

Article

A Simple Water Sample Storage Test for Water Isotope Analysis

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Abstract: Water is pivotal for human societies' sustainability and resilience. Isotope hydrology and hydrogeology research plays an important role in understanding and managing water resources. Reliable scientific results hinge on high-quality data. Preventing water sample evaporation is essential for accurate isotopic analysis. In this study, the impacts on the quality of isotopic data were tested for the storage of water samples and the repetitive opening of a laboratory reference material (LRM) sub-sample replica during daily operation. Twenty 15 mL water samples were stored in high-density polyethylene (HDPE) bottles at room temperature and humidity to simulate storage conditions. One 60 mL water sample was collected from the same starting batch to simulate the LRM sub-sample. Each 15 mL sample was analysed once over 80 days for the isotopic composition of oxygen ($\delta^{18}\text{O}$) and hydrogen ($\delta^2\text{H}$). The 60 mL sample was repeatedly analysed in the same period. The data were tested to identify shifts in the isotopic composition induced by evaporative processes. The main results of the work are the following: (i) storage of the 15 mL water samples did not cause detectable evaporation in the testing period; (ii) the 60 mL $\delta^{18}\text{O}$ values showed evidence of evaporation as proved by the positive shift of the isotopic data; (iii) the repetitive opening of the 60 mL sample was the main cause of evaporation; (iv) five openings can already cause detectable isotopic enrichment. Careful manipulation and frequent replacement of the LRM are thus necessary to prevent deterioration of the quality of the analyses.



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Keywords: isotopes; hydrogen; oxygen; evaporation; laboratory reference material; quality control; statistical evaluation

1. Introduction

Water plays a central role in the health and sustainability of human societies. Often, global initiatives to alleviate poverty and attain sustainable development focus on addressing challenges related to water availability and quality. United Nations' Sustainable Development Goal 6 acknowledges this significance by incorporating objectives for water security [1,2]. Progress in scientific research on isotopes within the water cycle, coupled with advancements in technologies for stable isotope analyses and applications, enhanced the utilization of isotope tracers for more effective management of water resources. Water stable isotopes (i.e., ^1H , ^2H , ^{16}O , ^{17}O , and ^{18}O) have become largely applied in hydrological studies to trace the water fluxes across different environmental matrixes and processes such as evaporation, precipitation, surface water, groundwater, critical zone, etc. [3–11]. Moreover, further advancements are expected for the application of water stable isotopes in tracing water-related processes in hydrology, hydrogeology, and ecohydrology [12–14]. Consequently, water stable isotope analyses are now widely used in environmental monitoring and research programs. With the substantial growth of produced isotopic data, their quality stands as a paramount issue, since reliable data are the foundation of all high-quality research.

For water stable isotopes, this primarily implies preventing any form of water evaporation and consequent isotopic enrichment from developing along the analytical procedure,

from collection to actual determination. Deleterious evaporative processes may happen in improperly stored samples due to leakage of the container cap or inappropriate bottle materials [15]. A few works have previously investigated the evaporative enrichment of water samples stored in bottles intentionally manipulated for gas exchange with the external atmosphere. They highlighted the importance of preventing any leakage from the sample bottles [16,17]. In addition, plastic bottles (depending on polymer and wall thickness) were proven to cause isotopic drifts over long storage periods due to water permeation through the plastic material [18,19]. The optimal sampling and conservation conditions are achieved with tight-seal double-capped (plastic/neoprene positive seals) glass bottles and refrigeration [9,15,19–21]. Storing the water samples in high density polyethylene (HDPE) bottles and at room temperature and humidity is then considered a sub-optimal storing procedure. In the opinion of the authors, further testing of the potential evaporation due to storage in HDPE at room temperature and humidity can contribute to best practices in isotope hydrology. Furthermore, the evaporation of laboratory reference material (LRM) sub-samples used in daily operation may represent a more subtle process potentially undermining analyses quality.

Thus, the main research questions of the work are the following:

- (i). Further testing the performances of HDPE bottles in avoiding water sample evaporation during multi-day storage at room temperature and humidity.
- (ii). Does the repetitive opening and closing of an LRM sub-sample for routine water stable isotope analyses cause its evaporation and consequent isotopic enrichment?

2. Materials and Methods

2.1. Experimental Design

Twenty-one Milli-Q water samples were collected from the same starting batch, stored in HDPE bottles with single caps, and filled to the top without air bubbles. One aliquot was poured into a 60 mL bottle, labelled Test0, while the remaining 20 samples were collected in 15 mL bottles, labelled from Test1 to Test20 (Figure 1, Tables 1 and 2). The samples were stored for about 80 days from 4 August to 20 October 2022 at laboratory room temperature and humidity. Every 15 mL sample was analysed once for water stable isotopes, extracting every time the necessary 3 mL from each bottle (Figure 1). The 60 mL sample was analysed repeatedly for water stable isotopes during the observation period, extracting 3 mL of water each time, to mimic the multi-day usage of an LRM sub-sample replica.

