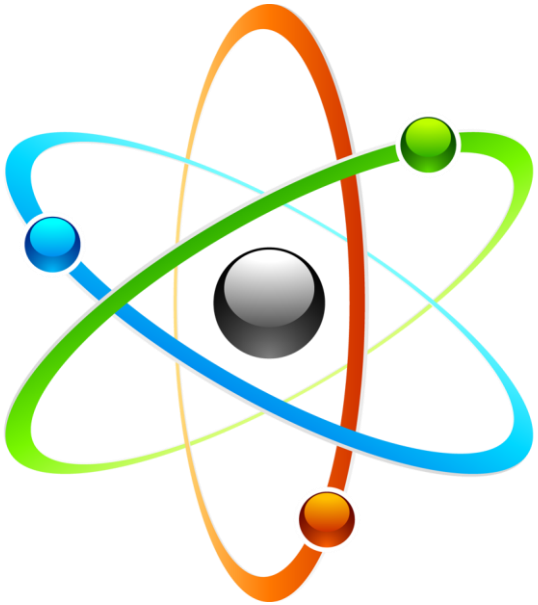


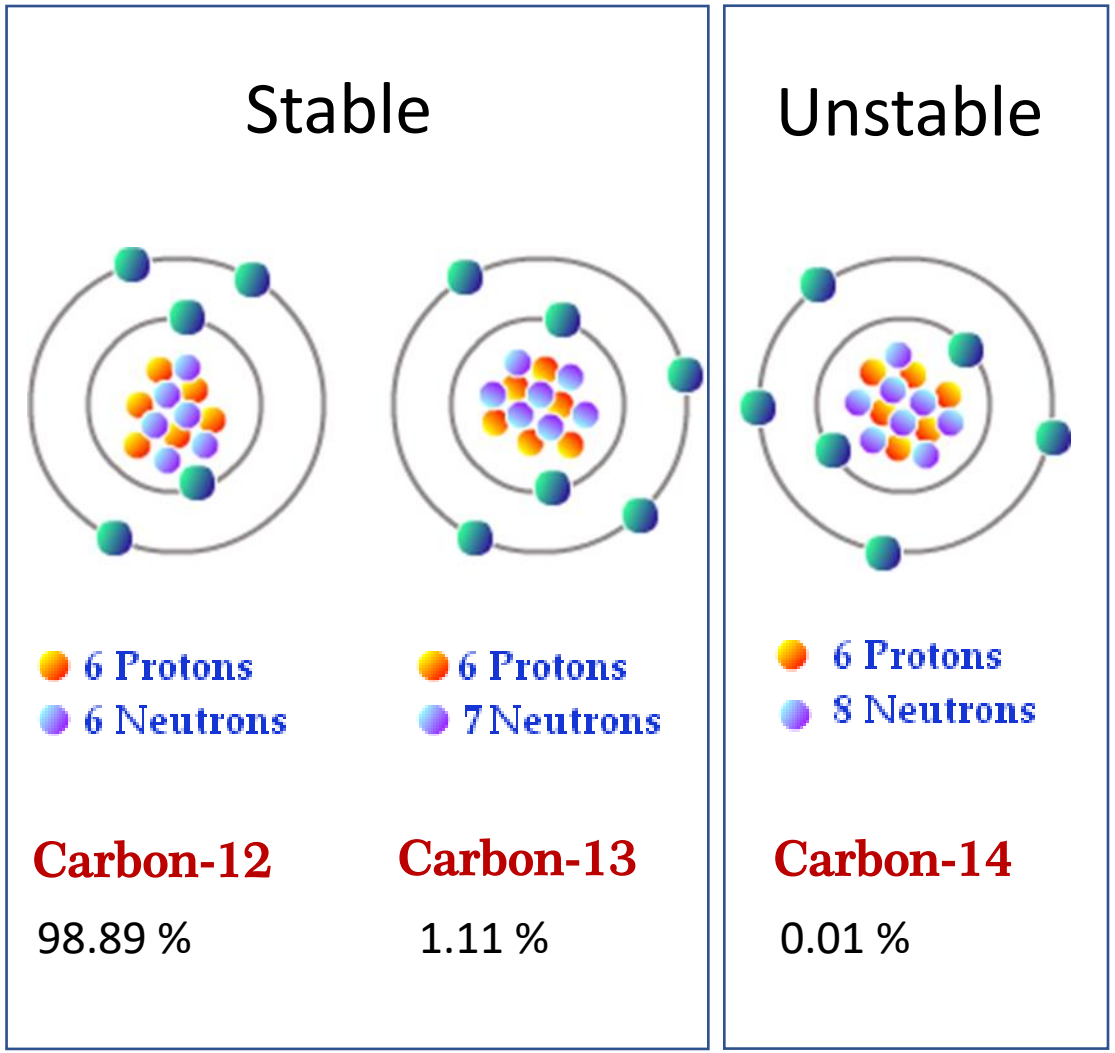
STABLE ISOTOPES

As a guide to authenticity and origin of food

Doris Potočnik, Lidija Strojnik, Nives Ogrinc
Jožef Stefan Institute

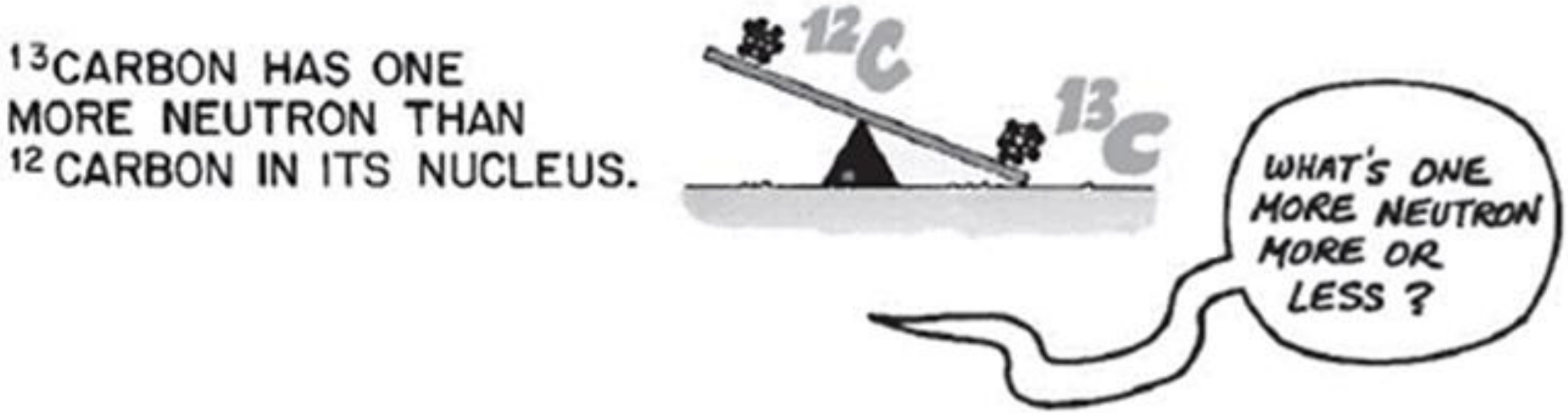
TUNTWIN Summer School, April 18-20, 2023

Isotopes



https://www.javatpoint.com/isotopes-and-isobars

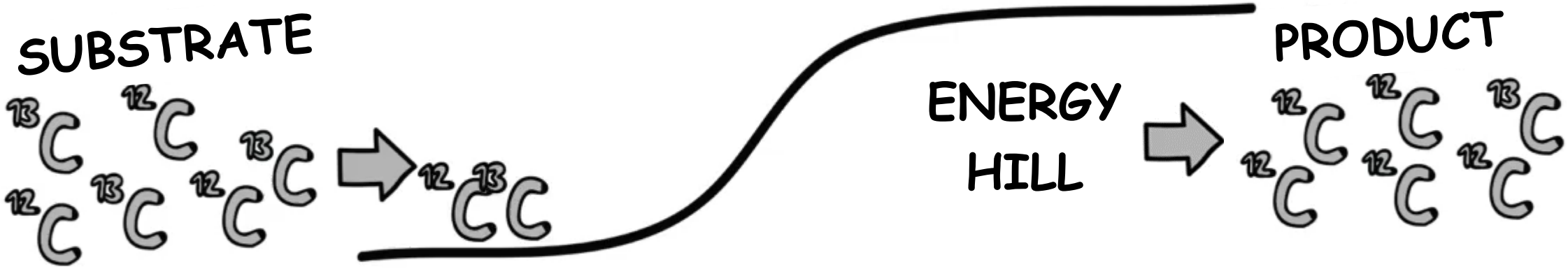
atoms of the element with the **same number of protons** but **different numbers of neutrons**



One neutron is important!

Source of picture: Fry,2006, Stable Isotope Ecology

SOMETIMES THE EXTRA NEUTRON MAKES A DIFFERENCE. IT'S HARDER TO PUSH THE HEAVY MOLECULES UP AN ENERGY HILL….

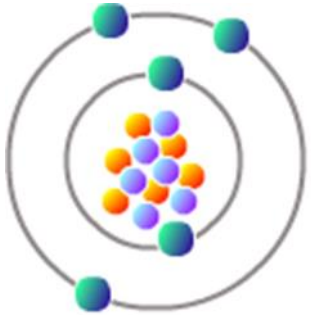


…SO THAT PRODUCTS HAVE MORE OF THE LIGHT ISOTOPE AND LESS OF THE HEAVY ISOTOPE.

Reactivity of isotope and their compounds

Chemical behavior of isotope is qualitatively SIMILAR.

Physical behavior of isotope is qualitatively DIFFERENT.



6 Protons
6 Neutrons

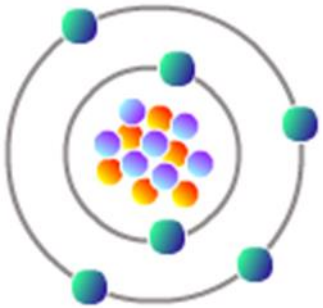
^{12}C has

Higher vibrational energy

Higher velocity

Higher reaction rate

compared to ^{13}C



6 Protons
7 Neutrons

Isotopes of common interest for environmental implications

^{2}H ^{13}C ^{15}N ^{18}O ^{34}S

Isotopes of the most abundant elements

H, C, N, O, S

Stable isotope fractionation

C N O H S

Stable isotope fractionation

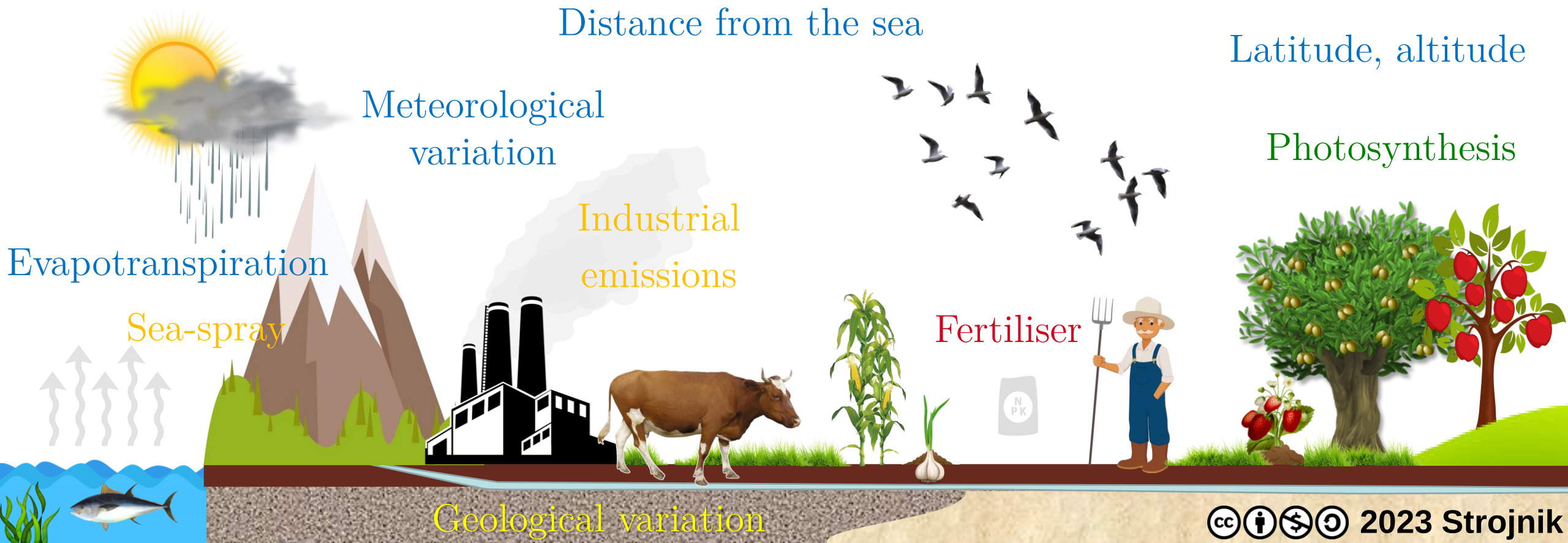
C N O H S

Stable isotope fractionation

C N O H S

Stable isotope fractionation

C N O H S



Variations in isotopic abundances

Element	Minor Isotope	Natural Abundance [%]
H	^{2}H	0.01557
C	^{13}C	1.11140
N	^{15}N	0.36630
O	^{18}O	0.20404
S	^{34}S	4.21500

Variations in many natural systems occur in the third to fifth decimal place.

Variations in isotopic composition

Element	Minor Isotope	Terrestrial range [‰]
H	^{2}H	$\delta^{2}H$ = -450 to +50
C	^{13}C	$\delta^{13}C$ = -120 to +10
N	^{15}N	$\delta^{15}N$ = -20 to +30
O	^{18}O	$\delta^{18}O$ = -50 to +40
S	^{34}S	$\delta^{34}S$ = -65 to +90

Hydrogen has a large terrestrial range, but also relatively low precision.

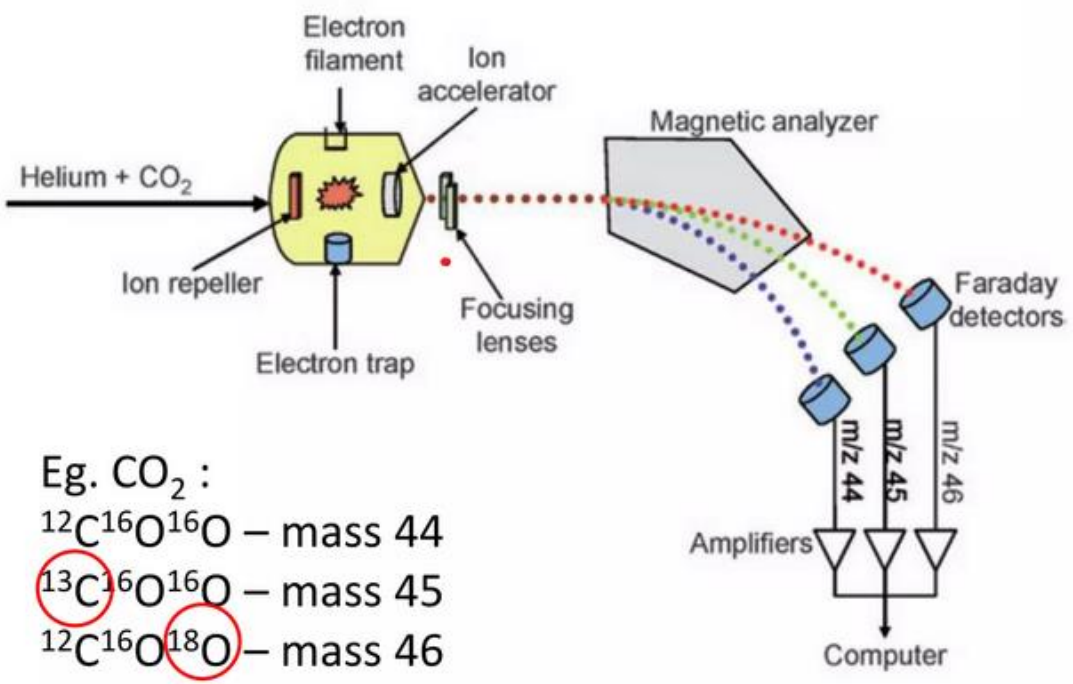
Nitrogen has a smaller terrestrial range, but better technical precision.

Isotope analysis

Element	Minor Isotope	Analyzed as
H	^{2}H	H_2
C	^{13}C	CO_2
N	^{15}N	N_2
O	^{18}O	CO_2, (CO)
S	^{34}S	SO_2 (SF6)

We analyse gases that contain the isotopes of interest!

Isotope Ratio Mass Spectrometer



Eg. CO_2 :

$^{12}C^{16}O^{16}O$ – mass 44

$^{13}C^{16}O^{16}O$ – mass 45

$^{12}C^{16}O^{18}O$ – mass 46

Adapted from Prdaeep Raja K.P.

Continuous Flow

Isotope Ratio Mass Spectrometer

On-line sample preparation

Smaller sample size

Faster and simpler analysis

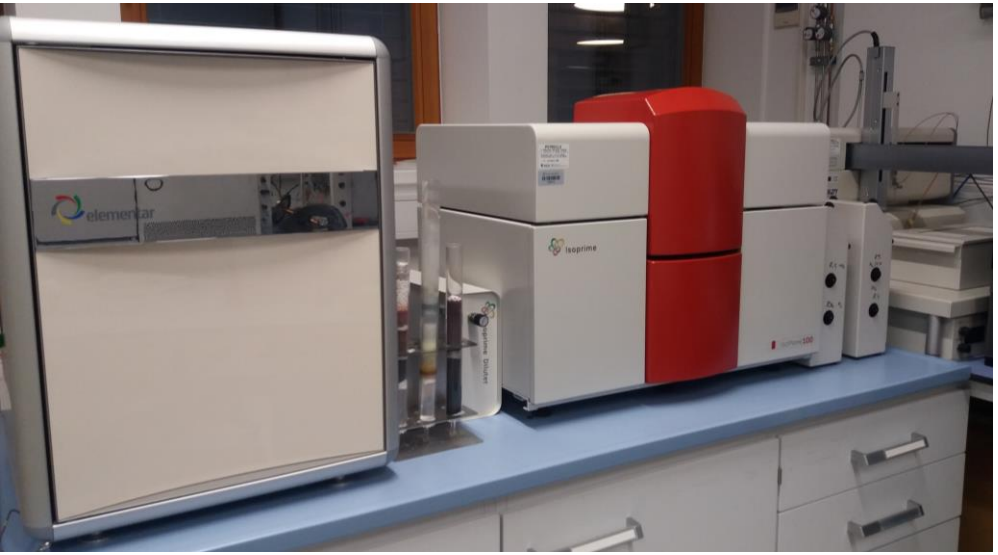
Cost effective

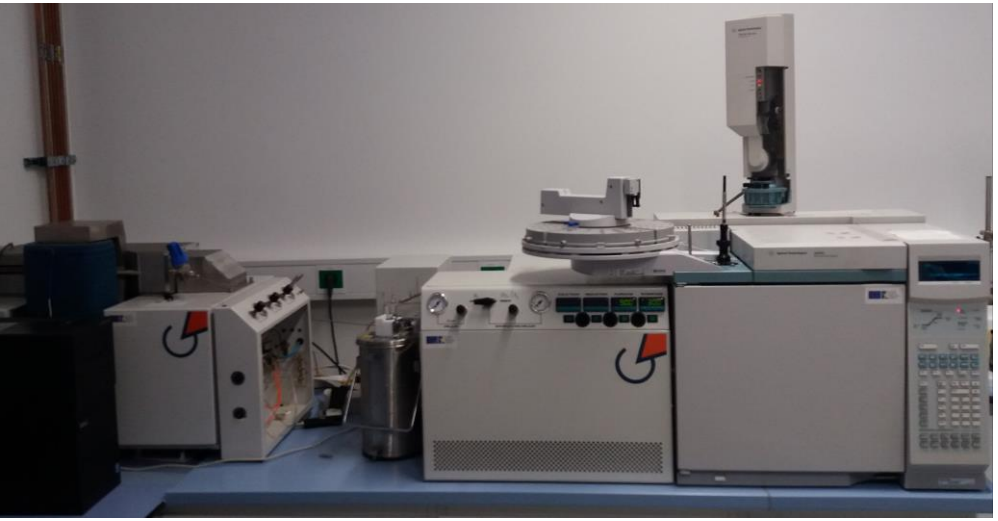
Dual Inlet

Isotope Ratio Mass Spectrometer

The most precise method

Continuous Flow IRMS





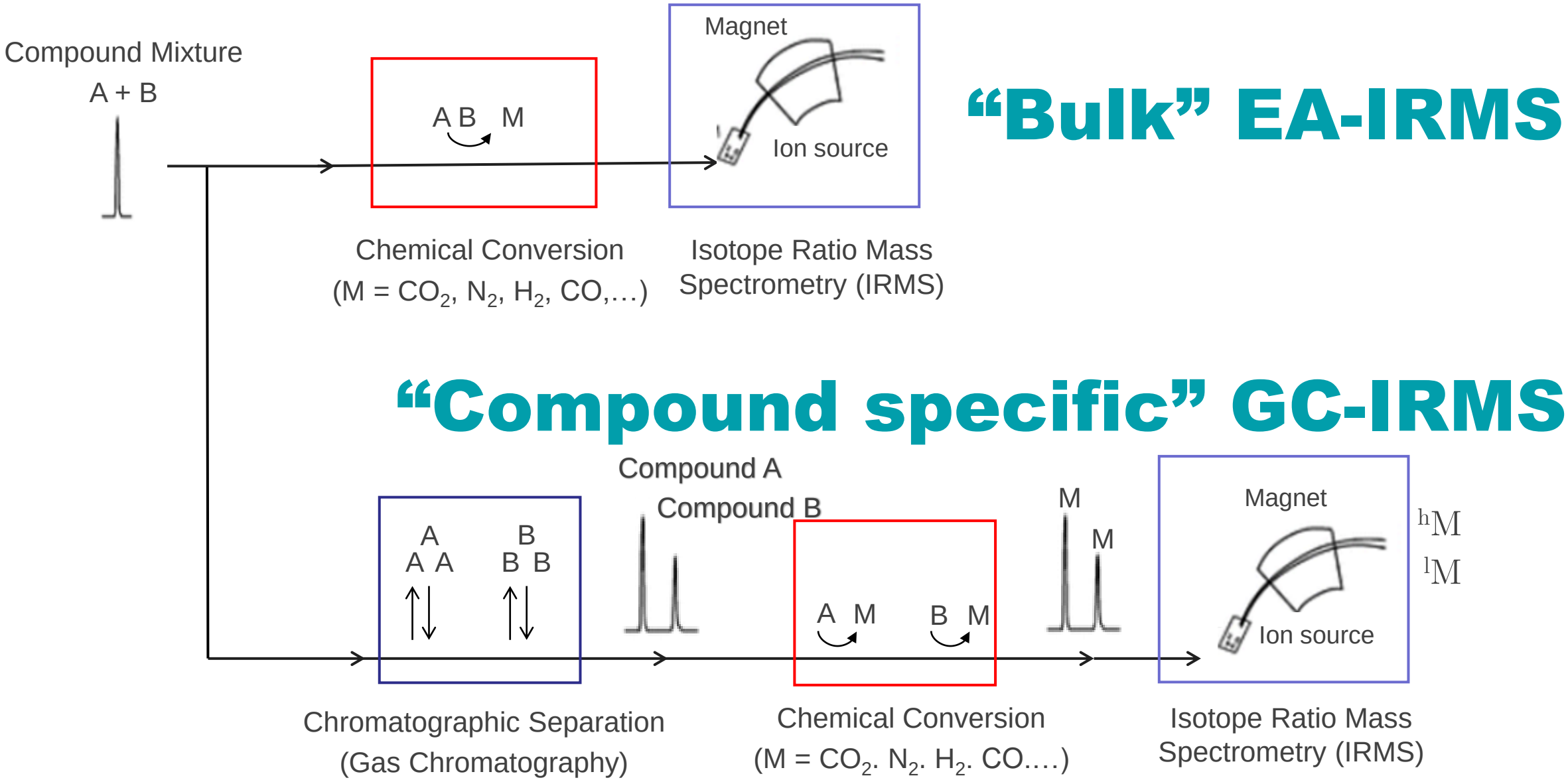
Can be interfaced with other preparation techniques

Elemental analyser (EA)

Gas chromatography (GC)

Liquid chromatography (LC)

From bulk to compound specific



Stable isotopes - δ notation

$$\delta^{i}E = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ [‰]$$

R is

… the isotope ratio of the HEAVY / LIGHT isotopes in either your sample or a standard

… $^{2}H/^{1}H$, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$

… a very small number

Stable isotopes - δ notation

$$\delta^{i}E = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ [‰]$$

This is a small number, because R_{sample} never deviates much from $R_{standard}$ (natural variation in isotope ratios is limited).

Stable isotopes - δ notation

$$\delta^{i}E = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \times 1000\ [‰]$$

This is a small number, because R_{sample} never deviates much from $R_{standard}$ (natural variation in isotope ratios is limited).

To make the variation more apparent, one multiplies the value by 1000

Thereby expressing the value in per mil (parts per thousand; ‰ notation).

Stable isotopes - δ notation

Delta notation indicates the isotope ratio in your sample relative to a known standard, defined as 0 ‰ (mUr – SI unit).

$$\delta^{i}E = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \text{ x } 1000 \text{ [‰]}$$

If the isotope ratio in your sample equals the standard, $R_{sample}/R_{standard}$ =1 and $\delta^{i}E$ = 0 ‰.

Stable isotopes - δ notation

Element	Reference standard
H	Vienna Standard Mean Ocean Water (V-SMOW)
C	Vienna Pee Dee Belemnite (V-PDB)
N	Atmospheric N_2 (AIR)
O	Vienna Standard Mean Ocean Water (V-SMOW)
S	Vienna-Canyon Diablo Troilite (V-CDT)

Stable isotopes - δ notation

$$\delta^{i}E = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \text{ x } 1000 \text{ [‰]}$$

A positive δ value means that the ratio of heavy to light isotope is higher in the sample than in the standard.

A negative δ value means that the ratio of heavy to light isotope is lower in the sample than in the standard.

Stable isotopes - δ notation

$$\delta^{i}E = \left[\frac{R_{sample}}{R_{standard}} - 1\right] \text{ x } 1000 \text{ [‰]}$$

A positive δ value means that the ratio of heavy to light isotope is higher in the sample than in the standard.

A negative δ value means that the ratio of heavy to light isotope is lower in the sample than in the standard.

A sample with a $\delta^{18}O$ value of +19.7 ‰ has an $^{18}O/^{16}O$ ratio that is 19.7 per mil, or 1.97 percent higher than in the standard.

Comparative terms

LIGHT vs HEAVY SAMPLES

LIGHTER sample contains more of the lighter isotope, relative to another sample.

HEAVIER sample contains more of the heavier isotope, relative to another sample.

DEPLETED vs ENRICHED SAMPLES

A sample **"DEPLETED" IN THE LIGHT ISOTOPE** contains less of the light isotope, and more of the heavy isotope, relative to another sample.

A sample **"ENRICHED" IN THE LIGHT ISOTOPE** contains more of the light isotope, and less of the heavy isotope, relative to another sample.

Example

$\delta^{15}N$ (‰)

More <u>negative</u>

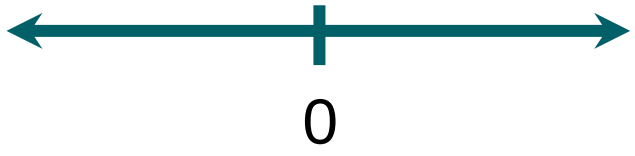
Isotopically <u>lighter</u>

<u>Depleted</u> in ^{15}N

More <u>positive</u>

Isotopically <u>heavier</u>

<u>Enriched</u> in ^{15}N



Sample A

$\delta^{15}N$ = -3.00 ‰

Sample B

$\delta^{15}N$ = +3.00 ‰

Sample B is more positive / enriched in ^{15}N / heavier compared to /relative to Sample A

Common mistakes

$\delta^{13}C$ composition

$\delta^{13}C$ value; carbon isotope composition

$\delta^{13}C$ values are numbers; a composition of numbers has no meaning

Common mistakes

Isotopically depleted water

^{18}O depleted water

A given sample of water is neither depleted nor enriched in isotopes

Common mistakes

Heavy (light) $\delta^{18}O$ values

High (low) $\delta^{18}O$ values

As numbers, δ values can be high or low, positive or negative, but not heavy or light.

Common mistakes

Isotopically negative

Relatively low δ values

Isotopic ratios are neither negative or positive; they are lower or higher than those of the standards.

Common mistakes

Depleted $\delta^{13}C$ value

Low $\delta^{13}C$ value (relative to another)

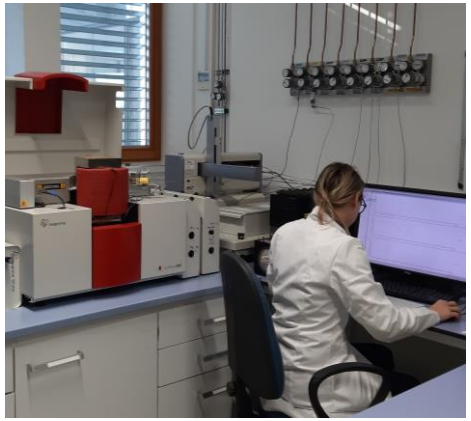
$\delta^{13}C$ values are numbers; as such, they cannot be depleted or enriched.

IRMS challenges

IRMS challenges

1

Instrument preparation



2

Reference materials

3

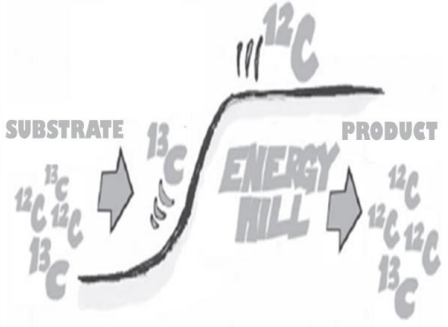
Data normalisation

4

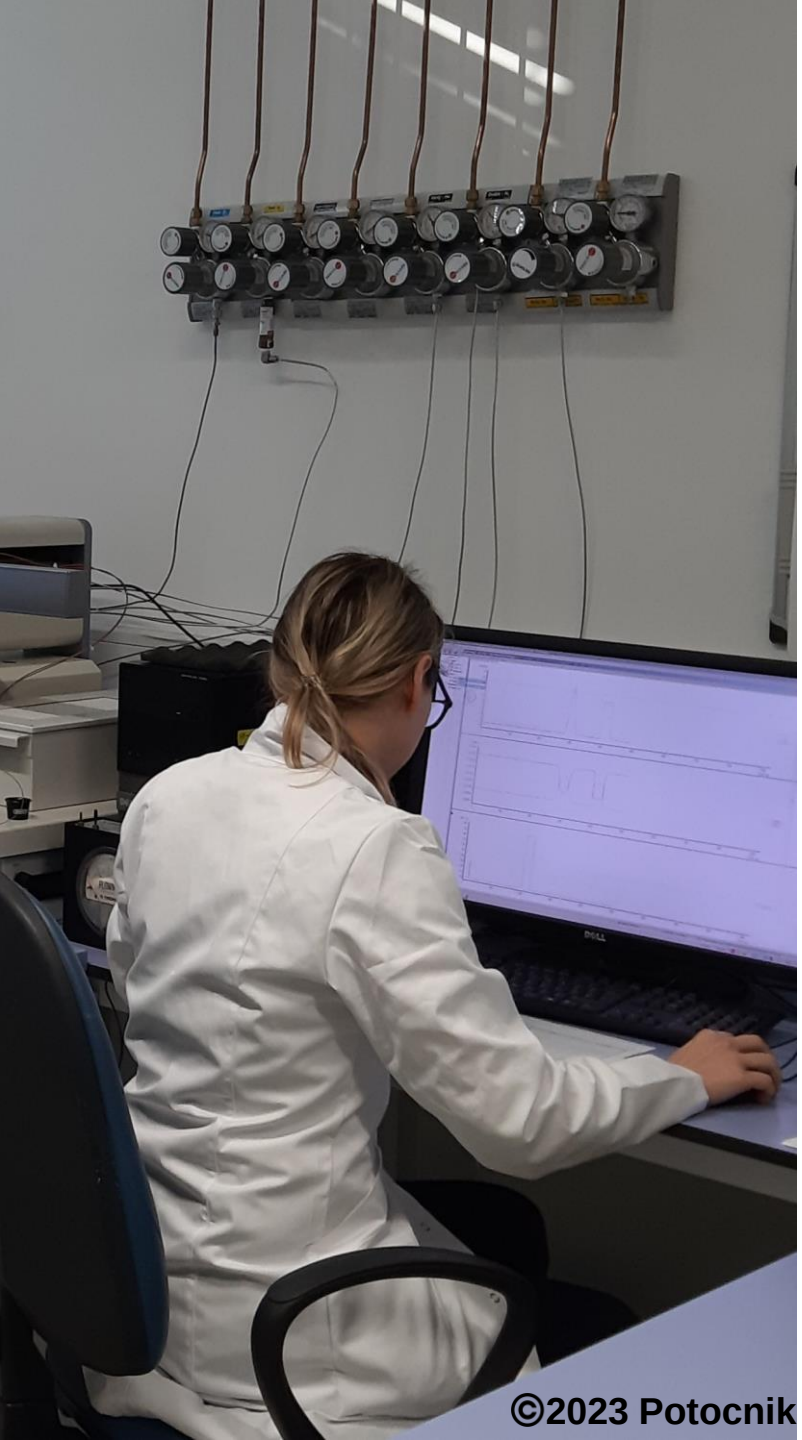
Measurement uncertainty

5

Isotope fractionation



Source of picture: Fry,2006, Stable Isotope Ecology

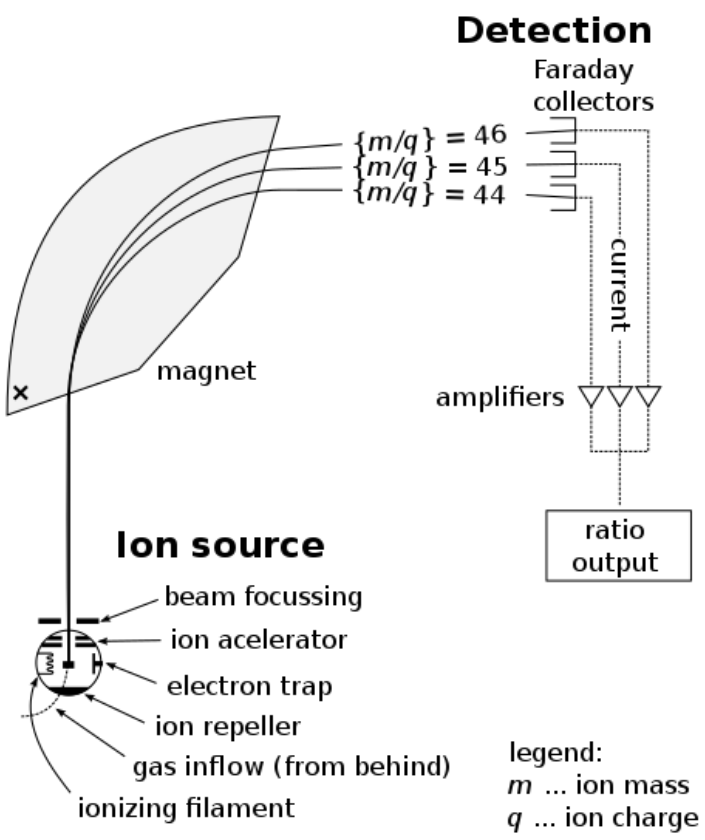


1 Instrument preparation

Instrument preparation

1. Laboratory gases
   bottle pressure, flow rate
2. Electrical supply
3. Vacuum system checks
   leak checking, backgrounds
4. Ion source tuning
5. Peak centering
6. Base performance
   working gas stability & linearity
7. Blank determination
8. Preconditioning

Ion source tuning

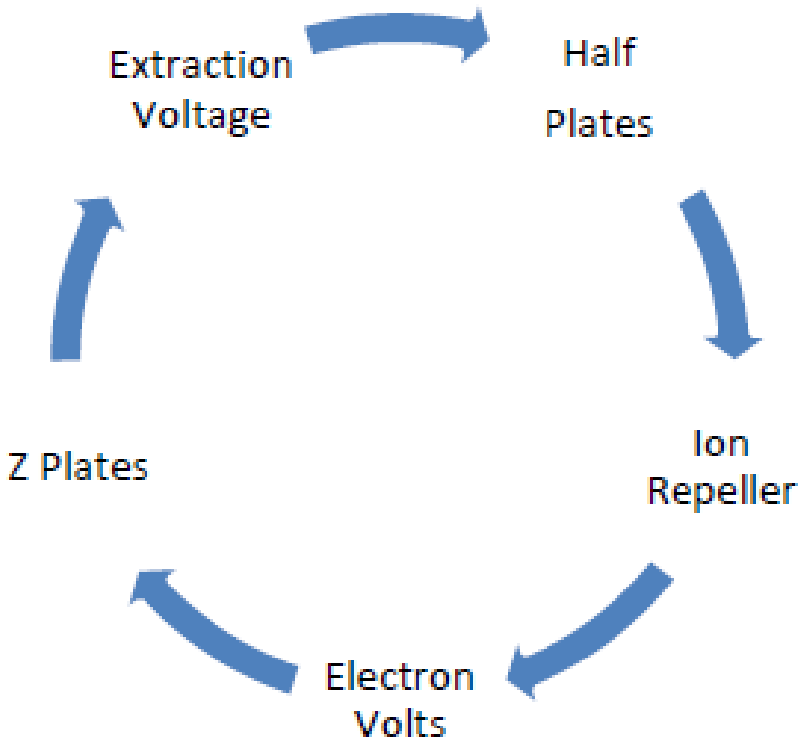


A poorly focused ion beam gives a divergent image at the detector.

Any small change in the energy of the ion beam would result in a change in amplitude of the ion beam.

Isotopic ratio of the beam varying drastically because of instrument inefficiency.

