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Interlaboratory Comparison of Different Analytical Techniques for the Determination of Oxygen-18 Abundance

J. R. Speakman,*[,1] K. A. Nagy,[2] D. Masman,[3] W. G. Mook,[3] S. D. Poppitt,[1] G. E. Strathearn,[2] and P. A. Racey[1]

Department of Zoology, University of Aberdeen, Aberdeen, Scotland, U.K. AB9 2TN, Laboratory of Biomedical and Environmental Sciences, University of California, Los Angeles, 900 Veteran Avenue, Los Angeles, California 90024, and Rijksuniversiteit Groningen, Centrum voor Isotopen Onderzoek, Westersingel 34, 9718 CM Groningen, The Netherlands

A series of six artificially enriched waters, containing between 2500 and 6500 ppm oxygen-18, was prepared by adding weighed portions of distilled water and highly enriched $H_2{}^{18}O$. These waters were analyzed by radio activation analysis (proton activation, PAA) and by gas isotope ratio mass spectrometry (gIRMS) with CO_2 as the analysis gas. Carbon dioxide was prepared from water samples either by using the guanidine reaction, with 3-, 5-, and 10-μL samples, or by isotopic equilibration, using small (10 μL) and large (1.5 mL) samples. The large samples were diluted to the natural abundance range prior to analysis as were the 3-μL guanidine samples. Precision was greatest with large sample CO_2 equilibration (mean relative standard deviation (RSD) = 0.108%). The other gIRMS gas preparation techniques had lower precision (3 μL guanidine, RSD = 0.529%, 5 μL guanidine, RSD = 0.364%, 10 μL guanidine, RSD = 0.48%; 10 μL equilibration, RSD = 0.43%) and the lowest precision occurred with PAA (RSD = 0.58%). For all the techniques except small sample equilibration, accuracy (percent deviation of mean evaluation from expected gravimetric mean) was worse than precision (RSD). Average deviations, including sign, were as follows: 3 μL guanidine, −0.97%, 5 μL guanidine, −1.43%, 10 μL guanidine, −1.30%; 10 μL equilibration, −0.33%; 1.5 mL equilibration, −1.55%; and proton activation, +0.68%. Gas IRMS, independent of the preparation technique, tended to underevaluate abundance, and PAA tended to overevaluate abundance. Both precision and accuracy were very much worse at the high levels of enrichment studied than have previously been reported for estimates at the natural abundance levels of 1800–2050 ppm ^{18}O.

Two distinct analytical techniques are available for the evaluation of the enrichment of ^{18}O in samples of water, radio activation analysis and gas isotope ratio mass spectrometry (gIRMS). In the former method samples are bombarded with particles of various types to effect nuclear reactions in the ^{18}O, the decay products of which can then be measured. The most useful technique involves proton bombardment (proton activation analysis, PAA) to form ^{18}F, which decays with a half-life of 110 min (*1*). This has the advantage that the product nuclide has a long half-life and does not therefore require very rapid product transfer systems which are necessary for analysis of other RAA products, for example ^{17}N (*2*) with a 4.14-s half-life or ^{19}O (*3*) with a 29-s half-life.

With gIRMS the water is converted to a gas and ionized at very low pressure. A mass spectrum is then measured for the major and minor beams which correspond to the appropriate masses for molecular ions containing either ^{16}O or ^{18}O. There are two problems with the direct analysis of gaseous water, firstly it is corrosive, and secondly it is difficult to remove from the inlet system of the analyser once analysis is complete (*4*). These problems lead to memory effects unless a long evacuation is performed between samples. Nevertheless a commercial machine is available (VG Isogas, Aquasira) which analyzes water directly (*5*) and is in widespread use (e.g. refs 6 and 7). The memory effects of this machine however, and the fact it is a single inlet device, restrict its usage to samples of relatively low ^{18}O enrichment (<1000δ‰ relative to V-SMOW) and to samples where the approximate range of the sample is known beforehand, so that appropriate standards may be run adjacent to the unknown.

