Forensic Science International 214 (2012) 135–141

Contents lists available at ScienceDirect

Forensic Science International

journal homepage: www.elsevier.com/locate/forsciint

USGS42 and USGS43: Human-hair stable hydrogen and oxygen isotopic reference materials and analytical methods for forensic science and implications for published measurement results☆

Tyler B. Coplen *, Haiping Qi

U.S. Geological Survey, 431 National Center, Reston, VA 20192, USA

ARTICLE INFO

Article history:
Received 22 March 2011
Received in revised form 6 July 2011
Accepted 17 July 2011
Available online 17 August 2011

Keywords:
Oxygen isotopes
Hydrogen isotopes
Keratin

ABSTRACT

Because there are no internationally distributed stable hydrogen and oxygen isotopic reference materials of human hair, the U.S. Geological Survey (USGS) has prepared two such materials, USGS42 and USGS43. These reference materials span values commonly encountered in human hair stable isotope analysis and are isotopically homogeneous at sample sizes larger than 0.2 mg. USGS42 and USGS43 human-hair isotopic reference materials are intended for calibration of δ^2H and δ^{18}O measurements of unknown human hair by quantifying (1) drift with time, (2) mass-dependent isotopic fractionation, and (3) isotope-ratio-scale contraction. While they are intended for measurements of the stable isotopes of hydrogen and oxygen, they also are suitable for measurements of the stable isotopes of carbon, nitrogen, and sulfur in human and mammalian hair. Preliminary isotopic compositions of the non-exchangeable fractions of these materials are

$$\text{USGS42(Tibetan hair)}\delta^2\text{H}_{\text{VSMOW-SLAP}} = -78.5 \pm 2.3‰(n = 62) \text{ and } \delta^{18}\text{O}_{\text{VSMOW-SLAP}} = +8.56 \pm 0.10‰(n = 18)$$
$$\text{USGS43(Indian hair)}\delta^2\text{H}_{\text{VSMOW-SLAP}} = -50.3 \pm 2.8‰(n = 64) \text{ and } \delta^{18}\text{O}_{\text{VSMOW-SLAP}} = +14.11 \pm 0.10‰(n = 18)$$

Using recommended analytical protocols presented herein for $\delta^2\text{H}_{\text{VSMOW-SLAP}}$ and $\delta^{18}\text{O}_{\text{VSMOW-SLAP}}$ measurements, the least squares fit regression of 11 human hair reference materials is

$$\delta^2\text{H}_{\text{VSMOW-SLAP}} = 6.085\delta^{18}\text{O}_{\text{VSMOW-SLAP}} - 136.0‰$$

with an *R*-square value of 0.95. The δ^2H difference between the calibrated results of human hair in this investigation and a commonly accepted human–hair relationship is a remarkable 34‰. It is critical that readers pay attention to the $\delta^2\text{H}_{\text{VSMOW-SLAP}}$ and $\delta^{18}\text{O}_{\text{VSMOW-SLAP}}$ of isotopic reference materials in publications, and they need to adjust the $\delta^2\text{H}_{\text{VSMOW-SLAP}}$ and $\delta^{18}\text{O}_{\text{VSMOW-SLAP}}$ measurement results of human hair in previous publications, as needed, to ensure all results on are on the same scales.

Published by Elsevier Ireland Ltd.

1. Introduction

The stable isotopic abundances (and thus the atomic weights) of many common elements vary as a result of physical, chemical, and biological processes [1]. The stable isotopic abundances of hydrogen (Fig. 1), carbon, nitrogen, oxygen (Fig. 2), and other elements differ because these elements have two or more naturally occurring stable isotopes that vary in abundance in natural materials [2]. Stable hydrogen and oxygen isotopic abundances of precipitation, rivers, and tap waters are known to vary geographically and form the basis of isotope hydrology [3–7]. Because the isotopic composition of water ingested by humans varies geographically and because the stable hydrogen and oxygen isotopic compositions of human hair reflect this geographic variation, in favorable cases, such measurements of human hair can provide a history of geographical movements of humans [8–12]. Consequently, measurements of stable hydrogen and oxygen isotopes in human hair have grown over the last decade in forensic science, anthropology, and other sciences.

The measurement of accurate isotopic compositions of human hair, as well as other keratin materials, is complicated by the fact that human hair has exchangeable hydrogen [13–16]; thus, measurement of the same homogeneous sample in various laboratories can yield different isotopic compositions. To ensure that measurement of the same homogeneous human hair sample by any laboratory

☆ This article is a U.S. Government work and is in the public domain in the U.S.A. Part of this work was presented at the Fourth FIRMS Network Conference, Washington, D.C., U.S.A., 2010.

* Corresponding author. Tel.: +1 703 648 5862; fax: +1 703 648 5832.
E-mail address: tbcoplen@usgs.gov (T.B. Coplen).

0379-0738/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.forsciint.2011.07.035

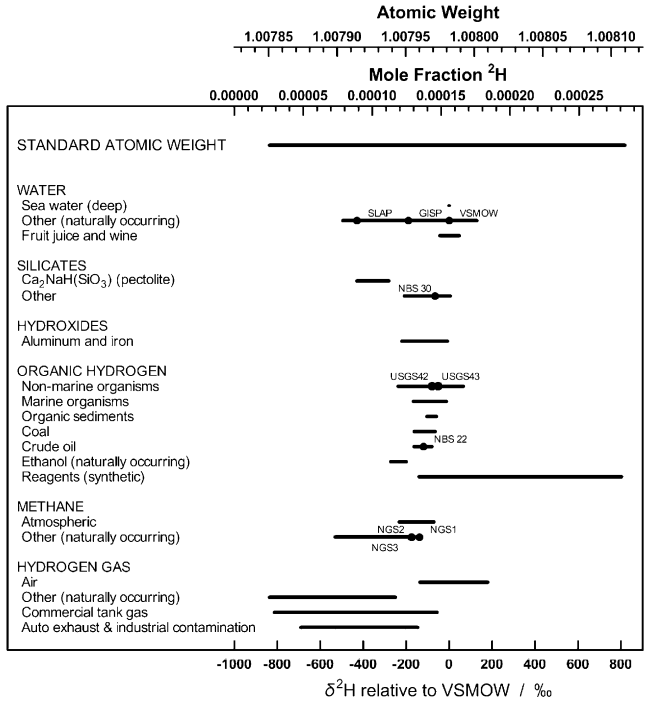

Fig. 1. Hydrogen isotopic composition and atomic weight of selected hydrogen-bearing materials; modified from [1,2]. Solid black dots denote internationally distributed isotopic reference materials. USGS42 and USGS43 are the first stable hydrogen and oxygen isotopic reference materials for human hair.

