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High-Precision δ^2H and δ^{18}O Measurement for Water and Volatile Organic Compounds by Continuous-Flow Pyrolysis Isotope Ratio Mass Spectrometry

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An on-line pyrolysis technique is described for analysis of δ^2H and δ^{18}O by continuous-flow isotope ratio mass spectrometry. A pyrolysis furnace packed with nickel, on which carbon is deposited as required, is shown to give efficient conversion of nanomole quantities of sample material to H_2 and CO. A water trap and postpyrolysis gas chromatograph separate analyte gases from traces of H_2O and CO_2. The isotope ratio mass spectrometer used gives adequate separation of $^2H^1H^+$ and $^4He^+$ to allow use of He as carrier gas. The pyrolysis technique provides a rapid method for analysis of water with a precision of ±2‰ for δ^2H and ±0.3‰ for δ^{18}O. Similar precisions are obtained for injections of 0.5 μL of urine and volatile organic compounds. Memory between samples is <2% for both δ^2H and δ^{18}O measurement. The technique is accurate over a wide range of enrichment for both δ^2H and δ^{18}O and is suitable for use in natural abundance and tracer studies of liquid samples.

High-precision measurement of the stable isotopes of H, C, N, O, and S by isotope ratio mass spectrometry (IRMS) has conventionally involved off-line conversion of samples to H_2, N_2, CO, CO_2, and SO_2 prior to isotopic analysis of sample and reference gases from a dual-inlet system. Continuous-flow isotope ratio mass spectrometry (CF-IRMS) methods for isotopic analysis of permanent gases have evolved rapidly since being first reported in the late 1970s.[1] By the early 1990s, CF-IRMS methods were available for rapid on-line conversion and measurement of δ^{13}C, δ^{15}N, δ^{18}O, or δ^{34}S.[2] Helium has been used exclusively as the inert carrier gas in continuous-flow systems. Poor separation of the $^2H^1H^+$ ion beam from the tail of the much more intense $^4He^+$ ion beam has until recently precluded high-precision measurement of δ^2H by CF-IRMS.[3,4] Tobias et al.[3] used heated palladium filters to physically separate H_2 from He carrier gas, while Prosser and Scrimgeour[4] have built a novel IRMS instrument with high dispersion to achieve high-precision measurement of δ^2H in He carrier gas.

Interfacing IRMS with gas chromatography (GC) has revolutionized compound-specific isotopic analysis. Most progress has been made in measurement of ^{13}C abundance by combustion of GC-separated compounds to CO_2 and the subsequent measurement of the $^{12}C^{16}O^{16}O$:$^{13}C^{16}O^{16}O$ ratio. Routine δ^{13}C analysis can now be perfomed for subnanogram quantities of sample material.[5] Compound-specific δ^{13}C analysis by GC-combustion-IRMS has found applications in areas as diverse as metabolite studies, food adulteration, archaeology, and geochemistry.[6] The direct measurement of δ^2H and/or δ^{18}O for individual compounds, other than water,[3,7,8] by GC-IRMS is as yet unreported. The combustion methods use CuO or NiO as an oxygen source for conversion of hydrocarbons to CO_2 and H_2O and hence do not easily lend themselves to either δ^2H or δ^{18}O measurement. On-line pyrolysis IRMS techniques have been developed for δ^{18}O measurement in milligram amounts of cellulose, carbohydrate, and some aromatics[9,10] on glassy carbon at a temperature of >1000 °C.

Hydrogen isotope ratio measurement has been most widely applied to water samples, at natural abundance in hydrology, geochemistry-, and food authenticity and at tracer level in physiology. Conversion of microliter volumes of H_2O to H_2 has generally involved reduction with zinc in a batch process[11,12] or on-line with uranium.[13] The zinc reduction method is inconvenienced by difficulties in handling and cleaning of the catalyst,[14] while the uranium reduction method is known to suffer from memory effects. Alternatively, submilliliter volumes of H_2O can be equilibrated with H_2, in the presence of a platinum catalyst, and the resultant $^2H^1H$:$^1H^1H$ ratio measured.[15,16] The platinum catalyst may be physically isolated from the water (platinum on alumina), giving a slow, temperature-insensitive equilibration,[15] or supported on a porous hydrophilic polymer (Hokko beads) to obtain rapid equilibration in under 1 h.[16] Measurement of δ^{18}O in water samples is also conventionally undertaken by equilibra-