The preferred gas for analysis by gIRMS is CO_2. Several different procedures are available for the preparation of CO_2 from water for gIRMS analysis (reviewed in ref 8). The most popular technique is that of equilibration (*9*), in which water and CO_2 are mixed at a controlled temperature and react as follows:

$$H_2{}^{18}O + C^{16}O_2 \rightarrow H_2{}^{16}O + C^{18}O_2 \quad (1)$$

After an equilibration period, which is dependent upon the system (7 h for large volume, well-agitated systems to 48–72 h for small volume equilibration), the CO_2 is removed and

[1] University of Aberdeen.
[2] University of California.
[3] Rijksuniversiteit Groningen.

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purified cryogenically and admitted to the mass spectrometer. The enrichment of ^{18}O in the original water is calculated (*10*), from the measured isotopic ratio in the carbon dioxide, the relative moles of each species originally present, and the equilibrium fractionation factor between CO_2 and H_2O (which equals 1.0412 at 25 °C, refs 11 and 12). Commonly, large samples and automated agitation units are used (e.g., ref 13), however, systems are in use for routine analysis of much smaller samples of 2–100 μL (*14*, *15*).

An alternative technique is chemical conversion, utilizing guanidine hydrochloride to convert water to CO_2 (*12*, *15*, *16*). This reaction involves a two-stage process. In the first stage 2–10 μL of water and 100 mg of anhydrous guanidine hydrochloride are reacted together in a sealed vessel at 250 °C for 3 h. The reagents react as follows:

$$NH_2C{:}(NH){\cdot}NH_2{\cdot}HCl + 2H_2O \xrightarrow{>250\ °C} 2NH_3 + CO_2 + NH_4{\cdot}Cl \quad (2)$$

On cooling the ammonia and CO_2 produced combine to form ammonium carbamate ($NH_4{\cdot}NH_2{\cdot}CO_2$). Then, the vessel containing the products is broken under vacuum inside a second evacuated and sealed container having in it 1 mL of 6 M (100%) phosphoric acid. Heating at 90 °C for 90 min yields CO_2 by the reaction

$$NH_4{\cdot}NH_2{\cdot}CO_2 + H_2PO_4 + NH_4{\cdot}CL \xrightarrow{90\ °C} CO_2 + (NH_4)_2{\cdot}PO_4 + NH_4{\cdot}Cl \quad (3)$$

The carbon dioxide released is then purified cryogenically and introduced into the mass spectrometer.

The accuracy and precision of all these procedures have been previously investigated (see ref 8 for review), generally at levels of natural abundance of ^{18}O, but more rarely at artificially high levels of enrichment. Even more infrequently comparison is made between two techniques for analysis of a given water (e.g., refs 1 and 17). To date however, no study has made a comparison of accuracy and precision of all three of the most widely used procedures (proton activation, equilibration, and guanidine conversion) across a series of water standards at high enrichment levels. This study presents such a comparison, across a nominal enrichment range of 2300–6500 ppm ^{18}O (equal to 150 to 2280δ‰ vs V-SMOW).

Enrichments in this range are of interest principally because they are used routinely in tracer studies of animal metabolism, in particular the doubly labeled water technique, in which artificially high enrichments of both ^{18}O and an isotope of hydrogen in the body water are produced by introducing the isotopes as water into the subjects body. The difference in the elimination rates of the isotopes is used to measure CO_2 production (*18–21*).

EXPERIMENTAL SECTION

Preparation and Distribution of Water Standards. A series of six solutions was prepared by dilution of a very high enrichment cocktail (VHEC) which contained both enriched levels of ^2H and ^{18}O at approximately 9 atom % excess (APE) and 19 APE, respectively (manufacturers values, Amersham International PLC, Yeda Isotopes). The dilutant consisted of double distilled tap water, henceforth called standard reference water (SRW).

The standards were prepared into glass vessels. Vessels and all other glassware involved in preparation were cleaned prior to use first by washing in trichloroethylene to remove grease, followed by complete immersion in a solution of Decon (Decon Labs Ltd.) in water, and ultrasound washed for 15 min. The vessels were finally sequentially rinsed in distilled water, a mix of 50:50 water and acetone, and lastly 100% acetone before air drying in an oven at 80 °C. During preparation all vessels were handled only with plastic gloves.

For all standards preparation involved mixing between 0.1 and 0.5 g of VHEC and 19 and 26 g of SRW. The detailed procedure was as follows: weigh vessel (mass A); add 19–26 g of SRW to vessel; weight vessel with SRW (mass B); calculate mass of VHEC to add, and add estimated quantity of VHEC using graduated syringe; weigh vessel containing SRW and VHEC (mass C); seal vessel. The vessels were sealed with rubber caps, and there was no evidence of fractionation of the standards over time after sealing.

