Rapid Commun. Mass Spectrom. 2009; **23**: 999–1019
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.3958

Comprehensive inter-laboratory calibration of reference materials for $\delta^{18}O$ versus VSMOW using various on-line high-temperature conversion techniques

Willi A. Brand[1]*, Tyler B. Coplen[2],‡, Anita T. Aerts-Bijma[3], J. K. Böhlke[2]‡, Matthias Gehre[4], Heike Geilmann[1], Manfred Gröning[5], Henk G. Jansen[3], Harro A. J. Meijer[3], Stanley J. Mroczkowski[2]‡, Haiping Qi[2], Karin Soergel[6]†, Hilary Stuart-Williams[7], Stephan M. Weise[8] and Roland A. Werner[6]**

[1]MPI-BGC, Max-Planck-Institute for Biogeochemistry, Beutenberg Campus, P.O. Box 100164, 07701 Jena, Germany
[2]USGS, U.S. Geological Survey, 431 National Center, Reston, VA 20192, USA
[3]CIO, Centre for Isotope Research, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands
[4]UFZ, Helmholtz-Centre for Environmental Research, Laboratory for Stable Isotopes, Permoserstrasse 15, 04318 Leipzig, Germany
[5]IAEA, International Atomic Energy Agency, Isotope Hydrology Laboratory, P.O. Box 100, A-1400 Vienna, Austria
[6]ETH Zurich, Institute of Plant Sciences, Universitätsstrasse 2, CH-8092 Zurich, Switzerland
[7]ANU, The Australian National University, Research School of Biological Sciences, Canberra, ACT 0200 Australia
[8]UFZ, Helmholtz-Centre for Environmental Research, Laboratory for Stable Isotopes, Theodor-Lieser-Strasse 4, 06120 Halle, Germany

Received 5 December 2008; Revised 21 January 2009; Accepted 21 January 2009

Internationally distributed organic and inorganic oxygen isotopic reference materials have been calibrated by six laboratories carrying out more than 5300 measurements using a variety of high-temperature conversion techniques (HTC)[a] in an evaluation sponsored by the International Union of Pure and Applied Chemistry (IUPAC). To aid in the calibration of these reference materials, which span more than 125‰, an artificially enriched reference water ($\delta^{18}O$ of +78.91‰) and two barium sulfates (one depleted and one enriched in ^{18}O) were prepared and calibrated relative to VSMOW2[b] and SLAP reference waters. These materials were used to calibrate the other isotopic reference materials in this study, which yielded:

Reference material	$\delta^{18}O$ and estimated combined uncertainty*
IAEA-602 benzoic acid	+71.28 ± 0.36‰
USGS35 sodium nitrate	+56.81 ± 0.31‰
IAEA-NO-3 potassium nitrate	+25.32 ± 0.29‰
IAEA-601 benzoic acid	+23.14 ± 0.19‰
IAEA-SO-5 barium sulfate	+12.13 ± 0.33‰
NBS 127 barium sulfate	+8.59 ± 0.26‰
VSMOW2 water	0‰
IAEA-600 caffeine	−3.48 ± 0.53‰
IAEA-SO-6 barium sulfate	−11.35 ± 0.31‰
USGS34 potassium nitrate	−27.78 ± 0.37‰
SLAP water	−55.5‰

*According to IUPAC rules delta is defined as $\delta_{B,R} = r_B/r_R - 1$ (with r_B being the isotope amount ratio in sample B and r_R in reference R, without the extraneous factor 1000). In this paper we express the corresponding delta values either as $10^3\ \delta_{B,R}$ or we use the ‰ sign behind the number, depending on space availability and readability.

*Correspondence to: W. A. Brand, MPI-BGC, Max-Planck-Institute for Biogeochemistry, Beutenberg Campus, P.O. Box 100164, 07701 Jena, Germany.
E-mail: wbrand@bgc-jena.mpg.de
**Correspondence to: T. B. Coplen, USGS, U.S. Geological Survey, 431 National Center, Reston, VA 20192, USA.
E-mail: tbcoplen@usgs.gov
† Present address: Leibniz Institute for Zoo and Wildlife Research (IWZ), Alfred-Kowalke-Strasse 17, 10315 Berlin, Germany.
‡The contributions of T. B. Coplen, J. K. Böhlke and S. J. Mroczkowski to this article were prepared as part of their official duties as United States Federal Government employees.
Contract/grant sponsor: International Union of Pure and Applied Chemistry (IUPAC); contract/grant number: 2005-022-1-200.

[a]Other terms and acronyms in common use include HTP (High-Temperature Pyrolysis), HTCR (High-Temperature Carbon Reduction), and TC/EA (Thermal Conversion Elemental Analysis).
[b]In 2007, VSMOW2 replaced the almost exhausted VSMOW as the primary reference material and anchor to the VSMOW scale (for details see: http://www-naweb.iaea.org/NAALIHL/ and http://www-naweb.iaea.org/NAALIHL/docs/ref_mat/InfoSheet-VSMOW2-SLAP2.pdf). For ^{18}O, VSMOW2 and VSMOW are indistinguishable. The scale itself remains unaltered and keeps its name ('VSMOW').

The seemingly large estimated combined uncertainties arise from differences in instrumentation and methodology and difficulty in accounting for all measurement bias. They are composed of the 3-fold standard errors directly calculated from the measurements and provision for systematic errors discussed in this paper. A primary conclusion of this study is that nitrate samples analyzed for $\delta^{18}O$ should be analyzed with internationally distributed isotopic nitrates, and likewise for sulfates and organics. Authors reporting relative differences of oxygen-isotope ratios ($\delta^{18}O$) of nitrates, sulfates, or organic material should explicitly state in their reports the $\delta^{18}O$ values of two or more internationally distributed nitrates (USGS34, IAEA-NO-3, and USGS35), sulfates (IAEA-SO-5, IAEA-SO-6, and NBS 127), or organic material (IAEA-601 benzoic acid, IAEA-602 benzoic acid, and IAEA-600 caffeine), as appropriate to the material being analyzed, had these reference materials been analyzed with unknowns. This procedure ensures that readers will be able to normalize the $\delta^{18}O$ values at a later time should it become necessary.

The high-temperature reduction technique for analyzing $\delta^{18}O$ and $\delta^{2}H$ is not as widely applicable as the well-established combustion technique for carbon and nitrogen stable isotope determination. To obtain the most reliable stable isotope data, materials should be treated in an identical fashion; within the same sequence of analyses, samples should be compared with working reference materials that are as similar in nature and in isotopic composition as feasible.

In contrast to the success and wide use of high-temperature conversion (HTC) techniques for measuring $\delta^{18}O$ and $\delta^{2}H$ stable isotope properties in a large variety of bulk materials,[1–10] the inter-laboratory comparability of such data is still poor primarily because the reference materials used for defining the $\delta^{18}O$ scale are either water (VSMOW2)[c,3] or carbonates (NBS-19), neither of which is well suited for this type of technique.[2,6,11] HTC has been used for a number of applications, with the analysis of natural cellulose being the most frequent. An inter-laboratory comparison was conducted recently for cellulose by Boettger *et al.*,[12] who concluded that ''*There is, however, a real present need for a number of reliable, new, internationally certified (IAEA) stable isotope (C, H, O) cellulose standards. Therefore, an international standardization of reference cellulose standards used in this study would be helpful for the entire stable isotope tree ring research community.*'' The same study emphasized that ''*standard materials that are of a nature similar to those of the samples being measured (cellulose nitrate, cellulose)*'' and a ''*two-point calibration method*'' should be used. The latter statements refer to the widely adopted principle of identical treatment,[13] which minimizes systematic errors by subjecting sample unknowns and reference materials to exactly the same chemical and other manipulation steps, including the transfer pathway to the mass spectrometer ion source.

A number of criteria must be rigorously satisfied before an internationally distributed reference material can be released. These criteria include demonstration of:

(1) Sample homogeneity down to the smallest amounts usually reacted in analytical methods,
(2) Sample purity to ensure that when new or different preparation techniques are used the same results (within analytical uncertainty) for the isotope ratio(s) can be obtained,
(3) Immunity against alteration during storage or handling (e.g. negligible exchange with air components that might affect isotopic composition, non-hygroscopic, etc.), and
(4) Ease of handling; this includes, for instance, how well behaved the material is during standard reactions, how easily it can be weighed into the reaction containers, etc.

In addition, the chemical nature of a reference material should be as similar as possible to the samples under investigation.[13] This is a strict requirement for daily working reference materials, but is an advantage also for internationally distributed reference materials.

For cellulose, some, but not all criteria can be met. In particular, criterion (3) is violated as it is difficult to remove moisture completely and thus keep samples dry.[12,14–17] As a consequence, cellulose, although already available as a reference material for carbon isotopes (IAEA-CH-3), has been rejected as a reliable international reference material that is able to provide a dependable anchor to the VSMOW $\delta^{18}O$ scale[d]. Instead, materials that meet the above requirements more closely have been selected for this study. These include two benzoic acid samples of different isotopic composition that decarboxylate quantititatively at moderately high temperatures. They serve the purpose of a general scale anchor for organic materials. We have also included a caffeine sample containing nitrogen in a reduced form. This material poses a number of experimental difficulties that might serve as a critical test for the high-temperature reaction conditions. Inorganic materials already available as international standards like sulfate and nitrate samples have been studied as scale anchors. These types of materials need to be analyzed rather frequently.

[c]In this work, the emphasis is on bulk material, usually wrapped or packed in Ag or Sn foil. The situation is different for liquids when directly injected into the reactor through a septum using a suitable syringe.

[d]As a local working reference material, however, cellulose is recommended to be used when cellulose samples are analyzed.

DOI: 10.1002/rcm

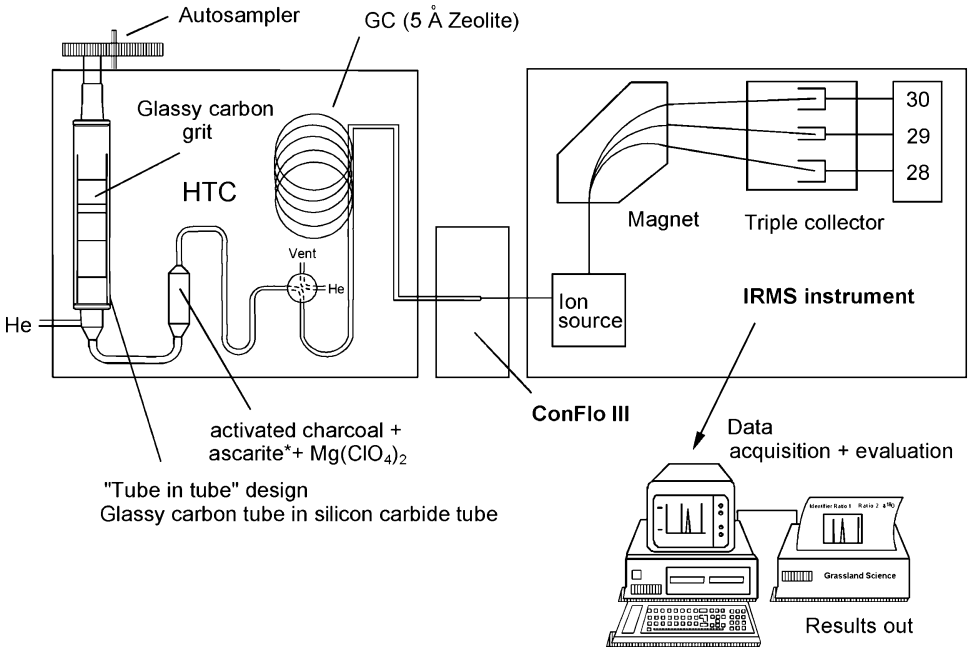

Figure 1. General layout of an HTC-EAMS system (High-Temperature Conversion Elemental Analysis-Mass Spectrometry; example from the ETH laboratory).
*Ascarite is used as a scrubber for a number of compounds, in particular for CO_2. It is comprised of NaOH on a large surface, usually pumice.

THE METHOD

A variety of experimental arrangements (for an example, see Fig. 1), data generation procedures, and evaluation schemes were used during this study in participating laboratories.

The common sample preparation method used by all partners was a high-temperature 'pyrolysis' (carbon reduction) technique[3,4,6–8,18] with the commonly used acronyms including TC/EA ('Thermal Conversion/Elemental Analysis'), HTC ('High-Temperature Conversion'), HTP ('High-Temperature Pyrolysis'), and HTCR ('High-Temperature Carbon Reduction').

The underlying principle is the Schuetze/Unterzaucher reaction[19–23] in which oxygen-bearing material (commonly organic material) is reacted with carbon at temperatures well in excess of 1000°C to produce H_2, CO, and a largely organic residue according to the reaction:

$$C_xH_{2y}O_z + nC \rightarrow zCO + yH_2 + (n + x - z)C \qquad (1)$$

Flushed by a continuous stream of high-purity He, the material under investigation is pyrolyzed in the presence of a large surplus of carbon at temperatures of typically 1400°C. Any CO_2 formed initially, for instance by a decarboxylation step, reacts further with excess carbon and forms CO. Following separation by gas chromatography (GC), the oxygen-isotopic composition ($\delta^{18}O$) is measured by feeding the exhaust of the TC/EA unit to an isotope ratio mass spectrometer (IRMS) via an open-split interface and measuring the ion currents at m/z 30 and 28 ($^{12}C^{18}O^+$ and $^{12}C^{16}O^+$).[10]

An illustration of reaction (1) for water ($x = 0$; $y = 1$; and $z = 1$) is:

$$H_2O + nC \rightarrow CO + H_2 + (n - 1)C \qquad (2)$$

This reaction is an example of a non-carbon-bearing material reacted at high temperature with carbon. It is not a pyrolysis reaction, which would require $\Delta n = 0$; i.e. carbon not participating in the reaction. An example for a pure pyrolysis reaction is given in equation (3), representing the thermal decomposition of glucose ($x = 6$; $y = 6$; $z = 6$):

$$C_6H_{12}O_6 + nC \rightarrow 6CO + 6H_2 + nC \qquad (3)$$

The surplus carbon in this case does not participate in the reaction stoichiometry, although it may act as a catalyst or as an exchange partner. However, it has been noted frequently that carbon–oxygen bonds already present in a molecule under investigation remain unaltered. This observation opens up the possibility of studying the $\delta^{13}C$ values of intramolecular carbonyl moieties.[8,24]

Reaction (1) needs modification when elements other than C, O, and H are involved. For nitrogen-bearing materials the reaction-product mix depends strongly on the N-oxidation state; nitrates generally are reduced quantitatively to CO and N_2, but the fate of nitrogen in compounds such as amino acids or caffeine, which might form $(CN)_2$ or other species containing CN^-, is more difficult to assess and depends on the parameters of the reaction[e].

Sulfur in sulfur-bearing materials probably ends up in a neutral to negative oxidation state, producing compounds such as CS_2 and similar carbon-sulfur compounds. Provided that the temperatures are in excess of 1350°C, sulfate oxygen in general reacts quantitatively to CO.

[e]The N_2 gained by carbon reduction of nitrates can be used for $\delta^{15}N$ measurement. The N_2 of ammonium compounds, in contrast, is useless for isotopic analysis (Kornexl *et al.*[25]).