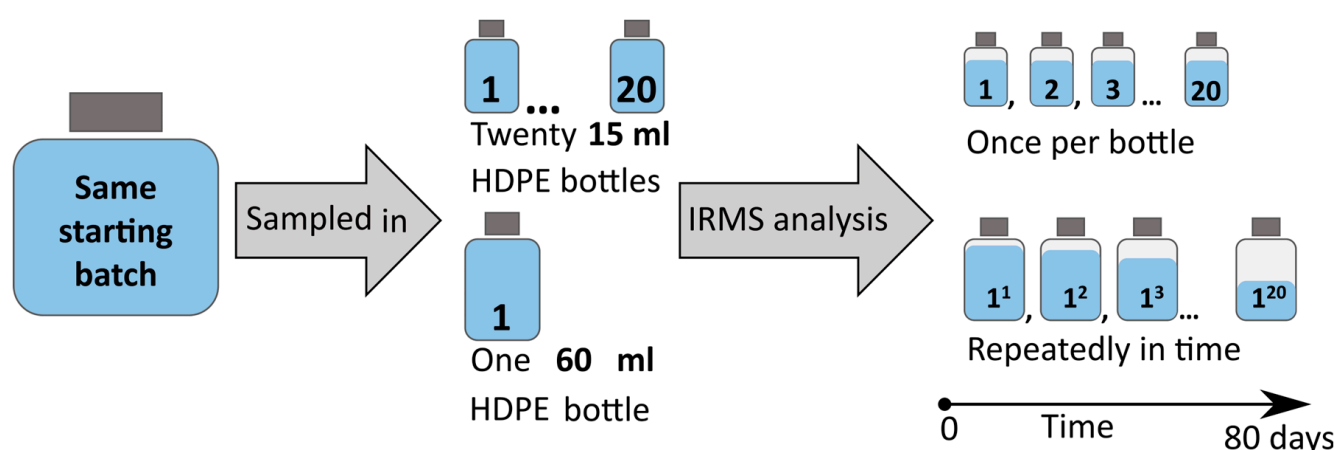


Figure 1. Experimental design.

Table 1. The date, days from the start of the experiment, number of openings of the bottle, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values, and respective σ values of each consecutive analysis of the 60 mL bottle are here reported. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values and respective σ values are expressed in ‰.

Sample ID	Date of Analysis	Days from the Start	N. Openings	$\delta^{18}\text{O}$	$\sigma \delta^{18}\text{O}$	$\delta^2\text{H}$	$\sigma \delta^2\text{H}$
Test0	4 August 2022	0	1	−9.20	0.015	−59.7	0.3
Test0	5 August 2022	1	2	−9.22	0.015	−60.7	0.3
Test0	20 August 2022	16	3	−9.22	0.015	−59.5	0.3
Test0	24 August 2022	20	4	−9.14	0.015	−60.0	0.3
Test0	26 August 2022	22	5	−9.20	0.015	−60.1	0.3
Test0	3 September 2022	30	6	−9.22	0.015	−60.3	0.3
Test0	9 September 2022	36	7	−9.22	0.015	−60.0	0.3
Test0	10 September 2022	37	8	−9.12	0.015	−60.9	0.3
Test0	13 September 2022	40	9	−9.19	0.015	−60.2	0.3
Test0	14 September 2022	41	10	−9.21	0.015	−59.6	0.3
Test0	21 September 2022	48	11	−9.20	0.015	−60.3	0.3
Test0	22 September 2022	49	12	−9.21	0.015	−59.4	0.3
Test0	13 October 2022	70	13	−9.18	0.015	−60.0	0.3
Test0	14 October 2022	71	14	−9.19	0.015	−59.4	0.3
Test0	18 October 2022	75	15	−9.18	0.015	−60.2	0.3
Test0	20 October 2022	77	16	−9.17	0.015	−61.3	0.3
Test0	20 October 2022	77	16	−9.20	0.015	−59.9	0.3
Average	-	-	-	−9.19	0.015	−60.1	0.3

Table 2. The date, days from the start of the experiment, $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values, and respective σ values of each consecutive analysis of the 15 mL bottles are here reported. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values and respective σ values are expressed in ‰.