All weighings were performed with a five-significant figure balance (Sartorius MP 2904, accuracy ± 0.01 mg) at 25 °C. Weighing was continued until stability was reached in the least significant digit, which was generally 45–60 s.

Samples of the standards from the stock in the vessels were flame sealed into 5-, 10-, or 100-μL pipets (Vitrex) or in the case of large sample equilibration transferred into small glass bottles sealed with rubber gromets in metal caps. Five- and ten-microliter samples were used directly in gIRMS analyses and 100-μL samples were subdivided and analyzed by PAA after distribution to the participant laboratories. Standards were accompanied only by an indication of the rank enrichments and an indication of the anticipated upper and lower limits of the entire series. All samples were therefore analyzed blind.

Gravimetric Evaluation of Enrichments and Sensitivity Analysis. The masses of VHEC and SRW in each vessel were calculated as mass C-B, and mass B-A, respectively. Given the mass of VHEC and SRW in each vessel the nominal enrichment of a given standard was calculated as follows:

$$E_S = \frac{(M_{VHEC}/MM_{VHEC})E_{VHEC} + (M_{SRW}/MM_{SRW})E_{SRW}}{(M_{VHEC} + M_{SRW})/MM_{MIX}} \quad (4)$$

where E_S, E_{VHEC}, and E_{SRW} are the enrichments of ^{18}O in the standard, the very high enrichment cocktail, and the standard reference water, respectively. M_{VHEC} and M_{SRW} are the masses of very high enrichment cocktail and standard reference water, respectively. MM_{VHEC}, MM_{SRW}, and MM_{MIX} are the molecular masses of the very high enrichment cocktail, standard reference water, and the final mix, respectively.

The unknown molecular masses were evaluated from the estimated isotopic abundances of $^1H/^2H$ and $^{16}O/^{18}O$ as follows:

$$MM_{SRW} = 2(1.000147) + 16.0039 = 18.00428$$

$$MM_{VHEC} = 2(1.08) + 16.38 = 18.54$$

$$MM_{MIX} = ((MM_{VHEC}M_{VHEC}) + (MM_{SRW}M_{SRW}))/(M_{VHEC} + M_{SRW})$$

The enrichment of standard reference water (E_{SRW}) was evaluated by using gIRMS (guanidine). While this appears to be an incestuous characterization, mass spectrometric abundance evaluations at natural abundance levels have been validated on many previous occasions (reviewed in ref 8) and therefore we do not feel this represents a serious source of bias. The mean enrichment of SRW (E_{SRW}) evaluated in this way equaled 1999.9 ppm ^{18}O (n = 8). The enrichment of ^{18}O in the VHEC was unknown and was unfortunately beyond the range of accurate evaluation by either gIRMS or PAA. The enrichment of the VHEC was therefore evaluated by solving eq 4 for E_{VHEC} and substituting the mean evaluated enrichment across all techniques (except 3 μL guanidine, which was analyzed at a later date) for the most dilute of the standard series (standard 1) for E_S. With this method the calculated value for E_{VHEC} equaled 187 140 ppm ^{18}O. Substituting this enrichment estimate into eq 4 with the appropriate masses of the SRW and VHEC in all the standard series except the most dilute yielded the nominal gravimetric evaluations for the series (Table I).

All the component parameters in eq 4 are measured with error. The sensitivity of the nominal enrichments to these errors was assessed by using a sensitivity analysis, by allowing the component variables to vary in the following ranges. The masses of SRW and VHEC were allowed to vary by ±0.0001 g, which is 10 times worse than the reported accuracy of the balance. Variation in the enrichment of the most dilute standard (I) equaled 4.04 ppm across all the techniques (s.e., n = 6 methods, see below). This variation results in a range for the estimate of E_{VHEC} of 185380–188930 ppm ^{18}O and this range was used in the sensitivity analysis. Empirically observed maxima and minima in the evaluations of E_{SRW} were also used in the analysis (minimum = 1995.8 ppm, maximum = 2009.0 ppm ^{18}O). The effects of these