Ion source tuning



Ion source must be well tuned in order to produced reliable isotope data

Starting with the default tuning parameters

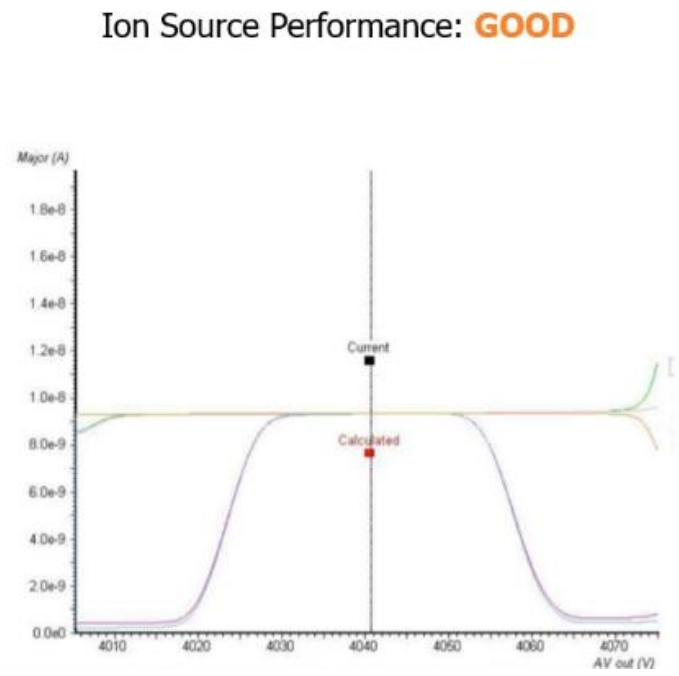
Optimise the Half Plates and Z Plates for steering the focus

Repeat the sequence above until the ion beam currents change only by small amounts

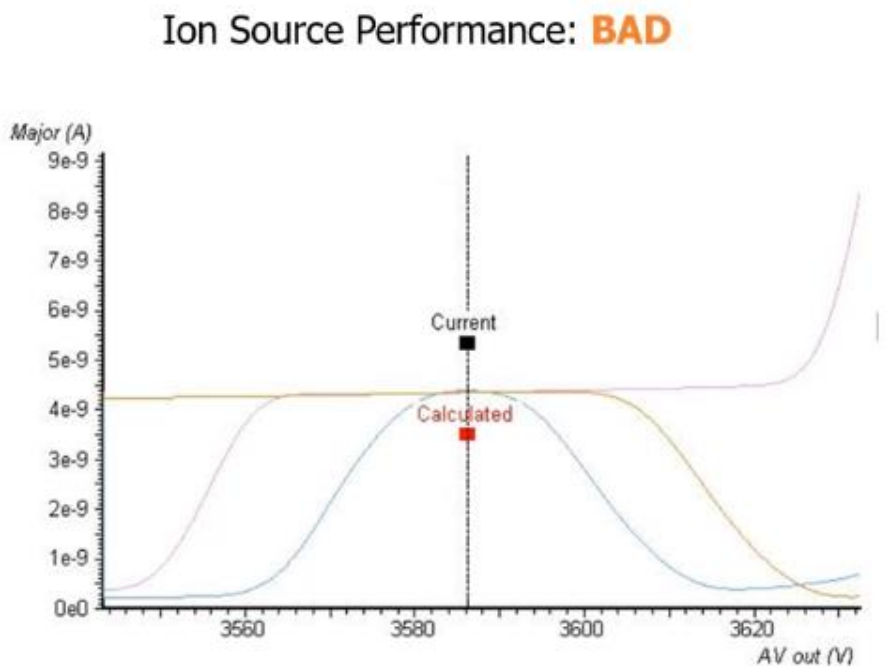
Peak centering

Good peak centre allow well focussed instrument and stable isotope ratios.

Peak centre scans should produce a symmetrical and flat peak across the top.



Adapted from Rees, 2021



Adapted from Rees, 2021

Base performance

After vacuum, tuning and peak center is OK.

Checks the quality of the IRMS prior to analysing samples.

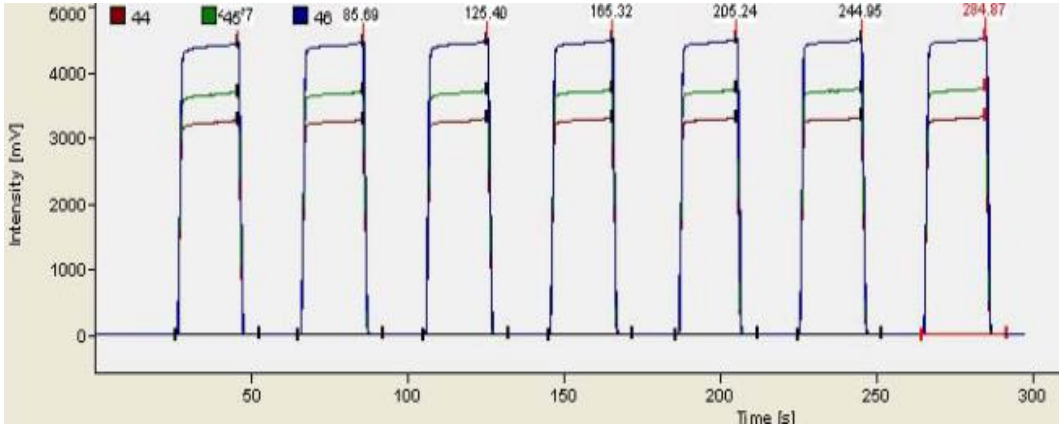
Performed by injecting pure working gas into the ion source.

Two major checks:

- Stability
- Linearity

Base performance: Stability

Determines the best reproducibility that can be achieved.



Adapted from FIRMS

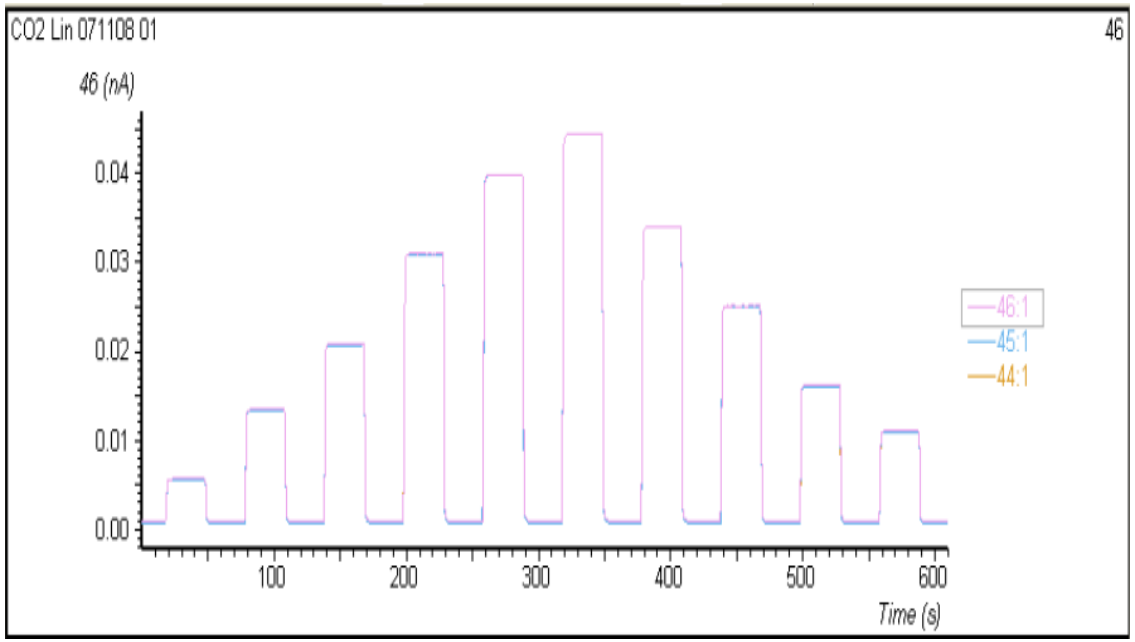
Repeat injection of working gas at the same pressure to check that the instrument returns the same ratio.

Monitor on a daily basis

For CO_2, N_2 and CO < 0.1‰, ≤ 1‰ for H_2

Base performance: Linearity

Attain consistent ion ratios over a range of signal intensities



Adapted from FIRMS

Performed with the intensity of the gas pulses encompassing the anticipated working range of sample heights.

Monitor before each sample batch.

Can also be determined from samples.

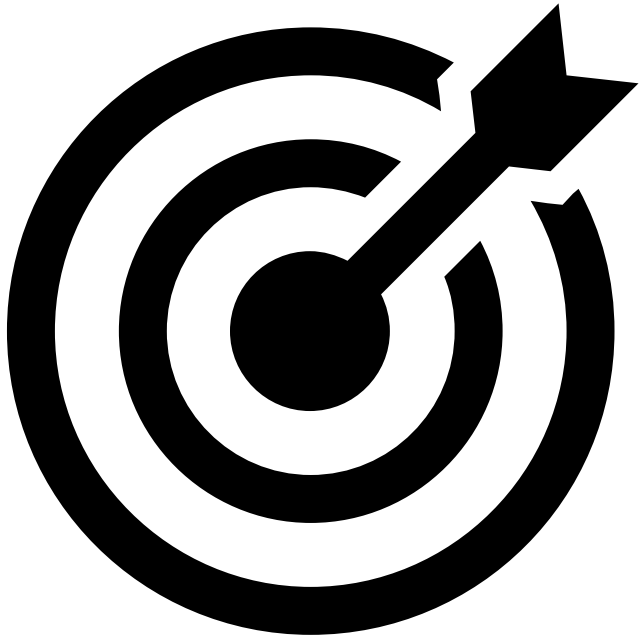
For CO_2, N_2 and CO ≤ 0.03‰ / nA, ≤ 0.1 for H_2

Most affected by the relationship between the extraction voltage (EX) and ion repeller (IR) .

2 Reference materials

The use of relevant reference material following principle of ”identical treatment”.

Why stable isotope RM?



All stable isotope laboratories must 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.

Hierarchy of RM

1

PRIMARY RM

2

SECONDARY RM

3

LOWER LEVEL RM

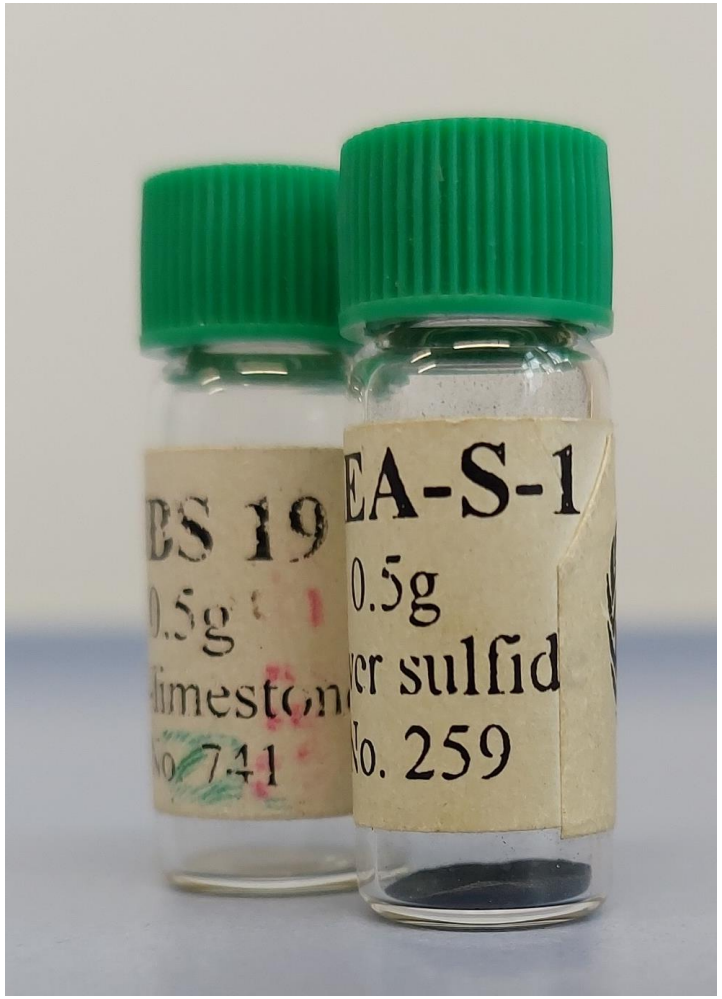
Hierarchy of RM: Primary

Physical materials which define the international δ-scale. Exact isotope δ values by consensus with no uncertainty

Limited supply - purchase every 3 years

Impractical for day-to-day use.

Hierarchy of RM: Primary



Most primary RMs are inorganic compounds or materials and cannot be directly comparable to the analysis of organic compounds.

Hierarchy of RM: Secondary

Natural or synthetic compounds that have been carefully calibrated relative to the primary RMs.

by IAEA, USGS, IRMM…

Hierarchy of RM: Secondary

Physical materials with internationally agreed *δ*-values and uncertainty.

Limited range of materials and *δ*-values.

Hierarchy of RM: Lower level

Materials calibrated against primary and/or secondary RMs.

Used on a daily basis for QA and QC.

Chemically and physically similar to typical laboratory samples.

3 Data normalisation

To ensure laboratory comparability measured data must be normalized to the appropriate international δ-scale.

Common normalisation methods

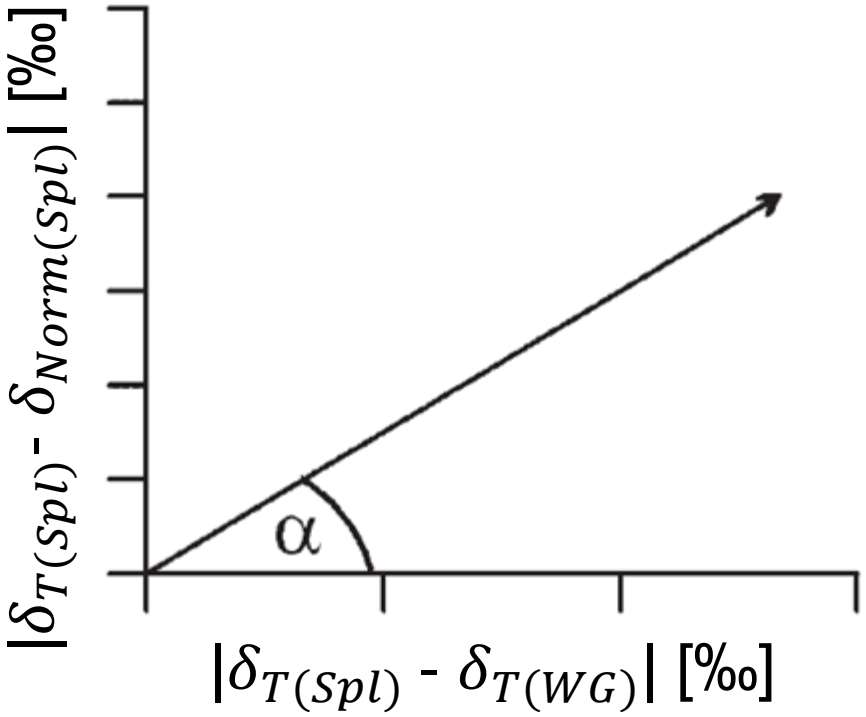
1. **Single-point normalisation:**
   - referencing versus the working gas (WG)
   - referencing versus a certified RM

2. **Two-point linear normalisation**

3. **Multiple-point linear normalisation**

Single - point normalisation

Referencing versus the working gas (WG)



Requires that the δ-values of working gas is known and constant over a period of time.

The stable isotope composition of a working gas may vary with time.

RM and the unknown samples **are not analysed** using the principle of identical treatment.

Single - point normalisation

Referencing versus the reference material (RM)

RM and the unknown sample are analysed using the principle of identical treatment.

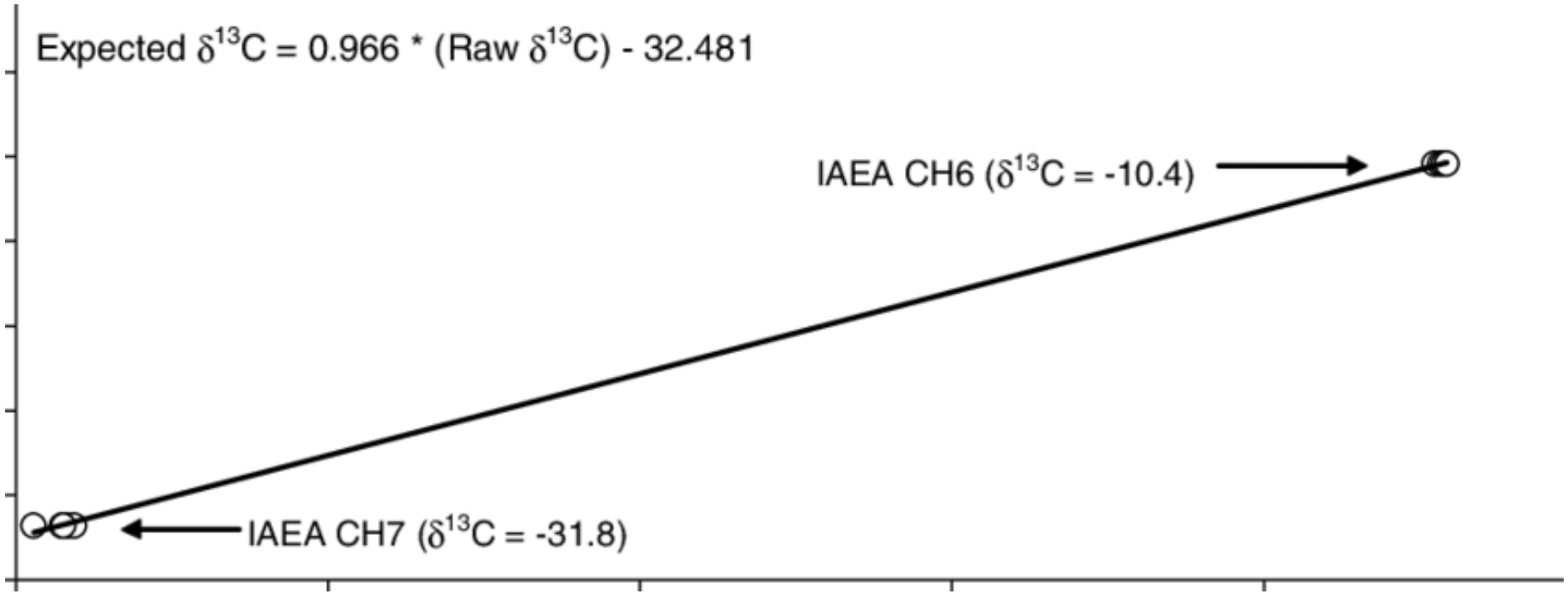
Acceptable where is a lack of RM and $\delta_{Sample} \sim \delta_{RM}$

$$\delta_{T(Spl)} = \left[\frac{(\delta_{raw(Spl)}+1000)(\delta_{true(RM)}+1000)}{(\delta_{raw(RM)}+1000)}\right] -1000$$

2-point normalisation

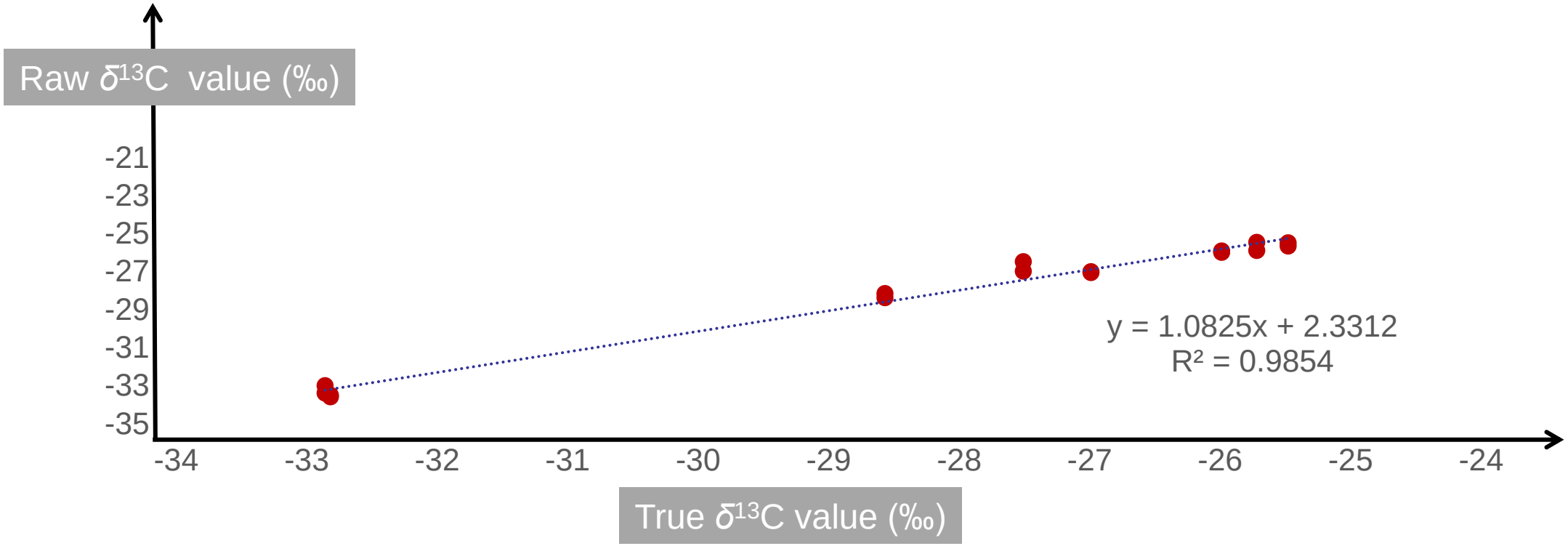
Using two RMs with contrasting isotopic compositions are needed for accurate isotopic measurements.

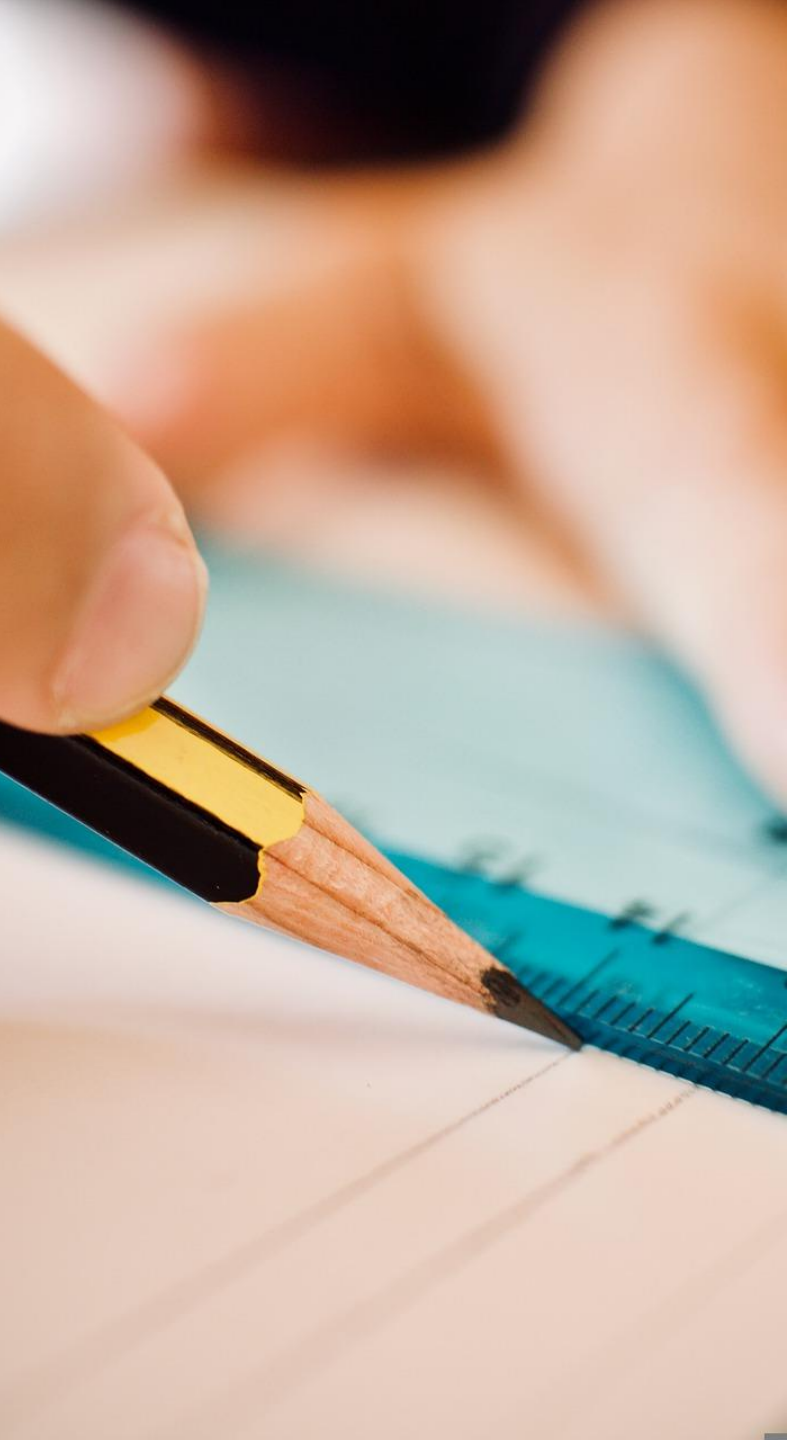
Suitable RMs should ideally be available as pairs.



Multiple-point isotopic linear normalisation

Reduce the random error associated with the analysis of a reference material used to anchor the linear scale.





4 Measurement uncertainty

Measurement uncertainty

All measurements are affected by a certain error.

The measurement uncertainty tells us what size the measurement error might be.

The laboratory to verifiy its´ own quality of measurement.

Customer needs it in order to be able to make correct decisions.

Required for accreditation to ISO 17025.

Where uncertainty arise in IRMS?

From all calculation stages from raw instrumental data onwards.

Sampling	heterogeneity, background variations
Sample preparation	weighing, extraction, derivatisation, etc
Instrument analysis	conversion to gas, separation, etc
Raw data	Integration parameters
Calculation of raw *δ* values	use of WG, correction for ^{17}O, H_3^+
Corrections to raw *δ* values	blank, drift, linearity, memory
Normalization	using suitable RM
Further correction	exchangeable H, derivative C

Adapted from Dunn, 2016

Corrections to data can significantly impact the source and magnitude.

Kragten spreadsheet approach

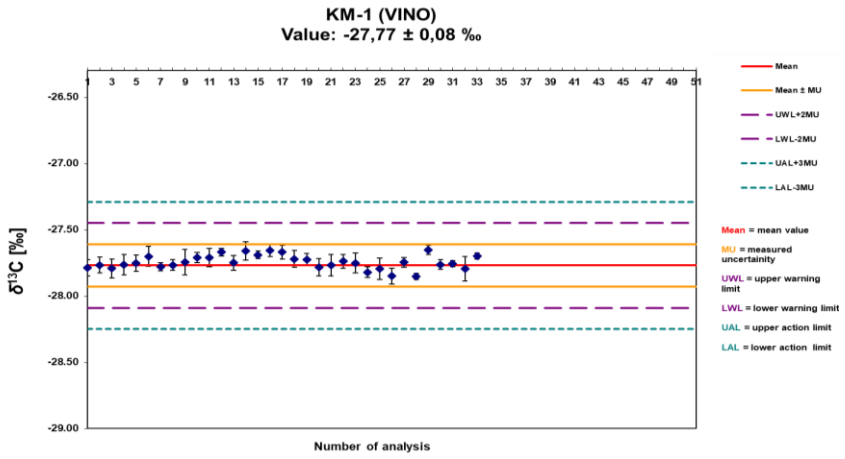
One of the most straightforward approaches with two-point scale calibration/normalization

Example provided in current FIRMS Good Practice Guide for IRMS:

	A	B	C	D	E	F	G	H
1								
2	**Parameter**	**value (δ^2H, ‰)**	**uncertainty (δ^2H, ‰)**					
3	$\delta_{true(VSMOW2)}$	0.0	0.3	B3+C3	B3	B3	B3	B3
4	$\delta_{true(SLAP2)}$	−427.5	0.3	B4	B4+C4	B4	B4	B4
5	$\delta_{raw(VSMOW2)}$	0.3	1.2	B5	B5	B5+C5	B5	B5
6	$\delta_{raw(SLAP2)}$	−420.7	1.2	B6	B6	B6	B6+C6	B6
7	$\delta_{raw(sample)}$	−189.0	1.5	B7	B7	B7	B7	B7+C7
8	$\delta_{true(sample)}$	Eqn. (13) applied to values above	$u(\delta_{true(sample)})$ = square root of sum of squared differences	Eqn. (13) applied to values above	Eqn. (13) applied to values above	Eqn. (13) applied to values above	Eqn. (13) applied to values above	Eqn. (13) applied to values above
9			Difference	D8-B8	E8-B8	F8-B8	G8-B8	H8-B8
			Squared differences	$(D9)^2$	$(E9)^2$	$(F9)^2$	$(G9)^2$	$(H9)^2$

Adapted from FIRMS

Estimation of MU from validation

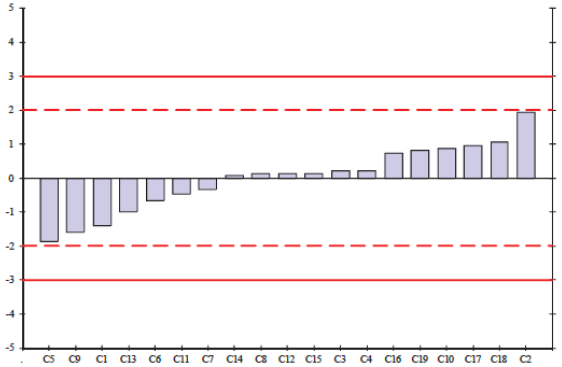


Measurement uncertainty within laboratory reproducibility ($u(R_w)$)

internal QC material

Size :	19
Mean :	-25.86
Robust Mean :	**-25.86**
Standard Deviation :	0.15
Robust SD :	**0.16**
Target SD :	**0.15**
Min :	-26.14
Max :	-25.57

Laboratory Code	Sample	Z-scores
C5	-26.14	-1.86
C9	-26.10	-1.59
C1	-26.07	-1.39
C13	-26.01	-0.99
C6	-25.96	-0.66
C11	-25.93	-0.46
C7	-25.91	-0.32
C14	-25.85	0.08
C8	-25.84	0.14
C12	-25.84	0.14
C15	-25.84	0.14
C3	-25.83	0.21
C4	-25.83	0.21
C16	-25.75	0.74
C19	-25.74	0.81
C10	-25.73	0.88
C17	-25.72	0.94
C18	-25.70	1.08
C2	-25.57	1.94



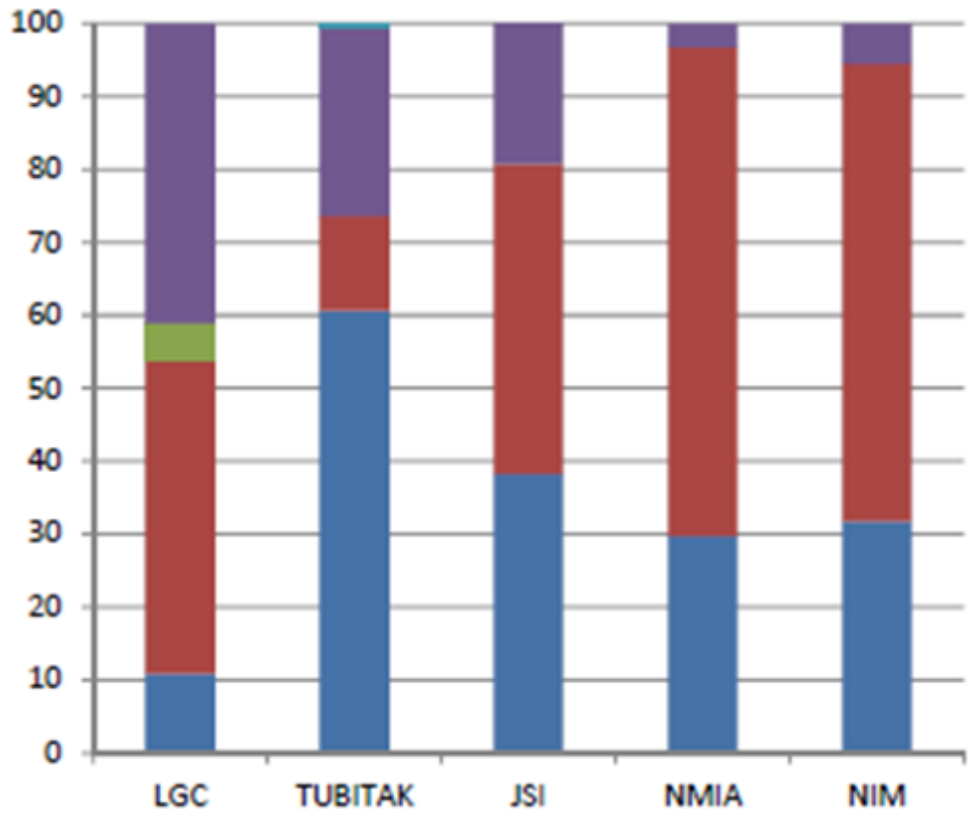
Measure uncertainty from laboratory bias ($u(bias)$)

proficiency testing (PT) (FIT-PTS, CCQM, FIRMS-PTS, IAEA)

$$u_c = \sqrt{u(R_W)^2 + (u(bias))^2}$$

Estimation of MU from validation

CCQM: key comparison on stable isotope ratio delta values



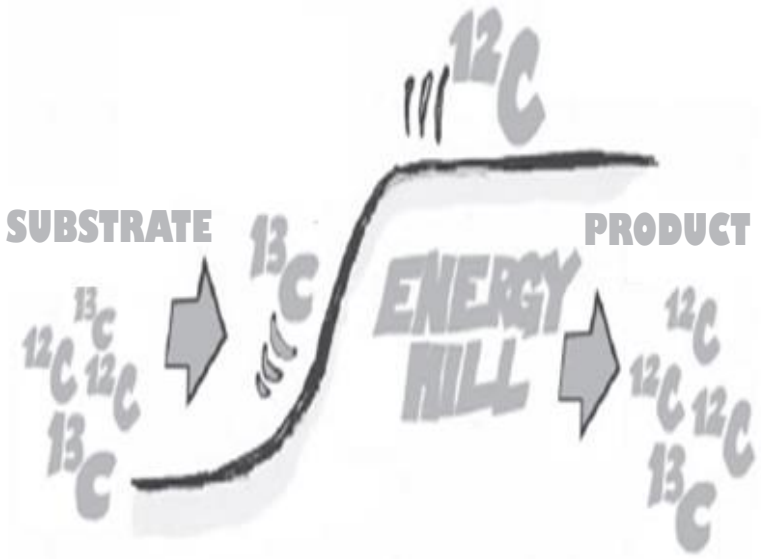
The uncertainty budget:

the uncertainty of the calibration curve

uncertainty of the used RM

the uncertainty of the sample material (repeatability)

5 Isotope fractionation

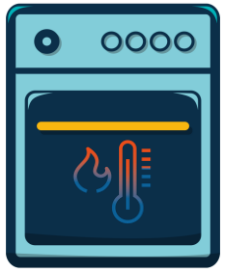


enrichment of one isotope relative to another in a chemical or physical process.

Sample preparation procedures

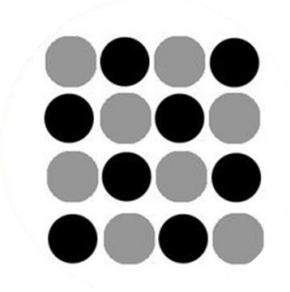
are of the utmost importance because errors that occur cannot be corrected subsequently.

sampling

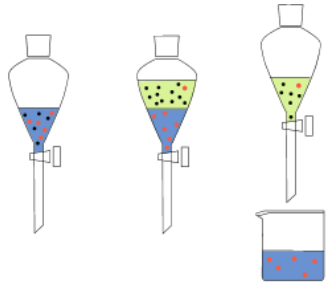


drying

grinding

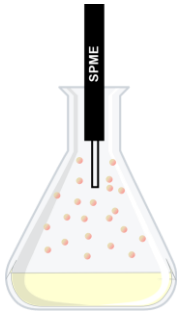


homogeneity



extraction

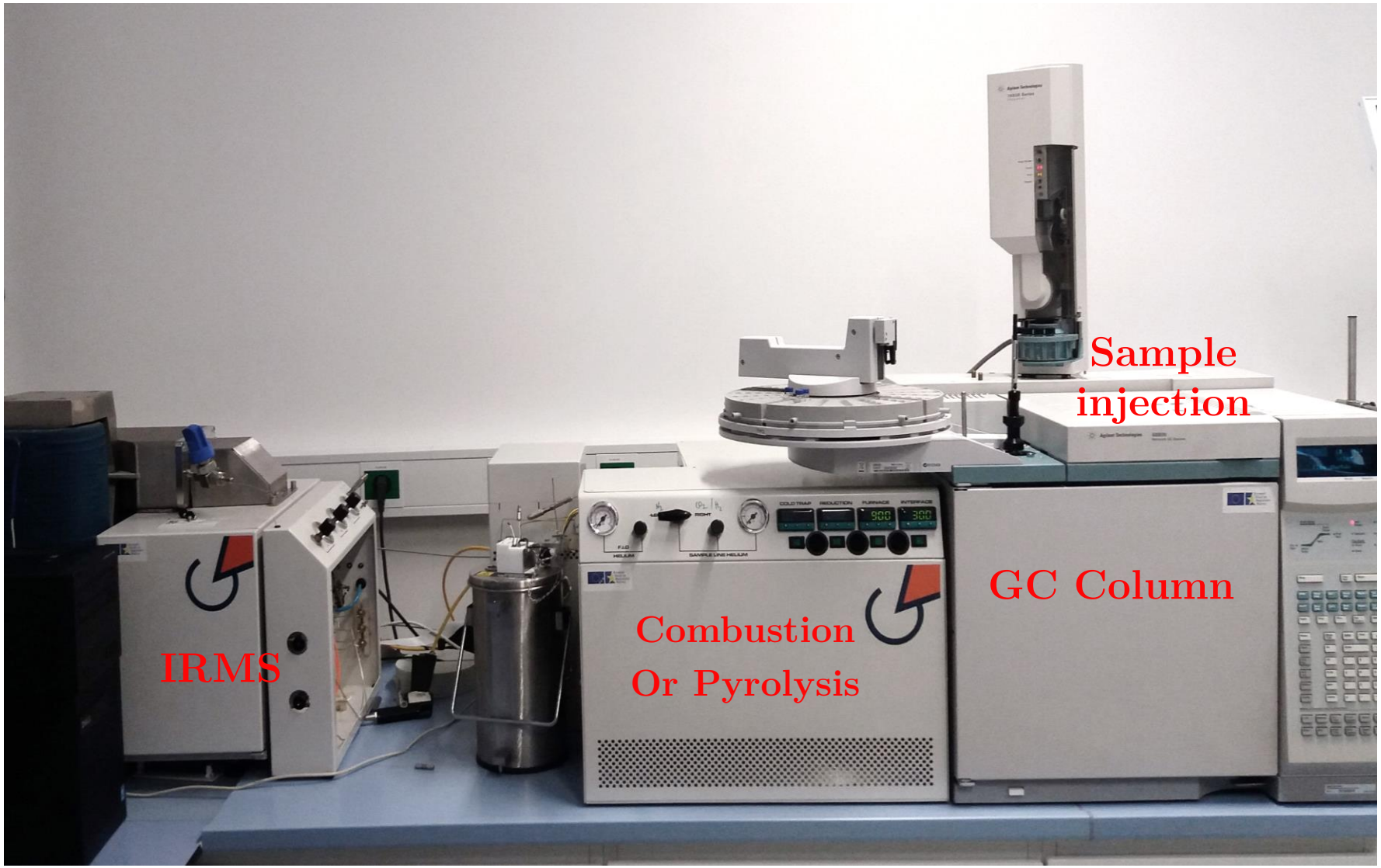
clean-up



preconcentration

derivatization

Instrumental conditions



May also cause irreproducible isotope fractionation.

