

Simple, Real-Time Measurement of Stable Isotope Ratios in H₂O and CO₂

Summary. Picarro WS-CRDS-based analyzers are the first instruments to combine simple, turnkey operation with sensitivity and precision better than or equal to the IRMS (isotope ratio mass spectrometer) systems traditionally used in stable isotope labs. This opens the door for widespread exploitation of stable isotope studies in fields such as carbon/water cycle research, GHG (greenhouse gas) monitoring, animal migration, and human metabolism.

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Introduction

Stable isotopes have long been used for carbon/water cycle studies, historical profiling of terrestrial temperatures, and research in plant biology. The reason is that every chemical and biological process fractionates isotopes with a characteristic ratio. For example, in the case of carbon, C3 type plants photosynthetically fix carbon dioxide with a shift in ¹³C/¹²C ratio that is different than C4 type plants. And in the case of greenhouse gases, “anthropogenic” CO₂ from burning fossil fuels has a much lower ¹³C/¹²C ratio than “biogenic” CO₂ derived from burning biofuels such as corn ethanol. But scientists in many other fields are now starting to recognize the potential information that can be obtained from measuring stable isotope ratios. Nascent applications range from tracking the origins of opium and other drugs, to mapping the migration of animals, and studying human metabolism. Unfortunately, this potential has never been fully explored or exploited because of the practical issues in making these measurements by traditional methods based on IRMS (isotope ratio mass spectrometry). These limitations stem from the high cost of these large, specialty instruments, together with their need for a trained, experienced operator and their requirement for extensive sample preparation – often both physical and chemical. As a result, stable isotope measurements have been confined to a few analytical laboratories with other researchers using their services on a contract basis. Needless to say, direct field measurements have been completely impractical with these ultra-high vacuum instruments; samples have to be collected and subsequently analyzed in a lab. Here we show how a new type of compact, rugged, benchtop instrument eliminates these drawbacks and is thereby completely changing the landscape by providing simple turnkey access to highly precise isotope ratios. These compact WS-CRDS-based isotope analyzers even support field applications, for example, for continuous GHG monitoring and source identification such as in leak detection at sequestration sites.

WS-CRDS Background

Nearly every small molecule (e.g., H₂O, CO₂) has a unique near-infrared absorption spectrum consisting of sharp well-resolved lines. However, conventional infrared spectrometers (and even those based on tunable laser diodes) don’t have the requisite combination of spectral resolution, sensitivity and large linear dynamic range that is necessary to use this spectral information to measure isotope ratios. Fortunately, WS-CRDS has all three, plus several other advantages, including speed, simplicity, long-term stability and a small footprint.

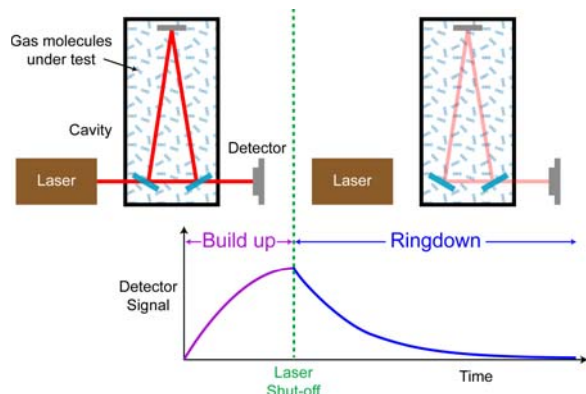


A vapor-phase isotope analyzer based on WS-CRDS is relatively compact – about the size of a briefcase.

In WS-CRDS (wavelength-scanned cavity ring down spectroscopy), light from a narrow-linewidth, wavelength-tunable laser diode enters the sampling cavity which contains three exceptionally high reflectivity (>99.999%) mirrors (see figure next page). This cavity is automatically and precisely temperature and pressure stabilized. When the signal from the detector reaches a steady state condition, the laser is switched off. Because the mirrors do not have 100% reflectivity, the light intensity inside the cavity slowly leaks out and this ring-down (decay) is followed in real-time by a quantitative photodetector.

