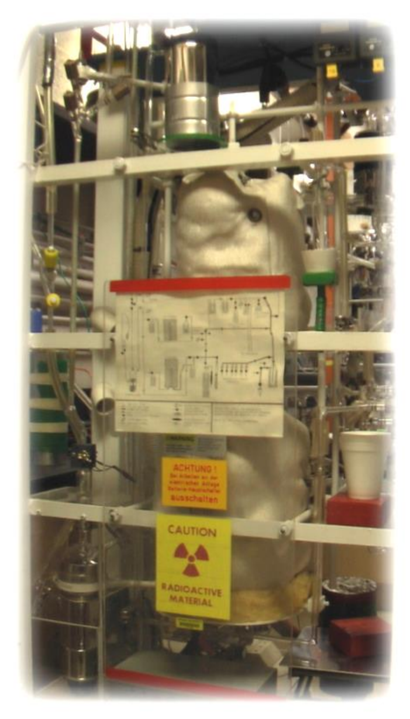
INDIANA UNIVERSITY
BLOOMINGTON

LESSONS LEARNED FROM THE DEVELOPMENT OF ORGANIC STABLE ISOTOPE REFERENCE MATERIALS



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in collaboration with many international colleagues

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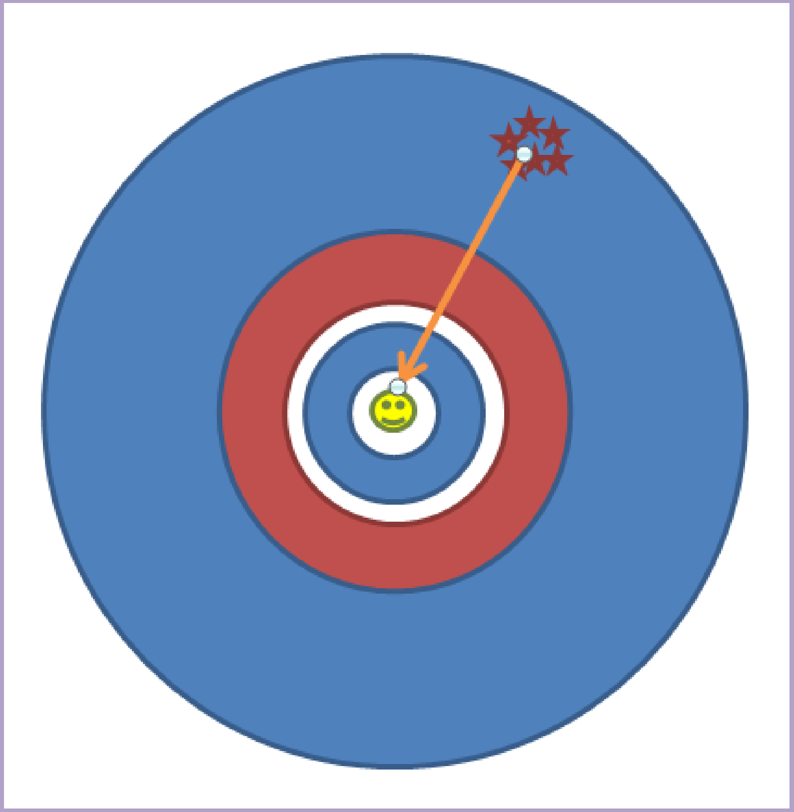
Slovenia, December 2016

Why stable isotope reference materials (RMs)?

- Any isotope laboratory worldwide should be able to measure the same sample and obtain the same δ-value within analytical uncertainty.
- Measured isotopic data with high precision but low accuracy need to be **normalized**.
- **2-point normalization using two RMs** with contrasting isotopic compositions are needed for accurate isotopic measurements. Hence, suitable **RMs should ideally be available as pairs**.



analytical chemistry

Article

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Organic Reference Materials for Hydrogen, Carbon, and Nitrogen Stable Isotope-Ratio Measurements: Caffeines, *n*-Alkanes, Fatty Acid Methyl Esters, Glycines, L-Valines, Polyethylenes, and Oils

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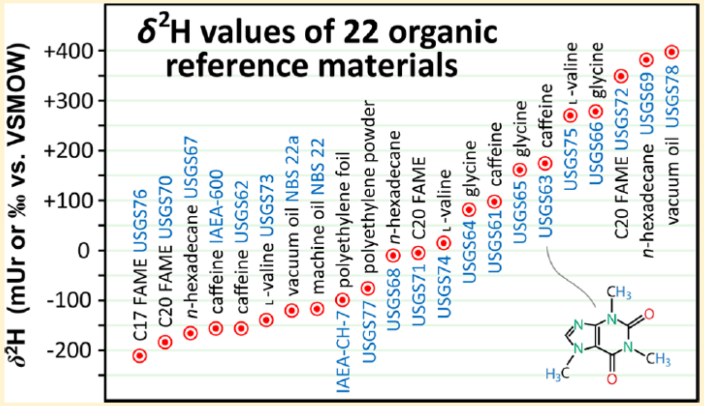
ABSTRACT: An international project developed, quality-tested, and determined isotope−δ values of 19 new organic reference materials (RMs) for hydrogen, carbon, and nitrogen stable isotope-ratio measurements, in addition to analyzing pre-existing RMs NBS 22 (oil), IAEA-CH-7 (polyethylene foil), and IAEA-600 (caffeine). These new RMs enable users to normalize measurements of samples to isotope−δ scales. The RMs span a range of $\delta^2H_{VSMOW\text{-}SLAP}$ values from −210.8 to +397.0 mUr or ‰, for $\delta^{13}C_{VPDB\text{-}LSVEC}$ from −40.81 to +0.49 mUr and for $\delta^{15}N_{Air}$ from −5.21 to +61.53 mUr. Many of the new RMs are amenable to gas and liquid chromatography. The RMs include triads of isotopically contrasting caffeines, C_{16} *n*-alkanes, *n*-C_{20}-fatty acid

continued...



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Lessons #1 and #2:
Haste is waste.
Always work in a team.

Choose raw materials wisely to approach ideal qualities of reference materials:

- high purity (important for GC and LC)
- homogeneity of solid compounds
- raw material available at low price
- chemical stability
- low vapor pressure
- suitable for storage at room temperature in air
- lack of toxicity, flammability
- no exchangeable hydrogen
- non-hygroscopic
- no electrostatic problems when weighing
- legality and ease of international distribution

Adequate funding and patience are prerequisites for successful RM development

Original
idea
2009

1 Mar 2011

28 Feb 2014

March 2016

Proposed to
NIST 2010

NSF Grant EAR-1052927

This project would not have been possible without the dedicated support of ***Dr. Marilyn Fogel***
who served as Program Director at NSF.

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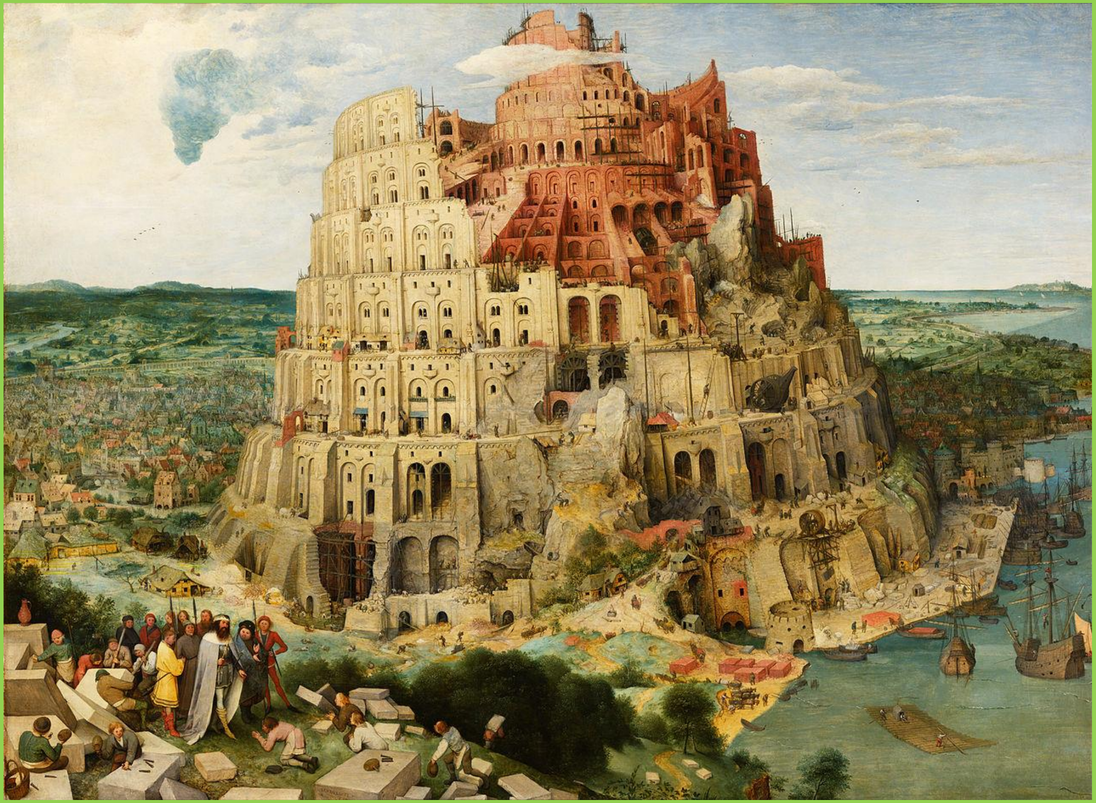


Lesson #3:

Plan big to satisfy long-term future demand

Lesson #4:
Victory relies on a variety of approaches

Overview on relevant analytical IRMS capabilities of expert laboratories.
*available, but probably not needed; **water analyses only.

