

REVIEW

Referencing strategies and techniques in stable isotope ratio analysis

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Stable isotope ratios are reported in the literature in terms of a deviation from an international standard (δ -values). The referencing procedures, however, differ from instrument to instrument and are not consistent between measurement facilities. This paper reviews an attempt to unify the strategy for referencing isotopic measurements. In particular, emphasis is given to the importance of identical treatment of sample and reference material ('IT principle'), which should guide all isotope ratio determinations and evaluations. The implementation of the principle in our laboratory, the monitoring of our measurement quality, the status of the international scales and reference materials and necessary correction procedures are discussed. Copyright © 2001 John Wiley & Sons, Ltd.

Roughly 15 years ago a new and relatively economic chromatographic method to measure stable isotope ratios with high precision became commercially available. Carrier gas or on-line isotope ratio analysis systems now cover a wide range of applications and have led to a dramatic increase in the number of stable isotope ratio measurements worldwide. Although this has been a benefit to this field of science as a whole, it has also created a number of problems. We feel that the depth of knowledge that the few original experts possessed has been diluted considerably over the years with the consequence that the overall precision and reliability of isotope ratio values may have declined. In particular, the accuracy of reported δ -values relative to an international standard may have suffered from the vast increase in analyses made.

On the other hand, such a large increase in the number of analyses offers the opportunity to establish fully automated analysis sequences that include a proper referencing strategy. This opportunity should enable the reliability of isotope ratio value assignment to be improved. Although this has happened to a certain extent in many laboratories, referencing strategies have certainly not been unified throughout the community.

This review will be of considerable value both in

educating students and also newcomers to the exciting field of high precision stable isotope ratio measurements. In particular, we want to emphasize techniques for reliable standardization in carrier gas or on-line techniques where an accepted protocol for assigning δ -values^a on an internationally accepted scale has not yet been defined. We feel particularly suited for this task because we were given the opportunity to start a large isotope ratio measurement facility from scratch two years ago and thus had to develop all the techniques and protocols for such an endeavor recently.

Stable isotope ratios and their international scales

The stable isotope ratios measured most widely include $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$, $^{18}\text{O}/^{16}\text{O}$ and $^2\text{H}/^1\text{H}$ (or simply D/H). Other less frequently measured ratios are inter alia $^{17}\text{O}/^{16}\text{O}$, $^{34}\text{S}/^{32}\text{S}$, $^{33}\text{S}/^{32}\text{S}$. The common feature of these ratios is that they can be determined using a few light gases (CO_2 , CO , N_2 , O_2 , H_2 , and SO_2). Hence they share a common technology termed (Gas) Isotope Ratio Mass Spectrometry.

The principle of the classical method is very easy to understand: Two gases are stored in containers connected via capillaries to a switching unit, the 'changeover valve'¹ (Fig. 1). The latter serves to direct one of the gases to the ion source of an isotope ratio mass spectrometer (IRMS)^{2,3,4,5,6} while the other gas flows to a waste vacuum line, and vice versa. The ion currents are measured separately from both gases and compared a number of times. The measured relative difference in ion current ratios is then calculated relative to an internationally agreed isotope ratio scale.

Table 1 is a compilation of the international stable isotope ratio scales in use together with the presently accepted absolute ratios and their errors. When comparing samples

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^a The variation of stable isotope ratios in nature is small. The small differences are conveniently expressed as delta values (δ) in per mill [‰] deviation from a reference^{2,3,8} according to

$$\delta[\text{‰}] = (R_{\text{sa}}/R_{\text{ref}} - 1) \cdot 1000 \quad (1)$$

R_{sa} and R_{ref} are the sample and reference isotope ratios, respectively.

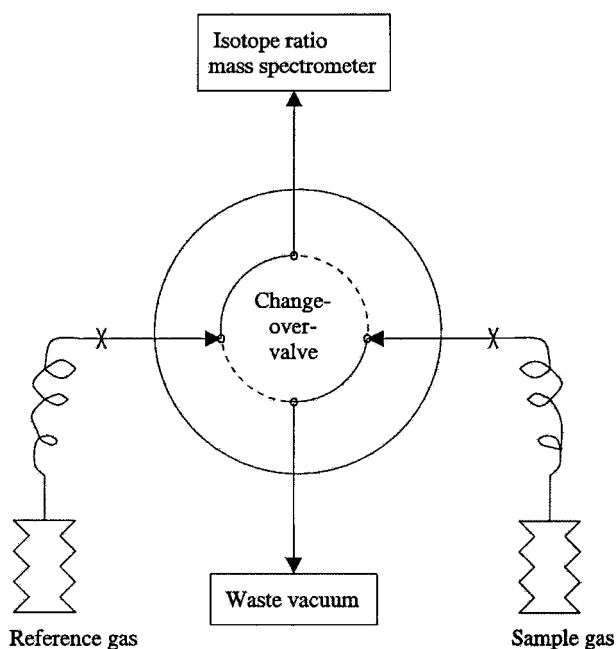


Figure 1. Schematic representation of a dual inlet system featuring the 'Changeover Valve' as the classical referencing technique in Stable Isotope Ratio Mass Spectrometry. While the gas in one volume is flowing to the mass spectrometer, the gas in other goes to a waste line and vice versa.

with one of the standard materials, however, the errors are generally considerably smaller owing to the fact that a relative measurement has far fewer sources of error than an absolute determination.

Carbon

The international scale for $^{13}\text{C}/^{12}\text{C}$ started as a carbonate laboratory standard for oxygen and carbon isotope ratios in the group of Harold Urey at the University of Chicago in the early 1950s.^{7,8} Being representative for carbon in the lithosphere that precipitated from the world oceans, PDB was later proposed as the international reference material for the carbon and oxygen δ -scale by Harmon Craig.⁹ The Cretac-

eous belemnite material was picked from the Pee Dee formation in South Carolina, hence 'PDB' for Pee Dee Belemnite. The original material no longer exists. It has been replaced by assigning exact δ -values (both $\delta^{13}\text{C}$ and $\delta^{18}\text{O}^b$) to another carbonate (NBS-19) relative to PDB.^{10,11} This new scale is termed 'VPDB' (Vienna PDB) in recognition of the role that the International Atomic Energy Agency (IAEA), located in Vienna, has played in redefining the PDB scale. The IAEA played a similar role for the other δ -scales that have a 'V' preceding the original scale name.

Unfortunately, isotope ratios of CO_2 must be determined using the molecular ion masses 44, 45 and 46. The ^{13}C information comes in disguise. It is available from the 45/44 ion current only after subtracting the $\sim 7\%$ contribution from $^{12}\text{C}^{17}\text{O}^{16}\text{O}^+$ (see 'Correction of isobaric interferences'). This complicates the exact comparability of $\delta^{13}\text{C}$ values between different laboratories.

Oxygen

High precision isotope ratio measurements of oxygen were first made on CaCO_3 , O_2 and H_2O . Again it was Urey's group that developed these applications for paleoclimate reconstruction.^{2,19,20,21} The two accepted international scales (VSMOW and VPDB) have developed over time. A variety of carbonate (including PDB) and water standards were used until 1961, when Harmon Craig proposed SMOW (Standard Mean Ocean Water). SMOW isotopically represents the hydrosphere with the world oceans as the largest reservoir of oxygen (and hydrogen). Interestingly, the standard that Craig proposed was not available as a reference material. It had a δ -value that was believed to represent average ocean water based on experience accumulated over time. It was precisely defined relative to the (now exhausted) water standard NBS-1 that was almost 8‰ off the new $\delta^{18}\text{O}$ -scale and 47.6‰ off the simultaneously defined δD -scale. Both Craig and the IAEA engaged in developing a reference material with an isotopic composition of SMOW, which led to the current VSMOW scale. This material presently is still available in limited amounts from the IAEA in Vienna. A new batch of reference water matching the VSMOW scale as closely as possible in all isotope ratios is currently being prepared.

$^{18}\text{O}/^{16}\text{O}$ ratios are measured using CO_2 after appropriate conversion from the original material. Due to the fact that the

^b $\delta^{18}\text{O}_{\text{NBS-19}} \equiv -2.2\text{‰}$ vs. VPDB; $\delta^{13}\text{C}_{\text{NBS-19}} \equiv +1.95\text{‰}$ vs. VPDB

Table 1. International isotope ratio scales¹²

Isotope ratio	International scale	Accepted ratio [$\times 10^6$]	Error of ratio	Error of ratio [‰]	Ref.
$^{13}\text{C}/^{12}\text{C}$	VPDB	11180.2 ^c	± 2.8	± 2.5	Chang and Li ¹³
$^{18}\text{O}/^{16}\text{O}$	VSMOW	2005.2	± 0.45	± 0.22	Baertschi ¹⁴
	VPDB	2067.2 ¹²			using +30.92‰
$^{17}\text{O}/^{16}\text{O}$	VSMOW	379.9	± 0.8	± 2.11	Li <i>et al.</i> ¹⁵
	VPDB	386.0 ¹²			with +30.92‰ and $\lambda = 0.52$
$^{15}\text{N}/^{14}\text{N}$	AIR-N ₂	3678.2	± 1.5	± 0.41	DeBievre <i>et al.</i> ¹⁶
$^2\text{H}/^1\text{H}$	VSMOW	155.75	± 0.08	± 0.51	De Wit <i>et al.</i> ¹⁷
$^{34}\text{S}/^{32}\text{S}$	VCDT	44150.9	± 11.7	± 0.27	Ding <i>et al.</i> ¹⁸

Isotope ratios are reported as δ -values in per mill (‰) deviations from the origin of the respective international scale. Please note that for oxygen two internationally accepted scales coexist.

^c Chang and Li report a $^{13}\text{C}/^{12}\text{C}$ value of 0.011202 for NBS-19. The $\delta^{13}\text{C}$ -value of NBS-19 is +1.95‰, defining the VPDB scale. The previously established $^{13}\text{C}/^{12}\text{C}$ PDB ratio value⁹ of 0.0112372 is 5‰ higher.

behavior of terrestrial ^{17}O and ^{18}O are closely linked via mass fractionation laws, $^{17}\text{O}/^{16}\text{O}$ ratios (measured using O_2 gas) are analyzed only occasionally.

When carbonates or CO_2 in air are measured, oxygen isotope ratio values are preferentially reported on the VPDB or VPDB_{gas} scale and this can lead to confusion when the scales are not stated explicitly. The reason for the coexistence of two oxygen scales is that measurements of δ -values against a chemically identical or similar reference can in principle be made with enhanced precision. VPDB has a $\delta^{18}\text{O}$ value of +30.92‰²² on the VSMOW scale. The CO_2 gas 'developed' from hypothetical VPDB (= VPDB_{gas}) at 25 °C using pure, water-free H_3PO_4 is 10.25‰ heavier;^{23,d} it has a $\delta^{18}\text{O}$ value of +41.5‰ on the VSMOW scale. It should be noted that δ -values can not be added or subtracted in a simple way when referring to different standards.^e

Hydrogen

The isotopes of hydrogen have the largest relative mass difference. Since most isotope fractionations in nature roughly scale with relative mass difference, the largest variations in terrestrial isotope ratios are found for hydrogen. Although the large mass difference makes the isotope signatures easier to detect, the low abundance of deuterium (only about 150 ppm in ocean water) can create problems during high-precision analysis. Consequently, two international reference materials are in frequent use to cover the natural range of isotope ratios for D/H. The basis of the international scale is the same as oxygen, VSMOW. The other reference material is a water sample named SLAP (Standard Light Antarctic Precipitation) that is, relative to VSMOW, more than 42% depleted in deuterium (−428‰). The difference between VSMOW and SLAP is used to correct for the nonlinear behavior of instrumentation and (or) sample preparation during analysis.

Nitrogen

Unlike the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values in atmospheric CO_2 , the $\delta^{15}\text{N}$ value of atmospheric N_2 does not change within measurement precision over time or space.^{24,25} This is due to the fact that the atmospheric pool of nitrogen is by far the largest of all nitrogen-bearing pools on earth and thus cannot be altered significantly by known processes. It participates in all natural processes as source and sink so that the net change over time is close to zero. Consequently, AIR- N_2 has been adopted as the international reference material for all nitrogen isotope ratio analyses. This does not imply that the isotope ratios are easy to measure. From air, N_2 must be isolated without isotopic fractionation for use as a primary reference. For convenience (and thus enable better data comparability) the IAEA has made secondary standards (mostly ammonium and nitrate salts) available for direct use in sample preparation lines involving combustion.