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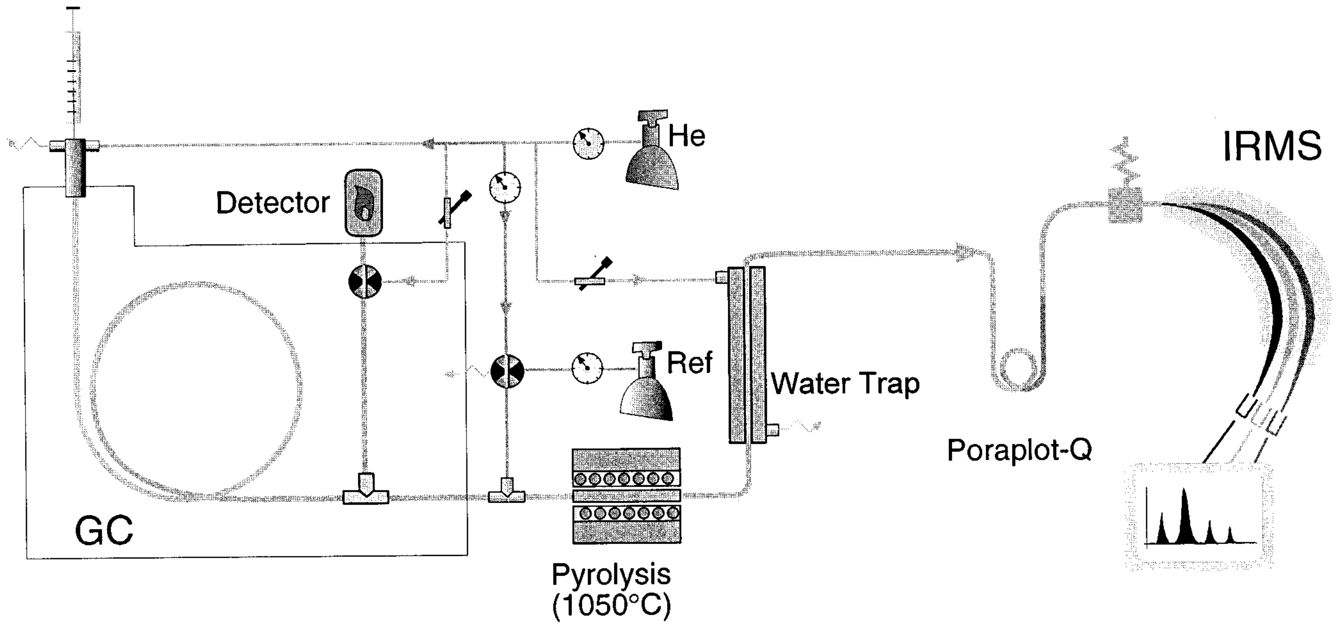

Figure 1. Schematic diagram of the continuous-flow pyrolysis isotope ratio mass spectrometer. A HP 5890 GC with a column of deactivated fused silica capillary is interfaced to a Europa Scientific 20-20 DH IRMS instrument via a pyrolysis furnace, Nafion water trap, and Poraplot Q column.

tion, with CO_2 and the resultant $^{12}C^{18}O^{16}O$:$^{12}C^{16}O^{16}O$ ratio measured.[17]

There is currently intense activity in the development of new methods for both batch processes and on-line conversion of water for δ^2H and/or $\delta^{18}O$ measurement by CF-IRMS.[3,4,7,8,18−21] Batch processes for reduction of microliter volumes of water for δ^2H measurement using manganese[18] and chromium[19] have been reported to give a precision of ±1‰. It is claimed that these reduction methods do not suffer from the surface inhomogeneity and H_2 exchange problems associated with zinc reduction. A batch process based on equilibration of water samples with H_2 and CO_2 for δ^2H and $\delta^{18}O$ measurement, respectively, has also recently become commercially available.[20] On-line reduction of nanoliter volumes of water for δ^2H measurement using nickel at 850 °C has been reported by Tobias et al.[3] to give a precision of <10‰.

In a recent study, we reported the use of a mixture of nickelized carbon and nickel catalysts in the on-line conversion of water for δ^2H and $\delta^{18}O$ measurement as H_2 and CO, respectively.[7] This method allowed accurate analysis of nanoliter volumes of water samples with a precision of ±4‰ for δ^2H and ±0.4‰ for $\delta^{18}O$. Between-sample memory was, however, significant, necessitating double washes between samples. These memory effects are envisaged as being limiting in future applications involving chromatographic separation of analytes prior to pyrolysis. We have, therefore, refined the on-line conversion to minimize memory while retaining a system capable of measuring both δ^2H and $\delta^{18}O$. By use of a nickel catalyst, onto which elemental carbon is deposited by pyrolysis of hexane as necessary, the degree of memory between samples is much reduced. This pyrolysis technique has been applied to the analysis of δ^2H and $\delta^{18}O$ in waters, urine, and volatile organic compounds at both natural abundance and enriched levels.