Table I. Nominal Enrichments of ^{18}O in a Series of Six Standard Solutions[a]

std	nominal enrichment (ppm ^{18}O)	range (ppm ^{18}O) min	range (ppm ^{18}O) max
I	2312.1	2303.0	2329.2
II	2730.6	2720.6	2740.6
III	3385.2	3367.3	3403.5
IV	4751.8	4716.3	4787.3
V	5449.6	5405.5	5493.8
VI	6504.1	6446.6	6561.7

[a] Enrichment for standard I was the mean enrichment evaluated across seven preparation techniques. The range for standard I was the range across these techniques. Nominal enrichments for standards II–VI were evaluated gravimetrically from the weighed quantities of a standard reference water, at natural abundance (1999.9 ppm ^{18}O), mixed with a very high enrichment cocktail (187 140 ppm ^{18}O). The ranges for standards II–VI were estimated by using a sensitivity analysis (refer to text and Table II).

Table II. Effects of Error in the Component Parameters of an Equation for Calculation of the Nominal Enrichment of ^{18}O in Standard VI, by Gravimetric Procedures

	manipulation	ppm ^{18}O original	ppm ^{18}O recalcd	error, %
1. mass of std ref water	+0.0001 g	6504.12	6504.10	−0.00035
	−0.0001 g	6504.12	6504.14	+0.00034
2. mass of VHEC[a]	+0.0001 g	6504.12	6504.96	+0.013
	−0.0001 g	6504.12	6503.24	−0.0134
3. enrichment of std ref water	set to 1995.8 ppm	6504.12	6500.47	−0.056
	set to 2009.0 ppm	6504.12	6512.96	+0.136
4. enrichment of VHEC[a]	set to 185 380 ppm	6504.12	6451.30	−0.812
	set to 188 930 ppm	6504.12	6560.83	+0.872

[a] Very high enrichment cocktail.

variations in the estimate of the nominal enrichment of the most enriched standard (VI) are shown in Table II. Clearly the most influential factor was variation in E_{VHEC}. Since E_{VHEC} was evaluated as a mean across all the methods, the calculated nominal enrichments will not be biased in favor of, or against, any one technique. Maximum and minimum limits to the gravimetric evaluations for the series were defined by setting the worst possible combinations of errors, and these limits are shown with the mean nominal enrichments in Table I.

Analyses. Analyses of the samples were made by proton activation analysis (*1*) (KAN) and by gIRMS using the guanidine preparation technique (*12*, *15*, *16*) for 5- and 10-μL samples (JRS/SDP/PAR) and 3-μL samples (GS/KAN) and the equilibration technique (*9*, *15*) for 10-μL (DM/WGM) and 1.5-mL samples (GS/KAN). In addition the most dilute solution was also analyzed by direct mass spectrometry of gaseous water (*22*) using an Aquasira machine (W. Scott, Scottish Universities Research and Reactor Centre, East Kilbride). Due to a laboratory accident standard II was analyzed only by PAA and by the small volume equilibrium technique. For large volume equilibrium and 3 μL guanidine, standard waters were further diluted (by a factor of 1:50 or 1:100) with a characterized water at background abundance (1989 ppm ^{18}O) prior to analysis. In the case of gIRMS all enrichments were calculated on the basis of the major to minor beam ratios (44:46 for CO_2) in comparison to known working standards using dual inlet machines (Finnigan-MAT and VG Isogas) and then normalized to the V-SMOW/SLAP scale (*23*). Absolute enrichments (ppm) were then calculated (*4*) by using 2005.2 ppm as the best estimate of the enrichment of V-SMOW (*24*).

RESULTS

Estimated mean isotope enrichments, standard deviation of replicate analyses, precision (relative standard deviation, standard deviation × 100/mean), and number of replicates for all techniques and all standards are summarized in Table III. In addition, accuracy, calculated as the percentage deviation of the technique's mean evaluation from the mean nominal enrichment (Table I) for standards II–VI, and for standard I as percent deviation from the average enrichment across all techniques, is also shown in Table III.

