

# Simple and Precise Dual Carbon–Hydrogen Isotope Analysis of Organic Materials Using Combustion–CRDS

PICARRO

## APPLICATION NOTE (AN044) STABLE ISOTOPE ANALYZERS COMBUSTION MODULE–CRDS (CM–CRDS)

*An integrated approach for joint  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  analysis from a single combustion event*

### Introduction

Picarro Cavity Ring-Down Spectroscopy (CRDS) analyzers are widely adopted for stable isotope measurements due to their operational simplicity, analytical stability, cost-effective operation, and suitability for high-throughput laboratory workflows. Picarro's Combustion Module–CRDS (CM–CRDS) system has enabled routine bulk  $\delta^{13}\text{C}$  analysis of solid and liquid organic materials, supporting applications including but not limited to environmental research, geochemistry, and food authenticity. Carbon and hydrogen isotopes are each well established as independent tracers, with  $\delta^{13}\text{C}$  reflecting carbon sources and processing pathways and  $\delta^2\text{H}$  capturing hydrological, climatic, and biosynthetic information. Combining the two provides a more robust approach to determining provenance, assessing authenticity, and identifying sources across a range of organic materials. Dual  $\delta^{13}\text{C}$ – $\delta^2\text{H}$  datasets are increasingly recognized as valuable for applications such as food and agricultural product authentication, soil and sediment studies, and biobased material verification, where single-isotope approaches may not fully resolve overlapping signatures.

Traditionally, bulk carbon and hydrogen isotope analyses have been performed using Elemental Analyzer–Isotope Ratio Mass Spectrometry (EA–IRMS) and Thermal Conversion Elemental Analyzer–Isotope Ratio Mass Spectrometry (TC–EA–IRMS) workflows, which provide high analytical performance but typically require independent reactor configurations, sequential analyses, and reliance on helium-based infrastructure. Building on the robustness of the existing CM–CRDS platform, we present a laboratory-developed extension that enables joint  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  analysis of bulk organic materials by integrating two dedicated CRDS analyzers, specifically a Picarro G2201-*i* for carbon isotopes from  $\text{CO}_2$  and a Picarro L2130-*i* for hydrogen isotopes from water vapor, within a coordinated combustion workflow. This approach preserves the established combustion and sample delivery pathway for carbon isotope analysis while enabling precise hydrogen isotope measurements from the same bulk material, providing a streamlined route to generating internally consistent dual-isotope datasets for research and applied science applications.

### Objectives

This application note aims to:

- 1 Describe the configuration and operating principles of a laboratory-developed combustion setup integrated with two CRDS analyzers for joint  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  analysis of bulk organic materials.
- 2 Evaluate the analytical performance of the system through repeated measurements of internationally recognized organic reference materials.

## System configuration, flow path and operational conditions (dual CM-CRDS setup)

The dual-isotope configuration is based on a standard Picarro Combustion Module (CM) workflow and extends it to enable joint  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  analysis of bulk organic materials from a single combustion event. The system combines a Picarro G2201-*i* analyzer for carbon isotope measurements with a Picarro L2130-*i* analyzer for hydrogen isotope measurements, operated in parallel and connected to a common combustion source (**Figure 1**). As an alternative configuration, the  $\text{CO}_2$ -only analyzer Picarro G2131-*i* may also be used in place of the G2201-*i* for carbon isotope analysis, depending on measurement requirements.

