

LIDI 2 Software Workflow: Overcoming challenges for clumped isotopes CO₂ analysis on carbonate samples

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Goal

Demonstrate how challenges of variable laboratory temperature and matching of crimping of sample and reference capillaries are overcome by the Thermo Scientific LIDI 2 Software Workflow. Show the improved accuracy and precision of $\Delta 47$ measurements from carbonates in limited sample sizes.

Introduction

Precision and accuracy on limited sample sizes are major challenges for clumped isotopes analysis. The introduction of the Long Integration Dual Inlet (LIDI) significantly improved the sample utilization by first measuring entirely the sample followed by the standard. With LIDI, higher sensitivity for small carbonate samples measured with the Thermo Scientific™ Kiel IV Carbonate Device interfaced to the Thermo Scientific™ 253 Plus™ 10 kV IRMS could be ensured. LIDI improved the statistical precision of $\Delta 47$ measurements from samples in the range of 80-120 μg of total carbonate sample weight.

Despite the progress in sensitivity which came along with LIDI, there are still two key challenges that limit further improvements to $\Delta 47$ determination with LIDI:

- The IRMS System must be in a stable lab environment during the measurement of sample and reference gas, which can take up to 45 minutes incl. sample preparation in the Kiel IV Carbonate Device. This requires a very stable laboratory temperature of ± 0.1 °C/h
- The crimping of the sample and reference capillaries must be precisely matched, if the classical dual inlet data evaluation mode is applied where the sample and the reference are strictly correlated in time. Otherwise, there would be unbalanced decays of reference and sample intensity over time resulting in inaccurate data at reduced precision

This technical note describes how the Thermo Scientific™ LIDI 2 Software Workflow overcomes these challenges. Data are shown demonstrating the improvement in data precision and accuracy.

Analytical setup

Clumped Isotope CO_2 analysis requires instrumentation of superior sensitivity and robustness, such as the 253 Plus 10 kV IRMS. For preparation of gaseous CO_2 from carbonate samples, the Kiel IV Carbonate Device is connected to a 253 Plus 10 kV IRMS.

The Kiel IV Carbonate Device employs acid digestion of carbonates using H_3PO_4 to release CO_2 gas, which is then purified from moisture and contaminants. The sample CO_2 gas is transferred to the 253 Plus 10 kV IRMS ion source via a dedicated capillary featuring an inert internal coating, thus preserving the clumped isotope distribution during transfer. $\Delta 47$ is determined on the 253 Plus 10 kV IRMS using 10^{13} Ohm current amplifier technology and automated online baseline correction. The sample CO_2 gas is referenced against a commercial CO_2 gas provided from the reference port of the Dual Inlet in the 253 Plus 10 kV IRMS.

Sample bracketing and its importance

In classical Dual Inlet mode, any instrument drift is corrected by referencing each sample integration interval by the reference data measured before and after the sample (pre- and post reference). This “bracketing” approach works perfectly for short Dual Inlet measurement cycles and requires only short term stability of the mass spectrometer. However, with LIDI, the reference measurement is only started once the full sample run has been completed, which might result in a delay of the reference measurement of about 20 minutes. This allows highest sample utilization and highest statistical precision but does not correct for instrumental drift. LIDI requires long term stability of the mass spectrometer. Small drifts in room temperature during the analysis could have an impact on the measured $\Delta 47$ and the precision.

Intensity matching and its importance

For a sample of 80 μg CaCO_3 , a measurement performed using LIDI might start at a signal intensity of around 20000 mV ($m/z=44$, 3×10^9 Ω amplification), going down to less than 10000 mV after 600 s of data recording. Ideally, the reference gas follows the same signal decay over time to get precise reference and sample matching and to follow the principle of identical treatment for sample and standard comparison.

With LIDI, the matching of reference to sample isotope ratios is solely time-based, i.e. the sample ratio integrated from 40 to 50 sec after starting the measurement is referenced against data from the same time interval of the reference gas measurement assuming identical start intensities and capillary crimping for sample and reference. Any difference in the starting beam intensity or the signal decay during the individual LIDI runs is not corrected for and will have an impact on the accuracy and precision of the data. The crimping procedure and the pressure adjust of the reference gas to match the start intensity of the sample is laborious and does not always deliver perfect results. Further challenges arise when the source tuning is optimized for high sensitivity, which is common in clumped isotope measurements.