Laboratory	*Analytical IRMS capability to be used for this proposed study*				
	Off-line	Oxid. GC	Reduct. GC	Oxid. EA	Reduct. EA
Indiana University (PI's lab)	Yes	Yes	Yes	Yes	Yes
USGS, USA (Coplen)	Yes*	Yes*	Yes*	Yes	Yes
Caltech, USA (Sessions)	No	Yes	Yes	Yes	No
IAEA, Austria (Gröning)	No	No	No	No	Yes**
MPI, Germany (Brand)	No	No	No	Yes	Yes
UFZ, Germany (Gehre)	No	Yes	Yes	Yes	Yes
JAMSTEC, Japan (Chikaraishi)	No	Yes	Yes	Yes	No
RUG, Netherlands (Meijer)	No	No	No	Yes	Yes
ETHZ, Switzerland (Werner)	No	No	No	Yes	Yes
SCRI, UK, (Meier-Augenstein)	No	Yes	Yes	Yes	Yes

Caffeine

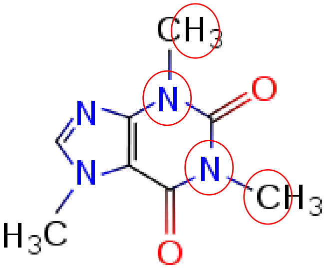
We tested and measured more than 10 batches from different suppliers. We finally chose two suppliers of the bulk raw materials plus several isotopically spiked caffeines from various suppliers, for example:

- Caffeine, 99.92 %, Alfa-Aesar δ^{2}H +96.6 ‰ δ^{13}C -35.05 ‰ δ^{15}N -2.87‰
- Caffeine, *Coffein Compagnie Bremen* δ^{2}H -172 ‰ δ^{13}C -27 ‰ δ^{15}N +1 ‰
- Caffeine 3-methyl-^{13}C, 99 atom % ^{13}C, Cambridge Isotope Laboratories, Inc.
- Caffeine 1,3-$^{15}N_2$, 99 atom % ^{15}N, Cambridge Isotope Laboratories, Inc.
- Caffeine-d3 (1-methyl-d3), 99.8 atom % ^{2}H, ICON

USGS61 caffeine	δ^{2}H +96.6 ‰	δ^{13}C -35.05 ‰	δ^{15}N -2.87 ‰
USGS62 caffeine	δ^{2}H -156.1 ‰	δ^{13}C -14.79 ‰	δ^{15}N +20.17‰
USGS63 caffeine	δ^{2}H +174.5 ‰	δ^{13}C -1.17 ‰	δ^{15}N +37.83 ‰

$C_8H_{10}N_4O_2$

odorless solid for GC, LC and EA applications.

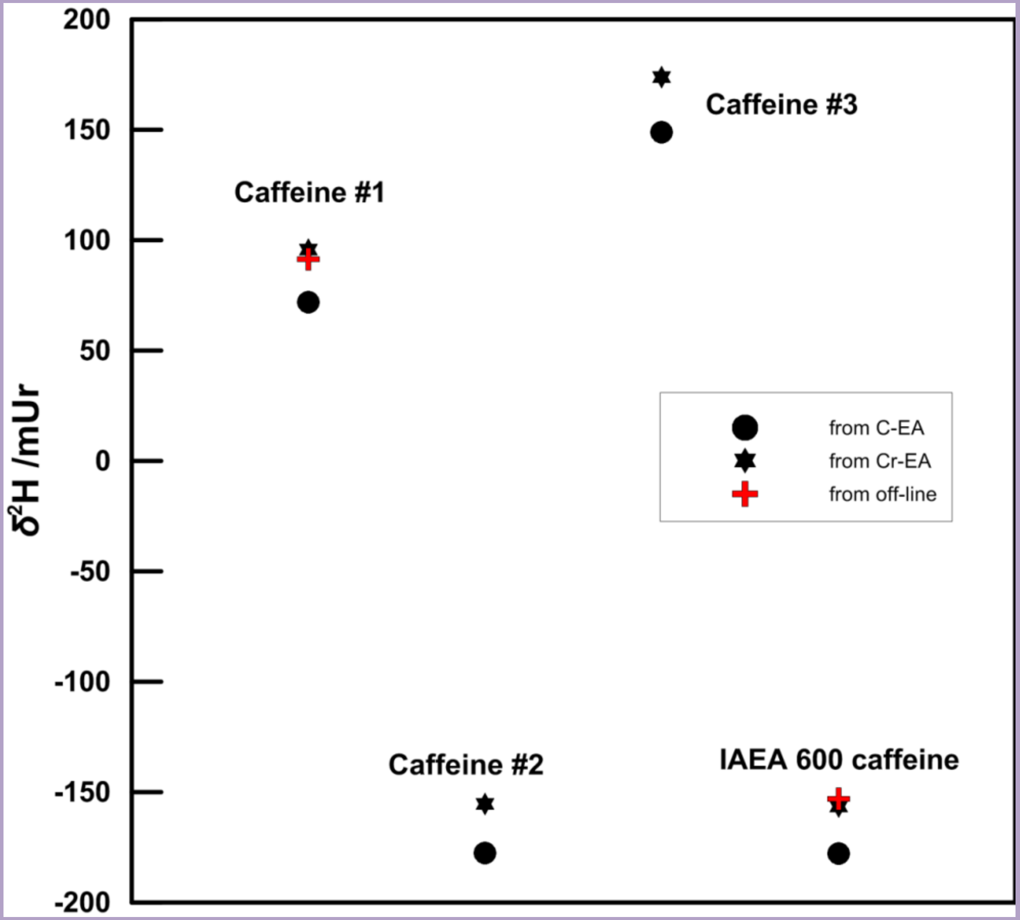


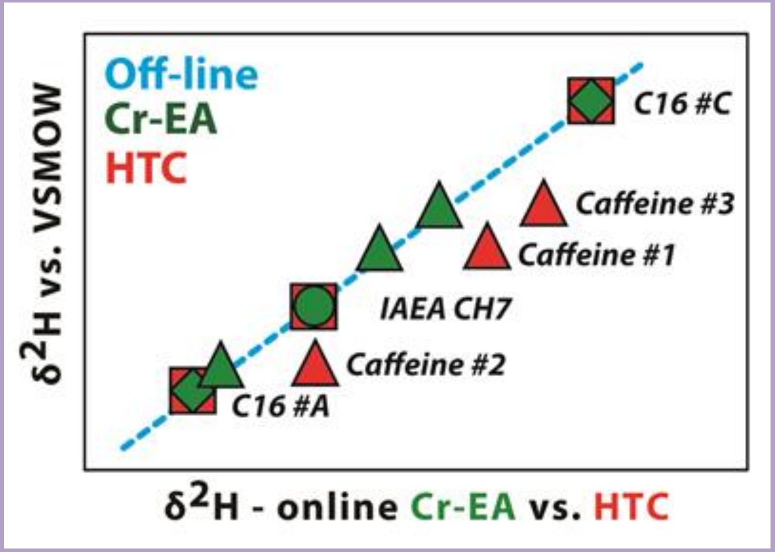
Distribution:

500 mg in sealed glass vial.

Complication: Fractionation *via* incomplete conversion

Measuring δ^2H values of organic materials—production of HCN in addition to H_2





Gehre et al., 2015. On-line hydrogen-isotope measurements of organic samples using elemental chromium—an extension for high temperature elemental-analyzer techniques. *Anal. Chem.* 87, 5198-5205.

Lesson #5:

Homogeneity of solids is at the end of the rainbow

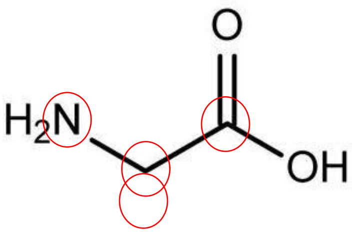
Glycine

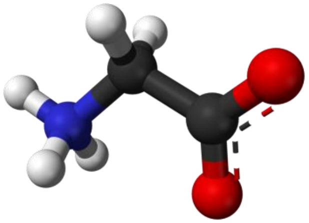
- Glycine, 99+ %,10 kg, (δ^{2}H ~ +275 ‰) δ^{13}C -40.81 ‰ δ^{15}N +1.76 ‰
- Isotopically spiked glycines with ^{13}C-enrichments at molecular sites 1 or 2. Our products employ spike ratios 1:2 in medium-enriched glycine and 2:1 in highly-enriched glycine for future use in site-specific carbon isotopic measurements. We also used 2,2-$^{2}H_2$ and ^{15}N-spiked glycines.