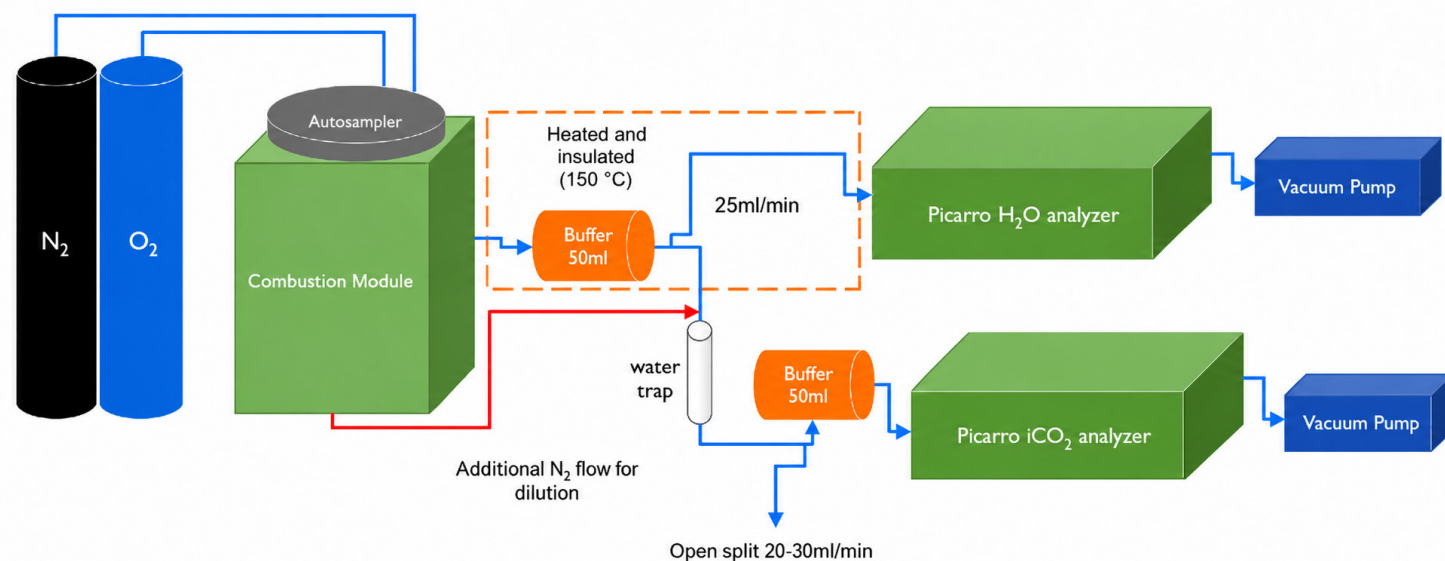


**Figure 1.** Schematic of the dual-isotope CRDS measurement setup: Samples are combusted using  $\text{O}_2$  with  $\text{N}_2$  as carrier gas and dilution, and combustion products are routed through buffered and conditioned flow paths to dedicated Picarro  $\text{H}_2\text{O}$  (L2130-*i*) and  $\text{CO}_2$  (G2201-*i*) CRDS analyzers.

Solid and liquid samples are combusted in an oxidizing environment, producing a gas stream composed primarily of carbon dioxide ( $\text{CO}_2$ ) and water vapor ( $\text{H}_2\text{O}$ ), together with carrier and combustion gases. The post-combustion stream is split and routed to the two analyzers for simultaneous isotope measurements.

The carbon isotope measurement pathway follows the established CM-CRDS configuration used for routine  $\delta^{13}\text{C}$  analysis. After combustion, the  $\text{CO}_2$ -bearing gas stream passes through a 50 mL buffer volume upstream of the G2201-*i* to condition the transient combustion pulse and improve peak shape and reproducibility. A three-way valve positioned upstream of the buffer allows excess combustion gas to be vented during purge steps, preventing sample overloading and supporting stable operation across samples.

The hydrogen isotope pathway is configured to deliver combustion-derived water vapor directly to the L2130-*i* without intermediate moisture removal. Transfer lines associated with this pathway are fully temperature controlled, and a heated buffer volume of 50 mL maintained at  $150^\circ\text{C}$  is used to ensure that water remains in the vapor phase during transport. This thermal control minimizes condensation and isotopic fractionation and supports reproducible hydrogen isotope measurements from combustion-generated water vapor. A summary of the operating parameters used in this study is provided in **Table 1**.