Precision (relative standard deviation) remained almost constant throughout the range of evaluations for any given technique. Mean and standard deviation precision across all six standards were as follows: guanidine 3 μL (0.528% standard deviation = 0.08%, n = 5), guanidine 5 μL (0.364%, standard deviation = 0.13%, n = 5); guanidine 10 μL (0.492%, standard deviation = 0.13%, n = 5); equilibrium 10 μL (0.435%, standard deviation = 0.33%, n = 6); equilibrium 1.5 mL (0.108%, standard deviation = 0.009%, n = 5); PAA (0.58%, standard deviation = 0.12%; n = 6). Large sample equilibration had by far the best precision and was almost 4 times better than the other gIRMS sample preparation techniques which had a precision of approximately 0.4%. Precision for PAA was significantly lower than for gIRMS with all preparation techniques combined (t = 2.87, p = 0.040, one-tailed) but not significantly lower than gIRMS excluding large sample equilibration (t = 1.94, p = 0.077, one-tailed).

Significant differences in mean isotopic enrichment evaluations both between techniques and in comparison of the techniques to the nominal gravimetric evaluation were apparent for all the standards. Mean evaluations using guanidine were significantly lower than the gravimetric evaluations (t = 2.15, p = 0.026, n = 10, one-tailed). However, the degree of difference was progressively greater as standard enrichment increased and was most marked in the standard of greatest ^{18}O enrichment (standard VI, Table III). In 11 out of 15 evaluations using guanidine, inaccuracy (percent deviation) was greater than precision (relative standard deviation). Small sample equilibration also significantly underestimated the nominal gravimetric enrichment (t = 2.25, p = 0.037, n = 6, one-tailed), however, the average deviation (−0.33%, standard deviation = 0.35%), was considerably smaller than the average deviation when using guanidine (−1.22%, standard deviation = 1.89%, n = 15). In addition when small sample equilibration was used, the inaccuracy (percent deviation from nominal) was always exceeded by the precision (relative standard deviation). Although precision was greatest with large sample equilibration, it was also the least accurate technique (average deviation = −1.54%, standard deviation = 1.00%, n = 5), and inaccuracy always exceeded precision. In addition the pattern of error was unusual in that for standards above the most dilute (III–VI) the accuracy progressively improved with increasing ^{18}O enrichment. With PAA, abundances were significantly overestimated (t = 5.09, p = 0.0017, n = 6, one-tailed). This contrasted gIRMS, where the pattern, independent of preparation technique, was to underestimate abundance, relative to the nominal enrichments. In four of six evaluations with PAA inaccuracy (percent deviation) exceeded precision (relative standard deviation). The average deviation was +0.68%, standard deviation = 0.30%, n = 6.

DISCUSSION

There are few validation comparisons in the literature in which accuracy and precision of these techniques have been examined at the levels of enrichment reported here, and none in which the three techniques are all compared across a standard series and analyzed blind.

Precision. The use of guanidine to prepare CO_2 was originally described by Boyer et al. (*16*), who reported a precision of 10δ‰ (standard deviation) at natural abundance (2000 ppm), which is equivalent to a relative standard deviation of 1%. This is 2 to 3 times worse than the precision reported

Table III. Evaluations of ^{18}O Enrichment of Six Standard Waters, Analyzed by Proton Activation Analysis (PAA) and by Gas Isotope Ratio Mass Spectrometry of CO_2 Prepared from the Water by Guanidine Conversion of 3-, 5-, and 10-μL Samples, and Equilibration of Gaseous CO_2 with 10-μL and 1.5-mL Water Samples[a]