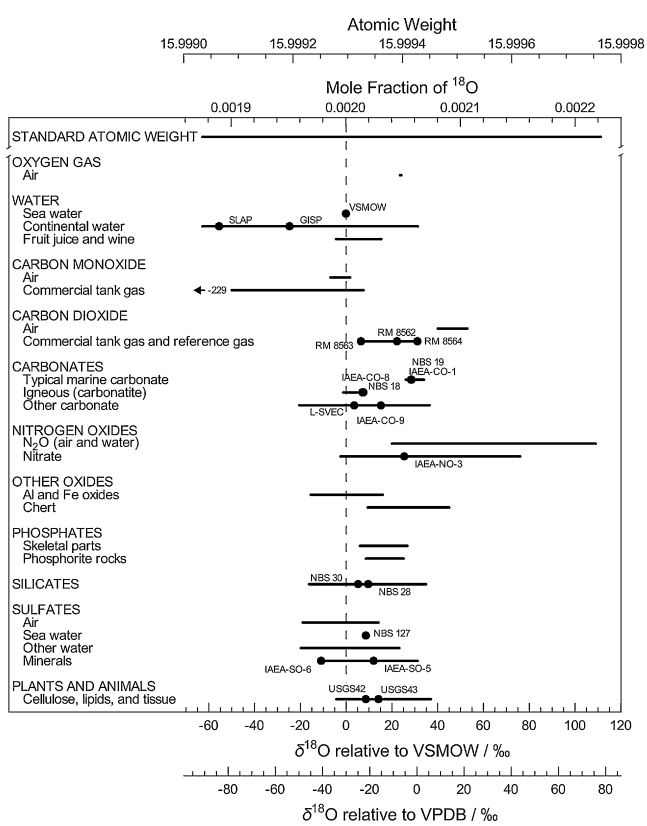

Fig. 2. Oxygen isotopic composition and atomic weight of selected oxygen-bearing materials; modified from [1,2]. Solid black dots denote internationally distributed isotopic reference materials. USGS42 and USGS43 are the first stable hydrogen and oxygen isotopic reference materials for human hair.

worldwide can yield the same isotopic composition within analytical uncertainty, isotope laboratories globally need at least one, and preferably two, stable human-hair isotopic reference materials available globally. Because there are no internationally distributed stable hydrogen and oxygen isotopic reference materials of human hair, the U.S. Geological Survey (USGS) has prepared two such materials, USGS42 and USGS43.

2. Preparation of human-hair isotopic reference materials

When considering the production of isotopic reference materials, it is convenient for the isotope community to have two materials with substantially different isotopic compositions that span values that might be encountered during analysis. It is desirable to prepare a kilogram of each material so that it can last for at least a decade. Two types of human hairs with different stable hydrogen and oxygen isotopic compositions were sought for this purpose. For the human hair relatively depleted in ^{2}H and ^{18}O, hair from 117 unidentified individuals from the Tibetan plateau of Yushu, Qinghai, People's Republic of China was obtained (named USGS42). For the second material, non-oriented strands of southern Indian, non-chemically treated, Remy human hair was purchased commercially (named USGS43).

Each material was washed with 1:200 Triton X-100 detergent (Sigma–Aldrich), rinsed with deionized water (DIW), soaked in ethanol overnight, rinsed with DIW, dried in air and vacuum oven at ambient temperature, cut in lengths less than 1 cm, and pulverized with a Retsch model MM301 ball mill. About 0.8 g of hair was pulverized at a time using cryogenic grinding with liquid nitrogen, which took about 20 min for each 0.8 g of hair. Once the hair was pulverized, it was homogenized using a sieve shaker (Retsch model AS200). Upper and lower sieves of 60 (0.25 mm) and 100 (0.15 mm) mesh, respectively, were used. Any material not passing through the 100 mesh sieve was pulverized again. For USGS43, due to the extreme difficulty of passing this material through the 100-mesh sieve, the final product is a 60-mesh powder. Aliquots of 200–400 g were sieved for 10 min. The material in the bottom collection tray was divided into quarters to emulate a fraction splitter. More than 10 rounds of sieving, splitting, and re-pulverizing were performed in order to ensure homogenization. This was an extremely arduous process. The final products are grey powders.

3. Experimental methods

In the Reston Stable Isotope Laboratory (RSIL; http://isotopes.usgs.gov/) of the USGS, measurement of accurate relative stable hydrogen isotope-ratio differences of water and other hydrogen-bearing substances is a critical endeavor of USGS programs. The quantity relative stable hydrogen isotope-ratio difference, δ^2H, is defined by the relation [17]

$$\delta^2\mathrm{H}_{\mathrm{VSMOW}} = \frac{(N(^2\mathrm{H})_\mathrm{P}/N(^1\mathrm{H})_\mathrm{P}) - (N(^2\mathrm{H})_{\mathrm{VSMOW}}/N(^1\mathrm{H})_{\mathrm{VSMOW}})}{N(^2\mathrm{H})_{\mathrm{VSMOW}}/N(^1\mathrm{H})_{\mathrm{VSMOW}}}$$

where $N(^2\mathrm{H})_\mathrm{P}$ and $N(^1\mathrm{H})_\mathrm{P}$ are the numbers of the two isotopes ^{2}H and ^{1}H of hydrogen in specimen P, and equivalent parameters follow for the *international measurement standard* VSMOW (Vienna Standard Mean Ocean Water). Because the isotope-number ratio, R, commonly shortened to isotope ratio [17], equals $N(^2\mathrm{H})/N(^1\mathrm{H})$, the quantity δ^2H also is defined by the relation

$$\delta^2\mathrm{H}_{\mathrm{VSMOW}} = \frac{R(^2\mathrm{H}/^1\mathrm{H})_\mathrm{P} - R(^2\mathrm{H}/^1\mathrm{H})_{\mathrm{VSMOW}}}{R(^2\mathrm{H}/^1\mathrm{H})_{\mathrm{VSMOW}}}$$

where $\mathrm{R}(^2\mathrm{H}/^1\mathrm{H})$ also is written in the literature as $\mathrm{R}(^2\mathrm{H},^1\mathrm{H})$. Commonly an extraneous numerical factor of 1000 is added to this definition. However, to be consistent with the Système International d'Unités, the SI (known in English as the International System of Units), which is published by the Bureau International des Poids et Mesures (BIPM), the factor 1000 should not appear in this equation so that this equation is written as a quantity equation and not as a numeric value equation [17,18].