^d More recently, a value of 10.44‰ has been reported by Kim and O'Neil.⁵²

^e The δ -value for a sample (sa) measured against a working standard (ws) on an international scale (is) is given by

$$\delta_{\text{sa/is}} = \delta_{\text{sa/ws}} + \delta_{\text{ws/is}} + \delta_{\text{sa/ws}} \times \delta_{\text{ws/is}}/1000 \quad (2)$$

Sulfur

Sulfur is most commonly measured as SO_2 gas on the molecular ion masses 64 and 66. Unfortunately, the isotopes of oxygen directly interfere with the sulfur isotopes (isobaric interferences) at these mass positions and thus cannot be measured independently (the ion current at m/z 66 comprises $^{34}\text{S}^{16}\text{O}_2^+$ and $^{32}\text{S}^{16}\text{O}^{18}\text{O}^+$). Using SF_6 as the measurement gas avoids this problem, but preparation of SF_6 requires fluorine gas as a reagent and preparing clean SF_6 is difficult. Hence, SF_6 has found only limited use. The international δ -scale is defined by VCDT (Vienna Cañon Diablo Troilite)²⁶ The original CDT,²⁸ adopted as the primary standard in 1960, was prepared from the FeS phase of a large iron meteorite found at Meteor Crater, Arizona. Improved measurement precision has revealed that CDT is not sufficiently homogeneous²⁷ to be continued as the primary reference material and, as a consequence, the CDT scale has been replaced with VCDT. VCDT is defined by assigning a $\delta^{34}\text{S}$ -value of −0.3‰ (exactly) to IAEA-S-1 (formerly NZ-1, Ag_2S).²⁶ It is worth noting that the absolute $^{34}\text{S}/^{32}\text{S}$ ratio in VCDT given in Table 1 differs from the previously established value (45004.5)²⁸ for CDT by about −20‰. This affects the correction for ^{18}O contribution (see 'Correction of isobaric interferences').

Referencing techniques in isotope ratio mass spectrometry and the principle of identical treatment

Dual inlet system and changeover valve. The breakthrough in classical isotope ratio mass spectrometry was the dual inlet mass spectrometer, introduced by Urey in 1948² and described by McKinney *et al.* in 1950.³ Despite significant improvements in electronics, vacuum system design and full computerization, the basic principles embodied in this mass spectrometer still form the basis of modern stable isotope ratio mass spectrometers for high-precision analysis of clean analyte gas.

A key feature of the McKinney instrument is the 'change-over valve'¹ (Fig. 1), which allows continuous flows of reference and sample gas to be alternated between the mass spectrometer and a waste line vacuum pump. The 'changeover valve' is the classical referencing technique. Together with the dual variable volume (or mercury piston) reservoir system for introduction of sample and reference gases, it allows a direct comparison of ion currents and ion current ratios. Instrumental effects like temporal fluctuation of sensitivity or temperature drifts cancel almost completely.

In order to avoid isotopic composition changes of the gas in either reservoir over time due to molecular effusion into vacuum, the connection between the gas reservoirs and the 'changeover valve' is made with thin capillaries (0.1–0.2 mm i.d.) of about 100 cm length that are crimped near the end.²⁹ The hole right at the capillary crimp also constitutes a molecular leak. However, provided the pressure in the capillary is sufficiently high (typically >50 mbar for hydrogen and >15 mbar for other gases), the flow through the capillaries is viscous, not molecular, and thus prevents the isotopically enriched gas at the crimp from diffusing back into the reservoirs.⁴ This viscous flow condition establishes a

Table 2. Measurement of two CO₂ gas samples by different laboratories

$\delta^{13}\text{C}$ [‰ VPDB]							
Sample	CIO	INSTAAR	CSIRO	Heidelberg	Scripps	mean	std. dev.
GS-19	-7.502	-7.517	-7.516	-7.539	-7.465	-7.508	0.0273
GS-20	-8.622	-8.624	-8.617	-8.635	-8.575	-8.615	0.0231
$\delta^{18}\text{O}$ [‰ VPDB-CO ₂]							
Sample	CIO	INSTAAR	CSIRO	Heidelberg	Scripps	mean	std. dev.
GS-19	-0.193	-0.790	-0.617	-0.426	-0.126	-0.430	0.280
GS-20	-0.991	-1.580	-1.380	-1.191	-0.915	-1.211	0.274

lower limit to the minimum amount of gas that can be determined with high precision. Small amounts of gas may be frozen into a small volume ('microvolume') in front of the capillary. Making this reservoir as small as is physically possible (~250 μL) and operating at 15 mbar results in a minimum sample size of about 4 $\text{b}\mu\text{L}^{\text{f}}$ gas.

Using modern dual inlet mass spectrometers it is possible to obtain a precision of 0.01‰ for $\delta^{13}\text{C}$ (and similar values for other isotope ratios) by comparing the ion current ratios of the gases in both reservoirs a number of times for several seconds. The mean is calculated and such measurements are compared a number of times during the course of a day. However, precision is not the same as accuracy. On the next day the results may again be very precise. The mean, however, may differ from the mean from the previous day, or from the previous week, month, or year.

The situation is illustrated by results from a recent intercomparison of CO₂ gas initiated by the Groningen Centre for Isotope Research (CIO).³⁰ The ring test involved two gases (GS-19 and GS-20) sent to five prominent laboratories for isotopic determination. The results from the ring test are given in Table 2.

The precision of the $\delta^{13}\text{C}$ -values is approx. 0.025‰ and appears acceptable, whereas the $\delta^{18}\text{O}$ data show an unacceptably large scatter. This finding is attributed to different referencing methods or materials in the laboratories. If GS-19 is used as the reference (δ -value set to 0.0‰) and GS-20 measured against it, the precision of the reported differences improves to 0.009‰ for $\delta^{13}\text{C}$ and 0.016‰ for $\delta^{18}\text{O}$. Through direct referencing, the $\delta^{13}\text{C}$ results are improved by a factor of 3 and the $\delta^{18}\text{O}$ situation improves almost 20-fold.

How can the precision that these mass spectrometers are obviously able to deliver be conserved and converted into an accuracy figure over long time periods? How can different mass spectrometers deliver results that can be compared reliably at a precision level of 0.01‰?

These questions require some discussion before a more

general conclusion can be reached. Several points need to be considered:

- The capillaries to the 'changeover valve' are carefully heated and the inlet system is conditioned until the ratios for the same gas, introduced to both reservoirs, is close to zero ('zero enrichment test').^g However, in the real world, the two gases are different and may even differ in their trace gas impurities. Traces of water or other protonation agents can give rise to an isobaric interference of CO₂H⁺ (m/z 45) resulting from ion/molecule reactions in the ion source. Similar protonation reactions are possible for other gases resulting in H₃⁺ or N₂H⁺.
- The isotopic composition of a sample and reference gas should not be very different; however, in reality, they always will be and, as a consequence, balancing the major ion beam means that the minor beams will have different readings. If the response of the measurement channels is not perfectly linear (and it never will be), this is a source of error that can vary with time.
- The measurement precision and results for $\delta^{18}\text{O}$ from CO₂ gases depend critically on the nature and the cleanliness of the internal surfaces of the reservoir and the transfer capillaries. Not only can CO₂ exchange oxygen with surface water, it may even exchange with other forms of loosely bound oxygen on the surface and hence cause uncontrolled isotopic fractionation. This may be different for the two capillaries and may change over longer time periods.
- Different mass spectrometers (even from the same manufacturer) can have several sources of error in determining a given isotopic difference. This has been shown for hydrogen where the isotope ratio differences are often large. It is also important for other gases like CO₂ when the measured δ -values differ by 10‰ or more and the required accuracy is high.

It is common practice in laboratories that strive for the highest long-term precision and accuracy to have sample and reference gas measured from the same (sample) reservoir of the dual inlet system. The gas in the reference volume serves as a temporary mediator between the different runs. Proceeding in this manner, sample and reference gas are handled in a highly comparable, almost equal, fashion and, as a result, some (but not all) of the errors discussed above tend to cancel out.

^f 1 $\text{b}\mu\text{L}$ ('bar microliter') is an amount of 1 μL gas at STP.

^g Please note that crimping of the capillaries will not necessarily result in the same signal height for the same gas pressure on both sides. In fact, when referencing through the sample line ('IT principle'), it is more important to have a long lifetime for the standard gas and thus make the crimp for the capillary tighter on this side.

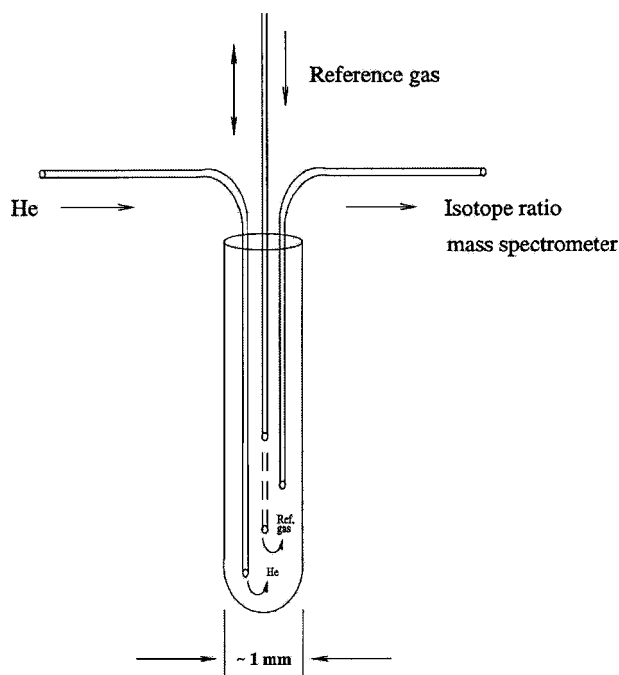


Figure 2. Referencing technique in isotope ratio monitoring applications. Helium enters a small glass tube through a fused-silica capillary. Another fused-silica 'sniffing' capillary (usually 1000 mm long and 0.1 mm i.d.) connects to the ion source of the mass spectrometer. Reference gas pulses are generated by moving a third capillary between two positions, one upstream and the other downstream from the sniffing point.

The IT principle

We will refer to 'Identical Treatment' of sample and reference material as the 'IT principle' for relative measurements. The IT principle, which does not apply exclusively to the dual inlet technique, serves excellently for maintaining long-term laboratory performance records for other isotope ratio measurement techniques as well (see 'Performance charts').

It is rare that the measurement gas is identical to the original sample. Typically, a carbonate, a water sample, an organic compound, a piece of bone, soil, hair or a gas mixture etc., is selected from which the measurement gas has to be produced or extracted. During this transformation it is critical to avoid isotopic alteration. In carbonate analysis or water equilibration the IT principle has been common practice for a long time. Carbonates are effectively standardized by running carbonate reference material alongside the samples on a daily routine basis. Water samples are equilibrated together with reference water standards in the same temperature-controlled bath. Because the isotopic fractionation occurring in the CO_2 liberation or the isotope transfer reaction to the gas phase is strongly temperature dependent, the reference material is subjected to the same reaction conditions. The isotopic fractionation cancels as long as the chemical nature of sample and reference is closely comparable. The same argument applies to water reduction methods for hydrogen isotope ratio analysis. Here, irrespective of the specifics of the reduction process, the

potential for isotopic fractionation is encountered in almost every detail of the reaction including the type of vessel, the temperature, the amount of reduction material, and the timing sequence of the reduction reaction. Hence, running reference water samples concurrently with unknown samples in every batch is required in order to obtain reliable results.

Isotope ratio monitoring techniques

Isotope ratio monitoring ('irm') encompasses all techniques for high-precision determination of stable isotope ratios that employ a helium carrier gas for transferring the analyte gas as a transient into the isotope ratio mass spectrometer.³¹ The term 'irm-GC/MS' was introduced by D.E. Matthews and J.H. Hayes in their pioneering paper in 1978.³² Popular acronyms in common use for specific irm couplings are GC/C/IRMS (GC/combustion/IRMS) and EA/IRMS (elemental analyzer/IRMS). The techniques were originally developed to measure $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$. More recently, D/H, $^{34}\text{S}/^{32}\text{S}$ and $^{18}\text{O}/^{16}\text{O}$ ratio determination has become available, accompanied by a host of new acronyms.

In irm systems the sample gas leaves the appropriate sample preparation and separation device as a peak entrained in helium. The chromatographic separation not only ensures that the analyte gas is pure or coming from a single precursor, it also separates the stable isotopes (i.e. isotopomeric molecules) of the analyte to a certain extent. In irm-GC/MS applications the separation often exceeds 100 ms between e.g. the ^{13}C moiety of the analyte, which elutes first, and its ^{12}C counterpart.^{31,33}

Referencing can be carried out in a number of ways. The most common method is to inject pulses of reference gas directly into or in parallel to the GC effluent stream.³⁴ The time window or windows for the reference gas pulses must be carefully selected in order to avoid interference with any analyte material entering the mass spectrometer concurrently. Other methods include the admixture of reference compounds to a GC mixture (internal standards) or, less favorably, the direct comparison of ratios from chromatogram to chromatogram.