TABLE 1

| Operating temperatures, volumes, and gas pressures used in this study |                     |                                                             |
|-----------------------------------------------------------------------|---------------------|-------------------------------------------------------------|
| Parameter                                                             | Setting             | Description                                                 |
| Carbon isotope buffer volume                                          | 50 mL               | Buffer volume upstream of $\delta^{13}\text{C}$ analyzer    |
| Heated buffer volume                                                  | 50 mL               | Temperature-controlled volume in $\delta^2\text{H}$ pathway |
| Heated buffer temperature                                             | 150°C               | Maintains combustion-derived water vapor                    |
| Hydrogen isotope transfer line                                        | Heated              | Prevents condensation during gas transfer                   |
| Oxygen supply pressure                                                | ~1 bar              | Oxidant gas supplied to combustion module                   |
| Compressed air supply pressure                                        | ~2.5 bar            | Combustion support gas                                      |
| Nitrogen supply                                                       | Carrier / purge gas | Used for flow control and purging                           |

Isotope ratios for both carbon and hydrogen were calculated from transient combustion peaks using a weighted-average integration approach, which accounts for the full peak shape while minimizing the influence of baseline noise and short-term flow variability.

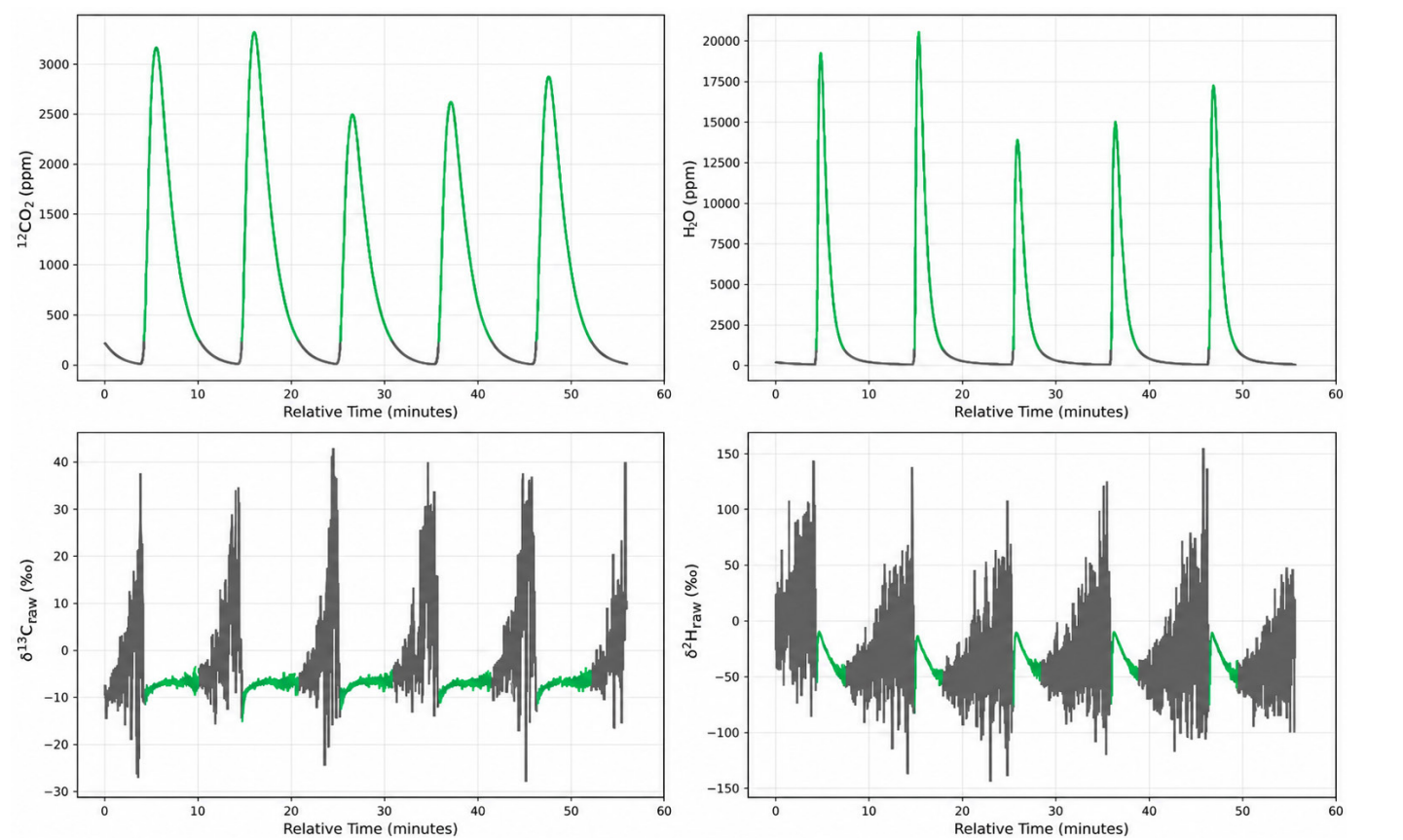


**Figure 2.** Example of time-series data during sequential measurements of a standard using the dual-CRDS workflow: Time-resolved  $\text{CO}_2$  and  $\text{H}_2\text{O}$  concentrations (top panels) and corresponding uncorrected  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  values (bottom panels) measured during repeat analyses ( $n = 5$  in this case) of a single standard, illustrating peak shape, signal stability, and isotope response over time.