		guanidine			CO_2 equilibrium		
std		3 μL	5 μL	10 μL	10 μL	1.5 mL	PAA
I	mean	2315.1	2313.7	2329.2	2307.6	2303.0	2316.0
	std dev	10.9	7.91	6.2	4.7	1.25	10.6
	RSD, %	0.47	0.34	0.26	0.20	0.05	0.46
	n	5	4	7	3	3	6
	error, %	+0.17	+0.06	+0.73	−0.19	−0.39	+0.16
II	mean				2723.6		2747.3
	std dev				8.4		17.3
	RSD, %				0.31		0.63
	n				3		6
	error, %				−0.31		+0.55
III	mean	3336.2	3329.9	3353.5	3392.1	3282.0	3413.5
	std dev	15.6	16.2	19.6	15.9	9.03	23.5
	RSD, %	0.46	0.48	0.58	0.47	0.27	0.69
	n	5	4	6	3	3	6
	error, %	−1.44	−1.69	−0.99	+0.14	−3.1	+0.77
IV	mean	4711.4	4705.8	4704.8	4719.6	4673.0	4807.6
	std dev	22.9	13.4	24.6	5.76	2.5	33.3
	RSD, %	0.48	0.20	0.52	0.12	0.05	0.69
	n	3	4	6	3	3	6
	error, %	−0.85	−1.02	−1.04	−0.73	−1.71	+1.11
V	mean	5475.7	5440.4	5490.11	5410.4	5382.0	5499.3
	std dev	32.8	28.6	27.7	57.4	6.0	36.5
	RSD, %	0.59	0.52	0.50	1.06	0.11	0.66
	n	4	3	4	3	3	6
	error, %	+0.47	−0.23	+0.68	−0.77	−1.30	+0.85
VI	mean	6295.9	6234.7	6124.2	6497.5	6426.0	6549.0
	std dev	40.6	17.9	36.9	29.2	3.9	25.6
	RSD, %	0.64	0.28	0.6	0.45	0.06	0.39
	n	4	3	4	3	2	6
	error, %	−3.2	−4.19	−5.89	−0.15	−1.25	+0.63

[a] Direct gIRMS of water using an aquasira machine for standard I gave a mean of 2303.2 ppm, std dev = 2.2 ppm, RSD = 0.46%, n = 6. In all cases mean enrichment, standard deviation of replicate analyses, precision (relative standard deviation), number of replicates, and accuracy, expressed as the percentage deviation from the nominal gravimetric abundance (standards two to six) or percentage deviation from the mean estimate across all techniques (standard one), are shown.

here. However, mass spectrometry instrumentation has improved significantly over the past 30 years and the difference in precision reflects this improvement in instrumentation rather than any differences in preparation. More recently precision of the technique at natural abundance (V-SMOW) was estimated at 0.19δ‰ (standard deviation) or 0.02% (relative standard deviation, n = 10) (*12*), which is very much better than with any technique reported here. A similar precision at natural abundance to that found by Dugan et al. (*12*), when analyzing biological samples (0.08–0.28δ‰, standard deviation, n = 4) was found by Wong et al. (*15*) but precision declined at higher enrichment (0.88–1.07δ‰, standard deviation, n = 4, at 250δ‰, equivalent to approximately 2.1 ppm at 2506.5 ppm ^{18}O or 0.08% relative standard deviation). This latter precision is better than that reported in this study. However this greater precision may depend upon the ability to utilize working standards closer to the unknown, which was not run blind. A precision of 60 ppm (standard deviation, n = 10) at 15700 ppm was found by Hoefs (*4*), which is equivalent to a relative standard deviation of 0.38%, which is almost equal to the precision reported for guanidine here. Over a range of solutions from 1980 to 12010 ppm ^{18}O using 1.5-μL water samples and the guanidine method for gas preparation (*1*), precision was highly variable between samples, and over the same enrichment range as used in the present study varied between 0 and 10% (relative standard deviation). To some extent this variation reflects the use of older mass spectrometric instrumentation but may also reflect the very small sample volume. This may indicate there is a minimum volume necessary for precise evaluation when using the guanidine technique. We did not however find any significant improvement in precision as sample size increased from 3 to 10 μL, and therefore if there is a critical minimum volume, it is certainly less than 3 μL.

The precisions of analyses with gIRMS, for gases prepared by the guanidine procedure and by small sample CO_2 equilibration, were lower than previous evaluations at natural abundance. One factor influencing this decline in precision is the inability to run appropriate comparison standards of similar abundance to unknowns. Machine parameters, for example the HT potential and beam focusing currents, must all be optimized therefore to measure enrichments at the natural abundance level of the comparison standard, and these may not necessarily also be the best settings for precise measurement at high abundance. This interpretation of the poor precision of gIRMS at high abundance is supported by the observation that the precision with large volume equilibration, in which the sample were diluted to near to natural abundance levels prior to analysis had significantly improved precision over the other preparation techniques, and in same range as previous measurements, also at natural abundance (*7*, *13*, *25–28*). An average precision of ±400 ppm for large volume sample equilibration for samples ranging up to 100000 ppm, analyzed without dilution, was found by Dostrovsky and Klein (*29*), which in the mid-range is equivalent to 0.8% (relative standard deviation), but again precision would be expected to be worse on old instrumentation, consequently the effect on analyzing large volume equilibration samples at

high enrichment levels without dilution cannot be judged from that study. However, the 3-μL guanidine samples were also prepared from diluted samples and these samples do not show improved precision. The reason for the low precision with these samples is obscure, but may reflect inexperience with the guanidine procedure in the laboratory which ran these samples (GS/KAN).