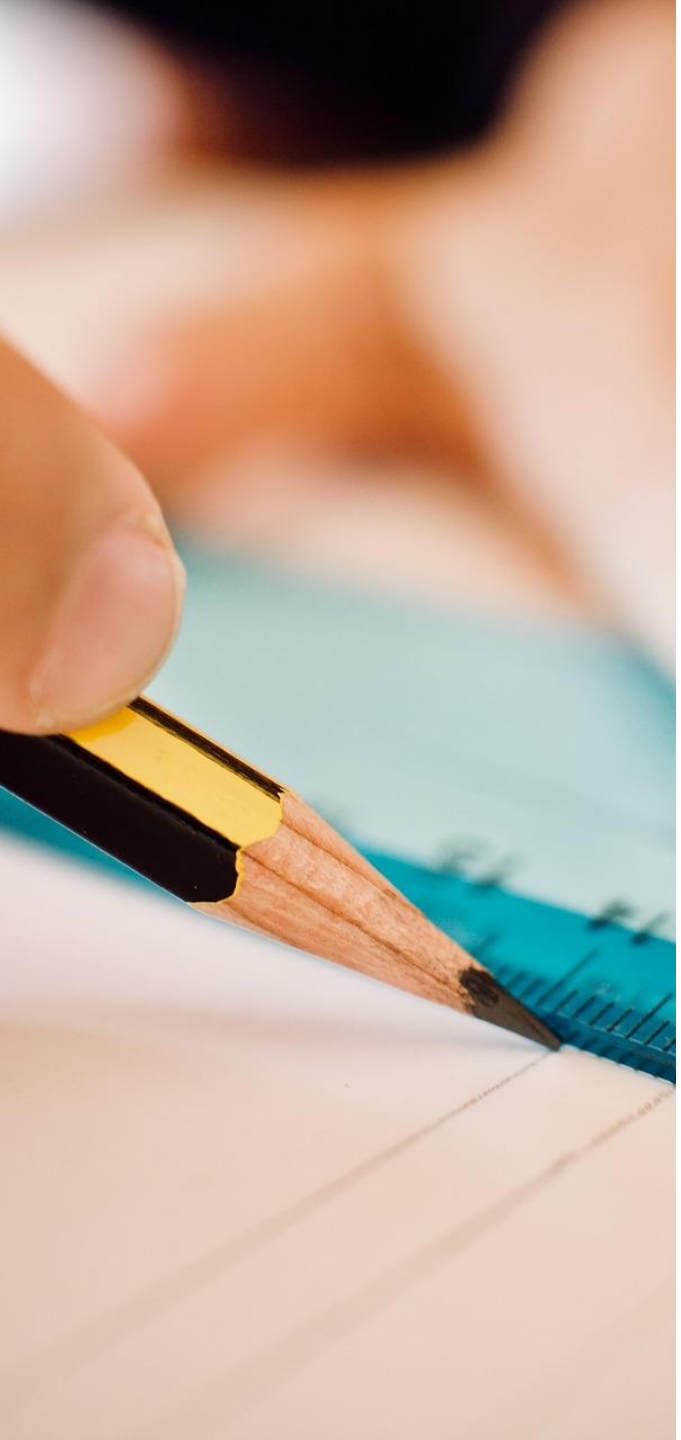
Recommendations
for isotope community

Metrological traceability

use of RM (names, their values and associated uncertainties)

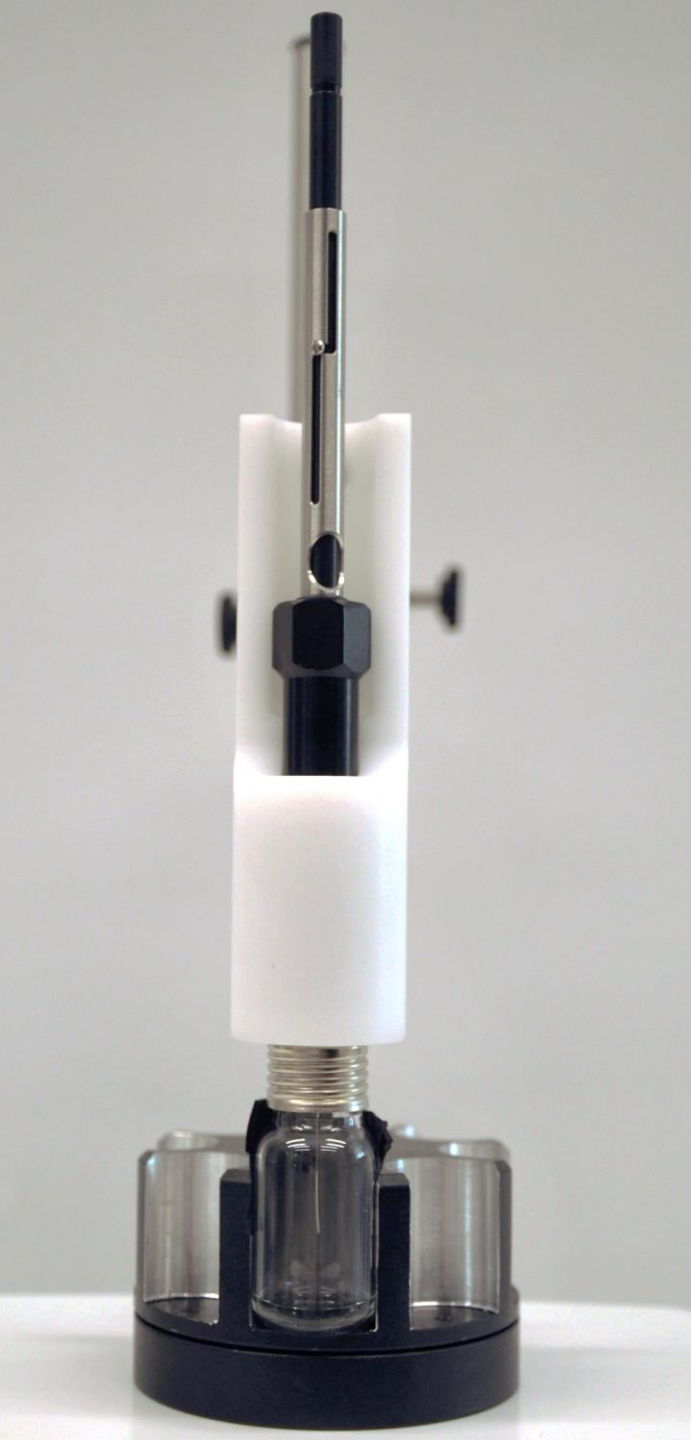
Data handling

how the measurement results were normalized to a stable isotope scale



Uncertainty evaluation

an associated uncertainty at a stated confidence level



Isotope fractionation

details on sample preparation protocol and analytical conditions

EA-IRMS

Doris Potočnik

Jožef Stefan Institute

TUNTWIN Summer School, April 18-20, 2023

1 Basic principle

Elemental analyser (EA) - IRMS

Combination of an inlet (EA) coupled to an IRMS.

Provide analysis of “bulk” or single-component material (required high purity and homogeneity of the sample).

Elemental analyser (EA): inlet

Liquid autosampler

for volatile liquid compounds (EtOH, VOC)

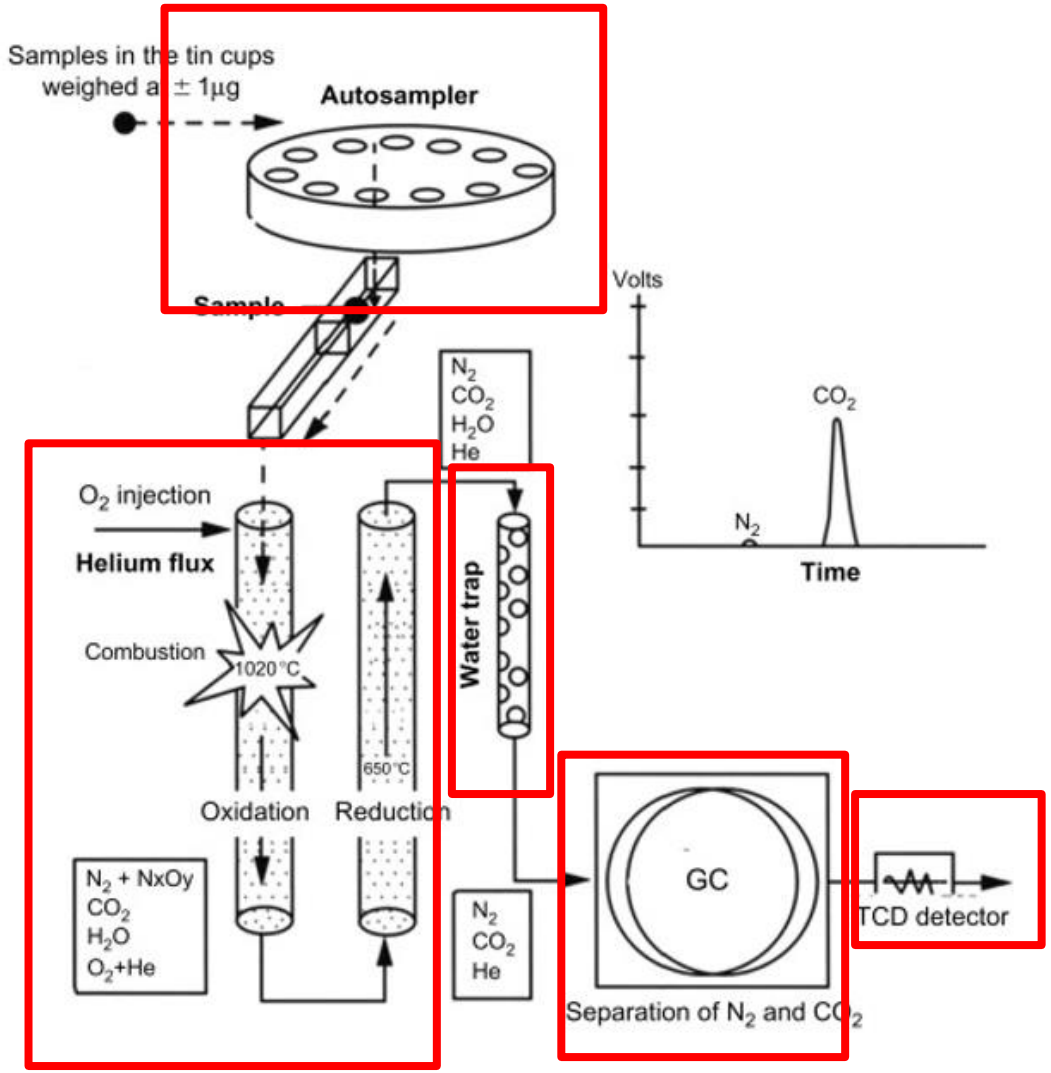
Solid autosampler (EA)

for CNS and OH analysis

Gas autosampler

for O and H analysis in water samples from foodstuff, C in DIC, O and C in carbonates

EA components



Adapted from Rees, 2021

Autosampler – introduce the sample into the EA

Heated furnace – combustion and reduction tube or pyrolysis furnace tube

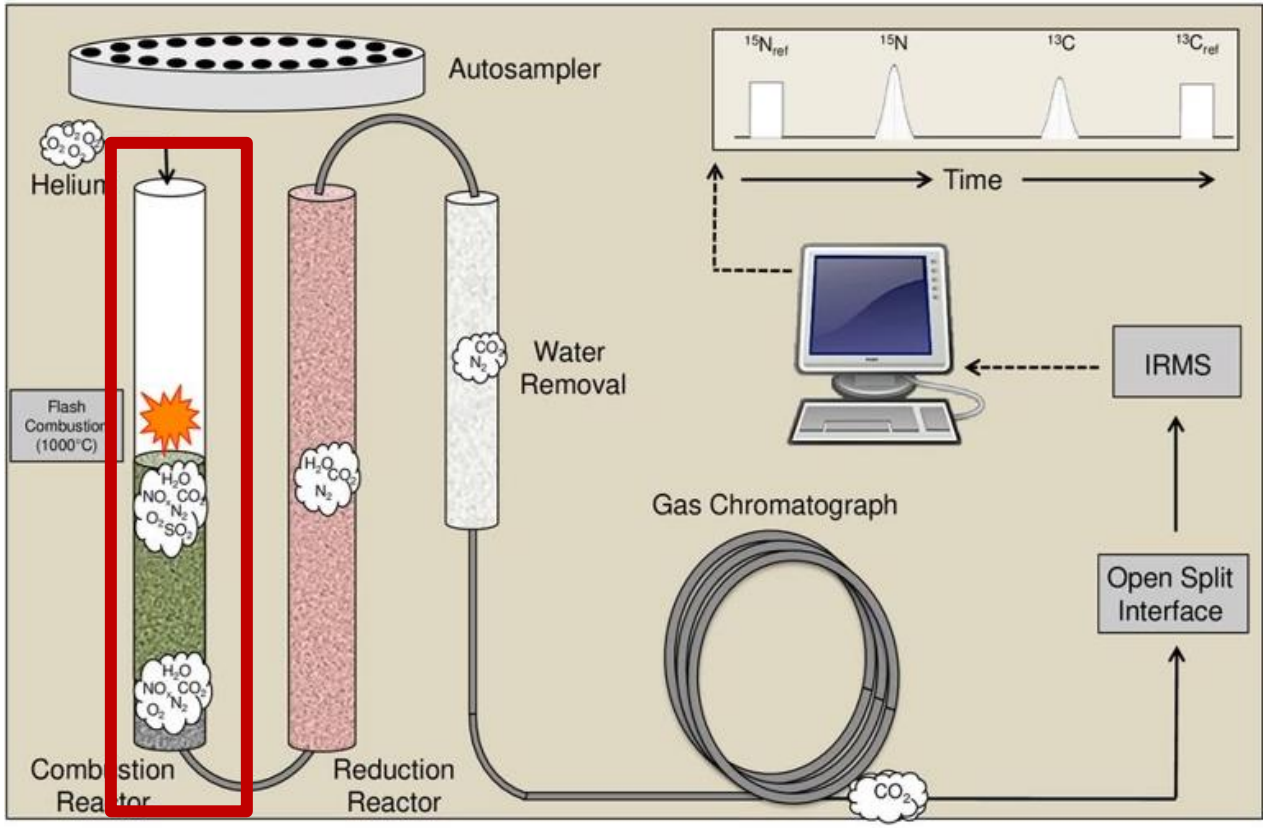
Water trap – contains desiccant

GC oven – separation of combustion gases

TCD detector for produced gases

EA-IRMS combustion mode

$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ via EA-IRMS



Adapted from Rees, 2021

$$CHNOS \xrightarrow[O_2]{1000°C} CO_2 + N_2 + SO_2$$

(^{13}C, ^{15}N, ^{34}S)

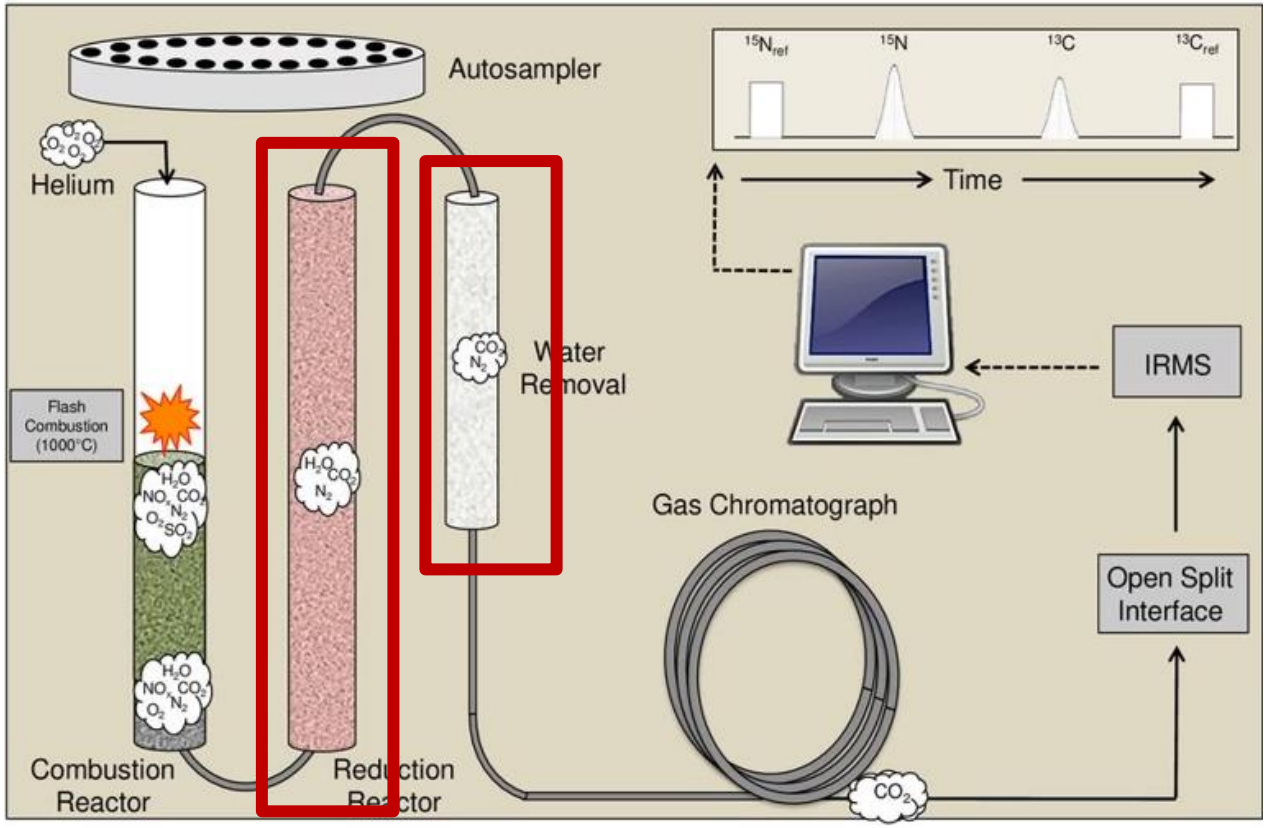
Routinely used for CNS analysis

Two-reactor system: a “combustion” reactor, followed by a “reduction reactor.

Combustion takes place in an O_2 atmosphere in a quartz reactor to produce CO_2, NO_x, SO_x and H_2O (T = 900-1050°C)

EA-IRMS combustion mode

$\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ via EA-IRMS



Adapted from Rees, 2021

$$CHNOS \xrightarrow[O_2]{1000°C} CO_2 + N_2 + SO_2$$

(^{13}C, ^{15}N, ^{34}S)

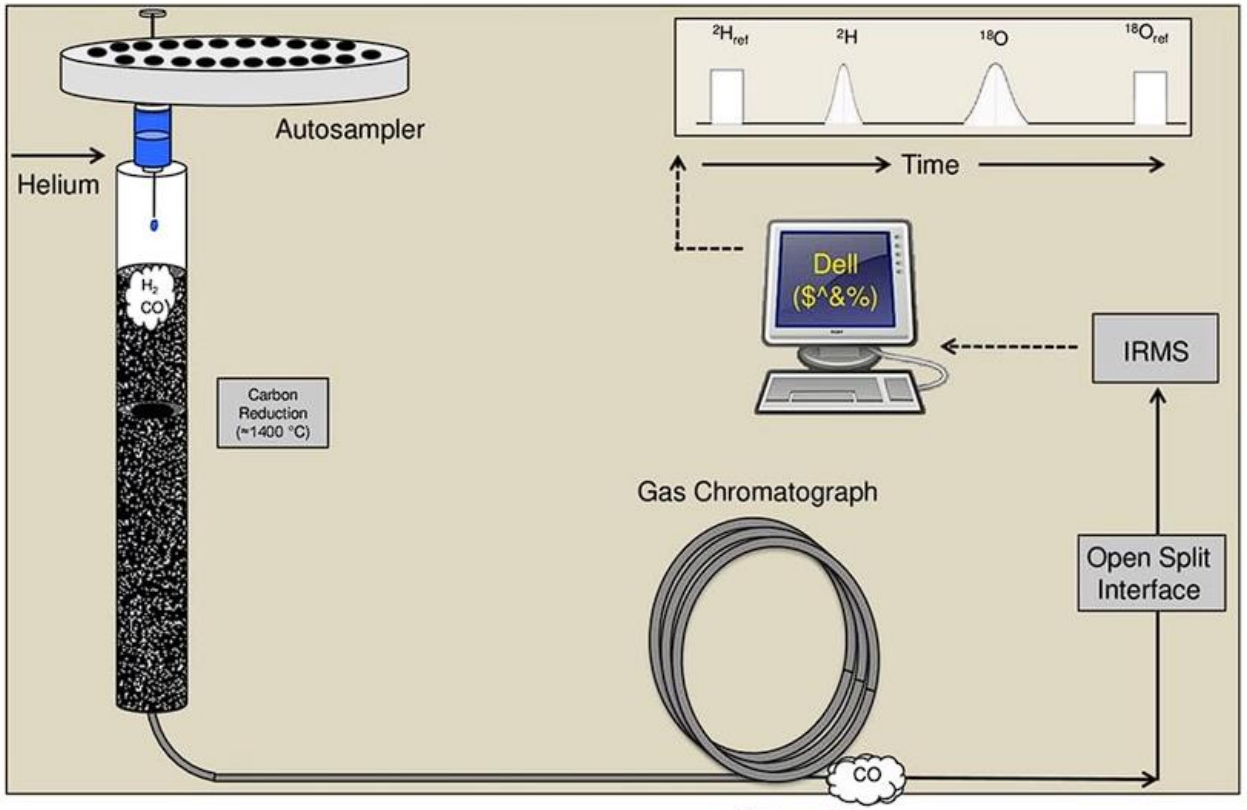
Removal of excess O_2 and reduction of the NOx to N_2 or SOx to SO_2 takes place in a reduction reactor.

Reduction reactor packed with high-purity copper.

The “water trap” contains magnesium perchlorate or phosphorus pentoxide.

EA-IRMS pyrolysis mode

δ^2H, $\delta^{18}O$ via TC/EA-IRMS



Adapted from Rees, 2021

$$CHNO \xrightarrow[\text{carbon}]{1400\,^\circ C} CO + H_2$$

(^{18}O → CO; ^2H → H_2)

High-temperature conversion between 1350 and 1450 °C

Single reactor – partially packed with glassy carbon and silver wool to bind the halogen atoms

Organic and inorganic compounds are converted to H_2, CO

Sample diluter

The concentration of a particular element in our sample is too large for the IRMS measurements.

The high amount of sample can damage the ion source and contaminate the IRMS.

High amount lead to inaccurate results

sample peak outside of the linear range

In food samples, large amounts of CO_2 and small amounts of N_2 or SO_2 are presented!

2 Sample preparation

Sample preparation for EA-IRMS

Plants' material

contains protein, lipids, tissue water & sugars

Honey

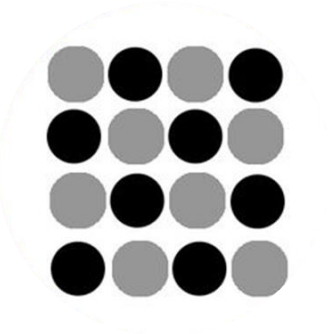
contains proteins, sugars & waters

Meat

contains proteins, lipids, and tissue water

Sample preparation for EA-IRMS

Required **high purity of the samples** (lipids, protein, water,…) since the **whole sample is introduced to the autosampler** (a mixture of components can lead to inaccurate measurements).

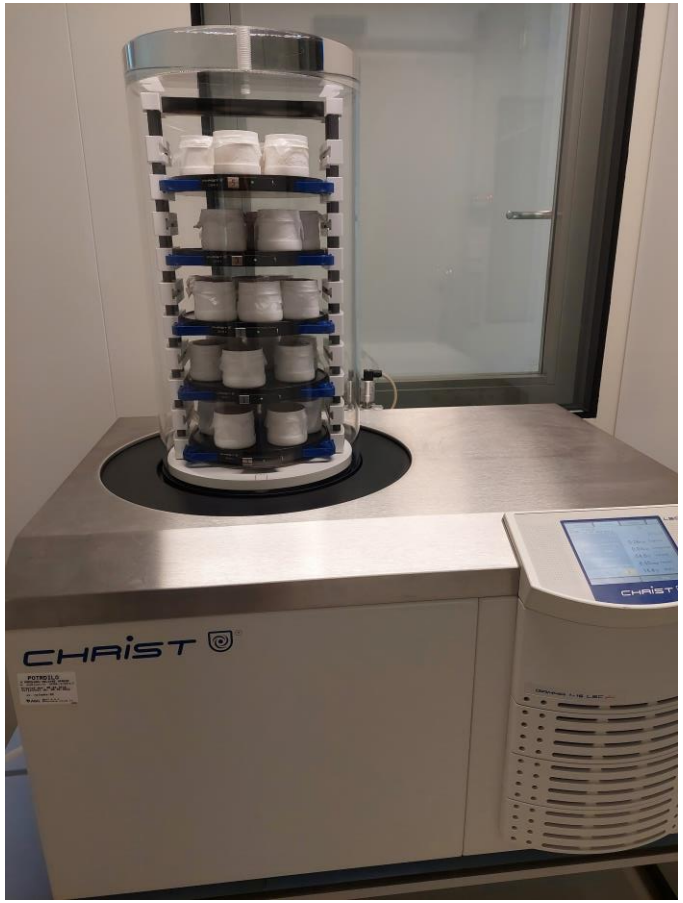


Required high homogeneity of the sample (non-homogeneous sample lead to inaccurate measurements).

Pre-treatment: drying

Depends on the type of the sample

meat, milk, plants, honey, sediments, bones, …



The water fraction needs to be removed to ensure a dry sample

oven or freeze-dryer

Pre-treatment: homogenisation

Depends on the type of the sample

meat, milk, plants, honey, sediments, bones, …

Samples must be turned into a homogenous fine powder

pestle and mortal, ceramic/stainless blade mill

Pre-treatment: extraction

Depends on the type of the sample

meat, milk, plants, honey, sediments, bones, …

Extraction of lipids fraction can be achieved with different solvent

Soxhlet extraction, extraction & homogenisation with Ultra-Turrax devices

Sample preparation: weighing

The sample can be weighted using a spatula into tin or silver capsules

CNS analysis – tin capsules

burns easily and has a lower melting point

OH analysis – silver capsules

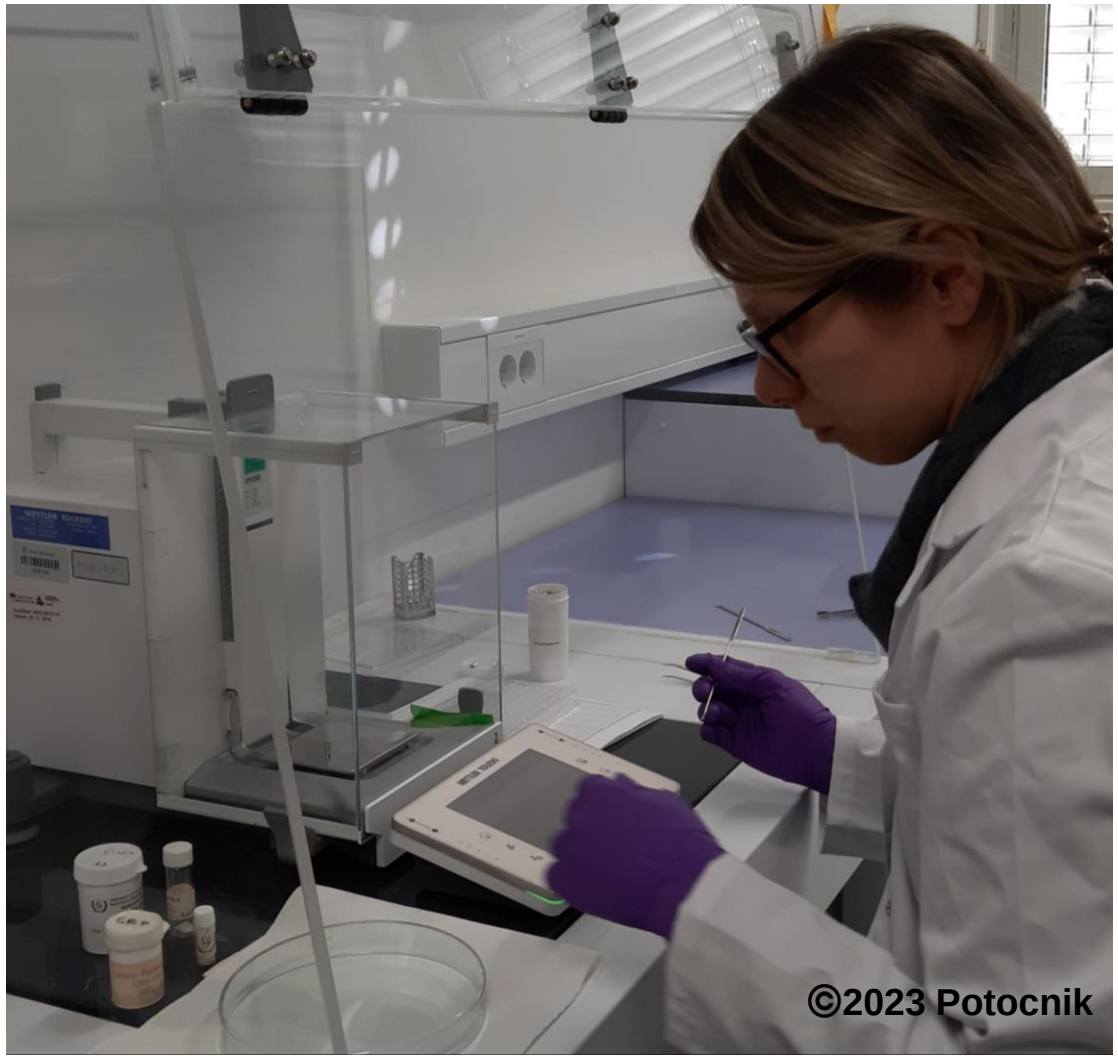
lower oxygen content

Sample preparation: weighing

The weight of the sample should be selected so that the resulting signal intensities are within the linear range of mass spectrometer and EA.

Capsules are sealed and stored

recommended 96 well plate



Sample preparation: liquid samples

Liquid samples

vegetable oils, juice, water solution, EtOH

Pipette into capsules

oven 40 °C during the night

Pipette into tin capsules with chromosorbe

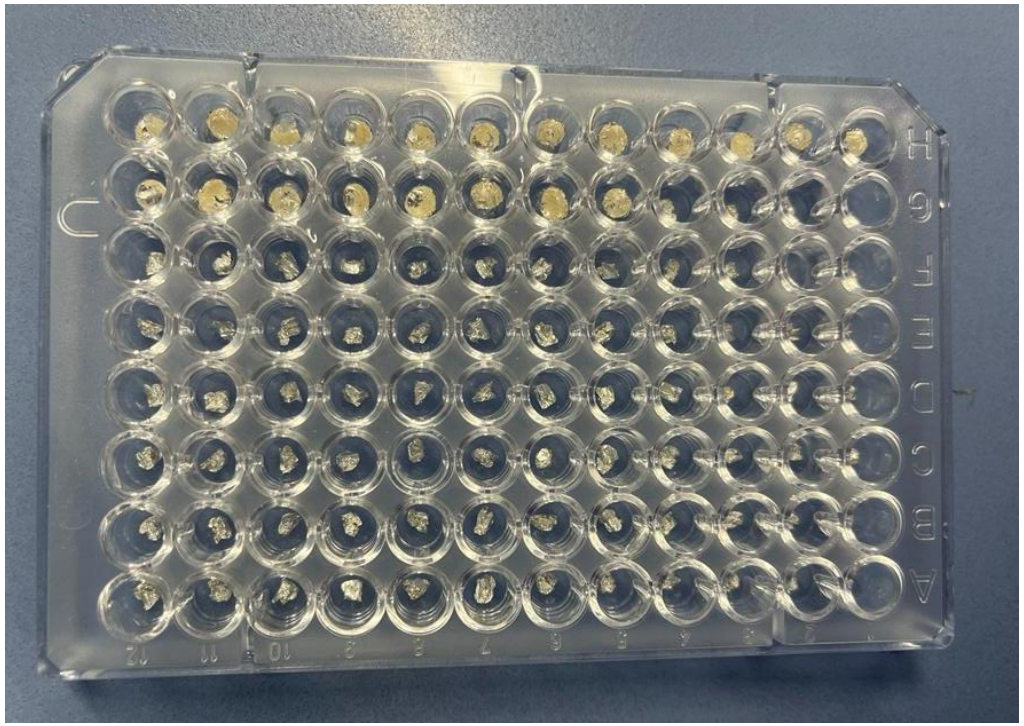
Sample preparation: hydrogen

Organic materials with hydroxyl group will **exchange hydrogen** atoms with atmospheric moisture

resulting in incorrect δ^2H values

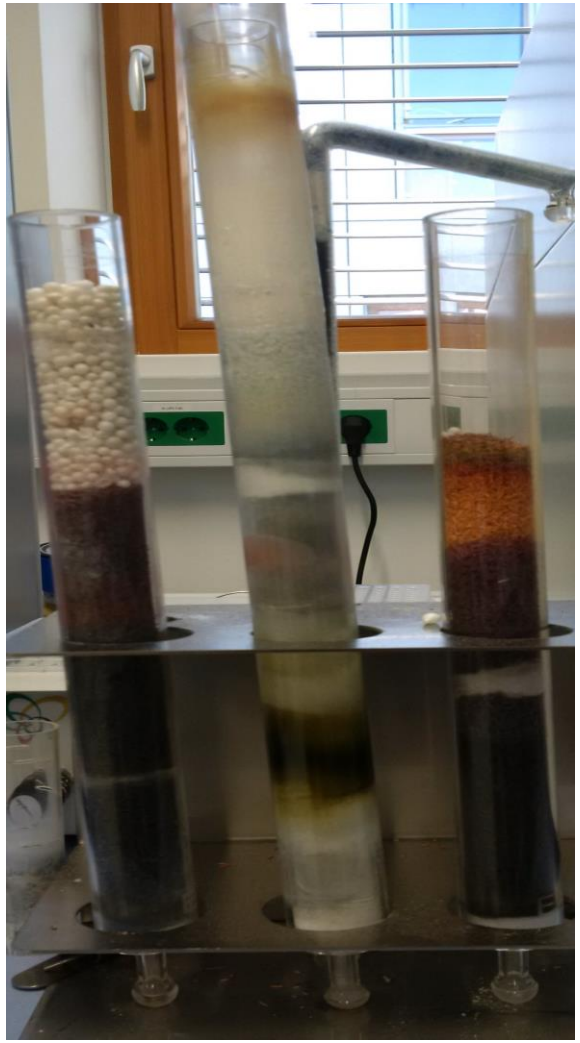
Samples and RM are equilibrated simultaneously with regard to atmospheric moisture

left unsealed in a well plate in a vacuum desiccator



3 Instrument preparation

For reliable functioning of EA



Replacing the oxidation and reduction column

Replacing the ash collector

Cleaning (baking) GC column

On daily basis…

Gas Species	Stability Levels
CO_2	≤ 0.08‰ 1σ on 10 pulses over 3 consecutive runs
N_2	≤ 0.08‰ 1σ on 10 pulses over 3 consecutive runs
H_2	≤ 0.2 ‰ 1σ on 10 pulses over 3 consecutive runs

Stability

Gas Species	Linearity
CO_2	≤ 0.03 ‰/nA
N_2	≤ 0.03 ‰/nA
CO	≤ 0.03 ‰/nA
SO_2	≤ 0.05 ‰/nA
N_2O	≤ 0.03 ‰/nA
H_2	≤ 0.1 ‰/nA

Linearity

When needed…

Tune of the instrument

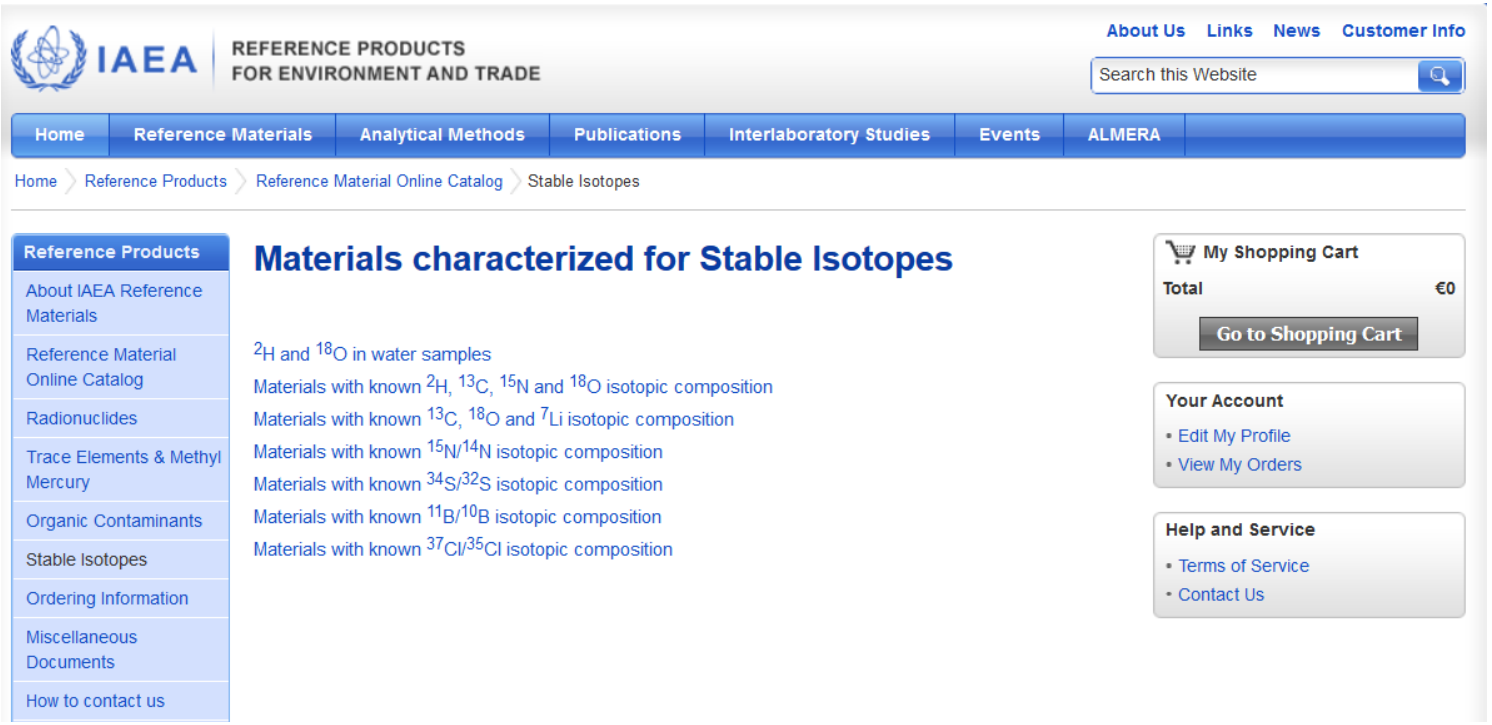
Source Parameter	CO_2	N_2	CO	SO_2	N_2O	H_2
Accelerating Voltage (V)	≈ 3500	≈ 3700	≈ 3700	≈ 2800	≈ 3500	≈ 4800
Extraction Voltage	≤ 75 %	≤ 75 %	≤ 75 %	≤ 75 %	≤ 75 %	≈ 75 %
Half Plate Differential (V)	Tuned for maximum sensitivity					
Z Plate Voltage (V)	Tuned for maximum sensitivity					
Electron Volts (eV)	70 to 100	95 to 100	95 to 100	95 to 100	95 to 100	95 to 100
Ion repeller Voltage (V)	-2 to -10	-2 to -10	-2 to -10	-2 to -10	-2 to -10	≈ +45
Trap Current* (μA)	200-800	200-400	200-600	400-600	≤ 400	400-800