EXPERIMENTAL SECTION

Instrumentation. A schematic of the continuous-flow pyrolysis IRMS instrument used is given in Figure 1. The isotope ratio mass spectrometer used was a Europa Scientific 20-20 (Crewe, Cheshire, UK) with flared flight-tube and additional collector spur, as described previously by Prosser and Scrimgeour.[4] The large physical separation between ion beams given by this geometry allows measurement of $^2H^1H^+$ in the presence of He, permitting δ^2H measurement. The upper limit for the abundance sensitivity of the tailing from $^4He^+$ (m/z 4) under the m/z 3 collector is 0.5 ppm.[4] The sensitivity of the amplifier on the additional collector (m/z 3) is 1000-fold that of the m/z 2 collector. The standard triple collector allows measurement of $\delta^{18}O$ for CO without system modification. An electromagnet, from Europa Scientific, allowed switching between the field strengths required for measurement of the $^2H^1H$:$^1H^1H$ and $^{12}C^{18}O$:$^{12}C^{16}O$ ratios. The IRMS was operated with a source pressure of $\sim 2 \times 10^{-6}$ mbar and an accelerating potential of ~4 kV. Eluent from the interface flowed via a crimp into the IRMS at a rate of ~ 0.05 mL min^{-1}.

A HP 5890 Series II gas chromatograph (Hewlett Packard) and high temperature microfurnace heating an alumina tube, 0.8 mm i.d. × 220 mm (Degussa, Macclesfield, UK), were interfaced with the IRMS. The injection port and oven of the gas chromatograph were heated to 160 °C for analysis of all samples other than ethyl benzene, for which both were raised to 180 °C. The injection port was used in the split mode (split ratio ≈ 100:1) for introduction of samples into a stream of high-purity He. A "column" of fused silica capillary (0.25 mm i.d. × 1 m) placed in the GC oven linked the injector with the microfurnace. The alumina tube was packed with 12 100-mm-long strands of 0.1-mm-diameter high-purity nickel wire (Johnson Matthey, Royston, UK) and heated to 1050 °C. A Nafion water trap, 0.6 mm i.d. × 300

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mm (Perma Pure, Toms River, NJ), was placed after the microfurnace to remove any residual water from the He carrier gas. A Poraplot Q column, 0.32 mm i.d. × 25 m, 10 μm film thickness (Chrompack, Middleburg, Netherlands), was used to separate any CO_2 formed during pyrolysis from H_2 and CO, as ~10% of CO_2 undergoes unimolecular decomposition in the ion source to form CO^+. At ambient temperature (21 ± 2 °C), H_2 and CO were unretained on the Poraplot Q column, while CO_2 had a retention time of ~60 s. Data acquisition, peak detection, and integration were performed using the ANCA-GC software suite (Europa Scientific).

Reagents. Isotopic reference standards, Vienna-Standard Mean Ocean Water (V-SMOW), Standard Light Antarctic Precipitation (SLAP), and Greenland Ice Sheet Precipitation (GISP), were obtained from the International Atomic Energy Agency, Vienna, Austria. Local tap water was calibrated against V-SMOW and used as a working standard in measurement of both δ^2H and $\delta^{18}O$. Labeled waters, D_2O (99 atom %) and $H_2{}^{18}O$ (10 atom %), were obtained from Europa Scientific and mixed with local tap water to provide the desired levels of enrichment. Deuterated volatile organic compounds, ethyl-d_5 alcohol-d (99+ atom %), acetone-d_6 (99.5 atom %), and ethylbenzene-d_{10} (98+ atom %), were obtained from Sigma Chemical Co. Urine samples from 16 individuals involved in a D_2O-labeling experiment were kindly provided by M. J. Rennie, Department of Anatomy and Physiology, University of Dundee.

Procedure. Samples were injected through the injection port of the gas chromatograph using a 1-μL liquid sampling syringe. The injection port was operated in the split mode with a split ratio of 100:1; hence, 5 nL of sample was carried to the pyrolysis furnace from an injection of 0.5 μL. The syringe was washed with acetone and dried thoroughly between samples to prevent cross contamination. The injection port was fitted with a straight-bore inlet sleeve containing a plug of nickel wool (Elemental Microanalysis, Okehampton, Devon, UK). When heated to 160 or 180 °C, the nickel wool ensured complete vaporization of samples and acted as a trap for removal of salts (e.g., NaCl) and organic matter (e.g., urea) from urine samples. For analysis of large numbers of urine samples, the inlet sleeve was cleaned and the nickel wool plug replaced at the end of every second day.

