Artigo

STABLE ISOTOPES OF OXYGEN AND HYDROGEN IN WATER: ANALYTICAL METHOD EVALUATION AND THE DETERMINATION OF $\delta^{18}O$ AND $\delta^{2}H$ ON A CONTROL SAMPLE

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Evaluation studies contribute to the identification of parameters that can affect the accuracy and precision of analytical methods. This study describes the analysis of $\delta^{18}O$ and $\delta^{2}H$ in water samples by the equilibrium method at the Isotope Geology Laboratory (LGI), Center for studies in Petrology and Geochemistry (CPGq) of the Federal University of Rio Grande do Sul (UFRGS). For that, six batches of analyzes were carried out under different ambient temperature conditions. For the reproducibility tests, four aliquots of the control sample were analyzed at the Polar Climate Center (CPC). Results showed that ambient temperature did not significantly affect the accuracy of the oxygen analysis. However, the mean result at 20 °C showed greater accuracy and acceptable precision. Hydrogen analyzes at a room temperature of 18 °C showed an external standard deviation and an internal precision exceeding the recommended, while at 20 and 22 °C the results were statistically acceptable, being the first more accurate. From that, it is possible to conclude that the determination of $\delta^{18}O$ and $\delta^{2}H$ in water at the LGI, employing the equilibrium method at an ambient temperature of 20 °C, showed satisfactory repeatability and reproducibility.

Keywords: stable isotopes; H and O; analytical method; water.

INTRODUCTION

There are three stable isotopes of oxygen and two stable isotopes of hydrogen in nature, ^{16}O, ^{17}O, ^{18}O, ^{1}H and ^{2}H, so that there will be nine possible combinations for the formation of water molecules, called isotopologues.[1] The mass variation of the isotopologues will be from 18 amu for $^{1}H_2{}^{16}O$ to 22 amu for $^{2}H_2{}^{18}O$.[2] The water species that are relevant to O and H stable isotope studies are: $^{1}H_2{}^{16}O$, $^{1}H_2{}^{17}O$, $^{1}H_2{}^{18}O$ and $^{1}H^{2}H^{16}O$ (Table 1). The double or triple labeled water species ($^{2}H_2{}^{16}O$, $^{1}H^{2}H^{17}O$, $^{1}H^{2}H^{18}O$, $^{2}H_2{}^{17}O$ and $^{2}H_2{}^{18}O$) are not relevant due to their low abundance in nature (Table 1).

Stable isotopes of oxygen (O) and hydrogen (H) in water are important tools with different applications in Earth Sciences. The isotope composition of water does not change as a result of rock/water interactions at low temperatures.[3] On the other hand, the water isotope composition is affected by the natural hydrological cycle, climatological parameters such as precipitation and temperature, and geological parameters such as altitude, latitude and continentality.[4] In environmental studies, these isotopes can help to understand the origin and movement of water throughout the hydrological cycle, sources of precipitation, aquifer recharge, seasonal variations in hydrological processes, contaminant tracking and associated hydroclimatic processes.[2-8] In addition, the isotope composition of a sample can be altered by different physical, chemical and/or biological phenomena, since lighter isotopes are more susceptible to certain physical variations and chemically react more easily, causing compositional variation.[9]

The application of O and H isotope data in water is also used as markers of geographic origin since, in general, groundwater has an isotope composition similar to the annual average precipitation of a given area, which, in turn, depends on geographic factors such as altitude, latitude, distance from the oceans or continentality.[10] This has recently become important also in forensic studies, because the application of O and H isotopes can also be used as markers of geographic origin for unknown human samples (e.g., bones, teeth, hair and nails); residence patterns of unidentified humans, or to determine the origin and provenance of food.[11-13]

Isotope analyses are possible using different mass spectrometry techniques. Thus, due to the importance that the use of these isotopes assume in scientific research, it is important for laboratories to guarantee the quality of the data acquired and the understanding of the different stages of this process.[14-18] Therefore, the objective of this study is to describe the analytical methods applied to acquiring O and H isotope data in water samples at the Isotope Geology Laboratory (LGI), Center for studies in Petrology and Geochemistry (CPGq) of the Federal University of Rio Grande do Sul (UFRGS). In addition, we describe the analytical method of these analyses, as well as the definition of $\delta^{18}O$ and $\delta^{2}H$ values on a control sample, used as one of the quality controls of the method.

Stable isotopes of O and H in water and the delta notation

The ratio between the amount of the rare isotope and the amount of the most abundant isotope in a given water sample is defined as the isotopic composition (R). For oxygen and hydrogen, R is given by $^{18}O/^{16}O$ and $^{2}H/^{1}H$ (or D/H), respectively.