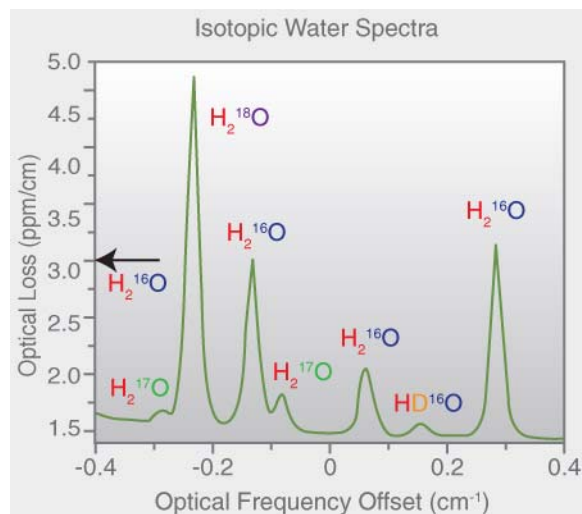
If the cavity contains a gas species that absorbs even weakly, this introduces a second light loss mechanism. This results in a shortened decay time, which forms the basis for a highly quantitative measurement. Even with a cavity of only 25 cm in length, the average pathlength that any photon effectively travels within the cavity can be over 20 kilometers, thus giving rise to high sensitivity. Also, because WS-CRDS measures decay rates, rather than transmitted (absorbed) intensity, any fluctuations in the laser intensity have no effect on the measurement, unlike conventional spectrometers and gas analyzers. Moreover, because the laser is systematically tuned over the target absorption line during each 1 Hz measurement cycle, the intensity of the line is very accurately determined. And the line intensity (i.e. extinction) in turn is linearly dependent

on the concentration of molecules in the cavity. This is the basis for Picarro's family of extremely sensitive WS-CRDS trace gas analyzers that now routinely deliver parts per trillion detection, with up to six orders of magnitude linear dynamic range.



The basic principles of WS-CRDS. Light from a tunable laser is trapped in a three-mirror cavity. When the laser is turned off the ring down time for the light to decay is faster if the cavity contains molecules that absorb at the laser wavelength.

Each of the sharp spectral absorption lines is due to the laser exciting vibrations and rotations in the target molecule. Because the frequency of these vibrations and rotations is dependent on the precise mass of the atoms, each isotopologue (e.g. H₂O, HDO) has lines at different frequencies (i.e. wavelengths). Furthermore, WS-CRDS instruments have the spectral resolution and absolute wavelength precision necessary to isolate and uniquely measure these spectral lines (see figure). And because WS-CRDS delivers both ppt sensitivity and high dynamic range, even weaker lines corresponding to molecular species containing low abundance isotopes can be measured with high signal to noise ratio. So, by measuring the concentration of each of the individual isotopologues in this way, the instrument records the isotopic concentration ratio. Just as important, the WS-CRDS instrument has very low long term absolute drift and the target isotopes are essentially measured simultaneously. Plus the final ratio only depends on the *relative* line intensities. All this adds up to extremely stable, drift-free, isotopic ratio measurements with no need for frequent calibration, unlike IRMS.



Each isotopologue gives rise to unique near-IR absorption lines as in this water sample. WS-CRDS can separately measure these lines because of the narrow-linewidth laser. It also has the requisite sensitivity and dynamic range to measure the absolute intensity of even the weaker lines with high precision.

In practice, the data can be recorded with arbitrary acquisition/integration time, from seconds through minutes, where longer times produce higher precision and sensitivity. Depending on the species, a few minutes data integration is all that is necessary to produce results equivalent to IRMS. The data can then be output in various formats including the normalized delta ratio (δ) that is referenced to a widely accepted standard. In the example of carbon isotopes in carbon dioxide, this is the normalized deviation from a standard value – the internationally accepted Peedee Belemnite (PDB) carbonate standard:

$$\delta^{13}\text{C} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$$

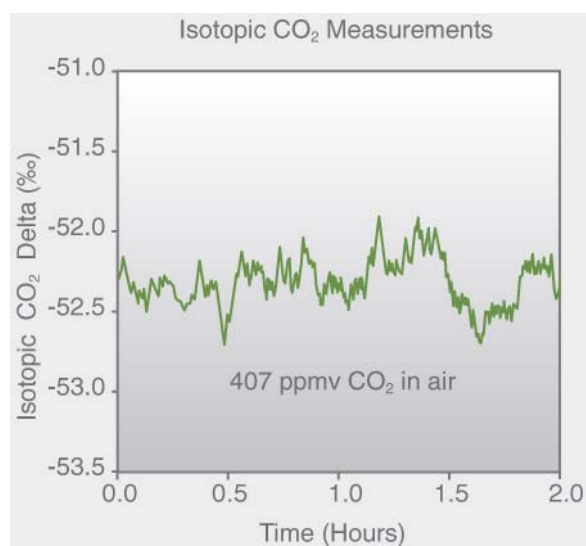
and is expressed in units of per mil (‰), where R is the raw concentration ratio ¹³C/¹²C. Delta ratios for other isotopes such as D are generated based on corresponding widely accepted standards.