In like manner, the quantity relative stable oxygen isotope-ratio difference, $\delta^{18}O$, is defined by the relation

$$\delta^{18}O_{VSMOW} = \frac{(N(^{18}O)_P/N(^{16}O)_P) - (N(^{18}O)_{VSMOW}/N(^{16}O)_{VSMOW})}{N(^{18}O)_{VSMOW}/N(^{16}O)_{VSMOW}} = \frac{R(^{18}O/^{16}O)_P - R(^{18}O/^{16}O)_{VSMOW}}{R(^{18}O/^{16}O)_{VSMOW}}$$

In 1978, it was recognized that measurement uncertainty could be substantially improved by normalizing δ^2H and $\delta^{18}O$ values of water samples relative to a second anchor, SLAP (Standard Light Antarctic Precipitation) [19], and it was recommended that isotopic results be normalized to VSMOW–SLAP isotope scales such that the δ^2H and $\delta^{18}O$ values of SLAP (Standard Light Antarctic Precipitation) international measurement standard are −428 and −55.5‰, respectively, relative to VSMOW (Vienna Standard Mean Ocean Water) [20]. Subsequently, it was recommended that δ^2H and $\delta^{18}O$ values of other materials, such as organic materials, be expressed relative to the VSMOW–SLAP scale [20], which can be denoted by writing $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$. Isotope-reporting guidelines appropriate for forensic science derive from publications of the Commission on Isotopic Abundances and Atomic Weights of the International Union of Pure and Applied Chemistry [17].

For δ^2H calibration, two identical sets of hair reference samples were prepared. One set was equilibrated with water depleted in ^2H and another set was equilibrated with water enriched in ^2H to perform isotope-mass-balance calculations. After equilibration, samples were dried in a vacuum oven at 60 °C. The dried human-hair samples were analyzed with a continuous-flow isotope-ratio mass spectrometer coupled with a Thermo Scientific thermal conversion elemental analyzer (TC/EA) [21,22] having an autosampler that can be sealed tightly (Costech Zero-blank autosampler, or equivalent). Hair samples were interspersed among calibrated water samples sealed in silver tubes using a semi-automated sealing technique [23]. The δ^2H and $\delta^{18}O$ measurements were performed individually because operating parameters of the TC/EA are different for δ^2H and $\delta^{18}O$ measurements. For δ^2H measurements, the method of Qi and Coplen [24] was used; He flow rate = 120 mL/min, reactor temperature = 1450 °C, GC temperature = 85 °C, and GC length = 1 m, 5 Å. For $\delta^{18}O$ measurements, the method of Qi and Coplen [25] was used; operating parameters included the following: mass of each human-hair sample = 460 ± 10 μg, which were weighed into 3.5 × 5-mm silver capsules (Costech 041066); He flow rate = 120 mL/min; reactor temperature = 1350 °C; GC temperature = 80 °C; and GC length = 1 m, 5 Å [25]. Because the introduction of N_2 into the source of a mass spectrometer during $\delta^{18}O$ TC/EA measurements interferes with the background of CO [22], use of an N_2 diverting valve was useful in producing high quality $\delta^{18}O$ measurements. The use of a relatively new (less than 6 months old) GC column is preferred because of the improved agreement between $\delta^{18}O$ measurements of water and of human hair. The water samples sealed in silver tubes and interspersed among hair samples had been previously calibrated for hydrogen isotopic composition by platinum equilibration and dual inlet isotope-ratio mass spectrometry [26,27]. Measurements of $\delta^{18}O$ on water were performed by CO_2–H_2O equilibration [28] and dual-inlet isotope-ratio mass spectrometry [29]. LIMS (Laboratory Information Management Program) for Light Stable Isotopes [30] was utilized to normalize [31] isotopic results to the VSMOW–SLAP isotope scale [19,20].

In addition to $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$ measurements, USGS42 and USGS43 also were analyzed for their $\delta^{13}C_{VPDB-LSVEC}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$ values using the analytical methods described in Coplen et al. [32], Révész and Qi [33], Qi et al. [34], and Qi and Coplen [35]. The $\delta^{13}C_{VPDB-LSVEC}$ values of internationally distributed isotopic reference materials analyzed with USGS42 and USGS43 are: $\delta^{13}C_{NBS19/VPDB-LSVEC}$ = +1.95‰, $\delta^{13}C_{LSVEC/VPDB-LSVEC}$ = −46.6‰ [32], $\delta^{13}C_{USGS40/VPDB-LSVEC}$ = −26.39‰ [32,36], and $\delta^{13}C_{USGS41/VPDB-LSVEC}$ = +37.63‰ [32,36]. The $\delta^{15}N_{AIR}$ values of internationally distributed isotopic reference materials analyzed with USGS42 and USGS43 are: $\delta^{15}N_{USGS40/AIR}$ = −4.52‰ and $\delta^{15}N_{USGS41/AIR}$ = +47.57‰ [34,36]. The $\delta^{34}S_{VCDT}$ values of internationally distributed isotopic reference materials analyzed with USGS42 and USGS43 are: $\delta^{34}S_{IAEA-S-1/VCDT}$ = −0.3‰, $\delta^{34}S_{IAEA-S-2/VCDT}$ = +22.67‰, and $\delta^{34}S_{IAEA-S-3/VCDT}$ = −32.55‰ [2,36,37].

4. Results, discussion, and conclusions

Ensuring the isotopic homogeneity of the prepared materials was a critical endeavor of this project. During early investigations of USGS42 and USGS43, USGS43 showed more consistent isotopic compositions. Therefore, we were more concerned with isotopic homogeneity of USGS42. Six aliquots, 0.5 mg each, were taken from two different size fractions of USGS42 (one 0.15 mm and the other 0.25 mm). The results in Table 1 show that the hair material with either powder size of 0.25 mm or 0.15 mm appear to be homogenized well.

Six aliquots with different masses were analyzed for δ^2H and $\delta^{18}O$ values to check the isotopic homogeneity at low mass levels. The results are shown in Table 2 and suggest that when sample mass is smaller than 0.2 mg, a larger uncertainty is obtained. This could be attributed to a combination of inhomogeneity and system blank, one source of which is atmospheric oxygen diffusing through the TC/EA reactor at high temperature.