Overcoming the challenges of clumped isotope analysis by using the LIDI 2 Software Workflow

Sample and reference bracketing

Challenges of temperature stability in the laboratory and the matching of crimping of the sample and reference capillaries are overcome by using the LIDI 2 Software Workflow, where sample bracketing becomes possible based on this four-step software-controlled approach:

1. Performing standard LIDI measurements, starting with a reference gas, as shown in Figure 1.

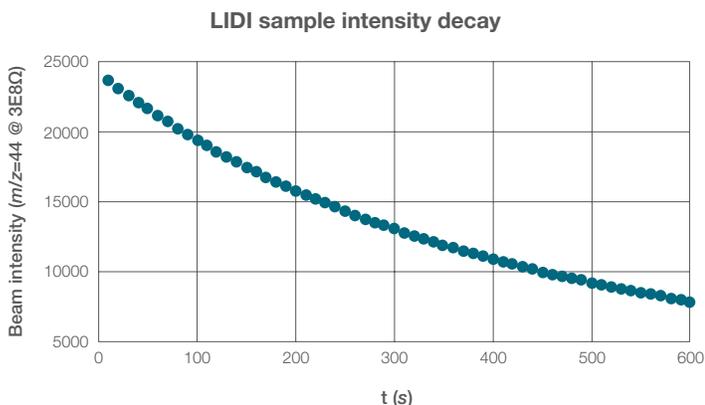


Figure 1. Standard LIDI measurement sequence and exemplary intensity decay for one sample.

2. Curve fitting of the reference isotope ratios¹, for pre- and post-references, as shown in Figure 2.

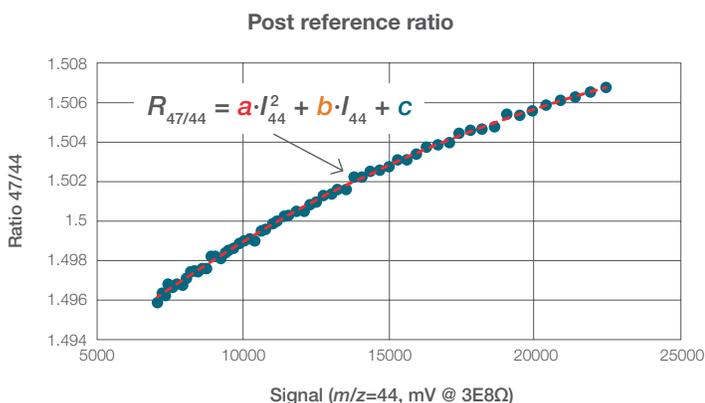


Figure 2. Curve fitting: 2nd order polynomial functions are applied to the reference isotope ratios.

1) Reference isotope ratios are calculated for all masses observed. The usage of $R_{47/44}$ is only exemplary.

3. Calculation of pre- and post- reference isotope ratios for each measured sample signal, as described in Table 1 for a post-reference:

Table 1. Transfer on sample beam intensity: Reference isotope ratios are calculated for each measured sample intensity using the fitting coefficients determined in step 1.

Calculated isotope ratios for post-reference		
LIDI cycle No.	Sample intensity (I_s , in mV)	Reference $R_{47/44}$ at I_s
1	20930.69	1.506777
2	20488.86	1.506544
3	20061.01	$= a \cdot I_{S44}^2 + b \cdot I_{S44} + c$
4	19644.36	...

4. Calculation of interpolated isotope ratios at the time of the sample measurement for each sample beam intensity, as shown in Figure 3.

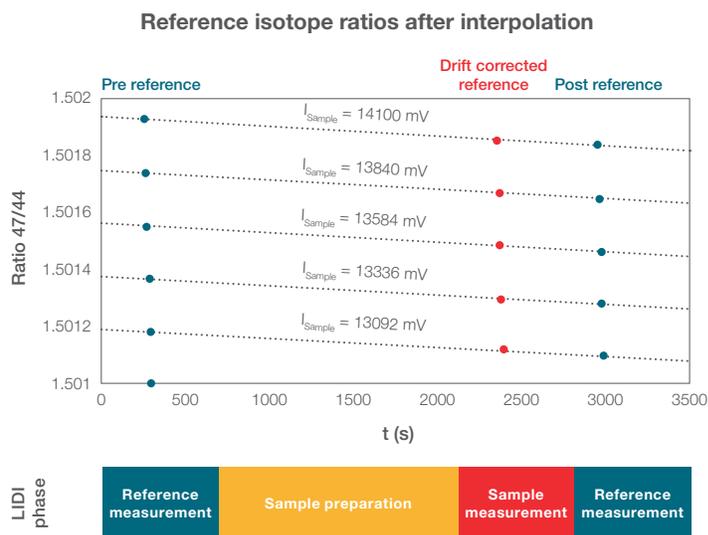


Figure 3. Bracketing: By applying a linear function, interpolated reference isotope ratios are calculated for each sample beam intensity and at the time of sample measurement.