Figure 2 shows schematically an example of a reference gas injection mechanism. Inside a closed, thin (<1 mm) glass tube, the effluent stream from the interface is fed in continuously with a fused-silica capillary. Downstream a second fused-silica capillary acts as the transfer line to the mass spectrometer. Usually, this capillary has an internal diameter of 0.1 mm and a length of 1000 mm. Together with the pressure drop from atmosphere to about 10^{-5} mbar, this capillary allows for a flow of about 400 μL per minute. The flow through long, thin capillaries under viscous flow conditions follows the law of Hagen-Poiseuille. For a given pressure difference the flow is linked to the 4th power of the radius and linearly to the length of the capillary. Hence, when doubling the radius, the flow will increase by a factor of 16 while keeping the other parameters constant. The third capillary (see Fig. 2) serves to inject pulses of reference gas. Using a piston mechanism it can be switched between two positions: one upstream and one downstream from the sniffing point of the transfer capillary. Only the upstream position will deliver reference gas to the sniffing point.

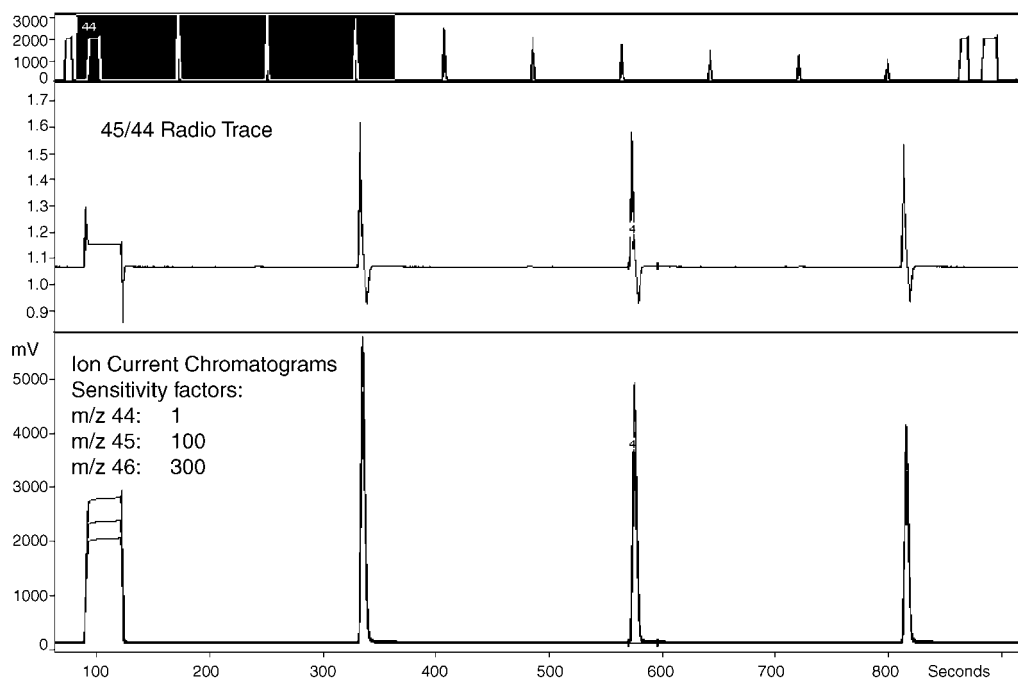


Figure 3. Graphical output of a irm-GC/MS system, in this case from a Gasbench II run (see $\delta^{18}\text{O}$ determination). Three GC traces are monitored simultaneously with different sensitivity factors. The upper trace is an overview over the whole run. The middle window ('45/44 Ratio Trace') represents the instantaneous 45/44 ion current ratio. The first, flat-topped peak represents a post column injection of reference gas. For the sample peaks, please note the isotope swing going positive first which is indicative of the partial separation of the isotopomers on the GC column. Reference pulses do not show this behavior. At the flanks they exhibit a reaction to the fast changing intensity due to differences in the amplifier time constants.

Downstream the helium carrier will sweep away any effluent from capillary three. Figure 3 shows an example of a simple GC run (in $\delta^{13}\text{C}$ measurement mode). The first, flat-topped peak is a reference gas injection lasting for about 30 s. The other peaks are from sample gas analyses. Their shape is typical for this type of gas chromatography. Please note the variation of the instantaneous (45/44)-ratio along each GC peak going positive first due to the ^{13}C moiety eluting earlier than the pure ^{12}C compound.

The principle of moving capillaries in the open split region has been utilized in a number of ways. Multiple reference gases can be introduced, the GC effluent gas can be diluted with helium further to cover a wider dynamic range³⁵ or the transfer capillary may be switched into a clean helium cushion for 'heartcutting' (i.e. selecting or selectively discarding) GC peaks.

Other means of injecting reference gas pulses include rotary valves equipped with an injection loop or carefully designed multiple valve systems. The drawback common to all of these systems is that during switching a short interruption of the continuous flow of carrier gas occurs which leads to problems in the background definition of the reference gas peaks and, hence, to reduced precision.

It is important to note that referencing solely relative to reference gas pulses is an immediate violation of the IT principle introduced above. Sample peaks are generated from original material going through a combustion/separation or separation/combustion step whereas the (working)

reference is pure gas. The principle governing long-term precision and, more importantly, accuracy requires that sample and reference be identical in nature and follow the same sample isolation and conversion pathway. Hence, a *n*-heptadecane sample should be referenced with a *n*-heptadecane reference.

Often gas chromatograms are very complex. The conversion efficiency of *n*-heptadecane should be very close to *n*-hexadecane or *n*-octadecane. Even an *n*-C25 alkane should be a valid reference point provided the stability of the mass spectrometer is high and the conversion unit reliably works close to 100% yield. Hence, even whole chromatograms can serve as references in pursuit of the IT principle. In this case, reference gas pulses again take the role of a mere mediator between runs, very much as the standard gas in dual inlet analyses. In principle, the number of reference runs is determined by the precision required for the experiment in question.

The workhorse in most modern isotope ratio laboratories is the combination of an elemental analyzer with isotope ratio mass spectrometry (EA-IRMS or irm-EAMS, sometimes termed BSIA⁴ for bulk sample isotope analysis). As with irm-GC/MS, the immediate or working referencing is commonly made through co-injected reference gas pulses into the effluent stream. Earlier commercial versions of this instrumentation relied solely on comparing from sample to sample, without reference gas peaks. However, precision of a single measurement is improved when comparing

standard and reference more closely in time. Data security is an issue when a single result relies on the measurement of the whole sequence.

In order to work under the rule of the IT principle individual sample results versus a gaseous working standard can be compared with results from reference material that has passed the full sample line in the same sequence of measurements.

Day-to-day working environment for high-precision isotope ratio analyses and generation of performance charts

In our laboratory we have implemented a number of routine measurement schemes for the following types of analyses:

- $\delta^{13}\text{C}$ from bulk solid (and liquid) material (irm-EAMS system)
- $\delta^{15}\text{N}$ from bulk solid (and liquid) material (irm-EAMS system)
- δD from water samples (Cr-reduction/dual inlet)
- $\delta^{18}\text{O}$ from water samples ($\text{CO}_2/\text{H}_2\text{O}$ equilibration followed by irm analysis)
- $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ from CO_2 in air samples (cryogenic separation/dual inlet, in development)
- O_2/N_2 ratios in air samples (changeover system with open split, in development)

The first step in setting up routine protocols is the establishment of effective laboratory standards that can be used and monitored on a daily basis. General rules for laboratory standards are difficult to fix, they largely depend on the type of analysis.

The second step is the development of consistent analysis sequences, i.e. the decision how on a daily basis samples, reference material, and blanks (if applicable) are positioned in a sample carousel or autosampler. In this respect, economic aspects, such as the number of samples per day and week, scheduling of routine maintenance, number of samples per reactor, etc., are of importance and must be considered with great care and separately for every type of analysis. In our laboratory we have tried to adjust the measurement cycles to the diurnal and weekly cycle of the human operators, to optimize throughput without sacrificing precision.

Selection of laboratory reference materials

After having obtained the relevant primary reference material from the IAEA or other agency, secondary or laboratory standards must be prepared for every day use. The IAEA recommends not using the original material on a

^h The previously recommended value of -29.74% was adjusted to -29.78% by the 8th Advisory Group Meeting on Future Trends in Stable Isotope Reference Materials and Laboratory Quality Assurance, IAEA, Vienna, Sept. 18–22, 2000.

ⁱ Carbonates can exchange oxygen isotopes with air- CO_2 . This effect depends on grain size, humidity, nature of the carbonate, and frequency of opening when the material is kept under inert gas. Water reference material can alter its isotopic composition through evaporation and transpiration through container walls. Hence, the frequency of opening the reference water container can affect the isotopic stability. GC injection mixtures may change their isotopic composition and concentration via evaporation when low boiling point material is used as a reference.

daily basis. Primary reference materials in general are in short supply and their commercial availability is restricted.

For $\delta^{13}\text{C}$ analysis using a combustion technique, NBS-22 (-29.78% ^h vs. VPDB) is a valid reference material for almost any purpose. The secondary laboratory standard should be chosen according to the following criteria:

- easy to handle during weighing or other preparatory steps.
- preferably a pure single chemical compound.
- isotopically homogenous down to the smallest amount used during analysis.
- stable and constant in isotopic content over a long time period. For liquids, in particular water, large quantities are advantageous in order to minimize evaporation losses. The isotopic composition should be stable versus frequent use.ⁱ
- easy to replace if exhausted.
- chemically identical or close to the samples to be measured. This point must be evaluated in terms of the corresponding analysis. It is probably easy to use some oily material like NBS-22 for referencing almost any bulk combustion ($\delta^{13}\text{C}$). However, using carbonates for referencing water samples is more difficult if not impossible in pyrolysis systems ($\delta^{18}\text{O}$).³⁶
- the isotopic composition should be in the range of the samples to be measured. Isotopic differences can be measured most precisely when small. Because most natural organic material is produced primarily through C_3 -photosynthesis, $\delta^{13}\text{C}$ values around -25% vs. VPDB are recommended for the laboratory standard.
- non-hygroscopic. This is of particular importance for oxygen and hydrogen isotope ratio analysis using high-temperature carbon reduction ('pyrolysis') systems.

irm-EAMS

We analyze any bulk material that can be combusted quantitatively to CO_2 , N_2 and H_2O (+ other oxides if applicable) using the combination of an elemental analyzer (NA 1110, CE Instruments, Milan, Italy) with an isotope ratio mass spectrometer (DELTA⁺XL, Finnigan MAT, Bremen, Germany). The coupling interface is a ConFlo II[®], modified in-house to a ConFlo III,³⁵ which includes two open splits, one for the coupling, the other one for reference gas introduction. The coupling split can be varied over a wide range from zero to 64-fold dilution of the effluent stream without causing detectable isotopic fractionation. Two reference gases (mostly CO_2 and N_2 , alternatively H_2 and CO) can be actuated under computer control. H_2 and CO reference gases are used when the system operates in high-temperature 'pyrolysis' mode with a Finnigan MAT TC/EA for D/H or $^{18}\text{O}/^{16}\text{O}$ isotope ratio analysis. The operation of the system generally follows a well-defined protocol. Samples, reference material aliquots, and blanks are weighed and filled into the 32-position autosampler carousel according to a loading list (Fig. 4). Please note that each list represents samples for three carousels (we use 96-position sample trays for storing samples in a desiccator before measurement). It serves as input for the sequence information in the mass spectrometer control software.

