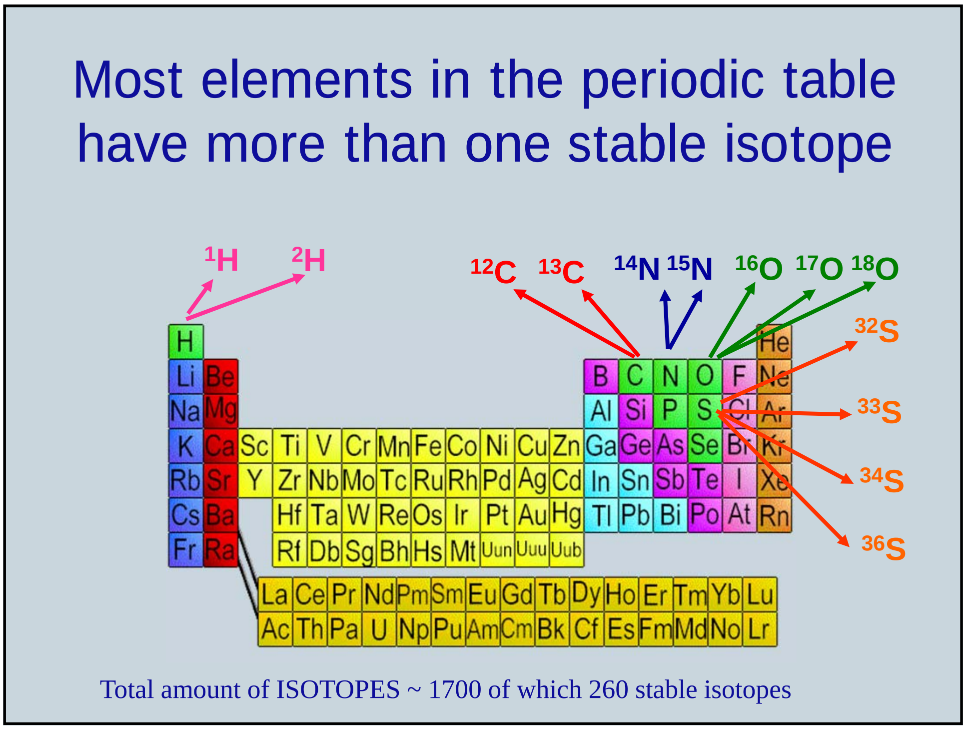
Isotope Ratio Mass Spectrometry

IRMS

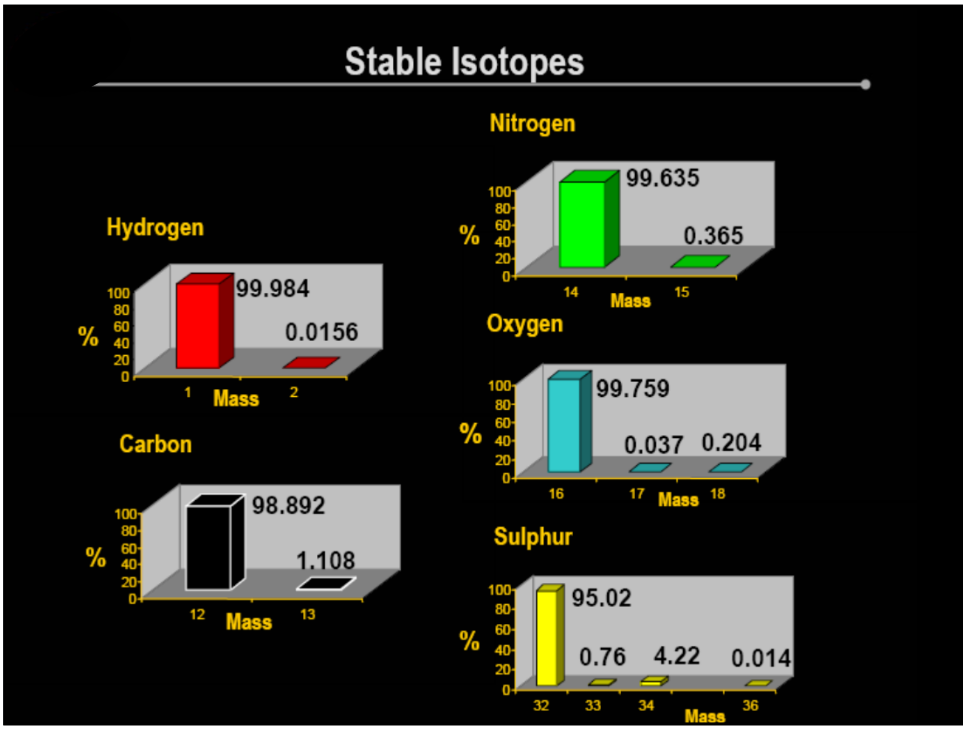
What are stable isotopes?

- ✓ Atoms that have same atomic number, but different atomic weight (different # of neutrons)
- ✓ Stable isotopes are not radioactive
- ✓ In nature, isotopic variations occur because substances (such as minerals, water, and gases) preferentially concentrate one isotope over another, or because organisms can more efficiently metabolize one isotope than another.



Most elements in the periodic table have more than one stable isotope

Total amount of ISOTOPES ~ 1700 of which 260 stable isotopes



Stable Isotopes

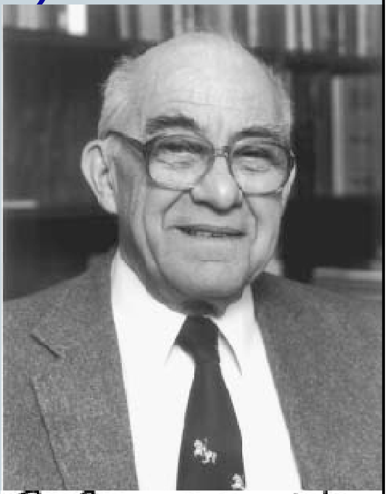
Alfred O. Nier
(1911-1994)

The Nier mass spectrometer:

crucial for stable isotope ratio
mass spectrometry of gases

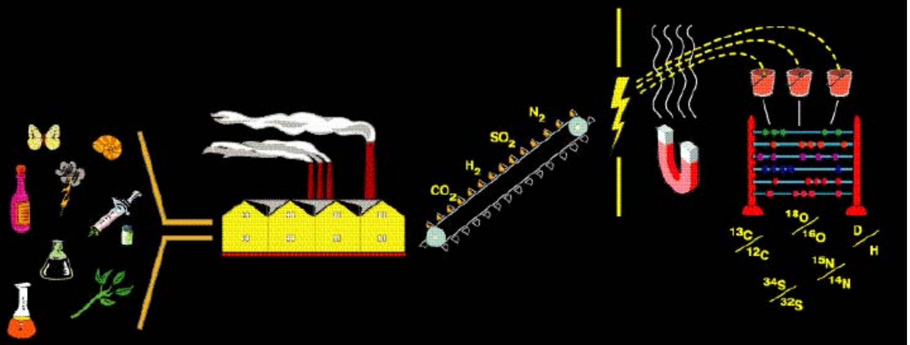
basis for modern mass spectrometers

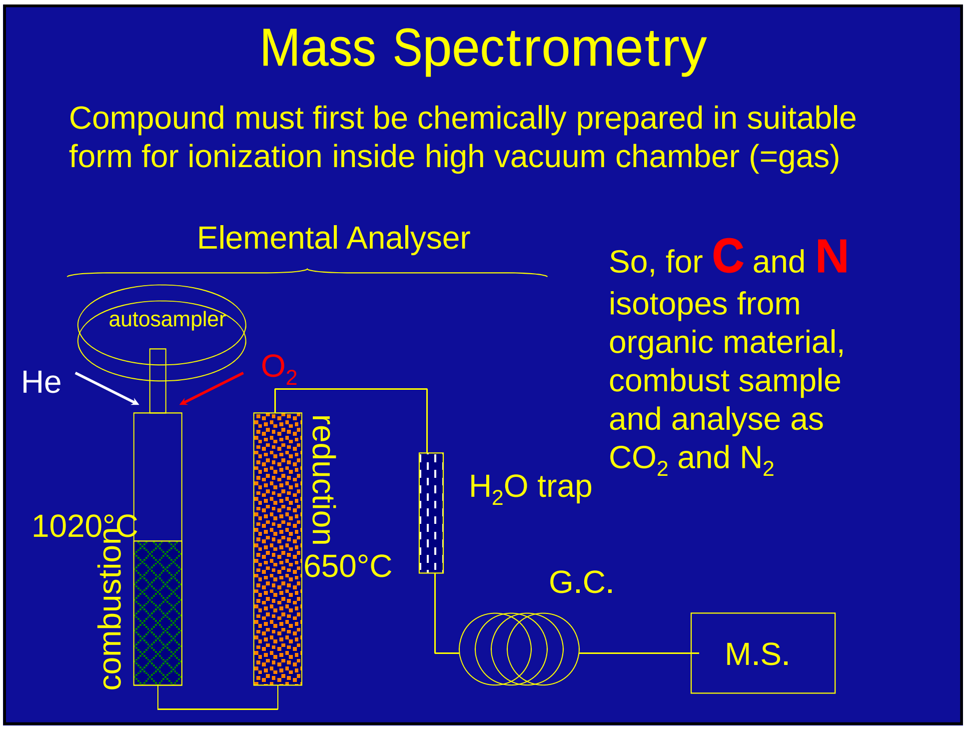
key elements:
- gas source
- double collectors
- dual inlet



Alfred O C Nier

What is Stable Isotope Ratio MS?

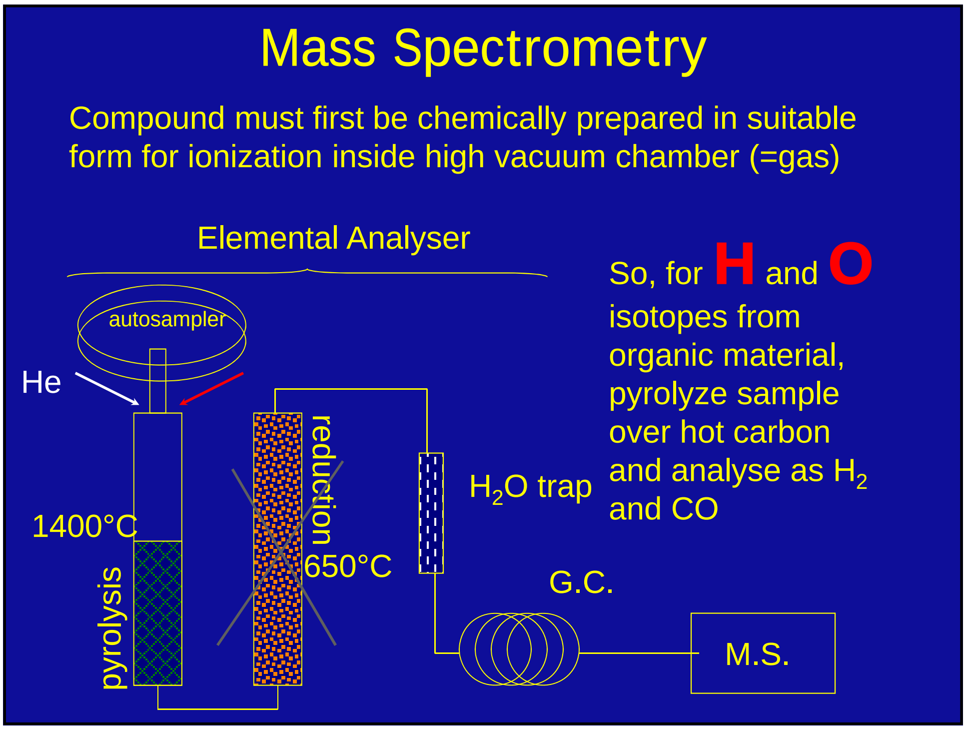




Mass Spectrometry

Compound must first be chemically prepared in suitable form for ionization inside high vacuum chamber (=gas)

So, for **C** and **N** isotopes from organic material, combust sample and analyse as CO_2 and N_2



Mass Spectrometry

Compound must first be chemically prepared in suitable form for ionization inside high vacuum chamber (=gas)

So, for **H** and **O** isotopes from organic material, pyrolyze sample over hot carbon and analyse as H_2 and CO

How does an isotope ratio mass spectrometer work?

Step 1

Sample must be converted to gas and gases purified

- δ^2H: H_2
- δ^{13}C: CO_2
- δ^{15}N: N_2
- δ^{18}O: CO or O_2
- δ^{34}S: SO_2

Step 2 Sample must be ionized e.g., $H_2 \dashrightarrow H_2^+$

Step 3 Ionic species are subjected to a magnetic field for separation

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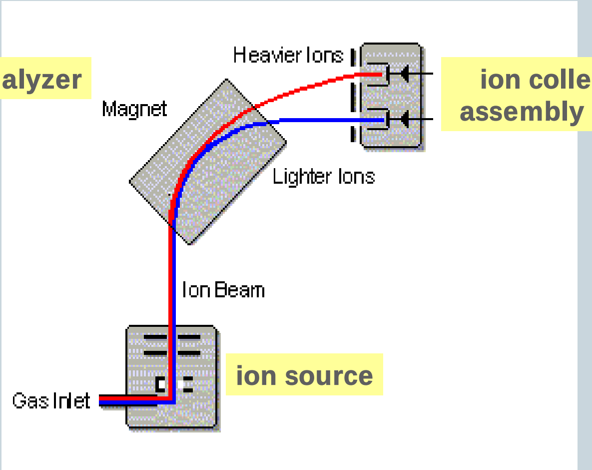
Isotope Ratio Mass Spectrometry

The mass spectrometers used for isotopic analysis generally comprise three basic sections:



mass analyzer

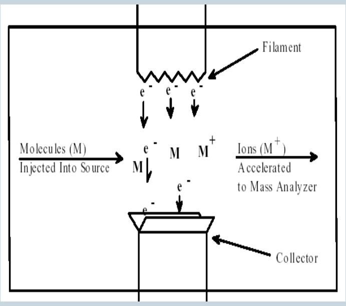
ion collection assembly (array)

ion source

Ionization

Ionization

Gaseous molecules are introduced into the ionization chamber where interaction with an ionization source causes electrons to be stripped from the molecules resulting in the formation of positive ions.