DOI: 10.1002/rcm

Salts containing halide anions are usually assumed to be inert; they remain in the reaction debris as halide anions. However, at very high temperature with an excess of carbon, formation of halogen-carbon compounds, such as CCl_4, CF_4, and their homologues, cannot be ruled out.[7] Precautionary measures, such as installing passive chemical traps containing charcoal or ascarite (see Fig. 1) between the reactor and the gas chromatograph, are desirable and commonly used.

Many other oxygen-bearing inorganic materials do not release their oxygen quantitatively under standard reaction conditions. These materials include silicates and other rather stable oxides like carbonates, in which two of the three oxygen atoms are released as CO_2, but the third oxygen may react only partially – the remaining metal oxide may tend to poison the reactor over time due to oxygen-exchange reactions with CO from other samples. Higher temperatures and (or) chemical additives, such as AgCl or CF, have been proposed as a remedy, with varying success.[2,7]

The situation is also rendered more complicated by interactions involving accumulated packing material (mostly Ag or Sn from capsules), residues from the bulk samples, and materials comprising the reactor. The complete stoichiometric reaction often is not known. In addition, the nature of the extra carbon in the on-line reactor can vary from pure glassy (vitreous) carbon over amorphous graphitic carbon to nickelized carbon, depending on the history of the reactor and the substances to which it has been exposed.

When compared with oxidative preparation of organic material for elemental or isotopic analysis ($\delta^{13}C$ or $\delta^{15}N$), the variety of interfering chemical processes in the HTC process is much richer and – to a large extent – not well characterized.

EXPERIMENTAL

Three different types of experiments were undertaken (water equilibration, HTC liquid injection, and HTC packet dropping) with the latter as the primary technique. HTC packet dropping was performed in six of the seven participating laboratories. The IAEA Isotope Hydrology Laboratory (Vienna, Austria) contributed to the calibration of a water reference enriched in ^{18}O, using the HTC-liquid injection technique. In addition, calibration of reference water using the classical equilibration technique[26] was made at USGS (Reston, VA, USA) and at UFZ (Leipzig, Germany). CIO (Groningen, The Netherlands) submitted data for water calibration using all three techniques. The installed equipment and experimental conditions in the participating laboratories for the reaction of samples in Ag or Sn capsules are described below.

The laboratories are listed by their full name in the headings below and are referred to elsewhere in this paper by the respective acronym.

U.S. Geological Survey, Reston, Virginia, USA [USGS]

Two sets of data were produced from two independent instruments. For the first set of data ('USGS'), solid samples, wrapped in silver (Ag) containers or liquid samples contained in crimped Ag capsules fabricated for liquids, are dropped into a TC/EA reduction unit (ThermoFinnigan, Bremen, Germany) equipped with a Costech Zero-Blank 50-position autosampler (Costech, Valencia, CA, USA). The helium carrier gas (90 mL/min) is fed from the top, as originally supplied. The samples drop into the hot zone with the temperature controller set at 1325–1380°C. The hot zone holds glassy carbon grit and a graphite crucible. The outer tube is made from Al_2O_3, the inner sleeve from glassy or vitreous carbon (HTW Thierhaupten, Germany). The gas chromatograph for separating H_2 and CO (and N_2, when applicable) is equipped with a ¼-inch o.d. 5-Å zeolite column. During analysis the gas chromatograph is maintained at a constant temperature of 30°C. The effluent from the gas chromatograph is coupled to a Delta$^+$XP IRMS via a ConFlo II interface (both ThermoFinnigan).

The original equipment has been enhanced by inserting an automated diverter valve (Valco Instruments Co. Inc., Houston, TX, USA). By actuating the valve nitrogen peaks can be diverted and thus prevented from entering the ion source, thereby alleviating problems with isobaric m/z 30 interference, as discussed below. For analyzing sulfate samples, finely powdered graphite (generated from used graphite crucibles) is intimately mixed with the sample material to enhance the reaction rate and improve the CO yield. Residual material is removed from the graphite crucible after each batch of $\leq$50 samples.

The second set of data ('USGS-II') was produced on a completely new TC/EA reduction unit (ThermoFinnigan) equipped with a Costech Zero-Blank 50-position autosampler. The helium carrier gas flow is 78 mL/min. The samples drop into the hot zone with the temperature controller set at 1330–1380°C. The GC temperature is set to 30°C. The effluent from the gas chromatograph is coupled to a Delta$^+$XP IRMS via a new ConFlo IV interface (both ThermoFinnigan). To divert nitrogen peaks produced from N-bearing materials, an automated diverter valve is installed as described above.

Equilibration of water samples with CO_2 and subsequent automated analysis are carried out on a classical equilibration line.[26] Aliquots of 2 mL of water are equilibrated with CO_2 at 25°C for 7 h prior to isotopic analysis with a double-focusing, double-collecting IRMS.[27] All results are normalized to VSMOW-SLAP,[28,29] assigning consensus values of 0 and −55.5‰ to VSMOW and SLAP reference waters, respectively.

Max-Planck-Institute for Biogeochemistry, Jena, Germany [MPI-BGC]

The Ag foils and capsules with sample material are positioned in a Costech Zero-Blank 50-position autosampler, mounted on top of a HTO high-temperature furnace (Hekatech, Wegberg, Germany), and flushed with He at a rate of ~80 mL/min. After loading samples, closing the autosampler lid, and before introducing the main He flow, the carousel is flushed for at least 2 h using a constant He flow of 50 mL/min. The m/z 28 ion current (N_2^+) is monitored to verify that complete flushing of atmospheric gases has taken place. To maintain a constant carrier gas flow through the core of the reactor (i.e. independent of increasing reactor resistance over time), the HTO unit has been fitted with a reversed He carrier gas feed from the bottom as described by Gehre *et al.*[3] The outer tube is made from SiC and the inner

DOI: 10.1002/rcm

tube from glassy carbon. SiC has the advantage of having a very small linear thermal expansion coefficient ($\sim 4 \times 10^{-6}$/K), which is similar to that of glassy carbon. The material can be operated in air at temperatures in excess of 1600°C. Its major advantage is that there is no build-up of background CO from contact with oxygen-bearing surfaces. This tube was introduced originally for use as a high-capacity reactor to eliminate the need for an internal tube.[30] However, without the inner glassy carbon tube, the linear flux in the reactor was too low for the required GC separation. In addition, a gradual, but significant, increase in the between-sample memory was observed, which probably results from the formation of Si–O bonds at the inner surface of the SiC tube. The inner glassy carbon tube is filled with glassy carbon chips to the level of the hot zone. These are held in place by an Ag-wool plug at the bottom of the tube. No graphite crucible is used. Instead, the reactor is vacuumed after each sample sequence to remove Ag and other residue, thus keeping the reaction conditions comparable for different batches of references and sample unknowns. Even with this protocol, the reactor deteriorates over time as can be inferred from the quality-assurance-reproducibility results. Therefore, the reactor is dismantled after every four sequences of samples (200 reactions in total) and the glassy carbon chips and Ag-wool plug are renewed.

The temperature profile of the reactor has been measured under conditions close to routine operation by using a septum at the top for insertion of a long thermocouple (type K) to various depths. Figure 2 displays the observed profile. The type K thermocouple did not allow measurement at full operating temperature; hence, the profile was measured at a set and regulated temperature of 1000°C. During normal operation the reactor is maintained at a temperature of 1430°C. There was no significant difference observable between the set value and the hot-zone temperature measured using the internal thermocouple. The length of the reaction zone with the temperature within $\sim \pm 25$°C of the set temperature is about 10 cm.

At 10 and 45 cm distance from the top, passively air-cooled heat sinks ensure that the top and bottom connectors (including their Viton seals) are maintained at a temperature of no more than 125°C, which is considered safe for this material and compatible with the analytical requirements.

Downstream from the reactor tube a chemical trap filled with ascarite is installed as a guard against fine graphitic dust particles and as a trap for acidic chemicals that might interfere with the GC separation or mass spectrometric measurement. The ¼-inch o.d. GC column is filled with 5-Å zeolite. It is maintained at 75°C during normal operation. About once per month the column is heated to 300°C over the weekend for reactivation. The column effluent is introduced into a ConFlo III interface[31] for transmission of analyte gas to a Delta$^+$XL IRMS (both ThermoFinnigan). The mass spectrometer analyzes the isotopic triplets *m/z* 28-29-30 and *m/z* 44-45-46 using a universal triple Faraday cup collector. A typical sequence of analyses comprises 50 specimens including working reference materials and quality assurance (QA) standards that are always interspersed in an identical fashion. This is done so that the raw data can be transferred to a master post-run evaluation spreadsheet and the same types of corrections are applied automatically.[3,13] The latter include corrections for memory and drift and for the isotopic relation between laboratory reference gas and working reference material.

Eidgenössische Technische Hochschule Zurich, Switzerland [ETH]

The equipment and procedures at ETH (Institut für Pflanzenwissenschaften) closely parallel those described for MPI-BGC. The minor differences are that the HTO (Hekatech) reaction unit can be equipped with a 50- or 100-position Zero-Blank autosampler carousel (Costech,

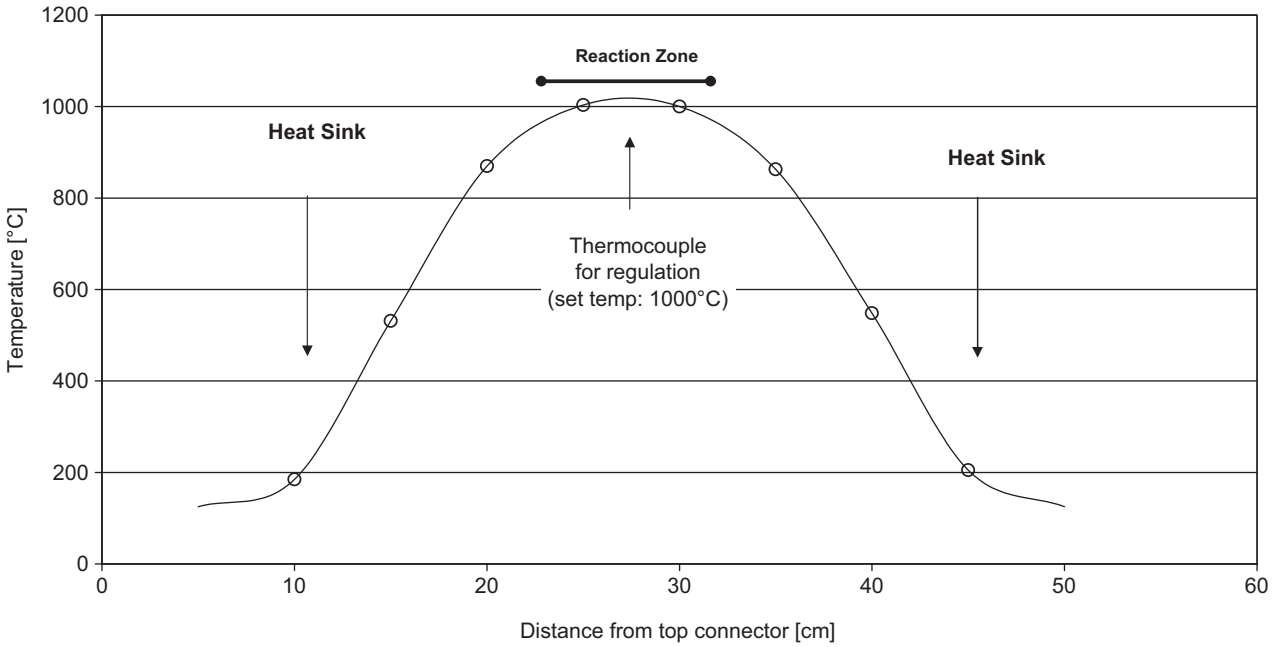

Figure 2. Temperature profile inside the MPI-BGC HTO unit (conditions close to normal operation except for the set temperature, which normally is greater than 1400°C).

DOI: 10.1002/rcm

Valencia, California, USA). The reaction tube also has a reversed He carrier gas feed from the bottom[3] and an outer SiC tube. The carrier gas flow is about 100 mL/min, and the reactor temperature is 1450°C during routine measurements. The mass spectrometer used is a Delta$^+$XP IRMS (Thermo-Finnigan). A typical sequence has 50 sample positions filled. After analysis of a complete sequence, residual reaction products are vacuumed from the reactor. The autosampler is initially evacuated before flushing with He (50 mL/min) for 0.5 h and then connected to the main carrier flow. The first half of the chemical trap is filled with charcoal and the last half is filled with ascarite. Evaluation of mass spectrometric data is also performed on a predefined spreadsheet. Final $\delta^{18}O$ values, expressed relative to VSMOW, are extracted from the raw data set using reference materials interspersed among unknowns to satisfy the requirements of identical treatment.[13]

Centre for Isotope Research, Groningen, The Netherlands [CIO]

The systems in operation at MPI-BGC and ETH have been used as a role model for the new HTC equipment installed at CIO, which includes a standard HTO (Hekatech) reaction unit equipped with an open carousel 40-position autosampler (Eurocap-DP; Hekatech), including a reversed He carrier gas feed from the bottom.[3] The outer tube is a standard Al_2O_3 alumina tube and the inner tube is the normal Hekatech glassy carbon reactor. The reactor is filled with glassy carbon to the hot zone. Samples are dropped onto a bed of nickelized carbon. Connection to the Isoprime IRMS (GVI Instruments, Manchester, UK) is made using the GVI gas box. The flow through the HTO unit is maintained at 90 mL/min. The reactor is operated at a nominal temperature of 1300°C, but measurements with an independent thermocouple indicated that the actual temperature in the hot zone is about 100°C higher. Samples are placed into Ag containers. A typical sequence of 50 samples and reference materials takes about 5 h to analyze.

The direct water injections were carried out using an AS 300 autosampler (Eurovector, Milan, Italy) with injected amounts of 0.15 μL per sample.

Data analysis, including quality checks, calibration and a sophisticated, three-pool memory correction,[32,33] is routinely applied using a sequence of spreadsheet-based programs. Equilibration of water with CO_2 is performed in a home-built automatic preparation system.[34] The capacity is 80 samples, arranged in five subsets of 16 reaction vessels each. Aliquots of 0.6 mL water and 0.2 mmol CO_2 are used. The water-CO_2 equilibrium is established statically during at least 24 h, at 25 ± 0.02°C, so that the CO_2 after establishment of the equilibrium should have a composition precisely traceable to the VSMOW-CO_2 scale. For analysis of the CO_2, the system is coupled to a dual-inlet IRMS (VG SIRA II series; VG Isotopes, Winsford, UK).

