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Analysis of Stability of Sudan I-D_5

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ABSTRACT
The stable isotopic labeling reference materials play an indispensable role in the verification and calibration measuring instruments, evaluation analysis methods, determining the material characteristic and the quality control in the process of production. Therefore, it has great economic and social benefits in international trade, environmental protection, human health and safety. This paper studied the analysis of stability of Sudan-I-D5. The isotopic abundance of D and chemical purity have been analyzed. Samples which chemical purity and isotopic abundance are more than 99%, were packed in a dry and clean laboratory. analysis of stability of the isotope abundance and chemical purity of Sudan-I-D5 has been performed. Outliers do not appear within the tracker（for 12 months）at the temperature of 20℃and avoid light conditions. The isotopic abundance and the chemical purity have been analyzed in the subsequent period of 12 days, after been saved ten hours in the (40±5) ℃ or (-20±5) ℃transport conditions. The results show that the isotopic abundance and the chemical purity of Sudan-I-D5 are stable.

Keywords: *Chemical purity, Isotopic abundance, Stability*

1. INTRODUCTION

The stable isotope reference materials play an important role in life science, food safety, resources and environment research. Developed countries have formed a high-tech industry with significant economic benefits in the preparation and analysis of the stable isotopic labeling reference materials. In contrast, the preparation of the stable isotopic labeling reference materials and its industrialization and analysis in china is out of blank. At present, the required stable isotope standard materials need to be imported from abroad, and the price is expensive. According to incomplete statistics, China needs tens of millions of dollars in foreign exchange for this purpose every year. Therefore, the research and development of the preparation and detection methods of stable isotope reference materials is of great significance for the development of stable isotope reference materials in China and the industrialization of stable isotope reference materials.

The stability of reference materials refers to the ability that the physical and chemical properties and characteristic values of reference materials remain unchanged under the influence of external environmental conditions when they are stored for a long time[1]. The longer the prescribed time interval, the better the stability of the substance. According to the requirements of the national standard material technical specification: The candidate should meet the principles of applicability, representativeness, and ease of reproduction[2]. The stability of the candidate shall be within a range suitable for the use of the reference substance. Standard substances shall be subjected to periodic stability tests for undetermined characteristic values under specified storage or use conditions. The time interval of stability test can be arranged in accordance with the principle of "first secrecy, then secrecy". The validity period of the reference substance should be more than 1 year or reach the validity period of the similar reference substance with advanced international level.

2. TEST METHOD AND JUDGMENT BASIS

The stability of a reference substance refers to the property that the characteristic value of a reference substance remains within a specified range within a certain time interval and under environmental conditions. The Sudan-I-D_5 reference material is a solid powder. We store it in a sealed container, the influence of humidity and other conditions on its stability can be ignored. Therefore, in this experiment, we mainly investigate the influence of temperature, time and other conditions on the stability of Sudan-I-D_5 reference material.

At the same time, considering that standard materials may encounter extreme hot or cold weather during sales and transportation, we also conducted stability experiments under short-term harsh transportation conditions to evaluate the feasibility of their transportation under short-term high or low temperature conditions.

When the standard value of the characteristic value of the reference substance is known, the t-test method is used to evaluate the stability of the reference substance, which is described by the formula as:

$$\left|\overline{x_i} - \overline{x}\right| \leq t_{(\alpha,n-1)}S \quad \text{……………..（2.1）}$$

When:

$$\left|\overline{x_i} - \overline{x}\right| \Big/ S \leq t_{(\alpha,n-1)} \quad \text{……………….（2.2）}$$

Among them: Xi: the ith measurement value; $\overline{\overline{x}}$: Arithmetic mean of the mean value of n measurements;

S: standard deviation of n measurements; $t_{(\alpha,n-1)}$: Critical value when significance level α and degree of freedom are n-1, upon inspection of form, T (0.05, 6) = 2.447.

If Equation 2.2 holds, then no significant change has occurred in the reference substance and the stability is good.