Sources of ionization

→ICP (inductively coupled plasma)

→**Thermal Ionization** (Thorium filament)

→Electron ionization

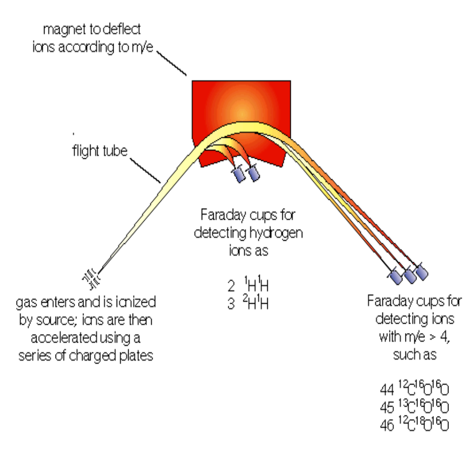
Separation/Collection

Electromagnet separation

The ions are then accelerated out of the chamber down a flight tube between the poles of an electromagnet where they are separated according to their mass-to-charge ratio (m/z).

Collection of ions

Faraday cups are lined up in fixed position along the beam to receive major ion currents of the gas (m/z 44, 45, 46 for CO_2). In the cups, the impact of the ions is translated into a recordable signal that is collected for data processing and analysis.



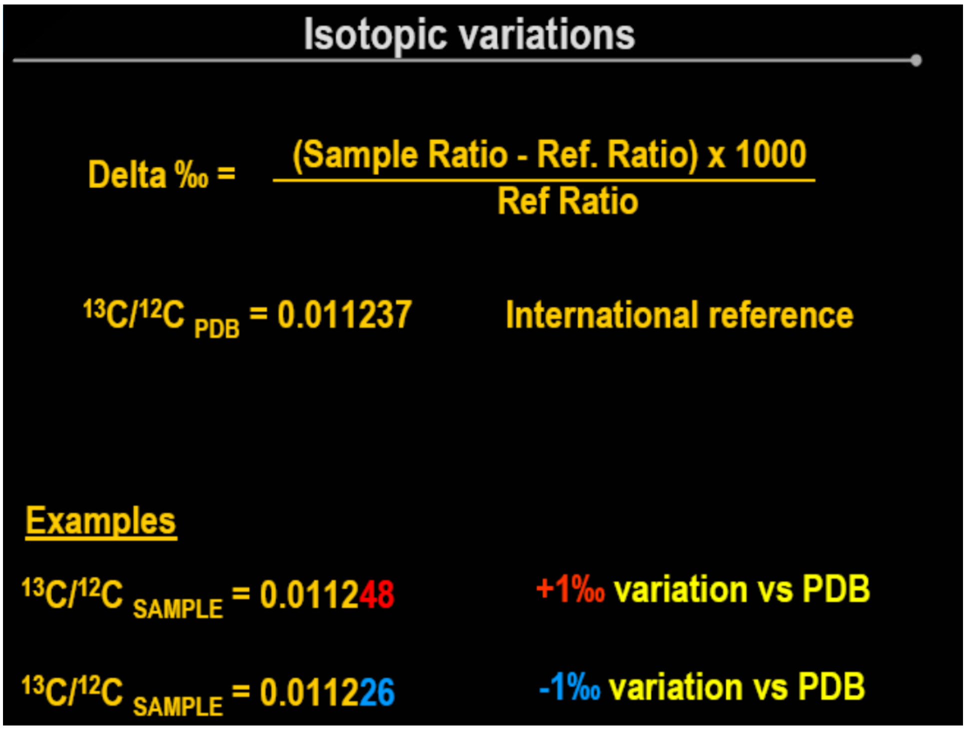
Some of the ionic gas species in the mass spectrometer

species	mol wt	m/z	
$^{12}C^{16}O^{16}O^{+}$	44	44	
$^{13}C^{16}O^{16}O^{+}$	45	45	
$^{12}C^{18}O^{16}O^{+}$	46	46	
$^{13}C^{18}O^{18}O^{+}$	49	49	rare, not measured
$^{14}N^{14}N^{16}O^{+}$	44	44	
$^{12}C^{16}O^{16}O^{++}$	44	22	rare, not measured
$^{14}N^{14}N^{+}$	28	28	
$^{15}N^{14}N^{+}$	29	29	
$^{15}N^{15}N^{+}$	30	30	rare, not measured

Isotope Ratio Mass Spectrometry

- The unit to express the data is $\delta^{13}C$ defined as the relative difference (‰) between the $^{13}C/^{12}C$ ratio of the sample and a reference standard
- As international reference standard has been selected a Cretaceous marine fossil, *Belemnitella americana*, from the PeeDee formation in South Carolina with $^{13}C/^{12}C$ ratio of 0.011237.

$$\delta^{13}C = \left(\frac{^{13}C/^{12}C_{(\text{sample})}}{^{13}C/^{12}C_{(\text{standard})}} - 1 \right) \times 1000$$



Isotopic variations

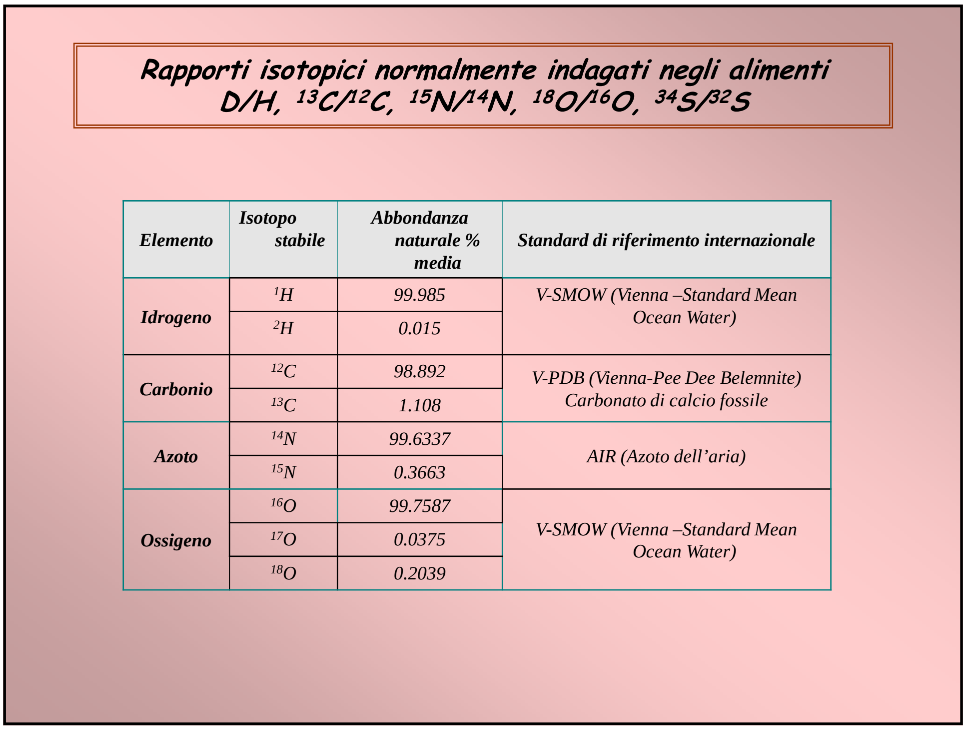
$$\text{Delta ‰} = \frac{(\text{Sample Ratio} - \text{Ref. Ratio}) \times 1000}{\text{Ref Ratio}}$$