Sample ID	Date of Analysis	Days from the Start	$\delta^{18}\text{O}$	$\sigma \delta^{18}\text{O}$	$\delta^2\text{H}$	$\sigma \delta^2\text{H}$
Test1	4 August 2022	0	−9.20	0.015	−59.9	0.3
Test2	5 August 2022	1	−9.20	0.015	−60.4	0.3
Test3	20 August 2022	-	-	-	-	-
Test4	24 August 2022	20	−9.19	0.015	−60.2	0.3
Test5	26 August 2022	22	−9.20	0.015	−60.4	0.3
Test6	3 September 2022	30	−9.17	0.015	−60.8	0.3
Test7	9 September 2022	36	−9.17	0.015	−60.2	0.3
Test8	10 September 2022	37	−9.17	0.015	−60.1	0.3
Test9	13 September 2022	40	−9.14	0.015	−60.6	0.3
Test10	14 September 2022	41	−9.21	0.015	−59.5	0.3
Test11	21 September 2022	48	−9.21	0.015	−60.1	0.3
Test12	22 September 2022	49	−9.15	0.015	−59.5	0.3
Test13	13 October 2022	70	−9.17	0.015	−59.5	0.3
Test14	14 October 2022	71	−9.16	0.015	−57.6	0.3
Test15	18 October 2022	75	−9.20	0.015	−60.8	0.3
Test16	20 October 2022	77	−9.21	0.015	−60.7	0.3
Test17	20 October 2022	77	−9.20	0.015	−59.3	0.3
Test18	20 October 2022	77	−9.20	0.015	−60.4	0.3
Test19	20 October 2022	77	−9.18	0.015	−60.8	0.3
Test20	20 October 2022	77	−9.21	0.015	−60.4	0.3
Average	-	-	−9.19	0.015	−60.1	0.3

2.2. Isotopes Analysis

The isotopic compositions of hydrogen ($\delta^2\text{H}$ expressed in per mil, ‰) and oxygen ($\delta^{18}\text{O}$ expressed in per mil, ‰) were determined using a water– H_2 (platinum—Pt) [22] and water– CO_2 [23,24] fully automated equilibration technique at the Jožef Stefan Institute (JSI, Ljubljana, Slovenia) [25,26]. Measurements were carried out on a dual inlet isotope ratio

mass spectrometer (DI IRMS) Finnigan MAT DELTA plus (Finnigan MAT GmbH, Bremen, Germany) with automated H₂-H₂O and CO₂-H₂O equilibrator HDOeq 48 Equilibration Unit (custom built by M. Jaklitsch). The equilibration of H₂-H₂O and CO₂-H₂O lasted for 2 and 6 h, respectively. The DI determines the isotope ratios from pure gases by alternately introducing 6 and 4 times the H₂ and CO₂ sample and working gas into the IRMS. The measured values are averaged and normalised to the VSMOW/SLAP scale to derive the final $\delta^2\text{H}$ and $\delta^{18}\text{O}$ values. All measurements were performed together with routinely measured samples and LRMs calibrated periodically against IAEA calibration standards to the VSMOW/SLAP scale that are stored in stainless steel containers under pressure with N₂ gas for long-term water storage [21] (V&F Handels KG, Absam, Austria). The results were normalized to VSMOW/SLAP using the Laboratory Information Management System for Light Stable Isotopes (LIMS) program. For normalisation (N) and independent quality control (QC), we used LRMs with defined isotope values and estimated measurement uncertainty calculated by the Kragten method [27] (Table 3) and commercial LRM provided by the USGS (i.e., USGS 47).

Table 3. $\delta^2\text{H}$ and $\delta^{18}\text{O}$ defined values of JSI laboratory reference materials with their associated combined standard uncertainties (1 σ level). N denotes normalization; QC for quality control.

JSI Code	Material	$\delta^2\text{H}/\text{‰}$	$\delta^{18}\text{O}/\text{‰}$	Comment
W-3869	Distilled see water	$+2.9 \pm 0.9$	$+0.36 \pm 0.04$	N, defined
W3871	Snow water Kranjska gora	-147.9 ± 0.6	-19.73 ± 0.02	N, defined
W-54	Snow water Kanin	-140.4 ± 0.7	-18.91 ± 0.03	N, defined
W-45	Milli-Q tap water Reaktor	-59.7 ± 0.7	-9.12 ± 0.03	QC, defined
W-53	Snow water Kanin	-140.4 ± 0.7	-18.91 ± 0.03	QC, measured
W-45	Milli-Q tap water Reaktor	-59.9 ± 0.7	-9.13 ± 0.03	QC, measured

2.3. Data Analysis

The data evaluation was conducted based on two assumptions: (i) all samples had the same starting $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values and (ii) all 15 mL bottles were identical and tightly closed after the filling. Sample evaporation, with the resulting enrichment in heavy isotopes, is considered the only possible consequence of not being stored in glass bottles under refrigeration and prolonged multi-day usage of the LRM sub-sample replica. Sample contamination during laboratory activities were carefully avoided.