Helmholtz Centre for Environmental Research, Leipzig and Halle, Germany [UFZ]

The high-temperature reaction system at the Leipzig facilities has been described in detail earlier.[6] Briefly, the reactor is an early (pre-production) model of the Hekatech system ('HTP' for High-Temperature Pyrolysis). Compared to the commercial system, it has a longer furnace (95 cm) and a larger distance between the injector/autosampler and the start of the heated zone. The system is equipped with a Euro Cap 40 autosampler (AS; Hekatech) with a straight He carrier gas feed (60 mL/min) from the top, allowing 80 analyses to be made within one sequence. The blank-effect of the AS is reduced with an additional He purge of ~60 mL/min. The reactor is made of an outer Al_2O_3 tube and an inner glassy carbon tube, filled with glassy carbon chips up to the hot zone (1400°C). The inner tube is mounted with special precaution to prevent contact between the ceramics and the glassy carbon. This helps to minimize the CO background. The samples (in Ag containers or wrapped in Ag foil) are dropped onto a small bed of nickel/carbon powder (ratio 1:9, ~20 mg; the carbon was produced as black carbon excess during an incomplete combustion process). The ~200 mesh powder is highly reactive. Downstream from the reactor, the sample gases are swept through an ascarite chemical filter, followed by the GC column (60 cm, ¼-inch, 5-Å zeolite) and maintained at a temperature of 90°C. Connection to the Finnigan 253 IRMS has been made via a ConFlo III interface,[31] both from ThermoFinnigan.

At the UFZ branch in Halle equilibration of water samples with CO_2 and subsequent automated analysis is carried out using a special equilibration interface developed and built by the IAEA Isotope Hydrology Laboratory[f]. Water aliquots of 4 mL are equilibrated with CO_2 for 4 h at 18°C. During this time the samples are stirred and the water bath temperature is kept within ±0.01 C. For $\delta^{18}O$ analysis a dual-inlet mass spectrometer (Delta S; Finnigan MAT, Bremen, Germany) is used and all results are normalized to the VSMOW-SLAP scale.[28,29]

Australian National University, Canberra, Australia [ANU]

At ANU (Research School of Biological Sciences) a laboratory-made reactor[35] is used for high-temperature reaction work. The system is an upgrade and builds on an earlier low-temperature version by Farquhar *et al.*[9] The reactor is equipped with an open-carousel AS200 autosampler (CE, Milan, Italy) with the He carrier gas feed down through the reactor. The carrier flow is regulated using mass-flow controllers and remains constant over a wide range of back pressures. The columns, with a molybdenum foil liner to prevent contact of the product gas with oxygen-bearing surfaces, do not require a glassy carbon liner and have a large cross-section. As a consequence of this and the mass-flow control, and because the columns deteriorate when cooled or exposed to air, they are not opened until their performance deteriorates, usually after 300 or more samples. The system can be operated at 1200–1400°C. The hot zone has a length of about 18 cm (for further details, see Stuart-Williams *et al.*[35]). The samples, wrapped in Ag foil, are dropped onto a bed of

[f]A description is available: HDOeq48/24 User's Manual version 1.2 (see, for instance, http://www-naweb.iaea.org/NAALIHL/equipment.shtml).

DOI: 10.1002/rcm

graphitic carbon. The system is connected via a reference injector box to an Isochrom IRMS (Fisons Instruments, Middlewich, UK) or to an Isoprime IRMS (GVI Instruments, Manchester, UK).

International Atomic Energy Agency, Vienna, Austria [IAEA]

The system at IAEA (Isotope Hydrology Laboratory) comprises a Heka-HTO high-temperature conversion unit, coupled on-line to an Isoprime IRMS (GVI Instruments) via a GVI diluter box. Water samples (2 μL) are injected by a GC-PAL autosampler (CTC Analytics, Zwingen, Switzerland) via a septum connector mounted directly on the head of the reactor. The latter is made of an Al_2O_3 outer tube and an inner glassy carbon tube. The system is operated at a temperature of 1450°C using a He carrier gas flow rate of 100 mL/min. The system is not equipped with an auto-sampler for dropping sample packets into the hot zone automatically. The IAEA, therefore, has contributed analytically to the on-line calibration of the water reference enriched in ^{18}O in this study.

Materials

For on-line $\delta^{18}O$ analysis and calibration using the HTC preparation techniques, the best-suited materials are organic materials composed of only C, H, and O (materials that do not contain N, S, or Cl, for example). In order to assign $\delta^{18}O$ values on the VSMOW scale, these materials either need to be converted into water quantitatively and analyzed using one of the more traditional methods, such as equilibration with CO_2, or the water samples need to be subjected to the same HTC preparation and analyzed on-line by measuring the ion currents at m/z 30 and 28. The latter technique has been used in this study with particular emphasis on using water samples for calibration to the VSMOW-SLAP scale. The reference materials analyzed during this study included:

- VSMOW2, SLAP, and W-89262 (water enriched in ^{17}O and ^{18}O, prepared by USGS for this study)
- IAEA-601 and IAEA-602 benzoic acids
- IAEA-CH-3 cellulose ($\delta^{18}O \sim +32.6‰$; H_2O-X-rejected[g])
- IAEA-CH-6 sucrose (H_2O-X-rejected[i])
- NBS 127, IAEA-SO-5, and IAEA-SO-6 $BaSO_4$ + S-4316 (depleted in ^{18}O) and S-4317 (enriched in ^{18}O) $BaSO_4$, prepared by USGS for this study
- IAEA-600 caffeine
- USGS40 and USGS41 L-glutamic acids (H_2O-X-rejected[g])
- IAEA-NO-3, USGS32, USGS34, and USGS35 nitrates

In addition, 3,4-dimethoxybenzoic acid, 3,5-dimethoxybenzoic acid, and 3,4,5-trimethoxybenzoic acid were studied as possible reference materials by some laboratories. The substituted benzoic acids were found to exchange only marginally with water. They were mainly employed as laboratory standards during this study.

[g]'H_2O-X'; Exclusion test for the material under investigation with water: 20 mL of water enriched in ^{18}O (+250‰) or from Antarctica (−50‰) were added to the material under investigation. Heating to ~ 80°C for up to 48 h promoted measurable change of $\delta^{18}O$ for the proposed reference material.

The USGS water-exchange test[g] was applied to all materials under consideration. Aliquots of the materials before and after the water-exchange tests were analyzed. Materials containing exchangeable oxygen were not selected for calibration in this project. Benzoic acid has two oxygen atoms that might be exchangeable. However, carboxylic acids are normally very resistant to oxygen exchange at neutral pH. In the case of benzoic acid, a pH value of less than 1 and elevated temperatures are needed to promote exchange.[36] In contrast, amino acids are easily exchangeable.[37] According to Sternberg *et al.*,[14,16,38] cellulose and sucrose can be autoclaved without exchanging oxygen isotopes. However, in this case the drying step is very critical for these hygroscopic compounds. Based on the water-exchange/drying test results, cellulose, sucrose and the glutamic acids exchanged oxygen and they were thus removed from the list of candidate materials for this study, irrespective of the possible causes of the observed shifts in $\delta^{18}O$.

The USGS laboratory also prepared ^{18}O-depleted $BaSO_4$ by heating one part sulfuric acid with water depleted in ^{18}O (δ^{18} of ~ −330‰) at 250°C, and then precipitating $BaSO_4$ by addition of $BaCl_2$. After an additional drying step, this resulted in the 'light' S-4316 $BaSO_4$ material. Similarly, water enriched in ^{18}O and ^{17}O was used to prepare 'heavy' S-4317 $BaSO_4$ material.

A 'heavy' water of approximately +80‰ was considered to be of vital importance for this study because most organic oxygen samples and many inorganic oxygen-bearing materials have $\delta^{18}O$ values substantially in excess of 0‰ on the VSMOW scale[7,39] (in contrast to meteoric water, which typically is negative). Because available calibrated water reference materials are all less than 0‰ and an extrapolation to $\delta^{18}O$ values greater than 30‰ was necessary, it was decided to prepare and calibrate a 'heavy' water sample with classical CO_2-H_2O equilibration. A heavy water sample (W-89262) was prepared by the USGS by mixing local deionized water with commercial ^{18}O-enriched water to arrive at about + 80‰ relative to VSMOW. As the ^{17}O-^{18}O relation in this water is far from natural, a small correction for the m/z 45 ion beam intensity was made for the determination of the $\delta^{18}O$ value. This reference water was disseminated to all participating laboratories for isotopic analysis.

The principle of identical treatment usually requires chemically identical or at least chemically similar materials. With the paucity of reference compounds suitable for HTC analysis, this requirement cannot be easily met, which was one of the motivations for this work. In the following, we report on experiences and difficulties during handling and HTC analyses of the various materials.

Waters (VSMOW2, SLAP, W-89262 (USGS))

In this study, reference water samples were loaded into Ag capsules and interspersed with solid reference materials for calibration by HTC. In this manner, one can transfer the international oxygen-isotope scale from the reference waters VSMOW and SLAP to other, more user-friendly, solid compounds. The masses of water and solid reference materials were selected so that their CO peaks had the same

DOI: 10.1002/rcm

magnitude. This transfer from the water scale to solid reference materials was a major challenge during this study.

Water samples and calibration

Water samples are particularly difficult to analyze with the HTC dropping technique. The liquid injection technique in comparison is easier to perform, and is an established method.[3] For this study, fresh ampoules of VSMOW2 (NIST-RM 8535a) and SLAP (RM 8537) were provided to each laboratory by the IAEA; W-89262 reference water enriched in ^{18}O was produced and distributed by the USGS.

A number of systematic effects during the handling and preparation of water samples need to be taken into consideration and accounted for in the results including:

(1) Evaporation during preparation: the capacity of the conversion reactor is limited and the sample mass cannot be made substantially larger than about 1 mg per reaction – some laboratories prefer substantially smaller CO amounts, necessitating masses of 0.25 mg. The transfer from the larger reference reservoir to the sample capsules is made using a syringe or micro-pipette. In order to avoid leaks during the sealing, the water sample should be placed at the bottom of the capsule. The syringe should not touch the walls of the Ag cup. Condensation of ambient water vapor during handling can be minimized by operating at ambient temperatures. Quick sealing minimizes exchange with humid laboratory air and evaporation, and subsequent alteration of isotopic composition. An enrichment in ^{18}O of roughly +0.05 to 0.2‰ (mainly owing to evaporation) has been estimated to affect measurements of water references (see below). Because no scale-compression of the $\delta^{18}O$ values of water was observed, the corresponding correction was made by shifting the results from the different laboratories using a lab-specific offset (depending on the size of water used for a routine sample). This uncertainty of the correction contributes to the remaining overall error budget of the data set.

(2) Evaporation during storage: the sealing of Ag capsules was determined to be a critical component of sample preparation. Prior baking of the Ag capsules in a muffle furnace at 500°C for 6 h is of great help to reduce blanks. Reliable and quick sealing of the capsules using a pair of pliers was also important for sample preparation. The pliers were made from a common pair of cranked wire cutters with grip reinforcement to apply a greater power for crimping. The capsules were weighed immediately after filling and again 24 h later to check for evaporative loss. Only those samples that exhibited no measurable weight loss were used for analysis. Even with these precautions, occasional (positive) $\delta^{18}O$ outliers were observed in some laboratories (10–20% of the water samples, depending on the laboratory). These outliers were excluded from the calibration program.

(3) Air inclusions in the crimped Ag capsules, both from the gas phase and from dissolved air in the sample water: a possible correction can be made by using the size of the preceding N_2 peak to quantify the amount of O_2 in the individual capsule, assuming a ratio of ~4:1 for N_2 to O_2. The corresponding amount of CO can then be calculated from the size of the CO^+ peak (m/z 28) using mass balance, assuming a $\delta^{18}O$ value of +23.8‰ for the atmospheric oxygen component. The size of the correction depends upon the isotopic composition of the water and the volume of the capsule; it is ~ −0.1‰ for SLAP, −0.03‰ for VSMOW, and 0‰ for a water with $\delta^{18}O$ of +23.8‰[h].[40]

Table 1. Oxygen isotopic composition of ^{18}O-enriched water W-89262

Analysis method	$\delta^{18}O$	n
Equilibration	+78.91 ± 0.08‰	5
Direct injection (HTC)	+78.87 ± 0.39‰	24
Sealed in Ag capsules (HTC)	+78.90 ± 0.38‰	23
Average	+78.89 ± 0.02‰	

The reference water W-89262 mentioned above was calibrated by different laboratories using different preparation techniques, including equilibration with CO_2, direct injection into a HTC unit with a syringe, and dropping packets of crimped Ag capsules into an HTC reactor. Consistent results were obtained from the three independent techniques (Table 1). As recommended by IUPAC, all values of solids and liquids have been scaled to the VSMOW-SLAP difference of −55.5‰.[29] Assuming that the standard reference technique (equilibration) provides accurate $\delta^{18}O$ values, the average offset of the HTC dropping technique is −0.01‰; for the HTC direct liquid injection, we observe −0.04‰. From these small differences, it can be concluded that $\delta^{18}O$ of pure water can be analyzed over a wide range (from +80 to −55.5‰) with a high degree of reliability using either technique. However, when using this information for calibrating other, non-aqueous materials, further sources of error might arise and need to be accounted for. These will be discussed individually for the different types of materials.

Benzoic acids (IAEA 601 and IAEA 602)

Initial positive experiences with benzoic acid when analyzing $\delta^{18}O$ using HTC techniques led to the introduction of this compound as an international reference material. During the tests for exchangeable oxygen at USGS (see above[i]), benzoic acid did not exhibit any significant oxygen exchange. Moreover, the compound is stable during weighing; significant hygroscopicity has not been detected.

The original material (purity grade '*pro analysi*', 3 kg) was acquired from Merck (Darmstadt, Germany). One of the batches was subjected to exchange with ^{18}O-enriched water at 80°C and pH 1 over a period of 3 weeks following a recipe by Wedeking and Hayes[36] (see also Refs. 41–43). The resulting two isotopically different materials were delivered to the IAEA for aliquoting and bottling and were labeled IAEA-601 (NIST-RM 8575) and IAEA-602 (RM 8576). In a

[h]The contribution of the small amount of dissolved O_2 in connection with the negligible isotopic difference of ~0.7‰ between gaseous and dissolved O_2 has been omitted in this first-order assessment.

DOI: 10.1002/rcm

pilot study, IAEA-601 and IAEA-602 were assigned preliminary values of +23.2‰ and +71.4‰ relative to VSMOW.[44] Two entirely different techniques were used for the preliminary assignment; one was the HTC technique of this study (made at MPI-BGC IsoLab) with direct analytical reference made to VSMOW. The other technique, employed by Schimmelmann at Indiana University (Bloomington, IN, USA), used low-temperature (550°C) decarboxylation[45] and manual dual-inlet analysis with analytical reference made to VPDB-CO_2. The $\delta^{13}C$ values[i] have been determined and agreed upon in a previous calibration exercise ($\delta^{13}C_{VPDB} = -28.81$ and −28.85‰, respectively), which also introduced a second anchor for the VPDB $\delta^{13}C$ scale.[46,47]

During material handling and comparison of results from different analytical runs over long periods of time, IAEA-601 appears to behave well without major problems (except for an increase in memory over time, see discussion below). The performance of IAEA-602 was somewhat different. The raw $\delta^{18}O$ data often showed larger deviations, which could be either due to sample inhomogeneity (following the ^{18}O exchange reaction, the material had to be crystallized, dried, and finely ground again) or to the fact that the $\delta^{18}O$ value is far away from that of the reference gas and the measurements may need adjustment for scaling and (or) background effects. Isotopic homogeneity was tested again by randomly selecting a number of aliquots and measuring those within the same sequence against a common reference. In order to exclude errors from recrystallization within a given sample bottle, all aliquots were thoroughly ground in a mortar before sub-sampling and weighing. We recommend to apply this treatment routinely when analyzing IAEA-602. With an average precision of 0.12‰ and a precision of the mean of 0.13‰, the data are comparable within and across the batches, suggesting that IAEA-602 is isotopically homogenous, at least at the sample amounts of ~5 mg used for the test (Table 2).