## Methods

### *Reference Materials and Calibration*

System performance was evaluated using a set of internationally recognized organic reference materials representing a range of bulk matrices commonly encountered in environmental and food-related use-cases. The materials analyzed were USGS88 (marine collagen), USGS89 (porcine collagen), USGS90 (millet flour), USGS91 (rice flour), and IAEA CH7 (polyethylene foil), with individual sample masses ranging from 1.56 to 2.03 mg. These standards were used primarily to assess overall system response, linearity, and consistency across different organic compositions for carbon isotope measurements.

### *Use of Hexamethylenetetramine (HMT)*

Organic reference materials containing exchangeable hydrogen, such as collagens and plant-derived flours, can exhibit changes in apparent  $\delta^2\text{H}$  values over time if sample vials are repeatedly opened. This behavior is associated with adsorption of atmospheric water and isotopic exchange at labile hydrogen sites, resulting in increased sensitivity to ambient laboratory moisture during handling and analysis. Consequently, variability in bulk  $\delta^2\text{H}$  values from these materials may reflect exchange processes rather than instrumental performance, which can complicate interpretation of hydrogen isotope precision under ambient laboratory conditions.

Hexamethylenetetramine (HMT) was therefore used as the primary reference material for assessing hydrogen isotope performance. HMT contains non-exchangeable hydrogen bound to carbon and does not readily undergo isotopic exchange with atmospheric moisture, making it well suited for precision testing under ambient laboratory conditions. For example, HMT is used to determine the carbon-bound non-exchangeable hydrogen in fructose and glucose in honey (Li et al., 2024). Repeated measurements of HMT, with individual sample masses ranging from 1.1 to 1.5 mg, were used to evaluate  $\delta^2\text{H}$  precision, peak reproducibility, and integration performance under transient combustion conditions, independent of hydrogen exchange effects.

## Results and Discussion

### *Calibration Performance for $\delta^{13}\text{C}$ and $\delta^2\text{H}$*

Calibration performance was evaluated by comparing CRDS-reported isotope values with certified values for USGS88, USGS89, USGS90, and USGS91, measured on three separate days (11, 12, and 16 December 2025; henceforth referred to as Day 1, Day 2, and Day 3, respectively). For both  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$ , strong linear relationships were observed between reported and certified values across the full isotopic range investigated (**Figure 3**).