3. THE EXPERIMENTAL METHOD

3.1. Stability under conventional storage conditions:

Store Sudan-I-D_5 reference material at room temperature and away from light. Due to the good stability of solid samples, after sampling and analysis in January, February and March respectively, the sampling interval was increased to 3 months, i.e., in June, September and December, the stability test of the two characteristic values was conducted.

3.2. Stability under short-term transport conditions:

The Sudan-I-D_5 reference substance was stored at (40±5)℃ for 10 hours, protected from light. The samples were randomly selected at 0, 1, 2, 3, 6, 9 and 12 days, respectively, and the chemical purity was determined by liquid chromatography. The isotopic abundance was measured by LC-MS mass spectrometry.

The Sudan-I-D_5 reference substance was stored at (-10±5)℃ for 10 hours, protected from light. The samples were randomly selected at 0, 1, 2, 3, 6, 9 and 12 days, respectively, and the chemical purity was determined by liquid chromatography. Isotope abundance of D was measured by electrospray mass spectrometry.

3.3. Instruments and chromatographic/mass spectrometry conditions used for the test[3-4]

Its purity was tested by liquid chromatography; The abundance of D (2H) (mol% or atom%) was determined by electrospray mass spectrometry and gas isotope mass spectrometry, respectively.

Method for chemical purity analysis of Sudan-I-D_5: High performance liquid chromatograph, Model: High performance liquid chromatography-mass spectrometer（HPLC: Shimadzu brand in Japan LCMS-2020）; Column: C18（150 mm×4.6 mm (I.D.)，5 μm，GL Sciences lnc）; Mobile phase A: aqueous solution of 0.3 % formic acid: acetone =3:1; Mobile phase B: acetonitrile; A:B=25:75；Sample quantity: 10 μL； Column temperature: 30℃; Flow rate: 1 mL·min-1，Shunt flow 0.8 mL·min-1；Characteristic wavelength: λ=478 nm；Mobile phases A and B are mixed in proportion (A:B=25:75)，Configuration concentration is 0.5，1，2，5 and 10 μg·mL-1 standard analysis of fluid. HPLC external standard method was used to analyze, draw the standard curve and find the corresponding relationship.

Isotope abundance analysis method of Sudan-I-D_5 : The abundance of deuterium-labeled isotopes was analyzed by TSQ Quantum Access high performance liquid chromatography coupled with tandem quadrupole mass spectrometry (Themo Scientific, USA). Electrospray voltage: 4 kV; Tuning filter voltage: 70 V; Dry gas flow rate: 10 L·min-1； Atomizing gas flow rate: 4 L·min^{-1}；Heating module temperature: 350℃; Temperature of desolventing tube: 250℃; Single scan time: 0.1s; Peristaltic pump flow rate: 10μL·min-1; Scanning mode: negative ion mode; Scan range: m/z=([M-H]-±10) dalton=(242~262) dalton；Number of spectral stacking: > 20; Number of parallel experiments: 3.

4. LONG-TERM STABILITY ANALYSIS OF THE CHEMICAL PURITY OF SUDAN-I-D_5

S11 and S14 sample units of Sudan-I-D_5 reference materials were selected for the long-term stability test of eigenvalue chemical purity.

Among them, the experimental results of chemical purity stability of Sudan-I-D_5 under conventional storage conditions are shown in Figure 1-1 series.

Table 1-1A Judgment 1 (12 months) of long-term stability of the purity

Label	The average purity (%)	The average	The standard deviation S	$\left\|\overline{x_i}-\overline{x}\right\|/S$	$t_{(0.05,6)}$
S11-0	99.47			0.688	
S11-1	99.40			0.304	
S11-2	99.51			1.255	
S11-3	99.37	99.42	0.07	0.729	2.447
S11-6	99.30			1.721	
S11-9	99.44			0.263	
S11-12	99.46			0.546	