For the purposes of this investigation, human hair can be considered to consist of three reservoirs: Reservoir A is absorbed water that can be removed with sufficient pumping in a vacuum oven at low or ambient temperature; Reservoir B is a reservoir containing exchangeable hydrogen, but no exchangeable oxygen, and the hydrogen cannot be removed, even with by evacuation; Reservoir C is hair that contains non-exchangeable hydrogen and oxygen. The stable hydrogen and oxygen isotopic compositions of the non-exchangeable fractions of USGS42 and USGS43, Reservoir C, are listed in Table 3. All of the quantities involving hydrogen were determined using the methods in reference [24]. An estimated combined uncertainty, μ_C, is provided that includes the uncertainty of the reference waters used for calibrations and the measurement uncertainty. The recommended $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$ values provide intervals that have a 68% probability of encompassing the true values. The δ^2H of non-exchangeable hydrogen are the mean values determined from water equilibrations at ambient temperature [24].

Dry hair is defined as hair that has been dried in a vacuum oven at 60 °C. The mass fraction of oxygen in dry hair, w_O, is presented in Table 3. The quantity w_O was determined by comparing the mole fractions of oxygen in IAEA-SO-6 barium sulfate, in dried USGS42, and in dried USGS43.

The mole fraction of absorbed water (as O) in the absorbed water layer (Reservoir A), $x_{O\text{-abs}}$, equals n(O of Reservoir A)/(n(O of Reservoir A) + n(O of Reservoir C)), where n is the amount of substance (O) expressed in units of mol. The quantity $x_{O\text{-abs}}$ is determined by comparing the amounts of oxygen atoms in moist samples of USGS42 and USGS43 and in dry samples of USGS42 and

Table 1
Measured $\delta^{18}O$ values and uncertainties of USGS42 for two size fractions.

Size fraction (mm)	$\delta^{18}O$
0.15	+7.40 ± 0.16‰ (n = 6)
0.25	+7.53 ± 0.12‰ (n = 6)

Table 2
Uncertainties of measured δ^2H and $\delta^{18}O$ values of USGS42 as a function of mass.

Mass (mg)	Uncertainty of δ^2H	Uncertainty of $\delta^{18}O$
0.1	±2.3‰ (n = 6)	±0.74‰ (n = 6)
0.2	±1.0‰ (n = 6)	±0.18‰ (n = 6)
0.4	±0.6‰ (n = 6)	±0.15‰ (n = 6)
0.5	±0.7‰ (n = 6)	±0.09‰ (n = 6)

Table 3
Stable hydrogen and oxygen isotopic composition of non-exchangeable fractions of USGS42 and USGS43; w_H = mass fraction of total hydrogen, where total hydrogen = exchangeable hydrogen + non-exchangeable hydrogen; w_O = mass fraction of oxygen; $x_{H\text{-ex}}$ = mole fraction of exchangeable hydrogen = n(H of Reservoir B)/((n(H of Reservoir B) + n(H of Reservoir C)); $x_{O\text{-abs}}$ = mole fraction of absorbed water (as O) in the absorbed water layer (Reservoir A) = n(O of Reservoir A)/(n(O of Reservoir A) + n(O of Reservoir C)). Estimated combined uncertainty, μ_C, includes uncertainty of reference waters used for calibration.

Description	$\delta^2H_{VSMOW-SLAP}$ and estimated combined uncertainty	$\delta^{18}O_{VSMOW-SLAP}$ and estimated combined uncertainty
USGS42 (Tibetan)	−78.5 ± 2.3‰ (n = 62) w_H = 6.1% $x_{H\text{-ex}}$ = 8.6 ± 2.0%	+8.56 ± 0.10‰ (n = 18) w_O = 22% $x_{O\text{-abs}}$ = ~8%
USGS43 (Indian)	−50.3 ± 2.8‰ (n = 64) w_H = 6.1% $x_{H\text{-ex}}$ = 9.0 ± 1.9%	+14.11 ± 0.10‰ (n = 18) w_O = 22% $x_{O\text{-abs}}$ = ~8%

Table 4
Stable carbon, nitrogen, and sulfur isotopic composition of USGS42 and USGS43; w_C = mass fraction carbon, w_N = mass fraction nitrogen, and w_S = mass fraction sulfur. Estimated combined uncertainty, μ_C, includes uncertainty of reference material values used for calibration.

Description	$\delta^{13}C_{VPDB-LSVEC}$ and estimated combined uncertainty	$\delta^{15}N_{AIR}$ and estimated combined uncertainty	$\delta^{34}S_{VCDT}$ and estimated combined uncertainty
USGS42 (Tibetan)	−21.09 ± 0.10‰ (n = 50) w_C = 45.7%	+8.05 ± 0.10‰ (n = 50) w_N = 15.3%	+7.84 ± 0.25‰ (n = 32) w_S = 4.4%
USGS43 (Indian)	−21.28 ± 0.10‰ (n = 50) w_C = 45.7%	+8.44 ± 0.10‰ (n = 50) w_N = 15.3%	+10.46 ± 0.22‰ (n = 35) w_S = 4.5%

USGS43. The mass fraction of absorbed moisture in hair can vary from 6.0 to 13.6% as laboratory relative humidity increases from 29.2 to 70.3% at a temperature of 23 °C [38]. In this study, typical values for the mole fraction of oxygen in absorbed water, $x_{O\text{-abs}}$, for USGS42 and USGS43 are 8%.

The mole fraction of exchangeable hydrogen, $x_{H\text{-ex}}$, equals n(H of Reservoir B)/((n(H of Reservoir B) + n(H of Reservoir C)), where n is the amount of substance (H) expressed in units of mol. The mole fraction of exchangeable hydrogen measured from these equilibrations ranged from 5.2 to 10.9% and was a function of the drying procedure used.

The average mole fraction of exchangeable hydrogen of 8.6 ± 2.0% for USGS42 is slightly lower than the value of 9.0 ± 1.9% for USGS43. This difference likely is due to the powder-size difference between the two reference materials. As indicated earlier, the powder size of USGS42 is smaller than 0.15 mm and that of USGS43 is larger. The fraction of exchangeable hydrogen is a function of the powder size or length of hair segments [13,14,24]. Thus, using powdered hair reference materials to calibrate non-powdered, cut hairs, could introduce an error to the final δ^2H values due to differences in the fraction of exchangeable hydrogen among reference powders and cut hair segments. Another uncertainty is introduced due to the fact that powders of different sizes might retain different amounts of absorbed water, which we did not investigate. An appropriate evaluation might be needed to quantify these factors.