For  $\delta^{13}\text{C}$ , linear regressions for all three days yielded slopes close to unity (1.000 on Day 1, 1.007 on Day 2, and 1.005 on Day 3) with coefficients of determination exceeding 0.999, indicating excellent linearity and highly reproducible carbon isotope measurements. The close agreement among measurement days demonstrates stable calibration behavior and minimal day-to-day variability.

For  $\delta^2\text{H}$ , similarly robust linearity was observed, with regression slopes close to unity (1.040 on Day 1, 1.074 on Day 2, and 1.017 on Day 3) and  $R^2$  values greater than 0.99 across all days. As expected for hydrogen isotope measurements, slightly increased variation was observed relative to carbon; however, the overall agreement confirms consistent hydrogen isotope response and reliable calibration performance across multiple analytical sessions.

Together, these results demonstrate that the dual CM-CRDS configuration provides accurate and reproducible bulk  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  measurements for organic reference materials when operated under stable analytical conditions.

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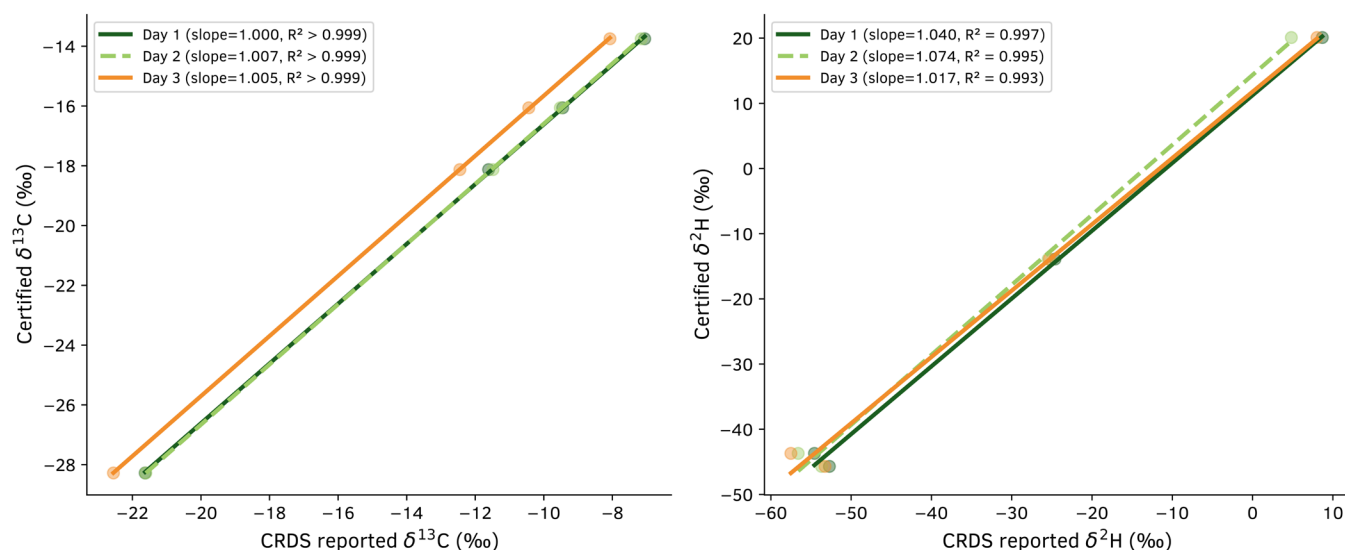


**Figure 3.** Calibration performance of CRDS  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  measurements: Side-by-side comparison of CRDS-reported versus certified  $\delta^{13}\text{C}$  (left) and  $\delta^2\text{H}$  (right) values for USGS88, USGS89, USGS90, and USGS91 standards measured on 3 separate days. Linear regressions show slopes near unity with  $R^2 \geq 0.99$ , demonstrating excellent linearity and day-to-day reproducibility for both carbon and hydrogen isotope measurements; the small offset observed in the  $\delta^{13}\text{C}$  plot reflects a minor calibration adjustment applied to the analyzer.