The prefilled positions in the loading list are mandatory. They are used in a post-run off-line evaluation on a

EA Loading List - C only

Total weight max.: 100 mg Sample, without Dil.: 30-210µg C, plus Dil.: max. 5000 µg C

Name: Date: C%: Tray identity:

	Line		Sample	[mg]Total Weight		Sample	[mg]Total Weight	Line	
	1	A1	Ali-j1		E1			49	
	2	A2	bl (blank)		E2			50	
	3	A3	Ali-j1		E3			51	
	4	A4	Ali-j1 [Ref] (71,09%C)		E4			52	
	5	A5			E5			53	
	6	A6			E6			54	
	7	A7			E7			55	
	8	A8			E8			56	
	9	A9			E9			57	
	10	A10			E10			58	
	11	A11			E11			59	
	12	A12	Ali-j1		E12			60	
	13	B1	bl		F1			61	
	14	B2	bl		F2			62	
	15	B3	Ali-j1		F3			63	
	16	B4	Ali-j1		F4	Ali-j1		64	
	17	B5			F5	Ali-j1 [Ref]		65	
	18	B6			F6			66	
	19	B7			F7			67	
	20	B8			F8	Caf-j1		68	
	21	B9			F9			69	
	22	B10			F10			70	
	23	B11			F11			71	
	24	B12	Caf-j1 (49,44%C)		F12			72	
	25	C1			G1			73	
	26	C2			G2			74	
	27	C3			G3			75	
	28	C4			G4	Ali-j1		76	
	29	C5			G5	bl		77	
	30	C6			G6	bl		78	
	31	C7			G7	Ali-j1		79	
	32	C8	Ali-j1		G8	Ali-j1		80	
	33	C9	Ali-j1 [Ref]		G9			81	
	34	C10			G10			82	
	35	C11			G11			83	
	36	C12	Caf-j1		G12			84	
	37	D1			H1			85	
	38	D2			H2			86	
	39	D3			H3			87	
	40	D4			H4			88	
	41	D5			H5			89	
	42	D6			H6			90	
	43	D7			H7			91	
	44	D8	Ali-j1		H8			92	
	45	D9	bl		H9			93	
	46	D10	bl		H10			94	
	47	D11	Ali-j1		H11			95	
	48	D12	Ali-j1		H12	Ali-j1		96	

Figure 4. Typical loading list for sequential isotopic analysis of samples and reference materials in a prescribed fashion. The 96 positions ('line') on this list represent samples for three carousels with 32 positions each.

sample #	spec.-no.	weight [mg]	area [Vs]	$\delta^{13}\text{C}$ vs.VPDB	area blk [Vs]	$\delta^{13}\text{C}$ -blk vs.PDB	$\delta^{13}\text{C}$ blk-corr	*for corr	avgStd	$\delta^{13}\text{C}$ ref.	Diff-Std	$\delta^{13}\text{C}$ vs.VPDB corr.	avgStd $\delta^{13}\text{C}$	std.dev.
All-11	794	0.15	198.36	-34.03	1.00	-26.84	-34.07					-33.99		
All-11	795	0.136	152.80	-34.07	1.00	-26.84	-34.12					-34.05		
All-11	796	0.117	142.76	-34.11	1.00	-26.84	-34.16	*				-34.09		
36-246	797	1.775	122.17	-24.50	1.00	-26.84	-24.48					-24.41		
36-246	798	1.741	116.34	-24.46	1.00	-26.84	-24.44					-24.37		
Caf-11	799	0.229	189.53	-52.05	1.00	-26.84	-52.19					-52.12		
36-246	800	1.731	114.68	-24.48	1.00	-26.84	-24.45					-24.38		
16-98-2	801	1.786	86.72	-22.85	1.00	-26.84	-22.80					-22.73		
16-98-2	802	1.804	86.26	-22.79	1.00	-26.84	-22.75					-22.68		
16-98-2	803	1.795	83.75	-22.84	1.00	-26.84	-22.79					-22.72		
114-806	804	1.776	170.37	-28.00	1.00	-26.84	-28.00					-27.93		
114-806	805	1.852	176.49	-28.08	1.00	-26.84	-28.09					-28.02		
114-806	806	1.795	176.36	-28.07	1.00	-26.84	-28.08					-28.00		
All-11	807	0.146	170.36	-33.99	1.00	-26.84	-34.04	*				-33.97		
blk	808	0	1.01	-27.48										
blk	809	0	0.98	-26.20										
All-11	810	0.144	165.17	-33.98	1.00	-26.84	-34.02	*				-33.95		
All-11	811	0.133	156.46	-33.89	1.00	-26.84	-33.93	*				-33.86		
129-504	812	1.837	157.67	-31.10	1.00	-26.84	-31.13					-31.06		
129-504	813	1.912	164.89	-31.09	1.00	-26.84	-31.12					-31.05		
129-504	814	1.843	157.83	-31.15	1.00	-26.84	-31.18					-31.11		
52-368	815	1.765	142.51	-27.28	1.00	-26.84	-27.28					-27.21		
52-368	816	1.837	153.82	-27.38	1.00	-26.84	-27.38					-27.31		
52-368	817	1.813	149.45	-27.29	1.00	-26.84	-27.29					-27.22		
93-668	818	1.813	288.98	-31.20	1.00	-26.84	-31.21					-31.14		
93-668	819	1.814	280.80	-31.10	1.00	-26.84	-31.11					-31.04		
93-668	820	1.784	290.18	-30.91	1.00	-26.84	-30.92					-30.85		
138-390	821	1.819	169.71	-27.40	1.00	-26.84	-27.41					-27.34		
138-390	822	1.931	179.12	-27.44	1.00	-26.84	-27.45					-27.38		
138-390	823	1.801	167.01	-27.39	1.00	-26.84	-27.39					-27.32		
100-727	824	1.946	265.80	-29.64	1.00	-26.84	-29.65					-29.58		
100-727	825	1.871	256.98	-29.17	1.00	-26.84	-29.18					-29.11		
100-727	826	1.956	255.76	-29.35	1.00	-26.84	-29.36					-29.29		
All-11	827	0.14	156.33	-33.86	1.00	-26.84	-33.90	*	-34.01	-33.94	0.07	-33.83	-33.94	0.10

Figure 5. Spreadsheet calculation of data from the irm-EAMS system showing the evaluation of the raw data to the final δ -values on an international scale. See text for further explanations.

$\delta^{13}\text{C}$ performance of Caf-j1
Scale based on Ali-j1 lab reference material

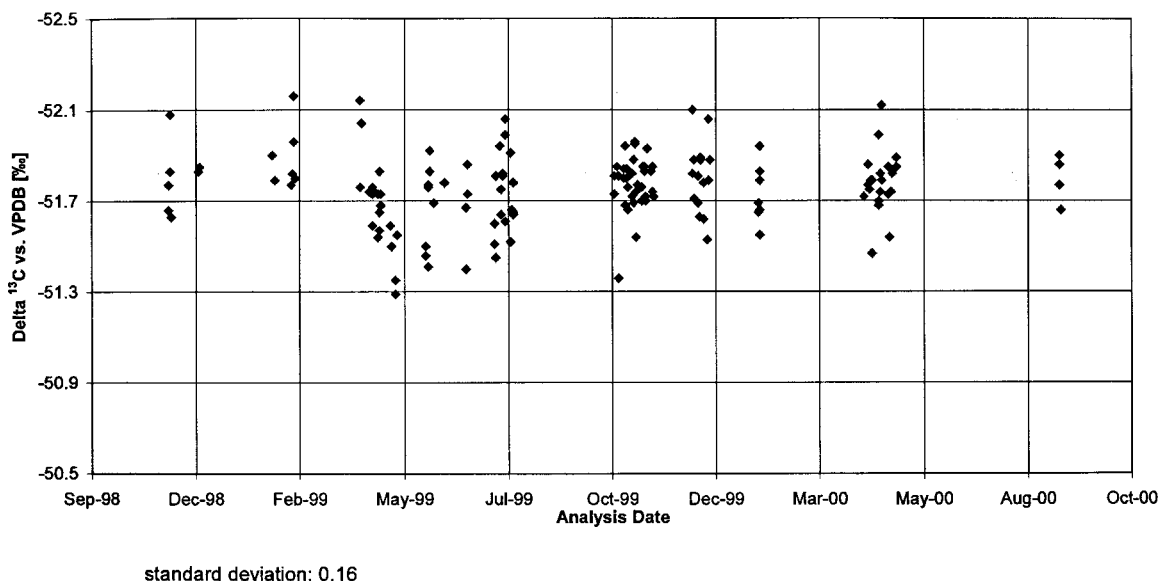


Figure 6. Performance Chart. $\delta^{13}\text{C}$ -values of a quality control standard as a function of measurement time. The scale is based on reference material that went through the same preparation channel as the QA standard. Each sequence of 32 samples measured holds one QA standard that is represented by a single point.

standardized spreadsheet (Fig. 5) for assigning the final δ -values on the respective international scale.

The spreadsheet evaluation³⁷ starts with the original values directly transferred from the host computer ('ISO-DAT' from Finnigan MAT). Columns 1 to 5 (Fig. 5) hold information about the sample including the sample name and weight, the integrated peak area and the measured $\delta^{13}\text{C}$ -value based on the injected reference gas. The average peak area (Vsec) and δ -value (‰) of the blank measurement^j are used to correct the data for blank contribution applying a simple mass balance correction.

$$\delta^{13}\text{C}_{\text{tot}} \times \text{area}_{\text{tot}} = \delta^{13}\text{C}_{\text{sa}} \times \text{area}_{\text{sa}} + \delta^{13}\text{C}_{\text{blk}} \times \text{area}_{\text{blk}} \quad (3)$$

The suffixes in Eqn. (3) refer to the total (tot), sample (sa) and blank (blk) properties, respectively.

Usually the correction is very small. The data in column 8 (Fig. 5) represent the blank-corrected preliminary $\delta^{13}\text{C}$ -values (‰) when the reference gas is used for standardization. The following columns denote the laboratory standards selected for further correction and final positioning of the data onto the VPDB scale. Ali-j1 (Acetanilide-Jena1) has been determined directly versus NBS-22 oil and USGS24 graphite a number of times and has been assigned a $\delta^{13}\text{C}$ -value of -33.94‰ on the VPDB scale. The measured average $\delta^{13}\text{C}$ -value of the selected Ali-j1 samples in Fig. 5 is -34.01‰ . Thus, there is an offset of 0.07‰ to the accepted δ -value that

^j We found that the carbon blank in our laboratory mostly has a $\delta^{13}\text{C}$ -value around -25‰ vs. VPDB. The major contributions are carbon in the tin containers and memory in the combustion reactor. Often the blank area is so small that the $\delta^{13}\text{C}$ measurement is erroneous (outside the natural abundance range). In such cases we replace the wrong $\delta^{13}\text{C}$ -value of the blank by our average value, -25‰ .

is used to correct all data (Eqn. (2)). The data in column 13 are the final $\delta^{13}\text{C}$ -values on the VPDB scale. The last two columns are average and precision of the selected Ali-j1 data.

In principle, this procedure represents a one-point calibration of the $\delta^{13}\text{C}$ -values; our scale expansion factor is 1.000. For measuring larger differences in isotopic composition it will be necessary to include a scaling factor in the calculations (see Fig. 10 and the discussion in 'Further corrections').

Not shown in Fig. 5 are further routine calculations of the carbon elemental contents based on the measured peak area and the known carbon content of the reference material (Ali-j1, 71.09% C). The $\delta^{15}\text{N}$ analyses are made in a completely analogous fashion.

Generation of performance charts

We use one dedicated sample position for determining a QA standard (quality assurance), in this case Caf-j1 (a caffeine sample from a 'Traube synthesis' in larger supply), in every sample carousel we run. We have chosen this material because it is off the usual $\delta^{13}\text{C}$ values for C_3 -plants by about -20‰ . With this QA standard, which does not enter the reference calculation, the long-term performance of the irm-EAMS line has been monitored (Fig. 6 for $\delta^{13}\text{C}$ and Fig. 7 for $\delta^{15}\text{N}$).

Figures 6 and 7 are representative for all measurements made on our irm-EAMS systems (in total from three different mass spectrometers and two different interfaces) since we started routine analyses. We measured both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ from the same sample until April 1999. From the performance charts we noticed that the accuracy, especially for $\delta^{15}\text{N}$, was not satisfactory and needed improvement. Careful analysis of the data indicated that the problems were

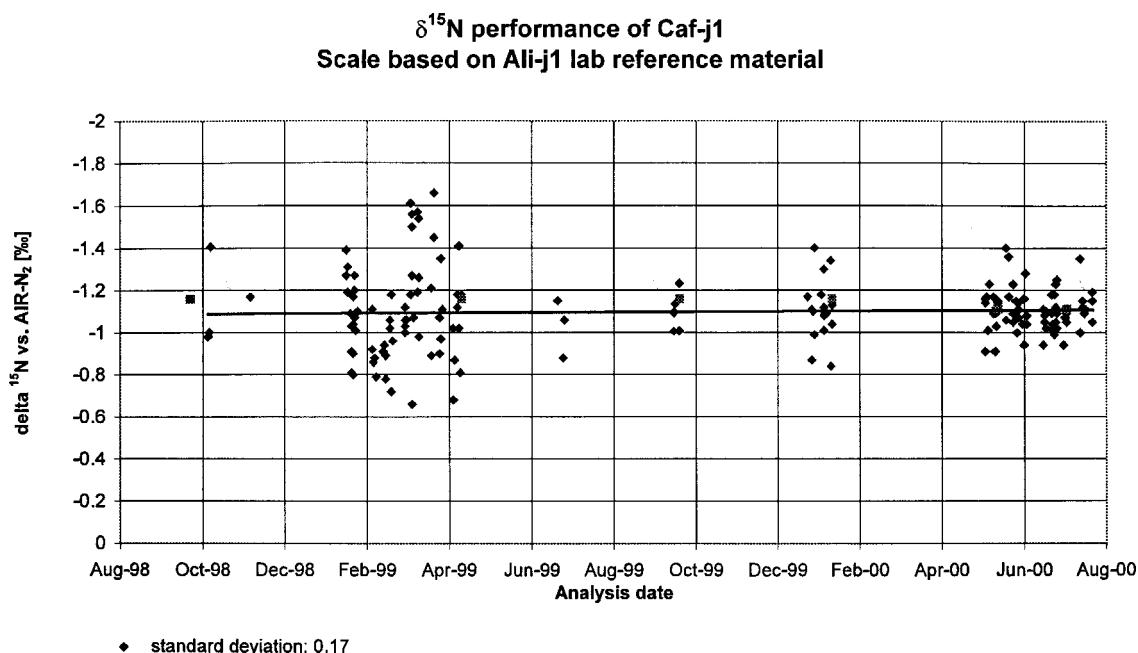


Figure 7. Performance Chart: Same as Fig. 6 for $\delta^{15}\text{N}$ -values

caused by tailing of the CO_2 peak into the nitrogen chromatogram of the subsequent sample. Most materials analyzed had an elemental content of $\leq 2\%$ nitrogen and $\geq 40\%$ carbon. For analysis the CO_2 had to enter the GC column (Porapak Q) and, due to the relatively large amount, was still present and declining steadily during the subsequent nitrogen analysis. CO_2^{++} ions undergo unimolecular decay in the ion source resulting in a 10% CO^+ contribution at m/z 28 and 29 that interferes with N_2^{++} and has a very different isotopic signature. The background assignments of all peaks in the nitrogen chromatogram were affected and hence precision and accuracy suffered. As a consequence, we decided to stop simultaneous isotope ratio measurement

using irm-EAMS altogether. For $\delta^{15}\text{N}$ determination we now trap CO_2 in a chemical (Ascarite) trap installed between the water trap and the GC column of the elemental analyzer.