Practical WS-CRDS Isotope Instruments for H₂O and CO₂ Vapors

All Picarro WS-CRDS instruments are configured for slow-flowing gas samples – the flow rate can be automatically or arbitrarily set. (The pressure within the WS-CRDS cavity is maintained at a constant value of 140 Torr, by automated control of the instrument's pump and flow valves.) Gas phase samples such as ambient air mixtures containing CO₂

and H₂O vapor are therefore particularly straightforward in terms of sample handling; the input port to the instrument can be connected directly to a sample canister or a gas/vacuum line. Alternately, it can just directly draw on the surrounding air in the case of ambient air monitoring applications such as for field-deployed GHG work. In the case of CO₂, it is not even necessary to dry the samples as there is no crosstalk between any water vapor and the CO₂ measurements.

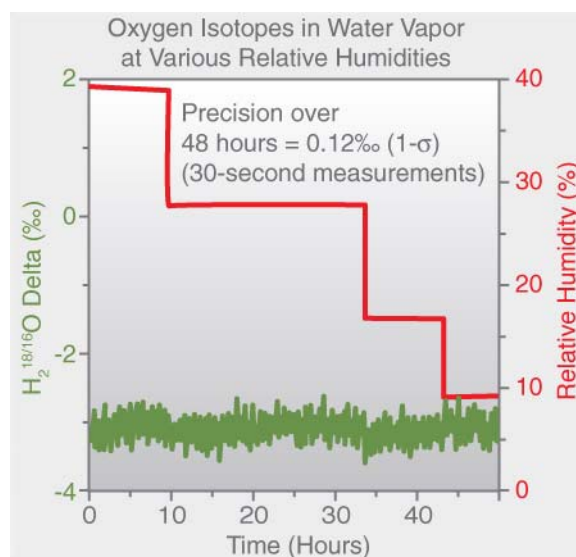
Easily transportable from site to site, Picarro isotopic water vapor analyzers can be set up and running within minutes. The gas concentration and isotope ratio(s) are displayed in real-time with no post-processing required, and are continuously archived to the analyzer's internal hard drive. Rugged and immune to changes in temperature and pressure, the same instrument can be used on a benchtop or remotely operated via its Ethernet connection or optional modem. Users can connect remotely with the analyzer's internal Windows-based PC and control it through a standard Remote Desktop connection or with similar remote login software. The software even includes a valve sequencer which can manually or automatically control up to six external solenoid valves. The data can be accessed in digital or analog format.



Data from a Picarro isotopic CO₂ analyzer measuring the isotopic carbon (¹³C/¹²C) ratio from a constant-concentration (407 ppmv) CO₂ gas stream. The measurement precision is 0.15 per mil (in δ¹³C units) in a 5-minute measurement.

In terms of performance, the Picarro G1101-i CO₂ analyzer can deliver the normalized carbon isotope ratio, δ¹³C from ambient air or pure CO₂ samples with a guaranteed

precision of better than 0.3 per mil (0.03%) in ambient air, with a total sampling time of only 5 minutes. The analyzer also measures the overall CO₂ concentration, from trace (ppbv) to the percent level. This performance is illustrated in the preceding figure which shows actual isotopic carbon (¹³C/¹²C) data from a constant-concentration (407 ppmv) CO₂ gas stream. The measurement precision in this data is 0.15 per mil (in δ¹³C units), which is typical WS-CRDS performance.



Typical data taken with the Picarro G1102-i isotopic water vapor analyzer showing the insensitivity of the isotopic ratio of ¹⁸O/¹⁶O (expressed here in units of δ¹⁸O) to changes in relative humidity.

For water vapor measurements the G1102-i simultaneously analyzes ambient air for both oxygen and hydrogen isotopes ratios, providing simple access to both δ¹⁸O and δD. This instrument is also both fast and precise; in 30 seconds or less it delivers sufficient precision for the majority of water isotope applications, with a guaranteed precision of δ¹⁸O < 0.2 ‰ and δD < 1.0‰, even in remote unattended locations and harsh operating environments. This precision is more than sufficient for the majority of water vapor isotope applications. The figure above shows typical data taken with this analyzer and illustrates the insensitivity of the isotopic ratio of ¹⁸O/¹⁶O (expressed here in units of δ¹⁸O) to changes in relative humidity.