Thus, in a substrate-product reaction, $R_{substrate}$ and $R_{products}$ are, respectively, the isotope ratios of the substrate and the product. Because the isotopes have slightly different reaction rates, due to their mass differences, at the end of the reaction, $R_{substrate}$ tends to be

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Table 1. Isotope species of waters and their relative abundances (adapted from reference 19)

Water isotopologues	Relative abundance (%)
$^{1}H_{2}^{16}O$	**99.73098**
$^{1}H_{2}^{18}O$	**0.199978**
$^{1}H_{2}^{17}O$	**0.037888**
$^{1}H^{2}H^{16}O$	**0.03146**
$^{1}H^{2}H^{18}O$	0.0000006
$^{1}H^{2}H^{17}O$	0.0000001
$^{2}H_{2}^{16}O$	0.00000002
$^{2}H_{2}^{17}O$	0.00000000001
$^{2}H_{2}^{18}O$	0.00000000005

different from the value of $R_{products}$. From this, it is possible to define the fractionation factor (α):

$$\alpha = (R_{substrate}/R_{products}) \quad (1)$$

The interpretation of the isotope ratio of a given sample is represented by its deviation from the R ratio of a standard.[19] The use of standard reference materials, in the analytical context, allows minimizing systematic errors during the analysis. From this the δ (delta) notation is given by:[19]

$$\delta = ((R_{sample} - R_{standard})/R_{standard})) \times 1000 \quad (2)$$

where R is the isotope ratio between the rare (heavy) isotope and the most abundant (light) isotope of the sample and/or standard. For oxygen and hydrogen, delta is represented by $\delta^{18}O$ and $\delta^{2}H$, respectively. Numerical values of δ are reported in permil (‰). Positive delta values mean that the isotope ratio between the rare isotope and the most abundant isotope of the sample is greater than the isotope ratio between the rare isotope and the most abundant isotope of the standard reference material. Likewise, negative delta values mean that the isotope ratio between the rare isotope and the most abundant isotope of the sample is less than the isotope ratio between the rare isotope and the most abundant isotope of the standard reference material.[2,19]

Isotope ratio mass spectrometry - IRMS

Mass spectrometry is an important analytical technique used for the determination of element concentration, especially in the trace (ppm) and ultra-trace (ppb) range, isotope ratio measurements and structural analysis of organic and bioorganic compounds. This analytical technique exhibits low detection limits and results with high precision and reproducibility.[20]

A spectrometer separates charged atoms and molecules based on their mass to charge ratio and their movements in magnetic and/or electric fields.[20] Generally, a mass spectrometer can be separated into five fundamental parts: an input system; an ion source; the mass analyzer; the ion detector and a registry system. In an IRMS applied to O and H isotope measurements, the input system is continuous or dual flow. In the continuous flow system, a carrier gas (He - ultrapure) is employed, which will lead, to the ion source, the CO_2 or H_2 that has reached isotope equilibrium with the sample or standard reference material. The dual input system allows the isotope ratio of two gases - the reference and the sample - to be measured progressively, providing more accurate results.[21]

From the inlet system, the gas, after equilibrated, or the reference standard gas is introduced directly into the electron ionization source. The ion source is applied for the formation of gas ions or volatile samples that readily form gases before or during the introduction into the mass spectrometer.[22] Electrons are emitted from a heated rhenium (Re) or tungsten (W) filament (cathode), with temperatures ranging between 1500 - 2000 K. The emitted electrons are accelerated towards the anode, which is in opposite position to the cathode, forming an electron beam. The atoms or gas molecules, as they pass through the ion source, are ionized and fragmented by collisions with electrons.

After ionization, the gas is focused into a beam and accelerated through the flight tube (analyzer). Afterwards, the beam is exposed to a magnetic field of specific intensity, according to the masses to be analyzed, where the ions will undergo a deviation according to their mass/charge ratio. Lighter ions are deflected more strongly than heavier ions of the same charge.[23] After separation, ions corresponding to different masses are conducted to collectors (Faraday cups) where they will be detected. The intensity of each detected beam, in the different Faraday cups, is proportional to the concentration of each isotope in the sample or in the reference gas. Due to the low signal intensity generated and the difference in concentration of each isotope in a sample, these different signals are amplified with different steps of signal enhancement. In summary, the electric current that comes from each collector is amplified differently and transformed into voltage in the amplifier, after which this analog signal is transformed into a digital signal and then passes through the integrator that will integrate the signal in certain variations of time (according to methodology). The signal originated from the integration step is directed to the equipment acquisition software.[22]

Analyses in the spectrometer are performed in batches. A batch is prepared and all samples from that batch are exposed to the same conditions during all stages of the process and are analyzed sequentially. Among the samples of a given batch, analytical reference standards must be included, which are necessary for the calibration and subsequent normalization of the results. In addition to the standards, it is suggested to place one reference (control) sample for every six unknown samples.

Measurements of $\delta^{18}O$ and $\delta^{2}H$ in water are reported by comparing the obtained data to the Vienna Standard Mean Ocean Water (VSMOW) reference material which, by definition, has $\delta^{18}O = 0‰$ and $\delta^{2}H = 0‰$. Because of the relatively large range of isotope ratios in the hydrological cycle, which often exceeds the linearity, a number of standards were prepared and are reported, such as GISP ($\delta^{18}O = -24.79‰_{VSMOW}$ and $\delta^{2}H = -189.7‰_{VSMOW}$) and SLAP ($\delta^{18}O = -55.5‰_{VSMOW}$ and $\delta^{2}H = -428.0‰_{VSMOW}$).[2]

Furthermore, for operational reasons, different laboratories and/or research centers prepare their own standards calibrating them with the VSMOW standard. An example of this are the ULW working standards ($\delta^{18}O = -4.33‰_{VSMOW}$ and $\delta^{2}H = -25.37‰_{VSMOW}$), Deplat ($\delta^{18}O = -12.37‰_{VSMOW}$ and $\delta^{2}H = -91.94‰_{VSMOW}$) and Brasília ($\delta^{18}O = -3.37‰_{VSMOW}$ and $\delta^{2}H = -13.92‰_{VSMOW}$), which were prepared and calibrated by the Polar Climate Center (CPC) of the Federal University of Rio Grande do Sul and available for this study.

