oxygen Exchange Reactions of Organic Compounds and Water'. *Journal of the American Chemical Society* 60 (3). American Chemical Society: 679–87. doi:10.1021/ja01270a052.

Coleman, Max L. 2004. 'Data Corrections for Mass-Spectrometer Analysis of SO_2'. In *Handbook of Stable Isotope Analytical Techniques*, edited by P.A. de Groot, 957–70. Elsevier.

Colón, Maria, Gloria Rodriguez, and Ramón Orlando. 1993. 'Representative Sampling of "Street" Drug Exhibits'. *Journal of Forensic Sciences* 38 (3): 641–48.

Coplen, Tyler B. 1994. 'Reporting of Stable Hydrogen, Carbon, and Oxygen Isotopic Abundances (Technical Report)'. *Pure and Applied Chemistry* 66 (2). Oxford; New York: Pergamon Press [etc.], 1960-: 273–76.

———. 1995. 'Discontinuance of SMOW and PDB'. *Nature* 375 (6529). Springer: 285–285.

———. 2000. 'A Guide for the Laboratory Information Management System (LIMS) for Light Stable Isotopes–Versions 7 and 8: US Geological Survey Open-File Report 00-345, 121 Pp'. US Geological Survey. http://water.usgs.gov/software/code/geochemical/lims/doc/ofr00345.pdf.

———. 2011. ‘Guidelines and Recommended Terms for Expression of Stable-Isotope-Ratio and Gas-Ratio Measurement Results’. *Rapid Communications in Mass Spectrometry* 25 (17): 2538–60. doi:10.1002/rcm.5129.

Coplen, Tyler B., Willi A. Brand, Matthias Gehre, Manfred Gröning, Harro A. J. Meijer, Blaza Toman, and R. Michael Verkouteren. 2006a. ‘After Two Decades a Second Anchor for the VPDB $\delta^{13}C$ Scale’. *Rapid Communications in Mass Spectrometry* 20 (21): 3165–66. doi:10.1002/rcm.2727.

———. 2006b. ‘New Guidelines for $\delta^{13}C$ Measurements’. *Analytical Chemistry* 78 (7). American Chemical Society: 2439–41. doi:10.1021/ac052027c.

Coplen, Tyler B., H. R. Krouse, and J. K. Böhlke. 1992. ‘Reporting of Nitrogen-Isotope Abundances (Technical Report)’. *Pure and Applied Chemistry* 64 (6). De Gruyter: 907–8. doi:10.1351/pac199264060907.

Coplen, Tyler B., and Haiping Qi. 2012. ‘USGS42 and USGS43: Human-Hair Stable Hydrogen and Oxygen Isotopic Reference Materials and Analytical Methods for Forensic Science and Implications for Published Measurement Results’. *Forensic Science International* 214 (1–3). Elsevier: 135–41. doi:10.1016/j.forsciint.2011.07.035.

———. 2016. ‘A Revision in Hydrogen Isotopic Composition of USGS42 and USGS43 Human-Hair Stable Isotopic Reference Materials for Forensic Science’. *Forensic Science International* 266. Elsevier: 222–25. doi:10.1016/j.forsciint.2016.05.029.

Coplen, Tyler B., Joe D. Wildman, and Julie. Chen. 1991. ‘Improvements in the Gaseous Hydrogen-Water Equilibration Technique for Hydrogen Isotope-Ratio Analysis’. *Analytical Chemistry* 63 (9). American Chemical Society: 910–12. doi:10.1021/ac00009a014.

Craig, Harmon. 1953. ‘The Geochemistry of the Stable Carbon Isotopes’. *Geochimica et Cosmochimica Acta* 3 (2–3): 53–92. doi:10.1016/0016-7037(53)90001-5.

———. 1957. ‘Isotopic Standards for Carbon and Oxygen and Correction Factors for Mass-Spectrometric Analysis of Carbon Dioxide’. *Geochimica et Cosmochimica Acta* 12 (1): 133–49. doi:10.1016/0016-7037(57)90024-8.

———. 1961. ‘Standard for Reporting Concentrations of Deuterium and Oxygen-18 in Natural Waters’. *Science* 133 (3467). American Association for the Advancement of Science: 1833–34. doi:10.1126/science.133.3467.1833.

Crivellari, Stefano, Patrícia Junia Viana, Marília de Carvalho Campos, Henning Kuhnert, Alyne Barros Machado Lopes, Francisco William da Cruz, and Cristiano Mazur Chiessi. 2021. ‘Development and Characterization of a New In-House Reference Material for Stable Carbon and Oxygen Isotopes Analyses’. *Journal of Analytical Atomic Spectrometry* 36 (6). The Royal Society of Chemistry: 1125–34. doi:10.1039/D1JA00030F.

Cueto Díaz, Sergio, Jorge Ruiz Encinar, Alfredo Sanz-Medel, and J. Ignacio García Alonso. 2013. ‘Liquid Chromatography, Chemical Oxidation, and Online Carbon Isotope Dilution Mass Spectrometry as a Universal Quantification System for Nonvolatile Organic Compounds’. *Analytical Chemistry* 85 (3). American Chemical Society: 1873–79. doi:10.1021/ac3032542.

Deming, William Edwards. 1943. *Statistical Adjustment of Data.* New York: Wiley.

Ding, T., S. Valkiers, H. Kipphardt, P. De Bièvre, P.D.P. Taylor, R. Gonfiantini, and R. Krouse. 2001. ‘Calibrated Sulfur Isotope Abundance Ratios of Three IAEA Sulfur Isotope Reference Materials and V-CDT with a Reassessment of the Atomic Weight of Sulfur’. *Geochimica et Cosmochimica Acta* 65 (15): 2433–37. doi:10.1016/S0016-7037(01)00611-1.

Doyle, Sean P. G., and Gerard van der Peijl. 2020. *FIRMS Guidance for the Forensic Interpretation of Isotope Ratio Data*. 1st ed. Bristol: FIRMS. https://www.forensic-isotopes.org/gpg.html.

Dugan, George. 1977. ‘Automatic Carbon, Hydrogen, Nitrogen, Sulfur Analyzer Chemistry of Sulfur Reactions’. *Analytical Letters* 10 (7–8). Taylor & Francis: 639–57. doi:10.1080/00032717708059229.

Dunn, Philip J. H., and Federica Camin. 2024. ‘The “LSVEC Problem” for the Vienna Peedee Belemnite Carbon Isotope-Delta Scale’. *Rapid Communications in Mass Spectrometry* 38 (17): e9841. doi:10.1002/rcm.9841.

Dunn, Philip J.H., Mine Bilsel, Adnan Şimşek, Ahmet Ceyhan Gören, Murat Tunç, Nives Ogrinc, Milena Horvat, and Heidi Goenaga-Infante. 2019. ‘Practical and Theoretical Considerations for the Determination of $\delta^{13}C_{VPDB}$ Values of Methylmercury in the Environment’. *Rapid Communications in Mass Spectrometry* 33 (13): 1122–36. doi:10.1002/rcm.8453.

Dunn, Philip J.H., James F. Carter, Lesley A. Chesson, Sean P. G. Doyle, John D. Howa, Wayne Gaunt, and Matthew Whetton. 2021. 'The FIRMS Network's PT Scheme: What Can Be Learned about Inter-Laboratory Performance?' *Forensic Chemistry* 22 (March): 100306. doi:10.1016/j.forc.2021.100306.