The δ^2H and δ^{18}O differences between USGS42 and USGS43 are 28 and 5.6‰. A larger difference is desirable, and production of another human-hair isotopic reference material more depleted in ^{2}H and ^{18}O than USGS42 is under consideration by the RSIL.

The $\delta^{13}C_{VPDB-LSVEC}$, $\delta^{15}N_{AIR}$, and $\delta^{34}S_{VCDT}$ values of USGS42 and USGS43 are shown in Table 4. An estimated combined uncertainty, μ_C, is given that includes the uncertainty of the reference material values used for calibrations and the measurement uncertainty. These values are provided for the interested reader. USGS42 and USGS43 are not intended for two-point isotopic normalization of δ^{13}C, δ^{15}N, or δ^{34}S scales. Users should instead use USGS40 and USG41 L-glutamic acid [32,34] for δ^{13}C and δ^{15}N scales. For the δ^{34}S scale, users should consider IAEA-S-1, IAEA-S-2, IAEA-S-3, and IAEA-S-4 [2,35–37].

5. Analyzing human hair for δ^2H and δ^{18}O values using USGS42 and USGS43

5.1. General guidelines for the use of USGS42 and USGS43

The following recommendations hold for USGS42 and USGS43:

- USGS42 and USGS43 human-hair isotopic reference materials are intended for calibration of δ^2H and δ^{18}O measurements of unknown human hair.
- It is suggested that the masses of USGS42, USGS43, and unknowns should be identical to minimize or cancel biases.
- It is also recommended that one should make the unknown sample powder size as close as possible to the size of USGS42 and USGS43, recognizing that in some studies the sample cannot be pulverized.
- Unknown hair samples and hair reference materials analyzed for both δ^2H and δ^{18}O values need to be equilibrated with laboratory air at ambient temperature for at least 5 days. In case δ^{18}O samples are not completely dried, the simultaneous 5-day equilibration of both reference materials and unknowns will minimize uncertainty caused by moisture that has not been removed because references and unknowns are treated identically. Unknowns and references for δ^{18}O measurements need to be thoroughly dried based on our observations [24]; otherwise, reproducibility is poor. However, based on our observations for δ^2H measurements, unknowns and references do not need to be thoroughly dried.
- **USGS42 and USGS43 should be interspersed among unknowns, treated in an identical manner as unknowns, and used for normalizing to the $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$ scales.**
- When reporting results, authors should always use text in their reports such as:

> **"The δ^2H and δ^{18}O values of human-hair samples are reported relative to the VSMOW–SLAP scale, and on this scale the δ^2H and δ^{18}O values of USGS42 and USGS43 are −78.5, +8.56, −50.3, and +14.11‰, respectively [reference]."**

In this manner, readers can re-normalize measurement results to current values of USGS42 and USGS43 as they are improved with new techniques. Improved, recommended values of USGS42 and USGS43 are posted on the website of the Commission on Isotopic Abundances and Atomic Weights of the International Union of Pure and Applied Chemistry [36].

5.2. Recommended protocol for determination of δ^{18}O of human hair

The recommended protocol for the determination of δ^{18}O of human hair is listed below.

1. Open unknown human-hair sample containers and allow it to equilibrate with laboratory atmospheric moisture for at least 5 days prior to isotopic analysis. Ensure that USGS42 and USGS43 are treated in an identical manner.
2. Weigh 460 ± 10 μg of each reference and unknown human hair sample into a silver capsule and wrap it.
3. Dry in a vacuum oven at 50 °C overnight.
4. Load unknown and reference samples into autosampler carousel that is capable of being sealed/evacuated. If the autosampler carousel is open to the air, reproducibility will be poor. The typical sequence of samples and references for δ^{18}O measurements is 4 USGS42, 3 USGS43, and 10 unknowns. Repeat this cycle for additional unknowns, and end with 3 USGS42 and 3 USGS43 samples. This sequence design allows scale correction and correction of drift with time.
5. Analyze samples [25] and make scale and drift corrections as needed. Normalize all measurement results to the VSMOW–SLAP scale.

Table 5
The $\delta^{18}O_{VSMOW-SLAP}$ values in selected human and horse hair determined by normalization to USGS42 and USGS43 using the protocol in Section 5.2. [Reproducibility listed is 1 sigma. Two aliquots of each unknown hair sample were analyzed in each equilibration run unless otherwise specified.].

Material	Equilibration 1	Equilibration 2	Equilibration 3	Mean
USGS42 (Tibetan hair)	+8.56 ± 0.14‰ $n = 6$	+8.56 ± 0.16‰ $n = 6$	+8.56 ± 0.24‰ $n = 6$	+8.56 ± 0.17‰, $n = 18$
USGS43 (Indian hair)	+14.11 ± 0.23‰ $n = 6$	+14.11 ± 0.23‰ $n = 6$	+14.11 ± 0.15‰ $n = 6$	+14.11 ± 0.19‰, $n = 18$
IAEA-085 (human hair)	+13.84 ± 0.10‰	+13.96 ± 0.16‰	+13.98 ± 0.04‰	+13.93 ± 0.08‰, $n = 18$
IAEA-086 (human hair)	+13.93 ± 0.13‰	+14.27 ± 0.19‰	+14.18 ± 0.46‰	+14.13 ± 0.18‰, $n = 6$
BCR No. 397 (human hair)	+12.15 ± 0.13‰	+11.62 ± 0.03‰	+11.94 ± 0.02‰	+11.90 ± 0.27‰, $n = 6$
GBW07601 (human hair)	+10.39 ± 0.04‰	+10.26 ± 0.08‰	+9.88 ± 0.01‰	+10.18 ± 0.27‰
GBW0901a (human hair)	+11.85 ± 0.05‰	+11.73 ± 0.01‰	+11.73 ± 0.25‰	+11.77 ± 0.07‰, $n = 6$
Canadian hair-1 (human hair)	+8.53 ± 0.16‰	+8.50 ± 0.20‰	+8.74 ± 0.10‰	+8.59 ± 0.13‰, $n = 6$
Canadian hair-2 (human hair)	+5.42 ± 0.24‰	+5.38 ± 0.16‰	+5.67 ± 0.43‰	+5.49 ± 0.16‰, $n = 6$
Canadian hair-3 (human hair)	+6.34 ± 0.06‰	+6.66 ± 0.38‰	+6.60 ± 0.16‰	+6.53 ± 0.17‰, $n = 6$
Canadian hair-4 (human hair)	+11.84 ± 0.12‰	+11.80 ± 0.29‰	+11.93 ± 0.01‰	+11.86 ± 0.07‰, $n = 6$
Utah horse hair	+3.60 ± 0.13‰	+3.31 ± 0.23‰	+3.57 ± 0.23‰	+3.49 ± 0.16‰, $n = 6$
Florida horse hair	+13.46 ± 0.20‰	+13.06 ± 0.01‰	+13.14 ± 0.06‰	+13.22 ± 0.21‰, $n = 6$