The USGS laboratory observed a slow increase in the $\delta^{18}O$ of benzoic acid samples over time with the HTC method. As the reactor ages, the peak width seems to increase and the results for subsequently processed samples seem to be affected. The reasons for this observation are not entirely clear. The USGS system might be more sensitive to the presence of fine carbonaceous residue than others. In this case the graphitic carbon buildup should occur with many organic materials that add new carbon to the reaction zone. It may also be related to the presence of the graphite crucible, which the other laboratories in this study did not use.

Table 2. Oxygen isotopic homogeneity of IAEA-602 benzoic acid. (All analyses were made at MPI-BGC using a single sample sequence with four capsules per aliquot of IAEA-602. The numbers in the sample description refer to the flask label.)

Sample description	$\delta^{18}O$	n
IAEA-602 No. 5	+71.18 ± 0.19‰	4
IAEA-602 No. 7	+70.85 ± 0.15‰	4
IAEA-602 No. 9	+70.86 ± 0.02‰	3
IAEA-602 No. 66	+71.06 ± 0.10‰	4
IAEA-602 (BGC in-house ref.)	+70.96 ± 0.15‰	4
Average (before calibration)	+70.98 ± 0.14‰	

Barium sulfates (NBS 127, IAEA-SO-5, IAEA-SO-6, + two local $BaSO_4$ samples)

Sulfate samples have been included in this study because they are analyzed commonly in a number of areas, including research on paleo-diets, paleo-climatic studies, aerosol investigations, and others. Results reported thus far for $\delta^{18}O$ measurements on sulfate samples suggest that analytical improvements and standardization to common reference materials are still a requirement for future progress. The materials in this test included NBS 127 (NIST-RM 8557), IAEA-SO-5 (RM 8533) and IAEA-SO-6 (RM 8534), and two sulfate samples with greatly differing $\delta^{18}O$ values prepared at the USGS specifically for this study. All materials are barium sulfates. Of particular importance is NBS 127, which is used commonly as a prime anchor for sulfates to the international $\delta^{34}S$ scale (VCDT) and to the VSMOW scale for $\delta^{18}O$.

Recent studies of NBS 127 published by Kornexl *et al.*,[5] Böhlke *et al.*,[48] Halas *et al.*[49] and Boschetti *et al.*[50] yield a mean $\delta^{18}O$ value of +8.6‰ with a variation of ±0.1‰. IAEA-SO-5 and IAEA-SO-6 have been included in the same studies, with a similar agreement of ±0.1‰ for IAEA-SO-5, but the reproducibility of IAEA-SO-6 is only ±0.4‰. In contrast, Bao and Thiemens[51] report a value of +9.4‰ for $\delta^{18}O$ of NBS 127 using a fluorination technique with O_2 as the measured gas.

When analyzing barium sulfates using the HTC technique, close contact with carbon is necessary for quantitative conversion (as is the case for most inorganic compounds). As a common observation, the CO peak originating from a sulfate sample is considerably broader than that from an organic material with a preformed CO bond. As an example, the peak width of an IAEA-SO-5 sample was 108 s, while the width of a dimethoxybenzoic acid sample was 71 s within the same sequence for comparable signal heights (Fig. 3, experimental setup at MPI-BGC). The CO peak from sulfate started later and it suffered from pronounced tailing, extending over more than 300 s, whereas the CO peak from organic samples returned to baseline values after about 120 s (visible when inspecting the signal close to background). Because GC also separates different isotopologues to a small extent (for organic materials, the heavier component in most cases elutes slightly ahead of the lighter one;[52–54] for CO this seems to be reversed), a systematic isotopic shift can arise from the selected tail-cutoff slope when comparing sulfates with organic materials. The effect can be diminished by reducing the GC resolution, i.e. by deliberately increasing peak width and degrading peak separation.

Another source of isotopic alteration during measurement can arise from non-linearity of the observed ion-current ratios. The height of a peak represents only the maximum intensity, but the delta value is obtained from integrating the whole peak. Each time slice of the peak has a different amplitude and, hence, ratio linearity. As an example, if the

[i] In IAEA-602 the ^{17}O-^{18}O relation is not that of naturally occurring terrestrial materials. Therefore, IAEA-602 should not be used for calibration of $^{13}C/^{12}C$ unless exchange of benzoic-acid-derived carbon oxides is achieved with excess oxygen via oxidative combustion (using, e.g. a CHN analyzer).

DOI: 10.1002/rcm

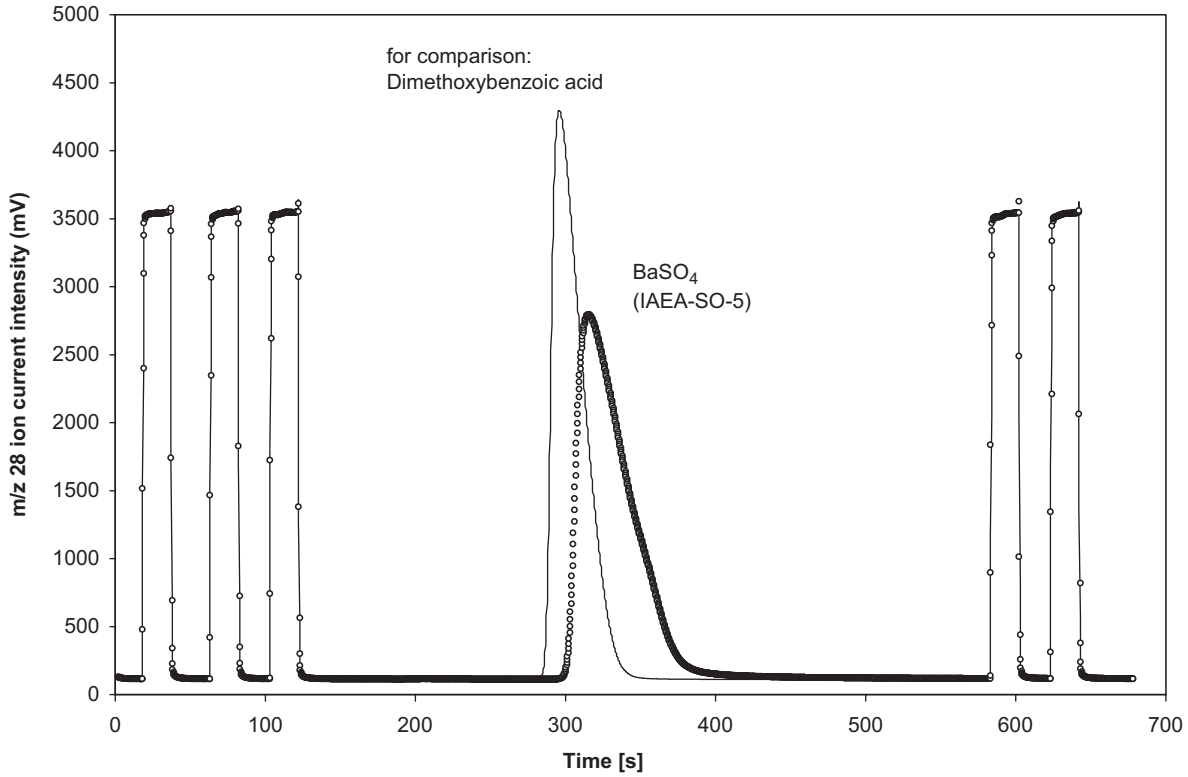

Figure 3. CO peak broadening for $BaSO_4$ samples. The panel shows the superimposed m/z 28 traces of a $BaSO_4$ and a dimethoxybenzoic acid sample, analyzed on the MPI-BGC system within the same sequence (samples have about the same amount of oxygen). Reference injections were made automatically at the same time intervals.

m/z 30/m/z 28 ratio varies by 0.1‰ per nano-ampere of ion current (a typical value), the resulting peak-shape details can play a major role when comparison is made between two materials reacting as differently as those depicted in Fig. 3.

Different experimental setups will show the described effects to varying degrees. In particular, systems with the carrier gas flow[3] reversed such as the instruments at MPI-BGC or ETH provide superior GC peak separation, enhancing the differences in peak shape. Hence, they might suffer more from these effects. The USGS laboratory mixed $BaSO_4$ samples intimately with glassy carbon from ground crucibles to enhance contact of the sample to promote reaction and minimize peak broadening. The UFZ laboratory used an admixture of nickel/reactive carbon powder (10:90, ~200 mesh) to reduce peak broadening in sulfates while ANU added ground graphite. The ETH laboratory also tested glassy carbon powder admixture to $BaSO_4$ samples without a noticeable effect on the analytical results. MPI-BGC tested admixtures of polyethylene (2:1 ratio of carbon per analyte-oxygen) with little apparent influence on peak shape; however, some (erroneous) alterations of isotopic results in the range of ±1‰ were observed.

The effects discussed above critically depend on the experimental details and the parameters used; they certainly vary from instrument to instrument, even for the same instrument type. The temperature in the hot zone probably always differs from the indicated temperature. The dwell time of the reactant and product gases depends on the linear flux and, thus, on the helium flow and the reactor dimensions. Moreover, the influence of reaction parameter details is likely to vary from compound to compound. As an example, it can happen that perfect results are observed for one type of material (e.g. for sulfates) while another class of compounds can exhibit further variations (e.g. nitrates, see below).

These effects are prominent examples of the necessity to use well-calibrated working reference materials of closely comparable chemical nature and similar amounts for accurate isotope-ratio measurements.[13]

Caffeine (IAEA-600)

The original material (~30 kg) for IAEA-600 was acquired in 2001 from a vendor in Bremen, where the first commercially successful decaffeination process was invented by Ludwig Roselius in 1903. Decaffeinated coffee has been produced there since that time. Homogeneity tests ($\delta^{13}C$ and $\delta^{15}N$) at MPI-BGC and at USGS revealed no detectable variations with sample sizes of about 500 μg. The material was ground and bottled at the National Institute of Standards and Technology (NIST, Gaithersburg, MD, USA) and is distributed by NIST as RM 8567 and by IAEA. A full calibration has been completed for $\delta^{13}C$ measurements (−27.77‰ relative to VPDB).[46,47] The $\delta^{15}N$ value of IAEA-600 has not yet been fixed by an inter-laboratory effort. Its $\delta^{15}N$ value is close to $+0.91 \pm 0.03$‰ relative to atmospheric N_2 (MPI-BGC, based on 30 separate analyses made in 2001 using $\delta^{15}N = +0.43$‰ for IAEA-N1 as scale anchor).

The $\delta^{18}O$ analysis of IAEA-600 in the participating laboratories exhibited rather mixed results. In one case,

DOI: 10.1002/rcm

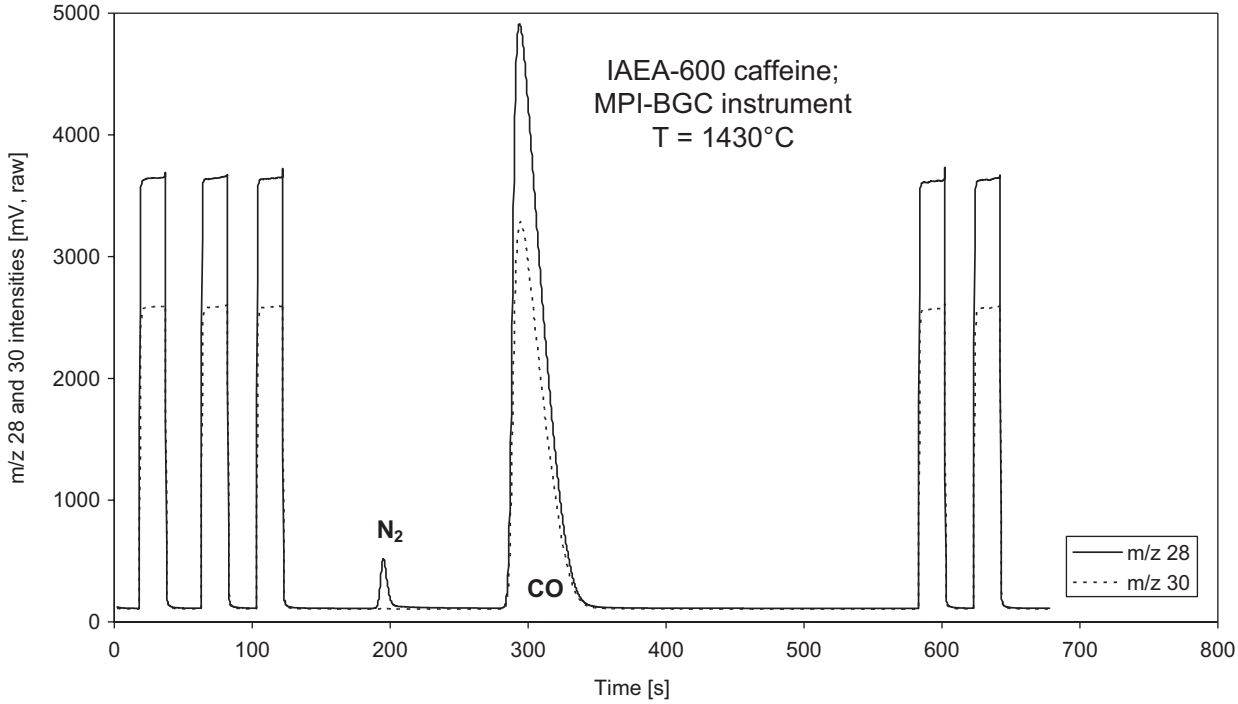

Figure 4. IAEA-600 caffeine $\delta^{18}O$ analysis on two different HTC systems: effect of nitrogen production on $\delta^{18}O$ results. The ratio of the peak areas of N_2 and CO at USGS is 0.51, compared with a stoichiometric value of 1.0.