Dunn, Philip J.H., Lu Hai, Dmitry Malinovsky, and Heidi Goenaga-Infante. 2015. 'Simple Spreadsheet Templates for the Determination of the Measurement Uncertainty of Stable Isotope Ratio Delta Values: Letter to the Editor'. *Rapid Communications in Mass Spectrometry* 29 (22): 2184–86. doi:10.1002/rcm.7376.

Dunn, Philip J.H., Sarah Hill, Simon Cowen, Heidi Goenaga-Infante, Mike Sargent, Ahmet Ceyhan Gören, Mine Bilsel, et al. 2019. 'Lessons Learned from Inter-Laboratory Studies of Carbon Isotope Analysis of Honey'. *Science & Justice* 59 (1): 9–19. doi:10.1016/j.scijus.2018.08.003.

Dunn, Philip J.H., Dmitry Malinovsky, and Heidi Goenaga-Infante. 2020. 'Calibration Hierarchies for Light Element Isotope Delta Reference Materials'. *Rapid Communications in Mass Spectrometry* 34 (9). doi:10.1002/rcm.8711.

Dunn, Philip J.H., Dmitry Malinovsky, Gill Holcombe, Simon Cowen, and Heidi Goenaga-Infante. 2021. 'Guidance for Characterization of In-House Reference Materials for Light Element Stable Isotope Analysis'. *Rapid Communications in Mass Spectrometry* 35 (20): e9177. doi:10.1002/rcm.9177.

Dunn, Philip J.H., Dmitry Malinovsky, Nives Ogrinc, Doris Potočnik, Lukas Flierl, Olaf Rienitz, Dipayan Paul, and Harro A. J. Meijer. 2024. 'Re-Determination of $R(^{13}C/^{12}C)$ for Vienna Peedee Belemnite (VPDB)'. *Rapid Communications in Mass Spectrometry* 38 (16): e9773. doi:10.1002/rcm.9773.

Dunn, Philip J.H., Helen Salouros, James F. Carter, and Sean P. Doyle. 2017. 'Forensic Application of Stable Isotope Delta Values: Proposed Minimum Requirements for Method Validation'. *Rapid Communications in Mass Spectrometry* 31 (17): 1476–80. doi:10.1002/rcm.7925.

Dunn, Philip J.H., and Grzegorz Skrzypek. 2023. 'Perspective: Hidden Biases in Isotope Delta Results and the Need for Comprehensive Reporting'. *Rapid Communications in Mass Spectrometry* 37 (20): e9623. doi:10.1002/rcm.9623.

Ellison, S. L. R., and A Williams. 2012. *EURACHEM/CITAC Guide: Quantifying Uncertainty in Analytical Measurement*. 3rd ed. www.eurachem.org.

Epstein, S, and T Mayeda. 1953. 'Variation of O18 Content of Waters from Natural Sources'. *Geochimica et Cosmochimica Acta* 4 (5): 213–24. doi:10.1016/0016-7037(53)90051-9.

Ettre, Leslie S. 2008. *Chapters in the Evolution of Chromatography*. London: Imperial College Press.

Fajgelj, Ales, and Sergey S. Assonov. 2016. 'Reference Sheet for Certified Reference Material IAEA-603 (Calcite)'. *International Atomic Energy Agency, Vienna*.

Farquhar, G. D., B. K. Henry, and J. M. Styles. 1997. 'A Rapid On-Line Technique for Determination of Oxygen Isotope Composition of Nitrogen-Containing Organic Matter and Water'. *Rapid Communications in Mass Spectrometry* 11 (14): 1554–60. doi:10.1002/(SICI)1097-0231(199709)11:14<1554::AID-RCM980>3.0.CO;2-I.

Fearn, Tom, and Michael Thompson. 2001. 'A New Test for "Sufficient Homogeneity"'. *Analyst* 126 (8). The Royal Society of Chemistry: 1414–17. doi:10.1039/B103812P.

Federherr, E., C. Cerli, F. M. S. A. Kirkels, K. Kalbitz, H. J. Kupka, R. Dunsbach, L. Lange, and T. C. Schmidt. 2014. 'A Novel High-Temperature Combustion Based System for Stable Isotope Analysis of Dissolved Organic Carbon in Aqueous Samples. I: Development and Validation'. *Rapid Communications in Mass Spectrometry* 28 (23): 2559–73. doi:10.1002/rcm.7052.

Federherr, E., H. J. Kupka, C. Cerli, K. Kalbitz, R. Dunsbach, A. Loos, M. de Reus, L. Lange, R. J. Panetta, and T. C. Schmidt. 2016. 'A Novel Tool for Stable Nitrogen Isotope Analysis in Aqueous Samples'. *Rapid Communications in Mass Spectrometry* 30 (23): 2537–44. doi:10.1002/rcm.7740.

Federherr, E., S. Willach, N. Roos, L. Lange, K. Molt, and T. C. Schmidt. 2016. 'A Novel High-Temperature Combustion Interface for Compound-Specific Stable Isotope Analysis of Carbon and Nitrogen via High-Performance Liquid Chromatography/Isotope Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 30 (7): 944–52. doi:10.1002/rcm.7524.

Federherr, Eugen, Robert J. Panetta, Lutz Lange, and Hans J. Kupka. 2015. 'Novel Tool for Simultaneous Carbon and Nitrogen Stable Isotope Analyses in Aqueous Samples'. *Procedia Earth and Planetary Science*, 11th Applied Isotope Geochemistery Conference AIG-11, 13 (January): 100–103. doi:10.1016/j.proeps.2015.07.024.

Filer, Crist N. 1999. 'Isotopic Fractionation of Organic Compounds in Chromatography'. *Journal of Labelled Compounds and Radiopharmaceuticals* 42 (2): 169–97. doi:10.1002/(SICI)1099-1344(199902)42:2<169::AID-JLCR178>3.0.CO;2-0.

Flierl, Lukas, and Olaf Rienitz. 2024. 'OCEAN—An EXCEL Add-in for ^{17}O Correction Using a Novel Approximation'. *Methods X* 12 (June). Elsevier. doi:10.1016/j.mex.2023.102529.

Fry, Brian. 2007. 'Coupled N, C and S Stable Isotope Measurements Using a Dual-Column Gas Chromatography System'. *Rapid Communications in Mass Spectrometry* 21 (5): 750–56. doi:10.1002/rcm.2892.

Fry, Brian, James F. Carter, Keita Yamada, Naohiro Yoshida, and Dieter Juchelka. 2018. 'Position-Specific $^{13}C/^{12}C$ Analysis of Amino Acid Carboxyl Groups – Automated Flow-Injection Analysis Based on Reaction with Ninhydrin'. *Rapid Communications in Mass Spectrometry* 32 (12): 992–1000. doi:10.1002/rcm.8126.

Gat, J. R., and R. Gonfiantini. 1981. 'Stable Isotope Hydrology. Deuterium and Oxygen-18 in the Water Cycle'. http://inis.iaea.org/Search/search.aspx?orig_q=RN:13677657.

Gat, Joel R., and Paul DeBievre. 2002. 'Making an Honest Measurement Scale out of the Oxygen Isotope *δ*-Values'. *Rapid Communications in Mass Spectrometry* 16 (23): 2205–7. doi:https://doi.org/10.1002/rcm.857.

Gehre, Matthias, Julian Renpenning, Heike Geilmann, Haiping Qi, Tyler B. Coplen, Steffen Kümmel, Natalija Ivdra, Willi A. Brand, and Arndt Schimmelmann. 2017. 'Optimization of On-Line Hydrogen Stable Isotope Ratio Measurements of Halogen- and Sulfur-Bearing Organic Compounds Using Elemental Analyzer–Chromium/High-Temperature Conversion Isotope Ratio Mass Spectrometry (EA-Cr/HTC-IRMS)'. *Rapid Communications in Mass Spectrometry* 31 (6): 475–84. doi:10.1002/rcm.7810.