Table 1-1B Judgment 2 (12 months) of long-term stability of the purity

Label	The average purity (%)	The average	The standard deviation S	$\left\|\overline{x_i}-\overline{x}\right\|/S$	$t_{(0.05,6)}$
S14-0	99.33			0.829	
S14-1	99.42			0.415	
S14-2	99.29			1.382	
S14-3	99.43	99.39	0.07	0.553	2.447
S14-6	99.51			1.659	
S14-9	99.39			0	
S14-12	99.36			0.415	

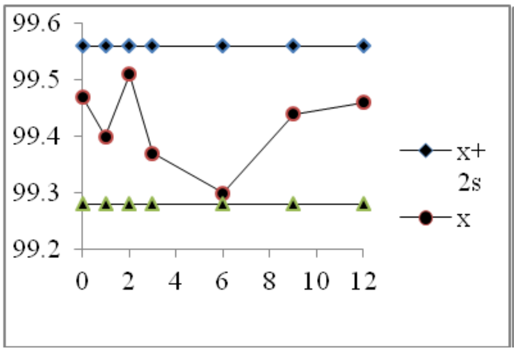

Figure 1.1A Curve 1 of long-term stability of purity

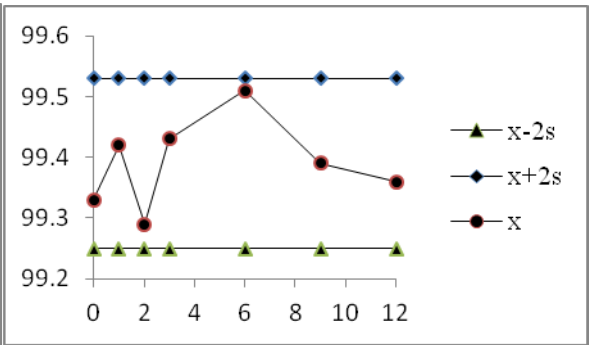

Figure 1.1B Curve 2 of long-term stability of purity

In Fig. 1.1a and Fig. 1.1b, x+2s represent the sum of the mean value of chemical purity and double standard deviation of the two Sudan-I-D_5 samples, respectively. x-2s denotes the difference between the mean chemical purity of two Sudan-I-D_5 samples and the standard deviation of two times, respectively. As can be seen from the figure, in 12 months, the chemical purity of Sudan-I-D_5 fluctuated within the purity range of x+2s and x-2s, with a small fluctuation. This indicates that the chemical purity of Sudan-I-D_5 is stable over 12 months.

As can be seen from the 1-1 series charts, the purity stability data of the two groups meet the requirements of $\left|\overline{x_i}-\overline{x}\right|/S \leq t(0.05,6)$. In other words, the purity of Sudan-I-D_5 standard substance did not change significantly after 12 months storage, and the stability was good.

5. LONG-TERM STABILITY ANALYSIS OF THE ISOTOPIC ABUNDANCE OF SUDAN-I-D_5

Sample units S11 and S14 of Sudan-I-D_5 reference material were selected for the experiment to conduct the long-term stability experiment of the isotopic abundance of eigenvalues. The experimental results of abundance stability of Sudan-I-D_5 reference substance under conventional storage conditions are shown in the series of Figure 1-2.

Table 1-2A Judgment 1 (12 months) of long-term stability of the isotopic abundance

Label	The average abundance (atom%D)	The average	The standard deviation S	$\|\overline{x_i}-\overline{x}\|/S$	$t_{(0.05,6)}$
S11-0	99.04			0.160	
S11-1	99.02			0.441	
S11-2	98.94			1.563	
S11-3	99.13	99.05	0.07	1.102	2.447
S11-6	99.11			0.822	
S11-9	99.00			0.721	
S11-12	99.12			0.962	

Table 1-2B Judgment 2 (12 months) of long-term stability of the isotopic abundance

Label	The average abundance (atom%D)	The average	The standard deviation S	$\|\overline{x_i}-\overline{x}\|/S$	$t_{(0.05,6)}$
S14-0	99.02			0.431	
S14-1	99.16			1.915	
S14-2	99.07			0.407	
S14-3	98.97	99.04	0.06	1.269	2.447
S14-6	99.04			0.096	
S14-9	99.01			0.598	
S14-12	99.05			0.072	