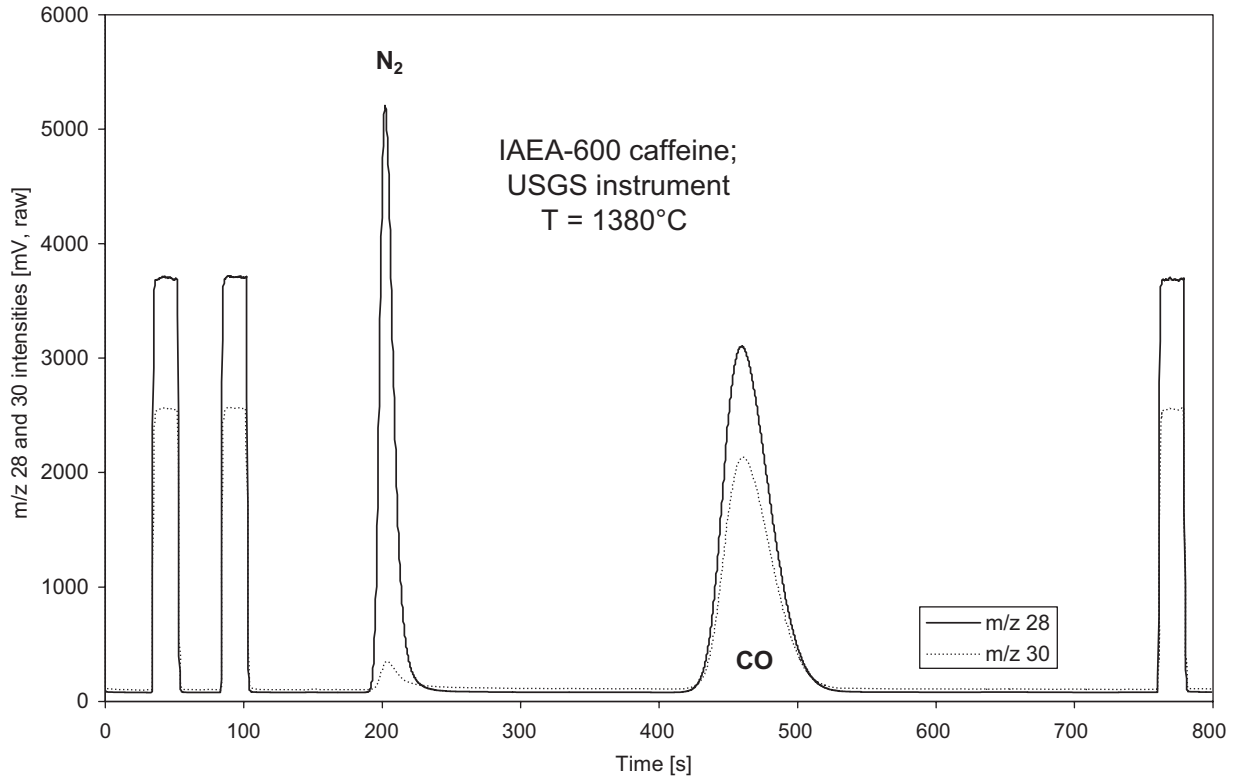

Figure 5. IAEA-600 caffeine $\delta^{18}O$ analysis on two different HTC systems: effect of nitrogen production on $\delta^{18}O$ results. The ratio of the peak areas of N_2 and CO at MPI-BGC is 0.045.

the results differed from the mean by −9‰. The within-laboratory precision in this case was also poor (±4.2‰). Other laboratories had to select their data for IAEA-600 and remove obvious outliers.

Analytical difficulties seem to arise from the 'nitrogen' peak in caffeine, which precedes the CO peak chromatographically. Figures 4 and 5 show two extreme examples of the phenomenon.

DOI: 10.1002/rcm

At USGS and ANU, a large N_2 peak eluted prior to the analyte CO, whereas N_2 production from caffeine was barely visible under the analytical conditions at ETH and MPI-BGC. The N_2/CO amount ratios as inferred from the integrated m/z-28 traces were 0.02 at ETH, 0.045 at MPI-BGC, 0.1 at CIO, and 0.51 at USGS. ANU observed the highest N_2/CO peak ratio of approximately 1.0, which would correspond to the stoichiometric value. The ratio of the N_2 and CO peak areas could be varied to a large extent by changing the reactor temperature. As a test experiment, the temperature of the USGS reactor was incrementally decreased to less than 1100°C with a correspondingly large loss in CO yield. Only then could a peak area ratio comparable with that in the MPI-BGC case be reproduced. However, the temperature distribution in the MPI-BGC reactor (see Fig. 2) excludes the possibility that this is solely an issue with temperature. Because the observed yields imply a quantitative conversion of oxygen, the origin of the discrepancy remains unclear. Within the caffeine molecule, nitrogen occurs in a reduced oxidation state. Hence, in a strongly reducing environment, such as the HTC reactor, N_2 cannot be formed from a pure redox reaction.[7,55,56] Instead, reactions of reduced forms of nitrogen with carbon at high temperatures probably lead to C–N bonds in components like cyanogen [$(CN)_2$], a common high-temperature product.[57] $(CN)_2$ can easily convert into a polymeric, highly inert form (para-cyanogen[58,59]) at high temperatures, which in the presence of a catalyst can decompose to N_2 and carbon, thus acting as a source for nitrogen. Decomposition to elemental N_2 and C occurs at temperatures in excess of 1200°C.[59,60] Moreover, cyanide anions, which are iso-electronic with halides, could be stable in the system when non-volatile salts are formed. With the presence of elemental Ag in the reactor, $CN^{\cdot}$ radicals originating from decomposing $(CN)_2$ can be stabilized by formation of AgCN, thus preventing formation of N_2. The temperature of the silver residues might play a role in the different types of reactors, with Ag available in colder regions of the MPI-BGC system. Furthermore, formation of higher homologues or polymers of tetra-cyanomethane[61] [$C(CN)_4$] also might be possible under the reaction conditions found in an HTC reactor.

The nitrogen peak preceding the elution of the CO peak can have two detrimental effects for the $\delta^{18}O$ stable isotope determination:

(1) By reaction of N_2 with residual oxygen on the filament, NO can be formed, which is ionized and $^{14}N^{16}O^+$ is detected on the m/z-30 Faraday cup where $C^{18}O^+$ is also measured. The formation of NO is a process with a relatively long time constant. NO continues to be observed long after gaseous N_2 has left the ion source, and it probably emanates from the filament. This effect will be discussed further for the case of analysis of nitrates.
(2) A variable background of non-analyte gas might be formed from $(CN)_x$ precursor molecules eluting slowly from the GC column. In such a case, the quantitative consequences are difficult to predict and alternative experimental solutions to eliminate this concealed interference need to be found.

The second scenario seems to apply when analyzing caffeine samples using the HTC technique (without excluding additional contributions from (1)). The experimental situation needs to be investigated and varied further, aiming at stabilizing the ratio of N and C as well as the oxygen yield. Caffeine HTC reactions need to be performed under more closely controlled conditions. In spite of the unresolved questions raised by the observations reported here, the stable isotopic composition of IAEA-600 could be determined with a satisfactory level of confidence using results from three laboratories. The results will be discussed in the respective section together with the results from the other materials.

Nitrates (IAEA-NO-3, USGS34, USGS35)

The nitrate materials under investigation in this study, IAEA-NO-3 (NIST-RM 8530), USGS34 (RM 8568), and USGS35 (RM 8569), have all been measured before with mixed results. Earlier, more 'classical' ways of preparing nitrate for $\delta^{18}O$ analysis have suffered greatly from contamination, most often from oxygen of the quartz/glass reactor walls.[62] The problem of NO formation mentioned above for caffeine also plays a major role in the $\delta^{18}O$ determination of nitrates.[48] The time behavior of NO^+ is very different from the time behavior of other nitrogen-related peaks. Nitrates have a constant N to O ratio with the corresponding N_2/CO peak-area ratio of 1:6, assuming identical ionization efficiency. However, this does not guarantee that the amount of NO in the mass spectrometer is automatically under control.

The rate of NO formation depends on the amount of N_2 introduced into the ion source and on the amount of oxygen in and around the filament that is available to produce NO. The presence of this oxygen might arise from the mass spectrometer backgrounds of O_2 and/or H_2O. Its amount depends on the recent short-term history of the filament and is highly variable.

Figures 6–8 show typical examples from the MPI-BGC system. The preceding m/z-30 peak clearly extends into the analyte $^{12}C^{18}O^+$ peak (Fig. 7), interfering with the determination of the background with the required high precision for on-line isotope ratio measurements. Because there are basically no foreign ion currents on the other lower mass positions (m/z 29 and m/z 28), the apparent ion current ratio, I_{30}/I_{28}, is too positive (Fig. 8) and declines strongly over time, making it difficult to quantify the CO^+ analyte m/z-30 peak with high precision ($\sim 5 \times 10^{-5}$).

Various approaches to discover a solution to these problems have been sought by the participating laboratories. At USGS and at ANU, the nitrogen peak has been diverted entirely using a four-way diverter valve to prevent the nitrogen peak from entering the mass spectrometer.[48] Make-up He is added when the stream containing the nitrogen peak is diverted. At MPI-BGC, CIO, and at the ETH, a manual correction of the background was made by selecting a representative section from the chromatogram having no interference from NO. At UFZ, the two concentric tubes are carefully positioned and the central flow is focused in a way that the split ratio is better than 9:1 through the reactor core. For attenuating the m/z-30 interference effect, the UFZ laboratory uses an additional pulse of CO reference gas

DOI: 10.1002/rcm

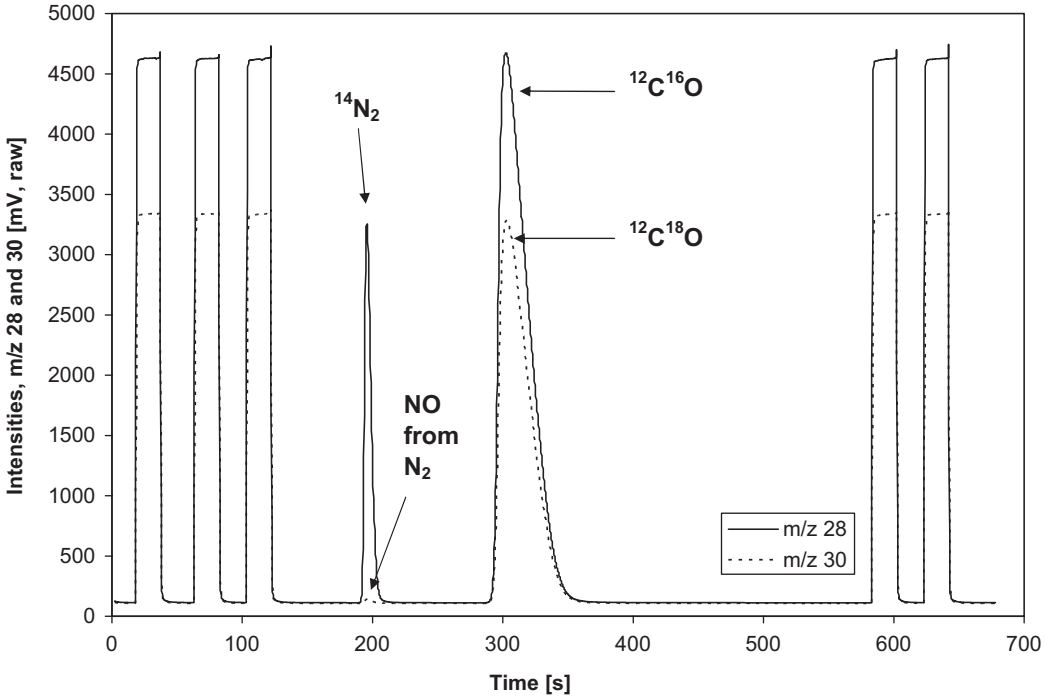

Figure 6. Ion current traces of *m*/*z* 30 and 28 for nitrates (example by MPI-BGC).

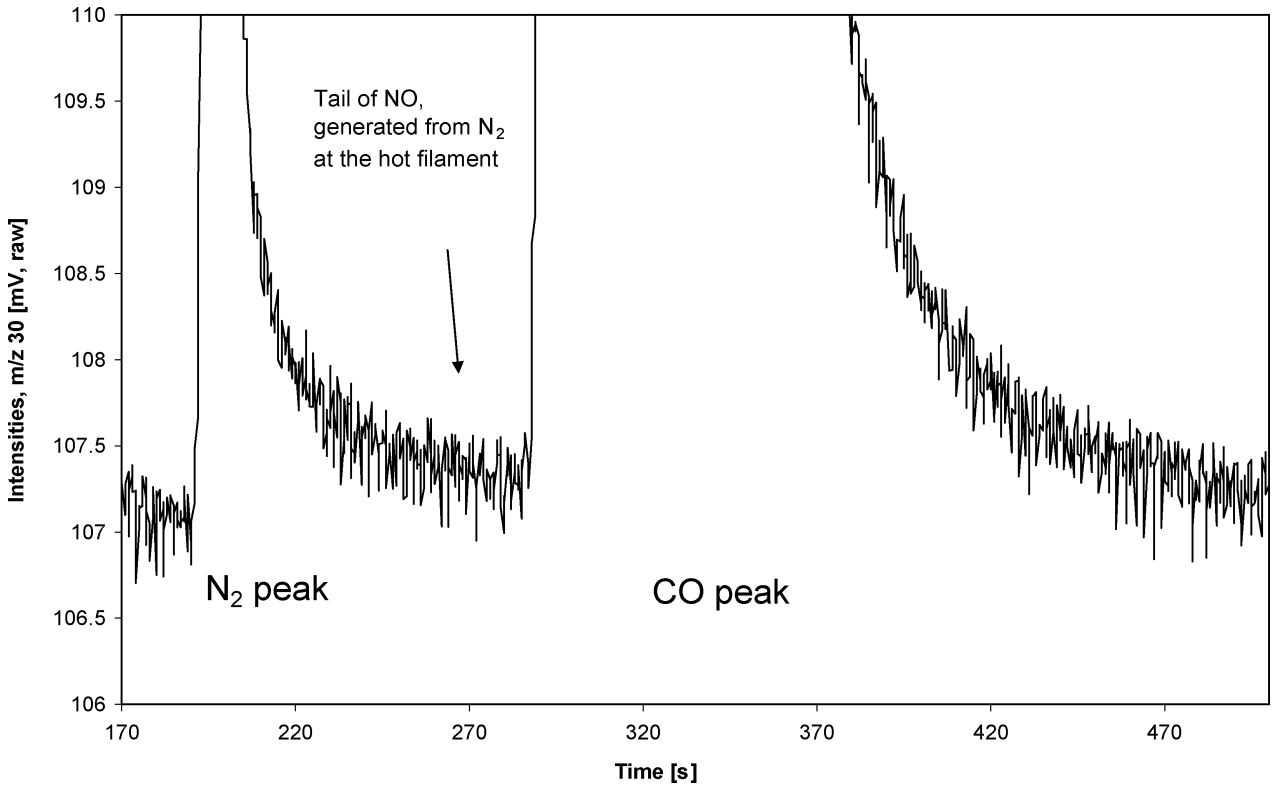

Figure 7. An interference on *m*/*z* 30 is observed when analyzing nitrate samples. This arises from traces of NO, generated from N_2 and a variable source of oxygen inside the ion source.

between the N_2 and the CO analyte peaks. Moreover, the N_2 peak is reduced further using He dilution.[2]

All laboratories routinely check chromatographic performance and restore it by regular GC baking as necessary. Chromatographic conditions directly influence the analytical results. The peak-separation requirements impose restrictions on the size of the reactor. If the reaction tube diameter is too large (or the respective carrier flow rate is too low), the time between the N_2 peak and the CO peak eluting afterwards is too short for heart-cutting N_2 and thus removing it from the recorded traces. Reverse-flow systems[3] and the ANU molybdenum liner[35] have an advantage