$^{13}C/^{12}C_{PDB} = 0.011237$ International reference

Examples

$^{13}C/^{12}C_{SAMPLE} = 0.011248$ +1‰ variation vs PDB

$^{13}C/^{12}C_{SAMPLE} = 0.011226$ -1‰ variation vs PDB

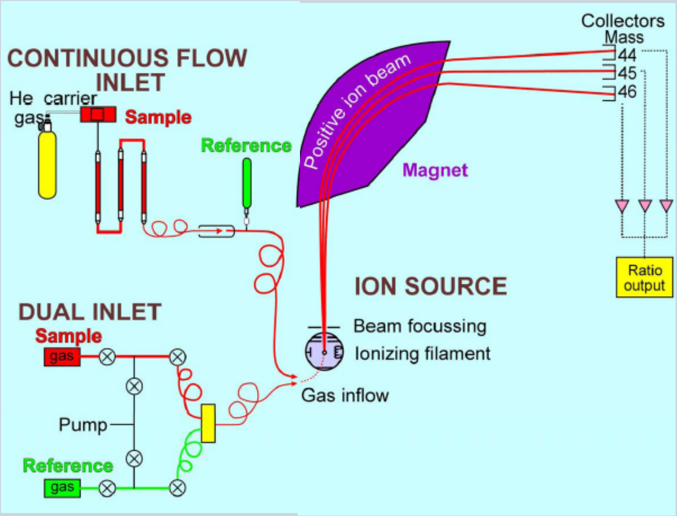


Rapporti isotopici normalmente indagati negli alimenti
D/H, $^{13}C/^{12}C$, $^{15}N/^{14}N$, $^{18}O/^{16}O$, $^{34}S/^{32}S$

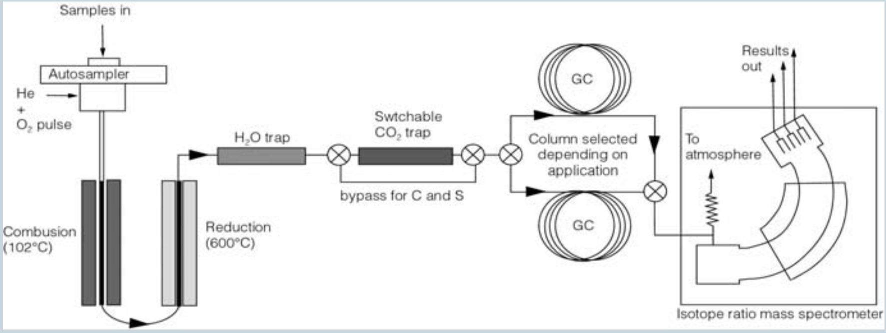
Elemento	**Isotopo stabile**	**Abbondanza naturale % media**	**Standard di riferimento internazionale**
Idrogeno	^{1}H	99.985	V-SMOW (Vienna –Standard Mean Ocean Water)
	^{2}H	0.015	
Carbonio	^{12}C	98.892	V-PDB (Vienna-Pee Dee Belemnite) Carbonato di calcio fossile
	^{13}C	1.108	
Azoto	^{14}N	99.6337	AIR (Azoto dell'aria)
	^{15}N	0.3663	
Ossigeno	^{16}O	99.7587	V-SMOW (Vienna –Standard Mean Ocean Water)
	^{17}O	0.0375	
	^{18}O	0.2039	