Overcoming the challenge of systematic drifts caused by laboratory temperature instability

Figure 4 shows $\Delta 47$ data from CO_2 gas repeatedly measured against itself (“Zero Enrichment measurement”) using established LIDI. Additionally, the data were reevaluated using the LIDI 2 Software workflow. A drift is visible in the original LIDI data correlating with laboratory temperature. By applying the LIDI 2 Software Workflow this drift can be fully corrected for (i.e. eliminated from the data), decreasing the standard deviation over $n=105$ data points from 32 ppm to 14 ppm.

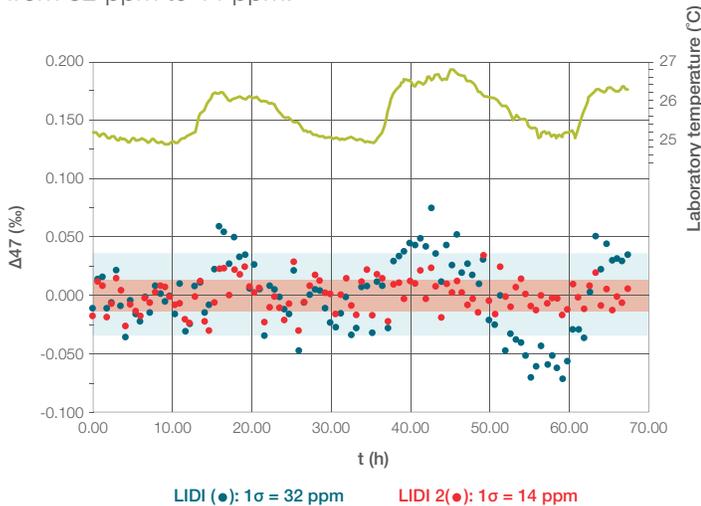


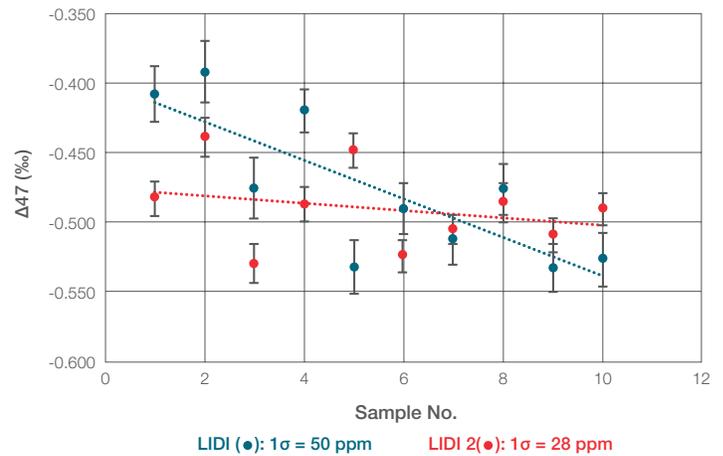
Figure 4. Three days Zero Enrichment data evaluated according to LIDI and using the LIDI 2 Software Workflow method. 1σ confidence intervals are indicated. Original LIDI data show correlation with laboratory temperature.

Table 2 and Figure 5 show the application of the LIDI 2 Software Workflow to Carbonate Standards measured using the Kiel IV Carbonate Device interfaced to the 253 Plus 10 kV IRMS demonstrating significantly improved data quality by using the LIDI 2 Software Workflow.

Table 2. Results from carbonate standard measurements showing that the LIDI 2 Software Workflow delivers more precise data than LIDI.