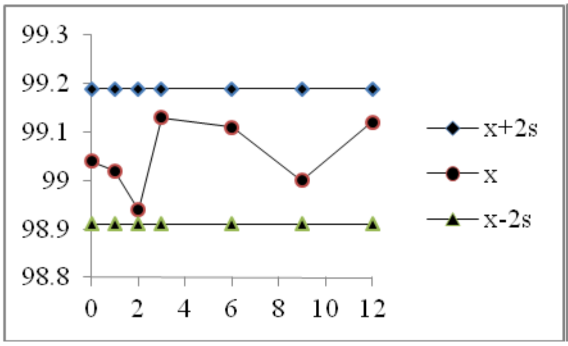

Figure 1.2A Curve 1of long-term stability of the isotopic abundance

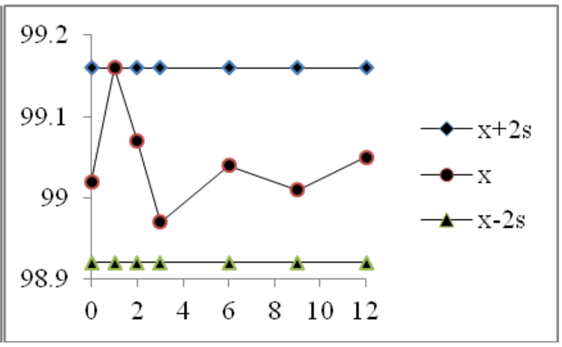

Figure 1.2B Curve 2 of long-term stability of the isotopic abundance

In Fig. 1.2a and Fig. 1.2b, x+2s represent the sum of the mean isotope abundance and double standard deviation of the two Sudan-I-D_5 samples, respectively. x-2s represents the difference between the mean isotope abundance of the two Sudan-I-D_5 samples and the standard deviation of two times, respectively. As can be seen from the figure, in 12 months, the isotope abundance of Sudan-I-D_5 fluctuated within the range of x+2s and x-2s, with a small fluctuation. This indicates that the isotopic abundance of Sudan-I-D_5 is relatively stable during 12 months.

It can be seen from the 1-2 series charts that the stability data of isotope abundance in the two groups are $\|\overline{x_i}-\overline{x}\|/S \le t(0.05,6)$, In other words, the isotope abundance of Sudan-I-D_5 reference material did not change significantly after preservation for 12 months, indicating good stability.

6. SHORT-TERM STABILITY ANALYSIS OF THE CHEMICAL PURITY OF SUDAN-I-D_5

①A sample unit of Sudan-I-D_5 reference material was selected and placed in (40±5)℃ (oven) for 10h. Chemical purity was measured at 0, 1, 2, 3, 6, 9, and 12 days, respectively.

②A sample unit of Sudan-I-D_5 reference material was selected and placed in an environment of (-20±5)℃ (refrigerated) for 10h. Chemical purity was measured at 0, 1, 2, 3, 6, 9, and 12 days, respectively.

(H- High temperature treatment, sample unit S1; C- Low temperature treatment, sample unit S7)

The experimental results of simulating the chemical purity stability of Sudan-I-D_5 under severe temperature conditions for short term transportation are shown in Table 1-3 series.

Table1-3A The results of stability experiments under high temperature of purity analysis

Label	The average purity (%)	The average	The standard deviation S	$\left\|\bar{x_i} - \bar{x}\right\| / S$	$t_{(0.05,6)}$
H-0	99.29			1.361	
H-1	99.37			0.256	
H-2	99.45			0.848	
H-3	99.36	99.39	0.07	0.394	2.447
H-6	99.50			1.538	
H-9	99.33			0.809	
H-12	99.42			0.434	

Table 1-3B The results of stability experiments under low temperature of purity analysis