Gehre, Matthias, Julian Renpenning, Tetyana Gilevska, Haiping Qi, Tyler B. Coplen, Harro A. J. Meijer, Willi A. Brand, and Arndt Schimmelmann. 2015. 'On-Line Hydrogen-Isotope Measurements of Organic Samples Using Elemental Chromium: An Extension for High Temperature Elemental-Analyzer Techniques'. *Analytical Chemistry* 87 (10). American Chemical Society: 5198–5205. doi:10.1021/acs.analchem.5b00085.

Gehre, Matthias, and Gerhard Strauch. 2003. 'High-Temperature Elemental Analysis and Pyrolysis Techniques for Stable Isotope Analysis'. *Rapid Communications in Mass Spectrometry* 17 (13): 1497–1503. doi:10.1002/rcm.1076.

Gentile, Natacha, Michel J. Rossi, Olivier Delémont, and Rolf T. W. Siegwolf. 2013. '$\delta^{15}N$ Measurement of Organic and Inorganic Substances by EA-IRMS: A Speciation-Dependent Procedure'. *Analytical and Bioanalytical Chemistry* 405 (1): 159–76. doi:10.1007/s00216-012-6471-z.

Gilevska, Tetyana, Matthias Gehre, and Hans Hermann Richnow. 2014. 'Performance of the Wet Oxidation Unit of the HPLC Isotope Ratio Mass Spectrometry System for Halogenated Compounds'. *Analytical Chemistry* 86 (15). American Chemical Society: 7252–57. doi:10.1021/ac501174d.

Gloël, J., C. Robinson, G. H. Tilstone, G. Tarran, and J. Kaiser. 2015. 'Technical Note: Could Benzalkonium Chloride Be a Suitable Alternative to Mercuric Chloride for Preservation of Seawater Samples?' *Ocean Science* 11 (6). Copernicus GmbH: 947–52. doi:10.5194/os-11-947-2015.

Gonfiantini, R. 1984. *Advisory Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations*. Vienna, Austria: IAEA. https://inis.iaea.org/collection/NCLCollectionStore/_Public/18/075/18075746.pdf.

Gröning, Manfred. 2004. 'International Stable Isotope Reference Materials'. In *Handbook of Stable Isotope Analytical Techniques*, edited by P.A. de Groot, 874–906. Elsevier.

———. 2011. 'Improved Water $\delta^{2}H$ and $\delta^{18}O$ Calibration and Calculation of Measurement Uncertainty Using a Simple Software Tool'. *Rapid Communications in Mass Spectrometry* 25 (19): 2711–20. doi:10.1002/rcm.5074.

———. 2023. 'Some Pitfalls in the Uncertainty Evaluation of Isotope Delta Reference Materials'. *Accreditation and Quality Assurance* 28 (3): 101–14. doi:10.1007/s00769-022-01527-6.

Groot, Pier A. de. 2004. *Handbook of Stable Isotope Analytical Techniques*. Elsevier.

Günther, Franklin R., and Antonio Possolo. 2011. 'Calibration and Uncertainty Assessment for Certified Reference Gas Mixtures'. *Analytical and Bioanalytical Chemistry* 399 (1): 489–500. doi:10.1007/s00216-010-4379-z.

Guyon, Francois, Laetitia Gaillard, and Dana Alina Magdas. 2018. 'Optimization of Oxygen-18 Ratio Measurements by Equilibration – Isotope Ratio Mass Spectrometry Using Various Water Content Matrices'. *Talanta* 189 (November): 653–56. doi:10.1016/j.talanta.2018.07.022.

Guyon, Francois, Laetitia Gaillard, Nathalie Sabathié, and Marie-Hélène Salagoity. 2015. 'Direct Determination of the Oxygen 18 Stable Isotope Ratio of in Situ Water Contained in Pasty Matrices'. *Analytical Methods* 7 (7). The Royal Society of Chemistry: 3211–14. doi:10.1039/C5AY00038F.

Hagemann, R., G. Nief, and E. Roth. 1970. 'Absolute Isotopic Scale for Deuterium Analysis of Natural Waters. Absolute D/H Ratio for SMOW'. *Tellus* 22 (6): 712–15. doi:10.1111/j.2153-3490.1970.tb00540.x.

Halsted, R. E., and Alfred O. Nier. 1950. 'Gas Flow through the Mass Spectrometer Viscous Leak'. *Review of Scientific Instruments* 21 (12). AIP Publishing: 1019–21.

Harms, A. V., and Manfred Gröning. 2017. *Reference Sheet for International Measurement Standards VSMOW2 and SLAP2*. rev1. Vienna, Austria: IAEA. https://nucleus.iaea.org/sites/ReferenceMaterials/Shared%20Documents/ReferenceMaterials/StableIsotopes/VSMOW2/VSMOW2_SLAP2.pdf.

Hélie, Jean-François, Agnieszka Adamowicz-Walczak, Paul Middlestead, Michelle M.G. Chartrand, Zoltán Mester, and Juris Meija. 2021. 'Discontinuity in the Realization of the Vienna Peedee Belemnite Carbon Isotope Ratio Scale'. *Analytical Chemistry* 93 (31). American Chemical Society: 10740–43. doi:10.1021/acs.analchem.1c02458.

Hélie, Jean-François, and Claude Hillaire-Marcel. 2021. 'Designing Working Standards for Stable H, C, and O Isotope Measurements in CO_2 and H_2O'. *Rapid Communications in Mass Spectrometry* 35 (5): e9008. doi:10.1002/rcm.9008.

Hilkert, Andreas W, and Hairigh Avak. 2016. *Isotope Analysis of Water, Fruit Juice and Wine Using the Thermo Scientific GasBench II IRMS*. Application Note 30048. Bremen, Germany: Thermo Fischer Scientific.

Hillaire-Marcel, Claude, Sang-Tae Kim, Amaëlle Landais, Prosenjit Ghosh, Sergey Assonov, Christophe Lécuyer, Marc Blanchard, Harro A. J. Meijer, and Hans Christian Steen-Larsen. 2021. 'A Stable Isotope Toolbox for Water and Inorganic Carbon Cycle Studies'. *Nature Reviews Earth & Environment* 2 (10). Nature Publishing Group: 699–719. doi:10.1038/s43017-021-00209-0.

Hitzfeld, Kristina L., Matthias Gehre, and Hans-Hermann Richnow. 2017. 'Evaluation of the Performance of High Temperature Conversion Reactors for Compound-Specific Oxygen Stable Isotope Analysis'. *Isotopes in Environmental and Health Studies* 53 (2). Taylor & Francis: 116–33. doi:10.1080/10256016.2016.1215983.

Horita, Juske, Akira Ueda, Kanae Mizukami, and Isao Takatori. 1989. 'Automatic δD and δ^{18}O Analyses of Multi-Water Samples Using H_2- and CO_2-Water Equilibration Methods with a Common Equilibration Set-Up'. *International Journal of Radiation Applications and Instrumentation. Part A. Applied Radiation and Isotopes* 40 (9): 801–5. doi:10.1016/0883-2889(89)90100-7.

Huber, C., and M. Leuenberger. 2003. 'Fast High-Precision on-Line Determination of Hydrogen Isotope Ratios of Water or Ice by Continuous-Flow Isotope Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 17 (12): 1319–25. doi:10.1002/rcm.1055.