The data derived from the experiment are the subsequent series of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values repeatedly determined for the 60 mL bottle and the series of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ values, one for each 15 mL bottle (Figure 1).

Before trend testing on isotopic data, Westgard rules were applied to the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ for verification of the measurements' quality [28,29].

A systematic enrichment, highlighted by a positive significant deviation in the isotopic values, was interpreted as an ongoing evaporative process. The presence of a monotonic trend in the data was tested with the Mann–Kendall non-parametric trend test. The Mann–Kendall test checks for monotonic trends and allows for missing values to be present in the series [30,31]. The Mann–Kendall trend was performed with the “rkt” function of the “rkt” package of the R 2023.03.0 software [32,33]. For those measurements sharing the same day of analysis (Tables 2 and 3), the average isotopic value was used in the trend testing in the “rkt” function. For those data series having a two-sided p-value equal or lower than 0.05 for the Mann–Kendall test, the null-hypothesis of the absence of a monotonic trend was rejected. Moreover, the possible linear trends in the isotopic values were tested with weighted linear regressions. The minimization of the sum of the squares of the weighted residuals (SSWR) was applied to compute the linear regression models [34]. This approach incorporates the different uncertainties on the isotopic measurements in the computation of the descriptive linear model. The residual is then the distance between the experimental point (y', x') and the corresponding estimated “best” point ($\hat{y}', x'$) belonging to the straight line $\hat{y}' = a + b \cdot x$ measured in units of experimental uncertainty. The “ y ” corresponds to the

$\delta^{18}\text{O}$ or $\delta^2\text{H}$ values and the “ x ” corresponds to time in days from the start of the experiment. The experimental uncertainties ($\sigma\delta^{18}\text{O}$ and $\sigma\delta^2\text{H}$) are derived from the analysis of repetitive measurements on the control samples (Tables 1 and 2). The $SSWR$ equation is then:

$$SSWR = \sum_{i=1}^N \left(\frac{y' - \hat{y}'}{\sigma_{y'}} \right)^2 = \sum_{i=1}^N \left(\frac{y' - (\alpha + \beta x')}{\sigma_{y'}} \right)^2$$

The weighted linear regression was performed with the “lm” function of the “stats” package of the R software [33]. The isotopic values, being the mean of the repetitive gas injection, do belong to normal distributions, as also confirmed by the repetitive analyses of single samples. The $SSWR$ can be then assumed to belong to a Chi-square (χ^2) distribution. Thus, for each regression model, the $SSWR$ 95% confidence intervals (C.I.) and its probability are derived from theoretical χ^2 distributions of the corresponding degrees of freedom [34]. The $SSWR$ 95% confidence intervals are used to evaluate the goodness of fit of the linear models. A linear model having an $SSWR$ within the confidence interval is considered as a good fit of the data. For those models with $SSWR$ within the confidence interval of the relative χ^2 distribution, the residuals’ normality was checked with the Shapiro–Wilk test executed with the shapiro.test with the R software [33]. The null hypothesis of normally distributed data was rejected for Shapiro–Wilk p -values equal or lower than 0.05. The Mann–Kendall trend testing and the linear model fitting were also computed for the $\delta^{18}\text{O}$ or $\delta^2\text{H}$ values of the 60 mL bottle against the number of openings of the bottle itself.

3. Results

During the experiment, the storage temperature and humidity varied between 22.5 °C and 25.3 °C and 42% and 58%, and were on average 24.2 °C and 50%, respectively. All isotopic data are reported in Tables 1 and 2 and presented in Figures 2 and 3.