Isotope Ratio Mass Spectrometry

Instrumental configuration



Elemental analyzer coupled to continuous flow IRMS



Simplified online reactions for continuous flow

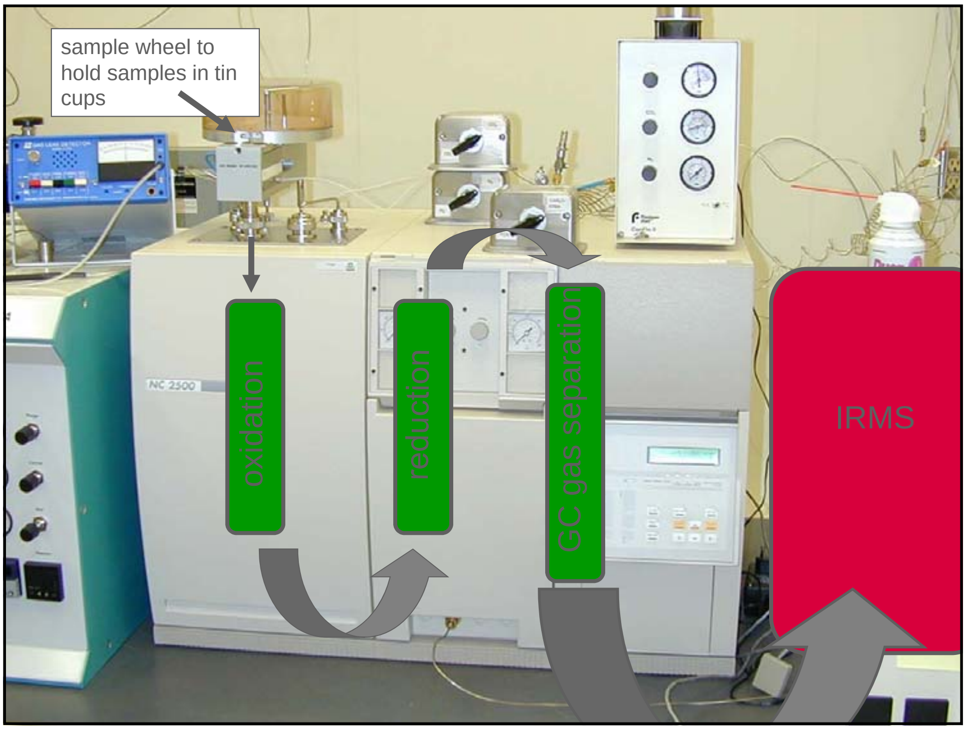
oxidation and reduction

$CH_2NO + O_2 \longrightarrow CO_2 + N_2$

pyrolysis

$CH_2NO + C \longrightarrow CO + H_2$

$H_2O + C \longrightarrow CO + H_2$



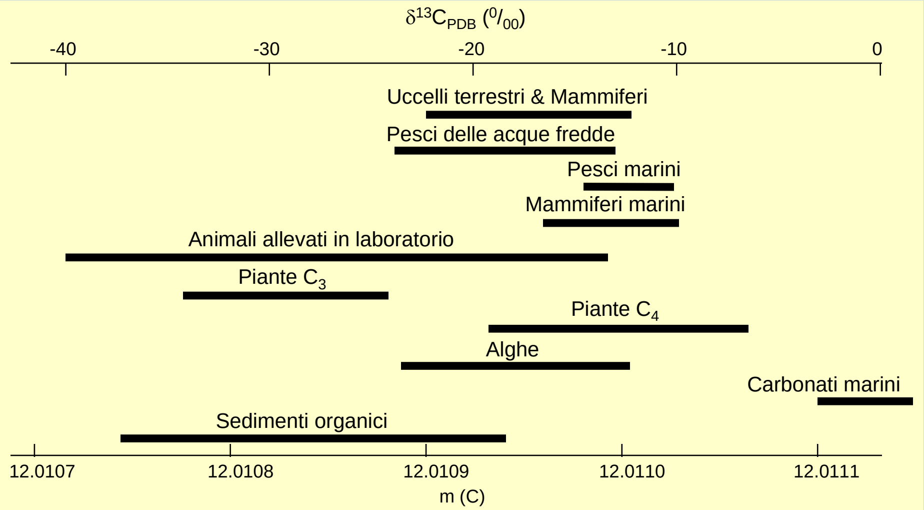
Comparison of IRMS to MS

- ✓ Resolution is more important in MS, while sensitivity is more important in IRMS
- ✓ Dynamic range is limited in IRMS to detect at specific isotope mass, while MS covers larger dynamic range
- ✓ → For MS, it's more important to have resolved peaks
- ✓ → For IRMS, it's more important to have accurate peak heights

CHE INFORMAZIONI DANNO I RAPPORTI ISOTOPICI ?

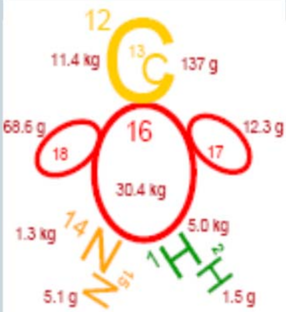
tipo di isotopo	è influenzato da….	e può perciò determinare…
$^{13}C/^{12}C$, D/H	Metabolismo (C3, C4, CAM)	**Aggiunta illecita di zucchero di canna e/o barbabietola** (ad esempio a vino e succhi di frutta) **Tenore di mais nella dieta animale**
$^{18}O/^{16}O$, D/H	Origine dell'acqua	**Annacquamento** (ad es. nel vino)
$^{13}C/^{12}C$, D/H	Sintesi chimica	**Aggiunta di sostanze di sintesi**
$^{18}O/^{16}O$, D/H, $^{15}N^{14}N$, $^{13}C/^{12}C$	Origine geografica	**Caratterizzazione di prodotti ad origine protetta e controllata**

Isotope ratio $^{13}C/^{12}C$ of proteins from different organisms and two class of minerals.



Isotopic abundance and geographical origin

- ✓ The relative abundances of isotopes of elements such as ^{2}H, ^{13}C, ^{15}N, ^{18}O and ^{34}S vary geographically as a result of **kinetic and thermodynamic fractionation processes** (evaporation, condensation, biochemical/chemical exchange).
- ✓ The same abundances are assimilated into plants and animals grown in the region.
- ✓ Isotopic abundances can be measured by total combustion using an elemental analyser coupled to an isotope ratio mass spectrometer IRMS and compared to values obtained for authentic materials.



Typical isotope composition of an average build adult

Geographical Variation of Isotopic Abundance

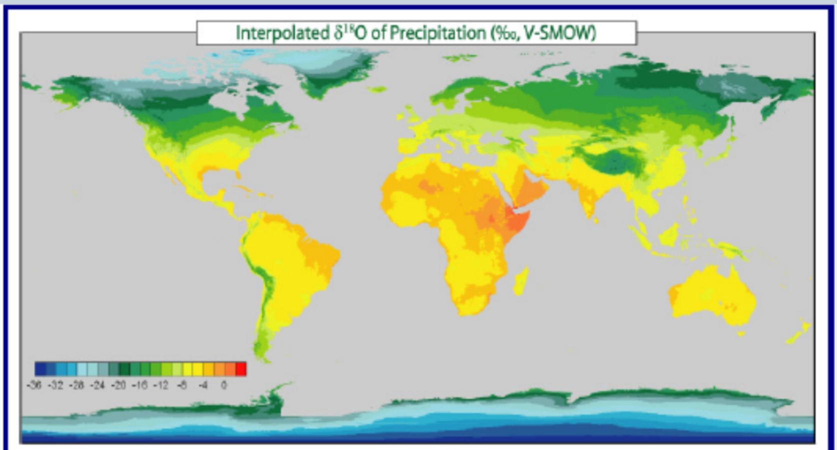
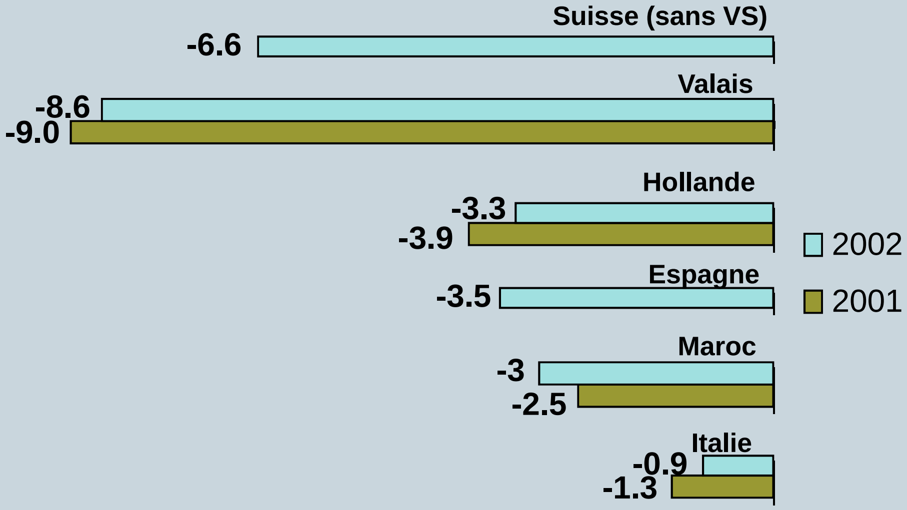


Figure 1: Diagram showing global variation of ^{18}O isotopic abundance in rain water [3].