The pyrolysis furnace consisted of an alumina tube packed with nickel wire and heated to 1050 °C. Alumina tubes were used in preference to quartz tubes, which were found to give rise to severe sample memory for both δ^2H and $\delta^{18}O$ measurement. Pyrolysis tubes were charged with hexane to provide a layer of elemental carbon, as required for pyrolysis of water by the Unterzaucher reaction. Injection of 2.5 μL of hexane was found to provide adequate elemental carbon for pyrolysis of ~50 aliquots of water to H_2 and CO. A similar quantity of hexane was pyrolyzed in the pyrolysis furnace prior to analysis of volatile organic compounds to ensure that carbon was available in excess. A furnace temperature of 1050 °C was chosen, as significant quantities of CO_2 are generated as a byproduct of pyrolysis at lower temperatures.[7] In excess of 95% of sample oxygen was converted to CO for all sample types investigated. However, even trace amounts of CO_2 are a potential source of ^{18}O fractionation, as unimolecular decomposition to CO^+ can occur in the ion source. To eliminate ^{18}O fractionation in the ion source, CO and CO_2 were separated chromatographically on a PoraPlot Q capillary column.

Measurement of δ^2H for H_2 is complicated by the formation of $H_3{}^+$ ions in the ion source. The $H_3{}^+$ and $^2H^1H^+$ ions are inseparable in the mass spectrometer and are both recorded by the m/z 3 collector. Measured values for $^2H^1H{:}^1H^1H$ are overestimated because of formation of $H_3{}^+$; as the number of $H_3{}^+$ ions formed is proportional to the square of the volume of H_2, there is a linear relationship between the measured $^2H^1H{:}^1H^1H$ ratio and the pressure of H_2 in the ion source. In dual-inlet IRMS, correction for $H_3{}^+$ formation involves calibration over a small range of signal size and accurate matching of the $^1H^1H^+$ beam intensities for both samples and standards. As CF-IRMS gives transient signals, it has been necessary to develop an alternative approach to $H_3{}^+$ correction. Mathematical correction methods have been proposed by Prosser and Scrimgeour[4] and Tobias et al.[3,21] Here, correction for $H_3{}^+$ formation has been applied using a slightly modified version of the "peakwise" linear regression method described by Prosser and Scrimgeour.[4]

The $H_3{}^+$ correction factor (k) was calculated on a daily basis from measured values of $^2H^1H{:}^1H^1H$ obtained for injection of 0.3, 0.5, and 0.7 μL of local tap water. Regression analysis through the data series of measured $^2H{:}^1H$ isotope ratio (R_m) vs major ion beam peak height (I_m) was used to find the value of k. Major ion beam peak intensity was used in determination of k, rather than major ion beam peak area as used previously, as it improved the linearity of the regression equations. The $H_3{}^+$-corrected $^2H{:}^1H$ isotope ratio (R_c) was calculated by

$$R_c = R_m - kI_m \qquad (1)$$

Tobias and Brenna[21] have shown that the linearity of the relationship between R_m and I_m is dependent on source pressure. The range of signal intensities for which linear regression may be used for correction of $H_3{}^+$ is limited by the rise in source pressure during ingress of H_2 through their Pd filter. In our CF-IRMS system, for which the source pressure is $\sim 2 \times 10^{-6}$ mbar due to continuous entry of He, only small changes in source pressure occur during analysis of H_2. Linear correction for $H_3{}^+$ formation (eq 1) is adequate for signal intensities in the range 2−8 nA. This method does, however, require that the peak shapes of both samples and standards are similar, as the method of peak integration in the ANCA-GC software (Europa Scientific) did not compensate for time shifts between m/z 2 and m/z 3.

The number of $H_3{}^+$ ions formed is additionally dependent on the magnitude of sample enrichment. If the sample size is kept relatively constant, the dependence of the $H_3{}^+$ effect on enrichment can be corrected using a simple linear scaling factor.[4] In dual-inlet IRMS, where beam intensities for sample and reference are closely matched, the necessary scaling factor is derived from a two-point calibration from V-SMOW and SLAP. Similarly, correction for $H_3{}^+$ formation (eq 1) is followed by a correction for sample enrichment based on a linear scaling factor derived from calibration of V-SMOW and SLAP to achieve the accepted difference.