DOI: 10.1002/rcm

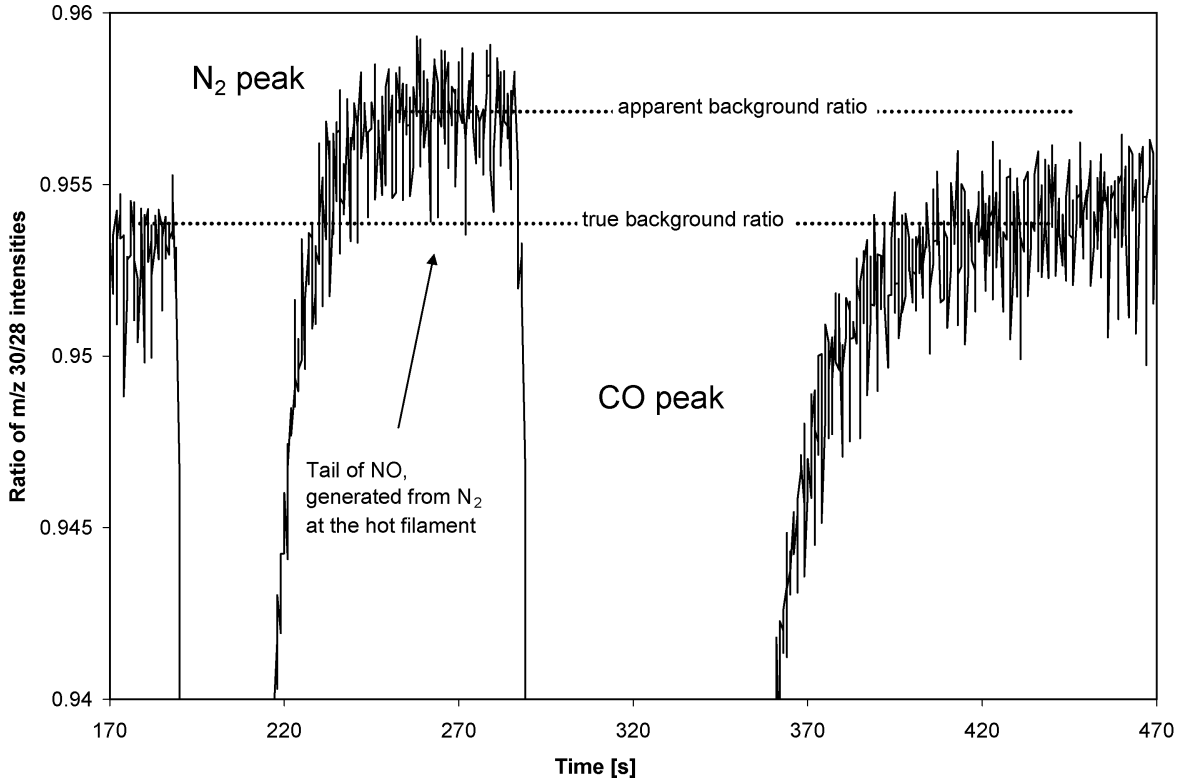

Figure 8. Close-up view of the ratio of the *m*/*z*-30 and *m*/*z*-28 intensities during measurements of nitrates. The delta value of the background is about 3‰ too positive due to isobaric interference by NO. Using the intensities from the figures above, an error of +0.33‰ in the assigned $\delta^{18}O$ value can be inferred for this example.

because the entire He carrier gas passes directly through the core of the reactor; hence, the peak shape is better than that of split-flow systems. In addition, the flow can be maintained over time, even when the internal reactor resistance builds up from accumulation of sample residue.

In a study involving some of the same materials as in this work, an improved background correction using extrapolation of the *m*/*z*-30 NO tail, and subtraction of the corresponding proportion from the analyte *m*/*z*-30 peak, has recently been described by Accoe *et al*.[63] In addition, a comparison was made between the diverter-valve technique discussed above and a simple switched He dilution technique using existing instrumental capabilities. The latter two correction options were found to eliminate the problem with roughly the same success. In light of these different approaches to correct for this contamination effect and considering the difficulties described for caffeine, it is advisable to calibrate nitrate samples with nitrate reference materials and sulfate samples with sulfate reference materials. This requires calibrated nitrate and sulfate materials as working and as international references, preferably with substantially different isotopic compositions in order to correct for scale-compression effects.[46,47,64] If laboratories are using sample masses of less than 1 mg and they require less than 100 mg per year of a specific internationally distributed reference material for regular use, it is advised that the internationally distributed reference material be used regularly, avoiding the need for another similar material. Nevertheless, it is emphasized that substantial calibration errors can result from differences in sample preparation prior to mass spectrometry.[62,65] It may be important to evaluate potential biases related to sample processing (e.g. incorporation of NO_3^- and H_2O in $BaSO_4$ prepared from aqueous solutions) and to prepare and process isotopic reference materials as samples when possible (e.g. dissolved NO_3^- reference materials paired with dissolved NO_3^- samples).

RESULTS AND DISCUSSION

Calibration strategy

The calibration strategy can be described as a scenario with two distinct tasks:

(1) Primary calibration of a representative working reference material, which is easy to handle and performs well under the HTC reaction conditions. This primary calibration enables the necessary extension of the international water (VSMOW-SLAP) scale to positive $\delta^{18}O$ values and, at the same time, enables scale transition to non-aqueous compounds.
(2) Secondary calibration of additional reference materials without the tedious and error-prone use of reference waters.

The scale extension to positive δ_{VSMOW} values has been necessary because most organic oxygen is substantially enriched in ^{18}O relative to ocean water[j].[7,39] In addition, atmospheric O_2 has a $\delta^{18}O$ value of +23.8‰ on the VSMOW scale.[66–68] Materials generated during combustion and

[j]Oxygen in naturally occurring organic material is derived from H_2O, O_2, and CO_2, often followed by oxygen-isotope exchange of the organic-bound oxygen with other water bodies (e.g. with ^{18}O-enriched leaf water).

DOI: 10.1002/rcm

Table 3. Oxygen isotopic composition of selected analyses of reference materials normalized to VSMOW-SLAP reference waters on a scale normalized to a $\delta^{18}O$ value of SLAP of −55.5‰. All values as reported by the participating laboratories ($n^{\#}$ = number of sequence runs; n = number of single analyses)

	USGS			USGS II			MPI-BGC			ETH			UFZ			ANU			CIO		
Material	$10^3\ \delta^{18}O$	$n^{\#}$	n	$10^3\ \delta^{18}O$	$n^{\#}$	n	$10^3\ \delta^{18}O$	$n^{\#}$	n	$10^3\ \delta^{18}O$	$n^{\#}$	n	$10^3\ \delta^{18}O$	$n^{\#}$	n	$10^3\ \delta^{18}O$	$n^{\#}$	n	$10^3\ \delta^{18}O$	$n^{\#}$	n
W-89262 water	78.82 ± 0.32	7	31				79.02 ± 0.19	4	32	79.02 ± 0.39	4	48	78.46 ± 0.73	6	35	79.84 ± 0.32	2	13			
VSMOW2 water	0.00 ± 0.14		50				0 ± 0.14	24	152	0		48	0	6	36				0.0 ± 0.17	1	8
SLAP water	−55.52 ± 0.24		46				−55.5 ± 0.18	23	148	−55.5		48	−55.5	6	36				−55.5 ± 0.13	1	8
evaporative loss (est.)	+0.1			0.0			+0.1			+0.1			+0.05			+0.1			+0.2		
S-4317 $BaSO_4$	73.49 ± 0.46	10	48				71.32 ± 0.55	4		73.71 ± 0.16	3	24	72.97 ± 0.82	6	36	72.96 ± 0.81	2	8	73.48 ± 0.09	1	8
IAEA-SO-5 $BaSO_4$	11.86 ± 0.11	3	12				12.00 ± 0.18	4	24	12.62 ± 0.19	5	40	12.43 ± 0.39	6	54	13.10 ± 0.37	2	8	11.57 ± 0.11	1	8
NBS 127 $BaSO_4$	8.37 ± 0.24	7	42				8.49 ± 0.12	10	60	8.57 ± 0.29	5	40	8.87 ± 0.38	6	54	9.44 ± 0.24	2	6	8.18 ± 0.09	1	8
IAEA-SO-6 $BaSO_4$	−11.55 ± 0.10	3	12				−11.18 ± 0.13	4	24	−10.44 ± 0.22	5	40	−10.97 ± 0.33	6	53	−10.00 ± 0.38	2	8	−12.14 ± 0.09	1	8
S-4316 $BaSO_4$	−57.13 ± 0.43	10	49				−55.71 ± 0.23	4	24	−56.25 ± 0.21	3	24	−55.79 ± 1.27	6	36	−54.92 ± 1.07	2	8	−56.56 ± 0.26	1	8
IAEA-602 benzoic acid	71.08 ± 0.39	11	49				71.06 ± 0.21	6	36	71.24 ± 0.43	6	48	71.84 ± 0.87	6	36	72.05 ± 0.53	2	4	71.97 ± 0.20	1	8
IAEA-601 benzoic acid	22.90 ± 0.13	11	48				22.93 ± 0.17	31	331	23.07 ± 0.08	6	48	23.24 ± 0.53	6	36	23.66 ± 0.65	2	4	22.83 ± 0.11	1	8
USGS35 $NaNO_3$	56.57 ± 0.23	7	31	56.85 ± 0.03	4	38	56.97 ± 0.29	4	18	56.57 ± 0.24	4	24	55.16 ± 1.23	3	18				58.02 ± 0.14	1	8
IAEA-NO-3 KNO_3	24.96 ± 0.17	7	29	25.50 ± 0.19	4	38	25.59 ± 0.15	4	18	24.60 ± 0.29	5	30	24.18 ± 0.98	3	13				25.75 ± 0.17	1	8
USGS34 KNO_3	−28.12 ± 0.33	7	30	−27.54 ± 0.08	6	54	−27.22 ± 0.29	4	18	−28.67 ± 0.30	5	30	−27.66 ± 0.65	3	18				−27.75 ± 0.10	1	8
IAEA-600 caffeine	−3.29 ± 0.26	3	12				−3.44 ± 0.04	3	24	−3.68 ± 0.23	6	48	−12.63 ± 4.23	3	18				−4.63 ± 0.10	1	8

DOI: 10.1002/rcm

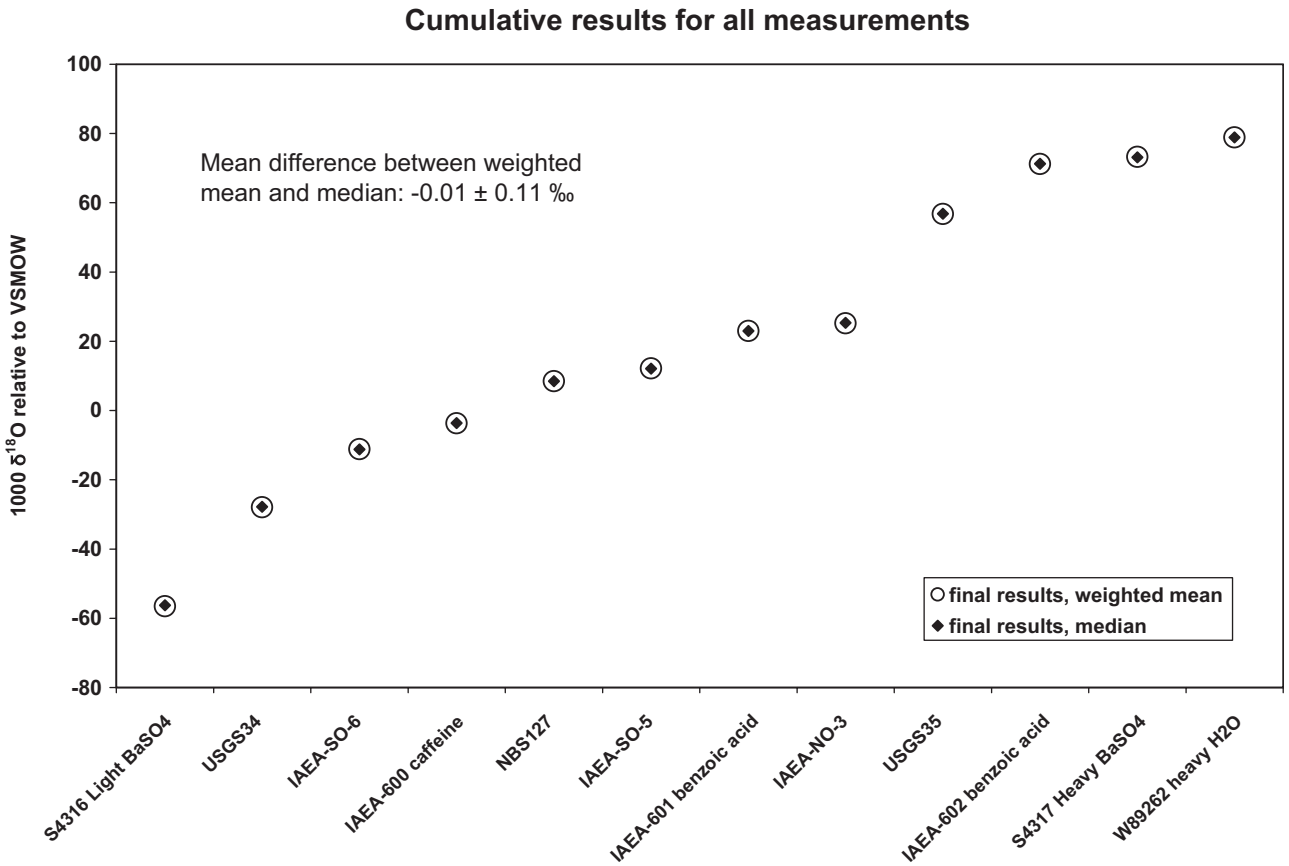

Figure 9. Weighted mean and median $\delta^{18}O$ values of laboratories in the study reported relative to VSMOW on a scale such that 10^3 $\delta^{18}O$ of SLAP reference water is -55.5.

respiration processes, hence, tend to maintain at least part of this atmospheric O_2 signature. For a review, see Schmidt *et al.*[39], in particular, and Fig. 8 therein.

In half of the laboratories, strategies (1) and (2) above were intertwined, whereas the other half followed a strategy of first calibrating a local organic working reference or reference pair using the primary water references, followed by a second step of calibrating the remaining compounds using the working reference materials from the first step. The number of analyses made for the different calibration materials varied greatly from laboratory to laboratory. This has been taken into account by weighting data in calculating mean values and respective uncertainties. In total, more than 5300 measurements (including blanks and co-reacted standards) were performed over roughly 2 years, most of them using the HTC technique. Protocols in the laboratories were subject to change and improved over time. After eliminating measurements with obvious sources of bias, the results of the remaining analyses are shown in Table 3.

Figure 9 provides a graphic overview of the materials and their respective mean and median $\delta^{18}O$ values on the VSMOW-SLAP scale as evaluated from the measurements in Table 3. The $\delta^{18}O$ values span almost 140‰, ranging from -56‰ for the 'light' $BaSO_4$ material S-4316 to $+79$‰ for the 'heavy' water W-89262. The mean difference between the $\delta^{18}O$ value of W-89262 and that of SLAP is 134.54 ± 0.49‰ (Table 4), in reasonable agreement with the expected value for the sum of $+78.91$‰ (from equilibration, see Table 1) and $+ 55.5$‰, which is 134.41‰.

In order to establish the bridge from water samples to non-aqueous materials, systematic errors such as the evaporation of water during sample preparation or storage had to be estimated for each laboratory. Because all water samples in Ag capsules, in particular those of the primary standards, VSMOW and SLAP, have similar evaporative losses (largely independent of the isotopic composition), the corresponding shift is similar for all non-aqueous materials. The effect does not alter the span of the scale. The amount of water per sample in each laboratory is a rough guide for the size of the necessary correction. In addition, the values reported for 'easy' organic materials like the benzoic acids are a guide to the size of the correction. From these criteria, the corrections applied to the data have been estimated to range between $+0.05$ and $+0.2$‰ for the different data sets ('evaporative loss (est.)', in Table 3).