MATERIALS AND METHODS

Determination of $\delta^{18}O$ in waters at the LGI

For the $\delta^{18}O$ determination in water samples, the method applied in this study involves the CO_2 – H_2O equilibrium and the equipment used is the Isotope Ratio Mass Spectrometry (IRMS) - Delta V Advantage - GasBench II, from Thermo Fisher Scientific®. Table 2 shows the specifications of purity, working pressure and gas flows used for the $\delta^{18}O$ determination in water.

Table 2. Gases used and specifications for determination of $\delta^{18}O$ in water

Type	Gas	Working pressure	Specification	Flow
Reference	CO_2	1.3 bar	4.5-99.99%	
Carrier	He	1.7 bar	5.0-99.999%	
Flush	He + CO_2	5.0 Kgf cm^{-2}	0.5% of CO_2 4.5 in He 4.6	100-150 mL min^{-1}

To carry out the $\delta^{18}O$ analysis in waters, the following steps are performed:

1. Add 500 µL of sample to a 10 mL borosilicate vial tube (Labco®). The tube is then closed with the cap containing a silicone septum. This step is performed on all samples and standards;
2. Afterwards, the samples and standards are placed in the autosampler tray. To obtain greater precision, it is necessary to control the temperature of the sampler, keeping it at 25 °C ± 0.1 °C. The fractionation factor α of the equilibrium $^{18}O/^{16}O_{CO2(g)}/^{18}O/^{16}O_{H20(l)}$ is 1.0412 at 25 °C,[24] and the temperature dependence is 0.2‰/°C, so the temperature control of 0.1 °C is suitable for more precise measurements. According to the equipment manufacturer, the recommendation is that the room temperature is 5 °C lower than the temperature of the sampler;[25]
3. The next step is to flush the system to replace the atmospheric air inside the tubes with a special gas mixture of CO_2/He (0.5% of CO_2 4.5 in He 4.6). This step is performed with a gas flow between 100 and 150 mL min^{-1} for 5 minutes, *per* tube;
4. Afterwards, it is necessary to wait 18 hours to reach equilibrium. The equilibrium is given according to the following reaction:[2]

$$H_2{}^{18}O_{(l)} + C^{16}O_{2(g)} \leftrightarrow H_2{}^{16}O_{(l)} + C^{16}O^{18}O_{(g)} \quad (3)$$

5. After the equilibration time, the control parameters of the equipment are reviewed, which are given in Table 3;
6. After validating the equipment control parameters, the automatic sequence of analyzes in the spectrometer is performed. The results are stored in the registry system and evaluated through statistical treatments (mean and standard deviation). The raw data are normalized to the VSMOW scale through calibration curves that are constructed from analytical standards analyzed together with the samples.

Determination of δ^2H in waters at LGI

The method applied in this study involves the $H_2 - H_2O$ equilibrium and the equipment used is the Isotope Ratio Mass Spectrometry (IRMS) - Delta V Advantage - GasBench II, from Thermo Fisher Scientific®. Table 4 shows the specifications of purity, working pressure and gas flows used for the determination of δ^2H analysis in water.

To carry out the determination of δ^2H in water, the following steps are used:

1. Add 200 µL of sample to a 10 mL borosilicate tube (Labco®). Afterwards, the platinum catalyst is added to prevent the formation of H_2S and to remove the water molecules adsorbed on the dissolved organic carbon (DOC). Then, the tube is closed with the cap containing a silicone septum;
2. Afterwards, the samples and standards are placed in the autosampler tray. To obtain greater measurement precision, it is necessary to have the temperature control of the sampler fixed at 25 ± 0.1 °C, since the temperature dependence is 6‰/°C for hydrogen analysis;[26]
3. The next step is to flush the system. This procedure is carried out by replacing the atmospheric air inside the sample tubes with the special gas mixture H_2/He (2% H_2 5.0 in He 4.6). This step is performed in a gas flow between 100 and 110 mL min^{-1} for 5 minutes, *per* tube;
4. After, it is necessary to wait 40 minutes for reaching equilibrium. The equilibrium reaction occurs according to the reaction below:[2]

$$^1H_{2(g)} + {}^1H^2HO_{(l)} \leftrightarrow {}^1H^2H_{(g)} + {}^1H_2O_{(l)} \quad (4)$$

5. After the equilibration period, the equipment control parameters are reviewed, given in Table 5;
6. After validating the equipment control parameters, the automatic sequence of analyzes in the spectrometer is performed. The results are stored in the registry system, evaluated through statistical treatments (mean and standard deviation), and normalized through calibration curves using the VSMOW scale.