The performance or quality chart is also the perfect tool to monitor the status of the laboratory reference materials. Any contamination in either the laboratory standard or the QA standard will show up as a step. Slow long-term alteration (e.g. evaporative loss in water standards) will show as a drift. We recommend using such charts as a general tool for maintaining and proving analytical performance in every isotope laboratory for every type of analysis.

In order to demonstrate the effect of the rigorous implementation of the IT principle, we have compiled the

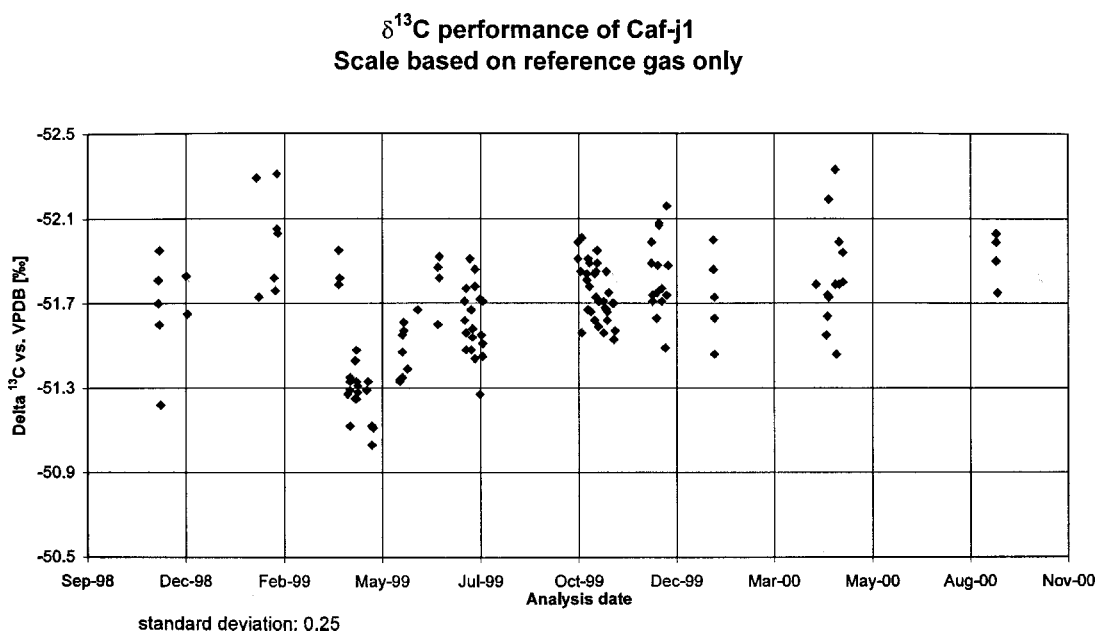


Figure 8. Performance Chart. $\delta^{13}\text{C}$ -values as in Fig. 6. Here, the scale is based on reference gas injections only. Please note the enhanced scatter of the data.

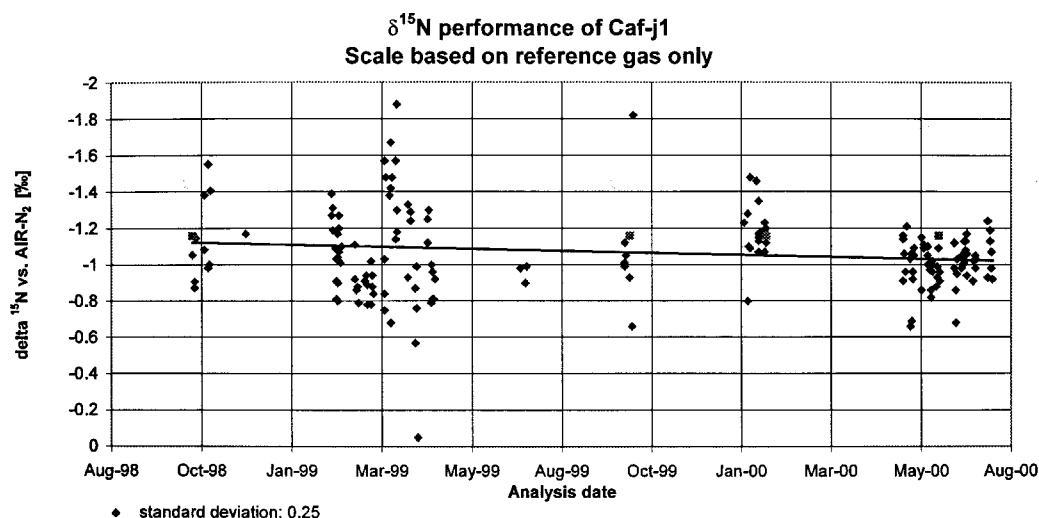


Figure 9. Performance Chart. $\delta^{15}\text{N}$ -values as in Fig. 7. The scale is solely based on reference gas. Compared with Fig. 7 a larger scatter and some outliers are evident.

results of our QA standard based exclusively on our reference gas (Figs. 8 and 9). The data clearly exhibit a larger scatter than the data compiled including the IT calculations. For both $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$, the precision has increased by about 50%. It is also worth noting that a number of marked outliers are present in Fig. 9 that are not seen in Fig. 7. These outliers might have escaped our attention if the IT principle had not been included in the measurement and evaluation.

The composition of the N_2 gas inside our 50-L high-pressure cylinders has not changed much over the reported time span. There is a slope of $0.04\text{‰}/\text{a}$ in the solid line in Fig. 9 that is not present in Fig. 7. This slope probably represents a change in the N_2 reference gas. To explain the differences between the graphs we suspect a number of sources of error that all contribute to the observed dependence of the data:

- the pressure regulator on the tank is critical. Most regulators alter the isotopic composition of the dispensed gas at least temporarily, the size of the alteration being dependent on the gas itself and on the surfaces inside the regulator.³⁸ The same applies to the second type of GC regulators (Porter) in the interface. The alteration for the isotopic composition of CO_2 is in the sub-per mill range.
- The combustion and (or) the reduction tube can be contaminated with previous sample material giving rise to memory effects.
- The combustion efficiency may vary slightly thereby altering the isotopic composition.
- The efficiency of the reduction tube for scavenging excess oxygen and for reducing nitrous oxides may change with time.
- When diluting the CO_2 effluent, a change in isotopic composition may occur due to diffusion effects.³⁵

As long as the reference material suffers from the same deficiencies during preparation, the errors will at least tend to cancel out and the long-term data monitored by a QA standard will exhibit much better laboratory performance than with pure standard gas referencing.

D/H determination from water

Measurement of δD -values from water samples is carried out on a fully automated chromium reduction system at 900°C ('H/Device', Finnigan MAT, Bremen, Germany) directly coupled to the sample inlet of the dual inlet system of our MAT 252 isotope ratio MS.³⁹ The samples, positioned in a GC autosampler tray (AS200, CTC Analytics, Switzerland), are transferred to the hot injection port via a gas-tight syringe (Model 702, Hamilton, Switzerland). The quartz reactor (Finnigan MAT) is filled with chromium powder (100 mesh, Alfa Aesar, Karlsruhe, Germany). The reaction of water vapor with the hot chromium is almost instantaneous. The valve at the end of the reactor is opened after a preset time and the H_2 gas is transferred to an intermediate equilibration volume. Again, after a preset equilibration time (in order to avoid diffusive alteration of the isotopic composition of the hydrogen gas), the reactor valve is closed and the H_2 gas is passed to the sample volume of the dual inlet for measurement. The equilibration and transfer times are critical and must be under computer control.

We routinely run 60 analyses (~ 20 h) in a sequence with the laboratory reference water and the QA standard distributed in a prescribed loading list similar to that presented in Fig. 4. All data enter a spreadsheet for final calculation of δD -values (Fig. 10).

The data in column 3 (Fig. 10) are the original results as automatically transferred from the mass spectrometer PC. All samples are run in triplicate. The data treatment is more elaborate owing to the fact that multiple effects must be accounted for. First, we apply a memory correction (column 4) to the δD -values against the working gas. The magnitude of the correction is 1% carry over from the previous sample plus 0.2% from the sample before. The effect of the memory correction can be judged from the triplets where the difference to the precursor sample is large.

Next is the IT calculation, that is, replacement of the hydrogen gas reference by the laboratory standard water