	ETH 1		ETH 3	
	LIDI	LIDI 2	LIDI	LIDI 2
Mean D47	-0.476	-0.490	-0.067	-0.090
SD over 10 samples	0.050	0.028	0.032	0.012
Median sample SE	0.019	0.012	0.019	0.012
$\Delta\Delta 47$ (LIDI 2)	$\Delta\Delta 47 = \Delta\text{ETH1} - \Delta\text{ETH3} = -0.400$			

ETH 1 carbonate standard



ETH 3 carbonate standard

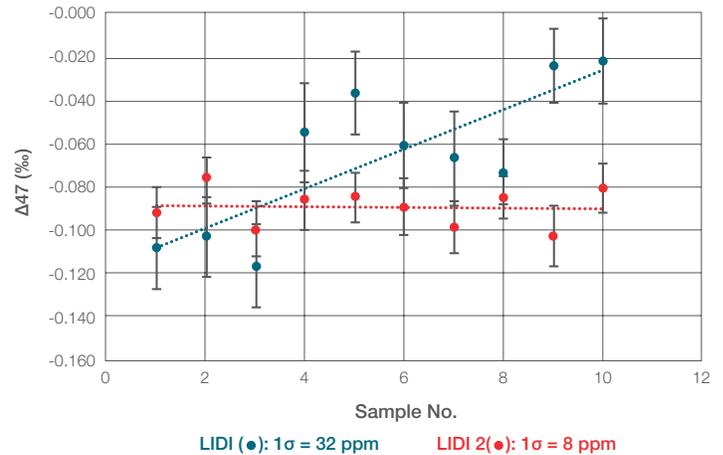


Figure 5. $\Delta 47$ data from CaCO_3 standards (ETH 1, ETH 3) measured using the Kiel IV Carbonate Device and 253 Plus 10 kV IRMS. Compared to LIDI evaluation, the LIDI 2 Software Workflow results in full drift correction and a significant improvement in precision.

Overcoming linearity problems by matching sample and reference intensities

With LIDI 2 Software Workflow, the reference isotope ratios are obtained using a mathematical fitting procedure, as described above. Therefore, reference isotope ratios are not only available at the beam intensities at which they were actually measured, but for any other intensity in between. This allows referencing the sample isotope ratios to interpolated reference isotope ratios with exactly the same beam intensity. Hence, the impact of signal decay becomes eliminated and the demand for matching the capillary crimping is further minimized. Figure 6 shows the impact of a crimping mismatch on the precision of the resulting $\delta^{47}\text{CO}_2$ data measurement using LIDI, and the improvement in data when the LIDI 2 Software Workflow is applied.

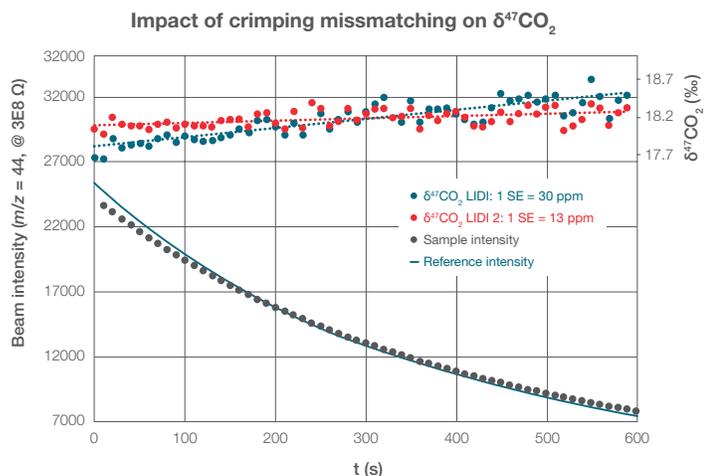


Figure 6. Sample and Reference decay in LIDI and resulting δ^{47} . With LIDI, the intensity decays of sample and reference signals show a mismatch due to non-perfect capillary crimping and pressure adjust. This causes a visible trend in the resulting $\delta^{47}\text{CO}_2$ data. With LIDI 2 Software Workflow, this artefact is removed, leading to significantly enhanced data precision.

Conclusion

This technical note illustrates how the LIDI 2 Software Workflow overcomes challenges of clumped isotope analysis, such as varying temperature and matching of crimping of sample and reference capillaries.

With the analysis performed on the Kiel IV Carbonate Device interfaced with 253 Plus 10 kV IRMS and by using the Thermo Scientific LIDI Software Workflow, precise $\Delta 47$ data can be obtained from small carbonate samples.

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