## Memory Effects during Sequential Standard Measurements

Memory effects were evaluated using sequential measurements of USGS reference materials acquired without intermediate conditioning steps. Measurement-to-measurement carryover was observed primarily following transitions between isotopically distinct standards, with isotope values converging rapidly thereafter (Figure 4).

Across all three days, both carbon and hydrogen isotope values stabilized within the first few measurements, with memory effects becoming negligible after approximately the third measurement for each standard. This behavior was consistent across all four standards and indicates minimal residual carryover under the applied operating conditions.

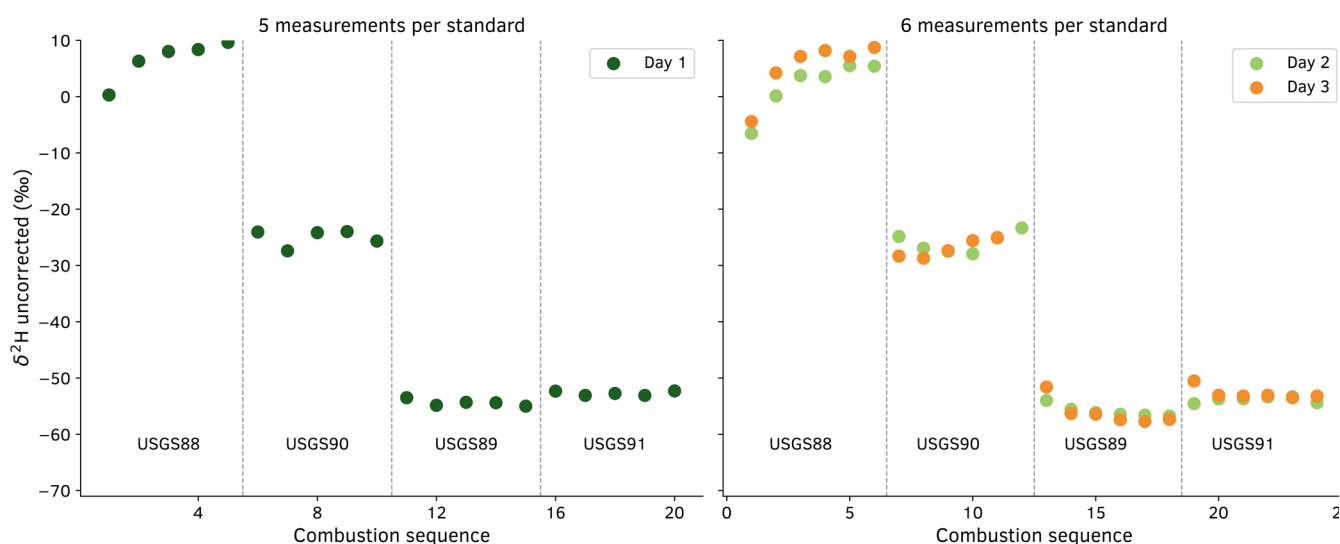


**Figure 4.** Memory effects during sequential  $\delta^2\text{H}$  measurements of USGS standards: CRDS-measured uncorrected  $\delta^2\text{H}$  values for USGS88, USGS90, USGS89, and USGS91 measured sequentially on Day 1 (5 measurements per standard; left panel) and on Day 2 and Day 3 (6 measurements per standard; right panel). Measurement-to-measurement carryover is most pronounced in the initial measurements, with memory effects becoming negligible after the third measurement across all standards.

## Instrument Stability and Precision Assessed Using HMT

Instrument stability and precision were assessed using repeated measurements of HMT. Time-series data show stable  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  values throughout extended measurement periods, with no observable systematic drift (**Figure 5**). The observed standard deviations were 0.04 ‰ for  $\delta^{13}\text{C}$  and 0.26 ‰ for  $\delta^2\text{H}$ , demonstrating high precision for both isotope systems under continuous operation.