Siberian Water Samples Spec# 7379-7438, H/Device, Feb. 22, 1999

#	Name	raw δ vs Heim(-220)	memory corrected ($\pm 0.2\%$)	average	$\Delta\delta$ ref.gas offset	δ VSMOW	average	1st drift correction	average	2nd drift correction	average	base adjusted	average	δ VSMOW / SLAP ($\pm 0.7\%$)	average	stdDev
1	WWJ-1A/Isolab/	-63.834	-63.83			-66.03		-66.41		-66.41		-66.41		-66.41		
2	WWJ-1A/Isolab/	-63.932	-63.93			-66.13		-66.38		-66.38		-66.38		-66.38		
3	WWJ-1A/Isolab/	-64.3018	-64.31			-66.50		-66.63		-66.63		-66.63		-66.63		
4	WWJ-1A/Isolab/	-64.3116	-64.31			-66.51		-66.51		-66.51		-66.51		-66.51		
5	WWJ-1A/Isolab/	-64.3122	-64.31			-66.51		-66.39		-66.39		-66.39		-66.39		
6	WWJ-1A/Isolab/	-64.3971	-64.40	-64.25	-2.35	-66.60	-66.45	-66.35	-66.45	-66.35	-66.45	-66.35	-66.45	-66.35	-66.45	0.11
7	142 A/E.D.Schulze/	-115.955	-116.57			-118.65		-118.28		-118.28		-118.28		-118.64		
8	142 A/E.D.Schulze/	-117.533	-117.66			-119.73		-119.23		-119.23		-119.23		-119.60		
9	142 A/E.D.Schulze/	-116.798	-116.79			-118.87	-119.08	-118.24	-118.58	-118.24	-118.58	-118.24	-118.58	-118.61	-118.95	0.56
10	150/E.D.Schulze/	-133.435	-133.63			-135.67		-134.92		-134.92		-134.92		-135.40		
11	150/E.D.Schulze/	-134.854	-134.90			-136.94		-136.07		-136.07		-136.07		-136.55		
12	150/E.D.Schulze/	-134.09	-134.08			-136.12	-136.24	-135.12	-135.37	-135.12	-135.37	-135.12	-135.37	-135.60	-135.85	0.61
13	131/E.D.Schulze/	-91.6047	-91.09			-93.23		-92.11		-92.11		-92.11		-92.29		
14	131/E.D.Schulze/	-91.9463	-91.87			-94.00		-92.75		-92.75		-92.75		-92.94		
15	131/E.D.Schulze/	-91.8027	-91.80			-93.93	-93.72	-92.57	-92.48	-92.57	-92.48	-92.57	-92.48	-92.75	-92.66	0.33
16	106 A/E.D.Schulze/	-129.781	-130.24			-132.28		-130.79		-130.79		-130.79		-131.24		
17	106 A/E.D.Schulze/	-130.974	-131.06			-133.10		-131.49		-131.49		-131.49		-131.94		
18	106 A/E.D.Schulze/	-131.055	-131.06			-133.10	-132.83	-131.36	-131.21	-131.36	-131.21	-131.36	-131.21	-131.81	-131.66	0.38
19	RWB-J1/Isolab/	-7.87838	-6.40			-8.73		-6.87		-6.87		-6.87		-6.45		
20	RWB-J1/Isolab/	-6.72937	-6.47			-8.80		-6.81		-6.81		-6.81		-6.40		
21	RWB-J1/Isolab/	-6.59651	-6.59			-8.93	-8.82	-6.81	-6.83	-6.81	-6.83	-6.81	-6.83	-6.39	-6.41	0.03
22	134 A/E.D.Schulze/	-119.087	-120.44			-122.50		-120.26		-120.26		-120.26		-120.64		
23	134 A/E.D.Schulze/	-120.452	-120.69			-122.76		-120.40		-120.40		-120.40		-120.77		
24	134 A/E.D.Schulze/	-121.628	-121.64			-123.71	-122.99	-121.22	-120.63	-121.22	-120.63	-121.22	-120.63	-121.60	-121.01	0.52
25	151 A/E.D.Schulze/	-120.951	-120.95			-123.01		-120.40		-120.40		-120.40		-120.78		
26	151 A/E.D.Schulze/	-121.147	-121.15			-123.21		-120.48		-120.48		-120.48		-120.85		
27	151 A/E.D.Schulze/	-121.266	-121.27			-123.33	-123.18	-120.47	-120.45	-120.47	-120.45	-120.47	-120.45	-120.85	-120.83	0.04
28	115/E.D.Schulze/	-92.6989	-92.36			-94.49		-91.50		-91.50		-91.50		-91.68		
29	115/E.D.Schulze/	-92.8674	-92.81			-94.94		-91.83		-91.83		-91.83		-92.01		
30	115/E.D.Schulze/	-92.8432	-92.84			-94.97	-94.80	-91.74	-91.69	-91.74	-91.69	-91.74	-91.69	-91.92	-91.87	0.17
31	144 A/E.D.Schulze/	-144.125	-144.74			-146.75		-143.39		-143.39		-143.39		-143.93		
32	144 A/E.D.Schulze/	-144.58	-144.69			-146.70		-143.21		-143.21		-143.21		-143.75		
33	144 A/E.D.Schulze/	-144.492	-144.49			-146.50	-146.65	-142.89	-143.17	-142.89	-143.17	-142.89	-143.17	-143.43	-143.70	0.25
34	WWJ-1A/Isolab/	-68.8824	-67.97			-70.16		-66.43		-66.43		-66.43		-66.43		
35	WWJ-1A/Isolab/	-68.45	-68.29			-70.48		-66.63		-66.63		-66.63		-66.63		
36	WWJ-1A/Isolab/	-68.0867	-68.08			-70.27	-70.31	-66.29	-66.45	-66.30	-66.45	-66.30	-66.45	-66.29	-66.45	0.17
37	145/E.D.Schulze/	-139.372	-140.23			-142.25		-138.14		-138.15		-138.15		-138.65		
38	145/E.D.Schulze/	-140.829	-140.99			-143.01		-138.78		-138.79		-138.79		-139.30		
39	145/E.D.Schulze/	-140.944	-140.95			-142.97	-142.74	-138.61	-138.51	-138.63	-138.52	-138.63	-138.52	-139.14	-139.03	0.34
40	1 NB 23/E.D.Schulze/	-72.4351	-71.61			-73.79		-69.32		-69.34		-69.34		-69.36		
41	1 NB 23/E.D.Schulze/	-71.4826	-71.33			-73.52		-68.91		-68.94		-68.94		-68.96		
42	1 NB 23/E.D.Schulze/	-71.5855	-71.58			-73.77	-73.69	-69.04	-69.09	-69.07	-69.12	-69.07	-69.12	-69.09	-69.14	0.20
43	144/E.D.Schulze/	-146.175	-147.07			-149.07		-144.22		-144.26		-144.26		-144.80		
44	144/E.D.Schulze/	-147.004	-147.16			-149.17		-144.19		-144.23		-144.23		-144.78		
45	144/E.D.Schulze/	-147.476	-147.48			-149.49	-149.24	-144.39	-144.27	-144.43	-144.31	-144.43	-144.31	-144.98	-144.85	0.11
46	1 NB 19/E.D.Schulze/	-59.8333	-58.78			-60.99		-55.77		-55.82		-55.82		-55.75		
47	1 NB 19/E.D.Schulze/	-58.7421	-58.55			-60.76		-55.42		-55.47		-55.47		-55.40		
48	1 NB 19/E.D.Schulze/	-58.7176	-58.72			-60.93	-60.89	-55.45	-55.55	-55.51	-55.60	-55.51	-55.60	-55.44	-55.53	0.19
49	145 A/E.D.Schulze/	-140.411	-141.39			-143.41		-137.81		-137.88		-137.88		-138.38		
50	145 A/E.D.Schulze/	-141.207	-141.38			-143.40		-137.68		-137.75		-137.75		-138.24		
51	145 A/E.D.Schulze/	-141.676	-141.68			-143.70	-143.50	-137.85	-137.78	-137.93	-137.85	-137.93	-137.85	-138.43	-138.35	0.09
52	150 A/E.D.Schulze/	-137.474	-137.42			-139.45		-133.48		-133.56		-133.56		-134.03		
53	150 A/E.D.Schulze/	-137.517	-137.51			-139.53		-133.44		-133.52		-133.52		-133.99		
54	150 A/E.D.Schulze/	-137.563	-137.56			-139.59	-139.53	-133.37	-133.43	-133.46	-133.51	-133.46	-133.51	-133.93	-133.98	0.05
55	112 A/E.D.Schulze/	-111.57	-111.26			-113.35		-107.00		-107.10		-107.10		-107.38		
56	112 A/E.D.Schulze/	-110.883	-110.82			-112.91		-106.44		-106.54		-106.54		-106.82		
57	112 A/E.D.Schulze/	-110.981	-110.98			-113.07	-113.11	-106.48	-106.64	-106.58	-106.74	-106.58	-106.74	-106.86	-107.02	0.31
58	WWJ-1A/Isolab/	-71.5395	-71.07			-73.25		-66.53		-66.64		-66.64		-66.64		
59	WWJ-1A/Isolab/	-71.0069	-70.92			-73.10		-66.26		-66.38		-66.38		-66.37		
60	WWJ-1A/Isolab/	-71.003	-71.00			-73.18	-73.18	-66.22	-66.34	-66.34	-66.45	-66.34	-66.45	-66.33	-66.45	0.17

Figure 10. Spreadsheet calculation of data from the H/Device sample preparation line showing the evaluation of the raw data to the final δ -values on an international scale. See text for further explanations.

samples in the run. The δD -value (-66.45%) of WWj-1A (working water-Jena1A) has been established by direct measurement against VSMOW and SLAP samples (freshly opened ampoules) obtained from the IAEA in Vienna. Using this δD -value and the actually measured average of WWj-1A, the apparent working reference gas offset (-2.35% , Fig.

10) is calculated according to:

$$\text{offset}_{\text{wgas}} = (-66.45 - \text{avg}_{\text{gwwj1}}) / (1 + \text{avg}_{\text{gwwj1}}/1000) \quad (4)$$

This offset is used to correct the raw memory corrected δD -values and to provide a preliminary positioning relative to VSMOW (Eqn. (2)).

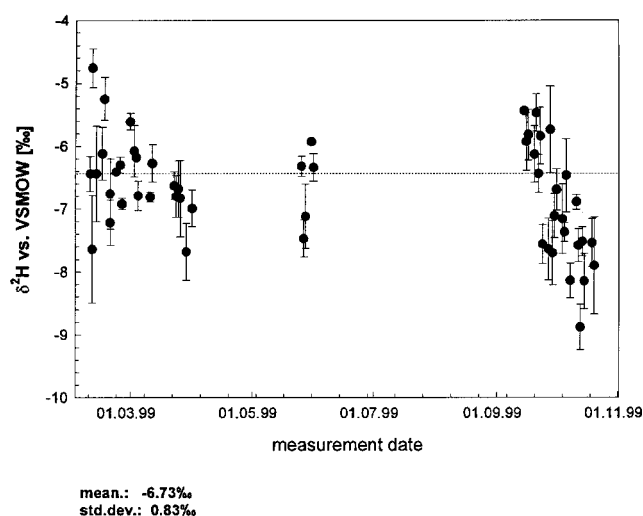
$\delta^2\text{H}$ Performance of RWB-J1

Figure 11. Performance Chart. $\delta^2\text{H}$ -values of the quality control standard RWB-J1 as a function of measurement time.

The next columns constitute a two-pass drift correction. The drift correction is necessary because the hydrogen reference gas in the dual inlet changes isotopic composition over time (a complete 60-sample sequence takes about 20 h). This is due to the fact that length and crimping of the capillaries are a compromise between CO_2 and H_2 work. To avoid back diffusion of isotopically altered gas into the variable volume, the capillaries would have to be twice as long for H_2 . Alternatively, a different crimp on reference vs. sample side could be used so that the reference could be operated at a much higher pressure. The magnitude of the drift depends on the filling state of the reference bellow volume at the beginning of the sequence. We have observed drifts of 3–5‰ in 20 h when starting with a full reservoir. Less reference gas results in larger drifts. Because the drift should follow an exponential function, we have implemented our analysis sequence with three reference water measurements in positions 34 to 36 and three more at the end of the sequence. The results for these reference water injections are used to correct the reference gas drift. A fine adjustment is made in column 13 (Fig. 10) that is only necessary for making the data mathematically consistent after drift correction (in the example shown the data are identical). The last correction involves scaling according to the observed scale contraction.⁴⁰ On average we have measured -425‰ instead of -428‰ for SLAP vs. VSMOW, hence all data are corrected by multiplying the difference from WWJ-1A by 1.007. The last two columns are the final δD -values on the VSMOW/SLAP scale and their precision.

^k Usually pure CO_2 gas is used for equilibration and a mass balance correction according to:

$$\delta_{\text{true}} = \delta_{\text{meas}} + \alpha x_{\text{gas}} (\delta_{\text{meas}} - \delta_{\text{gas}}) / x_{\text{w}} \quad (5)$$

is applied (α = exchange fractionation factor, δ_{gas} is the $\delta^{18}\text{O}$ -value of the added CO_2 gas before equilibration and x_{gas} and x_{w} are the mole fractions of gas and liquid, respectively).

In our case, due to the small amount of CO_2 in the gas phase, the correction can be neglected down to about $50 \mu\text{L H}_2\text{O}$. Equation (5) also applies to the equilibration of hydrogen gas with water.

In every 60-sample sequence we measure RWB-j1 (reference water B-Jena1) as a QA standard for generating the performance chart associated with the D/H procedure. Figure 11 shows the data as a function of measurement time. The overall precision of 0.83‰ vs. VSMOW is certainly acceptable. There is a tendency to more negative values at the end of the sequence that deserves further attention. During the short period of measurements this is not likely to be caused by a relative drift of our laboratory working waters. However, most of the internal precisions measured are better than 0.8‰ (see also Fig. 10). We attribute this finding to the fact that we do not determine the SLAP/VSMOW scaling factor for every sequence in contrast to frequent measurement of the H_3^+ factor. We started by using water samples with about 60‰ difference for implementing this correction. This difference turned out to be too small in relation to the measurement precision, leading to erroneous scaling factors. On the other hand, measuring water with about -400‰ δD in the same sequence affects the memory correction and the three samples following this negative water would have to be discarded. The potential for improving the performance chart precision to about 0.5‰ is within reach using the technique described.

Equilibration/irm-analysis for $\delta^{18}\text{O}$ determination

The ^{18}O abundance in water samples is usually measured with a modified $\text{CO}_2/\text{H}_2\text{O}$ equilibration technique. With a syringe we inject $400 \mu\text{L}$ water aliquots into 10-mL glass containers ('Exetainer') topped with a septum. Before water injection the glass vials are filled with a mixture of 0.5% CO_2 in helium.^k Equilibration takes place inside a 96-position autosampler rack held at 30°C ($\pm 0.1^\circ\text{C}$) that is part of the irm interface (GasBench II, Finnigan MAT). We operate the autosampler rack in two separate stages holding 48 sample tubes each. While one sequence of 48 samples is measured, the other batch of 48 samples is allowed to equilibrate for roughly 20 h. With this loading scheme we manage to have the system analyze samples almost continuously, i.e. 6 days per week.

Following equilibration, the sample vial is analyzed by piercing the septum with a double wall needle. The needle has a feed (He) and an exit (sample CO_2 in He). The flushing rate is approximately $0.3 \text{ mL}/\text{min}$. The sample gas flows over a Nafion[®] dryer and then through an injection loop from which the GC run (Poraplot Q) is started. From a single sample tube we inject about ten times. The ten CO_2 peaks are evaluated isotopically and the results are transferred to a spreadsheet for further data evaluation.