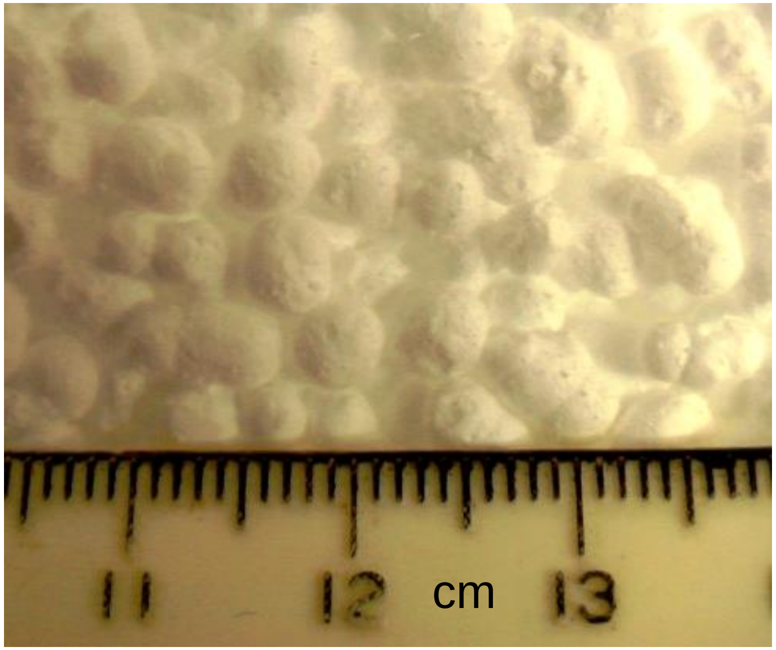
USGS64 glycine	(δ^{2}H ~ +275 ‰)	δ^{13}C -40.81 ‰	δ^{15}N +1.76 ‰
USGS65 glycine	(δ^{2}H ~ +400 ‰)	δ^{13}C -20.29 ‰	δ^{15}N +20.68 ‰
USGS66 glycine	(δ^{2}H ~ +600 ‰)	δ^{13}C ~ -0.67 ‰	δ^{15}N +40.83 ‰

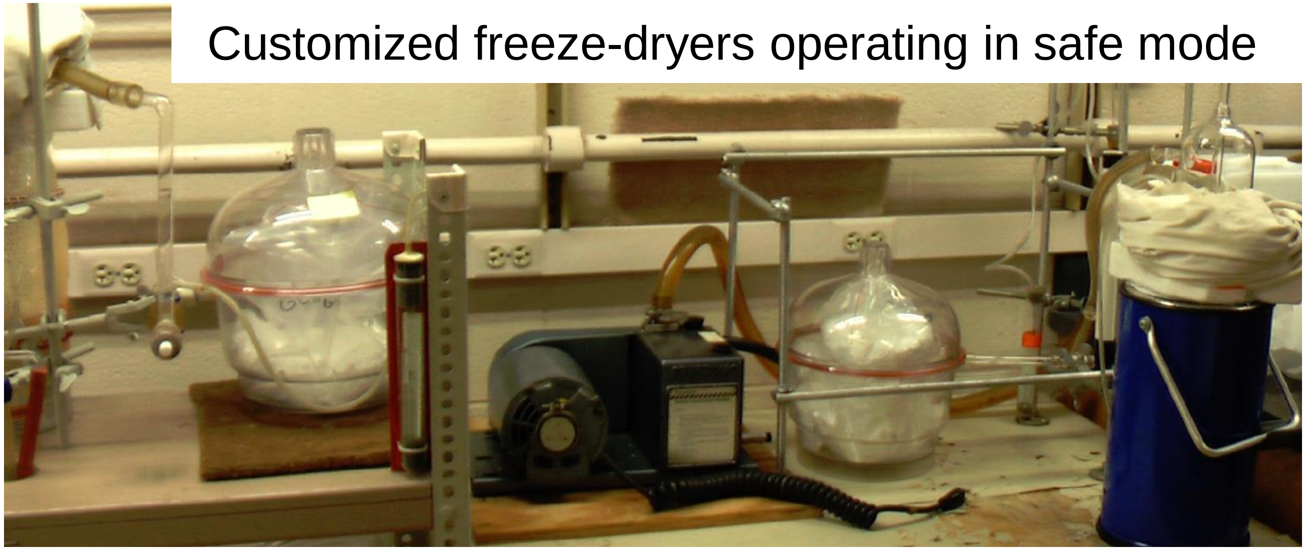
$C_2H_5NO_2$
odorless solid for GC, LC and EA applications.

Distribution:
0.5 g of powder in glass vial.



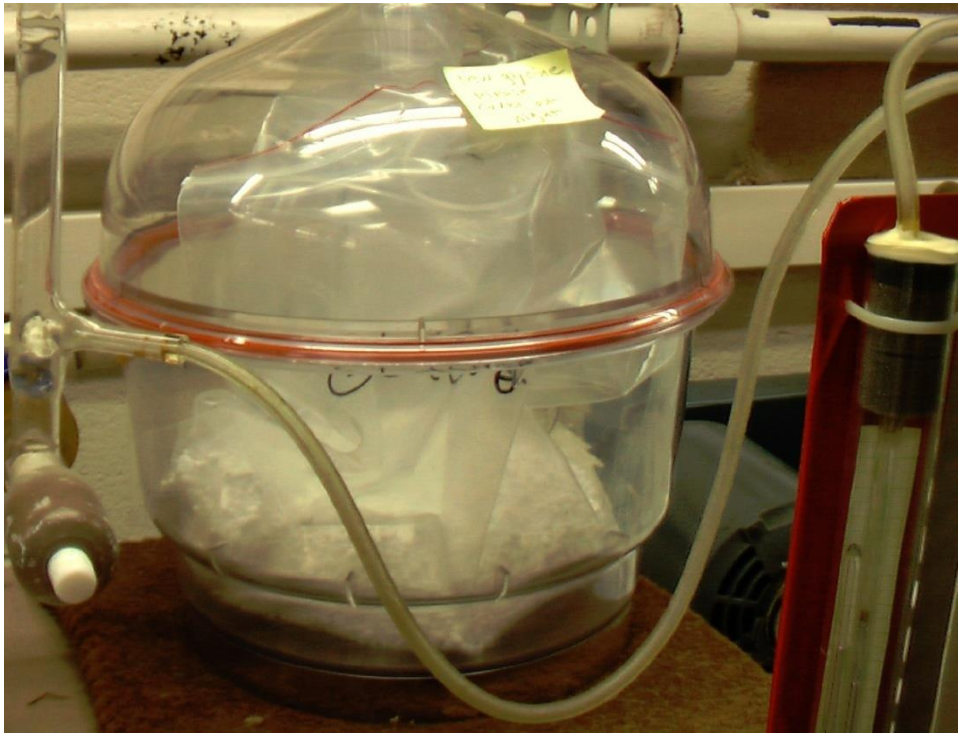






Customized freeze-dryers operating in safe mode

Dripping glycine solution



Lesson #6:

Work and store cool, because heat kills purity

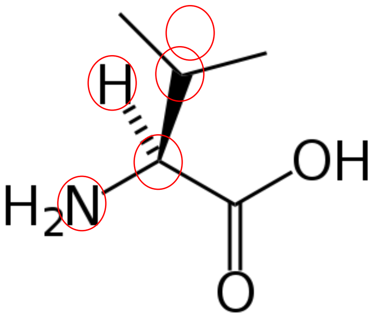
L-valine

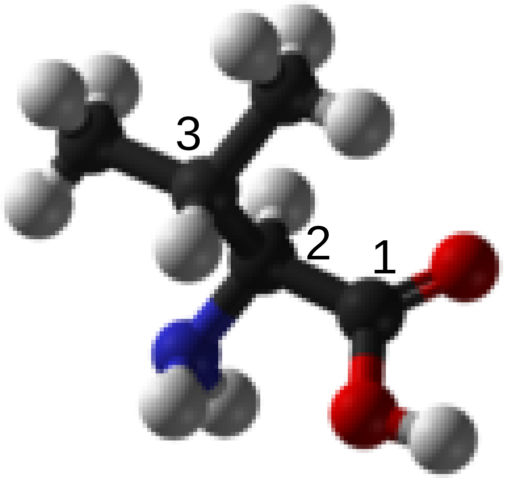
- L-valine #1, 99 %, 5 kg, δ^{13}C ~ -10 ‰ δ^{15}N ~ -5 ‰
- L-valine #2, 3 kg, *Amino GmbH*, δ^{13}C -24.03 ‰ δ^{15}N -5.21 ‰
- Site-specific ^{2}H- and ^{13}C-enrichments at different molecular sites 1, 2 and 3 are currently not measurable with high precision, but were incorporated in prospective RMs for future use in site-specific carbon and hydrogen isotopic characterization: L-valine #2: Spike ratio 2:1 for H-molecular positions 3 and 2; spike ratio 2:1 for C-molecular positions 2 and 1. L-valine #3: Spike ratio 1:2 for H-molecular positions 3 and 2; spike ratio 1:2 for C-molecular positions 2 and 1.