Label	The average purity (%)	The average	The standard deviation S	$\left\|\bar{x_i} - \bar{x}\right\| / S$	$t_{(0.05,6)}$
C-0	99.47			0.721	
C-1	99.45			0.492	
C-2	99.54			1.525	
C-3	99.38	99.41	0.08	0.311	2.447
C-6	99.40			0.082	
C-9	99.32			1.000	
C-12	99.29			1.345	

According to the series data in Table 1-3, all purity data are $\left|\bar{x_i} - \bar{x}\right| / S \leqslant t(0.05,6)$. In other words, Sudan-I-D_5 reference substance was treated at high temperature (40±5)℃ and low temperature (-20±5)℃ for 10 hours, and the chemical purity did not change significantly at 0, 1, 2, 3, 6, 9 and 12 days, showing good stability.

7. SHORT-TERM STABILITY ANALYSIS OF THE ISOTOPIC ABUNDANCE OF SUDAN-I-D_5

①A sample unit of Sudan-I-D_5 reference material was selected and placed in (40±5)℃ (oven) for 10h. Isotopic abundances were measured at 0, 1, 2, 3, 6, 9 and 12 days, respectively.

②A sample unit of Sudan-I-D_5 reference material was selected and placed in an environment of (-20±5)℃ (refrigerated) for 10h. Isotopic abundances were measured at 0, 1, 2, 3, 6, 9 and 12 days, respectively.

(H- High temperature treatment, sample unit S1; C- Low temperature treatment, sample unit S7)

The results of experiments simulating the stability of Sudan-I-D_5 isotope abundance under the condition of short term transportation and harsh air temperature are shown in Table 1-4 series.

Table 1-4A The results of stability experiments under high temperature of the isotopic abundance analysis

Label	The average abundance (atom%D)	The average	The standard deviation S	$\left\|\overline{x_i} - \overline{x}\right\| / S$	$t_{(0.05,6)}$
H-0	99.23			1.834	
H-1	99.10			0.357	
H-2	99.09			0.243	
H-3	98.96	99.09	0.09	1.233	2.447
H-6	99.00			0.779	
H-9	99.08			0.130	
H-12	99.02			0.552	

Table 1-4B The results of stability experiments under low temperature of the isotopic abundance analysis

Label	The average abundance (atom%D)	The average	The standard deviation S	$\left\|\overline{x_i} - \overline{x}\right\| / S$	$t_{(0.05,6)}$
C-0	99.07			0.642	
C-1	98.89			2.054	
C-2	99.09			0.941	
C-3	99.03	99.03	0.07	0.043	2.447
C-6	99.03			0.043	
C-9	99.01			0.257	
C-12	99.07			0.642	

As can be seen from the series data in Table 1-4, the abundance data of each isotope are $\left|\overline{x_i} - \overline{x}\right| / S$ ≤t(0.05,6). In other words, the isotope abundance of Sudan-I-D_5 reference material did not change significantly at 0, 1, 2, 3, 6, 9 and 12 days after treatment at high (40±5)℃ and low (-20±5)℃ for 10 hours, indicating good stability.

8. RESULTS AND DISCUSSION

It can be seen from the series of data in Table 1-1 and 1-2 that all the test data conform to Formula 1.2, which indicates that the developed Sudan-I-D_5 reference material is stable during the monitoring period of 1 year when stored at room temperature and protected from light, and meets the relevant requirements in JJG1006-94.

It can be seen from the series data in Table 1-3 and 1-4 that all the test data conform to Formula 1.2, which indicates that the developed reference substances are stable in the following 12 days after being stored at (40±5)℃ or transported at (-20±5)℃ for 10 hours.

9. CONCLUSION

Through these two stability experiments, we believe that the Sudan-I-D_5 reference material developed in this experiment can be stored for a long time at room temperature and under the condition of avoiding light and sealed. During short-term transportation, it can withstand (40±5)℃~(-20±5)℃ for 10 hours under harsh conditions. The results show that the isotopic abundance and the chemical purity of Sudan-I-D5 are stable.

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