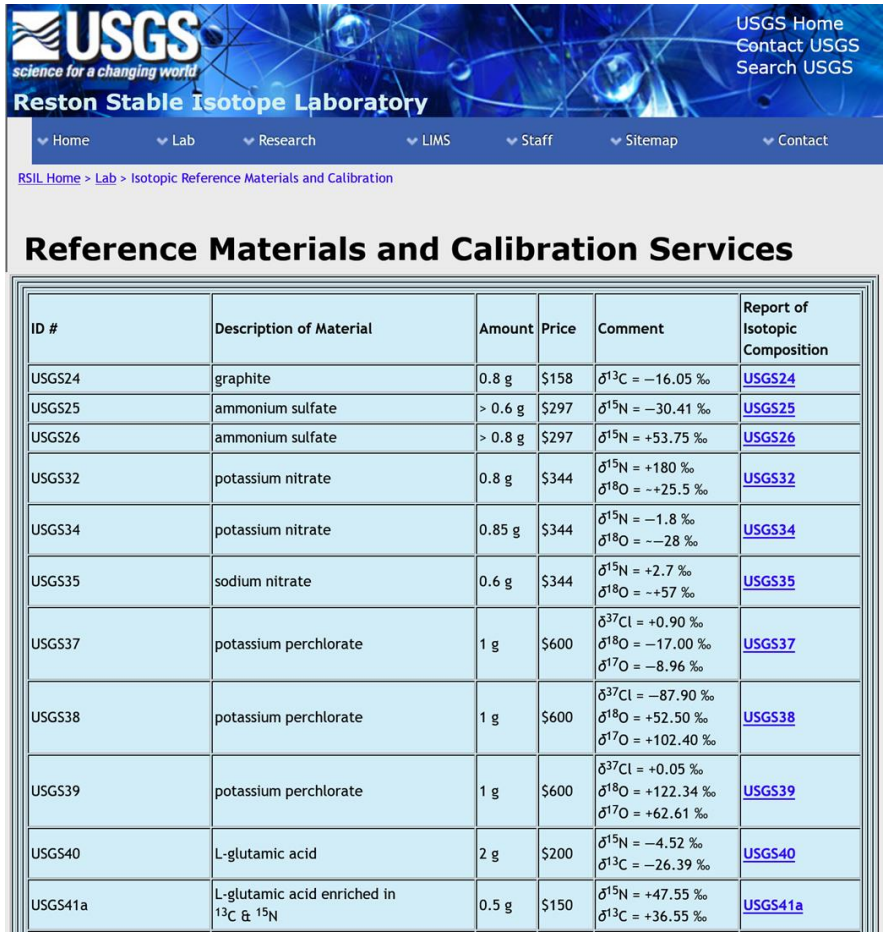
Must be in required ranges

4 Standard selection

Several international distributors

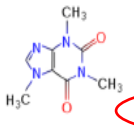
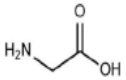
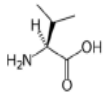
USGS, IAEA, NIST, IRMM,…





ID #	Description of Material	Amount	Price	Comment	Report of Isotopic Composition
USGS24	graphite	0.8 g	$158	$\delta^{13}C$ = −16.05 ‰	USGS24
USGS25	ammonium sulfate	> 0.6 g	$297	$\delta^{15}N$ = −30.41 ‰	USGS25
USGS26	ammonium sulfate	> 0.8 g	$297	$\delta^{15}N$ = +53.75 ‰	USGS26
USGS32	potassium nitrate	0.8 g	$344	$\delta^{15}N$ = +180 ‰ $\delta^{18}O$ = ~+25.5 ‰	USGS32
USGS34	potassium nitrate	0.85 g	$344	$\delta^{15}N$ = −1.8 ‰ $\delta^{18}O$ = ~−28 ‰	USGS34
USGS35	sodium nitrate	0.6 g	$344	$\delta^{15}N$ = +2.7 ‰ $\delta^{18}O$ = ~+57 ‰	USGS35
USGS37	potassium perchlorate	1 g	$600	$\delta^{37}Cl$ = +0.90 ‰ $\delta^{18}O$ = −17.00 ‰ $\delta^{17}O$ = −8.96 ‰	USGS37
USGS38	potassium perchlorate	1 g	$600	$\delta^{37}Cl$ = −87.90 ‰ $\delta^{18}O$ = +52.50 ‰ $\delta^{17}O$ = +102.40 ‰	USGS38
USGS39	potassium perchlorate	1 g	$600	$\delta^{37}Cl$ = +0.05 ‰ $\delta^{18}O$ = +122.34 ‰ $\delta^{17}O$ = +62.61 ‰	USGS39
USGS40	L-glutamic acid	2 g	$200	$\delta^{15}N$ = −4.52 ‰ $\delta^{13}C$ = −26.39 ‰	USGS40
USGS41a	L-glutamic acid enriched in ^{13}C & ^{15}N	0.5 g	$150	$\delta^{15}N$ = +47.55 ‰ $\delta^{13}C$ = +36.55 ‰	USGS41a

Inconsistent isotopic data

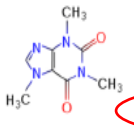
Reference ID	Chemical name	Structure or composition of material	Ring-test reference values with combined standard uncertainties (mUr or ‰) $\delta^2H_{VSMOW-SLAP}$	$\delta^{13}C_{VPDB-LSVEC}$	$\delta^{15}N_{Air}$
USGS61	caffeine		+96.9 ± 0.9	–35.05 ± 0.04	–2.87 ± 0.04
USGS62	caffeine		–156.1 ± 2.1	–14.79 ± 0.04	+20.17 ± 0.06
USGS63	caffeine		+174.5 ± 0.9	–1.17 ± 0.04	+37.83 ± 0.06
IAEA-600*	caffeine		–156.1 ± 1.3	–27.73 ± 0.04	+1.02 ± 0.05
USGS64	glycine		*no values indicated due to the presence of exchangeable hydrogen*	–40.81 ± 0.04	+1.76 ± 0.06
USGS65	glycine			–20.29 ± 0.04	+20.68 ± 0.06
USGS66	glycine			–0.67 ± 0.04	+40.83 ± 0.06
USGS67	*n*-hexadecane	$C_{16}H_{34}$	–166.2 ± 1.0	–34.50 ± 0.05	n.a.
USGS68	*n*-hexadecane		–10.2 ± 0.9	–10.55 ± 0.04	n.a.
USGS69	*n*-hexadecane		+381.4 ± 3.5	-0.57 ± 0.04	n.a.
USGS70	icosanoic acid methyl ester (C_{20} FAME)	$C_{20}H_{39}OOCH_3$	–183.9 ± 1.4	–30.53 ± 0.04	n.a.
USGS71	icosanoic acid methyl ester (C_{20} FAME)		–4.9 ± 1.0	–10.50 ± 0.03	n.a.
USGS72	icosanoic acid methyl ester (C_{20} FAME)		+348.3 ± 1.5	–1.54 ± 0.03	n.a.
USGS73	L-valine		*no values indicated due to the presence of exchangeable hydrogen*	–24.03 ± 0.04	–5.21 ± 0.05
USGS74	L-valine			–9.30 ± 0.04	+30.19 ± 0.07
USGS75	L-valine			+0.49 ± 0.07	+61.53 ± 0.14
USGS76	methylheptadecanoate (C_{17} FAME)	$C_{17}H_{33}OOCH_3$	–210.8 ± 0.9	–31.36 ± 0.04	n.a.
IAEA-CH-7*	polyethylene foil	$(C_2H_4)_n$	–99.2 ± 1.2	–32.14 ± 0.05	n.a.
USGS77	polyethylene powder (also extruded string)	$(C_2H_4)_n$	–75.9 ± 0.6	–30.71 ± 0.04	n.a.
NBS 22*	oil	n.a.	–117.2 ± 0.6	–30.02 ± 0.04	n.a.
NBS 22a	vacuum oil, regular	n.a.	–120.4 ± 1.0	–29.72 ± 0.04	n.a.
USGS78	vacuum oil, ^2H-enriched	n.a.	+397.0 ± 2.2	–29.72 ± 0.04	n.a.

Define new values of pre-existing RMs (Schimmellman et al., 2016)

caffeine & polyethylene foil

http://dx.doi.org/10.1021/acs.analchem.5b04392

Inconsistent isotopic data

Reference ID	Chemical name	Structure or composition of material	Ring-test reference values with combined standard uncertainties (mUr or ‰) $\delta^2H_{VSMOW-SLAP}$	$\delta^{13}C_{VPDB-LSVEC}$	$\delta^{15}N_{Air}$
USGS61	caffeine		+96.9 ± 0.9	–35.05 ± 0.04	–2.87 ± 0.04
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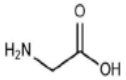
IAEA-600*	caffeine		–156.1 ± 1.3	–27.73 ± 0.04	+1.02 ± 0.05
USGS64	glycine		*no values indicated due to the presence of exchangeable hydrogen*	–40.81 ± 0.04	+1.76 ± 0.06
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USGS67	*n*-hexadecane	$C_{16}H_{34}$	–166.2 ± 1.0	–34.50 ± 0.05	n.a.
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USGS69	*n*-hexadecane		+381.4 ± 3.5	-0.57 ± 0.04	n.a.
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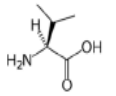
USGS73	L-valine		*no values indicated due to the presence of exchangeable hydrogen*	–24.03 ± 0.04	–5.21 ± 0.05
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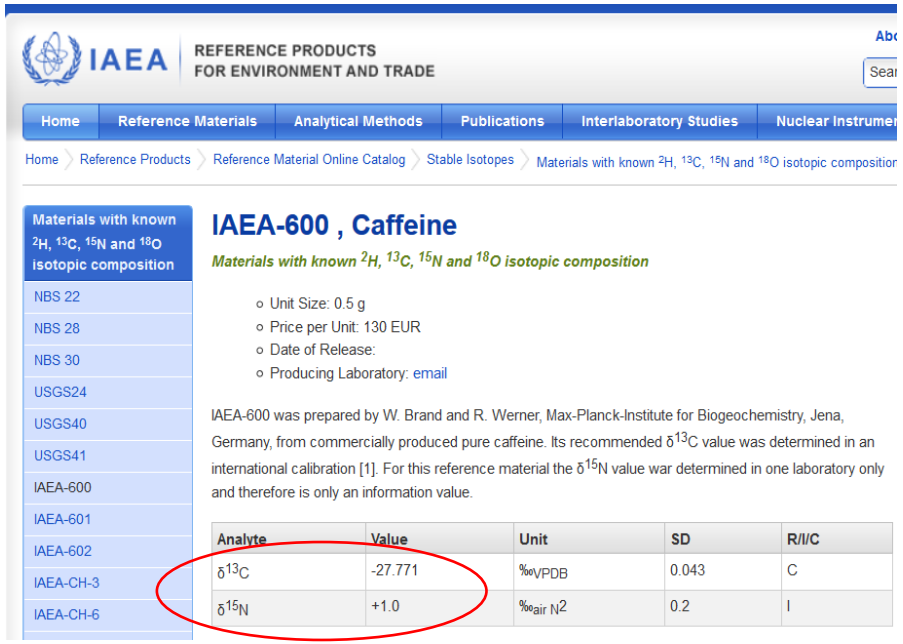
NBS 22*	oil	n.a.	–117.2 ± 0.6	–30.02 ± 0.04	n.a.
NBS 22a	vacuum oil, regular	n.a.	–120.4 ± 1.0	–29.72 ± 0.04	n.a.
USGS78	vacuum oil, ^2H-enriched	n.a.	+397.0 ± 2.2	–29.72 ± 0.04	n.a.

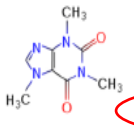
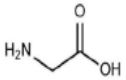
Define new values of pre-existing RMs (Schimmellman et al., 2016)

caffeine & polyethylene foil



http://dx.doi.org/10.1021/acs.analchem.5b04392

Inconsistent isotopic data

Reference ID	Chemical name	Structure or composition of material	Ring-test reference values with combined standard uncertainties (mUr or ‰) $\delta^2H_{VSMOW-SLAP}$	$\delta^{13}C_{VPDB-LSVEC}$	$\delta^{15}N_{Air}$
USGS61	caffeine		+96.9 ± 0.9	−35.05 ± 0.04	−2.87 ± 0.04
USGS62	caffeine		−156.1 ± 2.1	−14.79 ± 0.04	+20.17 ± 0.06
USGS63	caffeine		+174.5 ± 0.9	−1.17 ± 0.04	+37.83 ± 0.06
IAEA-600*	caffeine		−156.1 ± 1.3	−27.73 ± 0.04	+1.02 ± 0.05
USGS64	glycine		*no values indicated due to the presence of exchangeable hydrogen*	−40.81 ± 0.04	+1.76 ± 0.06
USGS65	glycine			−20.29 ± 0.04	+20.68 ± 0.06
USGS66	glycine			−0.67 ± 0.04	+40.83 ± 0.06
USGS67	*n*-hexadecane	$C_{16}H_{34}$	−166.2 ± 1.0	−34.50 ± 0.05	n.a.
USGS68	*n*-hexadecane		−10.2 ± 0.9	−10.55 ± 0.04	n.a.
USGS69	*n*-hexadecane		+381.4 ± 3.5	-0.57 ± 0.04	n.a.
USGS70	icosanoic acid methyl ester (C_{20} FAME)	$C_{20}H_{39}OOCH_3$	−183.9 ± 1.4	−30.53 ± 0.04	n.a.
USGS71	icosanoic acid methyl ester (C_{20} FAME)		−4.9 ± 1.0	−10.50 ± 0.03	n.a.
USGS72	icosanoic acid methyl ester (C_{20} FAME)		+348.3 ± 1.5	−1.54 ± 0.03	n.a.
USGS73	L-valine		*no values indicated due to the presence of exchangeable hydrogen*	−24.03 ± 0.04	−5.21 ± 0.05
USGS74	L-valine			−9.30 ± 0.04	+30.19 ± 0.07
USGS75	L-valine			+0.49 ± 0.07	+61.53 ± 0.14
USGS76	methylheptadecanoate (C_{17} FAME)	$C_{17}H_{33}OOCH_3$	−210.8 ± 0.9	−31.36 ± 0.04	n.a.
IAEA-CH-7*	polyethylene foil	$(C_2H_4)_n$	−99.2 ± 1.2	−32.14 ± 0.05	n.a.
USGS77	polyethylene powder (also extruded string)	$(C_2H_4)_n$	−75.9 ± 0.6	−30.71 ± 0.04	n.a.
NBS 22*	oil	n.a.	−117.2 ± 0.6	−30.02 ± 0.04	n.a.
NBS 22a	vacuum oil, regular	n.a.	−120.4 ± 1.0	−29.72 ± 0.04	n.a.
USGS78	vacuum oil, ^2H-enriched	n.a.	+397.0 ± 2.2	−29.72 ± 0.04	n.a.

Define new values of pre-existing RMs (Schimmelmann et al., 2016)

caffeine & polyethylene foil

You need to explain which RMs were used and which δ values were assigned to each RM.

http://dx.doi.org/10.1021/acs.analchem.5b04392

Standard selection

USGS88 $\delta^{13}C$ = -16.06 ± 0.14 ‰
collagen

IAEA-600 $\delta^{13}C$ = -27.71 ± 0.04 ‰
caffeine

USGS62 $\delta^{13}C$ = -14.79 ± 0.04 ‰
caffeine

BCR-656 $\delta^{13}C$ = -26.91 ± 0.07 ‰
ethanol

USGS91 $\delta^{13}C$ = -28.28 ± 0.08 ‰
rice flour

IAEA-CH-3 $\delta^{13}C$ = -24.72 ± 0.04 ‰
cellulose

IAEA-CH-6 $\delta^{13}C$ = -10.45 ± 0.03 ‰
sucrose

The chemical nature of the sample should be identical to that of the reference material.

At least 2 isotopic RMs with contrasting isotopic compositions.

RMs should bracket the δ value that you expect from your unknown sample.

5 Sequence preparation

Data processing – batch design

Position	Sample name
1	Dummy
2	Dummy
3	Dummy
4	Dummy
5	RM-1
6	RM-1
7	RM-2
8	RM-2
9	QC
10	QC
11	Sample 1
12	Sample 1
13	Sample 2
14	Sample 2
:	:
:	:
48	Sample 19
49	Sample 19
50	QC
51	QC
52	RM-2
53	RM-2
54	RM-1
55	RM-1

Blanks and dummy

at the beginning for condition purposes

RM for data normalisation

at least 2 different RMs at the beginning and at the end of the sequences

QC material

analysed periodically throughout the sequences

6 Drift correction

Data processing – drift correction

During an analytical sequence measured *δ* values can change:

- because of the changes in the isotopic composition of WG
- subtle changes within the ion source

The degree of drift should be determined by:

- analysis of QC or RMs at the beginning and at the end of the sequences

Data processing – drift correction

How calculated a drift correction?

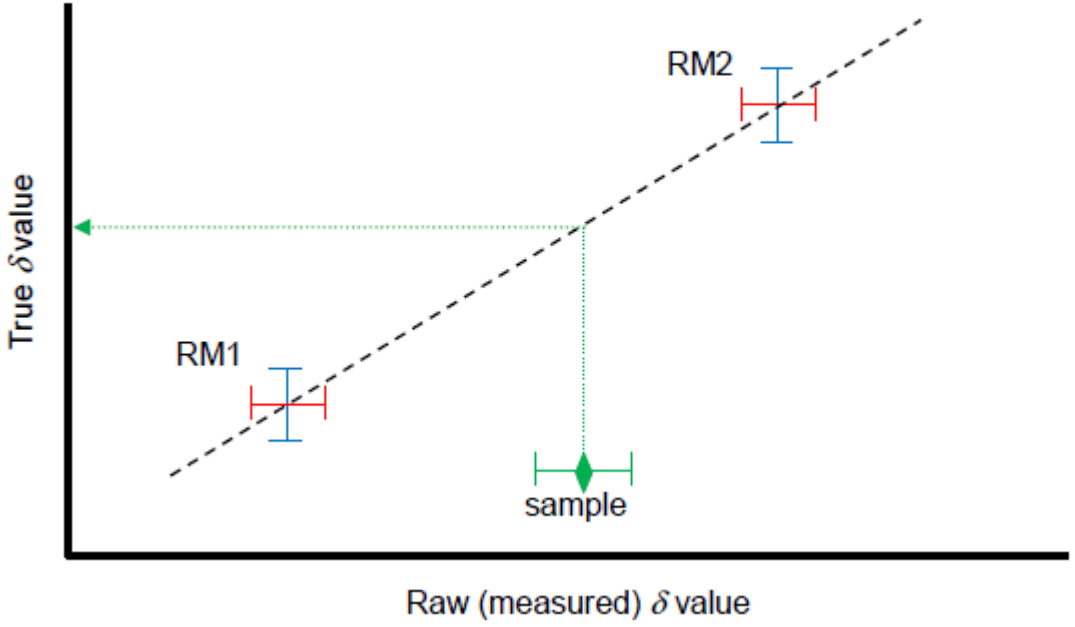
$$\delta_{drift\ corr} = \delta_{meas} - m \times position$$

$\delta_{\text{drift corr}}$	drift corrected δ value of the sample
δ_{meas}	determined (raw) δ value of the sample
m	*slope of linear drift curve (plot of* δ value versus autosampler position)
position	autosampler position within the sequences (assuming this is proxy of time)

7 Data normalisation

Data processing – normalisation

The true δ values when using **2 or more RMs** can be calculated using the following equation

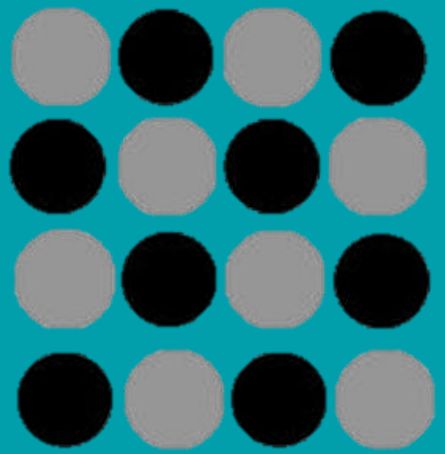


$$\delta_{true\,(Spl)} = m \times \delta_{raw\,(Spl)} + b$$

m **–** the slope of the regression line (“expansion factor”)

b **–** the intercept (“shift factor”)

Recommendations
for isotope community



High purity and homogeneity of the sample is required.

Correct selection and use of RMs.

Signal intensities of samples and RMs are within the linear range.

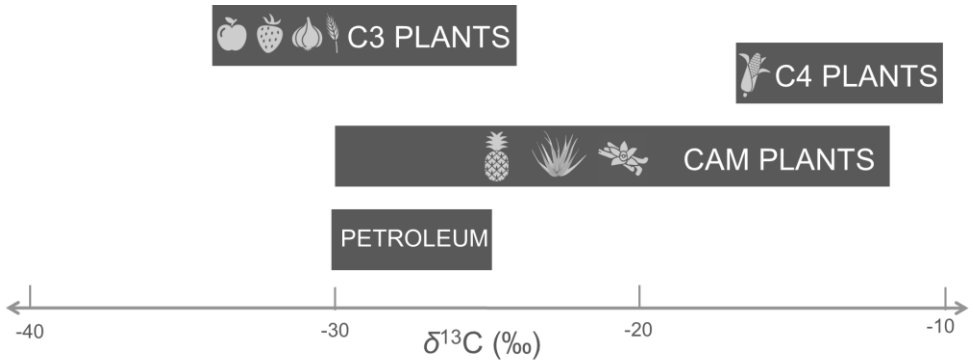
Appropriate data normalisation provide reproducible and accurate results.

GC-IRMS

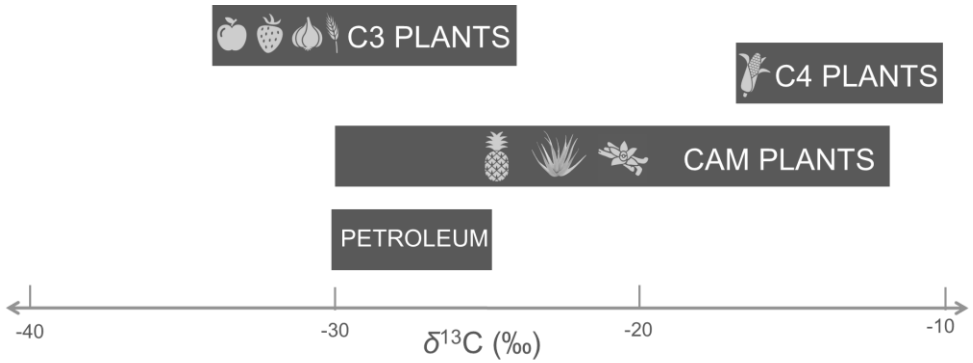
Lidija Strojnik
Jožef Stefan Institute

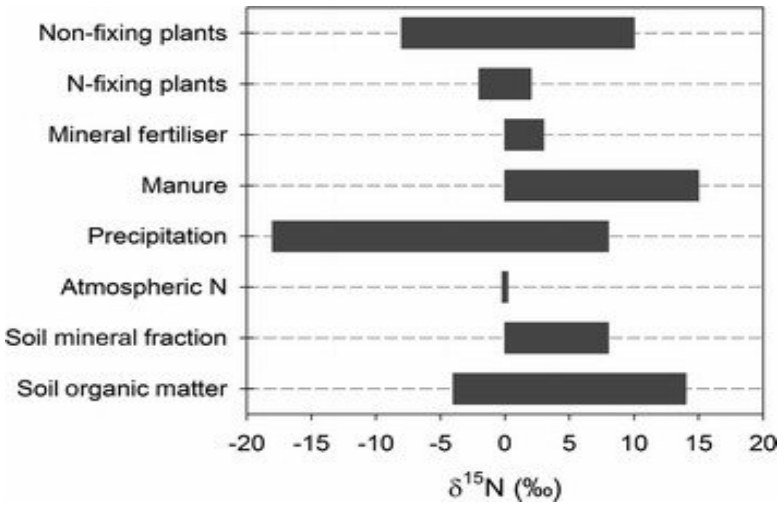
TUNTWIN Summer School, April 18-20, 2023

C N O H isotope as tracer



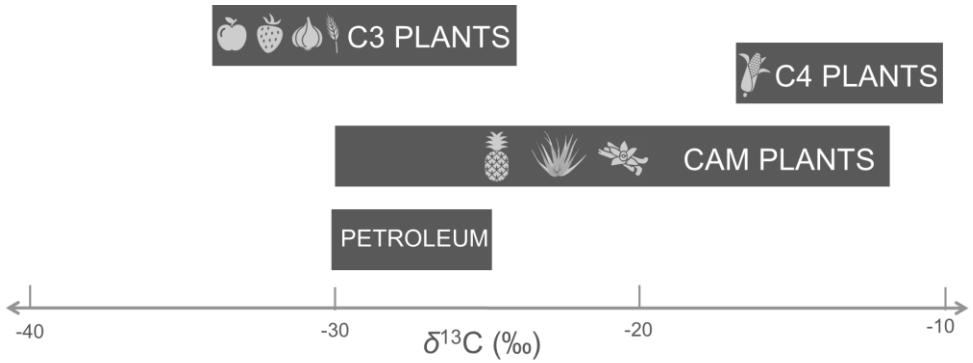
C N O H isotope as tracer

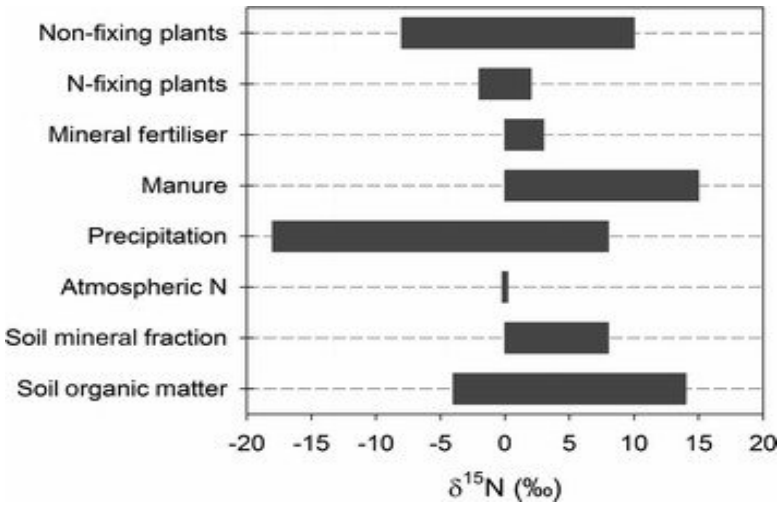




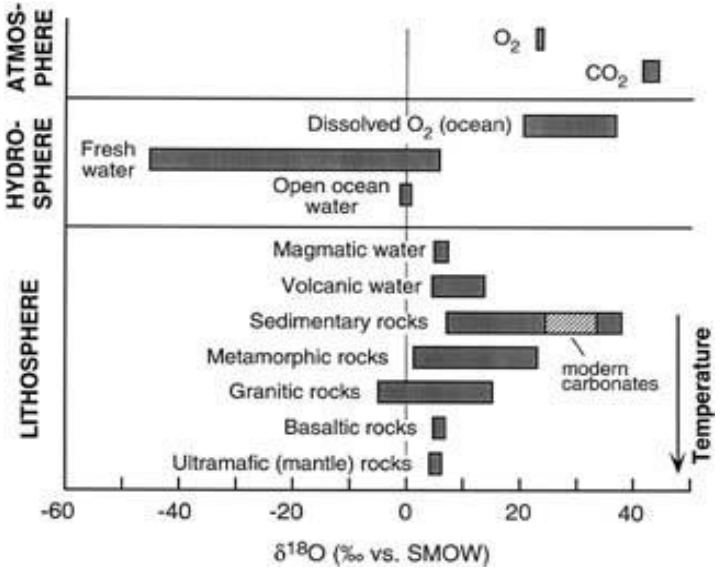
Fiorentino et al., 2014: DOI 10.1007/s00334-014-0492-9

C N O H isotope as tracer



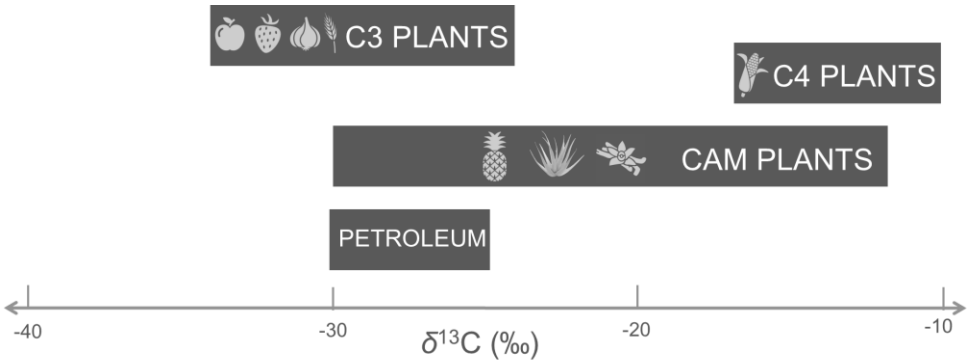


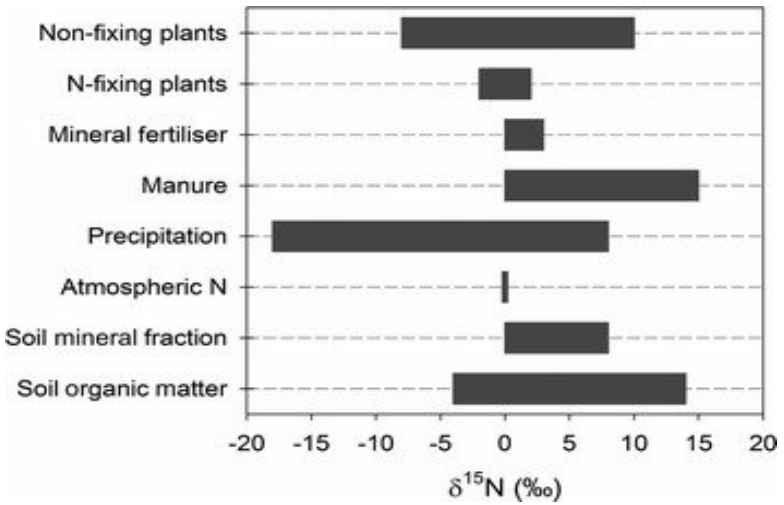
Fiorentino et al., 2014: DOI 10.1007/s00334-014-0492-9



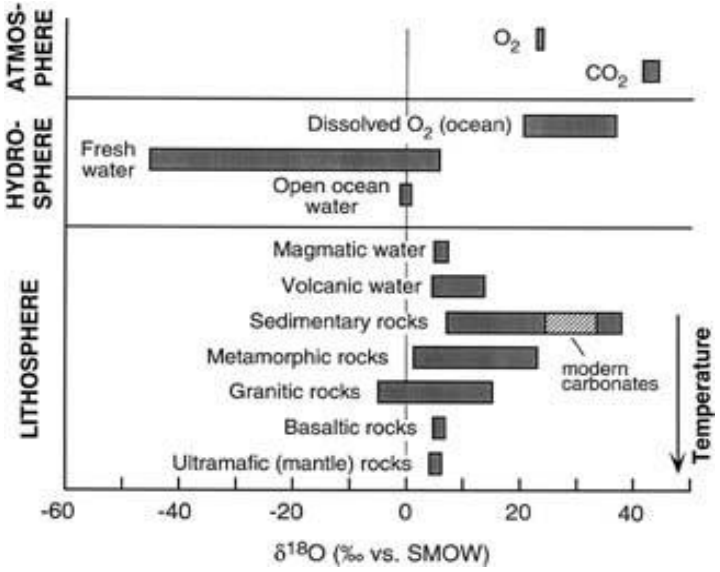
DOI: 10.1007/1-4020-4496-8_234

C N O H isotope as tracer





Fiorentino et al., 2014: DOI 10.1007/s00334-014-0492-9



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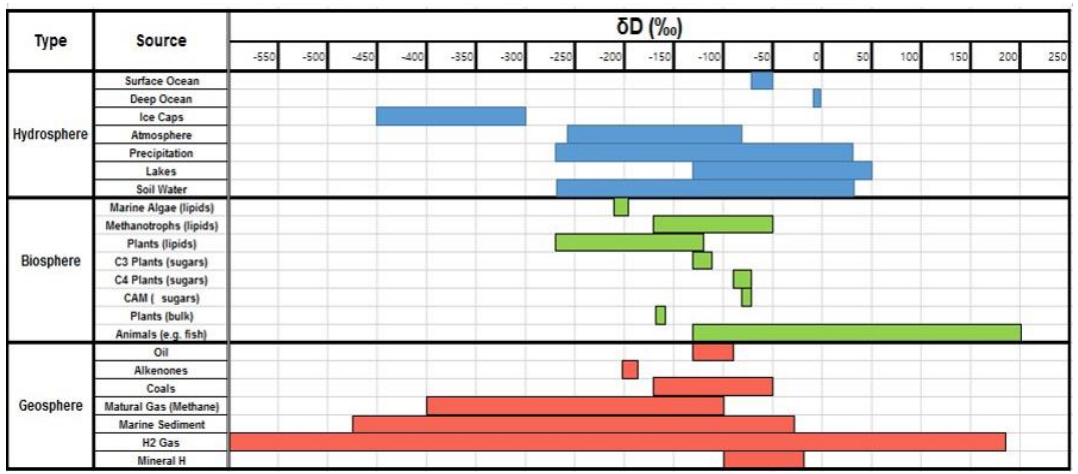
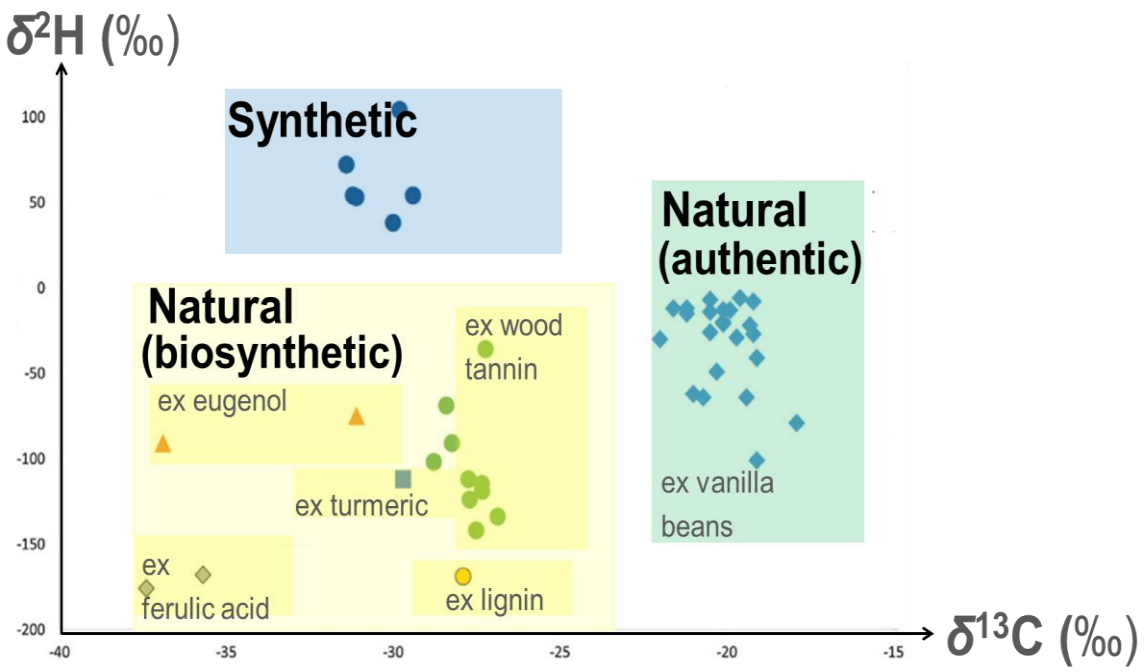
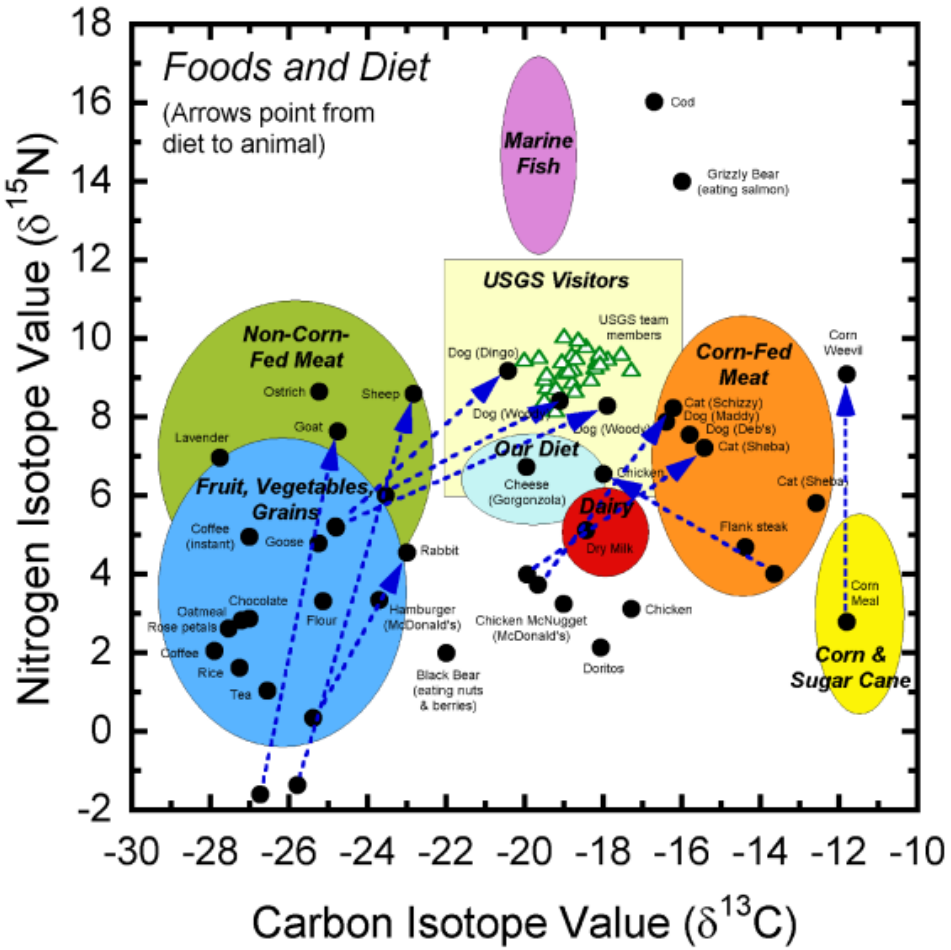


https://www.wikiwand.com/en/Hydrogen_isotope_biogeochemistry#Media/File:DD_Various_Sources.jpg