USGS73 L-valine	(δ^{2}H ~ 0 ‰)	δ^{13}C -24.03 ‰	δ^{15}N -5.21 ‰
USGS74 L-valine	(δ^{2}H ~ +200 ‰)	δ^{13}C -9.30 ‰	δ^{15}N +30.19 ‰
USGS75 L-valine	(δ^{2}H ~ +500 ‰)	δ^{13}C +0.49 ‰	δ^{15}N +61.53 ‰

$C_2H_5NO_2$
odorless solid for GC, LC and EA applications.

Distribution:
0.5 g of powder in glass vial.

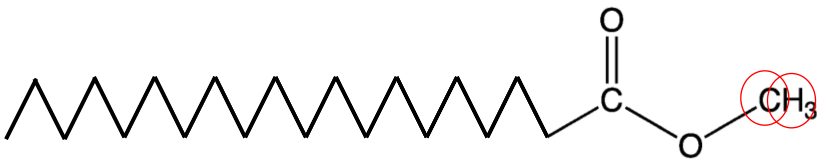




C_{20} fatty acid methyl ester

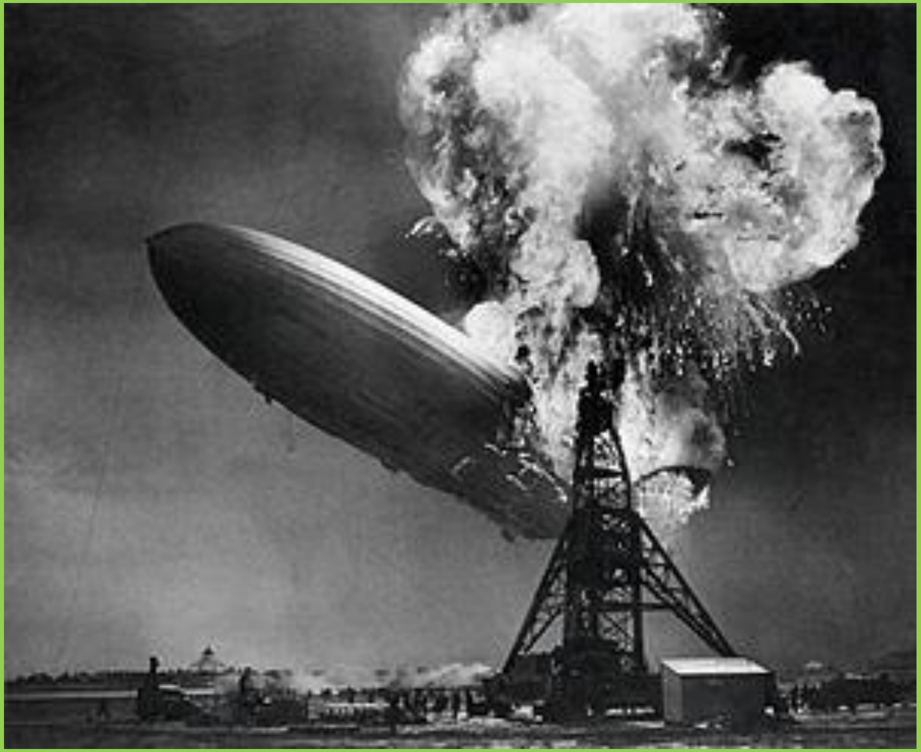
- Arachidic acid methyl ester, C_{20} fatty acid methyl ester, 2 kg, 99.9% custom-synthesized, δ^{2}H -183.9 ‰ δ^{13}C -30.53 ‰
- Isotopically $^{2}H_{1}$ and ^{13}C-spiked methanols for transesterification

USGS70 C20 *n*-alkanoic acid methyl ester	δ^{2}H -183.9 ‰	δ^{13}C -30.53 ‰
USGS71 C20 *n*-alkanoic acid methyl ester	δ^{2}H -4.9 ‰	δ^{13}C -10.50 ‰
USGS72 C20 *n*-alkanoic acid methyl ester	δ^{2}H +348.3 ‰	δ^{13}C -1.54 ‰



$C_{21}H_{42}O_{2}$
odorless solid for GC and EA applications.

Distribution:
100 mg of minibeads in glass vial.

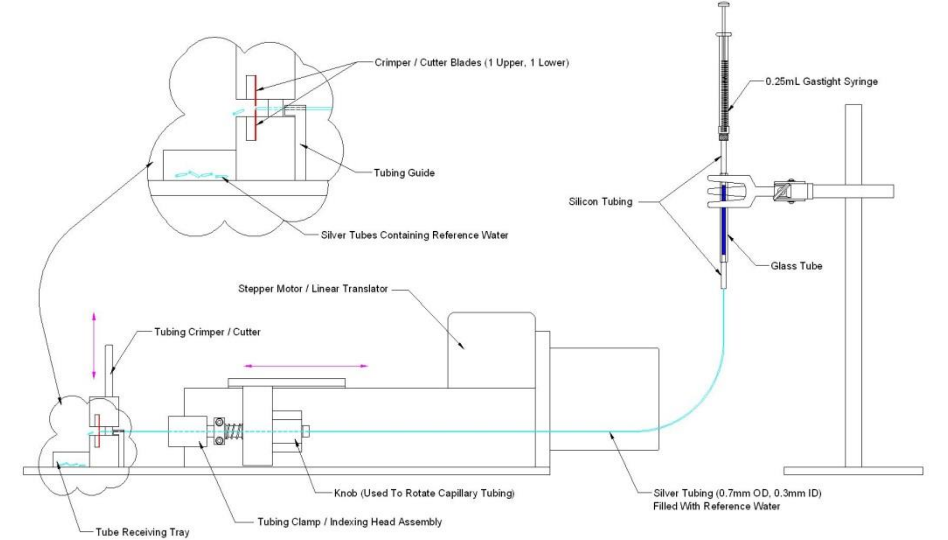


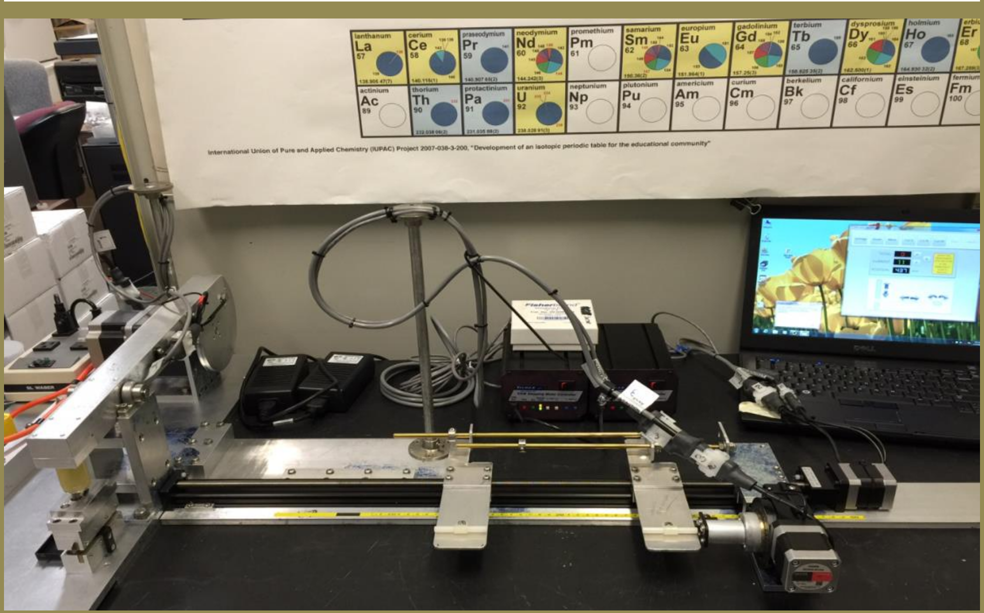
Lesson #7:
Always follow cautionary principles

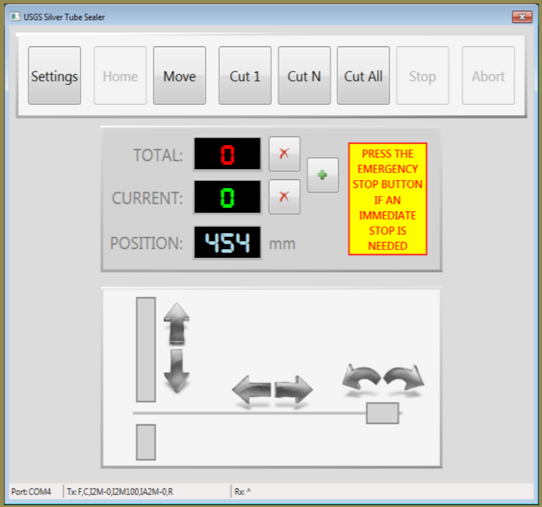
The Principle of Identical Treatment of unknown sample and standard

One fundamental rule in isotope-measurement science mandates that the analyte should be chemically and isotopically similar to the isotope standards that are used for calibration. Werner and Brand (2001) coined the expression ‘**principle of identical treatment**’ of unknowns and isotopic reference materials.