These results confirm that the dual-analyzer CM-CRDS configuration maintains stable performance for both isotope systems over multi-hour measurement intervals and that hydrogen isotope precision is governed primarily by material-specific exchange behavior rather than instrumental instability.

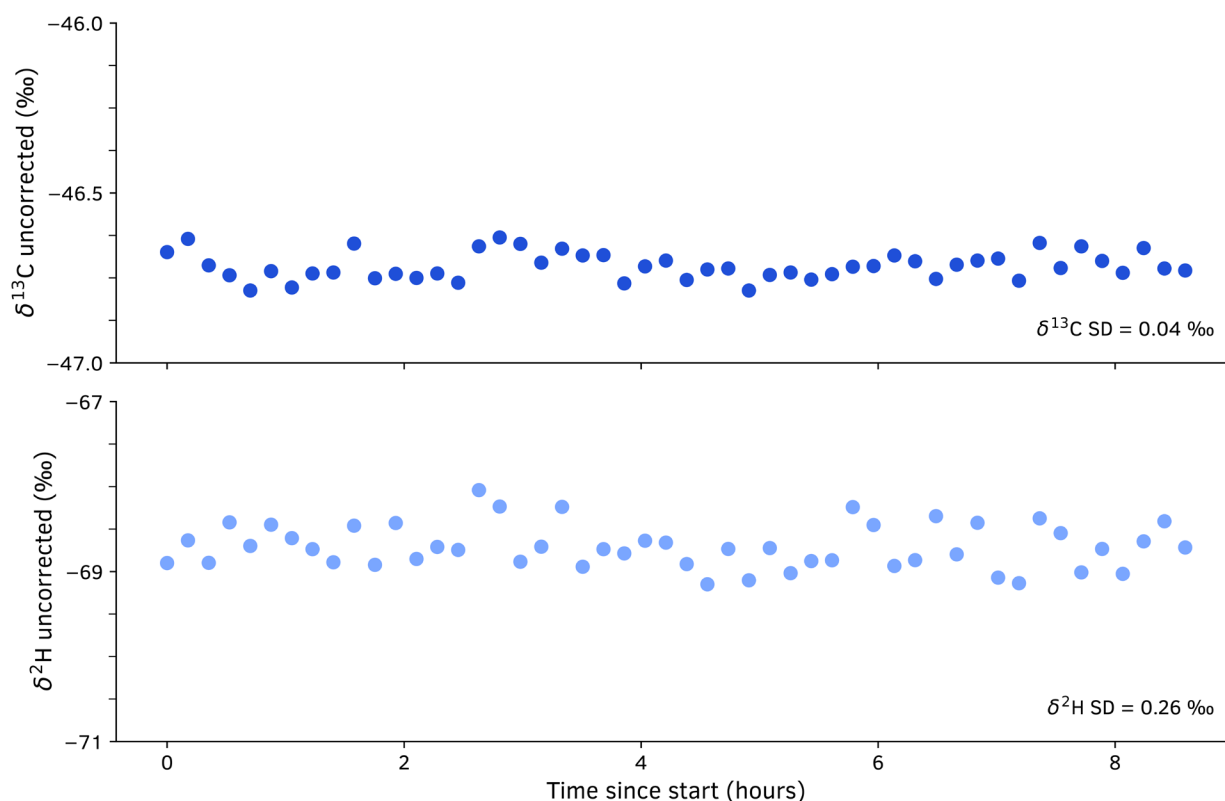


**Figure 5.** Temporal stability of  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  during repeated HMT analysis: Time-series scatter plots of CRDS-measured  $\delta^{13}\text{C}$  (top) and  $\delta^2\text{H}$  (bottom) values for HMT (hexamethylenetetramine) during repeated analysis, shown as hours since measurement start. Isotope values remain stable throughout the experiment, with standard deviations of 0.04 ‰ for  $\delta^{13}\text{C}$  and 0.26 ‰ for  $\delta^2\text{H}$ .