Isotope analysis of H_2 by mass spectrometry is based on measuring the current of ions with mass 2 and 3, simultaneously. The mass 2 ion current is related to the $^1H_2{}^+$ species, while the mass 3 ion current is related to the $^1H^2H^+$ e $^1H_3{}^+$ species.[2]

Table 3. Equipment control parameters evaluated before performing the determination of $\delta^{18}O$ in water

Equipment: IRMS Delta V Advantage - GasBench2		
Control	Acceptance criteria	Corrective measures
Stability	Difference between the values of 46/44 ratio SD ≤ 0.06	Adjust He source and/or flow parameters
Linearity	Ampl. 44 *vs.* $\delta^{18}O$ (graph: y = ax + b; x ≤ 0.066‰/V)	Adjust He source and/or flow parameters
Signal of mass 44	1 V-15 V	Check the concentration of the H_2 reference gas
Background CO_2 (44)	≤ 20 mV	Search for leaks
Intensity H_2O (18)	1000 mV-5000 mV	Check for humidity traps
Intensity Ar (40)	≤ 50 mV	Search for leaks
Vacuum	1-3 × 10^{-6} mbar	Search for leaks
Temp. sample tray	25 °C	Correct the temperature
Ambient temp.	20 °C	Correct the temperature
Temp. Gasbench column	70 °C	Correct the temperature

(Temp.): temperature. (Ampl.): amplitude.

Table 4. Gases used and specifications for determination of δ^2H in water

Type	Gas	Working pressure	Specification	Flow
Reference	H_2	1.9 bar	5.0-99.999%	
Carrier	He	1.3 bar	5.0-99.999%	
Flush	He + H_2	3.5 Kgf cm^{-2}	2% H_2 5.0 in He 4.6	100-110 mL min^{-1}

Table 5. Equipment control parameters evaluated before performing the determination of δ^2H in water

Equipment: IRMS Delta V Advantage - GasBench2		
Control	Acceptance criteria	Corrective measures
Stability	Difference between the values of 3/2 ratio SD ≤ 0.4	Adjust He source and/or flow parameters
^3H Factor determination	Ampl. 2 *vs*. 3 (area)/2 (area) (graph: y = ax + b; x = ^3H Factor)	Adjust He source and/or flow parameters
Linearity	After ^3H Factor determination, SD of analysis ≤ 0.4	Adjust source parameters
Signal of mass 2	1 V-15 V	Check the concentration of the H_2 reference gas
Background H_2 (2)	≤ 150 mV	Search for leaks
Intensity H_2O (18)	1000 mV to ≤ 5000 mV	Check for humidity traps
Intensity Ar (40)	≤ 50 mV	Search for leaks
Vacuum	1-3 × 10^{-6} mbar	Search for leaks
Temp. sample tray	25 °C	Correct the temperature
Ambient temp.	20 °C	Correct the temperature
Temp. Gasbench column	70 °C	Correct the temperature

(Temp.): temperature. (Ampl.): amplitude.

The $^1H_3^+$ ion is also produced at the source through collisions between $^1H_2$ and the $^1H_2^+$ ion according to the reaction below:

$$^1H_2 + {}^1H_2^+ \rightarrow {}^1H_3^+ + H^* \quad (5)$$

The formation of $^1H_3^+$ is a consequence of the use of H_2 (as reference gas) to carry out these measurements. In this case, the differentiation between the $^1H_3^+$ and $^1H^2H^+$ ions, both of mass 3, is not achieved, transforming the $^1H_3^+$ ion into an isobaric interference, requiring additional correction, since the signal of mass 3 *m/z* can be enriched by up to 30% (30 ppm mV^{-1}) of $^1H_3^+$ produced at source.

In conventional isotope ratio measurements, both the sample and the reference gas enter the ion source as H_2. Ion source pressures are typically 10^{-6} mbar or less during these measurements and H_2 is the only neutral species present in significant amounts. Under these conditions, collisions between $^1H_2$ and the $^1H_2^+$ ion are the main source of $^1H_3^+$ ion production. Therefore, the concentration of $^1H_3^+$ is proportional to the product of the concentrations between $^1H_2^+$ and $^1H_2$, according to the equation below:[27]

$$[^1H_3^+] \propto [^1H_2^+][^1H_2] \quad (6)$$
$$[^1H_3^+] = K\,[^1H_2^+][^1H_2]$$

The proportionality constant (K) is commonly known as the ^3H Factor.[27] The K is determined by measuring the ratio of (mass 3)/(mass 2) ions in the reference gas in a given pressure range. After carrying out these measurements, at different pressures, and as the number of $^1H_3^+$ and $^1H_2^+$ ions are proportional to the pressure of H_2 inside the ion source, the ^3H Factor can be calculated. In practice, the procedure to calculate the ^3H Factor is the acquisition of the hydrogen reference gas with pulses of different intensities generating the graph shown in Figure 1. From the resulting $^3H_2/^2H_2$ ratio (mass area 3/mass area 2) for each pulse *versus* the $^2H_{2+}$ signal intensity (mV), a linear regression can be performed (Figure 2) and the slope of the line is the ^3H Factor.

Thermo Scientific® isotope ratio mass spectrometers such as the Delta V Advantage generally operate with a ^3H Factor < 10 ppm mV^{-1}.[25] To reach this value it is necessary to follow some procedures:

1. Keep the extraction lens at extraction voltage values greater than 90%. This reduces the residence time of hydrogen ions inside the ionization chamber, repelling them before interacting with other neutral elements;[25]
2. Adjust the ionization energy (electron energy), since values greater than 100 eV can generate double charged He ions (He^{2+}). As these ions have a mass difference (Δm = 0.5%) in relation to $^2H_2^+$, this can lead to distortion of the hydrogen peak plateau.[25]

From the evaluation of the $^3H_2/^2H_2$ *versus* mass 2 signal amplitude (Figure 2), coupled to the data in Table 6, it is possible to observe the increase in the precision of the analysis after using the ^3H Factor. The black line in Figure 2 is a linear regression through the $^3H_2/^2H_2$ data of the reference gas acquired at different pressures, used for calculating the ^3H Factor. In Table 6, the $R^3H_2/^2H_2$ values corrected for isobaric interferences ($^1H_3^+$) are shown, which represent more precise δ^2H ($d^2H/^1H$) values. The red line in Figure 2 shows the same data points, but after applying the ^3H Factor.