Silver tube method for sealing of liquids





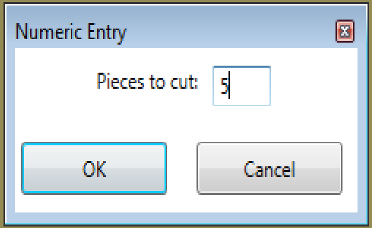


TOTAL: 0

CURRENT: 0

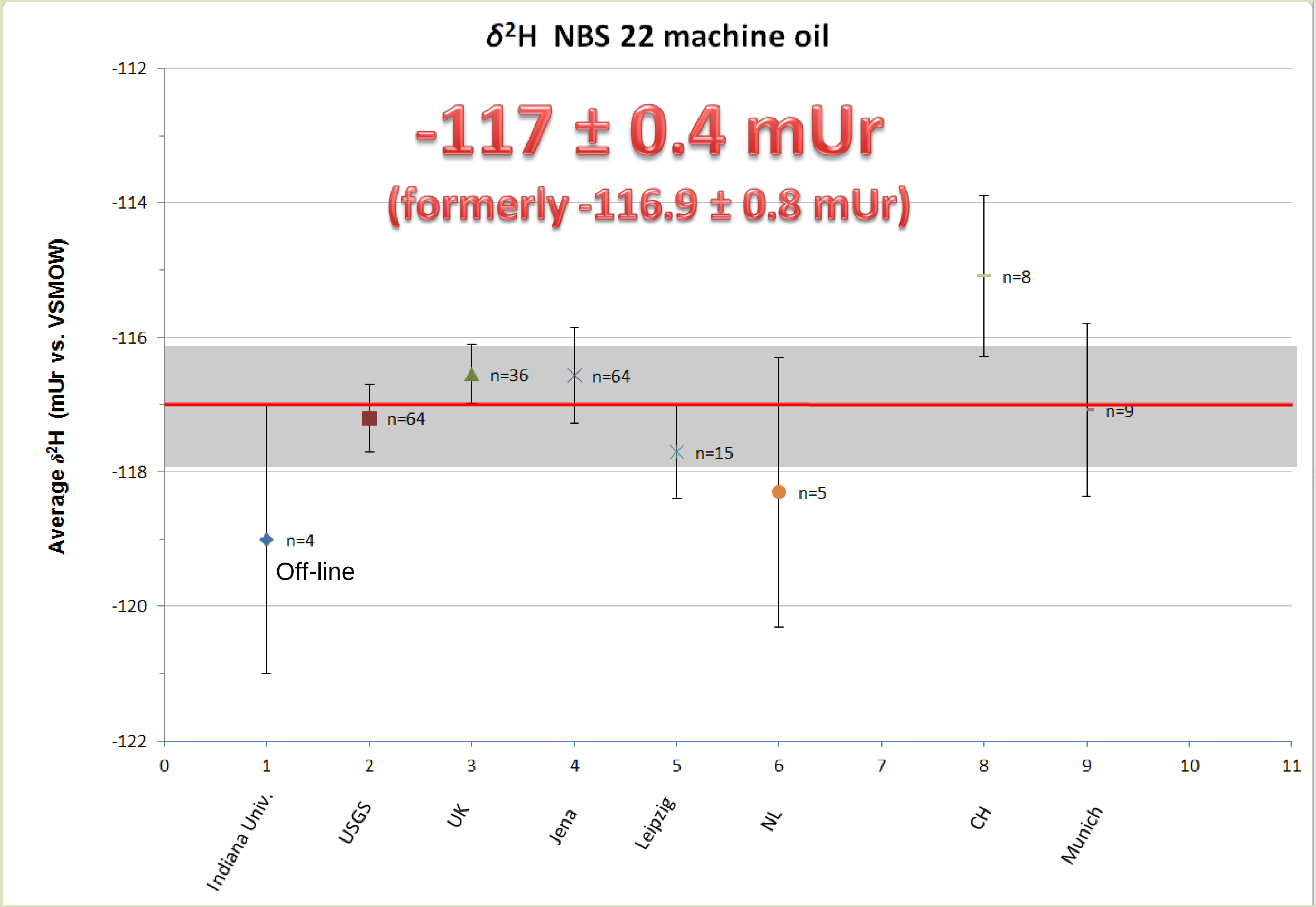
POSITION: 454 mm

Port: COM4 | Tx: F,C,I2M-0,I2M100,IA2M-0,R | Rx: ^



Pieces to cut: 5

Silver tube method enables adherence to IT-Principle in EA-HTC-IRMS



-117 ± 0.4 mUr

(formerly -116.9 ± 0.8 mUr)

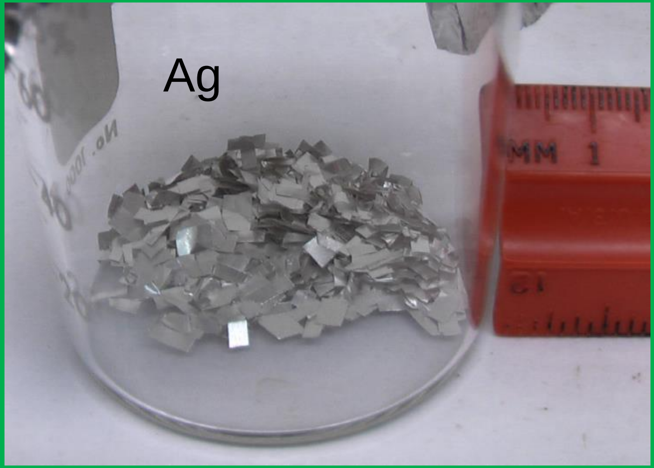
Lesson #8:

No single analytical method is infallible

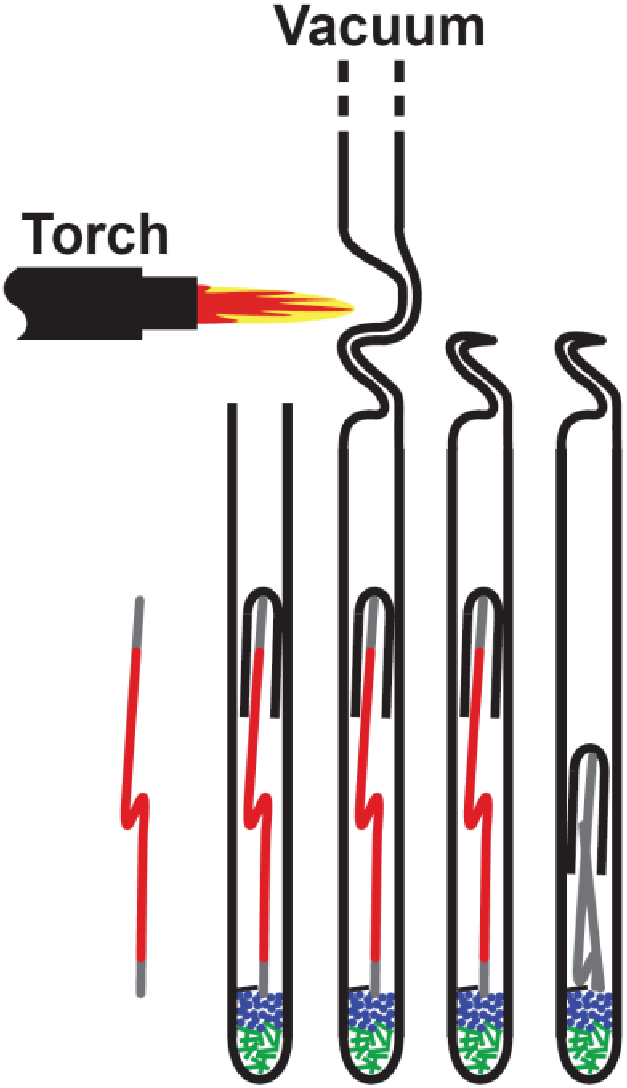
Preparation of **clean glassware and reagents** for processing and storage of reference materials:

Annealing of quartz and Pyrex® tubes, ampoules, and vials at 500°C burns off all organic contaminants.