RESULTS AND DISCUSSION

Water Samples. The accuracy and precision of the continuous-flow pyrolysis technique was determined for δ^2H_{V-SMOW} and $\delta^{18}O_{V-SMOW}$ of GISP (Table 1). The data presented in Table 1 have been scaled to a V-SMOW−SLAP linear calibration. Injected volumes of GISP in the range 0.3−0.7 μL were analyzed to show

Table 1. Accuracy and Precision of δ^2H and δ^{18}O for GISP

sample size (μL)	δ^2H_{V-SMOW}[a] (‰)	mean	SD	$\delta^{18}O_{V-SMOW}$[b] (‰)	mean	SD
0.3	−182.8					
0.4	−186.2					
0.5	−183.8					
0.6	−186.0					
0.7	−183.1	−184.4	1.6			
0.5	−187.9			−24.6		
0.5	−187.4			−24.6		
0.5	−186.0			−25.1		
0.5	−186.6	−187.0	0.8	−25.1	−24.9	0.3

[a] Calculated using eq 1 and then scaling to a V-SMOW−SLAP linear calibration. [b] Scaled to a V-SMOW−SLAP linear calibration.

Table 2. δ^2H for SLAP versus V-SMOW

day	δ^2H_{V-SMOW}[a] (‰)	mean	SD
5	−388.1		
	−390.4		
	−391.8	−390.1	1.9
6	−393.9		
	−396.9		
	−393.6	−394.8	1.8
17	−387.9		
	−391.6		
	−388.9	−389.5	1.9
19	−390.9		
	−393.0		
	−389.9	−391.3	1.6
22	−387.8		
	−389.5		
	−392.0	−389.8	2.1

[a] Calculated using eq 1 and then converting to the V-SMOW scale.

the effectiveness of linear regression correction for H_3^+ formation. The major ion beam peak height (I_m) was 1.97 nA for an injected volume of 0.3 μL, rising to 4.66 nA for 0.7 μL. A highly linear correlation ($r^2 = 0.9990$) was obtained between the measured ^{2}H:^{1}H isotope ratio (R_m) and I_m to give a proportionality constant (k) of 10 071. The corrected values of ^{2}H:^{1}H (R_c) given in Table 1 are indicative of the validity of the correction method. The measurement precision of δ^2H_{V-SMOW} for four replicate injections of 0.5 μL was 0.8‰ for GISP. Using the same continuous-flow pyrolysis system, the measurement precision for $\delta^{18}O_{V-SMOW}$ was 0.3‰ ($n = 4$). The measured values of δ^2H_{V-SMOW} and $\delta^{18}O_{V-SMOW}$ for GISP are in close agreement with the generally accepted values of −189.7 and −24.8‰, respectively.

The absolute accuracy of the continuous-flow pyrolysis IRMS system for determination of δ^2H_{V-SMOW} for SLAP was measured across a number of weeks during analysis of urine and volatile organic samples at both natural abundance and enriched levels (Table 2). The H_3^+-corrected values of δ^2H_{V-SMOW} given in Table 2 are in good agreement: mean values are in the range from −389.5 -to −394.6‰. A linear scaling factor of ~1.09 was required in order to obtain the accepted δ^2H_{V-SMOW} value for SLAP of −428‰. The $\delta^{18}O_{V-SMOW}$ value for SLAP was determined on two occasions, 8 days apart. The measured $\delta^{18}O_{V-SMOW}$ values were in excellent agreement: −53.7 ± 0.3‰ ($n = 3$) and −53.7 ± 0.0‰ ($n = 3$). To achieve the accepted $\delta^{18}O_{V-SMOW}$ value of −55.5‰, a linear scaling factor of 1.034 was required.