Source : W Maier-Augenstein, I Fraser & RM Kalin, Queens University Belfast

Base de donnée pour les tomates

(rapport $^{18}O/^{16}O$ dans le jus des tomates donnés en δ)



E.Pfammatter, V.Maury, Laboratoire Cantonal du VALAIS

Isotope ratio $^{13}C/^{12}C$ provide useful analytical information

- ✓ Natural Compounds greatly differ from those of synthetic origin: example

[Gioacchini A.M., Roda A., Parenti M., Cipolla A., Baraldini M.:
Differentiation between Natural and Synthetic Taurine using the $^{13}C/^{12}C$ isotope ratio.
Rapid. Comm. Mass. Spec., 9, 1106-1108, 1995.]

- ✓ All the compounds obtained from oil present a $\delta^{13}C$ of -31 ‰ , useful for identification.

ISOTOPE RATIO MASS SPECTROMETRY

Basandosi sui diversi rapporti isotopici permette di poter risalire alla

BIOGENESI e ORIGINE

del materiale in esame

Non-Equilibrium Fractionation in Biology

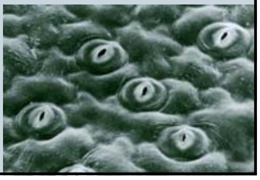
The $^{13}C/^{12}C$ isotope ratio is quite different in plant with C3 and C4 photosynthetic pathway and the substances extracted present values refered to the original plant;

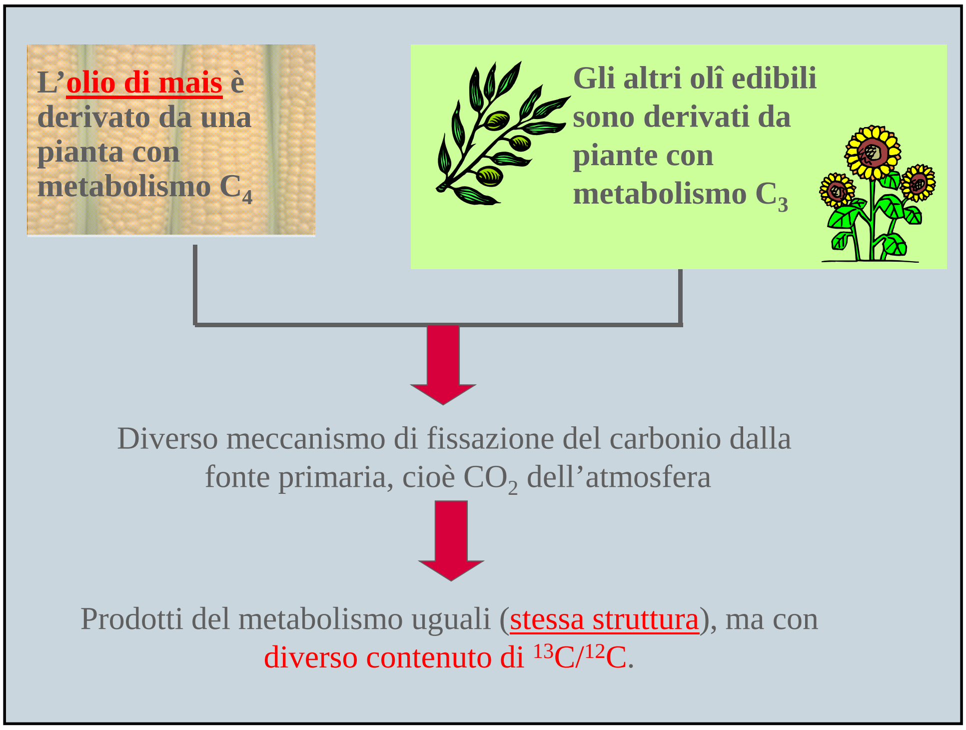
for example: the sucrose extracted from the sugar cane (plant C_4, δ=-11 ‰) and that extract from sugar beet (plant C_3, δ=-25 ‰)

Photosynthesis: green plants fixing C and if the primary step is diffusion of CO_2 into stomata they will be enriched in the lighter $^{12}CO_2$ in respect to the heavier $^{13}CO_2$

↓

Preferential ^{12}C in plant passed on to herbivores and up the food chain



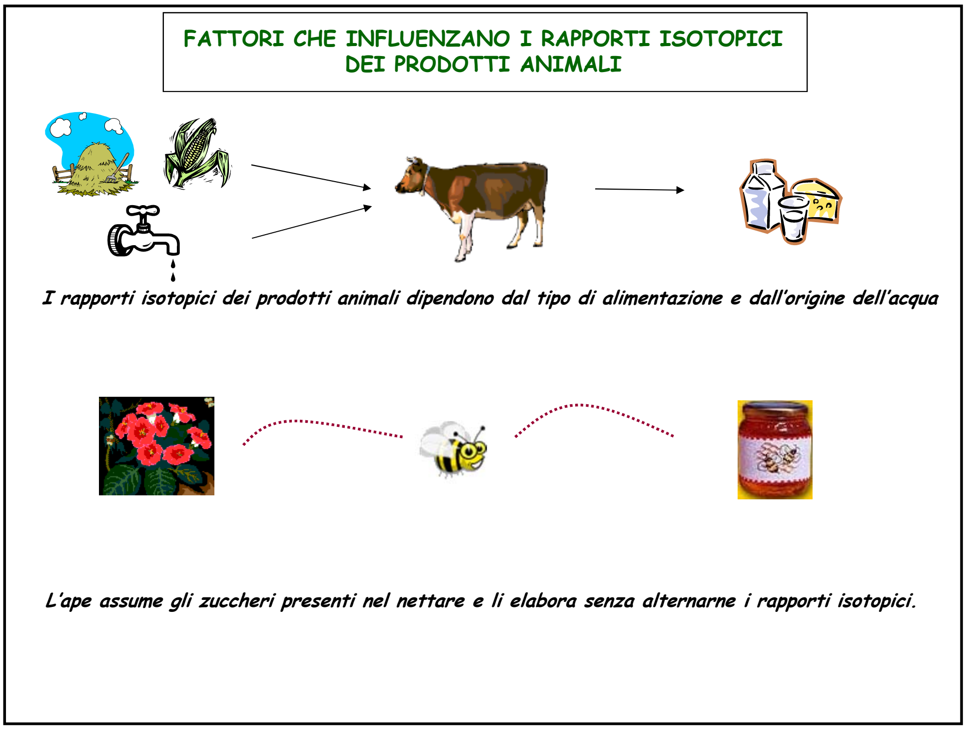


L'olio di mais è derivato da una pianta con metabolismo C_4

Gli altri olî edibili sono derivati da piante con metabolismo C_3

Diverso meccanismo di fissazione del carbonio dalla fonte primaria, cioè CO_2 dell'atmosfera

Prodotti del metabolismo uguali (stessa struttura), ma con diverso contenuto di $^{13}C/^{12}C$.