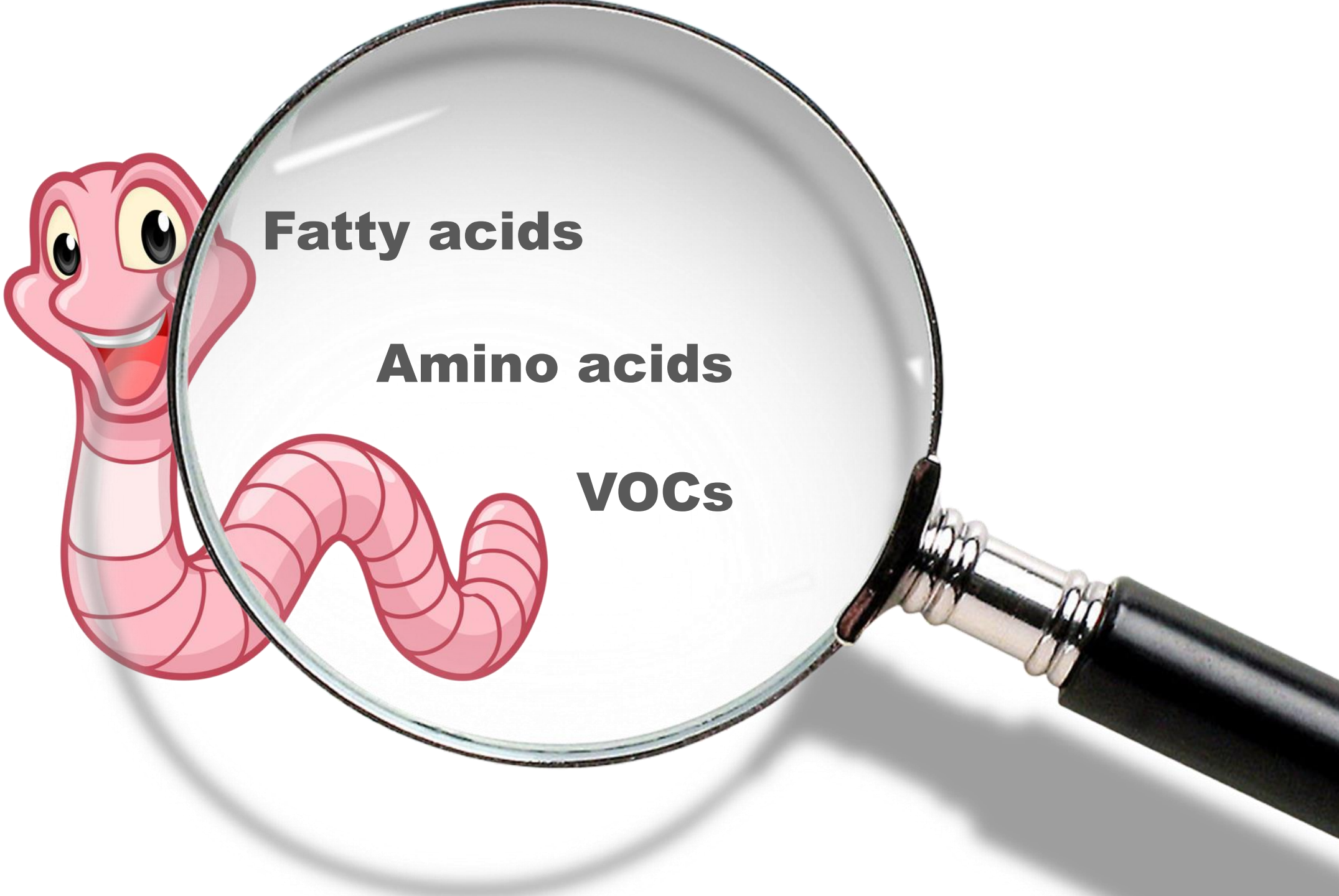
Combination of two or more isotopes may help





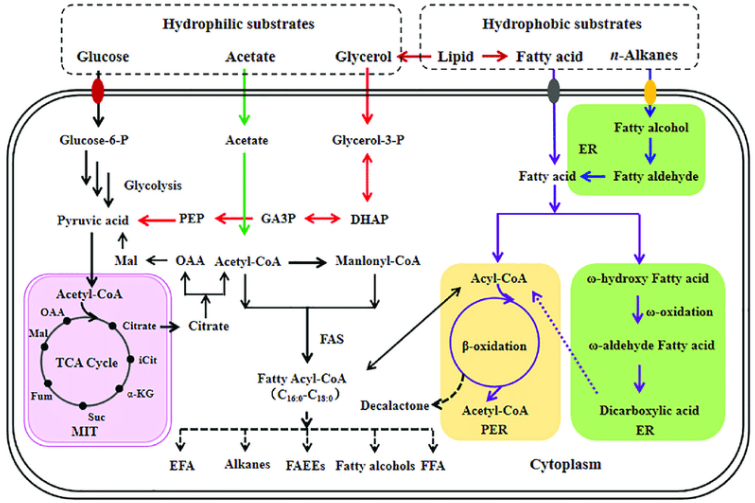
https://wwwrcamnl.wr.usgs.gov


Not working?


Fatty acids
Amino acids
VOCs

Metabolic pathways

Fatty acid metabolism



DOI: 10.3389/fmicb.2020.608662

Amino acid metabolism

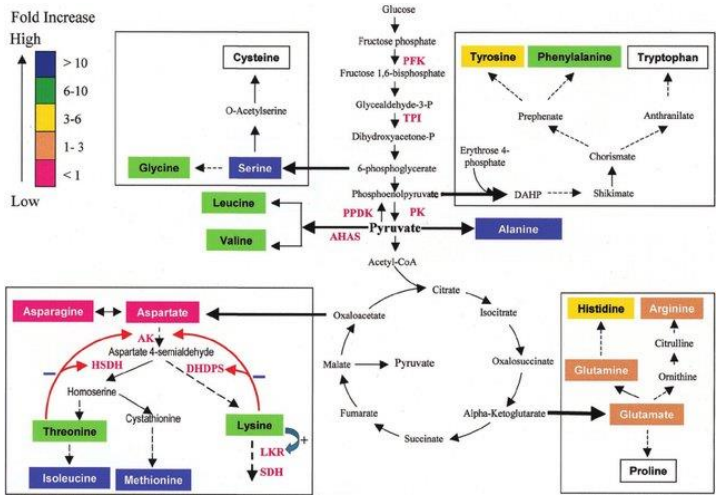


Figure adapted by Brian Larkins,
DOI: 10.1104/pp.125.4.1766

VOCs biosynthesis

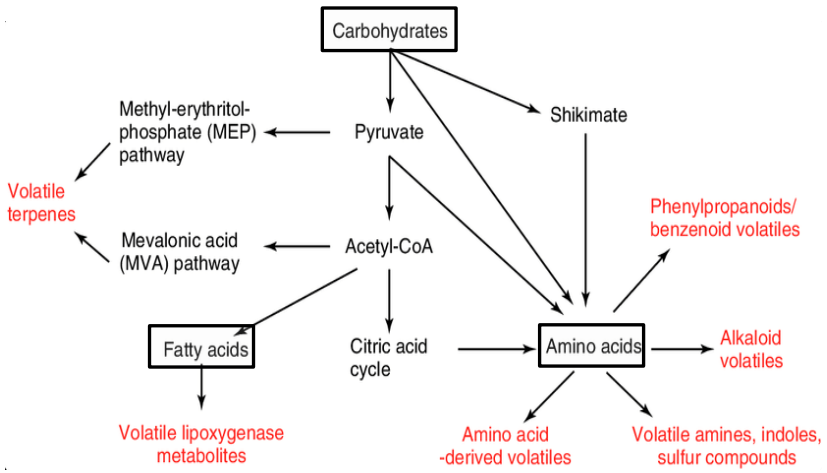
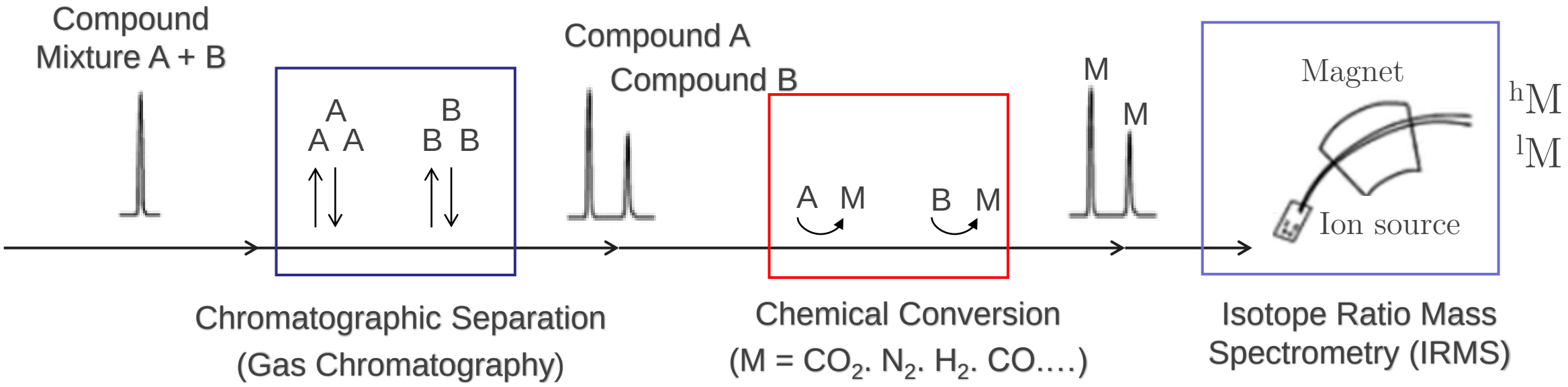
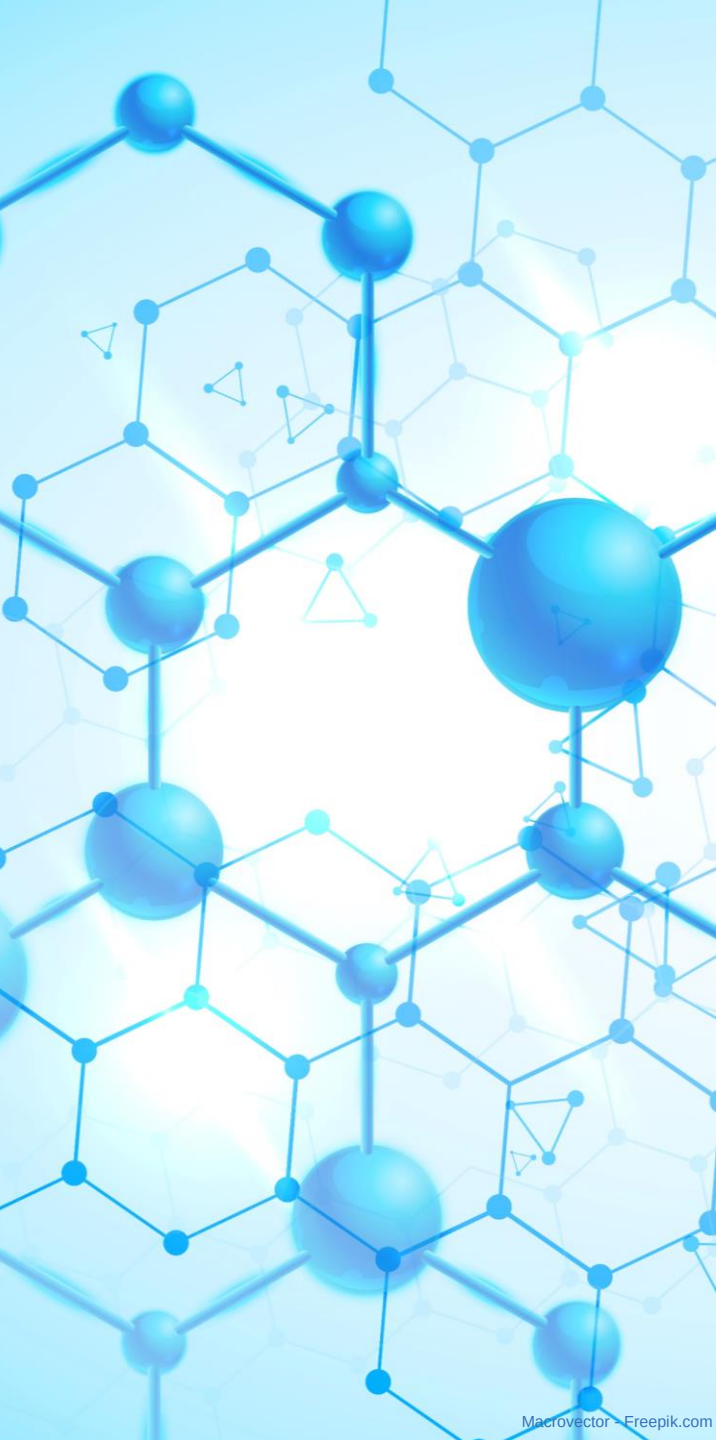


Figure adapted by Jörg-Peter Schnitzler,
DOI: 10.1002/9780470015902.a0000910.pub3

“Compound specific” GC-C/P-IRMS





When compound specific?

Complex materials or mixtures

Isotopic information accessible at the biochemical building-block

Linking molecular structure-stable isotope composition- source or process

At present, CSIA is the most specific and sophisticated method for determining food authenticity.

But...

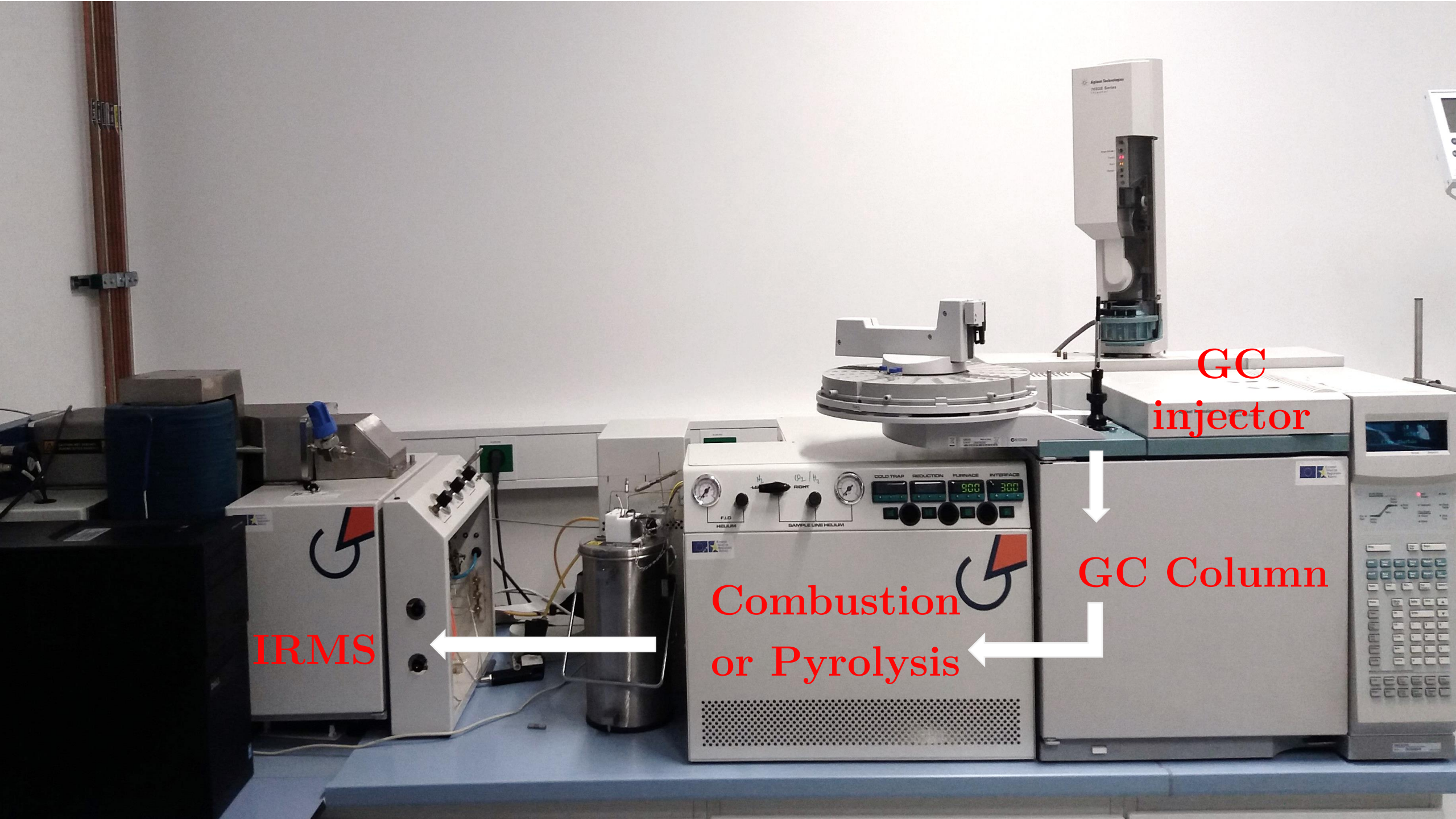
To obtain precise isotope ratios of analytes in complex sample matrices:

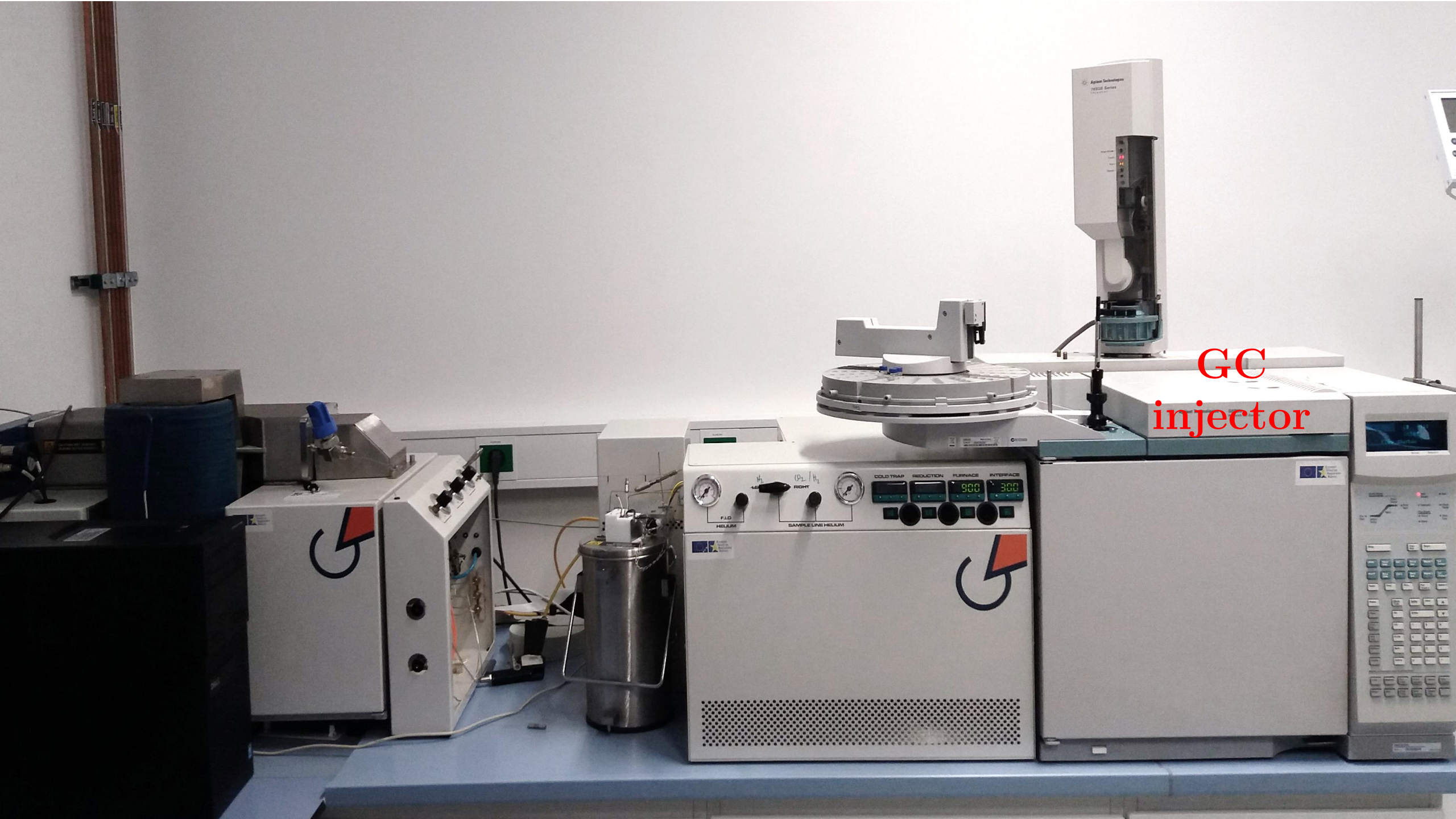
- Extensive method development
- Optimal instrument conditions
- Stability and linearity
- Tuning the injection volume
- Preventing carryover
- Appropriate RM and data normalisation

Don’t panic!

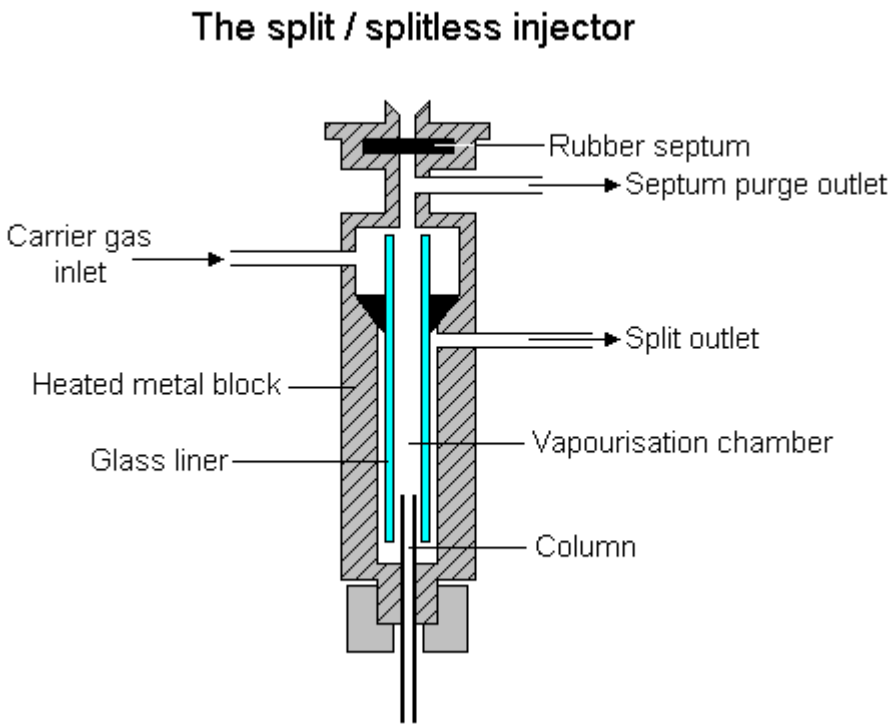

You will acquire new knowledge and skills

1 Basic principle


GC
injector
GC Column
Combustion
or Pyrolysis
IRMS


GC
injector
COLD TRAP
REDUCTION
FURNACE
INTERFACE
900
300
F.I.D
HELIUM
SAMPLE LINE HELIUM

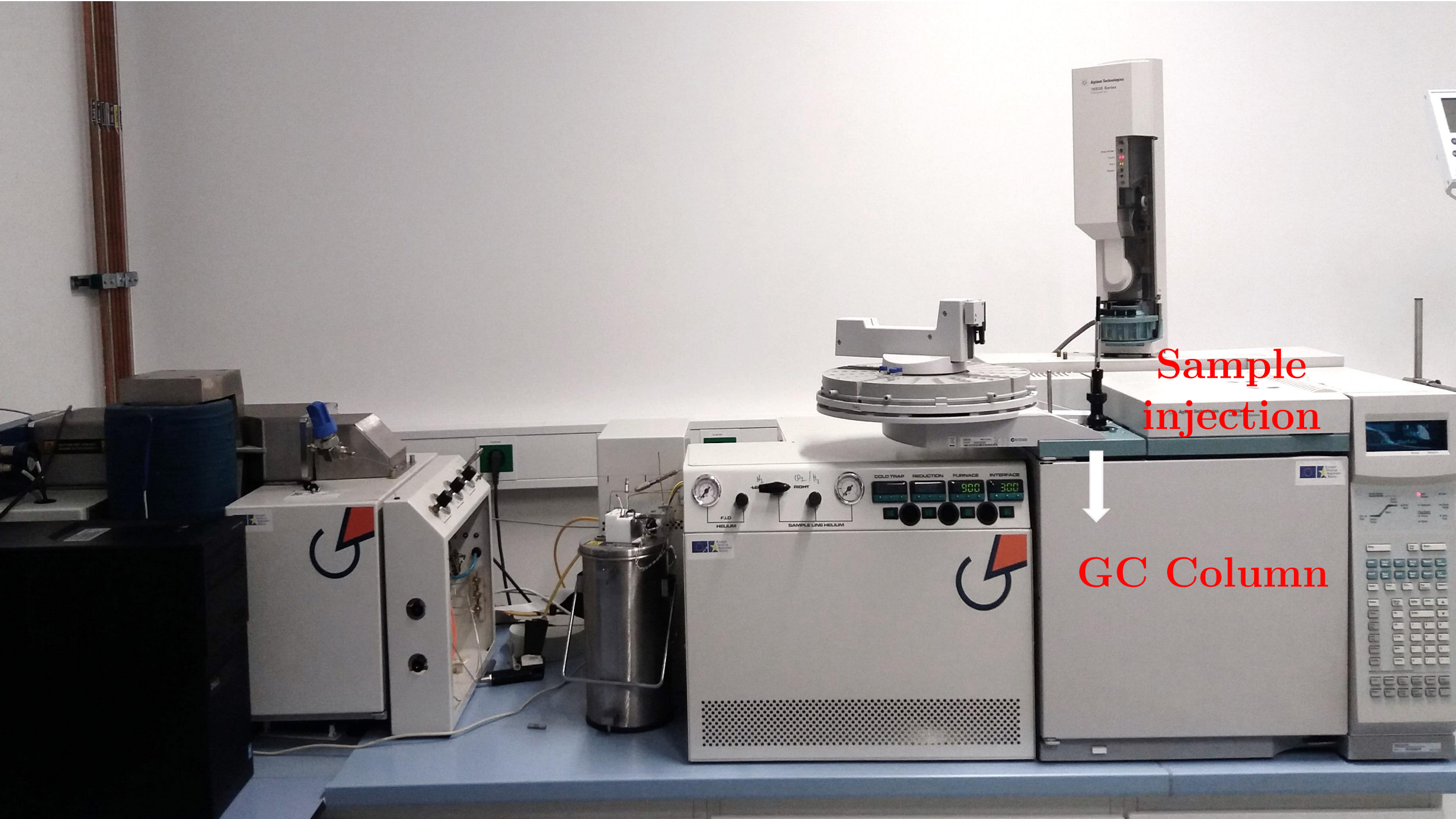
GC injector



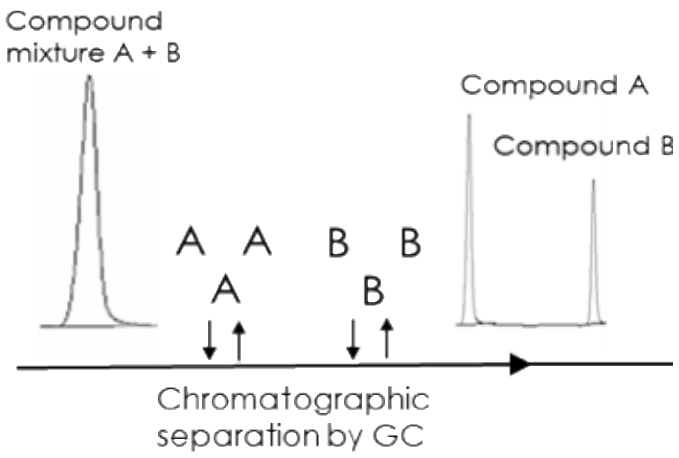
The injection port is where the liquid sample is injected, vaporised and transported onto the capillary column by the carrier gas.

It can be used in one of two modes:

split or splitless.

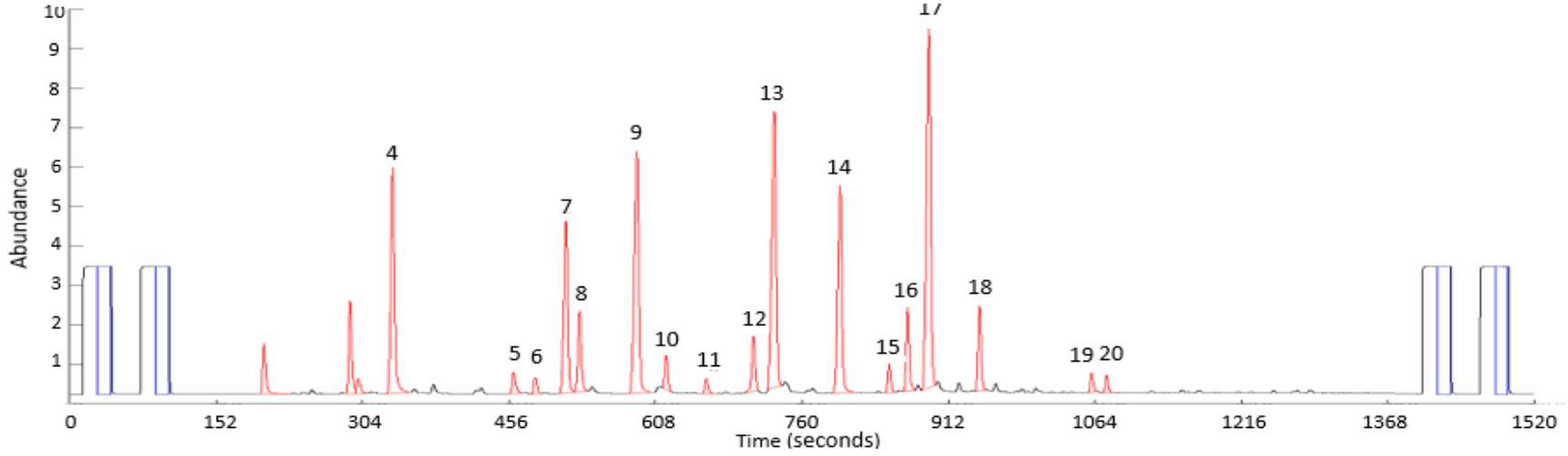

Sample
injection
GC Column

GC column



Chromatographic separation is of key importance in GC-IRMS.

Minor co-elutions and peak telling significantly influence on the correctness of delta value of a given compound.



GC column

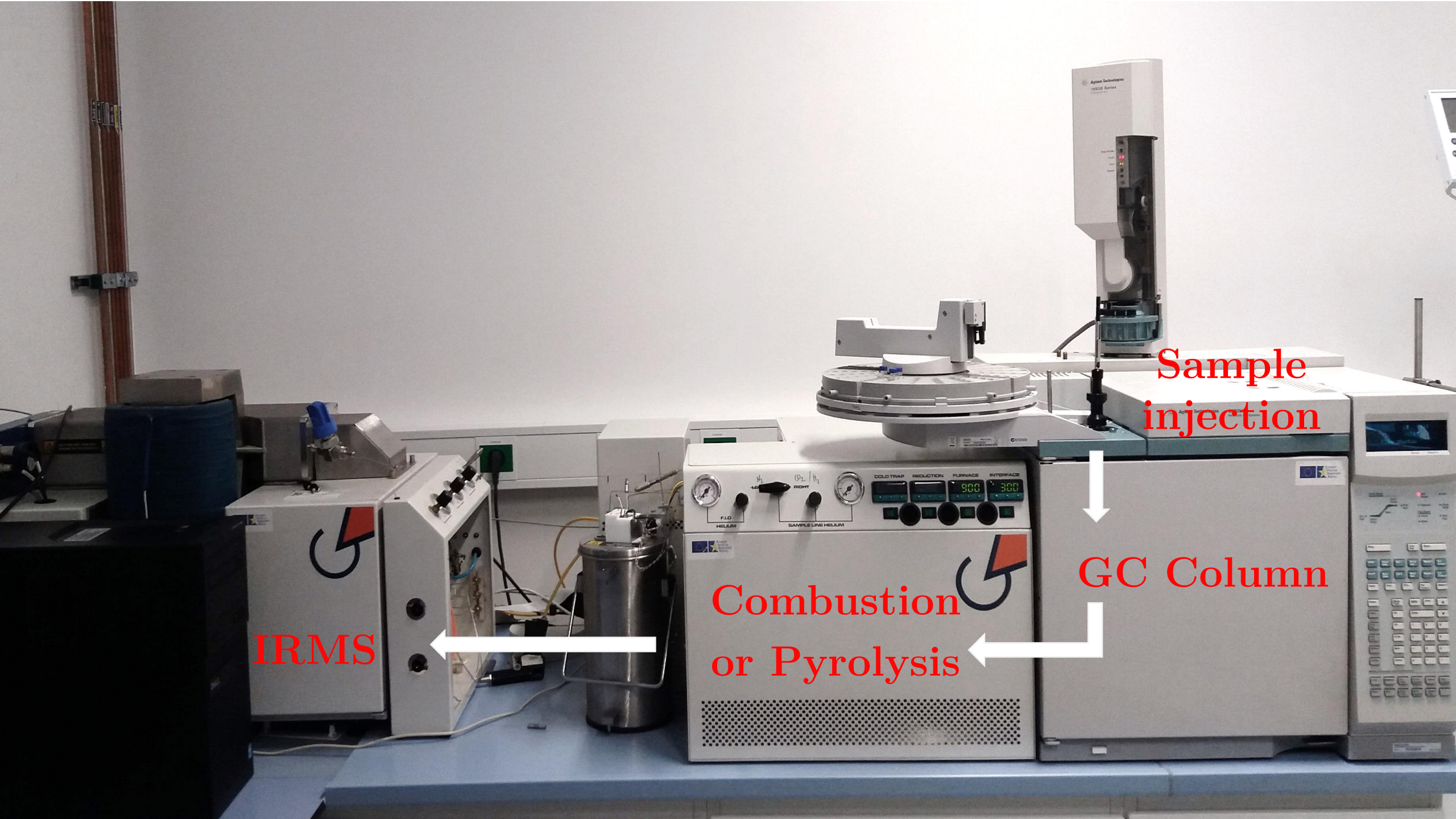
Typically have lengths from 20 to 60 m, internal diameters from 0.200 to 0.320 μm.

Long length

- improves peak separation
- broader peaks
- longer analysis time

Thicker stationary phase

- more sample onto the column
- good peak resolution of low molecular weight
- longer retention times
- higher column “bleed”


Sample
injection
GC Column
Combustion
or Pyrolysis
IRMS

Furnace tube interface

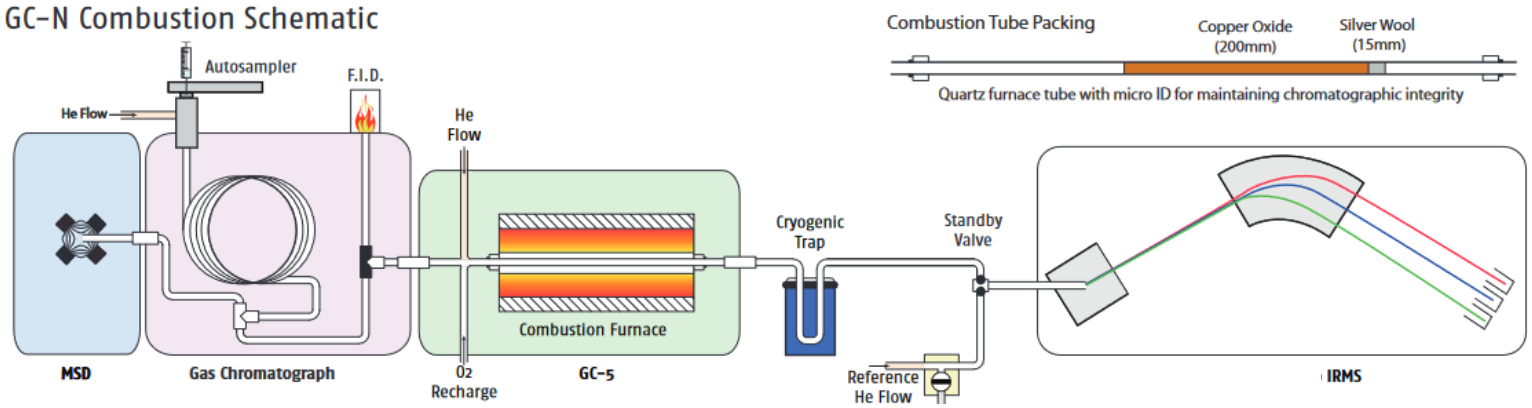
Enable complete conversion of components eluting from the GC column into target gases suitable for analysis by IRMS.