With a ^3H Factor of 4.97 ppm mV^{-1}, it was possible to reduce the standard deviation from 17.31 to 0.86 in the $d^2H/^1H$, showing the sensitivity and interference of $^1H_3^+$ (Table 6).

The effect of ambient temperature

Considering the importance of identifying parameters that affect the analysis of stable isotopes of oxygen and hydrogen in water, a ruggedness test was carried out with the objective of validating the methods of analysis assessing the stability of the IRMS, and the repeatability and reproducibility, as a function of ambient temperature.

For that, an amount of Milli-Q water was separated and fractionated in 12 mL borosilicate tubes with a lid and silicone septum, labeled as LGI control sample. The tubes were completely

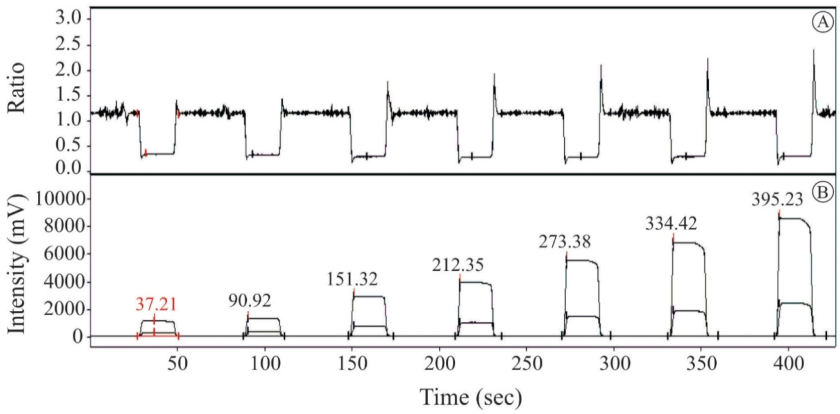

Figure 1. *Typical values for ^3H Factor determination. The graphic on top (A) shows the isotope ratio of masses 2 and 3 (3/2) over time; bottom graphic (B) shows the intensity (mV) of mass 2 and 3 signals over time varying the pressure of the reference gas H_2, with the upper plateau being mass 2 and bottom plateau mass 3*

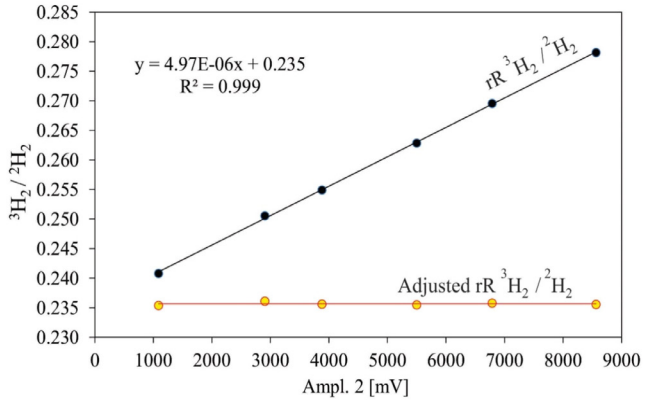

Figure 2. *Graphic of $^3H_2/^2H_2$ isotope ratio versus mass 2 signal amplitude for ^3H Factor determination*

filled (free from atmospheric air). Repeated determination of $\delta^{18}O$ and δ^2H of this sample were performed, with a total of six batches, labeled 1, 2, 3, 4, 5 and 6.

The equipment manufacturer recommends that the ambient temperature where the analyzes are carried out should be 5 °C below the temperature of the autosampler tray (25 °C for O and H analysis). In order to verify the stability of the equipment and the ability of the autosampler tray to maintain a stable temperature during the analyses, the different batches were analyzed under different ambient temperature conditions. Batch 1 was set to 18 °C, 2 to 20 °C and 3 to 22 °C for oxygen analysis, and batch 4 to 18 °C, 5 to 20 °C and 6 to 22 °C, for hydrogen analysis. All batches of analyses were performed on the same equipment, same method and with the same operator. The ambient temperature was stabilized for a period of 24 hours before running the different batches.

With the objective of evaluating the reproducibility of the analyses, four aliquots of the sample were also sent to the Laboratory of Stable Isotopes of the Polar Climate Center (CPC), so that different conditions of laboratory, equipment, operator, procedures, method, observer, instruments, conditions of use, location and time were analyzed. At the Polar Climate Center, located in Porto Alegre, data was also acquired by IRMS.

The standards used in the experiment were ULW ($\delta^{18}O$ = -4.33‰$_{VSMOW}$ and δ^2H = -25.37‰ $_{VSMOW}$), Deplat ($\delta^{18}O$ = -12.37‰$_{VSMOW}$ and δ^2H = -91.94‰ $_{VSMOW}$), Brasília ($\delta^{18}O$ = -3.37‰$_{VSMOW}$ and δ^2H = -13.92‰ $_{VSMOW}$) and VSMOW ($\delta^{18}O$ = 0.00‰, δ^2H = 0.00‰). The delta values of the ULW, Deplat and Brasília standards were determined by the CPC and made available for this study.