WS-CRDS for Liquid Water

With the possible exception of isotopic measurements of carbon dioxide and its associated use in TOC isotope analysis, no material has more applications for stable isotope studies than liquid water. These applications range

from water cycle studies to analyzing human urine for metabolic studies and even determining geographic history (recent travel). The Picarro L1102-*i* is the first turnkey instrument to directly measure isotope ratios in liquid water samples, in just minutes, with precision better than or equal to IRMS technology. Moreover, with no significant sample preparation and no special operator training, liquid samples can be simultaneously analyzed for both oxygen and hydrogen isotopes ratios, providing simple access to both $\delta^{18}\text{O}$ and δD .

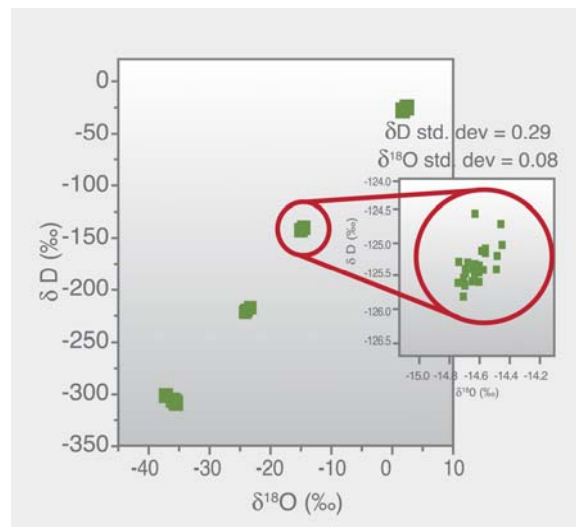
The L1102-*i* is based on the same WS-CRDS used in our gas phase analyzers. In addition, this benchtop analyzer (shown below) is equipped with a robust integrated robotic autosampler and vaporization accessory, which enables many liquid water samples to be vaporized and analyzed in a single automated, hands-free sequence. Moreover, the vaporization enables the WS-CRDS cavity to be loaded with a higher vapor pressure than might be found say in water-in-air samples. This enables an even higher level of sensitivity and precision; typical per mil precision is $\delta^{18}\text{O} < 0.1 \text{ ‰}$, $\delta\text{D} < 0.5 \text{ ‰}$.



*The Picarro L1102-*i* liquid water isotope analyzer is equipped with a robust integrated robotic autosampler and vaporization accessory, which enables many liquid water samples to be vaporized and analyzed in a single automated, hands-free sequence.*

The precision and stability of this approach is illustrated in the following figure. This shows data for samples from

groundwater locations, sequentially measured, with multiple instrument injections of each location. These four groundwater locations provide a range of both $\delta^{18}\text{O}$ and δD values which demonstrates the instrument's ability to transition with minimal memory. The expansion shows the excellent drift-free precision achieved for the multiple repeated injections, even through hours of operation, with no re-calibration whatsoever.



Typical liquid water data showing samples from groundwater locations with multiple injections of each location. The four locations provided a range of both $\delta^{18}\text{O}$ and δD values. The expansion shows the excellent precision achieved for the repeated injections, even through hours of operation.

Limitations of IRMS

IRMS has long been the “gold standard” analysis tool for stable isotope studies, in spite of its considerable practical limitations, because it delivers the requisite precision and because there was no viable competitive technology. Clearly, this equation has now completely changed with the advent of WS-CRDS-based isotope analyzers.

Today, the most widely-used IRMS instruments are of the continuous flow type. Typical performance is in the 0.2 - 0.3 per mil range, depending on the analyte. Where the analyte is a trace gas (CO_2 in air for example) the IRMS input may include pre-concentration and cryo-focusing of the analyte, in addition to the usual GC (gas chromatograph) used for separation. With this rather complex setup, precision as high as 0.05 per mil can sometimes be reached, but at a total hardware cost up to US\$250,000. However, this requires rigorous continuous calibration, where the sample and an isotopic reference gas

are alternately and repeatedly measured. In addition, both the IRMS and GC stage are instruments that require the presence of a skilled operator, as well as consumables. Moreover, because IRMS needs high sample purity, where a GC front-end is not used, the sample has to be carefully purified and often chemically transformed in a high-vacuum gas handling system, which is also operator-intensive. This is particularly true in the case of water. Specifically, water vapor should never be introduced to an IRMS machine as it will corrupt any measurements for a long time afterwards. This is why CO₂ samples, for instance, have to be carefully and thoroughly dehydrated before analysis.