6. In published reports, authors should state the $\delta^{18}O_{VSMOW-SLAP}$ values of USGS42 and USGS43 used for normalization to the $\delta^{18}O_{VSMOW-SLAP}$ scale.

An example of the use of this protocol is shown in Table 5. The inclusion of horse hair is not an endorsement of the use of horse hair as an isotopic reference material for $\delta^{18}O$ measurements of human hair, but is a demonstration that a reproducible value can be obtained with this protocol.

5.3. Recommended protocol for determination of δ^2H of human hair

The recommended protocol for the determination of δ^2H of human hair is listed below.

1. Open unknown human-hair sample containers and allow it to equilibrate with laboratory atmospheric moisture for at least 5 days prior to isotopic analysis. Ensure that USGS42 and USGS43 are treated in an identical manner.

Table 6
The $\delta^2H_{VSMOW-SLAP}$ of the non-exchangeable fraction of hydrogen in selected human and horse hair using the protocol in Section 5.3. [Reproducibility listed is 1 sigma. Two aliquots of each unknown hair sample were analyzed in each equilibration run unless otherwise specified.].

Material	Equilibration 1	Equilibration 2	Equilibration 3	Mean
USGS42 (Tibetan hair)	−78.5 ± 0.3‰ $n = 6$	−78.5 ± 0.6‰ $n = 6$	−78.5 ± 0.8‰ $n = 6$	−78.5 ± 0.6‰ $n = 18$
USGS43 (Indian hair)	−50.3 ± 0.8‰ $n = 6$	−50.3 ± 0.7‰ $n = 6$	−50.3 ± 1.1‰ $n = 6$	−50.3 ± 0.8‰ $n = 18$
IAEA-085 (human hair)	−49.5 ± 0.6‰	−49.9 ± 0.3‰	−46.8 ± 0.9‰	−48.7 ± 1.6‰ $n = 6$
IAEA-086 (human hair)	−48.1 ± 1.2‰	−47.8 ± 0.4‰	−46.4 ± 1.1‰	−47.4 ± 1.1‰ $n = 6$
BCR No. 397 (human hair)	−67.2 ± 0.9‰	−68.2 ± 0.3‰	−67.8 ± 1.7‰	−67.8 ± 1.0‰ $n = 6$
GBW07601 (human hair)	−76.6 ± 1.1‰	−76.0 ± 0.0‰	−76.3 ± 0.4‰	−76.3 ± 0.6‰ $n = 6$
GBW0901a (human hair)	−62.6 ± 0.6‰	−62.1 ± 0.1‰	−61.8 ± 0.3‰	−62.2 ± 0.5‰ $n = 6$
Canadian hair-1 (human hair)	−83.7 ± 0.7‰	−84.2 ± 0.5‰	−85.0 ± 0.3‰	−84.3 ± 0.7‰ $n = 6$
Canadian hair-2 (human hair)	−97.4 ± 0.5‰	−96.8 ± 0.7‰	−97.3 ± 0.1‰	−97.2 ± 0.5‰ $n = 6$
Canadian hair-3 (human hair)	−102.5 ± 1.4‰	−103.7 ± 0.1‰	−102.7 ± 0.0‰	−103.0 ± 0.9‰ $n = 6$
Canadian hair-4 (human hair)	−68.2 ± 0.4‰	−68.5 ± 0.2 ‰	−67.1 ± 1.1‰	−67.9 ± 0.8‰ $n = 6$
Utah horse hair	−116.0 ± 0.1‰	−117.4 ± 2.2‰	−117.7 ± 2.5‰	−117.0 ± 1.7‰ $n = 6$
Florida horse hair	−53.5 ± 0.2 ‰	−52.3 ± 0.1‰	−52.9 ± 0.7‰	−52.9 ± 0.6‰ $n = 6$

2. Weigh 400 ± 10 μg of each reference and unknown human hair sample into a silver capsule and wrap it.
3. Load unknown and reference samples into autosampler carousel. The typical sequence of samples and references for δ^2H measurements is 4 USGS42, 3 USGS43, and 10 unknowns. Repeat this cycle for additional unknowns, and end with 3 USGS42 and 3 USGS43 samples. This sequence design allows scale correction and correction of drift with time.
4. Analyze samples [24] and make scale and drift corrections as needed. Normalize all measurement results to the VSMOW–SLAP scale.
5. In published reports, authors should state the $\delta^2H_{VSMOW-SLAP}$ values of USGS42 and USGS43 used for normalization to the $\delta^2H_{VSMOW-SLAP}$ scale.

An example of the use of this protocol is shown in Table 6, which presents the δ^2H of human hair reference materials from several sources. The inclusion of horse hair in Table 6 is not an endorsement of the use of horse hair as an isotopic reference material for δ^2H measurements of human hair, but is a demonstration that a reproducible value can be obtained with this protocol.

The mean $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$ measurement results of the human hair reference materials presented in Tables 5 and 6 are shown in Fig. 3. Recognizing that the symbol ‰ is one part in one thousand parts, with value 10^{-3}, the least squares fit regression is $\delta^2H_{VSMOW-SLAP} = 6.085\ \delta^{18}O_{VSMOW-SLAP} - 136.0‰$ with an *R*-square value of 0.95. Also shown in Fig. 3 are measurement results of tap water and scalp hair published by Ehleringer et al. [11]. The scalp hair values lie below those of the reference materials analyzed in this study in Fig. 3. The mean human hair δ^2H difference between these two studies is 34‰. This is more than 15 times the $\delta^2H_{VSMOW-SLAP}$ analytical uncertainty of many laboratories. Therefore, readers need to pay attention to the $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$ of isotopic reference materials in publications, and they need to adjust the $\delta^2H_{VSMOW-SLAP}$ and $\delta^{18}O_{VSMOW-SLAP}$ measurement results of human hair as needed to ensure all results on are on the same scales.