Hunsinger, Glendon B., Christopher A. Tipple, and Libby A. Stern. 2013. 'Gaseous Byproducts from High-Temperature Thermal Conversion Elemental Analysis of Nitrogen- and Sulfur-Bearing Compounds with Considerations for Δ2H and Δ18O Analyses'. *Rapid Communications in Mass Spectrometry* 27 (14): 1649–59. doi:10.1002/rcm.6608.

Hut, G. 1987. *Consultants' Group Meeting on Stable Isotope Reference Samples for Geochemical and Hydrological Investigations*. Vienna, Austria: IAEA. https://inis.iaea.org/collection/NCLCollectionStore/_Public/18/075/18075746.pdf.

Jardine, Timothy D., and Richard A. Cunjak. 2005. 'Analytical Error in Stable Isotope Ecology'. *Oecologia* 144 (4): 528–33. doi:10.1007/s00442-005-0013-8.

Jardine, Timothy D., Karen A. Kidd, and Aaron T. Fisk. 2006. 'Applications, Considerations, and Sources of Uncertainty When Using Stable Isotope Analysis in Ecotoxicology'. *Environmental Science & Technology* 40 (24). American Chemical Society: 7501–11. doi:10.1021/es061263h.

Jasper, John P. 2001. 'Quantitative Estimates of Precision for Molecular Isotopic Measurements'. *Rapid Communications in Mass Spectrometry* 15 (17): 1554–57. doi:10.1002/rcm.414.

Junk, Gregor, and Harry J Svec. 1958. 'The Absolute Abundance of the Nitrogen Isotopes in the Atmosphere and Compressed Gas from Various Sources'. *Geochimica et Cosmochimica Acta* 14 (3): 234–43. doi:10.1016/0016-7037(58)90082-6.

Kaiser, Jan. 2008. 'Reformulated ^{17}O Correction of Mass Spectrometric Stable Isotope Measurements in Carbon Dioxide and a Critical Appraisal of Historic "Absolute" Carbon and Oxygen Isotope Ratios'. *Geochimica et Cosmochimica Acta* 72 (5): 1312–34. doi:10.1016/j.gca.2007.12.011.

Kaiser, Jan, and Thomas Röckmann. 2008. 'Correction of Mass Spectrometric Isotope Ratio Measurements for Isobaric Isotopologues of O_2, CO, CO_2, N_2O and SO_2'. *Rapid Communications in Mass Spectrometry* 22 (24): 3997–4008. doi:https://doi.org/10.1002/rcm.3821.

Kim, Sang-Tae, Seong-Sook Park, and Seong-Taek Yun. 2012. 'Influence of Dissolved Ions on Determination of Oxygen Isotope Composition of Aqueous Solutions Using the CO_2-H_2O Equilibration Method'. *Rapid Communications in Mass Spectrometry* 26 (17): 2083–92. doi:10.1002/rcm.6323.

Kirkels, F. M. S. A., C. Cerli, E. Federherr, J. Gao, and K. Kalbitz. 2014. 'A Novel High-Temperature Combustion Based System for Stable Isotope Analysis of Dissolved Organic Carbon in Aqueous Samples. II: Optimization and Assessment of Analytical Performance'. *Rapid Communications in Mass Spectrometry* 28 (23): 2574–86. doi:10.1002/rcm.7053.

Kirschenbaum, Isidor. 1951. *Physical Properties and Analysis of Heavy Water*. Vol. 4A. National Nuclear Enegy Series. New York: McGraw-Hill.

Klinken, Gert Jaap van, and Willem G. Mook. 1990. 'Preparative High-Performance Liquid Chromatographic Separation of Individual Amino Acids Derived from Fossil Bone Collagen'. *Radiocarbon* 32 (2). Cambridge University Press: 155–64. doi:10.1017/S0033822200040157.

Köster, Daniel, Irene M. Sanchez Villalobos, Maik A. Jochmann, Willi A. Brand, and Torsten C. Schmidt. 2019. 'New Concepts for the Determination of Oxidation Efficiencies in Liquid Chromatography–Isotope Ratio Mass Spectrometry'. *Analytical Chemistry* 91 (8). American Chemical Society: 5067–73. doi:10.1021/acs.analchem.8b05315.

Kragten, J. 1994. 'Tutorial Review. Calculating Standard Deviations and Confidence Intervals with a Universally Applicable Spreadsheet Technique'. *Analyst* 119 (10). The Royal Society of Chemistry: 2161–65. doi:10.1039/AN9941902161.

Krouse, H. R., and Tyler B. Coplen. 1997. 'Reporting of Relative Sulfur Isotope-Ratio Data (Technical Report)'. *Pure and Applied Chemistry* 69 (2). De Gruyter: 293–96.

Krummen, Michael, Andreas W. Hilkert, Dieter Juchelka, Alexander Duhr, Hans-Jürgen Schlüter, and Reinhold Pesch. 2004. 'A New Concept for Isotope Ratio Monitoring Liquid Chromatography/Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 18 (19): 2260–66. doi:10.1002/rcm.1620.

Kujawinski, Dorothea M., Lijun Zhang, Torsten C. Schmidt, and Maik A. Jochmann. 2012. 'When Other Separation Techniques Fail: Compound-Specific Carbon Isotope Ratio Analysis of Sulfonamide Containing Pharmaceuticals by High-Temperature-Liquid Chromatography-Isotope Ratio Mass Spectrometry'. *Analytical Chemistry* 84 (18). American Chemical Society: 7656–63. doi:10.1021/ac300116w.

Langel, Reinhard, and Jens Dyckmans. 2014. 'Combined ^{13}C and ^{15}N Isotope Analysis on Small Samples Using a near-Conventional Elemental Analyzer/Isotope Ratio Mass Spectrometer Setup'. *Rapid Communications in Mass Spectrometry* 28 (9): 1019–22. doi:10.1002/rcm.6878.

———. 2017. 'A Closer Look into the Nitrogen Blank in Elemental Analyser/Isotope Ratio Mass Spectrometry Measurements'. *Rapid Communications in Mass Spectrometry* 31 (23): 2051–55. doi:10.1002/rcm.7999.

Lin, Ying, Robert N. Clayton, and Manfred Gröning. 2010. 'Calibration of $\delta^{17}O$ and $\delta^{18}O$ of International Measurement Standards – VSMOW, VSMOW2, SLAP, and SLAP2'. *Rapid Communications in Mass Spectrometry* 24 (6): 773–76. doi:10.1002/rcm.4449.

Lott, Michael J., John D. Howa, Lesley A. Chesson, and James R. Ehleringer. 2015. 'Improved Accuracy and Precision in $\delta^{15}N_{AIR}$ Measurements of Explosives, Urea, and Inorganic Nitrates by Elemental Analyzer/Isotope Ratio Mass Spectrometry Using Thermal Decomposition'. *Rapid Communications in Mass Spectrometry* 29 (15): 1381–88. doi:10.1002/rcm.7229.

Lu, Feng H. 2016. 'How Long Is Enough: CO_2-H_2O Equilibration for $\delta^{18}O$ Analysis in Saline Formation Waters?' *Rapid Communications in Mass Spectrometry* 30 (13): 1647–52. doi:10.1002/rcm.7599.

Mactaggart, Douglas L, and Sherry O Farwell. 1992. 'Analytical Use of Linear Regression. Part II: Statistical Error in Both Variables'. *Journal of AOAC INTERNATIONAL* 75 (4): 608–14. doi:10.1093/jaoac/75.4.608.