The differences between the final weighted mean values and the median[k,69] values across the laboratories for the different compounds in general are small, with an average of -0.01 ± 0.11‰. The small difference and scatter are indicative of the reliability of the different results, suggesting negligible bias and absence of systematic errors that might

[k]The median is generated using the reported average values from the individual laboratories, whereas the weighted mean also accounts for the number of analyses made by each laboratory.

DOI: 10.1002/rcm

Table 4. Difference in $\delta^{18}O$ values of selected water, sulfate, benzoic acid, and nitrate isotopic reference materials. [Values expressed relative to VSMOW on a scale normalized to a $\delta^{18}O$ value of SLAP of -55.5‰.]

Laboratory	$\delta_{W\text{-}89262}(^{18}O) - \delta_{SLAP}(^{18}O)$	$\delta_{S\text{-}4317}(^{18}O) - \delta_{S\text{-}4316}(^{18}O)$	$\delta_{IAEA\text{-}602}(^{18}O) - \delta_{IAEA\text{-}601}(^{18}O)$	$\delta_{USGS35}(^{18}O) - \delta_{USGS34}(^{18}O)$
USGS	134.34‰	130.62‰	48.18‰	84.69‰
USGS II	n.d.	n.d.	n.d.	84.39‰
CIO	n.d.	130.04‰	49.14‰	85.77‰
ETH	134.52‰	129.96‰	48.17‰	85.24‰
UFZ	133.96‰	128.76‰	48.60‰	82.82‰
ANU	135.34‰	127.88‰	48.39‰	n.d.
MPI-BGC	134.52‰	127.03‰	48.13‰	84.19‰
Mean	134.54 ± 0.49‰	129.05 ± 1.40‰	48.43 ± 0.39‰	84.52 ± 1.01‰

Table 5. Oxygen isotopic composition of benzoic acid and caffeine isotopic reference materials. [Values expressed relative to VSMOW on a scale normalized to a $\delta^{18}O$ value of SLAP of -55.5‰.]

	$10^3\ \delta^{18}O$								
Material	USGS	ETH	CIO	MPI-BGC	UFZ	ANU	Mean	Median	Weighted mean $\pm 1\sigma$
IAEA-602	+71.26	+71.24	+72.17	+71.01	+72.30	+71.31	71.60	71.28	$+71.28 \pm 0.42$
IAEA-601	+23.02	+23.14	+23.03	+22.95	+23.42	+23.48	23.13	23.14	$+23.00 \pm 0.17$
IAEA-600	−3.19	−3.58	−4.43	−3.39	n.d.	n.d.	−3.65	−3.48	-3.47 ± 0.54

occur if results were based on reports from only one laboratory with a single optimized protocol on a single apparatus.

Benzoic acids

IAEA-601 benzoic acid was treated as a starting point in this study. The expectation was that water standards could be used to calibrate organic standards, which in turn could be used to calibrate all other solid reference materials. The original values for IAEA-601 reported from the different laboratories ranged from 22.90 to 23.66‰, with a weighted mean of 22.95‰ and a median of 23.07‰ relative to VSMOW, conveniently close to the air-O_2 $\delta^{18}O$ value. Taking the above-mentioned evaporative loss into account and correcting for span deviations using the individual measurements for the 'heavy' water W-89262, a median value of +23.14‰ (weighted mean = +23.00‰) was found for this material (see Table 5). The precision of the values across the different laboratories and, hence, across the different preparation conditions and mass spectrometric measurements was ±0.17‰. With the number of sequence analyses made, this figure can formally be converted into an error of the mean of 0.05‰. However, this requires the data set to be strictly normally distributed in a statistical sense, which we consider improbable.

The second benzoic acid sample, IAEA-602, had a weighted mean almost identical with the median value of $+71.28 \pm 0.42$‰ across the laboratories. Since the data for the heavy water sample has been normalized to the VSMOW-SLAP distance and the reported value for the HTC technique is very close to that of the classical equilibration technique, a scaling correction is already included in the assessment of IAEA-602. Based on the number of sequence runs made, an error of the mean of ±0.11‰ can be calculated. Again, this may under-represent the residual error of the mean. We estimate that a factor of 3 accounts for this uncertainty. Making provision for an extra evaporation correction error of ±0.1‰ the mean of IAEA-602 is assigned as +71.28‰ with an estimated overall uncertainty of ±0.36‰. Likewise, the value for IAEA-601 is $+23.14 \pm 0.19$‰.

Barium sulfates

The differences between the $\delta^{18}O$ values of S-4316 and S-4317 range from 127.03 to 130.62‰ (Table 4). The expectation of this study was that water standards could likewise be used to calibrate sulfate standards in a first step. Other materials could then be calibrated against the sulfates. However, the large variability in reported $\delta_{S\text{-}4317}(^{18}O) - \delta_{S\text{-}4316}(^{18}O)$ values indicates that some of the results are biased. The sulfate-only $\delta^{18}O$ scales of ANU and MPI-BGC appear compressed by as much as 3% (in relative terms) compared with those of USGS, ETH, UFZ, and CIO (Table 4). This variation in $\delta^{18}O$ values is thought to be the result of variations in the high-temperature chemistry and differences in instrumentation and methodology as discussed above[1].

Because it is less likely to expand than to compress isotope scales during measurement, it may be argued that the best values for calibration of sulfate isotopic references could be obtained by excluding the MPI-BGC and ANU results. On the other hand, the data for the other sulfate compounds are closer, so the results of the more extreme sulfates could suffer from experimental artifacts. The latter point is underlined by

[1]Some of the $\delta^{18}O$ variation of sulfates between laboratories may be attributed to different treatments. IAEA-SO-6, for instance, is suspected to contain included H_2O that may contribute as much as 7% to the CO^+ ion current if not removed. Fractional contributions to CO from H_2O in IAEA-SO-5 and NBS 127 are estimated to be ≤1% (Hannon *et al.*[65]). S-4317 and S-4316 also may contain minor amounts of H_2O. In this study, most laboratories used the materials as agreed (i.e. without pretreatment); UFZ applied a gentle heating step before measurement.

DOI: 10.1002/rcm

Table 6. Oxygen isotopic composition of selected measurements of sulfate reference materials. [All data adjusted to a common $\delta^{18}O_{NBS127}$ value of 8.59‰. Values expressed relative to VSMOW on a scale normalized to a $\delta^{18}O$ value of SLAP of −55.5‰. Values in parentheses are assigned as outliers.]

	$10^3\ \delta^{18}O$								
Material	USGS	ETH	CIO	MPI-BGC	ANU	UFZ	Average	Median	Weighted mean ± 1σ
S-4317	+73.79	+73.64	+73.89	+71.34	+71.37	+73.06	+73.14	+73.35	+73.43 ± 0.43
IAEA-SO-5	+12.09	+12.64	+11.98	+12.10	+12.21	12.17	+12.20	+12.13	+12.28 ± 0.20
NBS 127 (common)	+8.59	+8.59	+8.59	+8.59	+8.59	+8.59	+8.59	+8.59	+8.59 (± 0.2)
IAEA-SO-6	−11.35	(−10.39)	−11.73	−11.05	−10.62	−11.36	−11.37	−11.35	−11.24 ± 0.21
S-4316	−56.98	−56.14	−56.15	−55.52	−55.02	−56.44	−56.24	−56.14	−56.38 ± 0.42

Table 7. Oxygen-isotopic composition of selected nitrate reference materials. [Values expressed relative to VSMOW on a scale normalized to a $\delta^{18}O$ value of SLAP of −55.5‰. *1σ values designate the single-sample precision.]

	$10^3\ \delta^{18}O$								
Material	USGS	USGS II	ETH	CIO	MPI-BGC	UFZ	Average	Median	Weighted mean ± 1σ*
USGS35	+56.95	+56.67	+57.24	+57.59	+56.644	+56.47	+56.93	+56.81	+56.78 ± 0.19
IAEA-NO-3	+25.32	+25.32	+25.32	+25.32	+25.32	+25.32	+25.32	+25.32	+25.32 ± 0.19*
USGS34	−27.83	−27.73	−27.88	−28.18	−27.44	−26.82	−27.65	−27.78	−27.73 ± 0.30

the comparatively poor intra-lab precision of the S-4316 and S-4317 measurements for the majority of the laboratories. Moreover, scaling in the mass spectrometer has already been applied using the water results, which rendered further specific scaling unnecessary.

The best inter-laboratory agreement for the sulfate materials is found for NBS 127 (after correction for evaporative loss and scaling with W-89262) with identical weighted average and median of 8.59 ± 0.2‰. This is close to the majority of literature values of approximately 8.6‰.[5,48–50] Adjusting the results for the other sulfates accordingly to account for sulfate-specific effects (as discussed above) provides the results given in Table 6. The weighted mean $\delta^{18}O$ values and final estimated uncertainties (including evaporation offset errors) of IAEA-SO-5 and IAEA-SO-6 are +12.13 ± 0.33‰ and −11.35 ± 0.31‰, respectively. These values are similar to previously reported results from the USGS laboratory.[48]

Caffeine

The original $\delta^{18}O$ values reported by individual laboratories for IAEA-600 caffeine varied over a wide range from −3.29 (±0.26) to +12.63 (±4.23)‰. Possible reasons for this discrepancy have been discussed above, but further experiments would be necessary for a deeper understanding of the phenomenon. Values from four of the five reporting laboratories[m] were close together (Table 3) and the associated reproducibility values of the results were acceptable (0.04–0.26‰). After adjustment for the evaporative-loss effect, the weighted mean was −3.47 ± 0.54‰ with the median at −3.48‰ (Table 5). We use the median as the assigned value for $\delta^{18}O$ of IAEA-600 caffeine with a formal error of the mean of ±0.17‰. As before, we add ±0.1‰ evaporation loss uncertainty. Moreover, for the specific uncertainty arising from the NO^+ interference at the peak start (see above) we estimate another ±0.1‰. In order to account for the small number of valid analyses made on this material and for taking other unknown experimental uncertainties into consideration, we again multiply the standard error by a factor of 3 as a cautious and more likely error of the mean. Hence, the $\delta^{18}O$ value of IAEA-600 caffeine derived from the experiments in this study is −3.48‰ with a combined uncertainty of ±0.53‰.

Nitrates

In order to cope with the NO^+ interference on the m/z-30 channel (see discussion above), the raw data from the nitrate measurements have been treated in different ways by the different laboratories. The treatments include N_2 diversion during measurement (USGS), dilution of the N_2 peak (MPI-BGC), and manual background subtraction from non-contaminated time windows of the respective chromatograms (ETH, CIO, and MPI-BGC). The final reported $\delta^{18}O$ values for IAEA-NO-3, USGS34, and USGS35 as listed in Table 3 still exhibit large ranges, which may be attributed to non-consistent raw-data treatment and correction procedures. The weighted mean values are close to the respective raw averages; they are, however, more precise (0.2–0.3‰) and they are close to the median values.

Similar to the role of NBS 127 for the sulfates, IAEA-NO-3 has been used as the common scale anchor for the final evaporation loss and scale corrected values listed in Table 7. Using the median as the more probable,[69] accurate result, $\delta^{18}O$ values and estimated uncertainties (see footnote [n]) of

[m]The ANU laboratory was unable to remove the nitrogen yield effects in their system and declined to assert the correctness of their analyses.

[n]Derived as above and including ±0.1‰ provision for the NO^+ correction uncertainty.

DOI: 10.1002/rcm

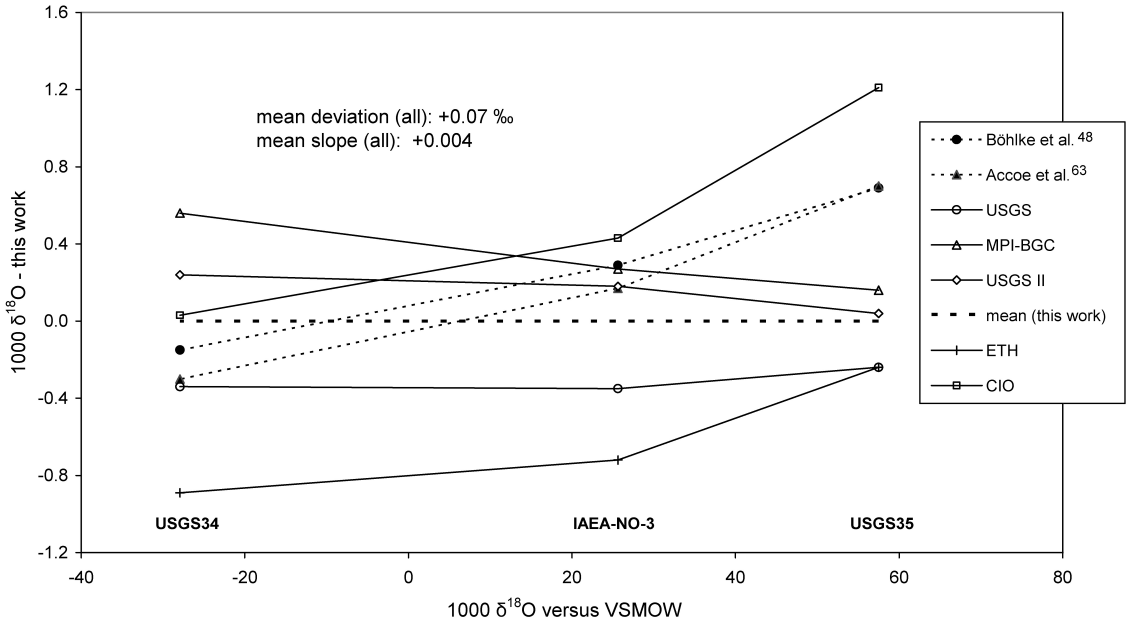

Figure 10. $\delta^{18}O$ deviations of nitrate compounds from the mean (individual laboratory values from this work and further literature sources[48,63]).

IAEA-NO-3, USGS34, and USGS35 then are $+25.32 \pm 0.29$‰, -27.78 ± 0.37‰, and $+56.81 \pm 0.31$‰, respectively.

These values differ from previous USGS results[48] by increasing amounts as $\delta^{18}O$ increases, with closest agreement for USGS34, where the $\delta^{18}O$ value is about half-way between those for VSMOW and SLAP. They can be made more consistent if the scale factors defined by normalization to VSMOW and SLAP were biased at one end by about 1 to 2% (e.g. if the measured value of Antarctic water were too high by about 0.5‰ in the previous study). This means that the $\delta^{18}O$ values of nitrate samples, when normalized to either set of calibration data, can be re-normalized to the other calibration scale without significant error (±0.2 to 0.3‰). Similarly, most other discrepancies between individual laboratory datasets from this and other studies vary systematically such that they can be reconciled to within small uncertainties by linear renormalization (Fig. 10).

Data summary

The final $\delta^{18}O$ values of materials in this study expressed relative to the VSMOW-SLAP scale are summarized in the Abstract and listed again in Table 8 together with the uncertainty (1σ), the error of the mean, and a comparison with older literature data. The error budget reflects the statistical inspection of the data; provision for systematic errors has not been made. To estimate these is not an easy task because they are in principle not known. From the good agreement of the weighted average and median for all compounds as discussed in connection with Fig. 9, it can be concluded that the systematic errors are probably small.

