Nitrous Oxide Isotopic Measurements of Discrete Gas Samples Using Sage Gas Autosampler and Picarro PI5131-*i* Analyzer

PICARRO

APPLICATION NOTE (AN042)
Picarro PI5131-*i* Analyzer
and Sage Gas Autosampler

Introduction and Objectives

The Picarro PI5131-*i* N_2O Isotopic Analyzer provides continuous, high-precision measurements of nitrous oxide (N_2O) mole fractions, as well as its $\delta^{15}N_\alpha$, $\delta^{15}N_\beta$, $\delta^{15}N$ and $\delta^{18}O$ isotopic compositions. To meet the growing demand for discrete sample analysis, Picarro has developed the Sage gas autosampler.

Integrated with the PI5131-*i*, Sage automates discrete gas sample analyses from field sites and laboratory experiments, extending the application of Picarro's CRDS technology. The Sage gas autosampler accommodates up to (12 mL) 150 vials/exetainers and is equipped with modern software that supports customizable sample sequencing, real-time data acquisition, calibration, and immediate data visualization. This combination provides flexible analytical parameters and robust high-quality data across a variety of research applications.

The current application note aims to:

- Demonstrate Compatibility: Show how effectively the Sage gas autosampler integrates with the Picarro PI5131-*i* analyzer.
- Assess Performance: Analyze the accuracy and reliability of N_2O isotopic measurements when using this setup.
- Provide Practical Recommendations: Deliver actionable guidelines for conducting N_2O concentration and isotopic analyses from discrete samples.

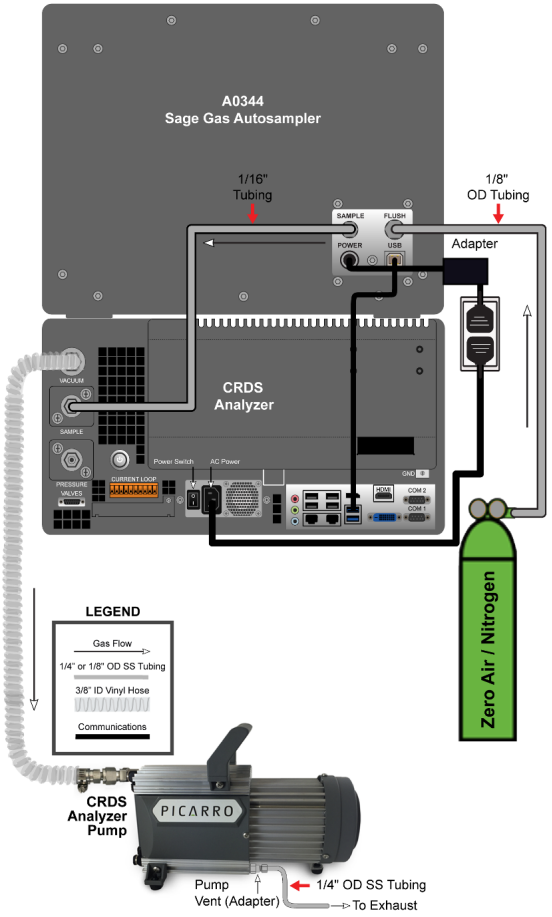

Figure 1. *Sage setup and installation*

Methods

Integrating Sage with Picarro analyzer

The Picarro PI5131-*i* analyzer was set up with its external A2000 pump and a monitor screen connected for data viewing. For further details on the setup procedures, refer to the PI5131-*i* user manual. The Sage–Picarro connection followed the standard configuration (see Figure 1). There were two main gas-line connections, one being the sample gas output from Sage to Picarro analyzer sample inlet port and the second one was the flush gas connection from a zero-air tank. The present experiments were performed in the Sage-PI5131-*i* setup with automatic evacuation (see below), and zero air was used for vial flushing. When integrating Sage with a previously purchased PI5131-*i* (or G5131-*i*) analyzer, Sage software needs to be installed on the analyzer (see Sage user manual, Section 3.1).