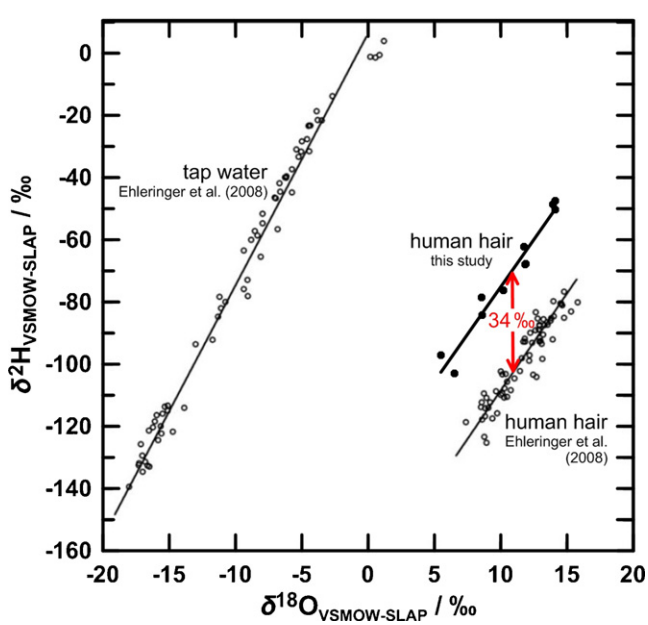

Fig. 3. Hydrogen versus oxygen isotopic compositions of tap water and human scalp hair. Open black circles [for human scalp hair, $\delta^2H_{VSMOW-SLAP} = 5.73\delta^{18}O_{VSMOW-SLAP} - 166‰$; *R*-square = 0.873] are from Ehleringer et al. [11], and solid black circles are from this study [$\delta^2H_{VSMOW-SLAP} = 6.085\delta^{18}O_{VSMOW-SLAP} - 136.0‰$; *R*-square = 0.95].

6. Source for USGS42 and USGS43

USGS42 and USGS43 human-hair isotopic reference materials are available from:

U.S. Geological Survey Reston Stable Isotope Laboratory
431 National Center
12201 Sunrise Valley Drive
Reston, Virginia 20192
USA
http://isotopes.usgs.gov/
Fax: +1 703 648 5832

Acknowledgements

This manuscript has benefited from careful reviews by L. Chesson (IsoForensics Inc., Salt Lake City, Utah, USA) and G. Singleton (U.S. Department of Energy). The support of the U.S. Geological Survey National Research Program made this report possible. 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] M.E. Wieser, T.B. Coplen, Atomic weight of the elements 2009, Pure Appl. Chem. 83 (2011) 359–396. , doi:10.1351/PAC-REP-10-09-14.
[2] T.B. Coplen, J.A. Hopple, J.K. Böhlke, H.S. Peiser, S.E. Rieder, H.R. Krouse, K.J.R. Rosman, T. Ding, R.D. Vocke, Jr., K.M. Révész, A. Lamberty, P. Taylor, P. De Bièvre, Compilation of minimum and maximum isotope ratios of selected elements in naturally occurring terrestrial materials and reagents, U.S. Geological Survey Water-Resources Investigations Report 01-4222, 2001, 98 p.
[3] W. Dansgaard, Stable isotopes in precipitation, Tellus 16 (1964) 436–468.
[4] K. Rozanski, L. Araguas-Araguas, R. Gonfiantini, Isotopic patterns in modern global precipitation, in: P.K. Swart, K.C. Lohmann, J. McKenzie, S. Savin (Eds.), Climate Change in Continental Isotopic Records, Geophysical Monograph 78, Washington, D.C., American Geophysical Union, 1993, p. 1.
[5] C. Kendall, T.B. Coplen, Distribution of oxygen-18 and deuterium in river waters across the United States, Hydrol. Proc. 15 (2001) 1363–1393.
[6] G.J. Bowen, J.R. Ehleringer, L.A. Chesson, E. Stange, T.E. Cerling, Stable isotope ratios of tap water in the contiguous United States, Water Resour. Res. 43 (2007) W03419, doi:10.1029/2006WR005186.
[7] J.M. Landwehr, T.B. Coplen, Spatial and seasonal variability of stable isotopic compositions (δ^2H and δ^{18}O) of tap waters throughout the United States, in: Fourth FIRMS Network Conference, Washington, D.C., 2010 April, 2010.
[8] Z.D. Sharp, V. Atudorei, H.O. Panarello, J. Fernández, C.C. Couthitt, Hydrogen isotope systematic of hair: archeological and forensic applications, J. Archaeol. Sci. 30 (2003) 1709–1716.
[9] I. Fraser, W. Meier-Augenstein, R.M. Kalin, The role of stable isotopes in human identification: a longitudinal study into the variability of isotopic signal in human hair and nails, Rapid Commun. Mass Spectrom. 20 (2006) 1109–1116. , doi:10.1002/rcm.2424.
[10] D.M. O'Brien, M.J. Wooller, Tracking human travel using stable oxygen and hydrogen isotope analyses of hair and urine, Rapid Commun. Mass Spectrom. 21 (2007) 2422–2430.
[11] J.R. Ehleringer, G.J. Bowen, L.A. Chesson, A.G. West, D.W. Podlesak, T.E. Cerling, Hydrogen and oxygen isotope ratios in human hair are related to geography, Proc. Natl. Acad. Sci. U.S.A. 105 (2008) 2788–2793.
[12] A.H. Thompson, L.A. Chesson, D.W. Podlesak, G.J. Bowen, T.E. Cerling, J.R. Ehleringer, Stable isotope analysis of modern human hair collected from Asia (China, India, Mongolia, and Pakistan), Am. J. Phys. Anthrop. 141 (2010) 440–451.
[13] G.J. Bowen, L. Chesson, K. Nielson, T.E. Cerling, J.R. Ehleringer, Treatment methods for the determination of δ^2H and δ^{18}O of hair keratin by continuous-flow isotope-ratio mass spectrometry, Rapid Commun. Mass Spectrom. 19 (2005) 2371–2378. , doi:10.1002/rcm.3018.
[14] L.A. Chesson, D.W. Podlesak, T.E. Cerling, J.R. Ehleringer, Evaluating uncertainty in the calculation of non-exchangeable hydrogen fractions within organic materials, Rapid Commun. Mass Spectrom. 23 (2009) 1275–1280. , doi:10.1002/rcm.4000.
[15] A. Schimmelmann, Determination of the concentration and stable isotopic composition of nonexchangeable hydrogen in organic matter, Anal. Chem. 63 (1991) 2456–2459. http://dx.doi.org/10.1021/ac00021a013.
[16] L.I. Wassenaar, K.A. Hobson, Comparative equilibration and online techniques for determination of non-exchangeable hydrogen of keratins for use in animal migration studies, Isotop. Environ. Health Stud. 39 (2003) 211–217.
[17] T.B. Coplen, Guidelines and recommended terms for expression of stable isotope-ratio and gas-ratio measurement results, Rapid Commun. Mass Spectrom. 25, doi:10.1002/rcm.5129.