Magozzi, Sarah, Clement P. Bataille, Keith A. Hobson, Michael B. Wunder, John D. Howa, Andrea Contina, Hannah B. Vander Zanden, and Gabriel J. Bowen. 2021. 'Calibration Chain Transformation Improves the Comparability of Organic Hydrogen and Oxygen Stable Isotope Data'. *Methods in Ecology and Evolution* 12 (4): 732–47. doi:10.1111/2041-210X.13556.

Mambelli, Stefania, Paul D. Brooks, Robin Sutka, Scott Hughes, Kari M. Finstad, Joey Pakes Nelson, and Todd E. Dawson. 2016. 'High-Throughput Method for Simultaneous Quantification of N, C and S Stable Isotopes and Contents in Organics and Soils'. *Rapid Communications in Mass Spectrometry* 30 (15): 1743–53. doi:10.1002/rcm.7605.

Mann, Jacqueline L., Robert D. Vocke Jr., and W. Robert Kelly. 2009. 'Revised $\delta^{34}S$ Reference Values for IAEA Sulfur Isotope Reference Materials S-2 and S-3'. *Rapid Communications in Mass Spectrometry* 23 (8): 1116–24. doi:10.1002/rcm.3977.

Mariotti, André. 1983. 'Atmospheric Nitrogen Is a Reliable Standard for Natural ^{15}N Abundance Measurements'. *Nature* 303 (5919). Springer: 685–87.

———. 1984. 'Natural ^{15}N Abundance Measurements and Atmospheric Nitrogen Standard Calibration'. *Nature* 311 (5983). Nature Publishing Group: 251–52.

Martin, P., and M. Gröning. 2009. 'Reference Sheet for International Measurement Standards VSMOW2 and SLAP2'. *International Atomic Energy Agency, Vienna*.

Mayer, B., and H. R. Krouse. 2004. 'Procedures for Sulfur Isotope Abundance Studies'. In *Handbook of Stable Isotope Analytical Techniques*, edited by P.A. de Groot, 538–96. Elsevier.

McKinney, C. R., J. M. McCrea, S. Epstein, H. A. Allen, and H. C. Urey. 1950. 'Improvements in Mass Spectrometers for the Measurement of Small Differences in Isotope Abundance Ratios'. *Review of Scientific Instruments* 21 (8): 724–30. doi:10.1063/1.1745698.

Meier-Augenstein, Wolfram. 1999. 'Applied Gas Chromatography Coupled to Isotope Ratio Mass Spectrometry'. *Journal of Chromatography A* 842 (1): 351–71. doi:10.1016/S0021-9673(98)01057-7.

Meier-Augenstein, Wolfram, and Arndt Schimmelmann. 2019. 'A Guide for Proper Utilisation of Stable Isotope Reference Materials*'. *Isotopes in Environmental and Health Studies* 55 (2). Taylor & Francis: 113–28. doi:10.1080/10256016.2018.1538137.

Meija, Juris, and Michelle M. G. Chartrand. 2018. 'Uncertainty Evaluation in Normalization of Isotope Delta Measurement Results against International Reference Materials'. *Analytical and Bioanalytical Chemistry* 410 (3): 1061–69. doi:10.1007/s00216-017-0659-1.

Meijer, H. A. J, R. E. M Neubert, and G. H Visser. 2000. 'Cross Contamination in Dual Inlet Isotope Ratio Mass Spectrometers'. *International Journal of Mass Spectrometry* 198 (1): 45–61. doi:10.1016/S1387-3806(99)00266-3.

Meyer, H., Schönicke ,L., Wand ,U., Hubberten ,H. W., and H. and Friedrichsen. 2000. 'Isotope Studies of Hydrogen and Oxygen in Ground Ice - Experiences with the Equilibration Technique'. *Isotopes in Environmental and Health Studies* 36 (2). Taylor & Francis: 133–49. doi:10.1080/10256010008032939.

Miller, M. F., T. Röckmann, and I. P. Wright. 2007. 'A General Algorithm for the ^{17}O Abundance Correction to $^{13}C/^{12}C$ Determinations from CO_2 Isotopologue Measurements, Including CO_2

Characterised by “Mass-Independent” Oxygen Isotope Distributions’. *Geochimica et Cosmochimica Acta* 71 (13): 3145–61. doi:10.1016/j.gca.2007.03.007.

Morrison, J., T. Brockwell, T. Merren, F. Fourel, and A. M. Phillips. 2001. ‘On-Line High-Precision Stable Hydrogen Isotopic Analyses on Nanoliter Water Samples’. *Analytical Chemistry* 73 (15). American Chemical Society: 3570–75. doi:10.1021/ac001447t.

Nelson, Daniel B., Ansgar Kahmen, Jochem Baan, Selina Hugger, Cristina Mercoli, and Meisha Holloway-Phillips. 2025. ‘Comparing Dual Water Equilibration Methods for Hydrogen Stable Isotope Analysis of Plant Carbohydrates and Proteinaceous Animal Samples’. *Rapid Communications in Mass Spectrometry* 39 (19): e10091. doi:10.1002/rcm.10091.

Nelson, Stephen T. 2000. ‘A Simple, Practical Methodology for Routine VSMOW/SLAP Normalization of Water Samples Analyzed by Continuous Flow Methods’. *Rapid Communications in Mass Spectrometry* 14 (12): 1044–46. doi:10.1002/1097-0231(20000630)14:12<1044::AID-RCM987>3.0.CO;2-3.

Nelson, Stephen T., and David Dettman. 2001. ‘Improving Hydrogen Isotope Ratio Measurements for On-Line Chromium Reduction Systems’. *Rapid Communications in Mass Spectrometry* 15 (23): 2301–6. doi:10.1002/rcm.508.

Newell, D. B., F. Cabiati, J. Fischer, K. Fujii, S. G. Karshenboim, H. S. Margolis, E. de Mirandés, et al. 2018. ‘The CODATA 2017 Values of *h*, *e*, *k*, and N_A for the Revision of the SI’. *Metrologia* 55 (1). IOP Publishing: L13. doi:10.1088/1681-7575/aa950a.

Niemann, Felix, Annika Gruhlke, Maik A. Jochmann, and Torsten C. Schmidt. 2025. ‘LC-IRMS Persulfate Oxidation: Case Study on Neonicotinoid-Related Structures’. *Rapid Communications in Mass Spectrometry* 39 (16): e10067. doi:10.1002/rcm.10067.

Nier, Alfred O. 1947. ‘A Mass Spectrometer for Isotope and Gas Analysis’. *Review of Scientific Instruments* 18 (6). AIP Publishing: 398–411.

———. 1950. ‘A Redetermination of the Relative Abundances of the Isotopes of Carbon, Nitrogen, Oxygen, Argon, and Potassium’. *Physical Review* 77 (6). American Physical Society: 789–93. doi:10.1103/PhysRev.77.789.

Ogawa, NO, T Nagata, and N Ohkouchi. 2010. ‘Ultra-Sensitive Elemental Analyzer/Isotope Ratio Mass Spectrometer for Stable Nitrogen and Carbon Isotope Analyses’. In *Earth, Life and Isotopes*, edited by N Ohkouchi, K I Tayasu, and K Koba. Kyoto University Press.

Ohlsson, K. E. Anders. 2013. ‘Uncertainty of Blank Correction in Isotope Ratio Measurement’. *Analytical Chemistry* 85 (11). American Chemical Society: 5326–29. doi:10.1021/ac4003968.