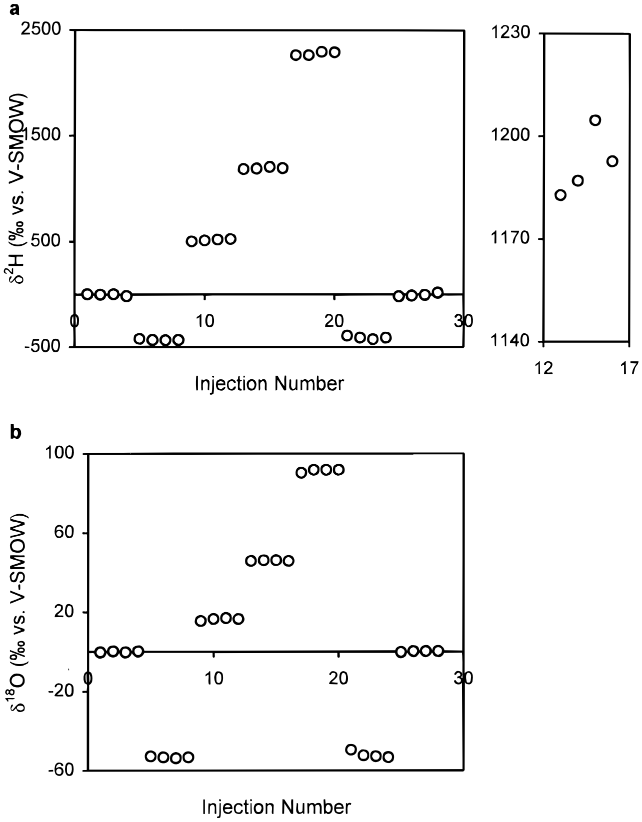

Figure 2. Instrumental response to consecutive injection of water samples to evaluate memory effects for pyrolysis (a) for δ^2H_{V-SMOW} measurement of waters extending from −428 to ~2200‰ and (b) for $\delta^{18}O_{V-SMOW}$ measurement of water extending from −55.5 to ~95‰.

Isotopic reference standards V-SMOW and SLAP were run together with doubly labeled waters extending to $\delta^2H_{V-SMOW} \approx$ 2200‰ and $\delta^{18}O_{V-SMOW} \approx$ 95‰ to determine the extent of memory effects resulting from pyrolysis on nickel wire coated with elemental carbon. Samples were ordered such that V-SMOW and SLAP were run before and after doubly labeled waters to give a transition of ~2600‰ for δ^2H and ~150‰ for δ^{18}O between injections 20 and 21 in Figure 2. The first injection of each new sample (injections 5, 9, 13, 17, 21, and 25 in Figure 2) was in error by <1.6% of the difference in isotopic abundance of consecutive samples (e.g., injections 20 and 21 in Figure 2) for both ^{2}H and ^{18}O. An error of 1.6% is equivalent to <1 SD (SD = 2 and 0.3‰ for δ^2H and δ^{18}O, respectively) for samples that differ by <125‰ for δ^2H or <20‰ for δ^{18}O. Prior to analysis of the enriched samples, the mean measured value of δ^2H_{V-SMOW} for SLAP averaged across injections 6−8 (Figure 2) was −434‰, while following analysis of the enriched samples, the mean measured value averaged across injections 22−24 (Figure 2) was −417‰. Similarly, the mean measured value of $\delta^{18}O_{V-SMOW}$ for SLAP, with no scaling applied, was −53.2‰ before and −52.9‰ after analysis of the enriched samples.

To assess the usefulness of the pyrolysis technique in analysis of natural abundance waters, a set of tap water samples extending across >100‰ for δ^2H and ~25‰ for δ^{18}O was prepared by evaporative concentration. As fractionation by evaporation is governed by both thermodynamic and kinetic processes, it was necessary to perform evaporation under carefully controlled conditions to obtain a linear relationship between enrichment in ^{2}H and ^{18}O. Equal volumes of local tap water were pipetted into

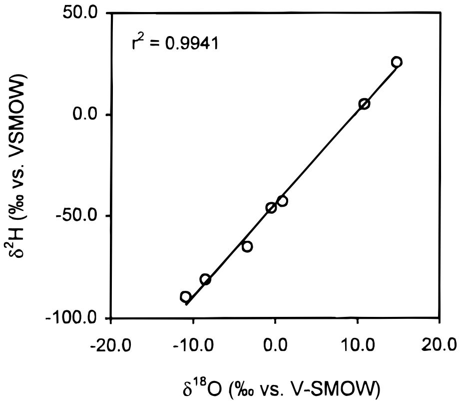

Figure 3. Relationship between measured ^{2}H and ^{18}O abundance for local water evaporation series ($r^2 = 0.994$).

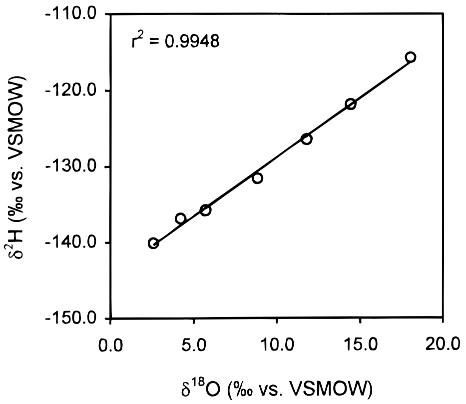

Figure 4. Relationship between measured ^{2}H and ^{18}O abundance for ethyl alcohol evaporation series ($r^2 = 0.995$).