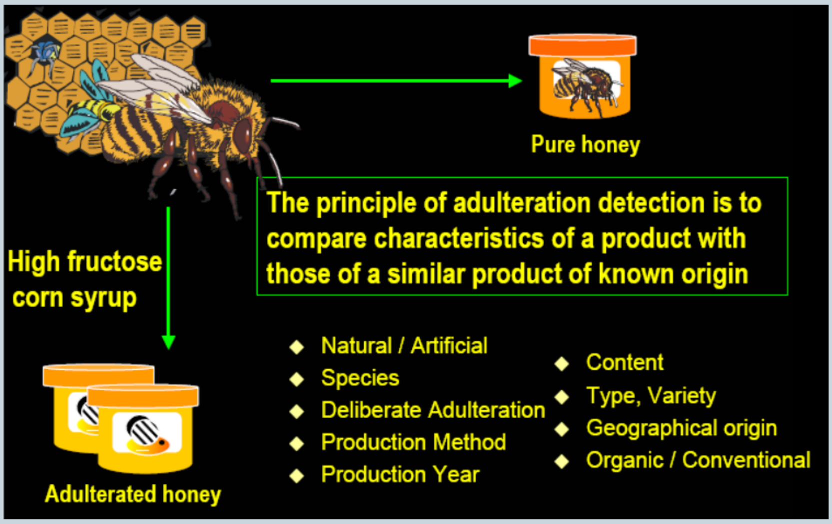


FATTORI CHE INFLUENZANO I RAPPORTI ISOTOPICI DEI PRODOTTI ANIMALI

I rapporti isotopici dei prodotti animali dipendono dal tipo di alimentazione e dall'origine dell'acqua

L'ape assume gli zuccheri presenti nel nettare e li elabora senza alternarne i rapporti isotopici.

Detection of adulterated honey using using the $^{13}C/^{12}C$ ratio



Pure honey

High fructose corn syrup

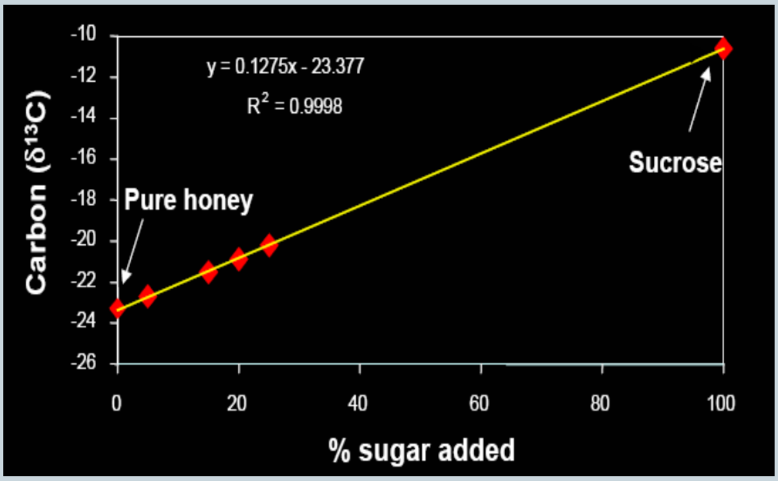
Adulterated honey

The principle of adulteration detection is to compare characteristics of a product with those of a similar product of known origin

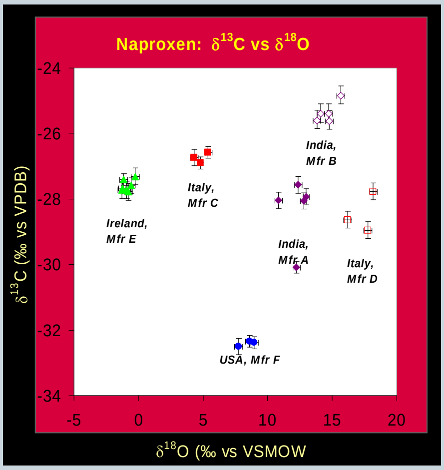
- Natural / Artificial
- Species
- Deliberate Adulteration
- Production Method
- Production Year

- Content
- Type, Variety
- Geographical origin
- Organic / Conventional

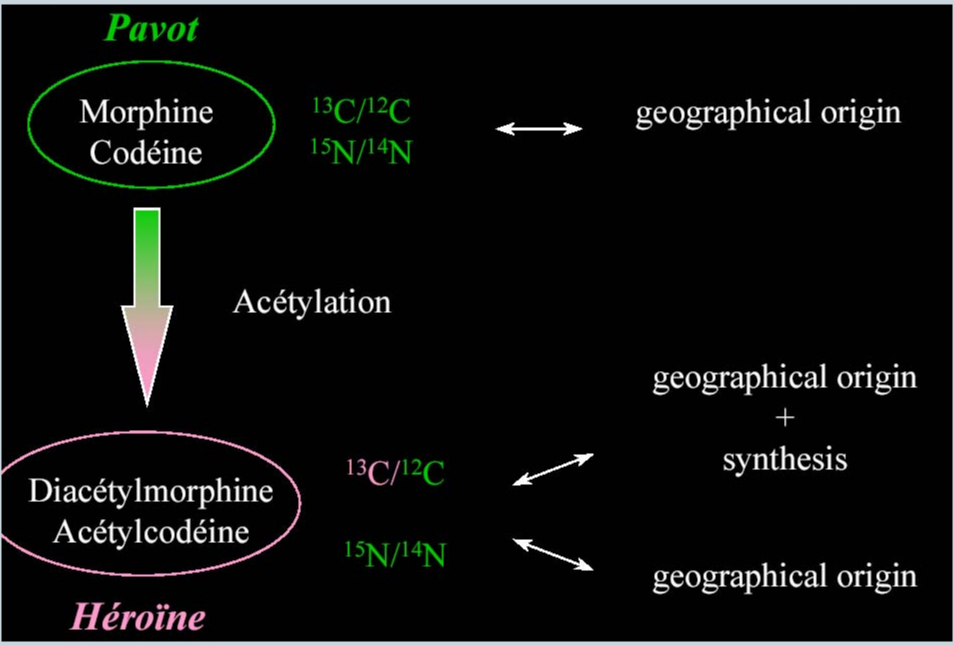
Detection of adulterated honey using using the $^{13}C/^{12}C$ ratio



IRMS- Isotope Ratio Mass Spectrometry



IRMS can provide the source of active pharmaceutical ingredients (APIs). In the bivariate isotope ratio graph shown, the typical clustering of the data is consistent with manufacturer-based isotopic provenance.