[18] BIPM, The International System of Units (SI), 8th edition brochure (in English), 2006, 88 p., http://www.bipm.org/.
[19] R. Gonfiantini, Standards for stable isotope measurements in natural compounds, Nature 271 (1978) 534–536.
[20] T.B. Coplen, New guidelines for the reporting of stable hydrogen, carbon, and oxygen isotope ratio data, Geochim. Cosmochim. Acta 60 (1996) 3359.
[21] A.W. Hilkert, C.B. Douthitt, H.J. Schlüter, W.A. Brand, Isotope ratio monitoring gas chromatography/mass spectrometry of D/H by high temperature conversion isotope ratio mass spectrometry, Rapid Commun. Mass Spectrom. 13 (1999) 1226–1230.
[22] W.A. Brand, T.B. Coplen, A.T. Aerts-Bijma, J.K. Böhlke, M. Gehre, H. Geilmann, M. Gröning, H.G. Jansen, H.A.J. Meijer, S.J. Mroczkowski, H. Qi, K. Soergel, H. Stuart-Williams, S. Weise, R.A. Werner, Comprehensive inter-laboratory calibration of reference materials for δ^{18}O versus VSMOW using various on-line high-temperature conversion techniques, Rapid Commun. Mass Spectrom. 23 (2009) 999–1019. http://dx.doi.org/10.1002/rcm.3958.
[23] H.P. Qi, M. Gröning, T.B. Coplen, B. Buck, S.J. Mroczkowski, W.A. Brand, H. Geilmann, M. Gehre, Novel silver tubing method for quantitative introduction of water into high temperature conversion systems for stable hydrogen and oxygen isotopic measurements, Rapid Commun. Mass Spectrom. 24 (2010) 1821–1827. , doi:10.1002/rcm.4559.
[24] H.P. Qi, T.B. Coplen, Investigation of preparation techniques for δ^{2}H analysis of keratin materials and a proposed analytical protocol, Rapid Commun. Mass Spectrom. 25 (2011) 2209–2222. , doi:10.1002/rcm.5095.
[25] H.P. Qi, T.B. Coplen, Improved online δ^{18}O measurements of nitrogen- and sulfur-bearing organic materials and a proposed analytical protocol, Rapid Commun. Mass Spectrom. 25 (2011) 2049–2058. , doi:10.1002/rcm.5088.
[26] T.B. Coplen, J.D. Wildman, J. Chen, Improvements in the gaseous hydrogen—water equilibration technique for hydrogen isotope-ratio analysis, Anal. Chem. 63 (1991) 910–912.
[27] K. Révész, T.B. Coplen, Determination of the $\delta(^{2}H/^{1}H)$ of water: RSIL lab code 1574, in: K. Révész, T.B. Coplen (Eds.), Methods of the Reston Stable Isotope Laboratory, US Geological Survey Techniques and Methods 10-C1, 2008, 27 p., http://pubs.usgs.gov/tm/2007/tm10c1/ (chapter C1).
[28] S. Epstein, T. Mayeda, Variation of ^{18}O content of waters from natural sources, Geochim. Cosmochim. Acta 4 (1953) 213–224.
[29] K. Révész, T.B. Coplen, Determination of the $\delta(^{18}O/^{16}O)$ of water: RSIL Lab Code 489, in: K. Révész, T.B. Coplen (Eds.), Methods of the Reston Stable Isotope Laboratory, U.S. Geological Survey Techniques and Methods 10-C2, 2008, 28 p., http://pubs.water.usgs.gov/tm10C2.
[30] T.B. Coplen, Laboratory information management system (LIMS) for light stable isotopes, U.S. Geological Survey Open-File Report 00-345, 2000, 121 p.
[31] T.B. Coplen, Normalization of oxygen and hydrogen isotope data, Chem. Geol. (Isotope Geosci. Sect.) 72 (1988) 293–297.
[32] T.B. Coplen, W.A. Brand, M. Gehre, M. Gröning, H.A.J. Meijer, B. Toman, R.M. Verkouteren, New guidelines for δ^{13}C measurements, Anal. Chem. 78 (2006) 2439–2441.
[33] K. Révész, H.P. Qi, Determination of the $\delta(^{15}N)$ and $\delta(^{13}C)$ of total N and C in solids: RSIL lab code 1832, in: K. Révész, T.B. Coplen (Eds.), Methods of the Reston Stable Isotope Laboratory, Reston, Virginia, U.S. Geological Survey, Techniques and Methods, Revised 2007, 31 p. http://pubs.water.usgs.gov/tm10C5/ (book 10, section C, chapter C5).
[34] H.P. Qi, T.B. Coplen, H. Geilmann, W.A. Brand, J.K. Böhlke, Two new organic reference materials for δ^{13}C and δ^{15}N measurements and a new value for the δ^{13}C of NBS 22 oil, Rapid Commun. Mass Spectrom. 17 (2003) 2483–2487.
[35] H.P. Qi, T.B. Coplen, Evaluation of the $^{34}S/^{32}S$ ratio of Soufre de Lacq elemental sulfur isotopic reference material by continuous flow isotope-ratio mass spectrometry, Chem. Geol. 199 (2003) 183–187.
[36] http://www.ciaaw.org/.
[37] B.W. Robinson, Sulphur isotope standards, in: Reference and Intercomparison Materials for Stable Isotopes of Light Elements, Vienna International Atomic Energy Agency IAEA-TECDOC-825, 1995, pp. 39–45.
[38] C.R. Robbins, Chemical and Physical Behavior of Human Hair, 4th edition, Springer, 2002.