RESULTS

The results for oxygen and hydrogen isotope determination in this study are presented in Appendix A and B (Supplementary Material), respectively. The average results for the determination of $\delta^{18}O$ of the LGI control sample from different batches and the results of the CPC analysis, for reproducibility tests, are compiled in Table 7. Figure 3 shows the control chart of the $\delta^{18}O$ analysis using data of this study.

Batch 1 showed a slightly more negative mean value of $\delta^{18}O$ compared to subsequent batches ($\delta^{18}O$ = -4.43‰). The external standard deviation (1 sigma) of the measurements was 0.10‰ and the internal precision among the 10 pulses analyzed *per* run ranged from 0.05-0.12‰. The results of batch 2 showed a mean value of $\delta^{18}O$

Table 6. Results obtained applying the ^3H Factor in an analysis. In bold are the adjusted results after correcting for isobaric interference

Peak nº	Ampl. 2 (mV)	r Area 2 (mVs)	r Area 3 (mVs)	rR $^3H_2/^2H_2$	**Adjusted rR $^3H_2/^2H_2$**	$d^2H/^1H$ (permil) *vs.* VSMOW	**Adjusted $d^2H/^1H$ (permil) *vs.* VSMOW**
1	1090	21012	5060	0.24081	**0.235393101**	-701.55	**-700.53**
2	1944	24299	5282	0.24208	**0.2358061**	-700.00	**-700.00**
3	2906	55554	13919	0.25055	**0.236094542**	-689.50	**-699.26**
4	3882	73903	18839	0.25492	**0.235606119**	-684.08	**-699.63**
5	5501	103954	27325	0.26286	**0.235494618**	-674.25	**-699.28**
6	6790	127751	34437	0.26956	**0.235789918**	-665.94	**-698.46**
7	8565	159850	44465	0.27817	**0.235564634**	-655.28	**-698.04**
Calculated H3-Factor: 4.97E-06 (ppm/nA)					Average	-681.51	**-699.31**
					SD	17.31	**0.86**

Table 7. Results of the determination of $\delta^{18}O$ for different batches performed at the LGI, and the results at the CPC

Isotope Geology Laboratory - LGI					
Batch	T (°C) Block	T (°C) Room	n	$\delta^{18}O$ (Average) ‰	External SD ‰
1	25	18	66	-4.43	0.10
2	25	20	65	-4.31	0.11
3	25	22	72	-4.20	0.07
Polar Climate Center - CPC					
Sample LGI	T (°C) Room	$\delta^{18}O$ ‰	Internal SD ‰	$\delta^{18}O$ (Average) ‰	External SD ‰
1	20	-4.25	0.02	-4.27	0.02
2		-4.25	0.02		
3		-4.3	0.03		
4		-4.28	0.05		

0.12‰ more positive than the mean value of batch 1. In addition, it has an external standard deviation similar to batch 1 (0.11‰). The internal precision among the 10 pulses analyzed *per* run also varied between 0.05-0.12‰.

The $\delta^{18}O$ determination of batch 3 showed an average result 0.11‰ more positive than the average value of batch 2. In addition, the external standard deviation of the analysis of this batch was the smallest compared to the previously analyzed (0.07‰). The internal precision among the 10 pulses *per* run varies between 0.04-0.10‰. The samples analyzed in the CPC showed mean value and an external standard deviation of -4.27‰ and 0.02‰, respectively.

From these data, a control chart for the determination of $\delta^{18}O$ was created (Figure 3). Therefore, it was established that the $\delta^{18}O$ value of the control sample is the representative mean values of batch 2 (4.31‰) and a confidence range of acceptable $\delta^{18}O$ values for the control sample (Figure 3), since this is the value that matches more closely the reported value. This range was established from the external precision data calculated for the batch 2 analyses performed at a 95% confidence interval ($\delta^{18}O_{bat.\ 2\ average} \pm 2$ sigma).[18] Therefore, the reference value of the sample plus the confidence interval are $\delta^{18}O = -4.31‰ \pm 0.22‰$ (Figure 4).

The averages results for the determination of $\delta^{2}H$ for batches 4, 5 and 6 of the control sample and the results of the CPC analysis, for reproducibility tests, are compiled in Table 8. Figure 4 shows the control chart of the determination of $\delta^{2}H$ from the data of this study.

According to the results of this study, the average value of the analysis of batch 4 is close to the average value between the subsequent batches (5 and 6). However, it showed the highest external standard deviation (2.95‰) compared to the other batches. The internal precision among the 10 pulses analyzed *per* run ranged from 0.30-1.18‰.