seven Vacutainer tubes, sealed, and weighed to a precision of ±0.001 g. Vacutainer tubes were incubated at 30 ± 1 °C, and evaporation of water was aided by passing a stream of nitrogen over the liquid surface. After varying periods of time, Vacutainer tubes containing concentrates were resealed to prevent any further evaporation and reweighed to allow calculation of the concentration factor. Evaporation gave concentration factors of up to ~70%. The enrichment in ^{2}H and ^{18}O abundances resulting from evaporation was determined separately by triplicate analysis of 0.5-μL aliquots of each concentrate. A good linear relationship ($r^2 = 0.994$) between measured δ^2H and δ^{18}O was observed for the series of tap water concentrates (Figure 3). The range of ^{2}H and ^{18}O abundances given in Figure 3 is similar to that obtained from local precipitation across the globe, confirming that the pyrolysis technique is suitable for use in hydrological studies.

Urine Samples. Accurate measurement of ^{2}H abundance in urine is required in a number of techniques used in human physiology. Deuterium oxide is used in measurement of total body water by isotope dilution and the water turnover rate required in total energy expenditure (TEE) studies. Determination of TEE by the doubly labeled water (DLW) method involves measurement of ^{2}H and ^{18}O abundances in urine collected daily for a period of 2 weeks after oral administration of DLW.[22] We have previously shown the pyrolysis technique to provide analytical precision for both δ^2H and δ^{18}O which is fully adequate for determination of TEE by the DLW method.[7]

The influence of the non-water content of urine on the pyrolysis and subsequent δ^2H and δ^{18}O measurements was investigated. To prevent compounds other than water from entering the pyrolysis furnace, we used a nickel plug in the inlet sleeve of the injection port. The injection port was heated to 160 °C, allowing vaporization of water but trapping salts and organic matter. Six replicate samples of urine of natural ^{2}H and ^{18}O abundance from a single subject were analyzed for both δ^2H and δ^{18}O. The mean δ^2H and δ^{18}O values for the urine sample were measured as +30.1 ± 1.2 and +8.5 ± 0.3‰ vs local tap water, respectively. These measurement precisions are equivalent to those obtained for water (Table 1).

As the amount and variety of non-water compounds found in urine can vary widely, we measured δ^2H in natural abundance and enriched urine samples from 16 different subjects. Each urine sample was analyzed in quadruplicate, the first injection in each case being used as a wash. As samples were ordered by subject, transitions of >2000‰ could occur between the last injection of one sample and the first injection of the next. The measurement precision for δ^2H in the natural abundance urines from 16 subjects ranged from 0.3 to 2.7‰ ($n = 3$) and averaged 1.7‰ ($n = 16$). The ^{2}H abundance of the enriched urines collected from the same 16 subjects, 4 h after oral administration of D_2O, averaged +2142‰ vs V-SMOW. The measurement precision for δ^2H ranged from 1.4 to 14.2‰ ($n = 3$) and averaged 6.5‰ ($n = 16$), or 0.3% if expressed as a relative standard deviation (RSD).

Volatile Organic Compounds. The natural ^{2}H and ^{18}O abundance of organic compounds is indicative of their geographical origin and biosynthesis. The differing biosynthetic pathways of organic compounds from C_3-plants (e.g., vine and beet), C_4-plants (e.g, cane and corn), CAM-plants, and their synthetic counterparts can be distinguished by ^{2}H isotope analysis.[23] The ^{2}H and ^{18}O abundances of volatile organic compounds in aromas and flavors are applied in authentication of their natural origin.[9,24] The ^{2}H:^{1}H isotope ratio in ethanol derived from the sugars of fruit juices can be used for detection of undeclared sugar addition.[25] Similarly, the ^{2}H:^{1}H isotope ratio of wine ethanol can be used to police addition of sugars during fermentation.[25]

We evaporated a series of seven ethyl alcohol samples, under the same controlled conditions as for local tap water, for varying periods of time to obtain evaporative concentrations of 0−80%. The ^{2}H and ^{18}O abundances for each ethyl alcohol concentrate were determined separately by triplicate analysis of 0.5-μL aliquots. A good linear relationship ($r^2 = 0.995$) was observed between measured δ^2H and δ^{18}O for the ethyl alcohol evaporation series (Figure 4). The linearity of the relationship, across a range of ^{2}H and ^{18}O abundances typical of those for wine ethanol of varying geographic origin, indicates that the pyrolysis technique ought to be applicable to the study of food authenticity. A similar evaporation series was prepared for methanol, which extended across ~40‰ for δ^2H and ~15‰ for δ^{18}O. Determination of δ^2H and δ^{18}O abundances based on triplicate analysis of 0.5-μL aliquots of each of the seven methanol samples gave a good linear relationship between measured ^{2}H and ^{18}O abundance ($r^2 = 0.988$). The measurement precision for δ^2H and δ^{18}O of methanol and ethyl alcohol at natural abundance levels was 2 and 0.3‰, respectively ($n = 6$).