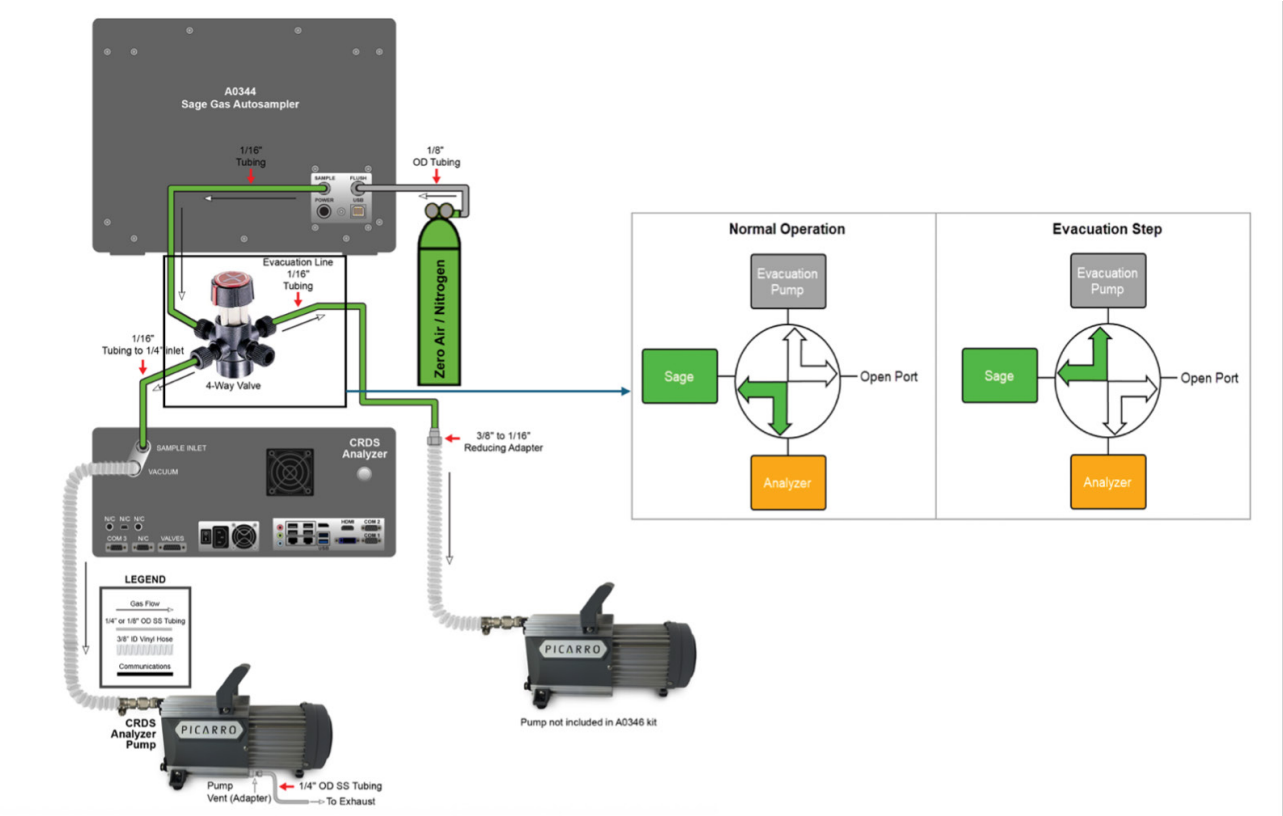

Figure 2. *Setup of Sage autosampler with A0346 Sample Preparation Kit's 4-way valve to run automated vial evacuation*

Evacuation of vials

Sage gas autosampler typically uses 12 mL LabCo exetainer vials with double-wadded septa. These vials need to be properly evacuated to perform contamination free experiments. Deviation from best practices on vial evacuation can lead to excess dilution and decrease in precision of vial measurements. The vials for this experiment were evacuated using A0346 Sage Sample Preparation Kit and an additional A2000 pump (refer to Figure 2 for the setup details). For this automated evacuation process, an external 4-way valve setup was used (Figure 2) as described in Appendix section A of Sage User Manual.