What about analyzing water itself by IRMS? This requires a two-part analysis. The oxygen isotopes are measured by first mixing the sample with isotopically pure CO₂ and allowing them to chemically equilibrate, transferring the oxygen isotope fractionation ratio to the CO₂. The CO₂ is then dried and measured. To measure hydrogen isotopes, the water must be purified and then passed over a hot zinc catalyst to pyrolyze it and thereby produce hydrogen. This hydrogen is then analyzed in an IRMS with its magnetic field and collection cups optimized to discriminate between masses 1, 2 (and 3). This requires a different IRMS setup than the CO₂ analysis where the cups are aligned to collect several masses around mass 46. So in high-throughput labs, two quite separate machines are usually employed for water, doubling the required capital investment.

In contrast, this new generation of WS-CRDS based isotope analyzers have none of these requirements and can perform measurements for an overall per-sample cost that is more than an order of magnitude lower than IRMS. And they can be operated within minutes of unpacking by a complete novice. However, just like an IRMS, a WS-CRDS isotope analyzer can also be directly coupled to the output of sample preparation products such as combustion devices, enabling isotopic analysis of complex, mixed samples by this straightforward technique.

And finally, IRMS instruments simply cannot be field deployed. In contrast, WS-CRDS analyzers are rugged and compact, and several available models offer complete portability and remote operation.

Applications and Opportunities

The advent of compact, turnkey, simple isotope analyzers is completely changing the ease with which stable isotopes are measured and is thereby significantly impacting and expanding the applications space for this type of

measurement. One group of nascent applications is in geographic memory; different locations will have unique isotope ratios in both the available drinking water as well as the natural vegetation foodsources. This already allows zoologists to study the migratory patterns of birds and even large land animals such as elephants. Now this aspect can be investigated for possible use by homeland security as well as drug enforcement. Specifically, the recent travel patterns of suspects potentially can be tracked by the drinking water they have ingested. And the trafficking patterns of illegal drugs that are plant-derived, such as heroin, can likely be mapped back to the point of origin by their isotopic composition.

There are also numerous applications for isotope analysis in the area of environmental science. For example, researchers studying carbon and water cycles can gain valuable information by tracking the oxygen and hydrogen ratios in water as well as the carbon (13/12) ratio in CO₂. Climate scientists already use this data to map historical temperatures using samples from ice cores. Greenhouse gas (GHG) studies represent another field that uses stable isotope data; because every source of CO₂ has a characteristic δ¹³C value, this data can be used to determine the origin of any increases (or decreases) in local GHG concentrations.

An important emerging GHG application is in carbon sequestration. This refers to the collection, liquefaction and deep terrestrial deposition of CO₂ gas, in abandoned oil/gas wells, porous rock layers, and deep saline aquifers. This will require long term monitoring of the geosequestration site for any leaks of CO₂, against a naturally varying (diurnal and seasonal) variation in CO₂ levels. Picarro isotope analyzers are already being used in field studies for this application.

Yet another area is in human metabolic studies. Stable isotopes have been used for some time in an application called hydrometry. Here, a patient is given a very precise dose of isotopically labeled water such as H₂¹⁸O. After several hours to allow for equilibration, a fluid sample is taken from the patient. By accurately measuring the diluted ¹⁸O concentration in this sample, the patient's total body water can be accurately determined. In a more sophisticated technique, doubly labeled water (D₂¹⁸O) is used to study metabolic rates. Specifically, the ¹⁸O can be expelled as water or turned into carbon dioxide by metabolic activity. But the deuterium can only be excreted as water. So measuring the decline in the ¹⁸O/D ratio provides a direct measurement of the patient's metabolic rate.

Conclusion

In conclusion, every so often a new analytical tool such as Fourier Transform InfraRed (FTIR) or the Atomic Force Microscope (AFM) comes along that produces a paradigm shift in the way certain key measurements are made. Although we are still in the early days of WS-CRDS isotope analyzers, we believe this technology is likely to have a similar landmark effect on stable isotope measurement. Indeed, by eliminating the severe practical limitations of existing IRMS technology, WS-CRDS may prove to be the key that finally opens the door to widespread exploitation of the unique information available from stable isotopes across many diverse branches of science.