GC-C (CO2), GC-N (N2), GC-H (H2), GC-O (CO)

GC configuration	Furnace tube / Packing / Temperature	Description
1. GC-Carbon ('High Capacity')	Quartz / Copper Oxide / 850°C	High Capacity All applications except natural gas
2. GC-Carbon ('Natural Gas')	Quartz / NiChrome, Copper, Platinum / 950°C	Natural Gas option Suitable for all GC-C applications, but with lower capacity
3. GC-Hydrogen ('Low Temperature')	Quartz / Chromium / 1050°C	All applications except natural gas analysis Suitable for N-containing compounds
4. GC-Hydrogen ('High Temperature')	Ceramic / Empty / 1450°C	Natural Gas option Suitable for most GC-H applications except N containing and halogenated compounds
5. GC-Nitrogen	Quartz / NiChrome, Copper, Platinum / 950°C	A one furnace tube solution Oxidation requires careful optimization
6. GC-Oxygen	Ceramic / Nickel tube / 1250°C	Suitable for all GC-O applications except N containing compounds

Furnace tube interface

C, N: oxidizing combustion reactor (Quartz / Copper Oxide / 850°C)



http://www.isotope.ac.cn/compound-N.pdf

H: low temperature thermal conversion reactor tube (TC) (Quartz / Chromium /1050°C)

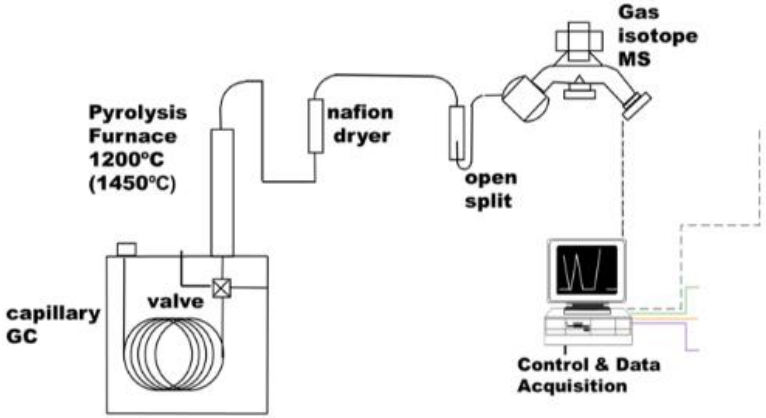
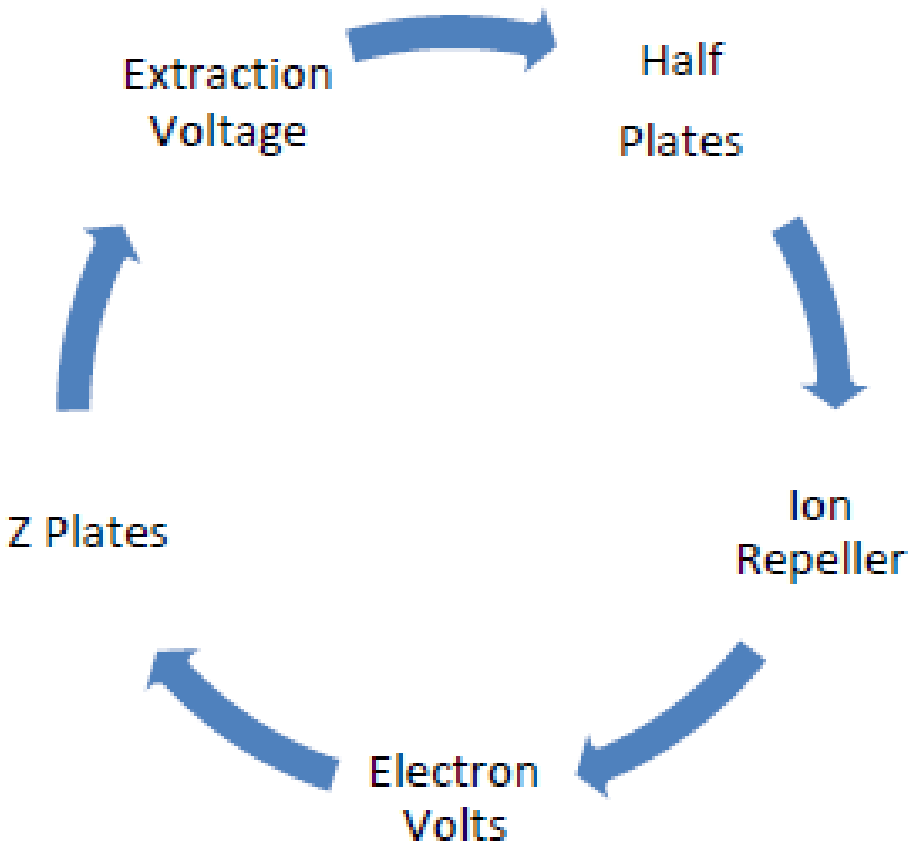


Figure adapted by Stear, 2008

2 Instrument preparation

The most important thing…



Ion source tuning

Peak centering

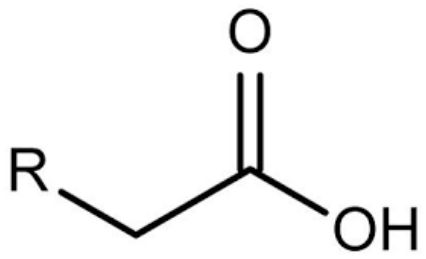
Stability

Linearity → determined from samples

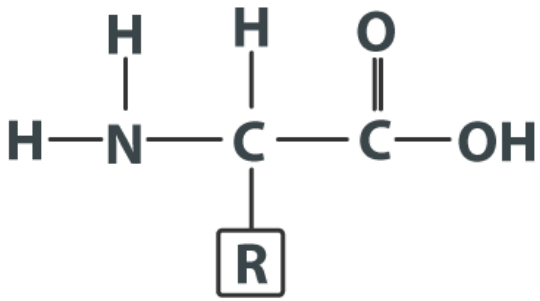
3 Sample preparation

Samples

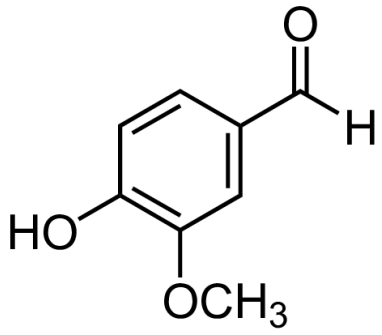
Food authenticity studies utilizing GC-IRMS often involve analysis of the following compounds:



Fatty acids

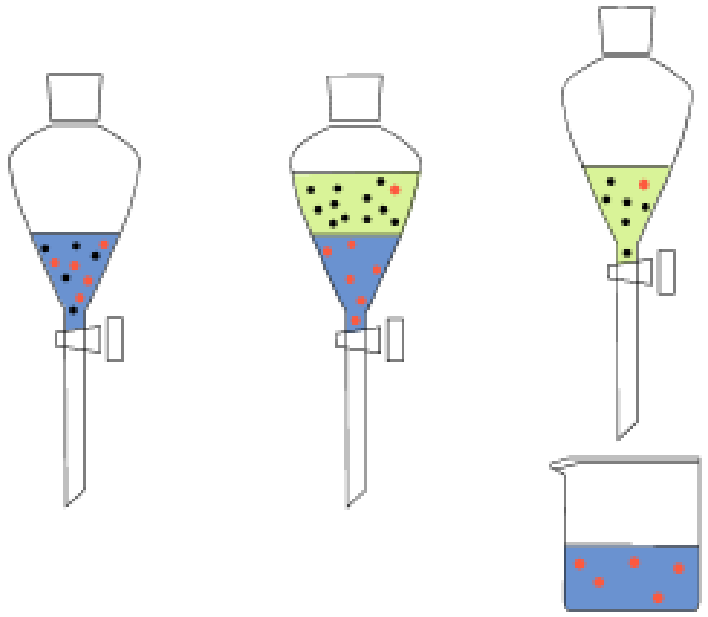


Amino acids



Flavour compounds

Sample extraction

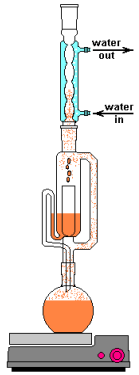


Extraction and purification of analytes from sample matrix are required before samples are ready for analysis by GC-IRMS.

Purity of extraction is not critical because analytes are separated on the GC column.

Sample preparation: Fatty acids

1 Lipid fraction should be extracted from the sample matrix



Soxhlet method

Folch method

Bligh and Dyer method

Microwave-assisted extraction

Supercritical fluid extraction

Ultrasonic-assisted extraction

The FA composition is determined as the methyl esters of FA by GC–IRMS.

2

The conversion process of FA into FAMEs is called derivatisation.

Source: https://doi.org/10.1016/j.arabjc.2020.06.039

Sample preparation: Amino acids

1 Amino acids extraction

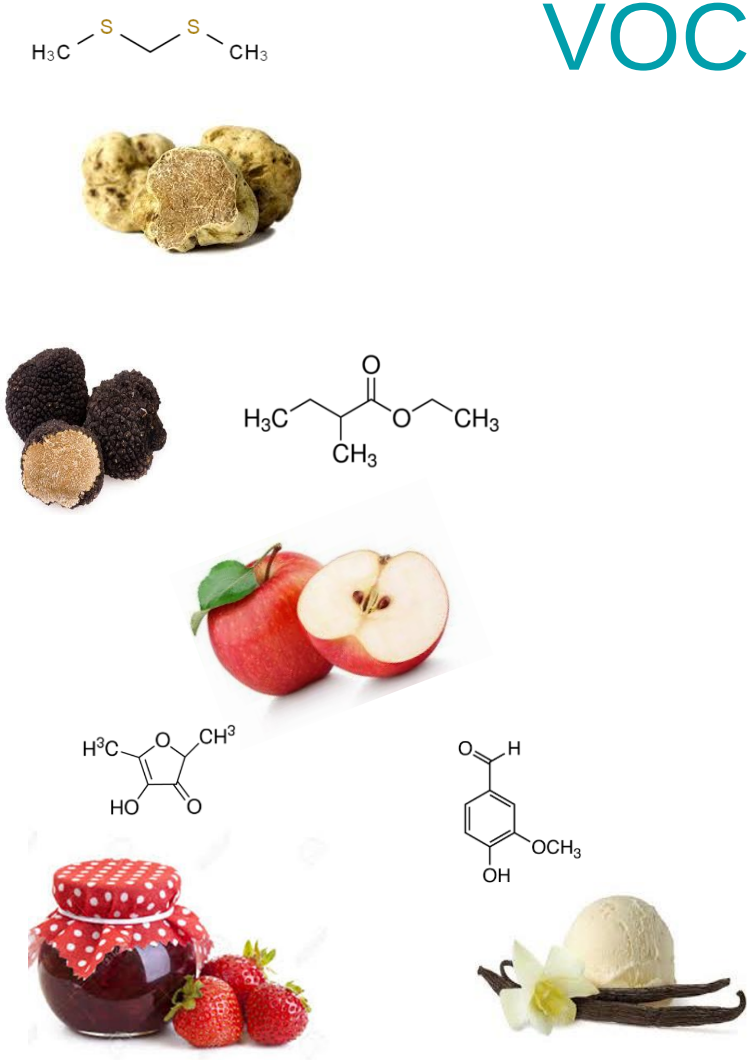
cation exchange resin Dowex 50W-X8 100mesh
40x2mm column
activation of resin
sample + IS
elution
3M NH_4OH
evaporate

2 Derivatisation procedure required to make amino acids sufficiently volatile for GC-IRMS measurement.

Esterification: 100µL butanol/HCl 3M for 1h, 110º C

Acetylation: 100µL TFAA, 80º C, 20 min; dry at 4º C; 1mL ethyl acetate

Sample preparation: Flavours



VOCs from sample can be extracted by:

- LLE (liquid-liquid extraction)
- SPE (solid-phase extraction)
- SFE (supercritical fluid extraction)
- DHS (dynamic headspace sampling)
- SPME (solid-phase microextraction)

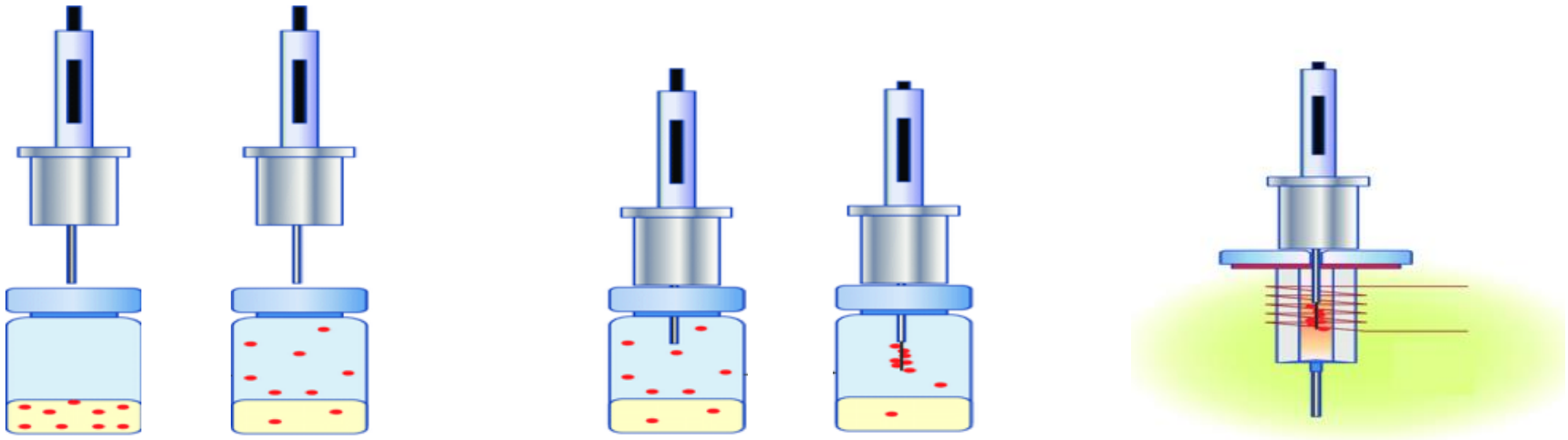
Sample preparation: Flavours

Solid-phase microextraction (SPME)

Sample preparation: Flavours

Solid-phase microextraction (SPME)

Equilibration → Extraction → Desorption



Adapted from Indelicato et al, 2014

4 Standard selection

The analytical procedures for organic stable isotope analysis are often non-standardised, and limited certified reference materials (CRMs) are available.

Reference materials that are compatible with GC-IRMS

Institute for Reference Materials and Measurements

CRM: BCR-656 absolute alcohol ($\delta^{13}C$)

INDIANA UNIVERSITY
BLOOMINGTON

n -alkane mixture ($\delta^{13}C$, $\delta^{2}H$)

A7 & B5 (C_{16} -C_{30}) and C4 (C_{17}-C_{25}) (mg in 0.5 mL hexane)

Fatty acid mixture ($\delta^{13}C$, $\delta^{2}H$)

F8-2 & F8-4 (C_{16} -C_{30}); 0.5 mL solution (mg in 0.5 mL cyclohexane / hexane)

Ethanol ($\delta^{13}C$)

C3 plant origin, C4 lant origin; 5 mL sealed in glass ampoule

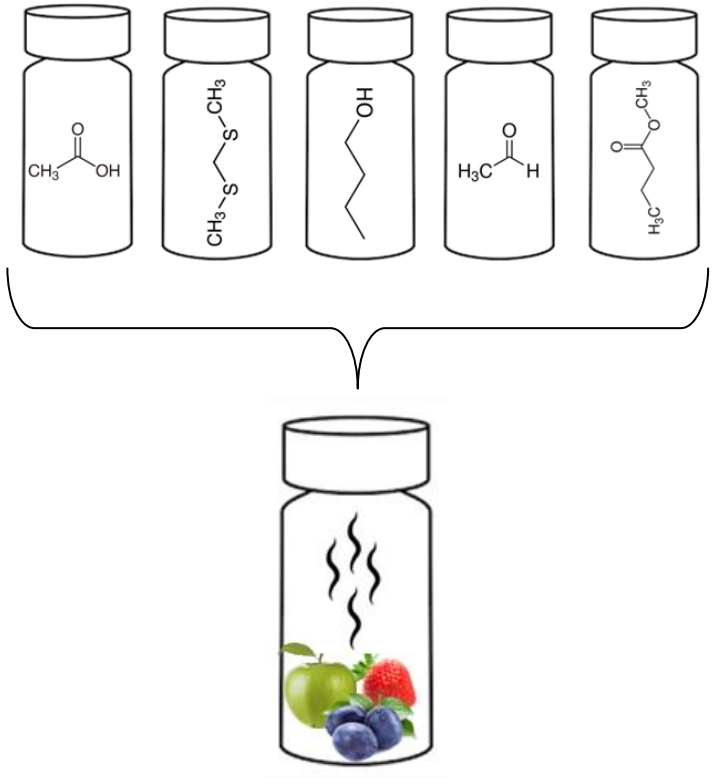
https://hcnisotopes.earth.indiana.edu/reference-materials/materials-descriptions/all-compounds.html

Reference materials that are compatible with GC-IRMS

INDIVIDUAL VOCs

EA-IRMS or TC/IRMS

„reference“ $\delta^{13}C$/ $\delta^{2}H$ values



REFERENCE MIXTURE

Materials (compounds) calibrated separately using EA-IRMS are suitable for GC-C-IRMS as a “reference material mixture” under identical treatment.

- Jochmann & Schmidt (2012) -

“Reference material mixture for VOCs”

In house standards

	EA-IRMS	HTC-IRMS
ethyl butanoate	-25.7 ± 0.01	-180 ± 1.8
hexanal	-25.5 ± 0.02	-40 ± 0.6
(*E*)-hex-2-enal	-27.5 ± 0.04	-152 ± 1.9
hexyl acetate	-27.0 ± 0.01	-113 ± 2.2
benzaldeyde	-26.0 ± 0.01	~ +470
ethyl 2-methylbutanoate	-24.7 ± 0.01	-185 ± 0.4
2-methylbutyl acetate	-32.9 ± 0.04	-156 ± 0.5
ethyl hexanoate	-32.8 ± 0.05	-290 ± 1.3
[(*Z*)-hex-3-enyl] acetate	-28.7 ± 0.03	-167 ± 2.6

	Ethyl butyrate	Ethyl-2-methyl butyrate	Butyl acetate	Hexanal	2-methyl buthyl acetate	Ethyl hexanoate	Hexyl acetate	cis-3-hexenyl acetate	Hexanol	trans-2-hexenol	Benzaldehyde
EA											
Average	-25.7	-24.7	-28.6	-25.5	-32.9	-32.8	-27.0	-28.7	-24.4	-27.5	-26.0
Stdev	0.01	0.01	0.06	0.02	0.04	0.05	0.01	0.03	0.05	0.04	0.01
Liquid injection/splitless/different concentration											
Difference from EA	0.13	-0.14	0.55	-0.69	0.07	-0.21	0.56	0.32	0.65	-0.12	0.29
Liquid injection/split											
Difference from EA	-0.66	-0.62	-0.14	-0.99	-0.44	-0.21	0.37	0.24	0.47	-0.22	0.39
SPME/split/different sample volume											
Difference from EA	-0.47	-0.22	-0.10	-0.02	-0.55	0.07	0.74	0.86	0.13	-0.38	0.19
SPME/splitless											
Difference from EA	-0.31	-0.11		-0.05	-0.13	0.10	0.53	0.18		0.96	-0.27

5 Sequence preparation

Sequence preparation

1. Dummy
2. RM1
3. RM2
4. QC material
5. Sample 1
6. Sample 2
7. Sample 3
8. QC material
9. RM2
10. RM1

* All in duplicate/triplicate

Dummy

at the beginning for condition purposes

RM for data normalisation

at least 2 different RMs or 1 reference mixture at the beginning and the end of the sequences in at least 2 different concentrations (0.5 nA – 15 nA)

QC material

analysed periodically throughout the sequence – separately or within reference mixture or sample

6 Drift correction

Drift correction

Time drift – individual measurement number taken as proxy for time

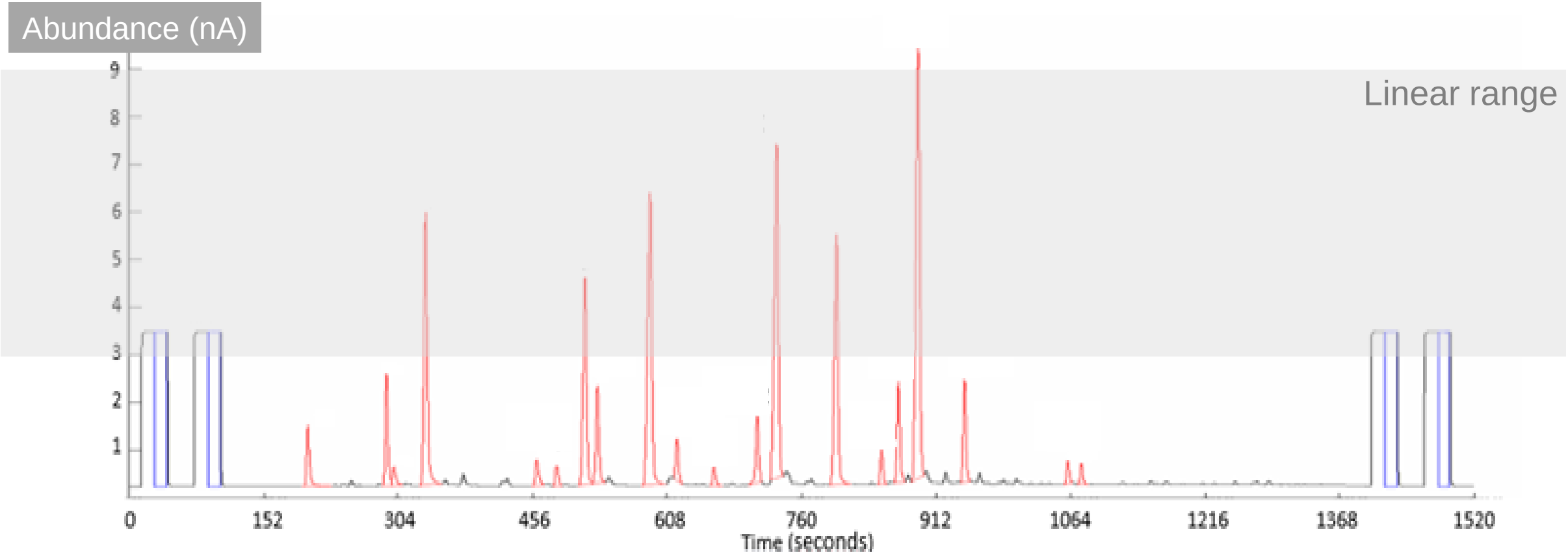
The only assumption is that each measurement takes approximately the same time.

The degree of drift should be determined by:

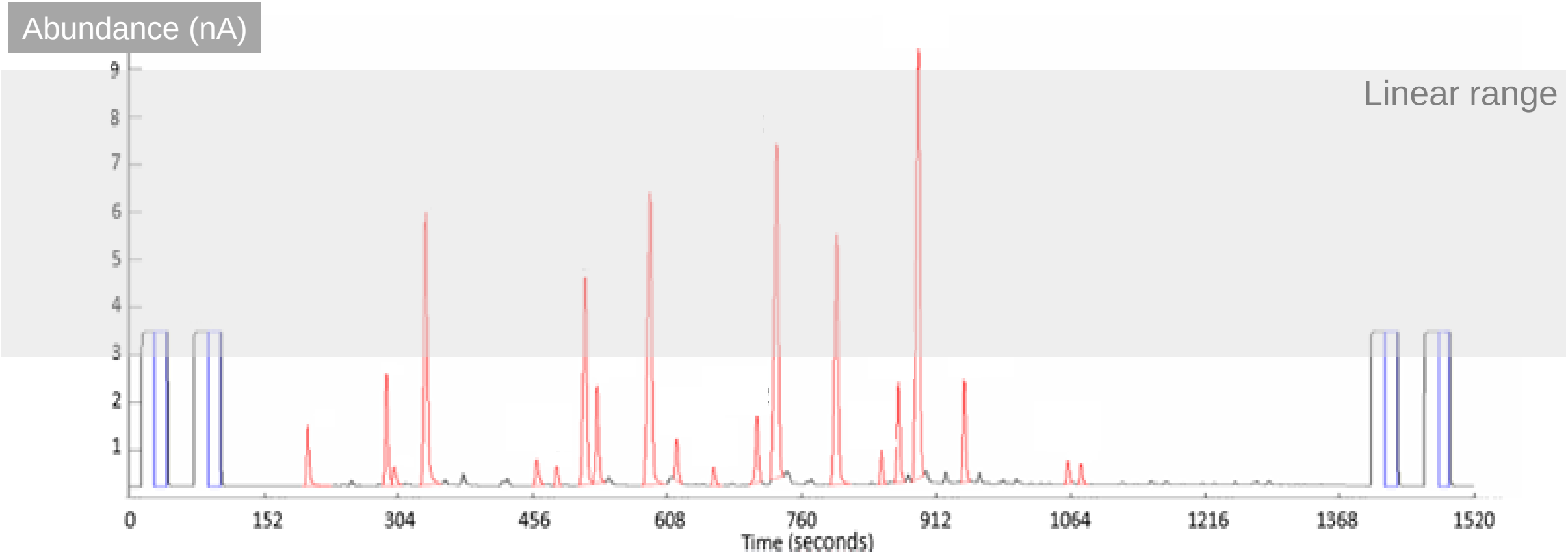
analysis of RMs at the beginning and the end of the sequences

7 Linearity correction

Many compounds often fall out of the linear range of instrument

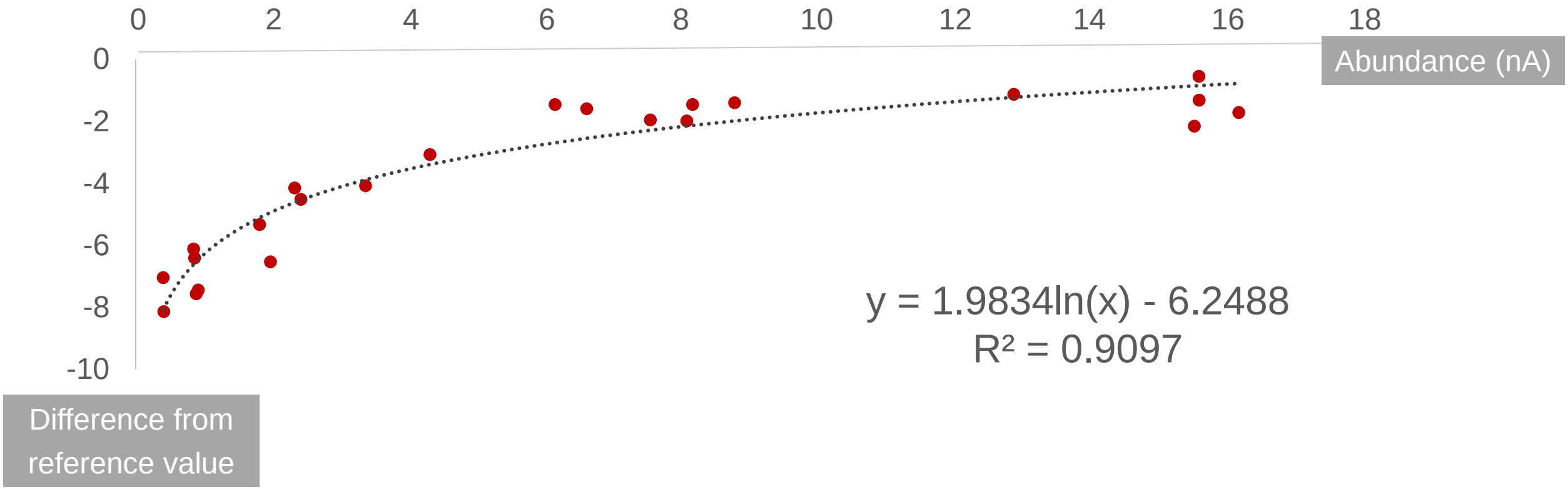


Only the values within a linear range should be reported



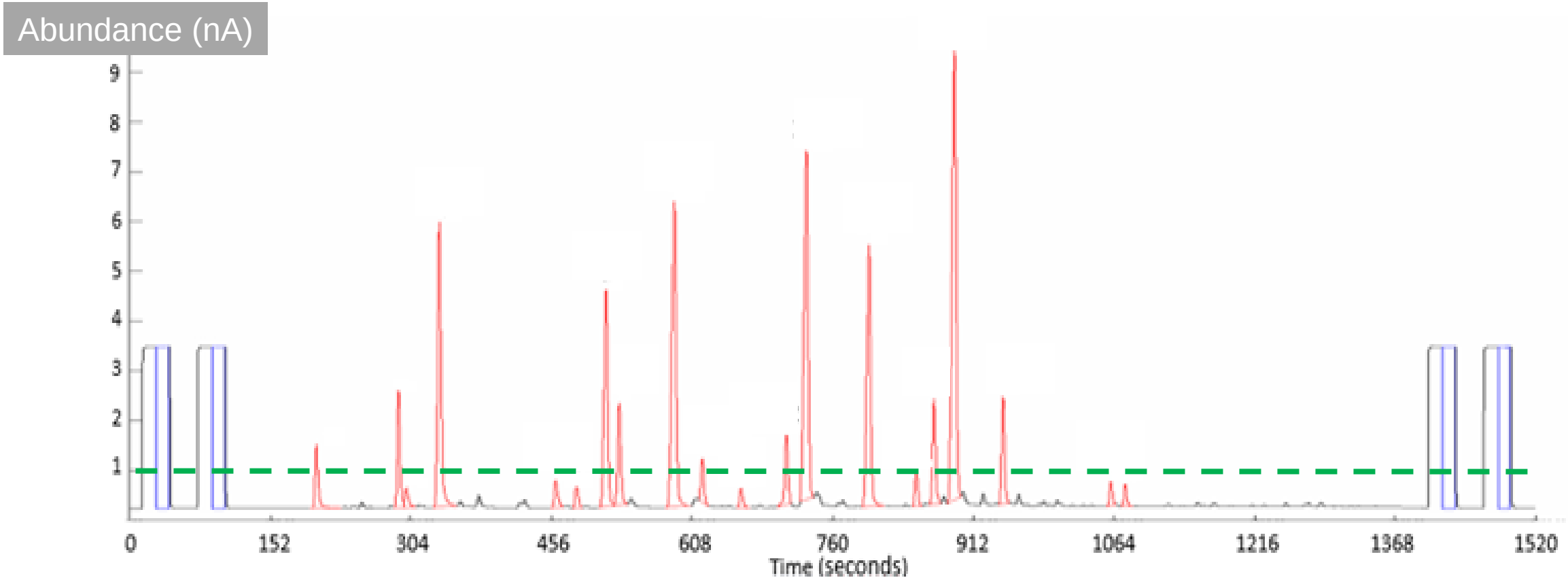
Peak size / linearity correction

Logarithmic trendline



Peak size / linearity correction

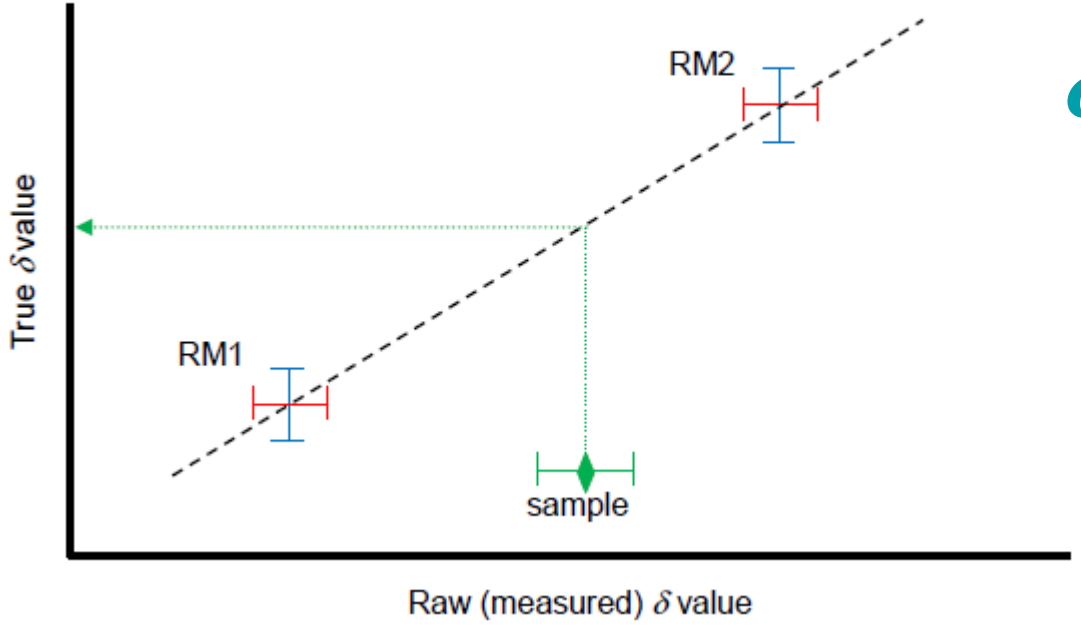
Significantly improved the measurement error of small peaks (below 1nA) from 3 ‰ to 0.5 ‰.



8 Data normalisation

Two-point normalisation

The true δ values when using **2 RMs** can be calculated using the following equation:



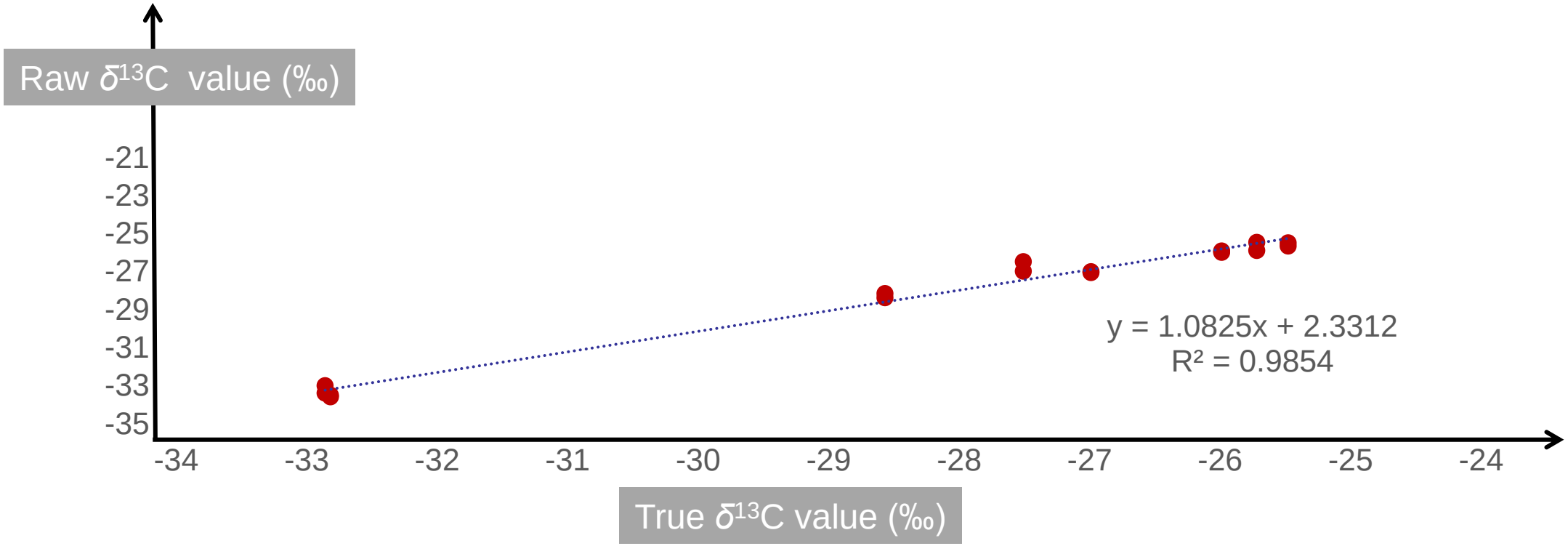
$$\delta_{true\ (Spl)} = m \times \delta_{raw\ (Spl)} + b$$

m **–** the slope of the regression line (“expansion factor”)

b **–** the intercept (“shift factor”)

Multiple-point isotopic linear normalisation

Reduce the random error associated with the analysis of a reference material used to anchor the linear scale.



9 Measurement uncertainty

Measurement uncertainty

In-house standard	Reference values (‰)	Combined uncertainty (u_c, k = 1)
ethyl butanoate	-25.7	0.47
hexanal	-25.5	0.32
(E)-hex-2-enal	-27.5	0.76
hexyl acetate	-27.0	0.64
benzaldehyde	-26.0	0.75
ethyl 2-methylbutanoate	-24.7	0.14
2-methylbutyl acetate	-32.9	0.25
ethyl hexanoate	-32.8	0.20
[(z)-hex-3-enyl] acetate	-28.7	0.23
Method		**0.42 ‰**

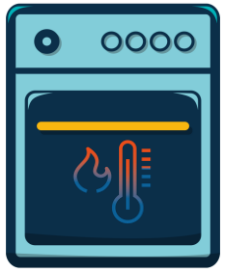
Perini et al., 2019: 1σ = ±0.8‰ (vanillin)
Hatorri et al., 2010: 1σ = ±0.4‰ (acetic acid)
Schipilliti et al., 2016: 1σ = ±1.0‰ (vanillin)

10 Isotope fractionation

Sample preparation procedures

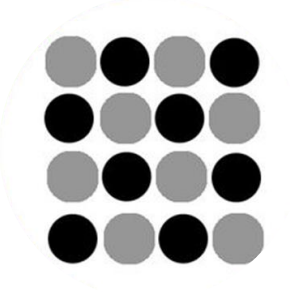
Are of the utmost importance because errors that occur cannot be corrected subsequently.

sampling

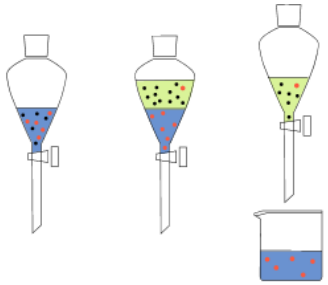


drying

grinding

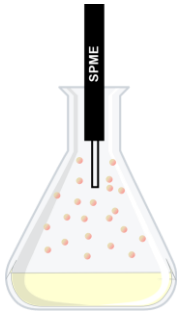


homogeneity



extraction

clean-up

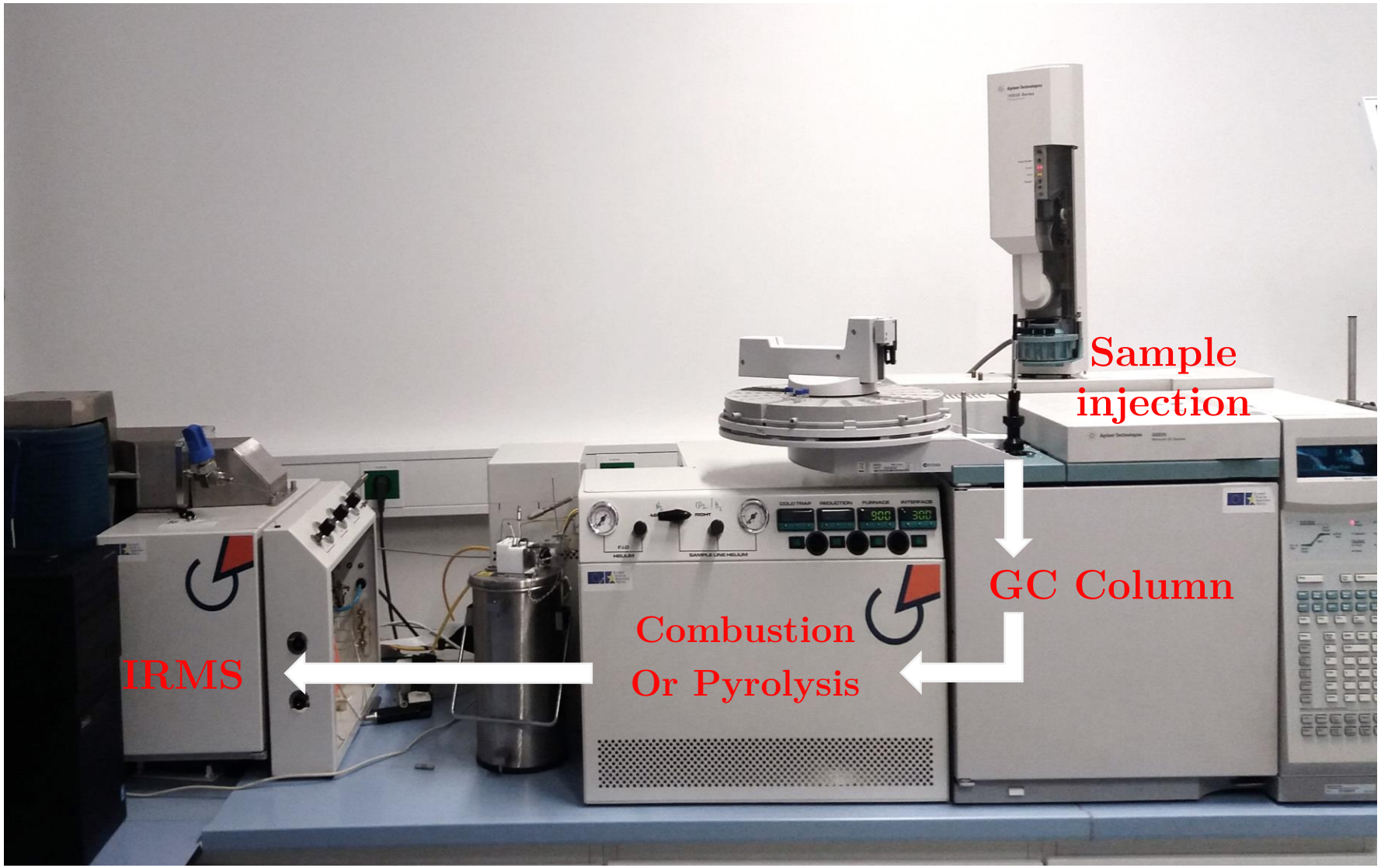


preconcentration

derivatization

At least one internal standard of a similar chemical nature and known isotopic composition should be added to the sample before sample preparation.