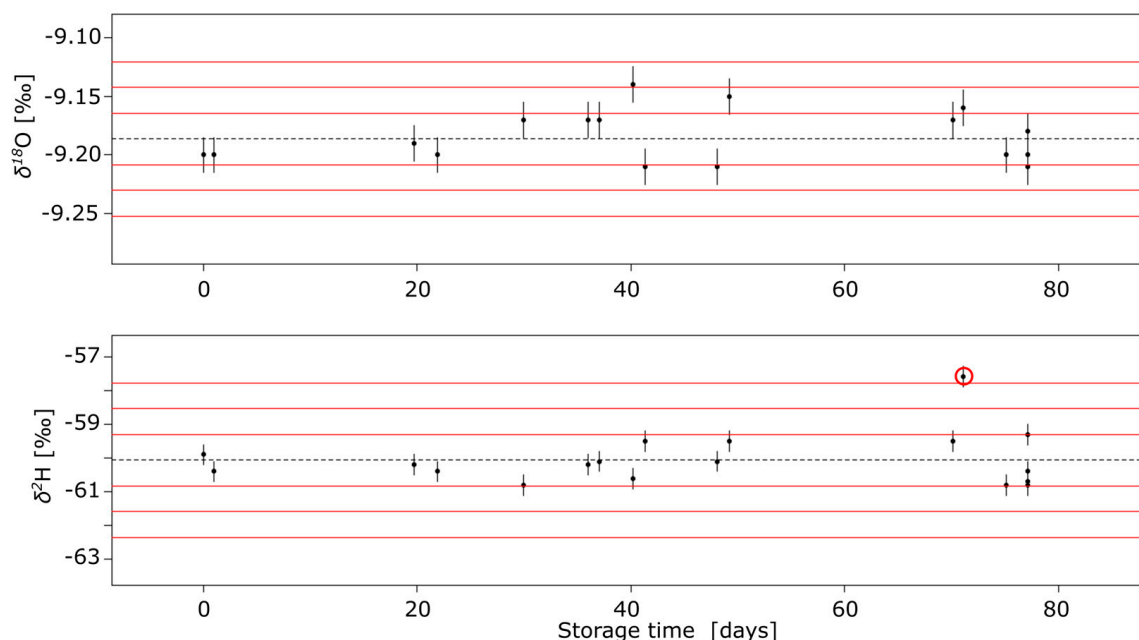


Figure 2. Shewhart control charts for the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ measurements vs. time of the single 15 mL bottles. The dashed black lines represent the mean values. The red lines represent the means $\pm 1\sigma$, $\pm 2\sigma$, and $\pm 3\sigma$. The red-circled data are considered outliers.

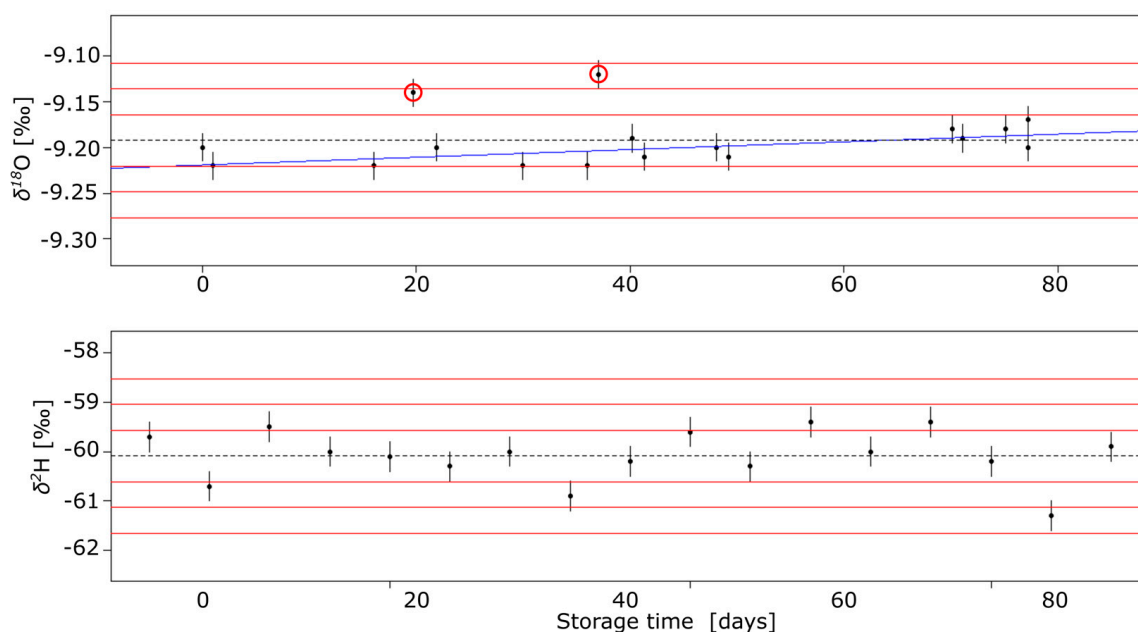


Figure 3. Shewhart control charts for the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ measurements vs. time of the 60 mL bottle. The dashed black lines represent the mean values. The red lines represent the means $\pm 1\sigma$, $\pm 2\sigma$, and $\pm 3\sigma$. The red-circled data are considered as outliers. The blue line represents the good linear fit model.

All isotopic values are reported in Tables 1 and 2. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ from the 15 mL bottles ranged from -9.21‰ to -9.14‰ and -60.8‰ to -57.6‰ , with an average value of -9.19‰ and -60.1‰ , respectively. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ from the Test 0 60 mL bottle ranged during the 80 days experiment from -9.22‰ to -9.12‰ and -61.3‰ to -59.4‰ , with an average value of -9.19‰ and -60.1‰ , respectively.

The majority of analyses were accurately conducted, with satisfactory results across most samples. However, the analysis of sample Test3 from the 15 mL bottles yielded unexpectedly low isotopic values. This discrepancy was likely due to a faulty connection between the equilibration ampulla and the vacuum line, resulting in unreliable data. Consequently, the results of this particular measurement were disregarded.

