

$\delta^{18}\text{O}$ analysis of organic solid samples (e.g. bulk plant and fungal tissues, cellulose, sugars, proteins; hair, nails, chitin and other animal tissues) and oils

Oxygen is one of the main constituents of all organic matter and its $\delta^{18}\text{O}$ value can be determined using pyrolysis-IRMS, whereby the organic molecules are disintegrated thermally, i.e. in very high temperatures above 1350 °C. The released oxygen combines with carbon in the reactor to form carbon monoxide, whose isotopic composition is determined with IRMS. Because $\delta^{18}\text{O}$ analysis is performed with a different type of reactor than the analyses of $\delta^{13}\text{C}$ and/or $\delta^{15}\text{N}$ values, these analyses always have to be performed separately, i.e. on a different subsample, from C/N analytics (but see below for exception: $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis of pure α -cellulose).

General considerations

As oxygen is one of the main building blocks of organic materials, the amount of material needed for $\delta^{18}\text{O}$ analysis is usually relatively low. Examples of typical oxygen Wt-% values in sample matrices are listed in the table below for reference. To find out the exact required weighing amounts for your samples, contact us at stableisotopes@helsinki.fi with the expected dry O weight-% values for your samples.

	O wt-%	sample mass, μg
Plant tissue	40-50	180 (90)
Hair, nails, hooves (keratin tissue)	20	350
Plant oils, animal fat	10	500
α -cellulose	47	180 (90)

Oxygen isotope analysis is generally more sensitive to disturbances in analytical conditions, and this often results in a larger standard deviation (i.e. less precise) around the $\delta^{18}\text{O}$ values. On top of the poorer internal analytical precision, additional uncertainty stems from the isotopic variability intrinsic in all natural materials and degree of sample homogenization. Regardless of the sample matrix, we recommend analyzing more than one replicate per sample to get a realistic estimate of the uncertainty for the isotope value. Note that this is a different thing than analytical uncertainty quoted in the analytical report, which only measures the precision of the instrument. If analyzing the whole set of samples in duplicate/triplicate is not feasible, we recommend choosing a representative set of samples, e.g. 10-30% of total sample number, to be analyzed in duplicate or triplicate. However, as prices are charged per analyzed replicate, the decision of replicate IRMS measurements is left up to the customer's discretion.

Sample preparation

The sample is weighed into high purity silver (Ag) 3.3 x 5 mm (w x h) capsules, closed and crimped for IRMS-measurement. Before weighing, solid samples must be completely dried, and usually they are ground into a homogenous powder facilitating weighing of replicates (see below).

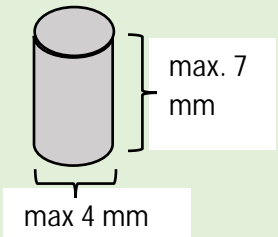

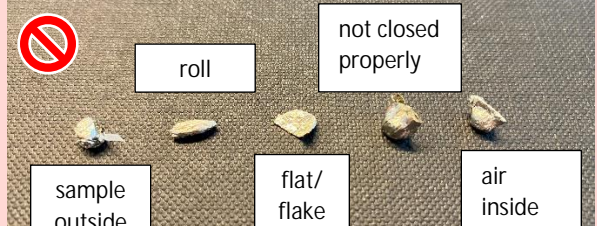
If you're weighing and closing your samples yourself, please see tips for encapsulating samples below, and note that the Wt-% results are dependent on the accuracy of your weighing. You should use a microbalance giving you 3 decimal digits below 1 mg, i.e. 0.001 mg / 1 μg precision. Aim at a constant weight with a precision of $\pm 10\%$ throughout your sample series. For example, aiming at a mean weight of 0.350 mg, you would accept sample weights from 0.315 to 0.385 mg.

Manufacturer	Product	ID	Size (mm, W x H)	Common for sample types
IVA Analysentechnik GmbH & Co. KG	Silver capsule (Ag 99,99)	176.9805.36	3.3 x 5	organic solids, Ag ₃ PO ₄ , oils

Table 1: Silver cups commonly used for O isotope analyses at the Laboratory of Chronology. Corresponding capsules are available from many other manufacturers.

Encapsulating samples:

The aim is to have all the sample material inside, all the air squeezed out, nothing leaking out, and have a shape that is bulky (3D) enough that it will not get stuck and wedged in the narrow spaces left between the moving and stationary parts of the autosampler.

YES	NO
☺ compress, squeeze out air from sample+capsule	☹ squeeze/fold only top part of tin cup closed
☺ make bulky shape: spherical, cubical, cylinder...	☹ folded into a flat/ flake-like/ roll-like shape
☺ fold and crimp in the opening	☹ sample is leaking or protruding out of cup
 <p>After closing and compaction, capsule must fit in a cylindrical shape of max 4 x 7 mm</p>	<p>NOTE THAT IF YOU'RE SAMPLES ARE SOMETHING DESCRIBED ABOVE OR PICTURED BELOW, WE WILL HAVE TO REPACK/ RESHAPE THEM AT AN ADDITIONAL CHARGE.</p>
 <p>sphere cylinder cube</p>	 <p>not closed properly roll sample outside flat/flake air inside</p>

IRMS-analysis and data normalization

The samples in Ag capsules are loaded into a Costech Zero Blank autosampler, which is then sealed and flushed with He to minimize the effects of atmospheric moisture and air blanks on analysis. The samples are pyrolyzed at temperatures > 1350 °C and the isotopic composition of oxygen is measured on a Thermo Scientific Flash IRMS EA coupled to a Thermo Scientific Delta V series isotope ratio mass spectrometer in continuous flow mode. For samples containing nitrogen, which is usually the case for natural organic materials, a longer (1.8 m) IRMS gas chromatography column is used for a full separation of the interfering nitrogen and carbon monoxide peaks (see e.g. Qi et al. 2011).