Ohlsson, K. E. Anders, and P. Håkan Wallmark. 1999. ‘Novel Calibration with Correction for Drift and Non-Linear Response for Continuous Flow Isotope Ratio Mass Spectrometry Applied to the Determination of δ^{15}N, Total Nitrogen, δ^{13}C and Total Carbon in Biological Material’. *Analyst* 124 (4). The Royal Society of Chemistry: 571–77. doi:10.1039/A900855A.

Ohsumi, Takashi, and Hiroshi Fujino. 1986. ‘Isotope Exchange Technique for Preparation of Hydrogen Gas in Mass Spectrometry D/H Analysis of Natural Waters’. *Analytical Sciences* 2 (5): 489–90. doi:10.2116/analsci.2.489.

Olsen, Jesper, Inger Seierstad, Bo Vinther, Sigfús Johnsen, and Jan Heinemeier. 2006. ‘Memory Effect in Deuterium Analysis by Continuous Flow Isotope Ratio Measurement’. *International Journal of Mass Spectrometry* 254 (1): 44–52. doi:10.1016/j.ijms.2006.05.004.

O’Neil, James R., and Samuel Epstein. 1966. ‘A Method for Oxygen Isotope Analysis of Milligram Quantities of Water and Some of Its Applications’. *Journal of Geophysical Research (1896-1977)* 71 (20): 4955–61. doi:10.1029/JZ071i020p04955.

Paul, Debajyoti, Grzegorz Skrzypek, and István Fórizs. 2007. ‘Normalization of Measured Stable Isotopic Compositions to Isotope Reference Scales – a Review’. *Rapid Communications in Mass Spectrometry* 21 (18): 3006–14. doi:10.1002/rcm.3185.

Piez, K. A., and Harry Eagle. 1956. ‘C^{14} Isotope Effect on the Ion-Exchange Chromatography of Amino Acids’. *Journal of the American Chemical Society* 78 (20). American Chemical Society: 5284–87. doi:10.1021/ja01601a035.

Pilecky, Matthias, Katharina Winter, Leonard I. Wassenaar, and Martin J. Kainz. 2021. ‘Compound-Specific Stable Hydrogen Isotope (δ^2H) Analyses of Fatty Acids: A New Method and Perspectives for Trophic and Movement Ecology’. *Rapid Communications in Mass Spectrometry* 35 (16): e9135. doi:10.1002/rcm.9135.

Polissar, Pratigya J., James M. Fulton, Christopher K. Junium, Courtney C. Turich, and Katherine H. Freeman. 2009. 'Measurement of ^{13}C and ^{15}N Isotopic Composition on Nanomolar Quantities of C and N'. *Analytical Chemistry* 81 (2). American Chemical Society: 755–63. doi:10.1021/ac801370c.

Pritzkow, W., J. Vogl, R. Köppen, and M. Ostermann. 2005. 'Determination of Sulfur Isotope Abundance Ratios for SI-Traceable Low Sulfur Concentration Measurements in Fossil Fuels by ID-TIMS'. *International Journal of Mass Spectrometry*, Isotope Ratio Measurements SI, 242 (2): 309–18. doi:10.1016/j.ijms.2004.10.024.

Prohaska, Thomas, Johanna Irrgeher, Jacqueline Benefield, J. K. Böhlke, Lesley A. Chesson, Tyler B. Coplen, Tiping Ding, et al. 2022. 'Standard Atomic Weights of the Elements 2021 (IUPAC Technical Report)'. *Pure and Applied Chemistry* 94 (5). De Gruyter: 573–600. doi:10.1515/pac-2019-0603.

Qi, Haiping, and Tyler B. Coplen. 2011. 'Investigation of Preparation Techniques for δ^2H Analysis of Keratin Materials and a Proposed Analytical Protocol'. *Rapid Communications in Mass Spectrometry* 25 (15). Wiley Online Library: 2209–22. doi:10.1002/rcm.5095.

Qi, Haiping, Tyler B. Coplen, Stanley J. Mroczkowski, Willi A. Brand, Lauren Brandes, Heike Geilmann, and Arndt Schimmelmann. 2016. 'A New Organic Reference Material, L-Glutamic Acid, USGS41a, for $\delta^{13}C$ and $\delta^{15}N$ Measurements − a Replacement for USGS41'. *Rapid Communications in Mass Spectrometry* 30 (7): 859–66. doi:10.1002/rcm.7510.

Qi, Haiping, Tyler B. Coplen, and Leonard I. Wassenaar. 2011. 'Improved Online $\delta^{18}O$ Measurements of Nitrogen-and Sulfur-Bearing Organic Materials and a Proposed Analytical Protocol'. *Rapid Communications in Mass Spectrometry* 25 (14). Wiley Online Library: 2049–58. doi:10.1002/rcm.5088.

Qi, Haiping, Heiko Moossen, Harro A.J. Meijer, Tyler B. Coplen, Anita T. Aerts-Bijma, Lauren Reid, Heike Geilmann, et al. 2021. 'USGS44, a New High-Purity Calcium Carbonate Reference Material for $\delta^{13}C$ Measurements'. *Rapid Communications in Mass Spectrometry* 35 (4): e9006. doi:10.1002/rcm.9006.

Ramsey, Michael H., S. L. R. Ellison, and Peter Rostron. 2019. *EURACHEM/EUROLAB/CITAC/Nordtest/AMC Guide: Guide: Measurement Uncertainty Arising from Sampling: A Guide to Methods and Approaches*. 2nd ed. www.eurachem.org.

Reinnicke, Sandra, Anat Bernstein, and Martin Elsner. 2010. 'Small and Reproducible Isotope Effects during Methylation with Trimethylsulfonium Hydroxide (TMSH): A Convenient Derivatization Method for Isotope Analysis of Negatively Charged Molecules'. *Analytical Chemistry* 82 (5). American Chemical Society: 2013–19. doi:10.1021/ac902750s.

Reinnicke, Sandra, Dieter Juchelka, Sibylle Steinbeiss, Armin Meyer, Andreas Hilkert, and Martin Elsner. 2012. 'Gas Chromatography/Isotope Ratio Mass Spectrometry of Recalcitrant Target Compounds: Performance of Different Combustion Reactors and Strategies for Standardization'. *Rapid Communications in Mass Spectrometry* 26 (9): 1053–60. doi:10.1002/rcm.6199.

Renpenning, Julian, Steffen Kümmel, Kristina L. Hitzfeld, Arndt Schimmelmann, and Matthias Gehre. 2015. 'Compound-Specific Hydrogen Isotope Analysis of Heteroatom-Bearing Compounds via Gas Chromatography–Chromium-Based High-Temperature Conversion (Cr/HTC)–Isotope Ratio Mass Spectrometry'. *Analytical Chemistry* 87 (18). American Chemical Society: 9443–50. doi:10.1021/acs.analchem.5b02475.

Revesz, Kinga, and Peter H. Woods. 1990. 'A Method to Extract Soil Water for Stable Isotope Analysis'. *Journal of Hydrology* 115 (1): 397–406. doi:10.1016/0022-1694(90)90217-L.

Ricci, Margaret P., Dawn A. Merritt, Katherine H. Freeman, and J. M. Hayes. 1994. 'Acquisition and Processing of Data for Isotope-Ratio-Monitoring Mass Spectrometry'. *Organic Geochemistry*, Compound-Specific Isotope Analysis in Biogeochemistry and Petroleum Research, 21 (6): 561–71. doi:10.1016/0146-6380(94)90002-7.

Rieley, Gareth. 1994. 'Derivatization of Organic Compounds Prior to Gas Chromatographic–Combustion–Isotope Ratio Mass Spectrometric Analysis: Identification of Isotope Fractionation Processes'. *Analyst* 119 (5). The Royal Society of Chemistry: 915–19. doi:10.1039/AN9941900915.