The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ results met the criteria outlined in the Westgard rules [28,29] for quality control in all cases except for one $\delta^2\text{H}$ data point of the multiple 15 mL bottles being higher than $+3\sigma$ from the mean (Figure 2 red circled). However, if the outlier is ignored, then Westgard rules are fulfilled also for the $\delta^2\text{H}$ data of the multiple 15 mL bottles experiment.

3.1. Trend Testing and Regression Linear Models vs. Storage Time

All $\delta^{18}\text{O}$ and $\delta^2\text{H}$ data series underwent Mann–Kendall trend detection tests and the results are reported in Table 4. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ series for the 15 mL bottles (Figure 2) and the $\delta^2\text{H}$ series for the single 60 mL bottle (Figure 3), did not show significant monotonic trends as proved by the p -values of the Mann–Kendall test being always above 0.05 (Table 4). Additionally, the SSWR for the derived linear models consistently approached or exceeded the upper limit of the 95% C.I. for χ^2 distributions (Table 4). This observation underscores the poor fitting of these linear models. Thus, for the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ series for the 15 mL bottles and the $\delta^2\text{H}$ series for the single 60 mL bottle, no significant changes were detected during the testing period and the analytical technique.

Table 4. Isotopic values trend testing and linear models against time. The reported statistics are p -values of Mann–Kendall trend testing, results of the weighted linear regression including coefficients (α , β), coefficient errors, sum of squared weighted residuals (SSWR), degrees of freedom (df), 95% confidence intervals for the χ^2 distribution of corresponding df, and probability for corresponding SSWR. In lines 2 and 5, the same results are reported for the data without the outliers highlighted in Figures 2 and 3. The last column reports the p -value for the Shapiro–Wilk normality test of the residuals.

	p -Value MK Test	α	α Error	β	β Error	SSWR	df	χ^2 95% C.I.	p -Value Residual S-W
$\delta^{18}\text{O}$ 60 mL bottle	0.11	−9.20	0.01	2.4×10^{-4}	1.5×10^{-4}	53.9	15	[7.26; 25.0]	-
$\delta^{18}\text{O}$ 60 mL bottle no outliers	0.01 *	−9.22	0.01	4.2×10^{-4}	1.5×10^{-4}	8.53 **	13	[7.96; 26.3]	0.27 ***
$\delta^2\text{H}$ 60 mL bottle	0.96	−60.0	0.1	-1.5×10^{-3}	2.9×10^{-3}	48.2	15	[5.89; 22.4]	-
$\delta^{18}\text{O}$ 15 mL bottles	0.57	−9.18	0.01	-3.7×10^{-5}	1.3×10^{-4}	38.3	17	[7.26; 25.0]	-
$\delta^{18}\text{O}$ 15 mL bottles no outlier	0.62	−60.2	0.1	-7.4×10^{-4}	2.7×10^{-3}	44.8	16	[8.67; 27.6]	-
$\delta^2\text{H}$ 15 mL bottles	0.34	−60.3	0.1	3.9×10^{-4}	2.7×10^{-3}	114	17	[8.67; 27.6]	-

* Significant monotonic trend, ** Good fit linear model, *** Residuals normally distributed.

The $\delta^{18}\text{O}$ measurements from the single 60 mL bottle (Figure 3) resulted in a p -value of 0.11 for the trend test. Two measurements of the dataset were, respectively, close to and more than $+2\sigma$ from the mean (red circled in Figure 3), while all other measurements remained within $\pm 1\sigma$ from the mean. Hence, the Mann–Kendall trend testing was repeated removing the two outliers, resulting in a p -value of 0.01, below the conventional 0.05 limit; thus, a positive monotonic trend was detected. The outlier removal was justified by the following observations. First, evaporation was the only possible cause of isotopic shift, and an abrupt change in isotopic values is not expected due to such a process. Second, if the shift in $\delta^{18}\text{O}$ values for the two outliers would have been real changes, then they should have propagated also to the next measurements, which is not the case.

The coefficient of the linear regression resulted in a positive value in both scenarios, with ($4.6 \times 10^{-4}\text{‰/day}$) and without ($3.2 \times 10^{-4}\text{‰/day}$) the identified outliers. The SSWR of the linear model for the $\delta^{18}\text{O}$ values after removing the two outliers was within the 95% C.I. for a χ^2 distribution with 13 degrees of freedom and residuals normally distributed (Table 4). Thus, the derived linear model can be considered a good fitting of the measured data, effectively modelling the ongoing evaporative process. In contrast, the SSWR value of the linear model obtained without outlier removal extended beyond the 95% C.I. for a χ^2 distribution with 15 degrees of freedom. This furthermore highlights the measurement errors associated with the excluded data points. Thus, it is reasonable to assume that an evaporative process occurred within the 60 mL LRM sub-sample replica during the experiment with a magnitude that caused an increase of the $\delta^{18}\text{O}$ value detectable with the DI IRMS measurement technique.