Alongside samples, each analytical run contains ca. 30% reference materials that are used for calibration (i.e. normalization) of isotope values and for quality control. The isotope values of the samples and QC materials are normalized using the known isotope values of at least *two different* calibration reference materials included in the run. We use several internationally recognized secondary reference materials for this purpose: IAEA-CH3 (cellulose), IAEA-CH6 (sucrose), IAEA-601 (benzoic acid), IAEA-602 (benzoic acid), USGS-42 (hair), USGS-43 (hair), USGS-46 (water), USGS-48 (water), USGS-86 (peanut oil), USGS-87 (corn oil), CBS and KHS (USGS; keratin). The calibration references and the QC materials are chosen to correspond to the analyzed sample matrix as closely as possible. Data normalization is performed using the 'LIMS for Light Stable Isotopes' software developed by Tyler Coplen of the Reston Stable Isotope Laboratory at the US Geological Survey. The long-term analytical precision is $\pm 0.3 \text{ ‰}$.

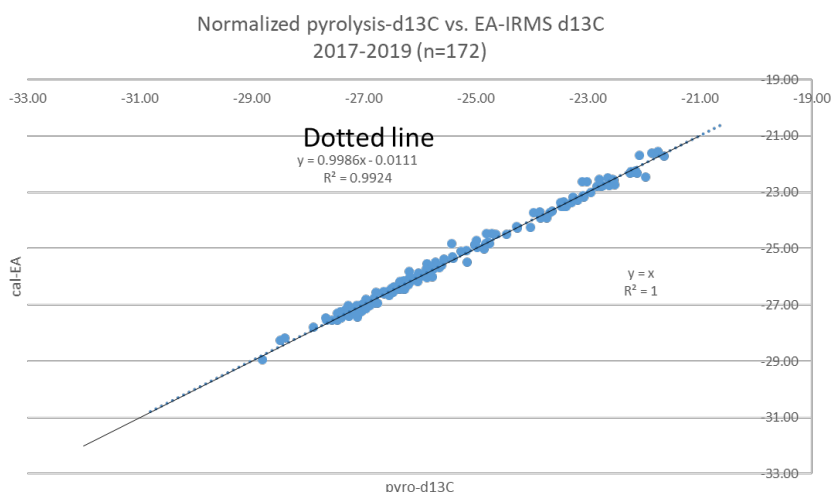
In addition to the requested isotope values, the report of analysis also includes the Weight-% of the element(s) in the samples, a method description and standard deviations of the reference materials to

evaluate internal precision. If the customer wishes to also receive a copy of the uncorrected, 'raw' data, this can be made available upon prior agreement.

Simultaneous $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analysis of α -cellulose

Our laboratory has a long history of isotopic tree-ring research. We have performed several thousands of $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ analyses of α -cellulose extracted from the annual growth rings of scots pine (e.g. Helama et al. 2018; Uusitalo et al. 2022). Traditionally, the carbon and oxygen isotope analyses are done separately using different techniques. However, our laboratory also performs simultaneous analyses of both the $\delta^{13}\text{C}$ and $\delta^{18}\text{O}$ values with *high temperature pyrolysis*, whereby the $\delta^{13}\text{C}$ values are obtained as a "side product" of $\delta^{18}\text{O}$ analysis. The approach is becoming increasingly favored over the more labor intensive separate analysis in most isotope laboratories focusing on tree-ring research, with typical correspondence between pyrolysis- $\delta^{13}\text{C}$ and traditional $\delta^{13}\text{C}$ data (EA-IRMS based) at $R^2 > 0.95$ (Woodley et al. 2012).

The analytical conditions are identical to those applied for single $\delta^{18}\text{O}$ analysis. The $\delta^{13}\text{C}$ values are normalized using the known values of isotope reference materials IAEA-CH3 (-24.72 ‰) and IAEA-CH6 (+10.45 ‰) as the low and high anchor-points, respectively. Accuracy is monitored with replicate analyses of laboratory in-house cellulose materials.



Graph shows the $\delta^{13}\text{C}$ values of samples ($n=172$) measured using both traditional EA-IRMS and pyrolysis-IRMS at the Laboratory of Chronology during years 2017-2019. A high correlation of data is demonstrated with $R^2 > 0.99$.

References:

Helama, S., Arppe, L., Timonen, M., Mielikäinen, K., & Oinonen, M. 2018. A 7.5 ka chronology of stable carbon isotopes from tree rings with implications for their use in palaeo-cloud reconstruction. *Global and Planetary Change* 170 20-33.

Qi H., Coplen T.B., Wassenaar L.I., 2011. Improved online $\delta^{18}\text{O}$ measurements of nitrogen and sulfur-bearing organic materials and a proposed analytical protocol. *Rapid Communications in Mass Spectrometry* 25, 2049-2058.

Uusitalo, J., Arppe, L., Helama, S., Mizohata, K., Mielikäinen, K., Mäkinen, H., Nöjd, P., Timonen, M. & Oinonen, M., 2022. From lakes to ratios: ^{14}C measurement process of the Finnish tree-ring research consortium. *Nuclear Instruments and Methods in Physics Research, Section B: Beam Interactions with Materials and Atoms* 519, 37-45

Woodley E., Loader N.J., McCarroll D. et al. 2012. High-temperature pyrolysis/gas chromatography/isotope ratio mass spectrometry: simultaneous measurement of the stable isotopes of oxygen and carbon in cellulose. *Rapid Communications in Mass Spectrometry* 26, 109-114.