Roether, Wolfgang. 1970. 'Water-CO_2 Exchange Set-up for the Routine ^{18}Oxygen Assay of Natural Waters'. *The International Journal of Applied Radiation and Isotopes* 21 (7): 379–87. doi:10.1016/0020-708X(70)90131-6.

Salouros, Helen, James F. Carter, Philip J.H. Dunn, and Sean P. G. Doyle. 2023. 'Letter to the Editor Regarding: A Review of Recent Compound-Specific Isotope Analysis Studies Applied to Food Authentication'. *Food Chemistry* 428 (December): 136776. doi:10.1016/j.foodchem.2023.136776.

Santrock, Jeffrey., and J. M. Hayes. 1987. 'Adaptation of the Unterzaucher Procedure for Determination of Oxygen-18 in Organic Substances'. *Analytical Chemistry* 59 (1). American Chemical Society: 119–27. doi:10.1021/ac00128a025.

Santrock, Jeffrey., Stephen A. Studley, and J. M. Hayes. 1985. 'Isotopic Analyses Based on the Mass Spectra of Carbon Dioxide'. *Analytical Chemistry* 57 (7). American Chemical Society: 1444–48. doi:10.1021/ac00284a060.

Sauer, Peter E., Arndt Schimmelmann, Alex L. Sessions, and Katarina Topalov. 2009. 'Simplified Batch Equilibration for D/H Determination of Non-Exchangeable Hydrogen in Solid Organic Material'. *Rapid Communications in Mass Spectrometry* 23 (7): 949–56. doi:10.1002/rcm.3954.

Schoell, Martin, Eckhard Faber, and Max L. Coleman. 1983. 'Carbon and Hydrogen Isotopic Compositions of the NBS 22 and NBS 21 Stable Isotope Reference Materials: An Inter-Laboratory Comparison'. *Organic Geochemistry* 5 (1): 3–6. doi:10.1016/0146-6380(83)90010-4.

Schönemann, Spruce W., Andrew J. Schauer, and Eric J. Steig. 2013. 'Measurement of SLAP2 and GISP $\delta^{17}O$ and Proposed VSMOW-SLAP Normalization for $\delta^{17}O$ and $^{17}O_{excess}$'. *Rapid Communications in Mass Spectrometry* 27 (5): 582–90. doi:https://doi.org/10.1002/rcm.6486.

Schütze, Max. 1939. 'The direct determination of oxygen in zinc oxide'. *Journal of Analytical Chemistry* 118 (7): 241–45. doi:10.1007/BF01592814.

Scrimgeour, C. M. 1995. 'Measurement of Plant and Soil Water Isotope Composition by Direct Equilibration Methods'. *Journal of Hydrology* 172 (1): 261–74. doi:10.1016/0022-1694(95)02716-3.

Sessions, Alex L. 2016. 'Factors Controlling the Deuterium Contents of Sedimentary Hydrocarbons'. *Organic Geochemistry* 96 (June): 43–64. doi:10.1016/j.orggeochem.2016.02.012.

Sessions, Alex L., Thomas W. Burgoyne, and John M. Hayes. 2001a. 'Correction of H_3^+ Contributions in Hydrogen Isotope Ratio Monitoring Mass Spectrometry'. *Analytical Chemistry* 73 (2). American Chemical Society: 192–99. doi:10.1021/ac000489e.

———. 2001b. 'Determination of the H_3 Factor in Hydrogen Isotope Ratio Monitoring Mass Spectrometry'. *Analytical Chemistry* 73 (2). American Chemical Society: 200–207. doi:10.1021/ac000488m.

Seth, Barbara, Claude Schneider, and Florian Storck. 2006. 'Improved Reliability of Oxygen Isotopic Analysis of Water Using the Finnigan GasBench II Periphery of a Continuous Flow Isotope Ratio Mass Spectrometer by Backflushing of the Sampling Line'. *Rapid Communications in Mass Spectrometry* 20 (6): 1049–51. doi:10.1002/rcm.2406.

Sharp, Zachary. 2017. 'Principles of Stable Isotope Geochemistry'.

Sherwood Lollar, Barbara, Sarah K. Hirschorn, Michelle M. G. Chartrand, and Georges Lacrampe-Couloume. 2007. 'An Approach for Assessing Total Instrumental Uncertainty in Compound-Specific Carbon Isotope Analysis: Implications for Environmental Remediation Studies'. *Analytical Chemistry* 79 (9). American Chemical Society: 3469–75. doi:10.1021/ac062299v.

Sieper, Hans-Peter, Hans-Joachim Kupka, Lutz Lange, Andreas Roßmann, Nicole Tanz, and Hanns-Ludwig Schmidt. 2010. 'Essential Methodological Improvements in the Oxygen Isotope Ratio Analysis of N-Containing Organic Compounds'. *Rapid Communications in Mass Spectrometry* 24 (19): 2849–58. doi:10.1002/rcm.4714.

Sieper, Hans-Peter, Hans-Joachim Kupka, Tony Williams, Andreas Rossmann, Susanne Rummel, Nicole Tanz, and Hanns-Ludwig Schmidt. 2006. 'A Measuring System for the Fast Simultaneous Isotope Ratio and Elemental Analysis of Carbon, Hydrogen, Nitrogen and Sulfur in Food Commodities and Other Biological Material'. *Rapid Communications in Mass Spectrometry* 20 (17): 2521–27. doi:10.1002/rcm.2619.

Skrzypek, Grzegorz. 2013. 'Normalization Procedures and Reference Material Selection in Stable HCNOS Isotope Analyses: An Overview'. *Analytical and Bioanalytical Chemistry* 405 (9). Springer: 2815–23. doi:10.1007/s00216-012-6517-2.

Skrzypek, Grzegorz, Colin E. Allison, J. K. Böhlke, Luana Bontempo, Paul Brewer, Federica Camin, James F. Carter, et al. 2022. 'Minimum Requirements for Publishing Hydrogen, Carbon, Nitrogen, Oxygen and Sulfur Stable-Isotope Delta Results (IUPAC Technical Report)'. *Pure and Applied Chemistry* 94 (11–12). De Gruyter: 1249–55. doi:10.1515/pac-2021-1108.

Skrzypek, Grzegorz, and Philip J. H. Dunn. 2020a. 'Absolute Isotope Ratios Defining Isotope Scales Used in Isotope Ratio Mass Spectrometers and Optical Isotope Instruments'. *Rapid Communications in Mass Spectrometry* 34 (20): e8890. doi:10.1002/rcm.8890.

Skrzypek, Grzegorz, and Philip J.H. Dunn. 2020b. 'Recalculation of Stable Isotope Expressions for HCNOS: EasyIsoCalculator'. *Rapid Communications in Mass Spectrometry* 34 (21): e8892. doi:10.1002/rcm.8892.

Skrzypek, Grzegorz, Rohan Sadler, and Debajyoti Paul. 2010. 'Error Propagation in Normalization of Stable Isotope Data: A Monte Carlo Analysis'. *Rapid Communications in Mass Spectrometry* 24 (18): 2697–2705. doi:10.1002/rcm.4684.

Socki, Richard A., and Tracey Jacksier. 2021. 'Isotope Fractionation of Gaseous-, Supercritical-, and Dual-Phase Carbon Dioxide in Pressurized Cylinders Exposed to Variable Temperatures'. *ACS Omega* 6 (12). American Chemical Society: 8625–29. doi:10.1021/acsomega.1c00474.