3.2. Trend Testing and Regression Linear Models vs. Number of Openings

Since the 60 mL bottle was used to mimic the results of a sub-sample of LRM, both $\delta^{18}\text{O}$ or $\delta^2\text{H}$ values were tested for monotonic trends and fitted with linear models based on the number of bottle openings. The $\delta^2\text{H}$ values exhibited no discernible monotonic trend, as proven by the high p -value (0.96) of the Mann–Kendall test (Table 5). Also, the SSWR value of the linear model for the $\delta^2\text{H}$ values fell beyond the 95% C.I. of the χ^2 distribution with 13 degrees of freedom. Conversely, the $\delta^{18}\text{O}$ values (excluding the identified outliers, red circled in Figure 4) revealed a significant trend having a p -value equal to 0.01 for the Mann–Kendall test (Table 5). Moreover, the SSWR of the corresponding linear model fell within the 95% C.I. of the χ^2 distribution with 13 degrees of freedom and residuals being normally distributed, indicating a satisfactory fit of the linear model to the $\delta^{18}\text{O}$ data (Figure 4).

Table 5. Isotopic values trend testing and linear models against the number of openings for the 60 mL bottle. The reported statistics are p -values of Mann–Kendall trend testing, results of the weighted linear regression including coefficients (α , β), coefficient errors, sum of squared weighted residuals (SSWR), degrees of freedom (df), 95% confidence intervals for the χ^2 distribution of corresponding df, and probability for corresponding SSWR. The last column reports the p -value for the Shapiro–Wilk normality test of the residuals.

	p -Value MK Test	α	α Error	β	β Error	SSWR	df	χ^2 95% C.I.	p -Value Residual S-W
$\delta^{18}\text{O}$ 60 mL bottle vs. openings no outliers	0.01 *	−9.22	0.01	2.1×10^{-3}	7.8×10^{-4}	8.95 **	13	[5.89; 22.4]	0.12 ***
$\delta^2\text{H}$ 60 mL bottle vs. openings	0.96	−60.0	0.2	-8.6×10^{-3}	1.5×10^{-2}	48.1	15	[7.26; 25.0]	-

* Significant monotonic trend, ** Good fit linear model, *** Residuals normally distributed.

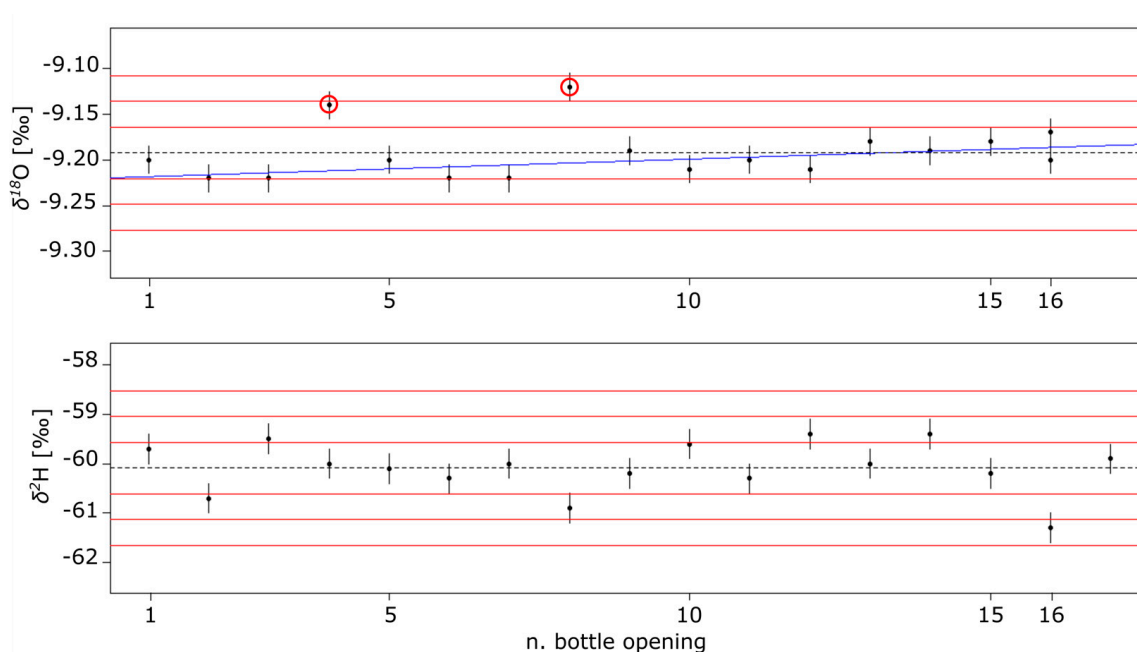


Figure 4. Shewhart control charts for the $\delta^{18}\text{O}$ and $\delta^2\text{H}$ measurements vs. the number of openings of the single 60 mL bottle. The dashed black lines represent the mean values. The red lines represent the means $\pm 1\sigma$, $\pm 2\sigma$, and $\pm 3\sigma$. The red-circled data are considered outliers. The blue line represents the good linear fit model.