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Table 3. δ^2H for Deuterium-Enriched Volatile Organic Compounds

sample	δ^2H_{V-SMOW}[a] (‰)	^{2}H abundance (ppm excess)	^{2}H expected (ppm excess)	r^2	slope
ethyl alcohol					
dil 1	4291.2 ± 7.1[b]	692.0	710.2		
dil 2	2109.7 ± 2.4	352.2	361.9		
dil 3	979.7 ± 5.6	176.8	181.8		
dil 4	384.2 ± 2.5	84.1	87.1		
dil 5	140.1 ± 0.5	46.1	47.6	1.000 00	0.975
ethyl benzene					
dil 1	5253.2 ± 18.5	837.6	839.9		
dil 2	2334.4 ± 10.3	383.6	388.7		
dil 3	1330.3 ± 7.6	227.4	233.3		
dil 4	501.9 ± 5.5	98.4	103.6		
dil 5	321.3 ± 0.7	70.3	71.1	0.999 97	1.001
acetone					
dil 1	4833.9 ± 3.2	776.3	782.7		
dil 2	2273.3 ± 12.4	378.0	388.2		
dil 3	1038.2 ± 1.7	85.8	189.1		
dil 4	456.3 ± 1.8	95.2	96.8		
dil 5	144.3 ± 2.6	46.6	47.3	0.999 95	0.991

[a] Calculated using eq 1 and then scaling to a V-SMOW−SLAP linear calibration. [b] Mean 1 ± SD ($n = 3$).

A fast, accurate method for measurement of δ^2H in organic compounds would allow metabolic studies using low levels of inexpensive deuterated tracers. Deuterated glucose is currently used in investigation of glucose turnover in humans. Deuterium dilution series for ethyl alcohol, acetone, and ethylbenzene were prepared by mixing 99+ atom % ethyl-d_5 alcohol-d, 99.5 atom % acetone-d_6, and 98+ atom % ethylbenzene-d_{10} with their corresponding natural ^{2}H abundance compounds. Each dilution series consisted of six samples extending across a range of ~0.075 atom % excess. Pyrolysis of 10-nL volumes of the ^{2}H-labeled volatile organic compounds was performed in quadruplicate. Samples from each dilution series were analyzed in order of ascending enrichment. The first injection of each new sample was used as a wash, and the three subsequent measurements were averaged to give the mean ± SD for δ^2H_{V-SMOW} (Table 3). Excellent linearity ($r^2 > 0.9999$) between measured and expected ^{2}H abundance was obtained for ethyl alcohol, ethylbenzene, and acetone (Table 3). The slopes given in Table 3 are for the best-fit linear relationship between measured and expected ^{2}H abundance and are indicative of the level of enrichment in the ^{2}H-labeled compounds.

CONCLUSIONS

A new approach for direct pyrolysis of samples has been described which allows precise measurement of δ^2H and δ^{18}O by CF-IRMS. By minimizing the quantity of elemental carbon within the pyrolysis furnace, associated memory effects have been much reduced, giving improved precision and accuracy. Memory between injections of samples of differing enrichment is ~1.6% of the difference in isotope abundance for both ^{2}H and ^{18}O. Measurement precision is adequate for many natural abundance studies: water samples can be routinely measured with a precision of ~2‰ for δ^2H and ~0.3‰ for δ^{18}O, for a sample volume of 5 nL. The pyrolysis technique has also been shown to be a rapid method for high-precision δ^2H and δ^{18}O measurement for urine and alcohols at natural abundance. The method is linear over several orders of magnitude of enrichment for both ^{2}H and ^{18}O, allowing use in tracer studies such as the doubly labeled water method for measuring energy expenditure. The precision for δ^2H measurement of ^{2}H-labeled urine samples from a tracer study was, on average, 0.3% RSD. Accurate δ^2H measurement has been made for a variety of volatile organic compounds, a significant advance toward the compound-specific ^{2}H analysis of organic compounds by GC-pyrolysis-IRMS. The true realization of compound-specific ^{2}H analysis by GC-pyrolysis-IRMS will, however, require new data integration and correction algorithms appropriate to a wide range of peak shapes and signal intensities.

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