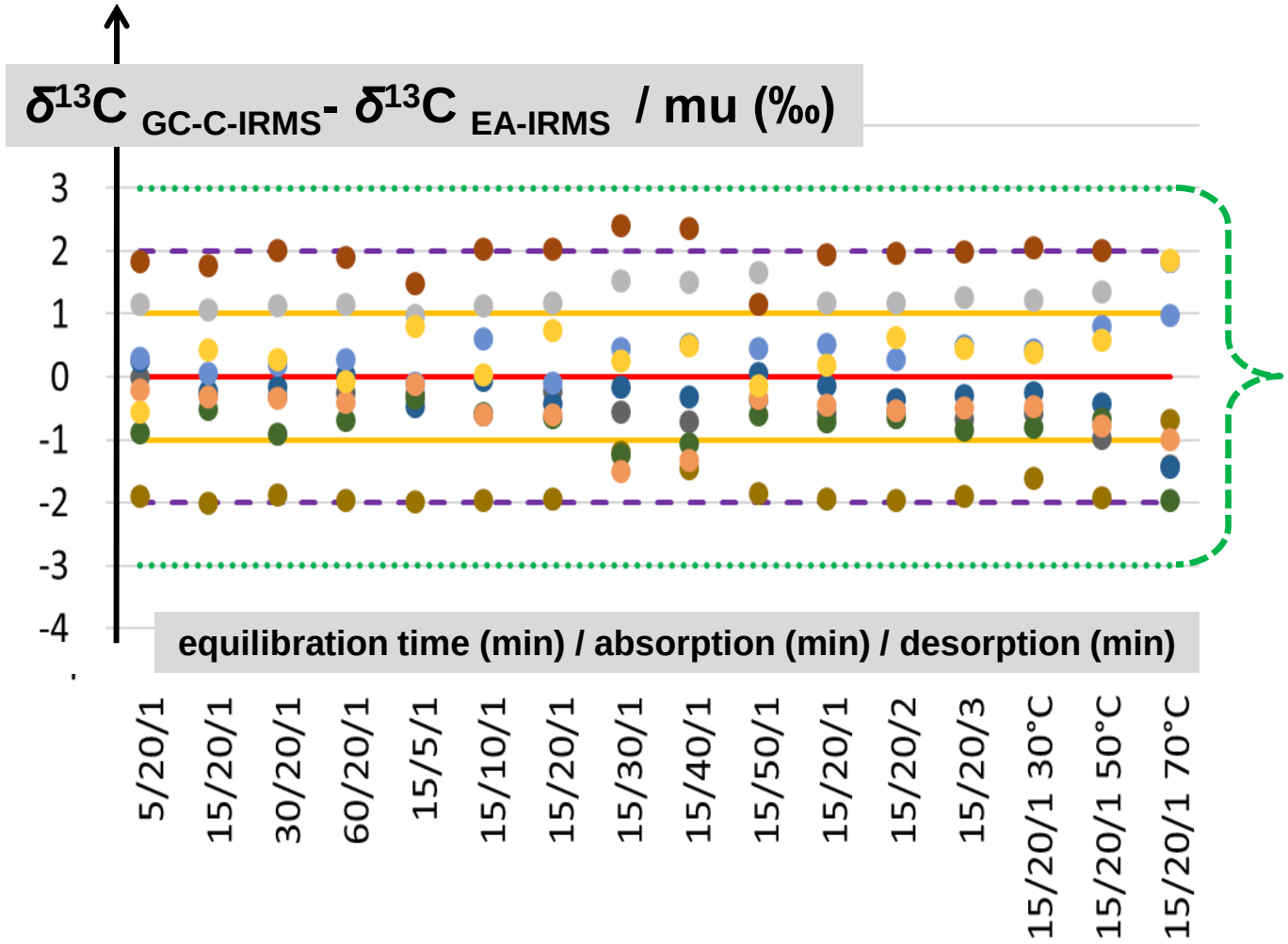
Instrumental conditions



May also cause irreproducible isotope fractionation.

Compound specific $\delta^{13}C$ measurements

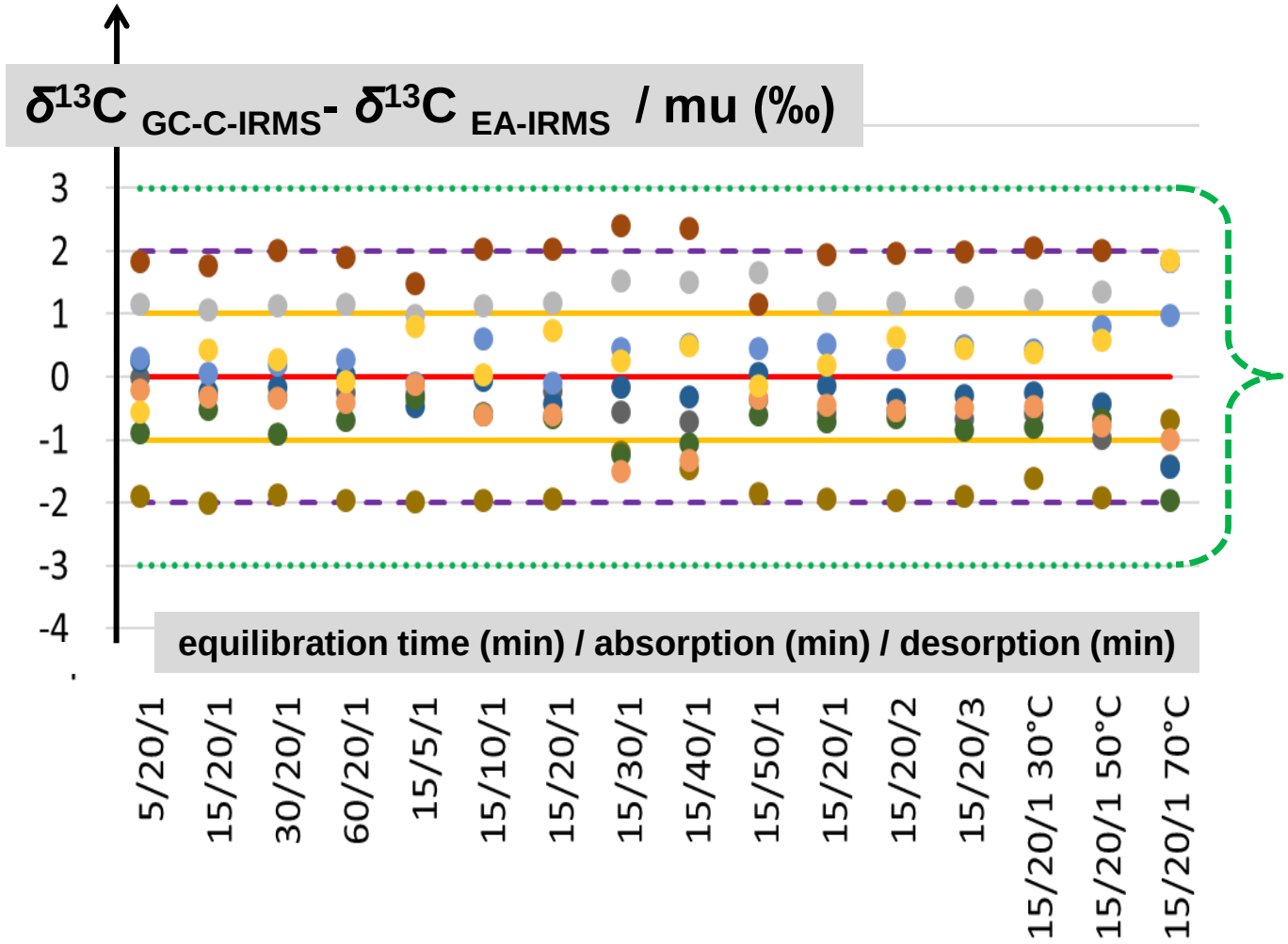
effects of equilibration, adsorption, desorption times and temperatures



All the $\delta^{13}C$ values were below ± 3*MU.

Compound specific $\delta^{13}C$ measurements

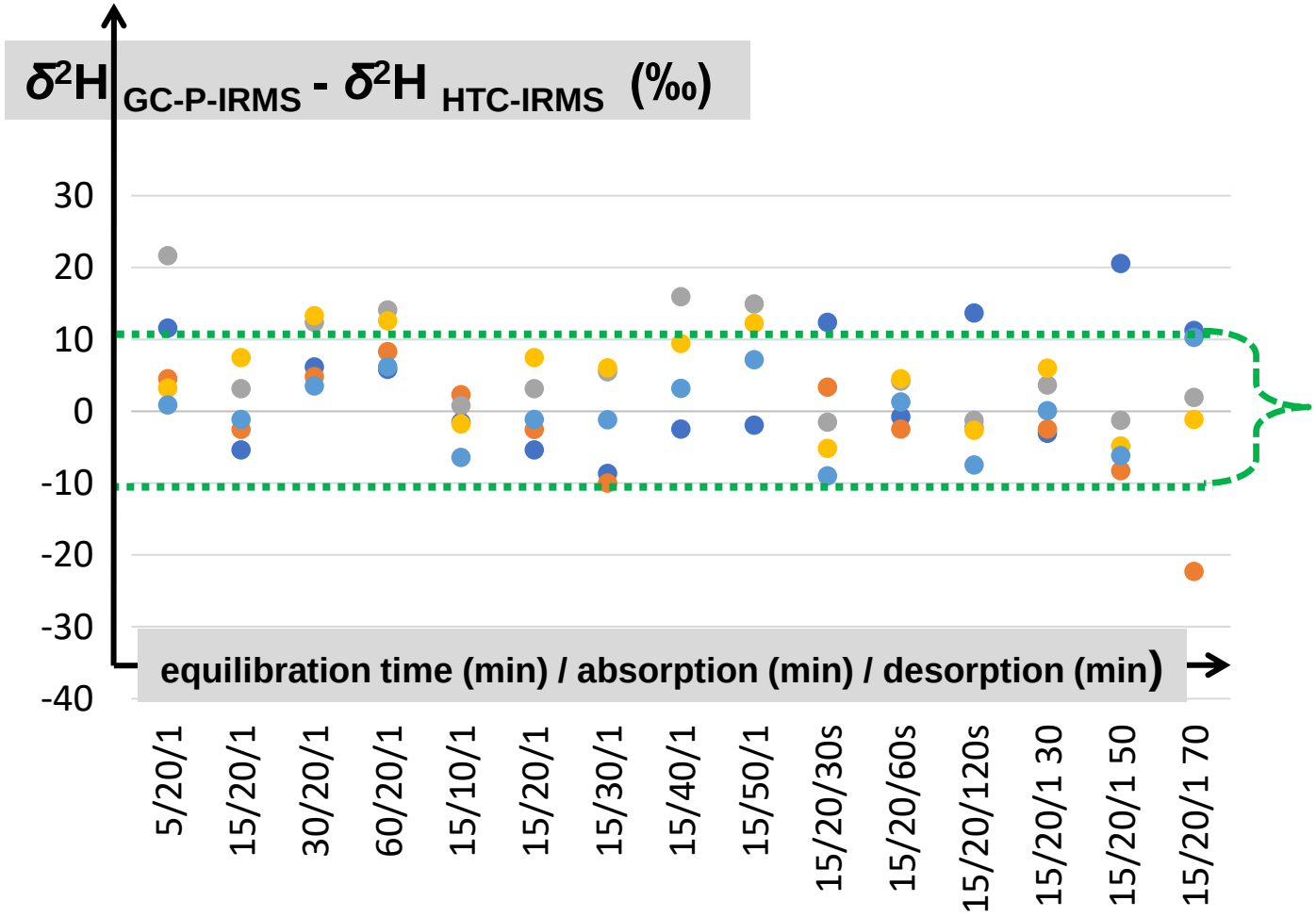
effects of equilibration, adsorption, desorption times and temperatures



No isotopic fractionation was observed.

Compound specific δ^2H measurements

effects of equilibration, adsorption, desorption times and temperatures



Method error < 10 ‰ can be obtained by optimising measurement conditions.

Perini et al., 2019: 1σ = ±7‰ (vanillin)

Hatorri et al., 2010: 1σ = ±5‰ (acetic acid)

Recommendations
for isotope community

Extraction and purification of analytes from sample matrix are required before analysis.

Appropriate data normalisation provides reproducible and accurate results.

No isotope fractionation

when optimised

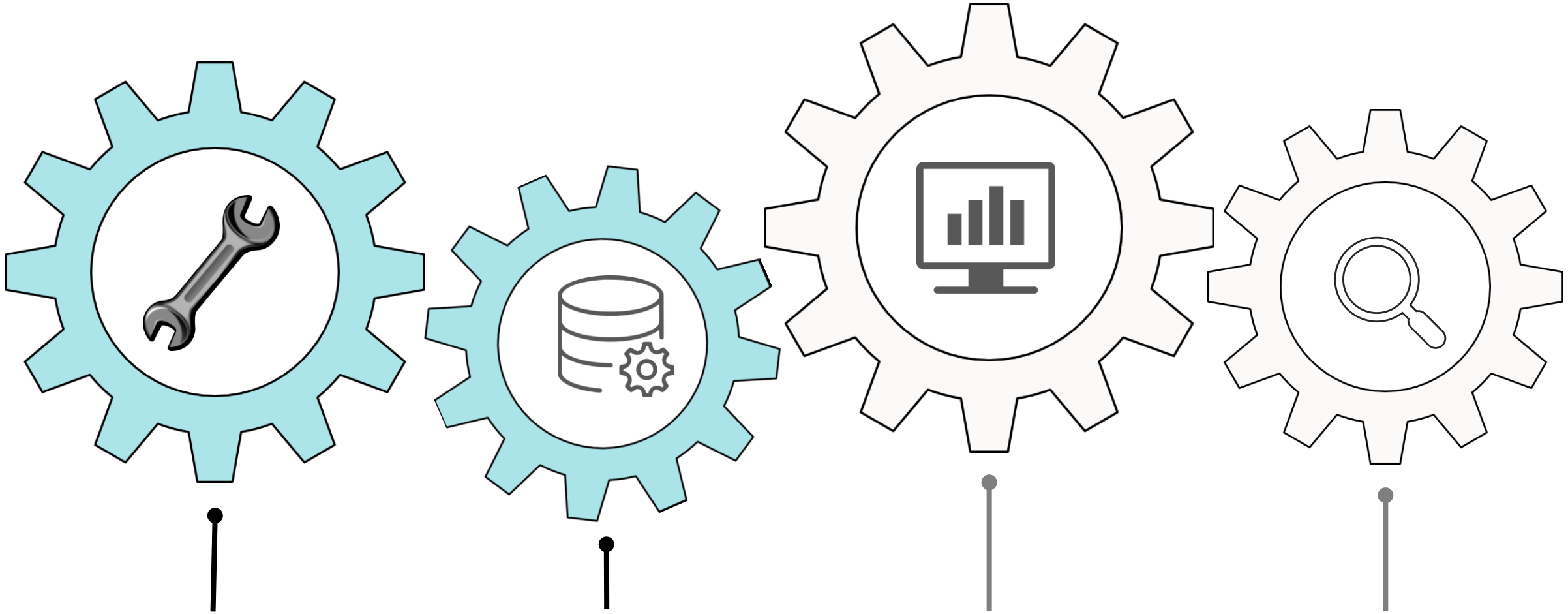
parameters are used.

Information about method uncertainty is needed for appropriate data evaluation.

The four gears building trust in our food

Methodology ??? ??? ???

The four gears building trust in our food



Methodology **Database** **???** **???**

Data interpretation requires extensive reference data set of authentic food samples against which a sample under investigation can be compared.

General requirements

Samples within database are authentic

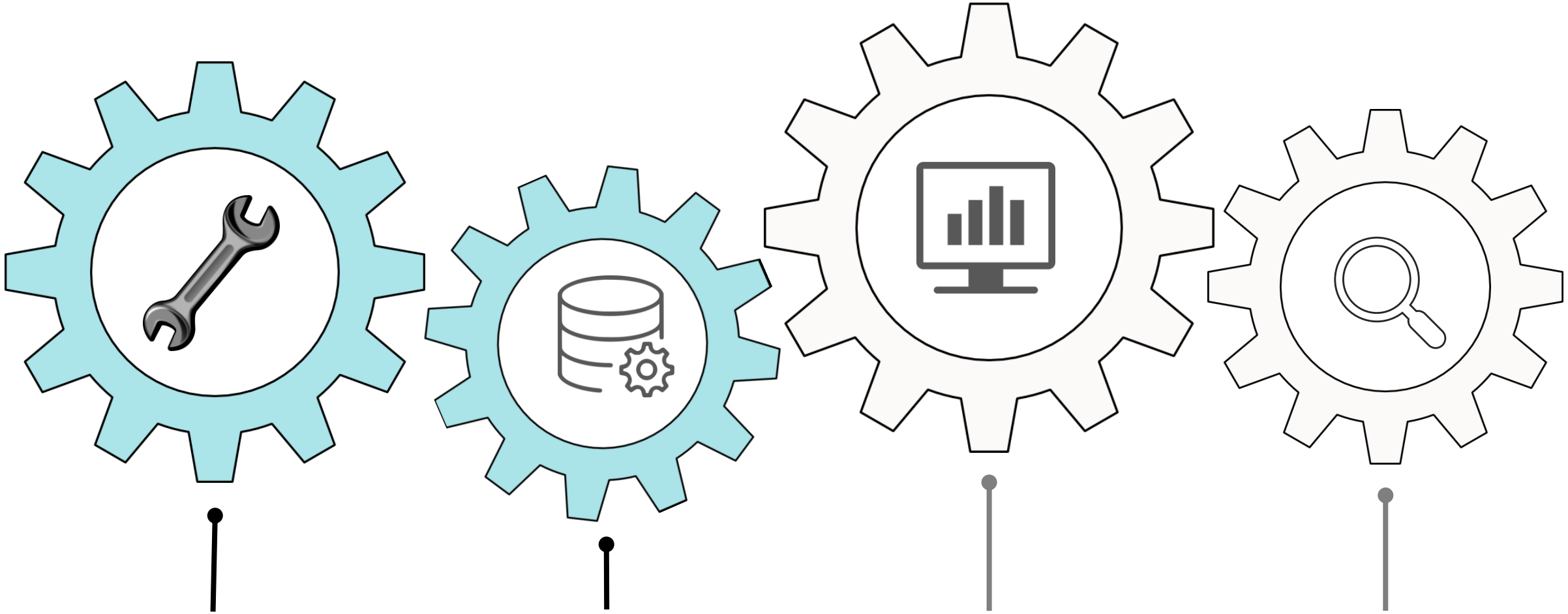
Collected from primary producers by impartial collectors

Adequate number of samples

Sufficiently representative

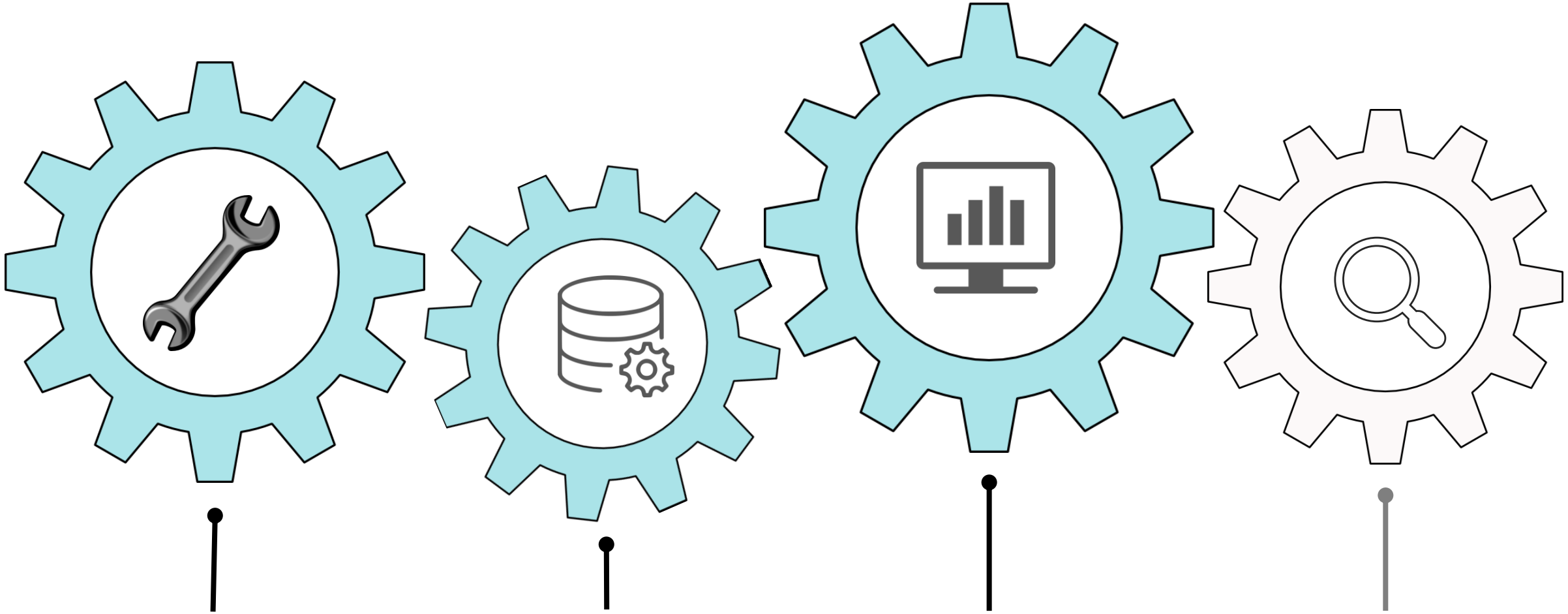
Cover natural variation

The four gears building trust in our food

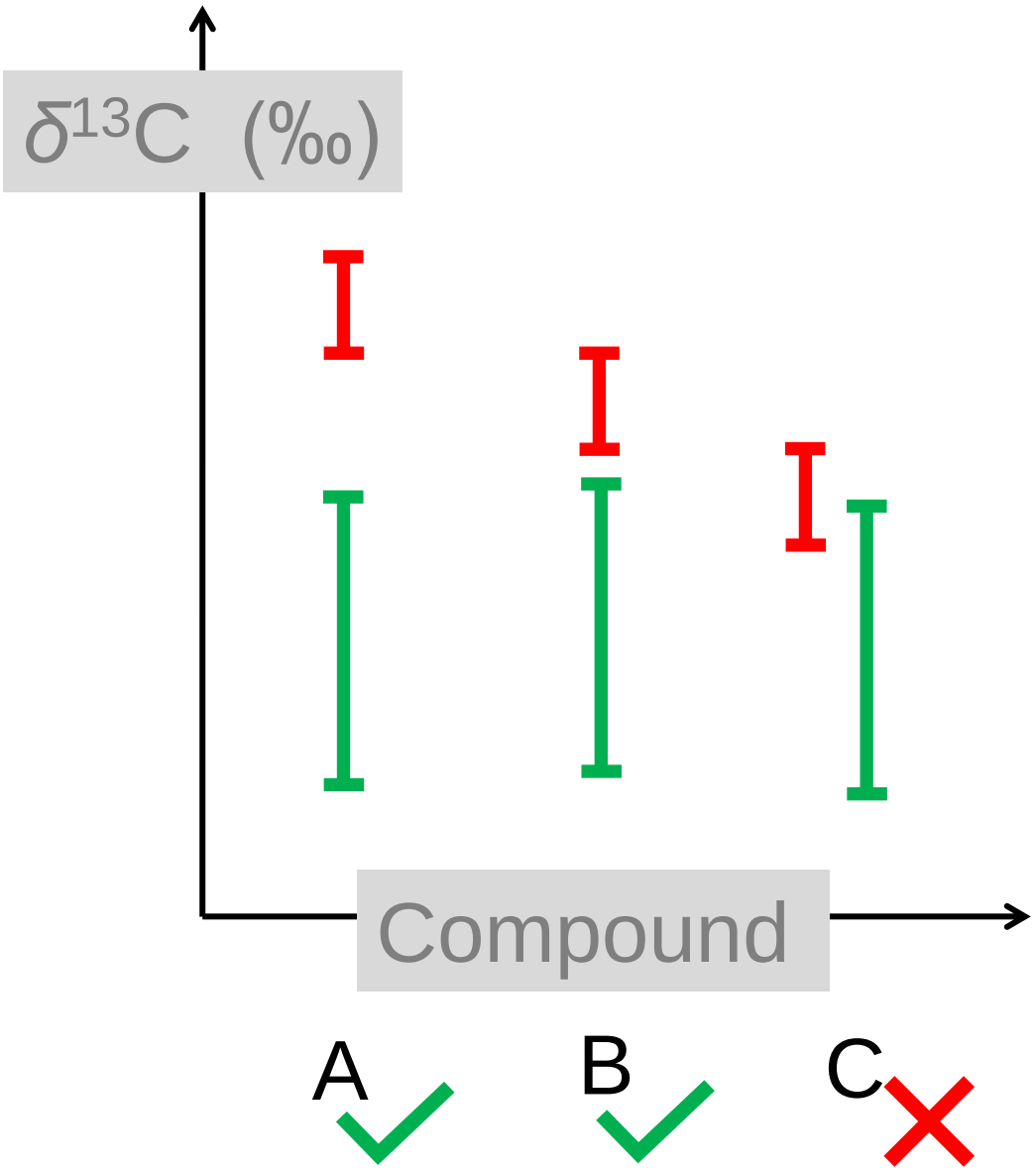


Methodology **Database** **???** **???**

The four gears building trust in our food

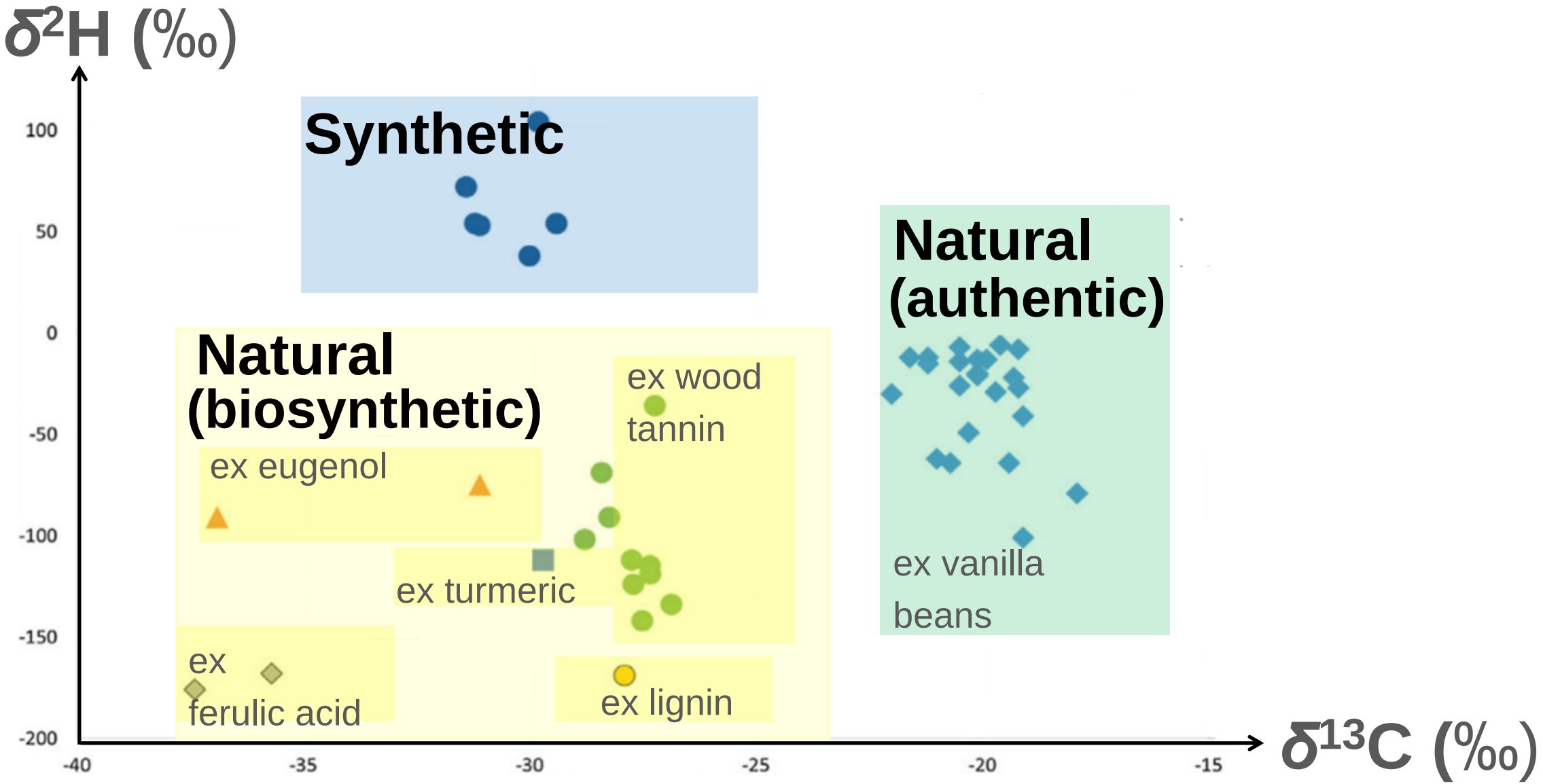


The choice of statistical method depends on the study goal and the characteristics of the objects and variables included.



Comparative analysis of (natural and synthetic) isotope ranges.

Combination of two isotopes



3 general chemometric approaches

Explorative analysis

Classification

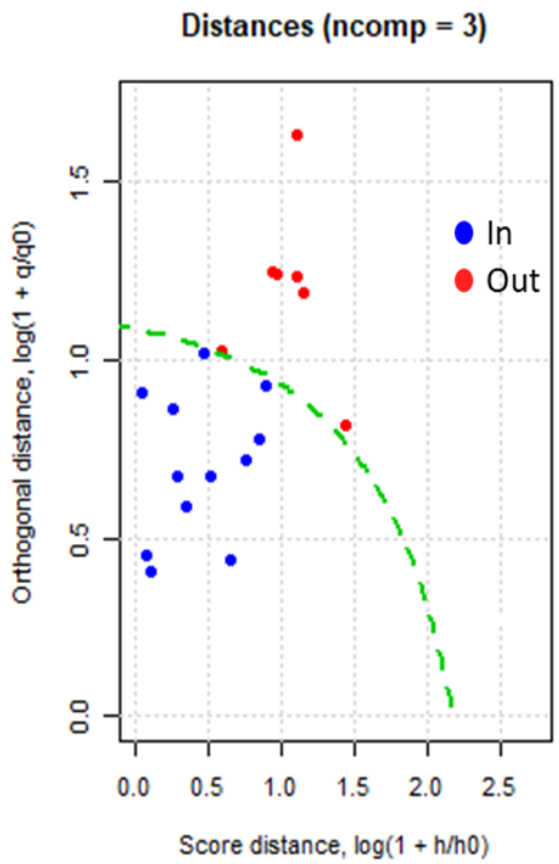
Class-modelling

Confirmation that a specific sample originates from a particular country.

One class classification problem

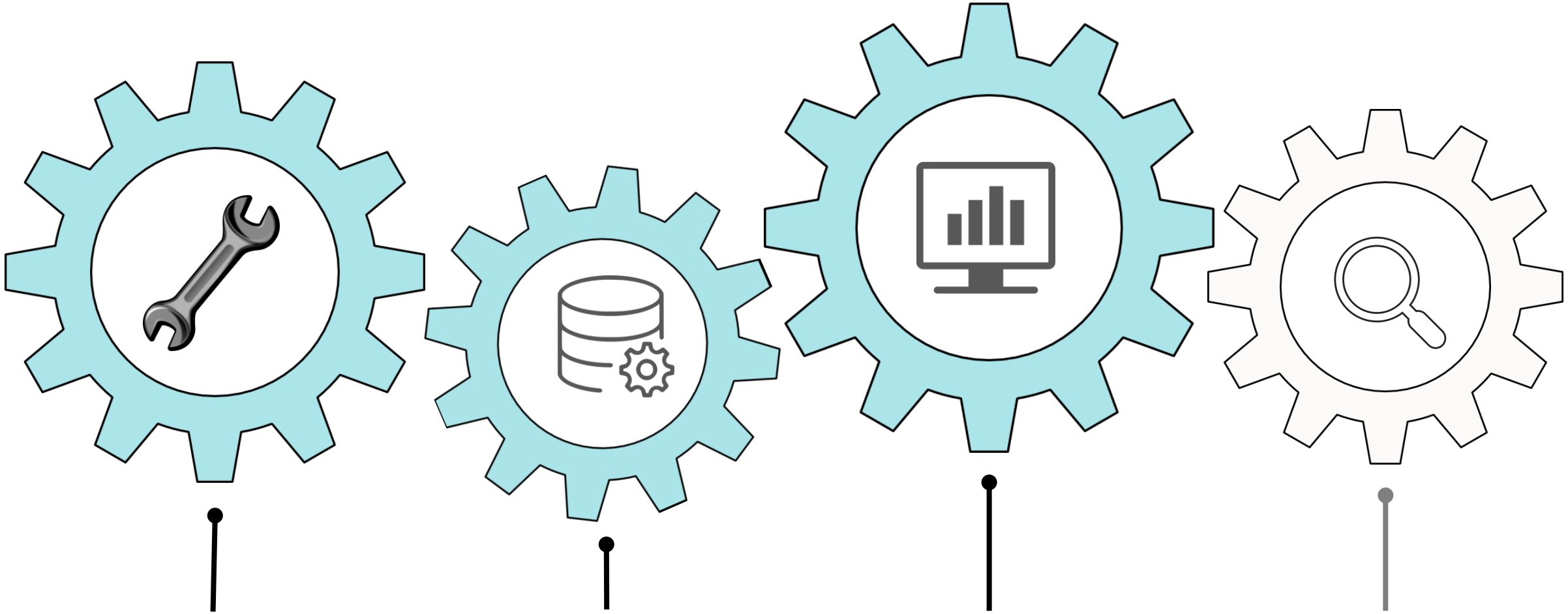
Class-modelling or one-class classifiers

Data Driven Soft Independent Modelling of Class Analogy (DD-SIMCA)

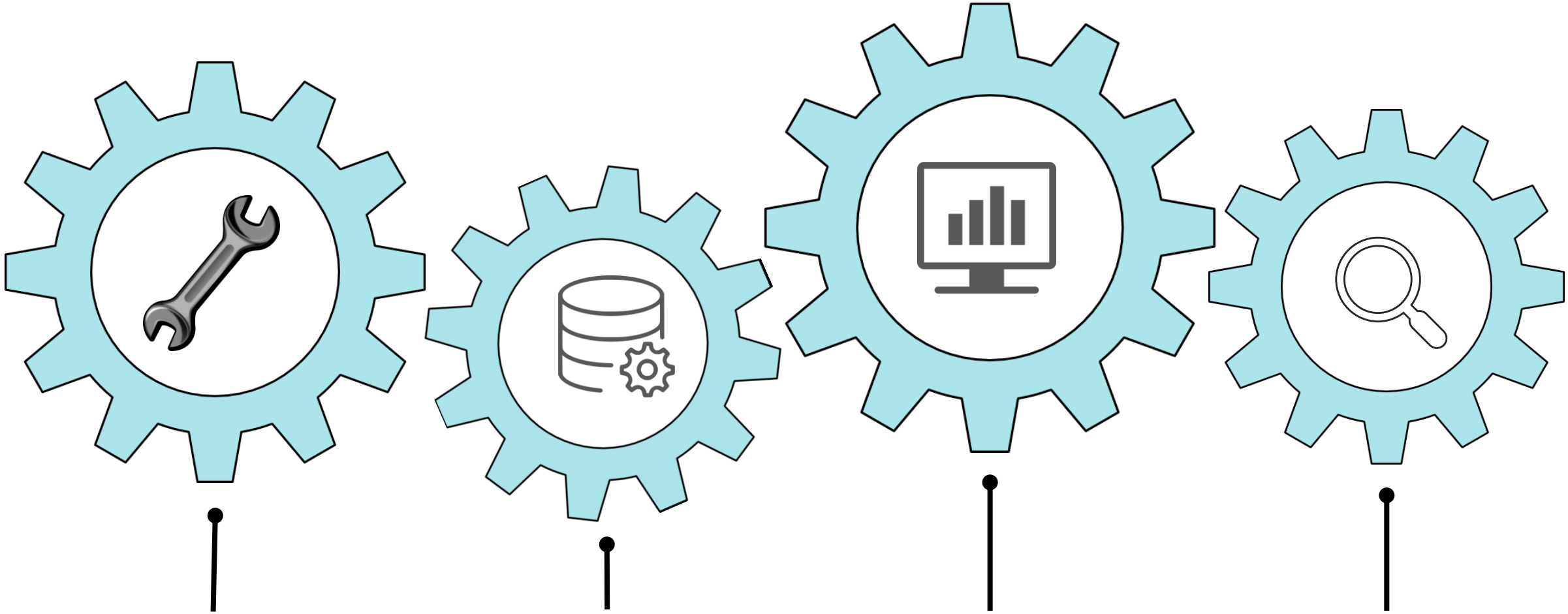


Meant to distinguish objects of one particular class from all other objects and classes.