4. Discussion and Best Practice Considerations

The absence of any discernible trend in the isotopic values of the 15 mL bottles suggests that the HDPE bottles with single caps effectively prevented the evaporation of the samples throughout the nearly 80-day testing period under room temperature and humidity conditions, in accordance with previous results [19]. While these conditions may not correspond to the optimal storing in glass bottles and under refrigeration, the findings indicate that HDPE bottles can be used for storing samples without undesired evaporation. Indeed, HDPE bottles offer advantages over glass bottles, particularly in terms of durability and resilience to impact, making them preferable for situations involving long transportation, shipments, or rough field conditions. Concerning our analysis, the HDPE bottles can prevent evaporation at room temperature and humidity for at least 80 days. However, HDPE bottles should reliably store samples for up to two years [19].

The effectiveness of the HDPE bottles in preventing sample evaporation, as shown by the 15 mL bottle tests and by previous studies [19], suggests that the primary cause of evaporation detected in the 60 mL bottle was the opening and closing required for sub-sample

analysis, instead of the storage time. In the worst-case scenario, such an evaporation mechanism can potentially cause a shift of $2.9 \times 10^{-3} \text{‰}$ /opening (the determined coefficient plus the error). With such a coefficient, the expected enrichment in $\delta^{18}\text{O}$ values following 5, 10, and 20 openings would be 0.02 ‰, 0.03 ‰, and 0.06 ‰, respectively. These values are already comparable to the analytical precision (average 0.015 ‰, Table 3) for $\delta^{18}\text{O}$ with the applied analytical technique. Such isotopic drifts would correspond to a very small mass loss when reported on the isotopic enrichment versus the fraction of remaining water presented in previous works [16,17,35]. However, despite the small magnitude, evaporation led to a significant shift in the $\delta^{18}\text{O}$ values of the 60 mL bottle, underscoring the importance of careful usage and monitoring of LRM sub-samples. We suggest that under similar working conditions, the LRM sub-sample should not be used more than five times, thus accordingly choosing the most appropriate bottle volume. The recommended threshold should be adjusted according to the volume of the sub-sample itself since smaller water volumes would experience greater enrichment with a similar number of repetitive openings. Also, shifts in the isotopic values of the LRM sub-sample should be tested accordingly for each laboratory-specific condition and procedure used.

5. Conclusions

The foundation of reliable and enduring scientific outcomes lies in the quality of data. Evaporation of water samples can undermine the quality of stable hydrogen and oxygen isotope analyses. This study assesses the influence on data quality resulting from the storage of water samples in HDPE bottles at room temperature and humidity, as well as from the repetitive analysis of an LRM sub-sample replica. Consequently, it contributes valuable insights for the appropriate management of water samples and laboratory procedures in isotope hydrology.

Overall, the study highlighted the practical utility of HDPE bottles in preserving sample integrity. In fact, the 15 mL HDPE bottle without double cap samples did not exhibit significant isotopic variation over the study period, thus proving to be a reliable and handy storing method for a reliable price. Therefore, HDPE bottles can be considered for short-term sample storage needs, recognizing their effectiveness and durability in maintaining sample quality.

Conversely, the findings highlighted the importance of cautious handling of the LRMs sub-samples and the impact of repetitive bottle opening on evaporation rates and subsequent isotopic enrichment. Indeed, a significant and positive linear trend was detected for the $\delta^{18}\text{O}$ values of the LRM sub-sample replica. This suggested that evaporation was occurring over the study period, most likely due to repeated opening of the bottle. Thus, careful manipulation and frequent replacement of the LRM should be performed to prevent any evaporation of water, which can subsequently undermine the analysis' reliability.

A better understanding of the water cycling under changing environmental conditions that is crucial for sustainable development can be supported also by water stable isotope data. By gaining reliable isotope data, we can help to ensure more responsible water resource management and promote sustainable practices in various sectors such as climatology, hydrology, hydrogeology, agriculture, urban areas, forensic studies, etc. Therefore, reliable and traceable isotope data in combination with water quantity and quality data are vital for securing a sustainable future.

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