## Practical Considerations for Storage, Analysis and Calibration

During multi-day measurements, it was observed that USGS organic reference materials exhibited variable contributions from exchangeable hydrogen, which can influence bulk  $\delta^2\text{H}$  results under ambient laboratory conditions. In addition, the reported  $\delta^2\text{H}$  uncertainties for these materials are relatively large compared to carbon isotope uncertainties, with values of  $\pm 6.3$  ‰ (USGS88),  $\pm 7.8$  ‰ (USGS89),  $\pm 2.4$  ‰ (USGS90), and  $\pm 7.4$  ‰ (USGS91), whereas corresponding  $\delta^{13}\text{C}$  uncertainties are much smaller ( $\pm 0.06$ – $0.11$  ‰). These characteristics limit the suitability of USGS organic standards for evaluating intrinsic hydrogen isotope precision, particularly when high-resolution  $\delta^2\text{H}$  performance is of interest.

## BEST PRACTICE

To minimize variability associated with hydrogen exchange, USGS reference materials and samples containing exchangeable hydrogen should be stored in sealed containers under dry conditions or, where appropriate, frozen, to reduce interaction with atmospheric moisture. During analysis, minimizing sample exposure to ambient laboratory air is strongly recommended.

Measurement consistency may be further improved through the use of a zero-blank autosampler, which reduces background contributions, limits conditioning requirements, and minimizes opportunities for hydrogen isotope exchange during sample handling and transfer. Together, appropriate storage practices and optimized sample introduction strategies significantly enhance the reliability of bulk  $\delta^2\text{H}$  measurements for organic materials and provide opportunities for further refinement of the workflow across a broader range of samples and analytical conditions.

## Conclusion

From this study, it is evident that the demonstrated calibration stability, low instrumental drift, and manageable memory behavior support reliable routine operation of the dual-CRDS combustion setup with minimal analytical overhead. The key findings of this work are summarized below:

- Stable and reproducible bulk  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  measurements were achieved over multiple days of operation. Calibration against USGS reference materials analyzed at sample masses of ~1.5 to 2.1 mg showed strong linearity for both isotopes, while continuous monitoring using hexamethylenetetramine (HMT) confirmed excellent measurement repeatability, with observed standard deviations of 0.04 ‰ for  $\delta^{13}\text{C}$  and 0.26 ‰ for  $\delta^2\text{H}$ , and no measurable instrumental drift during extended measurements.
- Memory effects were limited to the initial measurements following transitions between isotopically distinct materials and became negligible after a small number of conditioning measurements. Based on these observations, performing at least five repeat measurements per sample and excluding the first three is recommended, enabling efficient daily throughput of ~20 samples plus standards under routine operating conditions.

The dual-CRDS combustion configuration enables joint  $\delta^{13}\text{C}$  and  $\delta^2\text{H}$  measurements from a single combustion event using nitrogen as the carrier gas, eliminating the need for chromatographic separation or helium-based infrastructure. Simplified gas handling and a reduced number of interfaces lower operational complexity, maintenance requirements, and potential sources of analytical variability, supporting streamlined analytical workflows with minimal user intervention. As a result, the setup is well suited for routine bulk isotope measurements across applications including food and beverage authenticity testing, environmental and ecological studies, pharmaceutical and chemical quality control, forensic screening, and academic research. Building on the performance demonstrated here, continued evaluation across additional reference materials and sample matrices will further expand the scope and applicability of the dual-isotope workflow.

## Reference

[1] Li et al., 2024, A new approach to detecting sugar syrup addition to honey: Stable isotope analysis of hexamethylenetetramine synthesised from honey monosaccharides (fructose and glucose). Food Chemistry 434.