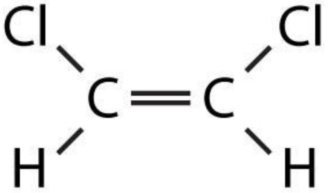
Copper(II)oxide, copper granules and silver are also annealed prior to their use in quartz combustion ampoules.

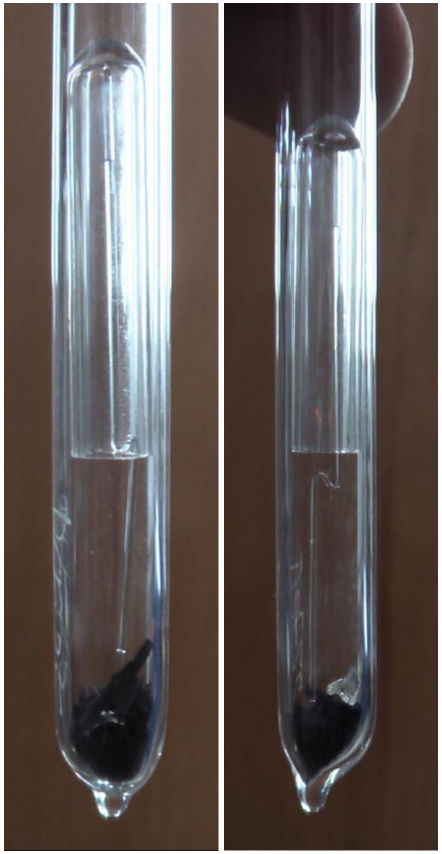


Liquids are first sealed in quartz capillaries with a Z-bend. Multiple 2 to 5 mg **aliquots of prospective reference material are loaded** in 9 mm o.d. quartz combustion ampoules, together with CuO, Cu and Ag.



This example shows cis-dichloroethylene in sealed capillaries.

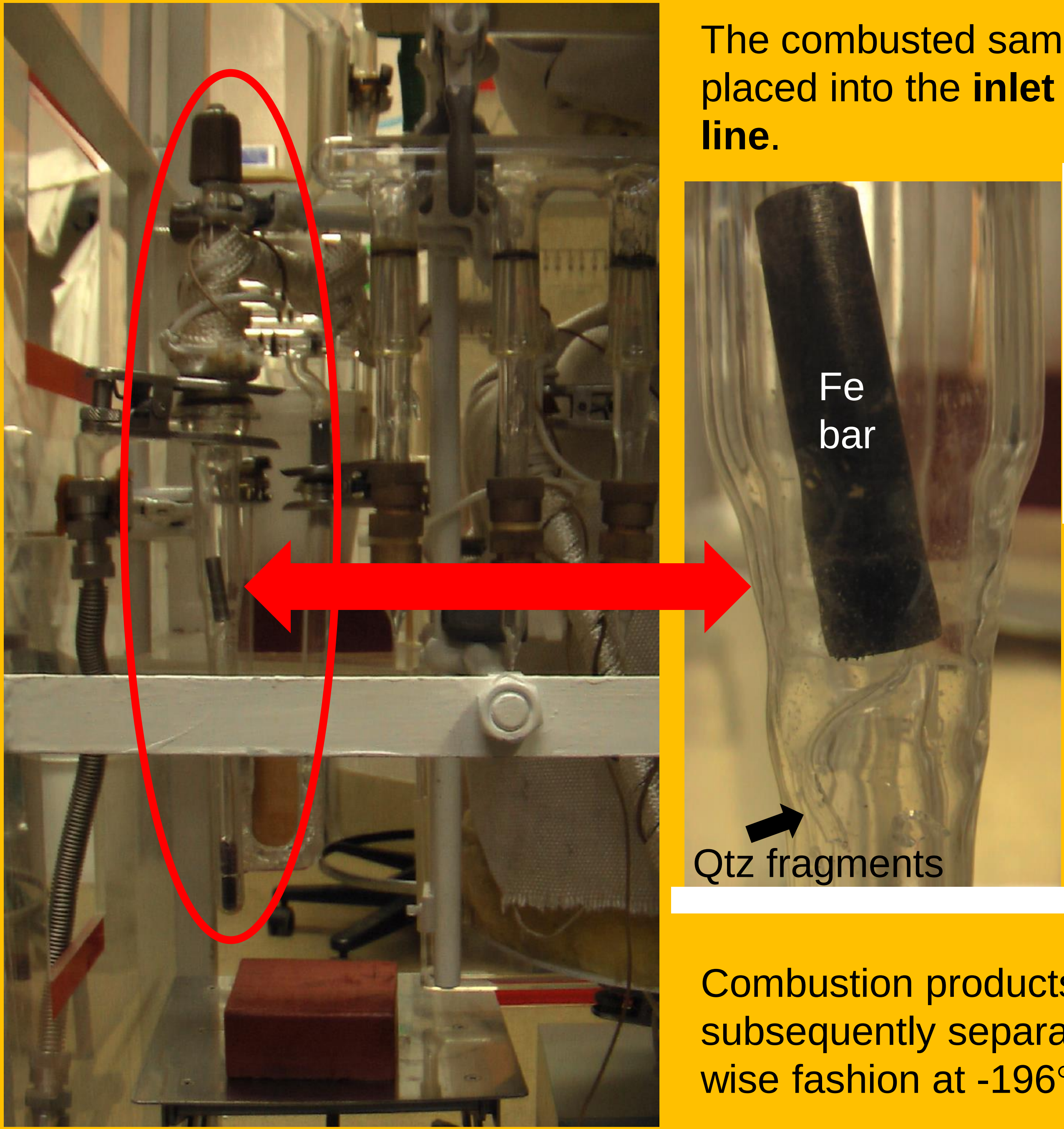


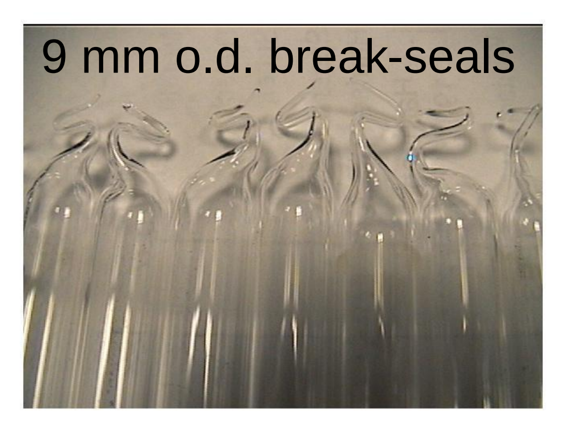


unbroken

broken

The combusted sample (still at ca. 200°C) is placed into the **inlet system of a vacuum line**.