The four gears building trust in our food



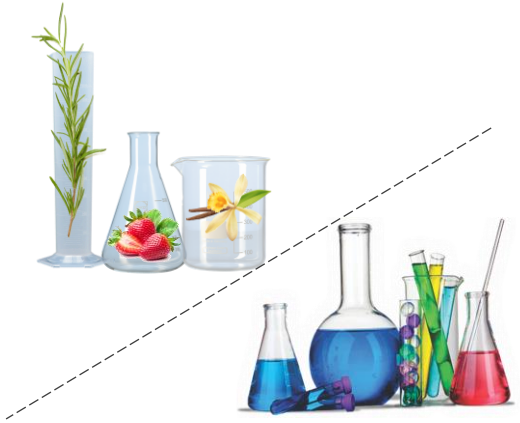
The four gears building trust in our food



Methodology **Database** **Data analysis** **Market testing**

Stable isotopes

what can we achieve?



Natural or synthetic

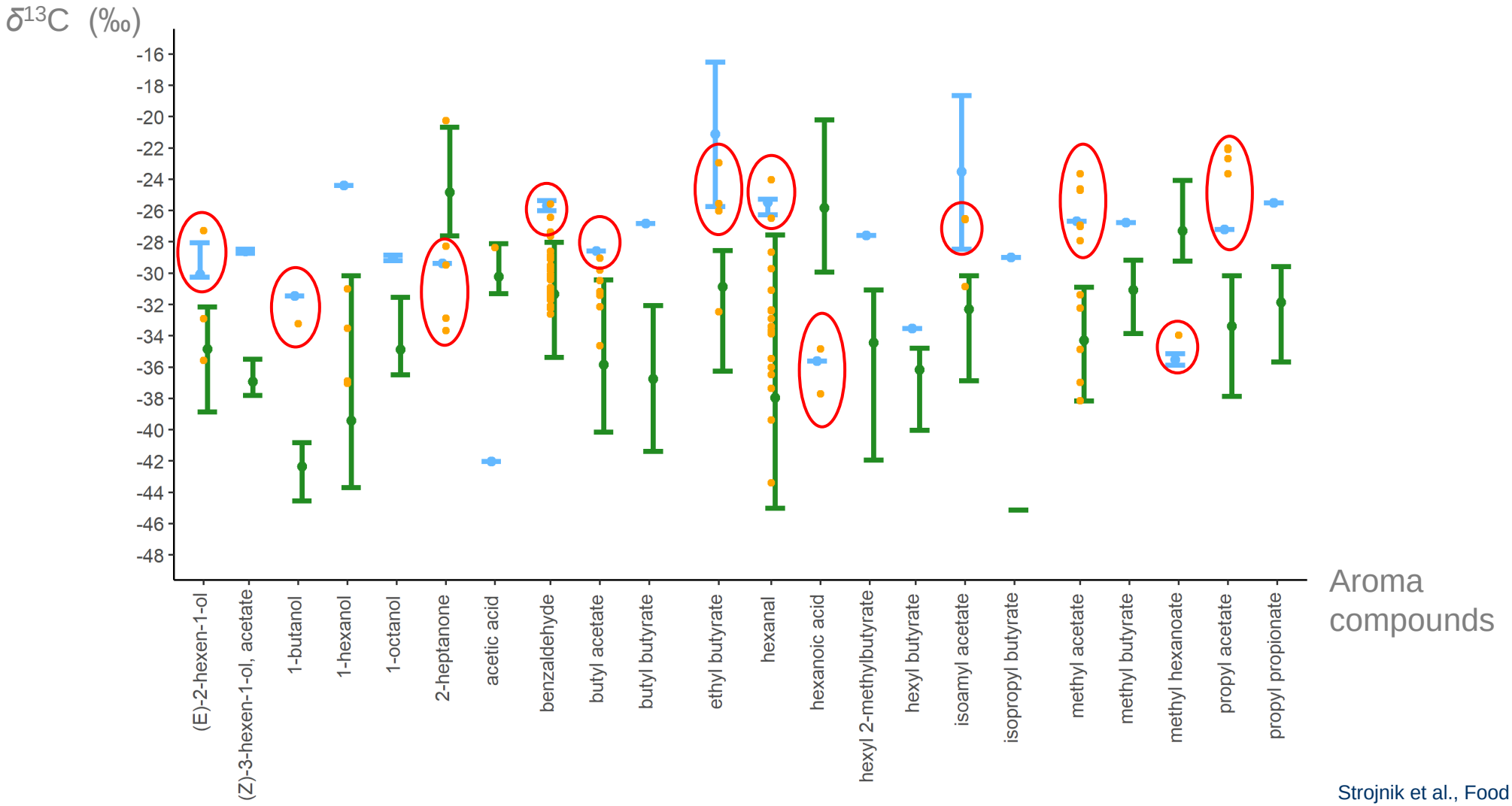
Authentic or adulterated

Organic or conventional

Geographical origin

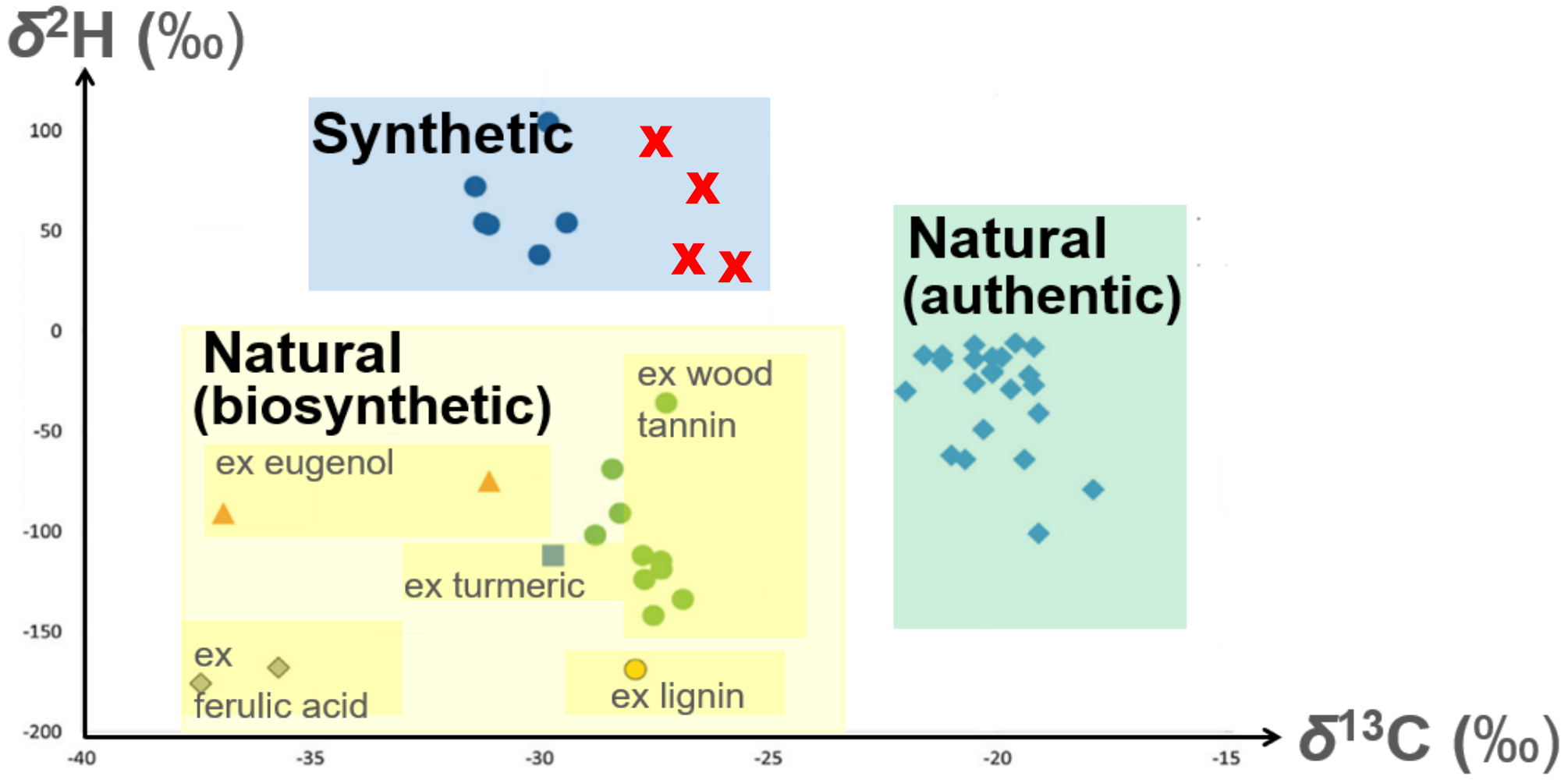
Naturalness of Food Flavourings

Fruit flavourings on the market can be questioned



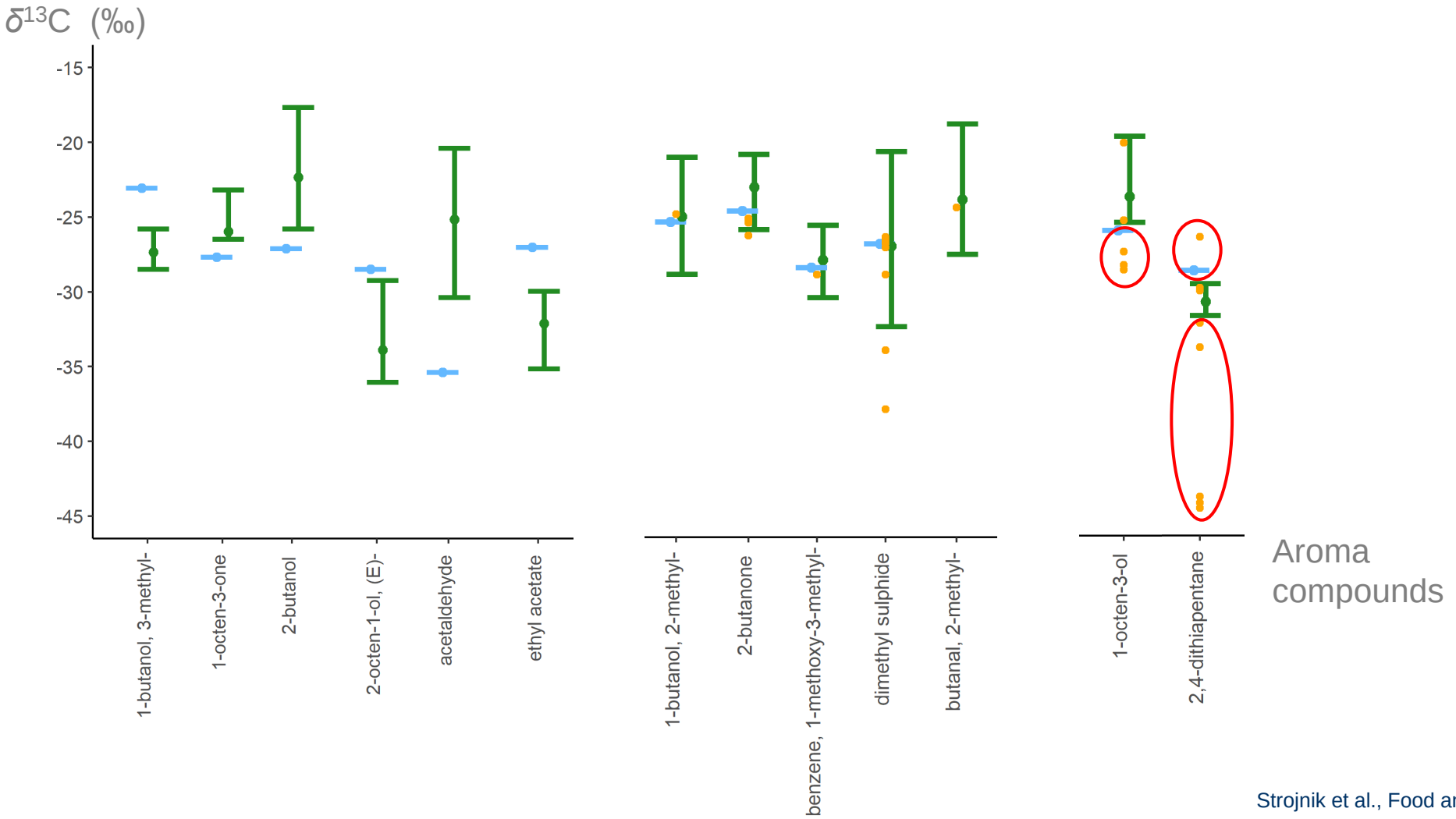
Strojnik et al., Food Chemistry 277, 766-773, 2019

All 4 samples contain synthetic vanillin



Perini et al., J Chrom. A. 21, 168-173, 2019

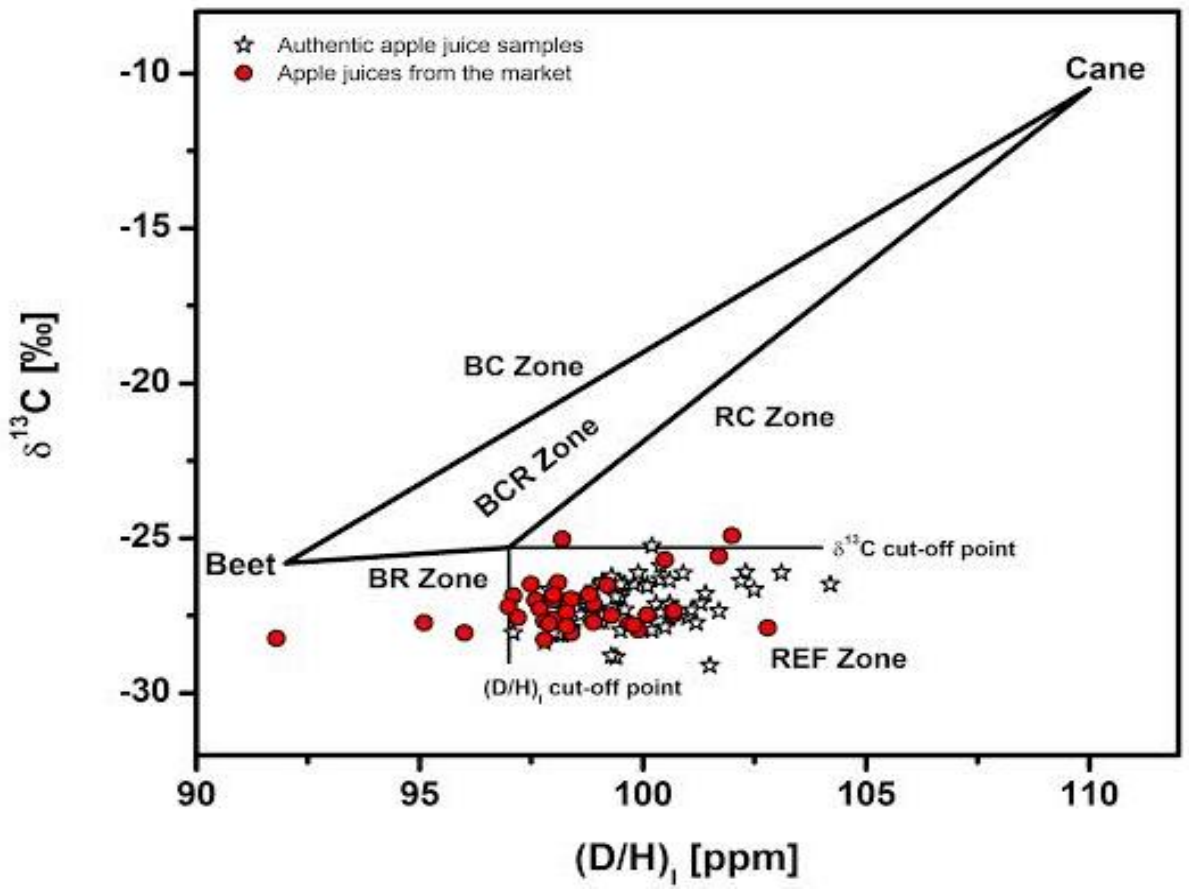
2 compounds indicate presence of synthetic flavour in truffle samples



Strojnik et al., Food anc Chemical Toxicology 142, 2020

Food authenticity

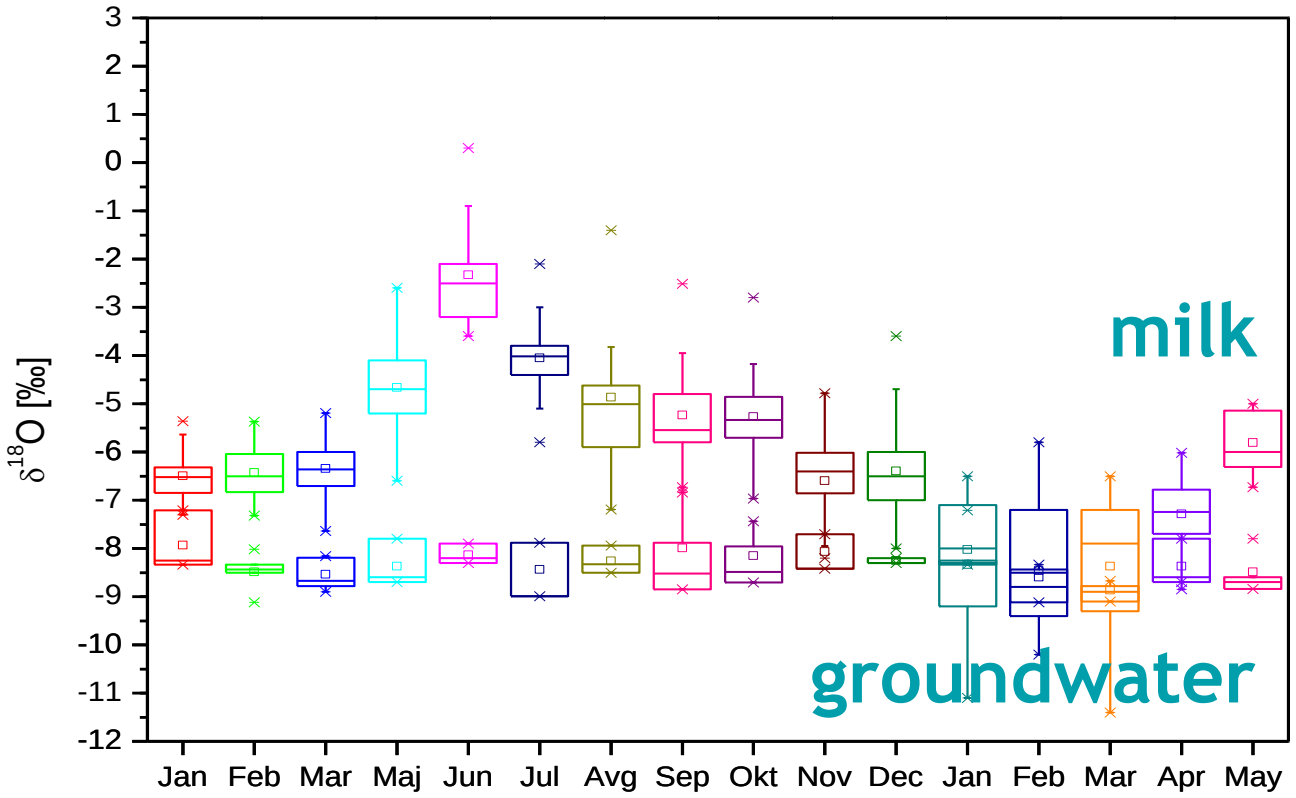
Undeclared addition of sugar in apple juice



10% of the samples

K. BIZJAK BAT *et al.*: Botanical and Geographical Origin of Slovenian Apples, *Food Technol. Biotechnol.* 50 (1) 107–116 (2012)

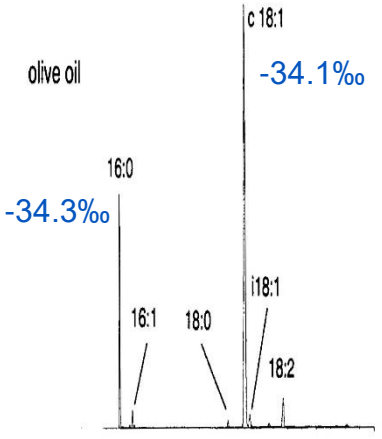
Undeclared addition of water in milk



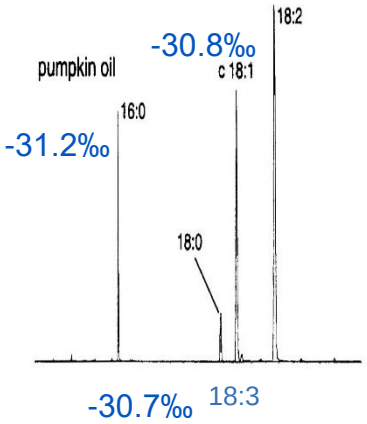
From 7 to 30% added water

Gregorčič et al. Milk Authentication: Stable Isotope Composition of Hydrogen and oxygen in Milks and their Constituents. Molecules, 2020

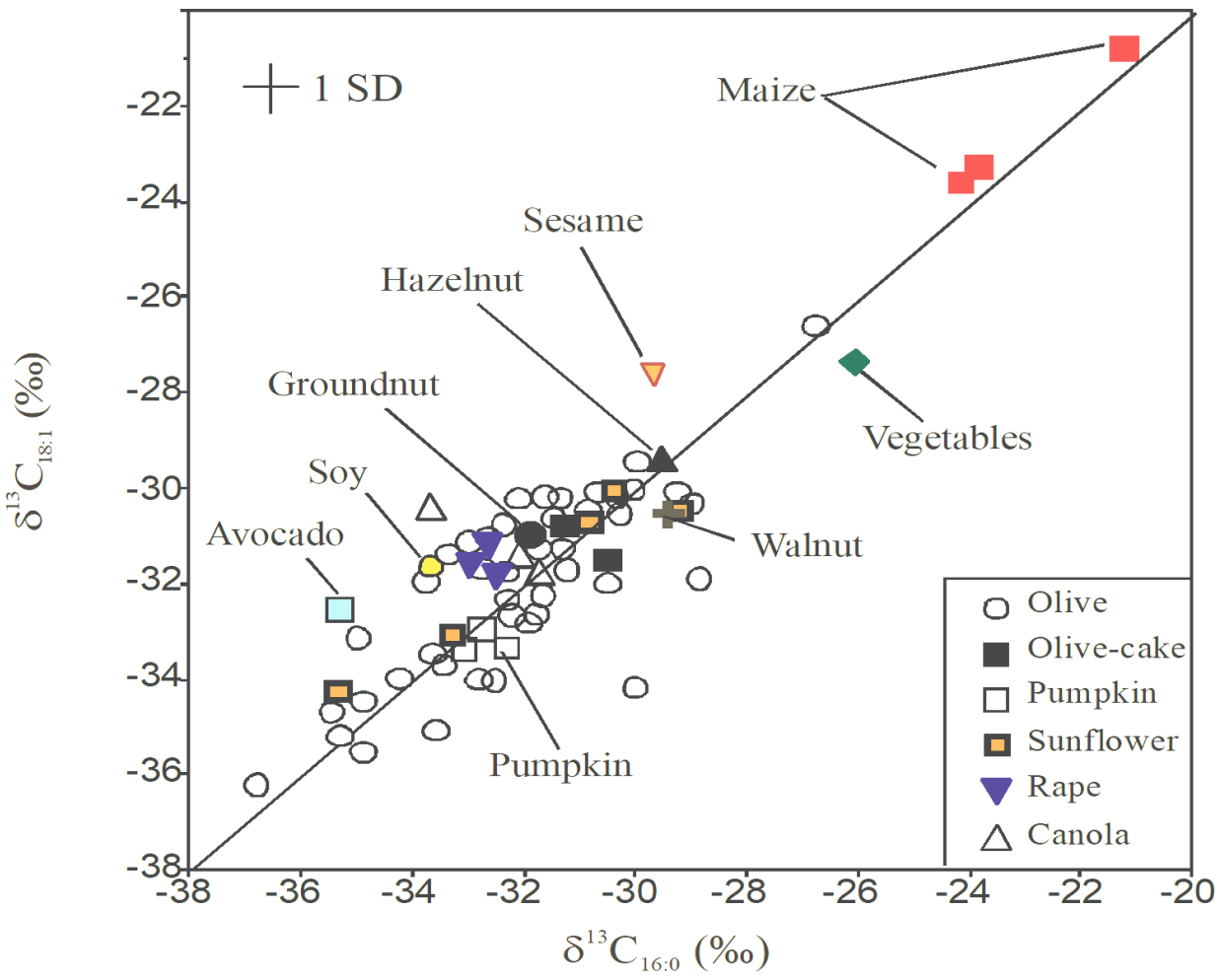
Adulteration of olive and pumpkin oil



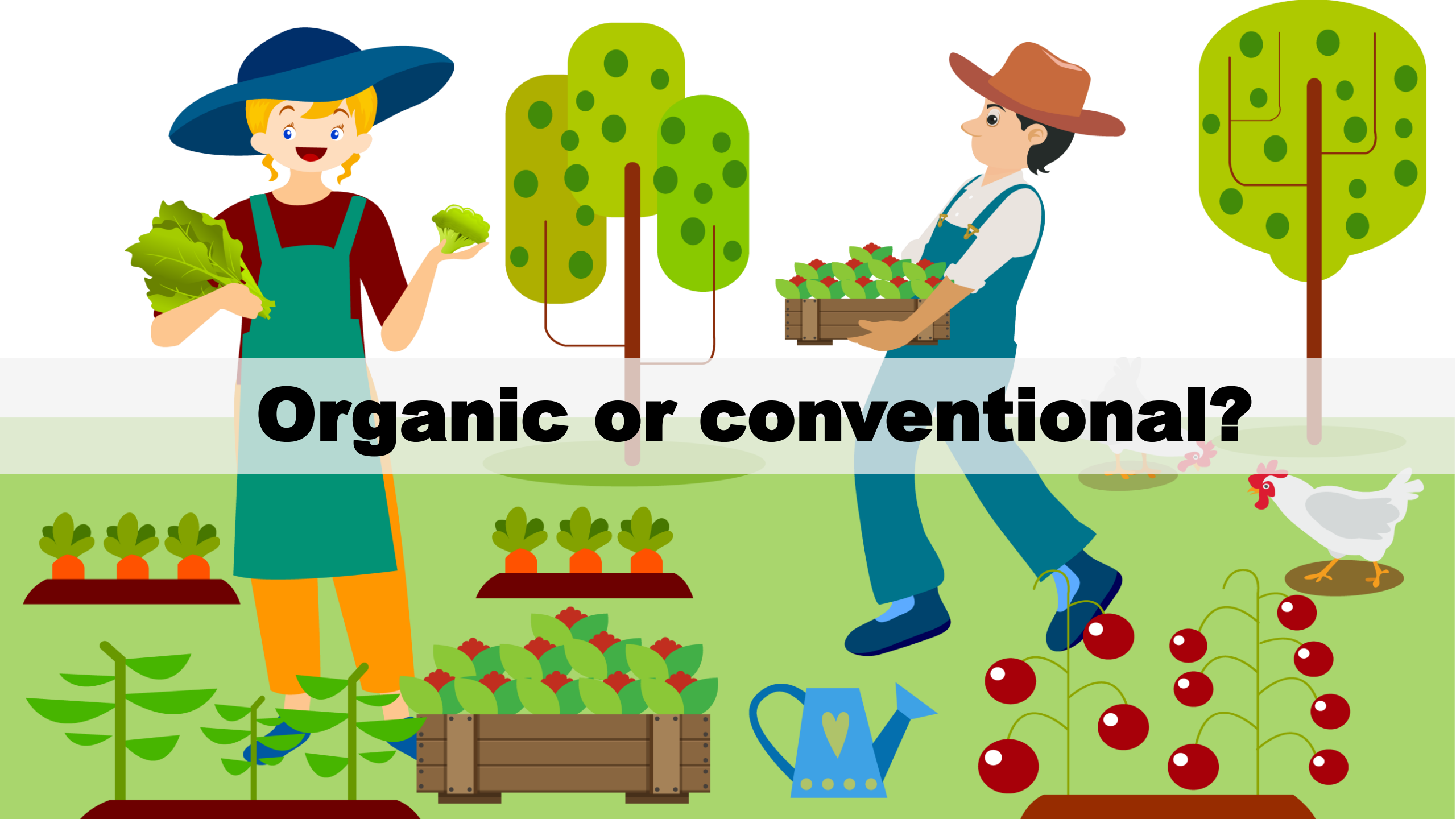
Spangenberg & Ogrinc, JAFC, 2001



Potočnik et al., JFAC 2016

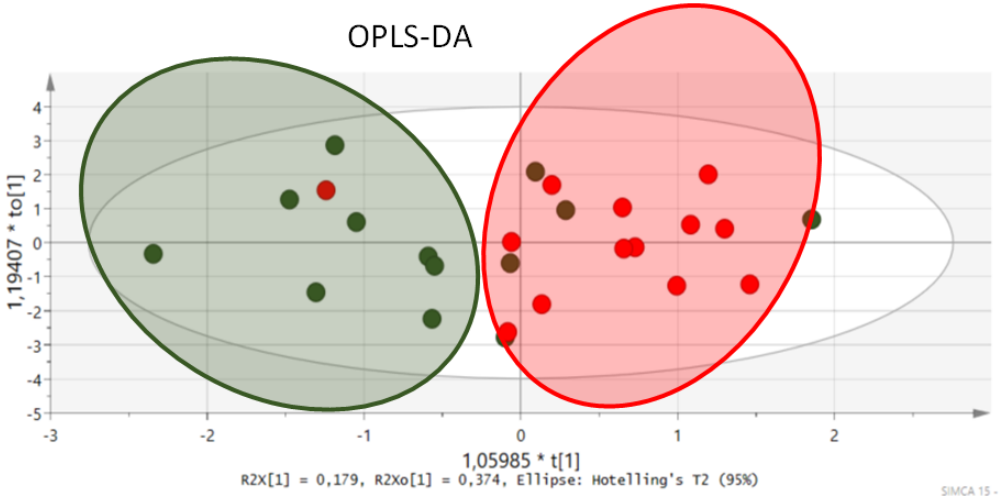


Organic or conventional?



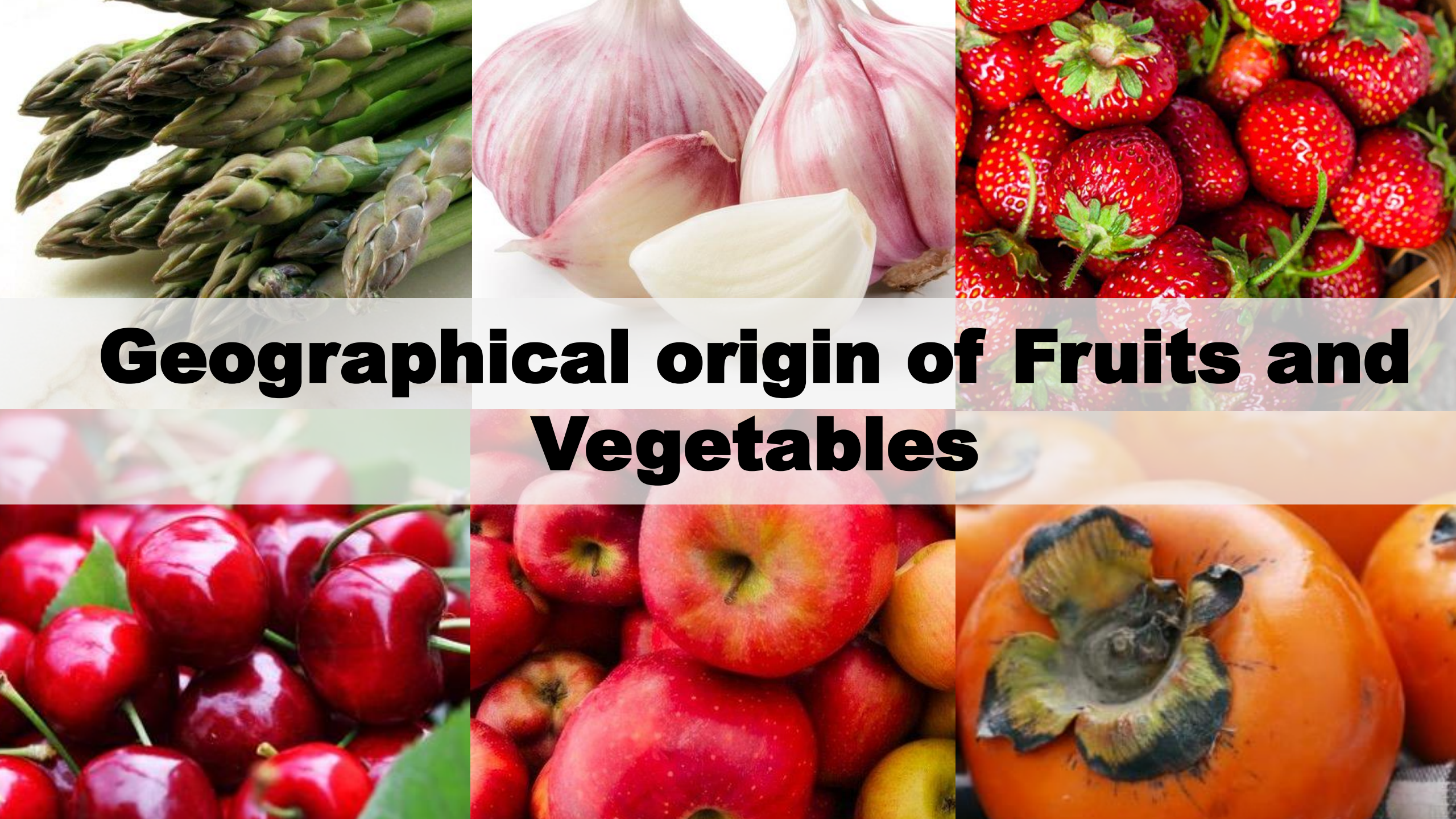
Comparison between different production practices for hops

$^{15}N/^{14}N$



$^{13}C/^{12}C$

Golubovič et al, Foods, 2021



Geographical origin of Fruits and Vegetables



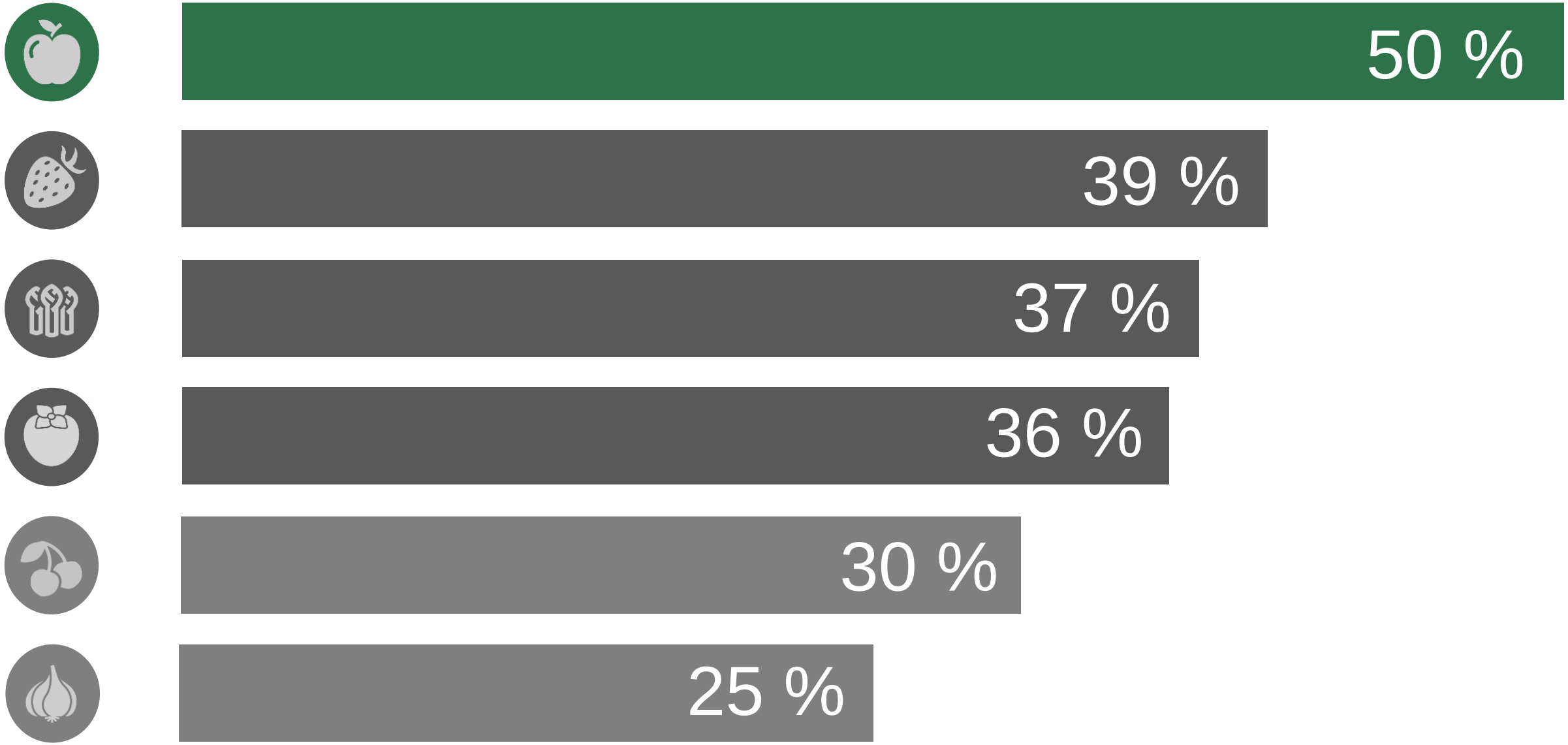
REPUBLIC OF SLOVENIA
MINISTRY OF AGRICULTURE, FORESTRY AND FOOD

THE ADMINISTRATION OF THE REPUBLIC OF SLOVENIA
FOR FOOD SAFETY, VETERINARY AND PLANT PROTECTION

Slovenian origin?



% of samples non compliant with declaration



Acknowledgements

Implementation of research

FULBRIGHT

INDIANA UNIVERSITY
BLOOMINGTON

METRO FOOD
INFRASTRUCTURE FOR PROMOTING
METROLOGY IN FOOD AND NUTRITION

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FNS - Cloud
Food Nutrition Security

PROmedLIFE
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THANK YOU!

Any questions?