The measurement sequence is assembled as before, i.e. following a rigid structure determined by a dedicated loading list. Hence, the evaluation follows a similar pattern: a mean $\delta^{18}\text{O}$ is calculated for all CO_2 peaks from a single sample. The δ -values are referenced at first to co-injected CO_2 standard gas peaks that serve as a mediator between the samples. WWJ-1A water samples strategically located in the 48-sample sequence again serve to position the preliminary $\delta^{18}\text{O}$ -values onto the VSMOW scale. As in deuterium analysis, a light reference water sample is used to establish

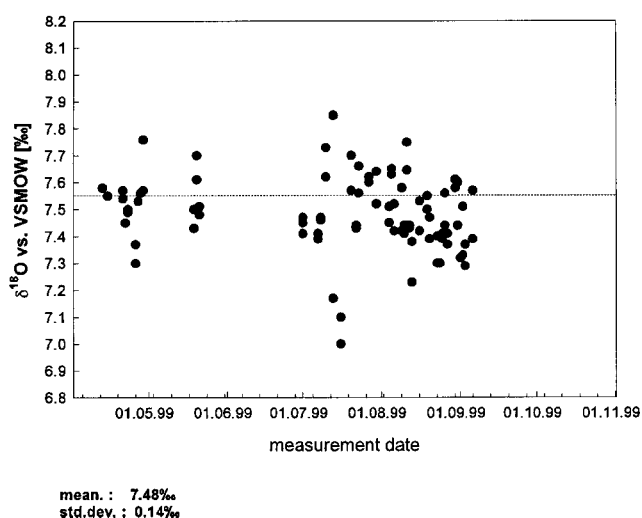
$\delta^{18}\text{O}$ Performance of RWB-J1

Figure 12. Performance Chart. $\delta^{18}\text{O}$ -values of the quality control standard RWB-J1 as a function of measurement time. Analysis is made using an equilibration/irm-GC/MS system (GasBench II, Finnigan MAT).

the final scale normalization so that SLAP returns a value of -55.5‰ vs. VSMOW.

Figure 12 shows the long-term $\delta^{18}\text{O}$ -performance of our RWB-J1 QA reference water. Each point corresponds to a single RWB-J1 sample in a 48-sample sequence. Apart from the scatter of the individual sample measurements, the

¹ If the reference material has been determined ten times in the sequence and the precision of the measurement is 0.1‰ then the isotopic value of the reference is determined with an error of $t \cdot (0.1\text{‰}) / \sqrt{10} = 0.063\text{‰}$. The Student factor t (2.0 in our case) reflects the statistically low number of measurements.

overall precision of 0.14‰ also includes the error of the reference water determination in the respective analysis sequence.¹

Isotopic analysis of CO_2 in air

The isotopic composition of CO_2 in air is important for constraining the sources and sinks of this important greenhouse gas in the atmosphere. The average $\delta^{13}\text{C}$ -value of CO_2 in the atmosphere has remained fairly constant over millions of years at about -6.5‰ vs. VPDB. During the last 200 years anthropogenic input from fossil fuel burning has led to a decline with an average $\delta^{13}\text{C}$ -value of about -8‰ at present. The decline continues at a rate of about $0.03\text{‰}/\text{a}$. The seasonal cycle of $\delta^{13}\text{C}$ varies with latitude and is a function of photosynthesis as well as human activity. At the South Pole the size of the cycling is about 0.05‰ (peak to peak) and increases to a maximum of 0.8‰ at high northern latitudes.⁴¹ These rather small isotopic signatures comprise multiple components including fossil fuel burning. Their measurement requires a high precision and accuracy analytical setup.

Our extraction line is schematically depicted in Fig. 13. From a Multiport valve the air passes through a capillary with a crimp, over a water trap at -70°C and a trap kept at -196°C . Here, CO_2 is frozen out at a pressure of about 400 mbar. The air is pumped through the pumping lines of the dual inlet system of our MAT 252 mass spectrometer.⁴² Sample CO_2 is measured directly from the sampling reservoir via a crimped capillary to the 'changeover valve'. The system is under full computer control for reliable timing and unattended operation. The precision of the system is aimed at and close to 0.01‰ $\delta^{13}\text{C}$ vs. VPDB and 0.025‰ $\delta^{18}\text{O}$ vs. VSMOW. It is very difficult to routinely prepare CO_2 from the primary reference materials (carbonates) across different laboratories at such levels of accuracy. Hence, the atmospheric CO_2 isotope ratio scales are refined scales that

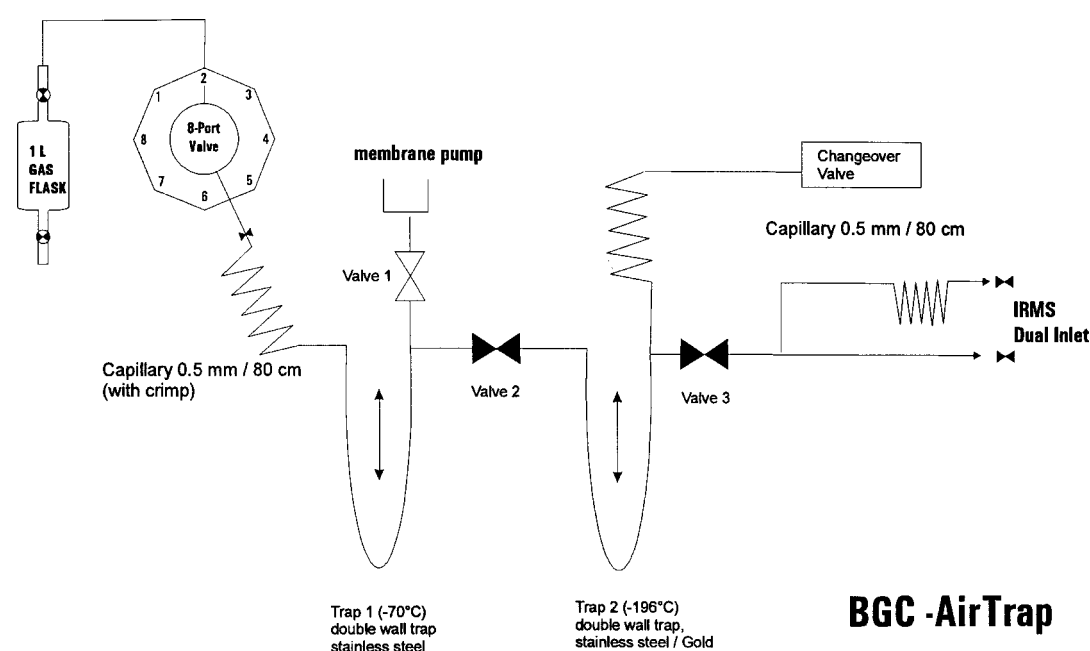


Figure 13. Schematic layout of the automated extraction system ('BGC-Airtrap') for high precision measurement of CO_2 isotope ratios in air.

use air samples and air in high-pressure cylinders as reference material. Consequently, the referencing in our system is made by a combination of permanently attached reference air (Multiport valve positions 1 and 2) as well as air in 1-L flasks that are exchanged routinely with other laboratories. The major problem here derives from the fact that gases are volatile. To keep them as reliable references requires a hierarchy of reference gases and a strategy that automatically detects mutual drifts in order to take corrective action. Without internal referencing high-precision measurement and international comparability of data would not be possible.

O₂/N₂ ratios in air

Complementary to the increase of CO₂ in the atmosphere there is a decrease of O₂. For every carbon atom burnt from fossil fuel one O₂ molecule is lost to CO₂. O₂ has sources and sinks that differ greatly from those of CO₂. Thus, by studying the decrease of O₂, i.e. measuring the O₂/N₂ ratio with high precision, a great deal can be learned about the global carbon cycle including the partitioning of sinks for CO₂ between the oceans and the land biosphere.

We have built a mass spectrometric inlet system for measuring O₂/N₂ ratios with a precision at the 5 perMeg^m level that is capable of monitoring small changes in atmospheric O₂ concentration. The system includes a 16-connection Multiport valve and an open split which is fed alternately from a sample and a reference gas, both switched on and off from a common transfer point, to a Delta⁺XL isotope ratio mass spectrometer (Finnigan MAT, Bremen, Germany). Referencing is made in a very similar fashion to that for CO₂ in air by measuring versus an air reference and implementing a multiple referencing hierarchy system. There is no international scale for this kind of work. Currently, consistency in the data is achieved by matching the scales of longer-term records measured by different laboratories. An intensive cooperation to overcome these limits to the comparability of data is under way by a group of laboratories involved in this type of analysis.

Correction of isobaric interferences

A review of referencing in isotope ratio measurements would not be complete without a discussion of the necessary corrections to the raw data. The precise measurement of isotope ratios is often hampered by interfering ion currents from other species hitting the same Faraday cup detectors. Among the most prominent examples are the contribution of

H₃⁺ on the mass 3 channel where δD is measured and the isobaric ¹²C¹⁸O¹⁷O⁺ ion current at the mass 45 position where $\delta^{13}C$ from CO₂ is determined. Here we intend to summarize some of the more important corrections. Further information for in-depth treatment is given in the cited literature.

¹⁷O correction for $\delta^{13}C$ determination

Using CO₂ gas, $\delta^{13}C$ cannot be measured independent of the oxygen isotope ratio unless the mass spectrometer used provides a working mass resolution of >52000. CO₂ gas contains a number of species with different masses ('isotopomers'). All combinations of stable isotopes are present, some of them very low in abundance. The list of isotopomers includes the major species ¹²C¹⁶O¹⁶O (*m/z* 44), ¹³C¹⁶O¹⁶O and ¹²C¹⁷O¹⁶O (*m/z* 45), ¹²C¹⁶O¹⁸O (*m/z* 46), as well as the minor species ¹³C¹⁷O¹⁶O and ¹²C¹⁷O¹⁷O (*m/z* 46), ¹³C¹⁷O¹⁷O, ¹³C¹⁸O¹⁶O and ¹²C¹⁷O¹⁸O (*m/z* 47), ¹²C¹⁸O¹⁸O and ¹³C¹⁷O¹⁸O (*m/z* 48) and ¹³C¹⁸O¹⁸O (*m/z* 49). When working in the natural abundance range of the isotopes the contributions of the minor species is small enough to be neglected. The ¹⁷O moiety at *m/z* 45, however, has a 7% contribution to the mass 45 ion current and must always be corrected for when determining $\delta^{13}C$ -values.

The square-root formula for correcting the ¹⁷O contribution to the 45/44 ratio is sometimes referred to as the Craig correction. Harmon Craig⁹ assumed a tight relationship between ¹⁷O and ¹⁸O with a fractionation coefficient λ of 0.5 exactly and devised a simple correction expression:

$$\delta^{13}C = 1.0676^{45} \delta_{\text{meas}} - 0.0338 \delta^{18}O \quad (6)$$

The numerical values in this expression are the ratio terms ⁴⁵R/¹³R and ¹⁷R/(2¹³R) of the reference gas evolved from the PDB standard as determined or used by Craig. As explicitly pointed out in the paper these numerical values do not apply to other standards and must therefore be used with great care.ⁿ Moreover, the average relation of ¹⁷O and ¹⁸O on earth (Craig equation: (¹⁸R_{sa}/¹⁸R_{st})^λ = ¹⁷R_{sa}/¹⁷R_{st}) cannot be described exactly using a fractionation factor λ of 0.5. Instead, 0.516⁴³ seems to be closer to the correct value for λ .^o The debate about the exact relationship found on earth is ongoing with new experimental values of 0.524 and 0.528 reported for λ .^{44,45}

PDB has been superseded by VPDB and the absolute ratios of the reference scale have been refined. In order to keep literature data consistent, Allison *et al.*⁴⁶ have compiled a recommendation for the ¹⁷O correction that keeps the 0.5 fractionation factor and provides exact values for all ratios involved. On the other hand, Santrock *et al.*⁴⁷ have shown in a H₂O/CO₂ equilibration experiment that data for $\delta^{13}C$ were not independent of the $\delta^{18}O$ -values of the equilibration water when using the Craig equation. The correction proposed includes a fractionation factor of 0.516 and an iterative correction procedure because the exact equations cannot be solved analytically for ¹³C. The dispute is not yet settled. Whatever correction algorithm is chosen (the mass spectrometer software should provide a choice), it is important to make sure that the reference gas isotope ratios

^m 1 perMeg is 0.001‰ in δ -units. It corresponds to a concentration change of 0.2 ppm O₂ in air.