PAA was used by Wood et al. (*1*) to evaluate a range of solutions from 1980 to 12 010 ppm ^{18}O. Precision over the range used in that study averaged 2.62% (relative standard deviation, standard deviation = 0.66%, n = 11), which is more than 4 times worse than in the current study. The reasons for this improvement include better γ-counting techniques, improved data analysis software, increased control of the cyclotron beam focus and intensity, and practice.

Accuracy. For all techniques, except small sample equilibration, inaccuracy exceeded precision, and many evaluations fell outside the calculated range for the gravimetric nominal enrichments (Tables I and III). The accuracy reported here for guanidine preparation is much worse than that reported previously for evaluations at background levels (*12*). The tendency for guanidine preparation to underestimate at high enrichments, relative to gravimetric estimates, has been previously noted (*1*); however in that study no large reduction in accuracy above 6000 ppm was found. The dramatic fall off in accuracy above 6000 ppm found in the present study is unlikely to be a consequence of isotopic fractionation during the reaction at high enrichments, since it was also evident in the 3-μL samples which were diluted prior to analysis. Neither can it be attributed to a bias in a particular mass spectrometer. Indeed the consistency of the estimates between the 3- and 5/10-μL guanidine preparations is remarkable, considering the 3-μL samples were prepared from diluted samples and measured on a different mass spectrometer. The causes of the consistency remain unknown.

The consistent underevaluation of on average 1% with all the gIRMS techniques indicates some fractionation is occurring, possibly during cryogenic capture of the evolved products, since this is the only common point across the different techniques performed in different laboratories and analyzed on different machines.

The relatively poor accuracy when employing large volume equilibration was unexpected, especially since small sample equilibration showed the best precision. Two factors may have influenced this error. Firstly the large sample equilibrium samples were sealed in small bottles rather than flame-sealed in capilliaries, and fractionation may have occurred during storage and transit (UK to USA) due to exchange with atmospheric water. Secondly errors may have been introduced at dilution. The sensitivity analysis in this study (Table II), for a similar dilution process however indicates this is unlikely to contribute much to the error unless gross weighing errors were made. Both of these factors can be eliminated however since the same inaccuracy was not apparent with 3-μL guanidine preparation which was performed on the same waters. The most probable cause of this accuracy error was contamination in the automatic manifold, whereby water from one sample was not fuly purged from the system and could then exchange with the subsequent sample.

Consistent overestimation of the theoretical abundance of samples by PAA was also reported by Wood et al. (*1*). In that case inaccuracies in the method may have reflected inaccuracies in the natural abundance comparison samples. In the current study that source of error seems unlikely to be of importance since the natural abundance comparator was checked prior to calculations by gIRMS. Over evaluation of abundance at high enrichments by on average 0.68% (standard deviation = 0.3%, n = 6) would appear therefore to be a feature of the PAA technique. One possible cause of this error was fractionation during distillation of the water into microcapillaries, as described by Nagy (*30*), although Wood et al. (*1*) found no evidence of fractionation at this stage of the process in their study.

CONCLUSIONS

Current techniques for the evaluation of isotopic abundance of ^{18}O in artificially enriched water varied in both precision and accuracy over the range 2500–6500 ppm ^{18}O. All gIRMS techniques studied underestimated the abundance, while PAA overestimated abundance. However, the least significant differences were found when using small sample CO_2 equilibration. Where absolute enrichment estimates are required, as opposed to relative abundance estimates, which are used in, for example, the doubly labeled water technique (*18–21*), small sample equilibration would appear to be the best technique. Precision was greater with large volume equilibration on samples which had been diluted to close to the natural abundance range prior to analysis. However these samples also had a decreased accuracy, which offset the greater precision. Undiluted gIRMS analyses involved lower precision and PAA the lowest precision. Generally however precision was better than accuracy, suggesting that evaluations of analysis techniques based upon precision estimates of replicate samples alone are insufficient.

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Registry No. ^{18}O, 14797-71-8; water, 7732-18-5.

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