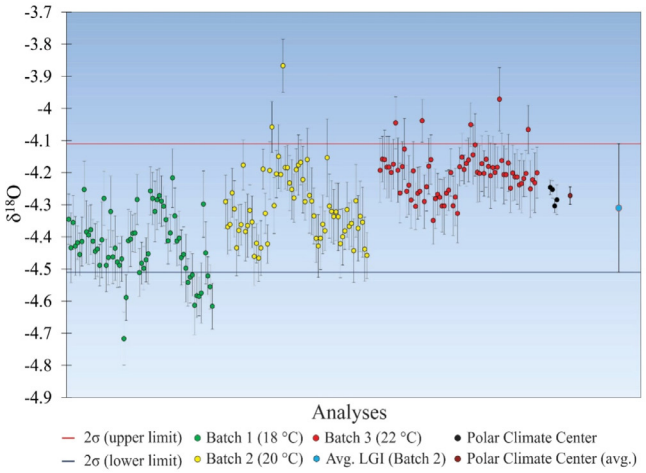

Figure 3. *Diagram of $\delta^{18}O$ values versus the number of analyses in the control sample - control chart*

The values for $\delta^{2}H$ of batch 5 showed an average of 1.01‰ more positive than the average value of batch 4, the smallest external standard deviation between batches (1.04‰) and the average value closest to the average value of the batches aliquots analyzed by the CPC ($\delta^{2}H = -19.26‰ \pm 0.27‰$). The internal precision among the 10 pulses analyzed per run ranged between 0.12-0.38‰. Results for the $\delta^{2}H$ of batch 6 showed the most negative mean result among all batches (-21.61‰) and an external standard deviation of 2.04‰. The internal precision among the 10 pulses analyzed *per* run ranged from 0.17-0.52‰.

In a similar fashion to the $\delta^{18}O$, a control chart using the $\delta^{2}H$ data of the control sample was drawn (Figure 4). Therefore, it was established

Table 8. Results of the determination of $\delta^{2}H$ of the different batches on the control sample at the LGI and at the CPC

Isotope Geology Laboratory - LGI					
Batch	T (°C) Block	T (°C) Room	n	$\delta^{2}H$ (Average) ‰	External SD ‰
4	25	18	25	-20.8	3.0
5	25	20	24	-19.8	1.0
6	25	22	30	-21.6	2.0
Polar Climate Center - CPC					
Sample LGI	T (°C) Room	$\delta^{2}H$ ‰	Internal SD ‰	$\delta^{2}H$ Average ‰	External SD ‰
1	20	-19.42	0.31	-19.3	0.3
2		-19.54	0.11		
3		-18.95	0.11		
4		-19.13	0.07		

that the δ^2H value of the control sample is the representative value of the average batch 5 analysis, with the associated acceptable range of δ^2H values. This range was established from the external precision data calculated from this batch at a 95% confidence interval ($\delta^2H_{bat.\ 5\ average}$ ± 2 sigma).[18] Therefore, the reference value of the sample plus the confidence interval are δ^2H = -19.8‰ ± 2.1‰ (Figure 4).

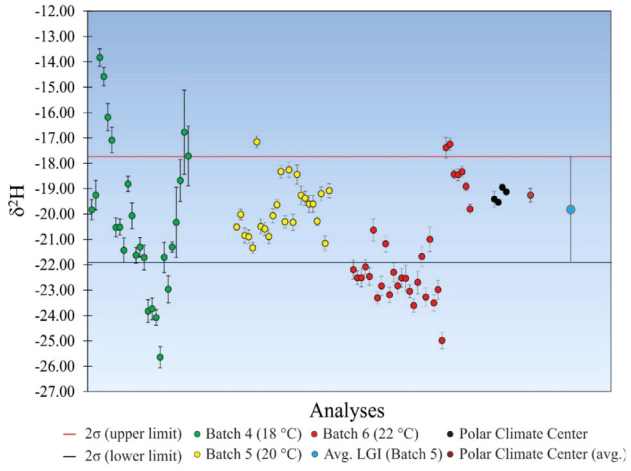

Figure 4. *Diagram of δ^2H values versus the number of analyses in the LGI control sample - control chart*

DISCUSSION

The implementation of analytical methods is important in the search for reliable analytical results. Effectiveness of methods produces standard operating procedures that incorporate a set of instructions for performing a measurement and defines parameter values that must remain stable during the analysis process and that can be used for interlaboratory studies.[28] Precision measures (repeatability and reproducibility) and linearity are important parameters for a method evaluation. Linearity is an important factor for measurements over a concentration range and is generally not quantified but verified, and can be corrected by using calibration functions or by choosing a narrower concentration range.[17] The main measures of accuracy estimated within a laboratory or by interlaboratory studies include: the standard deviation of repeatability and reproducibility.[14-18,28,29] These are important parameters, as they identify counter effects that need to be eliminated before the analysis, either by modifying the method, or by reducing the variation caused by the effect. For example, the minimization of these effects is done by establishing a control range, specifying a certain operating temperature or temperature range that reduces the variation.[28]

Temperature is one of the critical factors for isotope analysis of oxygen and hydrogen by the equilibrium method. Temperature variations influence the stability and linearity of the IRMS, impairing the focus of the beams, causing ion deviations in the collectors.[10] Furthermore, the stability temperature for determination of $\delta^{18}O$ and δ^2H by the equilibrium method is ± 0.1 °C, since the fractionation resulting from the temperature oscillation is approximately 0.2‰/°C for oxygen and 6.0‰/°C for hydrogen.[21,24,26] Thus, maintaining the laboratory's ambient temperature constant is required.[10]

Considering the results obtained under three different conditions (18 °C, 20 °C and 22 °C) of laboratory ambient temperature, no significant variation was observed in the external standard deviation for the oxygen data (Table 7 and Figure 3). Therefore, regardless of the ambient temperatures used in this study, the three batches analyzed, in general, met the external deviations of the manufacturer's specification (≤ 0.10‰). The fractionation was far below what would be expected for a temperature variation of 4° C (18 °C-22 °C). If the autosampler tray did not remain with a stable temperature during the analyses the oxygen results would tend to fractionate 0.8‰. Thus, the ambient temperature variation did not interfere significantly for the oxygen analyses.