An evacuation method was set up on Sage software, an example of such method is noted below (users can create their own evacuation methods as needed).
Flush: Not Applicable
Sample: 70 seconds
Stabilization: 5 seconds
Measurement: 60 seconds
Post Measurement: 5 seconds

In the “Run” page of the software, the vial positions were selected from the “Sample List” tab, and the method defined above was loaded from the “Sampling Method” dropdown option. For information on adding vial information and specifying positions, see section 7.5 titled “Add Experiment Details” of the Sage User Manual. Before starting the step, the evacuation pump was powered on. After the evacuation process, the external pump was immediately powered off to avoid any impact of excess and prolonged vibrations.

Filling of gas samples

As soon as the vials were evacuated, they were filled with gas samples. For the current experiment, sample filling was done directly from standard gas tanks. A needle with a Luer connection to a 1/8” Bev-A-Line tubing was used to connect to standard gas tanks and fill up evacuated vials. When filling up from the gas tank, it is recommended to use a 2-stage regulator and adjust the delivery pressure to >9 psi. Once the gas was flowing through the needle, the septum of the evacuated vial was punctured by pushing the needle through the septum in a perpendicular direction. Once inserted, the needle was held steady for ~20s. This ensured a proper filling of the vial, and after that, slowly the needle was pulled out from the vial, and then the regulator of the gas tank was closed at the end of this process. This step was repeated to fill each vial.

Figure 3. *Vial filling*

Samples used

For this study, four certified standard gas tanks were used, hereafter referred to as Tank 1, Tank 2, Tank 3, and Tank 4, with respective N_2O concentrations of 330 ppb, 693 ppb, 1066 ppb, and 1437 ppb covering the measurement range (300-1500 ppb). To evaluate measurement precision, multiple vials were filled from each gas tank and analyzed under identical conditions. A total of 18, 24, 28, and 10 replicate measurements were conducted for Tanks 1, 2, 3, and 4, respectively.

Sample analysis

Vials filled with gas samples were analyzed on Sage using the Sage software method which can be

defined by users based on their application, sample type and variability. An example is given below:

Flush: 90 seconds
Sample: 320 seconds
Stabilization: 120 seconds
Measurement: 180 seconds
Post Measurement: 20 seconds

Figure 4. *Method for sample analyses and explanation for the phases within a sample injection*

During the experimental run, some outliers were identified using the real-time data viewing screen, which helped to sync valuable species data with that of sensors (example: outlet valve and needle pressure) while the experiment was running. Refer to the Troubleshooting section of the Sage user manual for more related information.

Results and Discussion

Reproducibility

Based on the above experiment sets, we report standard deviation of each of the test samples and its respective species in Table 1. Each of the standard deviation values was based on the number of replicate samples that were analyzed. The standard deviation for all isotopic values were found to be within 1‰. The highest precision with standard deviation <0.5permil for all isotopic parameters was achieved at 1000ppb, see Fig. 5.

Index	Tank / Sample Description	N_2O concentration SD [ppb]	$\delta^{15}N$ N_2O Standard Deviation [‰]	$\delta^{15}N_\alpha$ N_2O Standard Deviation [‰]	$\delta^{15}N_\beta$ N_2O Standard Deviation [‰]	$\delta^{18}O$ N_2O Standard Deviation [‰]
1	Tank 1 (330 ppb)	3.4	0.5	0.7	0.8	0.9
2	Tank 2 (693 ppb)	7.6	0.5	0.6	0.5	0.7
3	Tank 3 (1066 ppb)	8.6	0.3	0.4	0.4	0.5
4	Tank 4 (1437 ppb)	12.3	0.6	0.8	0.5	0.6

Table 1. *Compilation of Standard Deviation (SD) values for all parameters listed according to respective tanks.*