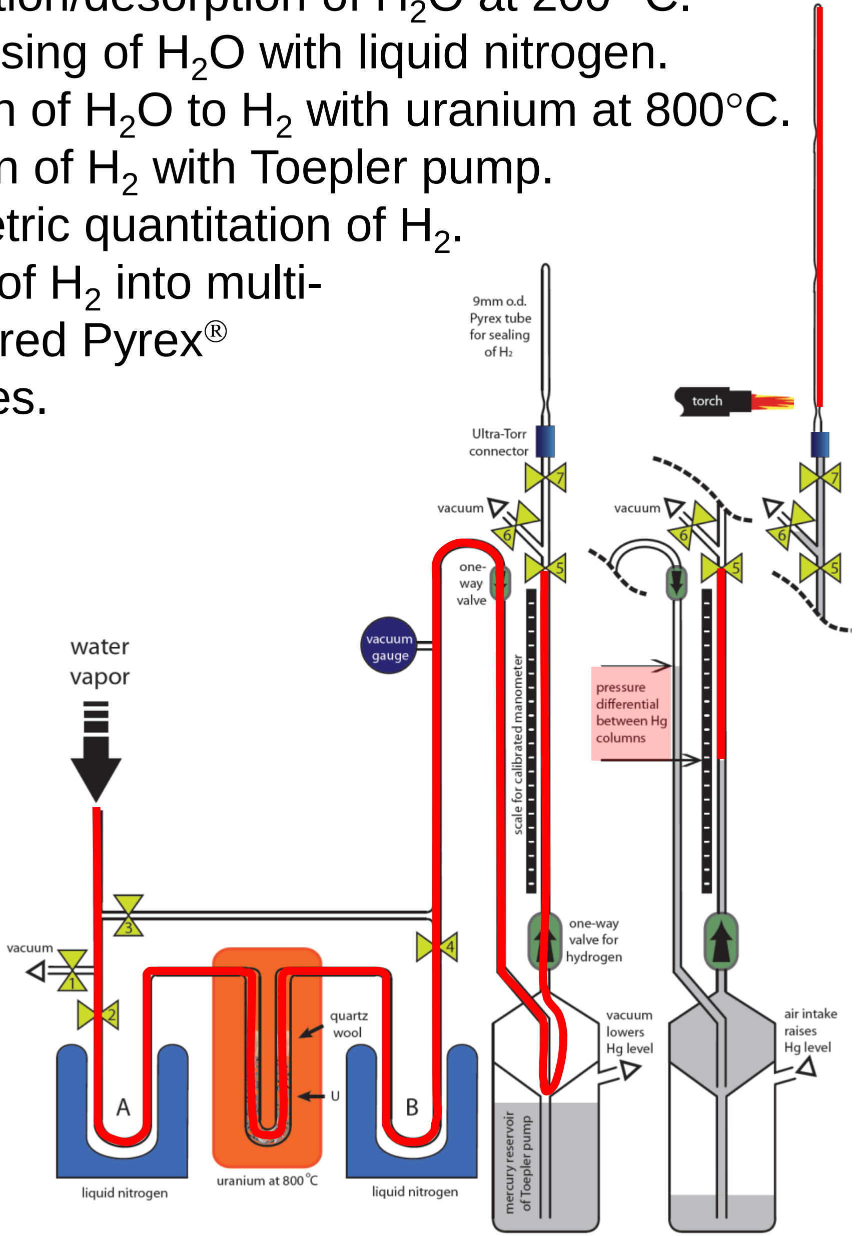


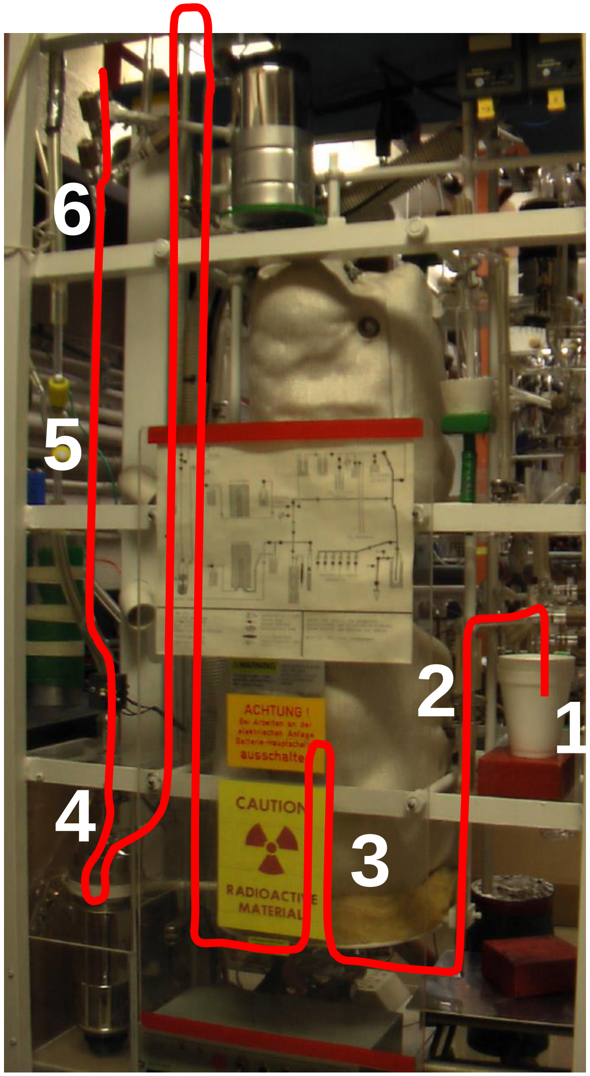
The break-seal is opened by a magnetically operated iron bar, releasing N_2, CO_2, and H_2O into vacuum. Cooling with liquid nitrogen freezes all gases except N_2.

Combustion products N_2, CO_2, and H_2O are subsequently separated/mobilized in a step-wise fashion at -196°C, -80°C, and +200°C.

Reduction of H_2O and collection of incondensible H_2 with a Toepler pump:

(1) evaporation/desorption of H_2O at 200 °C.
(2) cryofocusing of H_2O with liquid nitrogen.
(3) reduction of H_2O to H_2 with uranium at 800°C.
(4) collection of H_2 with Toepler pump.
(5) manometric quantitation of H_2.
(6) transfer of H_2 into multi-chambered Pyrex® ampoules.





Lesson #9:

Store RMs with utmost care in more than one location

Fisher Scientific Maxima C Plus ***Vacuum Pump Oil***. This oil is a triple-distilled hydrocarbon fraction that has been hydrogenated to reduce the abundance of aromatic components and to increase chemical stability. The vapor pressure at 25°C is 0.000133 Pa (or 1 x 10^{-6} Torr), the viscosity at 40°C is 65 CentiStokes (cSt) (305 SUS), and the specific gravity is 0.78 g/cm^3.

NBS 22a vacuum pump oil δ^2H -120.4 ‰ $\delta^{13}C$~ -29.72 ‰

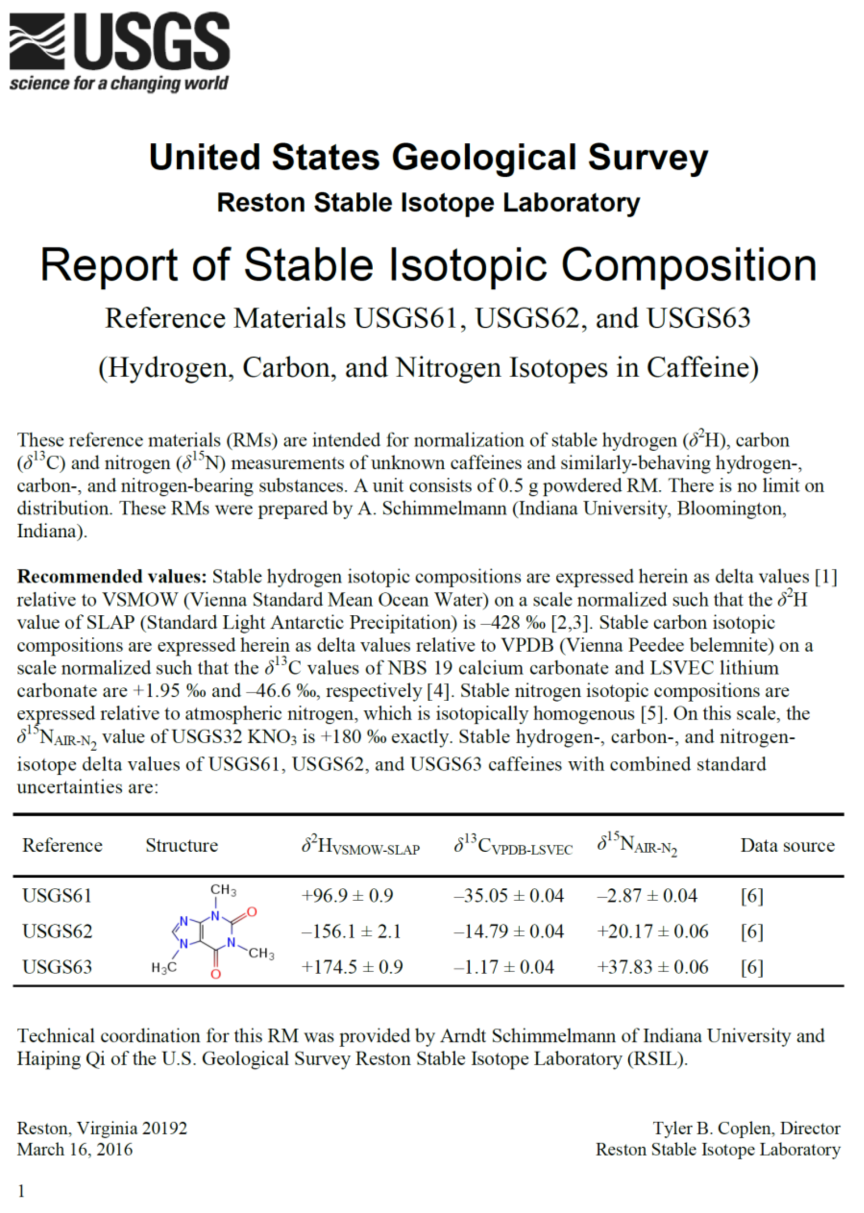
USGS78 vacuum pump oil δ^2H +397.0 ‰ $\delta^{13}C$ ~ -29.72 ‰

^2H-enriched with perdeuterated *n*-tetracosane – d_{50}, $C_{24}{}^2H_{50}$, MSD Isotopes


C-14 tetradecane $C_{14}H_{30}$
δD = -71.7 ± 1.4‰
$\delta^{13}C$ = -30.69 ± 0.03‰
C-21 heneicosane #2 $C_{21}H_{44}$
δD = -177.8 ± 1.5‰
$\delta^{13}C$ = -28.83 ± 0.02‰


n-alkane mixt
solution in he

Lesson #10: Publicize and facilitate international distribution of RMs



USGS
science for a changing world

United States Geological Survey

Reston Stable Isotope Laboratory

Report of Stable Isotopic Composition

Reference Materials USGS61, USGS62, and USGS63

(Hydrogen, Carbon, and Nitrogen Isotopes in Caffeine)

These reference materials (RMs) are intended for normalization of stable hydrogen (δ^{2}H), carbon (δ^{13}C) and nitrogen (δ^{15}N) measurements of unknown caffeines and similarly-behaving hydrogen-, carbon-, and nitrogen-bearing substances. A unit consists of 0.5 g powdered RM. There is no limit on distribution. These RMs were prepared by A. Schimmelmann (Indiana University, Bloomington, Indiana).