Table 8. Final $\delta^{18}O$ values of reference materials and a comparison with previous values. [Materials prepared for and used in this study only are indicated with an asterisk (*). 1-σ values are standard deviations (SDs) of the weighted averages, representing single analysis precision. The combined uncertainty values are derived from 3 × standard error (mean) plus ±0.1‰ evaporation correction. Values with a # have an added estimated uncertatinty of ±0.1‰ due to NO^+ interference.]

Material type/name	NIST-RM	$10^3\ \delta^{18}O$	SD (1σ) for single analysis	Combined uncertainty (see text)	Previous values[ref]	Change
VSMOW2	RM 8535a	0				
SLAP	RM 8537	−55.5				
IAEA-601 benzoic acid	RM 8575	+23.14	0.17	0.19	+23.2[44]	−0.06
IAEA-602 benzoic acid	RM 8576	+71.28	0.42	0.36	+71.4[44]	−0.12
W-89262 'heavy' H_2O*		+78.91	0.39	0.40		
IAEA-600 caffeine	RM 8567	−3.48	0.54	0.53#		
USGS35	RM 8569	+56.81	0.19	0.31#	+57.5[48,63]	−0.69
USGS34	RM 8568	−27.78	0.30	0.37#	−27.93[48]	+0.15
IAEA-NO-3	RM 8530	+25.32	0.19	0.29#	+25.6[48]	−0.28
IAEA-SO-5	RM 8533	+12.13	0.20	0.33	+11.99[48]	+0.14
IAEA-SO-6	RM 8534	−11.35	0.21	0.31	−11.34[48]	−0.01
S4317 'heavy' $BaSO_4$*		+73.35	0.43	0.39		
S4316 'light' $BaSO_4$*		−56.14	0.42	0.41		
NBS 127	RM 8557	+8.59	0.20	0.26	+8.59[48]	+0.0
(NBS 127)					+9.3[70]	−0.71

DOI: 10.1002/rcm

There are some principal reservations as to the absolute values of the nitrogen-bearing compounds and the effectiveness of the m/z-30 tail correction. For the sulfates, a small systematic effect due to the slower reaction might require an adjustment of the final $\delta^{18}O$ data. Moreover, the calibration with reference waters bears some limitations, which need to be accounted for in the error budget.

We estimate that the given mean errors can form a basis for an error budget that includes systematic errors when multiplied by a factor of 3. In addition we make an extra provision for the evaporative-loss correction uncertainty (±0.1‰). This results in an assigned error of ±0.3–0.5‰ for the nitrogen-bearing materials with the highest uncertainty and ±0.2‰ for IAEA-601 benzoic acid with the lowest uncertainty. This range of errors seems realistic and in line with the experience gained during this exercise. Comparison with literature data representing knowledge about the reference materials before this study reveals that some rather large corrections are necessary. In particular the previous NBS 127 $BaSO_4$ value (+9.3‰) needs substantial revision (by −0.71‰). In addition, USGS35 exhibits a significant shift by −0.69‰ and is in need of review.

CONCLUSIONS AND OUTLOOK

The analysis of $\delta^{18}O$ values using a modified Schuetze/Unterzaucher reaction[19–23] in an HTC reactor is not as simple a process as might be deduced from the frequent use of the technique. The sibling technique using oxidation to CO_2 and N_2 for analyzing $\delta^{13}C$ and $\delta^{15}N$ is much easier to perform; it is applied more often, and its chemistry apparently is simpler and more predictable. The HTC reaction is complicated by the fact that the reaction partner (carbon) is a solid, not a gas, and that the reaction product, CO, can exchange oxygen with oxygen-bearing materials and surfaces at the high temperatures employed. In addition, the complexity of carbon bonding and the mass overlaps of CO^+ and N_2^+ isotopologues create extra difficulties. Some of these difficulties are largely overcome by use of an oxygen-free shield (glassy carbon, molybdenum), but exchange with oxygen from residues of previous samples can still lead to substantial experimental errors. Regular replacement of the reactor filling and careful monitoring of results with reference materials are necessary to produce reliable analytical results. The traditional and scale-defining materials, water and carbonates, are particularly difficult to analyze using the HTC technique. With emphasis on the details of analytical protocols, we have calibrated a set of oxygen isotopic reference materials on the VSMOW-SLAP scale. These reference materials should aid in the oxygen-isotopic analysis of organic and inorganic oxygen-bearing materials. The $\delta^{18}O$ values of some internationally distributed isotopic reference materials are significantly different from previously reported values, including the $\delta^{18}O$ values for NBS 127 $BaSO_4$ and USGS35 $NaNO_3$.

A primary conclusion of this study is that nitrate samples analyzed for $\delta^{18}O$ should be analyzed with internationally distributed isotopic nitrates and the measured values of the nitrate reference materials should be published with sample results so that readers can normalize the $\delta^{18}O$ values at a later time, should it be necessary. Sulfate samples should be treated in an analogous fashion, as should organic samples.

The HTC technique has greatly facilitated oxygen-isotope analysis of non-aqueous and non-carbonate materials, in spite of the described experimental difficulties. The bridge to the water-isotope world has been constructed. New efforts might lead to a more intimate comparison with carbonate isotopic reference materials.[2,5] Moreover, a similar effort will be needed for the analysis of δ^2H values of hydrogen-isotopic reference materials to achieve a high level of confidence in value assignment, although in this case trusted off-line methods (i.e. with uranium, where we can process both waters and organics equally) are available. This is different from oxygen where off-line methods are less well developed.

Acknowledgements

This work was supported by the International Union of Pure and Applied Chemistry (IUPAC), under project number 2005-022-1-200. Initial tests for the production and measurement of IAEA-601 and IAEA-602 benzoic acids were made in collaboration between MPI-BGC and the Friedrich-Schiller-University, Institute of Chemistry in Jena, Germany. Preliminary assessment of the isotopic composition was made at MPI-BGC and by Arndt Schimmelmann, Indiana University, Bloomington, USA, with financial support from the IAEA Hydrology section under contract number 11792/R0. The raw material for IAEA-600 caffeine was supplied by MPI-BGC, and bottling was performed at NIST, Gaithersburg, MD, USA. W-89262 (H_2O enriched in ^{18}O), as well as $BaSO_4$ materials depleted in ^{18}O and enriched in ^{18}O, were prepared at USGS (Reston). The support of the U.S. Geological Survey National Research Program made this report possible. All other materials were made available by the IAEA (Vienna). The manuscript has benefited from careful reviews by Glenda Singleton (USGS), Barbara Kornexl (Zürich), Arndt Schimmelmann (Univ. of Indiana), Magnus Wendeberg (MPI-BGC), and an anonymous reviewer. Any use of trade, product or firm names in this publication is for descriptive purposes only and does not imply endorsement by the U.S. Government.

REFERENCES

1. Santrock J, Hayes JM. *Anal. Chem.* 1987; **59**: 119.
2. Gehre M, Strauch G. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1497.
3. Gehre M, Geilmann H, Richter J, Werner RA, Brand WA. *Rapid Commun. Mass Spectrom.* 2004; **18**: 2650.
4. Koziet J. *J. Mass Spectrom.* 1997; **32**: 103.
5. Kornexl BE, Werner RA, Gehre M. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1248.
6. Kornexl BE, Gehre M, Hofling R, Werner RA. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1685.
7. Werner RA. *Isot. Environ. Health Stud.* 2003; **39**: 85.
8. Werner RA, Kornexl BE, Rossmann A, Schmidt HL. *Anal. Chim. Acta* 1996; **319**: 159.
9. Farquhar GD, Henry BK, Styles JM. *Rapid Commun. Mass Spectrom.* 1997; **11**: 1554.
10. Brand WA, Tegtmeyer AR, Hilkert A. *Org. Geochem.* 1994; **21**: 585.
11. Crowley SF, Spero SP, Winter DA, Sloane HJ, Croudace IW, Steele DF. *Rapid Commun. Mass Spectrom.* 2008; **22**: 1703.
12. Boettger T, Haupt M, Knoller K, Weise SM, Waterhouse JS, Rinne KT, Loader NJ, Sonninen E, Jungner H,

DOI: 10.1002/rcm

Masson-Delmotte V, Stievenard M, Guillemin MT, Pierre M, Pazdur A, Leuenberger M, Filot M, Saurer M, Reynolds CE, Helle G, Schleser GH. *Anal. Chem.* 2007; **79**: 4603.
13. Werner RA, Brand WA. *Rapid Commun. Mass Spectrom.* 2001; **15**: 501.
14. Sternberg L, Pinzon MC, Anderson WT, Jahren AH. *Plant Cell Environ.* 2006; **29**: 1881.
15. Hoshino E, Wada Y, Nishizawa K. *J. Biosci. Bioeng.* 1999; **88**: 519.
16. Sternberg L, Deniro MJ. *Science* 1983; **220**: 947.
17. Lang ARG, Mason SG. *Can. J. Chem.-Rev. Can. Chim.* 1959; **37**: 1829.
18. Farquhar GD, Henry BK, Styles JM. *Rapid Commun. Mass Spectrom.* 1997; **11**: 1554.
19. Schuetze M. *Zeitschr. Analyt. Chemie* 1939; **118**: 245.
20. Unterzaucher J. *Berichte Der Deutschen Chemischen Gesellschaft* 1940; **73**: 391.
21. Unterzaucher J. *Anal. Chem.* 1952; **24**: 1523.
22. Unterzaucher J. *Analyst* 1952; **77**: 584.
23. Belcher R, Davies DH, West DD. *Talanta* 1965; **12**: 43.
24. Dennis MJ, Wilson P, Kelly S, Parker I. *J. Anal. Appl. Pyrolysis* 1998; **47**: 95.
25. Kornexl B, Medina R, Schmidt HL. *Isotopenpraxis* 1994; **30**: 215.
26. Epstein S, Mayeda T. *Geochim. Cosmochim. Acta* 1953; **4**: 213.
27. Coplen TB. *Int. J. Mass Spectrom. Ion Phys.* 1973; **11**: 37.
28. Coplen TB. *Chem. Geol.* 1988; **72**: 293.
29. Coplen TB. *Pure Appl. Chem.* 1994; **66**: 273.
30. Boner M, Hecker K, Foerstel H. *GASIR Meeting*, Jena, October 2005.
31. Werner RA, Bruch BA, Brand WA. *Rapid Commun. Mass Spectrom.* 1999; **13**: 1237.
32. Olsen J, Seierstad I, Vinther B, Johnsen S, Heinemeier J. *Int. J. Mass Spectrom.* 2006; **254**: 44.
33. Guidotti S, Verstappen BMMA, Aerts-Bijma AT, Jansen HG, van Dijk G, Meijer HAJ. 2009; in preparation.
34. Meijer HAJ. *Isot. Environ. Health Stud.* 2009; in press.
35. Stuart-Williams H, Wong SC, Farquhar GD, Keitel C, Clayton S. *Rapid Commun. Mass Spectrom.* 2008; **22**: 1117.
36. Wedeking KW, Hayes JM. *Isot. Geosci.* 1983; **1**: 357.
37. Murphy RC, Clay KL. *Biomed. Mass Spectrom.* 1979; **6**: 309.
38. Sternberg LDL, Deniro MJ, Savidge RA. *Plant Physiol.* 1986; **82**: 423.
39. Schmidt HL, Werner RA, Rossmann A. *Phytochemistry* 2001; **58**: 9.
40. Benson BB, Krause DJ. *Oxygen: Limnology and Oceanography* 1980; **25**: 662.
41. Maltsev KA, Galimov EM. *Geokhimiya* **1986**: 394.
42. Stalker L, Farrimond P, Larter SR. *Org. Geochem.* 1994; **22**: 477.
43. Sternberg L, Deniro MJD. *Geochim. Cosmochim. Acta* 1983; **47**: 2271.
44. Schimmelmann A. *Final Report to the International Atomic Energy Agency (IAEA)*, Contract No. 11792/R0 2002.
45. Santrock J, Hayes JM. *Anal. Chem.* 1985; **57**: 1441.
46. Coplen TB, Brand WA, Gehre M, Gröning M, Meijer HAJ, Toman B, Verkouteren RM. *Rapid Commun. Mass Spectrom.* 2006; **20**: 3165.
47. Coplen TB, Brand WA, Gehre M, Gröning M, Meijer HAJ, Toman B, Verkouteren RM. *Anal. Chem.* 2006; **78**: 2439.
48. Böhlke JK, Mroczkowski SJ, Coplen TB. *Rapid Commun. Mass Spectrom.* 2003; **17**: 1835.
49. Halas S, Szaran J, Czarnacki M, Tanweer A. *Geostandards Geoanal. Res.* 2007; **31**: 61.
50. Boschetti T, Iacumin P. *Rapid Commun. Mass Spectrom.* 2005; **19**: 3007.
51. Bao HM, Thiemens MH. *Anal. Chem.* 2000; **72**: 4029.
52. van Hook WA. Isotope separation by gas chromatography. In *Isotope Effects in Chemical Processes*, Gould RF (ed). *Advances in Chemistry Series*, ACS Publications: Washington DC, 1969; 99–118.
53. Hayes JM, Freeman KH, Ricci MP, Studley SA, Schoell M, Moldowan JM, Carlson R, Gallegos E, Habfast K, Brand W. A new approach to isotope-ratio-monitoring gas chromatography mass spectrometry. In *Advances in Mass Spectrometry*, vol. 11B, Longevialle P (ed). Heyden and Son: London, 1989; 1108.
54. Brand WA. *J. Mass Spectrom.* 1996; **31**: 225.
55. Belcher R, Ingram G, Majer JR. *Microchim. Acta* **1968**: 418.
56. Pella E. *Anal. Chim. Acta* 1966; **35**: 96.
57. Brotherton TK, Lynn JW. *Chem. Rev.* 1959; **59**: 841.
58. Johnston. *Trans. Roy. Soc. Edinburgh* 1840; **1**: 30.
59. Bircumshaw LL, Tayler FM, Whiffen DH. *J. Chem. Soc.* 1954; 931.
60. Meyer V, Goldschmidt H. *Berichte der Deutschen Chemischen Gesellschaft* 1882; **15**: 1164.
61. Meyer E. *Monatshefte fiir Chemie* 1969; 462.
62. Revesz K, Böhlke JK. *Anal. Chem.* 2002; **74**: 5410.
63. Accoe F, Berglund M, Geypens B, Taylor P. *Rapid Commun. Mass Spectrom.* 2008; **22**: 2280.
64. Gonfiantini R. *Nature* 1978; **271**: 534.
65. Hannon JE, Böhlke JK, Mroczkowski SJ. *Rapid Commun. Mass Spectrom.* 2008; **22**: 4109.
66. Kroopnic P, Craig H. *Science* 1972; **175**: 54.
67. Barkan E, Luz B. *Rapid Commun. Mass Spectrom.* 2003; **17**: 2809.
68. Luz B, Barkan E. *Geochim. Cosmochim. Acta* 2005; **69**: 1099.
69. Muller JW. *J. Res. Natl. Inst. Standards Technol.* 2000; **105**: 551.
70. Gonfiantini R, Stichler W, Rozanski K. *IAEA TECDOC* 1995; **825**: 13.

DOI: 10.1002/rcm