Furthermore, the average $\delta^{18}O$ results of all analyses (-4.31‰), at different temperatures, reproduces the results obtained by the Polar Climate Center (average $\delta^{18}O$ at 20 °C = -4.27‰) (Table 7), with a slightly difference of only 0.04‰ among these mean values. Thus, the method used in the LGI, in addition to presenting repeatability, proved to be reproducible, since the analyses performed presented results statistically consistent with those performed under different conditions, such as laboratory, equipment, operator, procedures, method, observer, instrument, conditions of use, location and time.[15,28]

It is important to highlight also that results were satisfactory at the different ambient temperatures analyzed and the average result of batch 2 was the closest to the average result analyzed by the CPC (Table 7). Although the results of batch 3 were statistically more accurate, the average result was less accurate (Table 7). From these data, we can consider that the optimal working ambient temperature (the best balance between repeatability and reproducibility) was 20 °C, in agreement with the manufacturer's guidelines.[25]

Considering the results obtained under three different conditions (18 °C, 20 °C and 22 °C) of ambient laboratory temperature, a significant variation was observed in the results of the hydrogen analysis of batch 4 (Table 8, Figure 4). This variation resulted in an external standard deviation of 2.95‰, a value higher than that recommended by the equipment manufacturer (external SD ≤ 2‰ for hydrogen isotopic analysis), and internal deviations, for some analyses, above 1‰, values also higher than recommended by the manufacturer (internal SD ≤ 0.4‰).[25] However, the average value of δ^2H of batch 4 was the closest to the average value of all analyses performed at different temperatures (average δ^2H of batches = -20.75‰) (Table 8).

The average δ^2H value of batch 6 (-21.61‰) is the most negative among the batches and the external standard deviation showed a satisfactory result (2.04‰), considering the manufacturer's recommendation. The internal standard deviation of the individual analyzes presented, in general, satisfactory results, despite presenting values greater than 0.4‰ for some samples.

The average δ^2H result from batch 5 was the most positive among batches (-19.82‰). In addition, it presented the lowest external standard deviation (1.04‰) and the lowest internal standard deviations considering the individual analyses (≤ 0.4‰). Also, the average result of batch 5 was the closest to the average result analyzed by the CPC (Table 8, Figure 4) with a difference of 0.56‰. Thus, the optimal working ambient temperature, that is, the one that resulted in the best balance between repeatability and reproducibility of the data, was also 20 °C, in agreement with the manufacturer's determination.[25]

Even though the results of batch 4 were discrepant in relation to the other batches, the fractionation was far below what would be expected for a temperature change of 4 °C (18 °C-22 °C). For this temperature variation, the hydrogen results would tend to fractionate 24‰ if the autosampler temperature did not remained stable, since the expected fractionation is 6.0‰/°C for hydrogen.[26] Thus, the results showed that the method used in the LGI for determining δ^2H, in addition to presenting repeatability, is also reproducible, since the analyses performed in the LGI presented results statistically similar to those performed under different conditions.[15,28]

Although temperature controls and analytical parameters (gas flows, control of isobaric interferences, stability and linearity) are essential to obtain reliable and reproducible results, the use of

standards correctly calibrated by laboratories and data normalization also play an important role.[30] For this study, calibration curves obtained from the analysis of standards (VSMOW, ULW, Deplat and Brasília) were used together with unknown samples for later normalization of the raw data, by linear regression, for the VSMOW scale. This feature is important, as it reduces associated sources of errors and make calibrating the reference gases unnecessary, since small changes in the gas composition, over time, do not affect the analytical results.[10,30]

CONCLUSIONS

Obtaining reliable and quality analytical results poses different challenges for analysis laboratories. Thus, method evaluation studies, such as the one presented in this article, contribute to the identification of effects that can affect the accuracy of methods, which are removed and/or modified to produce accurate and precise results (repeatable and reproducible). Different factors can contribute to obtaining quality data, including systematic control of analytical parameters such as stability and linearity of the equipment, determination of the ^{3}H Factor for hydrogen analysis, monitoring of interfering mass signals, background monitoring of masses of interest, vacuum control, temperature control (extraction column, autosampler tray and environment). In addition, the use of calibrated analytical standard reference materials, as well as raw data normalized to VSMOW scale is also important to obtain quality data.

This study contributed to the definition of the optimal ambient temperature of 20 °C for carrying out the isotope analyses of oxygen and hydrogen isotopes in water, using the equilibrium method. Under different conditions, the analyses performed at 18 °C and 22 °C were influenced by the ambient temperature, presenting average values that were either less accurate or less precise, when compared with the results of the CPC analyses.

Thus, at an ambient temperature of 20 °C, analyses performed at the LGI control sample presented satisfactory repeatability and reproducibility results. From this, we can conclude that the methods of $\delta^{18}O$ and $\delta^{2}H$ determination in water, by the equilibrium method, performed in the LGI, are valid analytical techniques.

SUPPLEMENTARY MATERIAL

The data for $\delta^{18}O$ and $\delta^{2}H$ obtained for each batch of analysis is available in the supplementary material at http://quimicanova.sbq.org.br in pdf format, with free access.

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