Recommended values: Stable hydrogen isotopic compositions are expressed herein as delta values [1] relative to VSMOW (Vienna Standard Mean Ocean Water) on a scale normalized such that the δ^{2}H value of SLAP (Standard Light Antarctic Precipitation) is –428 ‰ [2,3]. Stable carbon isotopic compositions are expressed herein as delta values relative to VPDB (Vienna Peedee belemnite) on a scale normalized such that the δ^{13}C values of NBS 19 calcium carbonate and LSVEC lithium carbonate are +1.95 ‰ and –46.6 ‰, respectively [4]. Stable nitrogen isotopic compositions are expressed relative to atmospheric nitrogen, which is isotopically homogenous [5]. On this scale, the $\delta^{15}\mathrm{N}_{\mathrm{AIR\text{-}N_2}}$ value of USGS32 KNO_3 is +180 ‰ exactly. Stable hydrogen-, carbon-, and nitrogen-isotope delta values of USGS61, USGS62, and USGS63 caffeines with combined standard uncertainties are:

Reference	Structure	$\delta^{2}\mathrm{H}_{\mathrm{VSMOW\text{-}SLAP}}$	$\delta^{13}\mathrm{C}_{\mathrm{VPDB\text{-}LSVEC}}$	$\delta^{15}\mathrm{N}_{\mathrm{AIR\text{-}N_2}}$	Data source
USGS61		+96.9 ± 0.9	–35.05 ± 0.04	–2.87 ± 0.04	[6]
USGS62		–156.1 ± 2.1	–14.79 ± 0.04	+20.17 ± 0.06	[6]
USGS63		+174.5 ± 0.9	–1.17 ± 0.04	+37.83 ± 0.06	[6]

Technical coordination for this RM was provided by Arndt Schimmelmann of Indiana University and Haiping Qi of the U.S. Geological Survey Reston Stable Isotope Laboratory (RSIL).

Reston, Virginia 20192
March 16, 2016

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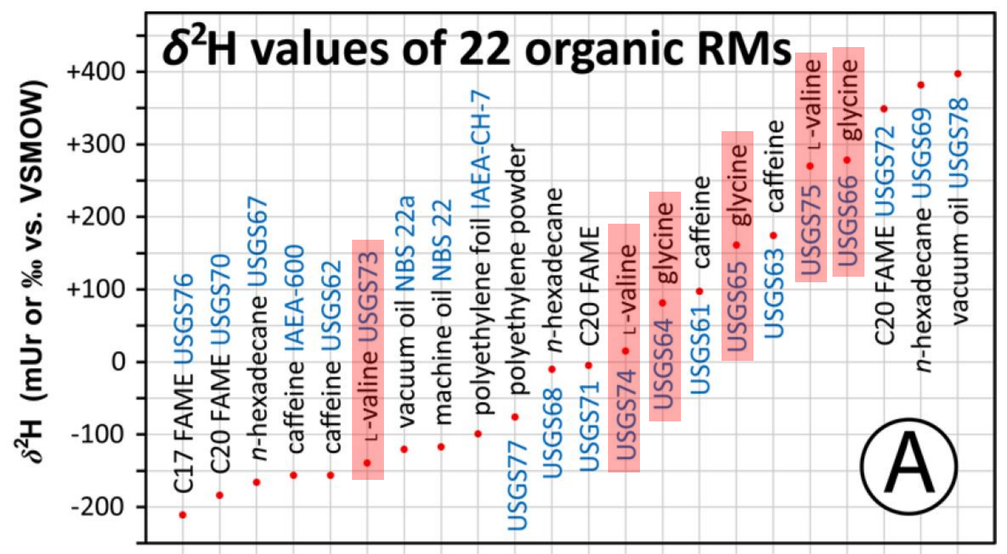
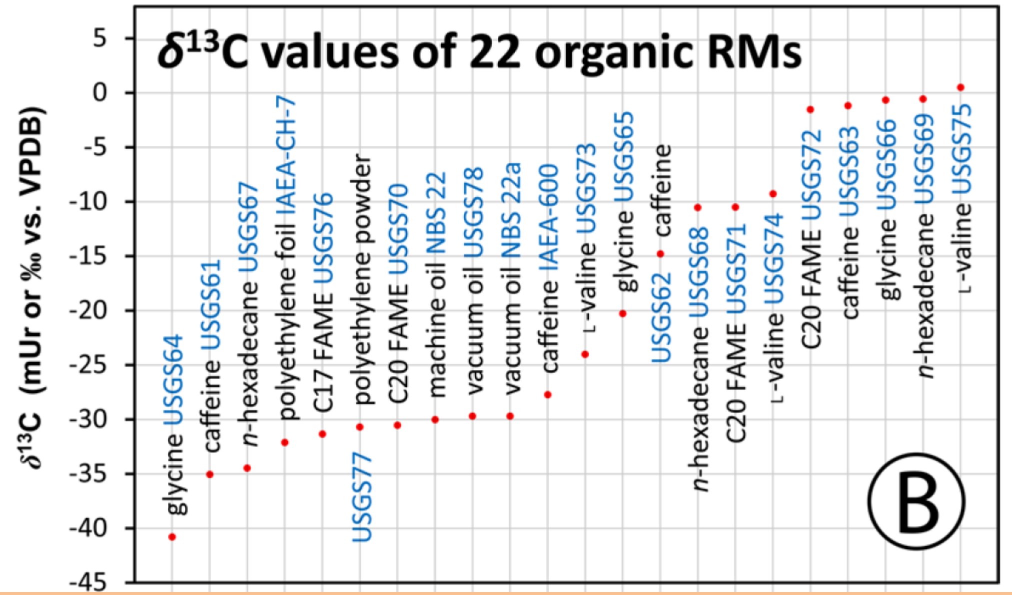
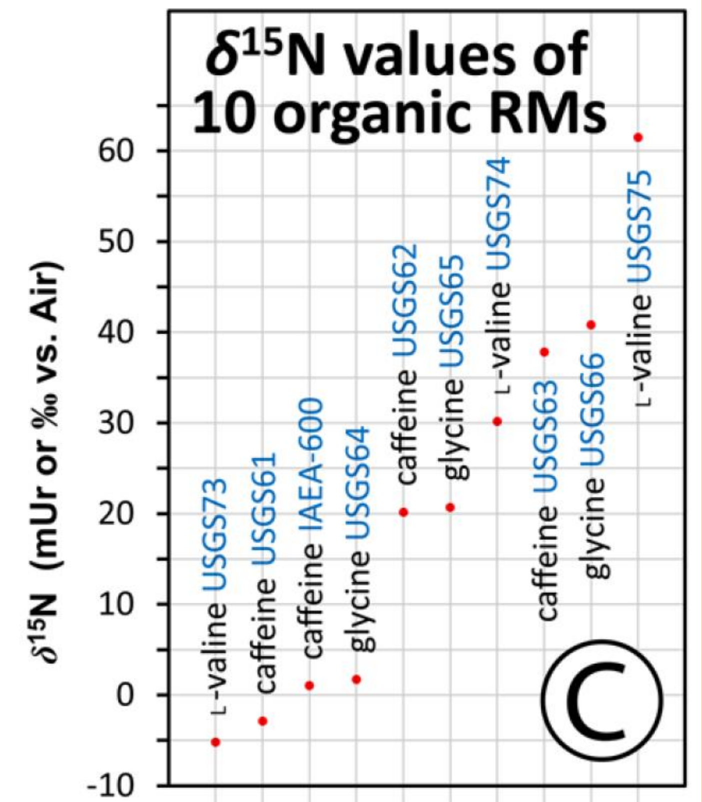


δ^{2}H values of 22 organic RMs



δ^{13}C values of 22 organic RMs



δ^{15}N values of 10 organic RMs

Most important lessons learnt:

- Choose starting materials wisely. Document purity.
- Start with large amounts of raw materials.
- Select an experienced team that uses multitudinous analytical approaches for recognition of biases.
- Set clear rules and expectations from the start. Rigorously apply the IT principle and 2-point normalization using a common set of RMs.
- Use gentle methods for homogenization. Test homogeneity using small sample sizes.
- Continually compile analytical data. Involve an experienced statistician.
- Spend as much effort on carefully storing RMs as you did on RM development. Dark and refrigerated storage in flame-sealed glass vessels under vacuum or a dry and inert gas is recommended.

THANK YOU FOR YOUR ATTENTION