ⁿ The numerical values in Eqn. (6) may be treated as variables. In this case, a set of water equilibration experiments with CO₂ gas that has identical carbon but different oxygen isotope ratios and vice versa can be made to determine the values of these variables. A correction based on these values will result in correct $\delta^{13}C$ -values irrespective of the exact knowledge of the oxygen isotope fractionation factors (T.B. Coplen, private communication).

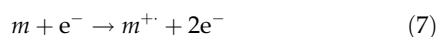
^o If the kinetics of the reactions causing isotopic fractionation are governed entirely by the respective zero point energy differences it can be shown that $\lambda = [(\mu_{16})^{-0.5} - (\mu_{17})^{-0.5}] / [(\mu_{16})^{-0.5} - (\mu_{18})^{-0.5}]$, with μ being the reduced mass. Using exact masses for a C-O bond the result is $\lambda = 0.5273$ which represents an upper limit for kinetic processes. In equilibrium processes, the value for λ can be as high as 0.531.⁴³

used in the calculations are consistent with the $\delta^{13}\text{C}$ -values assigned to the primary reference material.

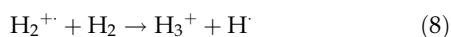
Please note that the correction is only valid for CO_2 from terrestrial sources, not for tracer studies. In addition, there is a strong ^{17}O anomaly in ozone in the stratosphere that affects other trace gases including CO_2 sampled from the atmosphere.

H_3^+ correction

Inside the ion source of the mass spectrometer ions are produced from neutral molecules m by 70 eV electron impact according to:



The molecular radical cation m^+ is produced in a variety of excited states. If the internal energy is high enough, molecular ions may decompose in a unimolecular decay reaction resulting in a daughter ion and a neutral (mostly a radical) giving rise to the observed mass spectrum. Another process can take place when ions within the ion source hit a neutral molecule. In this case, secondary ions may be formed via ion/molecule reactions. The formation of H_3^+ follows such a mechanism:



The reaction constant of Eqn. (8) is proportional to the number of both H_2^+ and H_2 . For a given sensitivity of the mass spectrometer, the number of H_2 molecules is proportional to the H_2^+ ion current, hence:

$$[\text{H}_3^+] = k \times [\text{H}_2^+]^2 \quad (9)$$

and the ratio $[\text{H}_3^+]/[\text{H}_2^+]$ is a linear function of the mass 2 ion current ($= k \times [\text{H}_2^+]$). The proportionality constant k is called the ' H_3^+ factor'. It is conveniently expressed in (ppm/nA) units.

In practice, the ion source is tuned to a large acceleration field across the ionization volume in order to minimize the time for the ion/molecule reaction (8) and hence suppress H_3^+ production. The H_3^+ factor is measured by observing the mass 3 ion current as a function of mass 2 ion intensity; a linear function is fitted through the data points. The correction involves a simple point-to-point subtraction of a linear portion of the mass 2 ion current from the signal observed at mass position 3. This is usually performed using the (clean) working reference gas. In particular, when helium carrier gas techniques are used, there may be other sources of non-linearity of m/z 3/2 including isobaric interference of He^{2+} ions at m/z 2 that should be studied and evaluated separately or traces of hydrocarbons that constitute a different source for H_3^+ ions.

There are other means of correcting the contribution of H_3^+ to the measured 3/2 ion current ratio.⁴⁰ The simplest correction is no correction at all which will obviously result in differences between reference and sample that are too small. Provided there are several isotopically known samples interspersed with the analyte material, a correction can be deduced from the results of these that may fully cover the requirements. As a general guideline, however, we feel that all effects should be studied and quantified as precisely

as possible in order to apply a valid correction and keep track of the details.

^{18}O correction for $\delta^{34}\text{S}$ determination using SO_2 gas

As for carbon isotope ratio analysis, the ion currents of SO_2 are influenced by the oxygen isotopes. In order to extract $\delta^{34}\text{S}$ the $^{32}\text{S}^{16}\text{O}^{18}\text{O}^+$ contribution to the mass 66 ion current (about 8.3%) needs to be taken into account. Unfortunately, there is no independent measurement of the other oxygen isotope, ^{17}O , which would enable a strategy similar to that for carbon isotope ratios. Instead, the established correction aims at keeping the oxygen isotopes in the sample and the reference SO_2 identical. In this case, the measured δ^{66} -values can be converted to $\delta^{34}\text{S}$ -values according to:⁴⁸

$$\delta^{34}\text{S} = {}^{66}\delta_{\text{meas}} + 2^{18}\text{R}/^{34}\text{R} \times ({}^{66}\delta_{\text{meas}} - \Delta\delta^{18}\text{O}) \quad (10)$$

In Eqn. (10) $^{18}\text{R}/^{34}\text{R}$ refers to the isotope ratio values of the standard gas and $\Delta\delta^{18}\text{O}$ is the ^{18}O difference between sample and reference SO_2 . Since these values cannot be measured independently in a simple way the expression $^{18}\text{R}/^{34}\text{R}$ is usually computed using the ratio values of VSMOW ($^{18}\text{R} = 0.0020052$, see Table 1) and VCDT ($^{34}\text{R} = 0.0441509$) and $\Delta\delta^{18}\text{O}$ is set to zero. Hence, the correction simply involves multiplying ${}^{66}\delta_{\text{meas}}$ with 1.091 for obtaining $\delta^{34}\text{S}$.

The simplification of Eqn. (10) applies strictly only when the oxygen in both sample and reference SO_2 are isotopically identical ($\Delta\delta^{18}\text{O} = 0$) and equal to VSMOW and the sulfur is isotopically equal to VCDT. Hence, this can only be regarded as a first-order correction. Fortunately, small errors in the adopted ratios do not alter the analytical result significantly. A difference in ^{18}O content in terms of $\delta^{18}\text{O}$, however, translates into an error of the analytical result that is 9% of the $\Delta\delta^{18}\text{O}$ -value.

Due to the 'stickiness' of SO_2 on surfaces, further corrections are necessary and recommended.⁴⁹ A systematic scaling factor of about 1.035 between sulfur measurements using SF_6 and others using SO_2 has been observed by many laboratories. The simplest overall correction is scaling the measured and first-order corrected SO_2 data with known isotopic compositions of reference material prepared together with the unknown samples. For sulfides, IAEA-S-2 (+22.66‰ VCDT) and IAEA-S-3 (−32.30‰ VCDT) provide good references for the scaling exercise. For analyzing barium sulfates, IAEA-SO-5 (+0.49‰ VCDT) and IAEA-SO-6 (−34.18‰ VCDT) will serve the purpose. Please note that the latter two values are provisional.^h

For firm techniques, where the SO_2 reference gas comes from a high-pressure cylinder, calculation of data should be made exclusively in terms of ${}^{66}\delta_{\text{meas}}$ -values up to the point where the reference is a sample processed exactly as the other samples (identical treatment again). Only then is the oxygen isotope signature similar enough to use the first-order correction given above.⁵⁰

Further corrections

Drift corrections

During analysis of a series of measurements one often observes a drift of the results as a function of time (or sample number). Drifts can have multiple causes including isotopic change of

the reference gas in the dual inlet system during an analysis sequence, build up of water or other contaminants during analysis, changing conditions of the mass spectrometer for instance following a fresh pump down of the vacuum system, deterioration of ion source conditions and many more. Many of these causes can and should be fixed by changing the conditions of the analysis. However, this can only be accomplished reliably when the overall design of the analytical sequences allows easy detection and provides means for correction of the drifting results. The design rules are simple: A sufficient number of identical samples are interspersed with the analyte samples and used to correct the drift. For an example of drift correction please refer to the spreadsheet in the hydrogen isotope analysis section (Fig. 10).

Linearity corrections

Similar to drift in time is a drift with size, i.e. the measured ratio is a function of the size of the sample (or a reagent). Commonly observed size or linearity effects are the dependence of the measured ratio of the analyte gas on the size of the major ion beam ('pressure effect'), the observed fractionation of hydrogen isotopes as a function of the amount of reduction reagent used, or the dependence of measured $\delta^{18}\text{O}$ -values on temperature during an equilibration experiment. The first and obvious choice is to eliminate the causes of the linearity problems by improving temperature stability or reducing the dwell time of the ions in the high-pressure region of the ion source. This is often successful for reducing the size of the effect. However, a small fraction will almost always remain and thus will have to be corrected for. The general correction strategy is similar to the one described above: A sufficient number of known samples are measured together with the analyte samples. In this case the amount of material is varied in order to detect the size of the required linearity correction.

Correcting cross contamination in dual inlet measurements (η -correction)

Following a switching action of the 'changeover valve' from one gas to the other a delay time ('idle time') is necessary to await complete exchange of the gases in the ion source. Depending on the gas this can be rather short (~ 4 s) or it may take up to several minutes for a complete replacement. Gases like H_2 , N_2 , SF_6 or N_2O have little surface activity and, hence, are pumped away fast whereas SO_2 and CO_2 can take considerably longer to exchange completely. The time depends on the material of the sputtered surfaces, on the sensitivity of the instrument, on the tightness of the ion source design and other parameters. The true δ -value can be calculated from the measured value according to:⁵¹

$$\delta_{\text{true}} = \delta_{\text{meas}} / (1 - 2\eta - \eta\delta_{\text{meas}}/1000) \quad (11)$$

with η describing the cross contamination affecting the background of the subsequent measurement. (Please note that Eqn. (4) in Ref. 51 is cast in absolute terms of δ , not in‰ 'units'). Meijer *et al.*⁵¹ also suggested a correction strategy which comprises (1) experimental determination of the cross contamination parameter η by introducing natural isotope abundance and enriched gas followed by (2) application of Eqn. (11) to the raw analytical results. For CO_2 , the numerical

value for η mostly ranges between 0.001 and 0.01. In some cases (when the ion source is rather tight, the sensitivity is high and the sputtered surface area is large) the η correction can be as large as 1‰ for $\delta^{13}\text{C}$ in CO_2 when the isotopic difference exceeds 40‰.

An alternative and recommended correction for cross contamination is scaling to precisely known (large) differences of reference materials as is routinely done in hydrogen and oxygen isotope ratio analysis by scaling to the SLAP/VSMOW difference. Besides hydrogen and oxygen isotope ratio determination this scale normalization has proven mandatory for sulfur (measured as SO_2) and might also turn out to be required for carbon, in particular when the measured isotopic differences are large and (or) the precision requirements are high.

Reporting of experimental data

Isotope ratio data often enter into a multidimensional puzzle from which further conclusions are drawn. This is the case in almost any carbonate or precipitation study; it is obvious also in CO_2 in air analyses. Sometimes isotope ratio data are valued by other researchers in a way that the original author had not anticipated. As a consequence, isotope ratio data reported in the scientific literature should be presented together with the necessary context that accompanies the pure number. Wherever the source of the scale that was used for referencing experimental values to an international standard may have arisen from, the procedure should and must be described.

For instance, most combustion analyses are calibrated with NBS-22 oil using a published $\delta^{13}\text{C}$ -value of, for example, -29.74 ‰ on the VPDB scale. This value, however, has been revised a number of times; the latest revision to -29.78 ‰ happened only recently.^h Thus, when published data are compared with other data reported vs. VPDB based on a different value or even based on a different material, adjustments can be made, provided the basis of the scale is clear. In addition to the final δ -values, precision ($\equiv 1\sigma$, standard deviation) as well as sample preparation and measurement techniques should accompany the data in order to judge the relative merits of published data.

Similarly, for reporting $\delta^2\text{H}$ -values of substances other than water, it is recommended that the author's measured $\delta^2\text{H}$ of NBS-22 oil, NBS-30 biotite, IAEA-CH-7 polyethylene foil, or other internationally distributed reference material be reported, as appropriate to the analytical method. Please note that normalization to the -428 ‰ difference of SLAP and VSMOW, as is mandatory for water analysis, requires the availability of new internationally distributed reference materials. The normalization procedure should be stated in the author's report.

For reporting $\delta^{18}\text{O}$ -values of substances other than water or carbonates (including the measurement of pure CO_2 gas), it is recommended that the author's measured $\delta^{18}\text{O}$ of NBS-28 quartz, NBS-30 biotite, NBS-127 barium sulfate, atmospheric oxygen or other internationally distributed reference material be reported, as appropriate to the analytical method. The $\delta^{18}\text{O}$ scale should be normalized such that the $\delta^{18}\text{O}$ of SLAP reference water is -55.5 ‰ VSMOW exactly, and so stated in the author's report.

CONCLUSIONS

We have described some of the recently developed techniques for referencing stable isotope ratio values in routine operation in our laboratory. The quality control for long-term accuracy is built into the daily cycles. Performance or quality charts help detect errors in high-precision referencing early and provide means for correction. It is common practice in many laboratories to treat sample and reference material in an identical manner. We propose to adopt this practice as the general principle guiding the positioning of measured isotope ratios on international scales.

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