Socki, Richard, Matt Matthew, James McHale, Jun Sonobe, Megumi Isaji, and Tracey Jacksier. 2020. 'Enhanced Stability of Stable Isotopic Gases'. *ACS Omega* 5 (29). American Chemical Society: 17926–30. doi:10.1021/acsomega.0c00839.

Song, Xin, and Margaret M. Barbour. 2016. 'Leaf Water Oxygen Isotope Measurement by Direct Equilibration'. *New Phytologist* 211 (3): 1120–28. doi:10.1111/nph.13962.

Soto, David X., Geoff Koehler, Leonard I. Wassenaar, and Keith A. Hobson. 2017. 'Re-Evaluation of the Hydrogen Stable Isotopic Composition of Keratin Calibration Standards for Wildlife and Forensic Science Applications'. *Rapid Communications in Mass Spectrometry* 31 (14). Wiley Online Library: 1193–1203. doi:10.1002/rcm.7893.

Srivastava, Abneesh. 2022. 'Physical Model for Multi-Point Normalization of Dual-Inlet Isotope Ratio Mass Spectrometry Data'. *Analytical and Bioanalytical Chemistry* 414 (19): 5773–79. doi:10.1007/s00216-022-04137-w.

Szpak, Paul, Jessica Z. Metcalfe, and Rebecca A. Macdonald. 2017. 'Best Practices for Calibrating and Reporting Stable Isotope Measurements in Archaeology'. *Journal of Archaeological Science: Reports* 13 (June): 609–16. doi:10.1016/j.jasrep.2017.05.007.

'The International System of Units (SI)'. 2019. Bureau International des Poids et Mesures. https://www.bipm.org/documents/20126/41483022/SI-Brochure-9-EN.pdf.

Thompson, Michael, Stephen L. R. Ellison, and Roger Wood. 2006. 'The International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (IUPAC Technical Report)'. *Pure and Applied Chemistry* 78 (1). De Gruyter: 145–96. doi:10.1351/pac200678010145.

Thompson, Michael, and Roger Wood. 1995. 'Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories (Technical Report)'. *Pure and Applied Chemistry* 67 (4). De Gruyter: 649–66.

Tripp, Jennifer A., James S. O. McCullagh, and Robert E. M. Hedges. 2006. 'Preparative Separation of Underivatized Amino Acids for Compound-Specific Stable Isotope Analysis and Radiocarbon Dating of Hydrolyzed Bone Collagen'. *Journal of Separation Science* 29 (1): 41–48. doi:10.1002/jssc.200500247.

Unterzaucher, Josef. 1952. 'The Direct Micro-Determination of Oxygen in Organic Substances'. *Analyst* 77 (920). The Royal Society of Chemistry: 584–95. doi:10.1039/AN9527700584.

Urey, H. C., H. A. Lowenstam, S. Epstein, and C. R. McKinney. 1951. 'Measurement of Paleotemperatures and Termperatures of the Upper Cretaceous of England, Denmark and the Southeastern United States'. *GSA Bulletin* 62 (4). GeoScienceWorld: 399–416. doi:10.1130/0016-7606(1951)62[399:MOPATO]2.0.CO;2.

Vadibeler, Devakunjari, Michael P. Stockinger, Leonard I. Wassenaar, and Christine Stumpp. 2022. 'Influence of Equilibration Time, Soil Texture, and Saturation on the Accuracy of Porewater

Water Isotope Assays Using the Direct $H_2O_{(liquid)}$–$H_2O_{(vapor)}$ Equilibration Method'. *Journal of Hydrology* 607 (April): 127560. doi:10.1016/j.jhydrol.2022.127560.

Vasileva, E., and Sergey Assonov. 2020. 'Reference Sheet for Reference Materials for δ^{34}S-Isotope Values: IAEA-S-1 (NIST RM 8554), IAEA-S-2 (NIST RM 8555) and IAEA-S-3 (NIST RM 8529)'. *International Atomic Energy Agency, Vienna*, no. Rev 0 original version (April).

Wang, Daobing, Qiding Zhong, Guohui Li, and Zhanbin Huang. 2015. 'Rapid Method for the Determination of the Stable Oxygen Isotope Ratio of Water in Alcoholic Beverages'. *Journal of Agricultural and Food Chemistry* 63 (42). American Chemical Society: 9357–62. doi:10.1021/acs.jafc.5b00636.

Wassenaar, Leonard I., and Keith A. Hobson. 2000. 'Improved Method for Determining the Stable-Hydrogen Isotopic Composition (δD) of Complex Organic Materials of Environmental Interest'. *Environmental Science & Technology* 34 (11). American Chemical Society: 2354–60. doi:10.1021/es990804i.

Wassenaar, Leonard I, and Keith A. Hobson. 2003. 'Comparative Equilibration and Online Technique for Determination of Non-Exchangeable Hydrogen of Keratins for Use in Animal Migration Studies'. *Isotopes in Environmental and Health Studies* 39 (3). Taylor & Francis: 211–17. doi:10.1080/1025601031000096781.

Wassenaar, Leonard I., Keith A. Hobson, and Leonardo Sisti. 2015. 'An Online Temperature-Controlled Vacuum-Equilibration Preparation System for the Measurement of δ^{2}H Values of Non-Exchangeable-H and of δ^{18}O Values in Organic Materials by Isotope-Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 29 (5): 397–407. doi:10.1002/rcm.7118.

Werner, R. A., and Willi A. Brand. 2001. 'Referencing Strategies and Techniques in Stable Isotope Ratio Analysis'. *Rapid Communications in Mass Spectrometry* 15 (7): 501–19. doi:10.1002/rcm.258.

Wieser, Michael E., and Michael Berglund. 2009. 'Atomic weights of the elements 2007 (IUPAC Technical Report)'. *Pure and Applied Chemistry* 81 (11). De Gruyter: 2131–56. doi:10.1351/PAC-REP-09-08-03.

Williamson, J. H. 1968. 'Least-Squares Fitting of a Straight Line'. *Canadian Journal of Physics* 46 (16). NRC Research Press: 1845–47. doi:10.1139/p68-523.

Wong, William W., and Lucinda L. Clarke. 2012. 'A Hydrogen Gas-Water Equilibration Method Produces Accurate and Precise Stable Hydrogen Isotope Ratio Measurements in Nutrition Studies'. *The Journal of Nutrition* 142 (11): 2057–62. doi:10.3945/jn.112.167957.

Yang, Lu, Shuoyun Tong, Lian Zhou, Zhaochu Hu, Zoltán Mester, and Juris Meija. 2018. 'A Critical Review on Isotopic Fractionation Correction Methods for Accurate Isotope Amount Ratio Measurements by MC-ICP-MS'. *Journal of Analytical Atomic Spectrometry* 33 (11). The Royal Society of Chemistry: 1849–61. doi:10.1039/C8JA00210J.

York, Derek. 1968. 'Least Squares Fitting of a Straight Line with Correlated Errors'. *Earth and Planetary Science Letters* 5. Elsevier: 320–24.

Zhang, Lijun, Dorothea M. Kujawinski, Eugen Federherr, Torsten C. Schmidt, and Maik A. Jochmann. 2012. 'Caffeine in Your Drink: Natural or Synthetic?' *Analytical Chemistry* 84 (6). American Chemical Society: 2805–10. doi:10.1021/ac203197d.

Zhang, Lijun, Dorothea M. Kujawinski, Maik A. Jochmann, and Torsten C. Schmidt. 2011. 'High-Temperature Reversed-Phase Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry'. *Rapid Communications in Mass Spectrometry* 25 (20): 2971–80. doi:10.1002/rcm.5069.

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