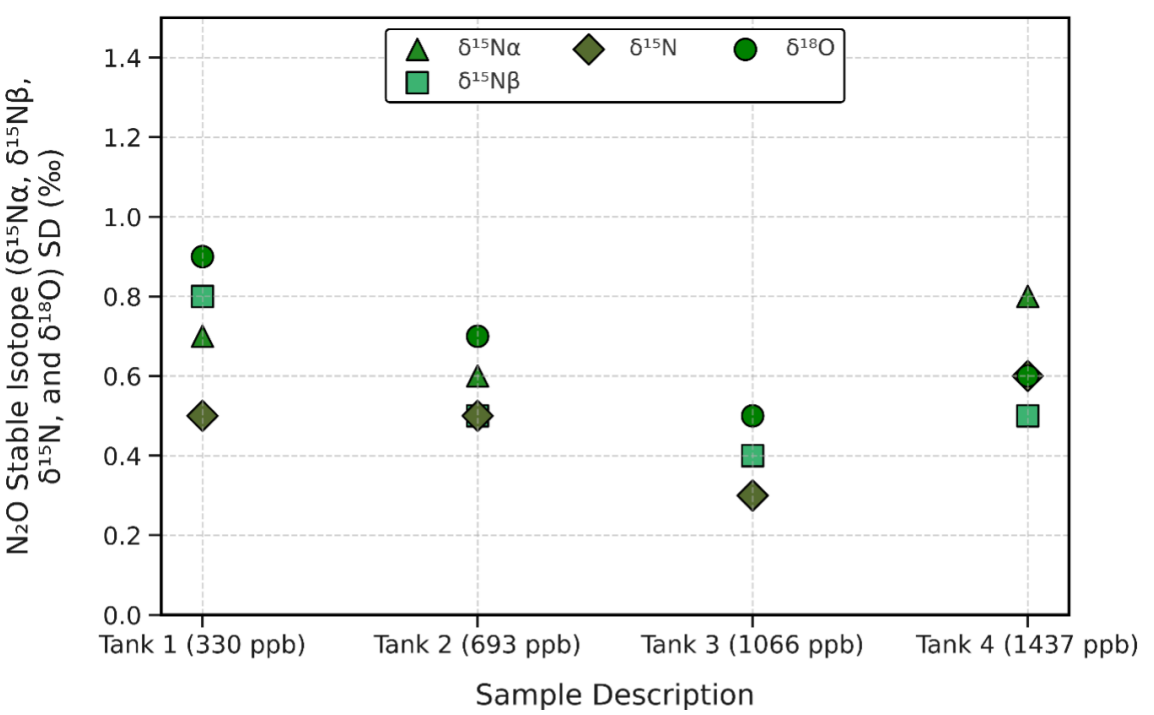

Figure 5. *Scatter plot showing N_2O stable isotope ($\delta^{15}N_\alpha$, $\delta^{15}N_\beta$, $\delta^{15}N$ and $\delta^{18}O$) SD (‰) on the Y-axis across the four tanks with varying N_2O concentrations. SD = Standard Deviation*

Commonly asked questions about best practices with Sage gas autosampler

How much recovery to expect for concentration measurements with well- prepared vials?

When working with discrete gas samples using a sample delivery peripheral such as an autosampler, users should expect some loss of sample. When sample vials are correctly prepared, users can generally expect >90% recovery of gas concentration measurements.

What is the recommended sample volume?

We recommend filling the vial with >20ml of sample volume to reach highest recovery of samples particularly for concentration focused measurements.

What is the optimal time of "sampling" time in the UI?

A higher stabilization time is required to achieve better precision. In our in-house results, 180 s of stabilization was seen to provide good precision.

What is the impact of delivery pressure from tank regulator on accuracy?

When using a standard gas tank to directly fill vials, >7.5 psi is recommended (no less than 7 psi and no greater than 11 psi). We get good results using 9 psi delivery pressure.

How to manually evacuate vials before analysis?

An external vacuum pump and tubing setup is needed to manually evacuate each exetainer vial. With the tubing setup ready and pump turned on, insert the needle perpendicularly through the exetainer septum and hold it steady for ~45 seconds. Once done, steadily pull the needle out in a perpendicular direction from the septum base.

How to optimize flushing time based on sample characteristics?

Adjustment of flushing time may be required to improve data quality depending on the concentration and isotopic variability of the gas samples. For example, longer flushing durations are recommended for samples with elevated N_2O concentrations or substantial isotopic heterogeneity to minimize memory effects and ensure stable, representative measurements.

How long can we reuse the DW septa and vials?

It is best to not reuse the septa. However, the data quality degrades significantly more after 2 rounds of usage. The glass vials can be reused multiple times when cleaned and evacuated properly.

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