Pavot

Morphine
Codéine

$^{13}C/^{12}C$
$^{15}N/^{14}N$

↔ geographical origin

Acétylation

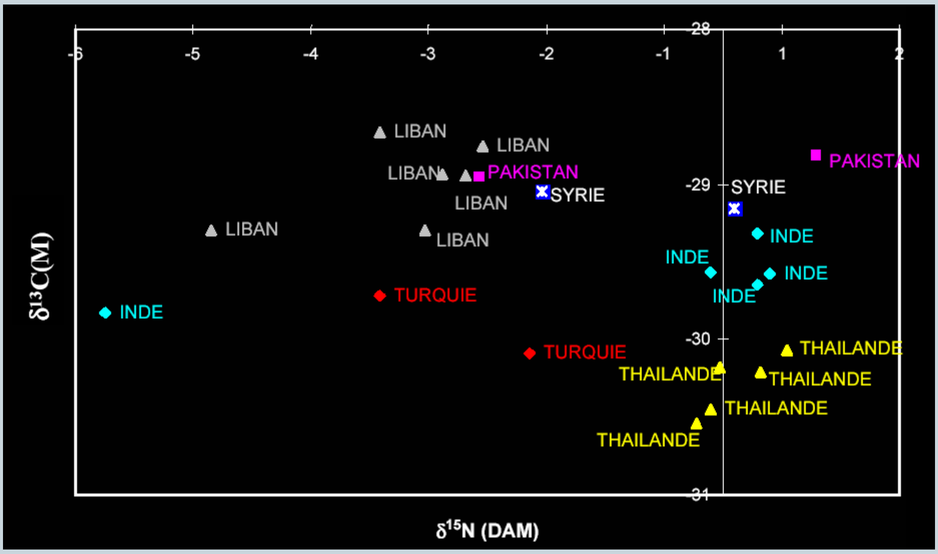
Diacétylmorphine
Acétylcodéine

Héroïne

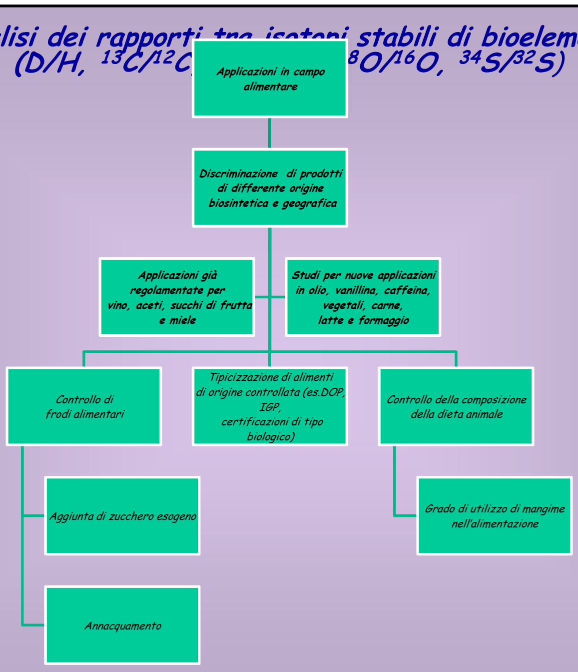
$^{13}C/^{12}C$ ↔ geographical origin + synthesis

$^{15}N/^{14}N$ ↔ geographical origin

Heroin



Analisi dei rapporti tra isotopi stabili di bioelementi (D/H, $^{13}C/^{12}C$ $^{8}O/^{16}O$, $^{34}S/^{32}S$)



Applicazioni in campo alimentare

Discriminazione di prodotti di differente origine biosintetica e geografica

Applicazioni già regolamentate per vino, aceti, succhi di frutta e miele

Studi per nuove applicazioni in olio, vanillina, caffeina, vegetali, carne, latte e formaggio

Controllo di frodi alimentari

Tipicizzazione di alimenti di origine controllata (es.DOP, IGP, certificazioni di tipo biologico)

Controllo della composizione della dieta animale

Aggiunta di zucchero esogeno

Annacquamento

Grado di utilizzo di mangime nell'alimentazione

13C-Urea Breath Test

Test per la diagnosi di infezione da Helicobacter pylori

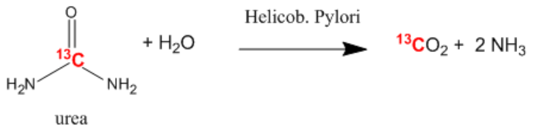
Il test del respiro (urea breath test - UBT) si basa sulla capacità di *H. pylori* di metabolizzare rapidamente l'urea somministrata per bocca, fino a ottenere acqua e anidride carbonica.

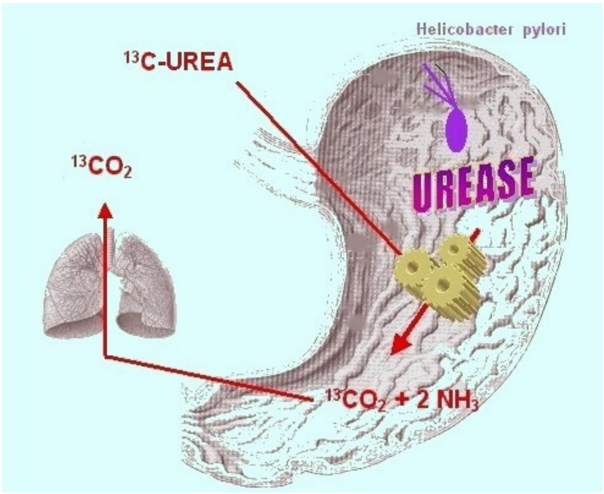
Se l'urea viene marcata con l'isotopo 13 del carbonio, non radioattivo e presente in natura, si può misurare l'eliminazione attraverso il respiro di anidride carbonica marcata.

Un suo aumento tra due prove consecutive (prima e mezz'ora dopo la somministrazione dell'urea) è quindi un indice indiretto della presenza di infezione da elicobatterio a livello gastrico.

Se il batterio non è presente, l'urea marcata passa intatta attraverso lo stomaco e, di conseguenza, non si ritroverà anidride carbonica marcata nei campioni di respiro.

L'UBT è in genere una metodica che presenta una alta sensibilità e specificità anche se vi è sempre la possibilità di avere dei risultati falsamente negativi quando il paziente è stato recentemente sottoposto a somministrazione di antibiotici o, più comunemente di farmaci inibitori della pompa protonica. Questi farmaci sopprimono l'*H. pylori*, senza però eradicarlo completamente. Ancora, risultati falsamente negativi possono essere ottenuti se il tempo di contatto dell'urea con la mucosa gastrica non è sufficiente a permettere l'idrolisi.





Basta comprare lo strumento.
Poi è tutto automatico!

È FALSO !!!

Lo strumentino che si autogestisce

NON ESISTE !!!

Basta il kit!

È FALSO !!!

L’era del

“click e lo spettro è fatto”

NON VERRÀ MAI !!!

Identificare X / Determinare la quantità di Y

CAMPIONE → ESTRAZIONE → PURIFICAZIONE

ANALISI → GC-, HPLC- MS

Quale informazione voglio ottenere? Qual'è l'esperimento più appropriato (sensibilità, selettività, velocità, ecc…) ?

S

nico-pratica

Identificare X / Determinare la quantità di Y

CAMPIONE → ESTRAZIONE → PURIFICAZIONE

ANALISI → RISULTATO

IL COMPUTER HA DETTO …. !!!!

INTERPRETAZIONE

Ipse dixit !

Identificare X / Determinare la quantità di Y

CAMPIONE → ESTRAZIONE → PURIFICAZIONE → ANALISI → RISULTATO ↔ INTERPRETAZIONE

Un **risultato affidabile** richiede:

scelta corretta del tipo di esperimento

corretta esecuzione → **AZIONE**

corretta interpretazione dei dati