"Forensic Applications in Continuous Flow IRMS Using the IsoPrime”

F. FOUREL

Menu

1/ Introduction to stable isotopes

2/ Dual inlet / Continuous Flow

3/ Example of applications

6/ Conclusions

Stable Isotope Ratio MS

Introduction

Atoms of a particular element having the same number of protons but differing number of neutrons are ISOTOPES.

Stable IRMS is concerned with determining the isotope content in the elements C,N,O,S,H found in light gases (eg: CO_2, N_2, N_2O, H_2, SO_2).

IRMS instruments measure the relative abundance of these isotopes to a reference gas (cf: TIMS which determines the absolute isotope ratio).

Stable Isotope Ratio MS

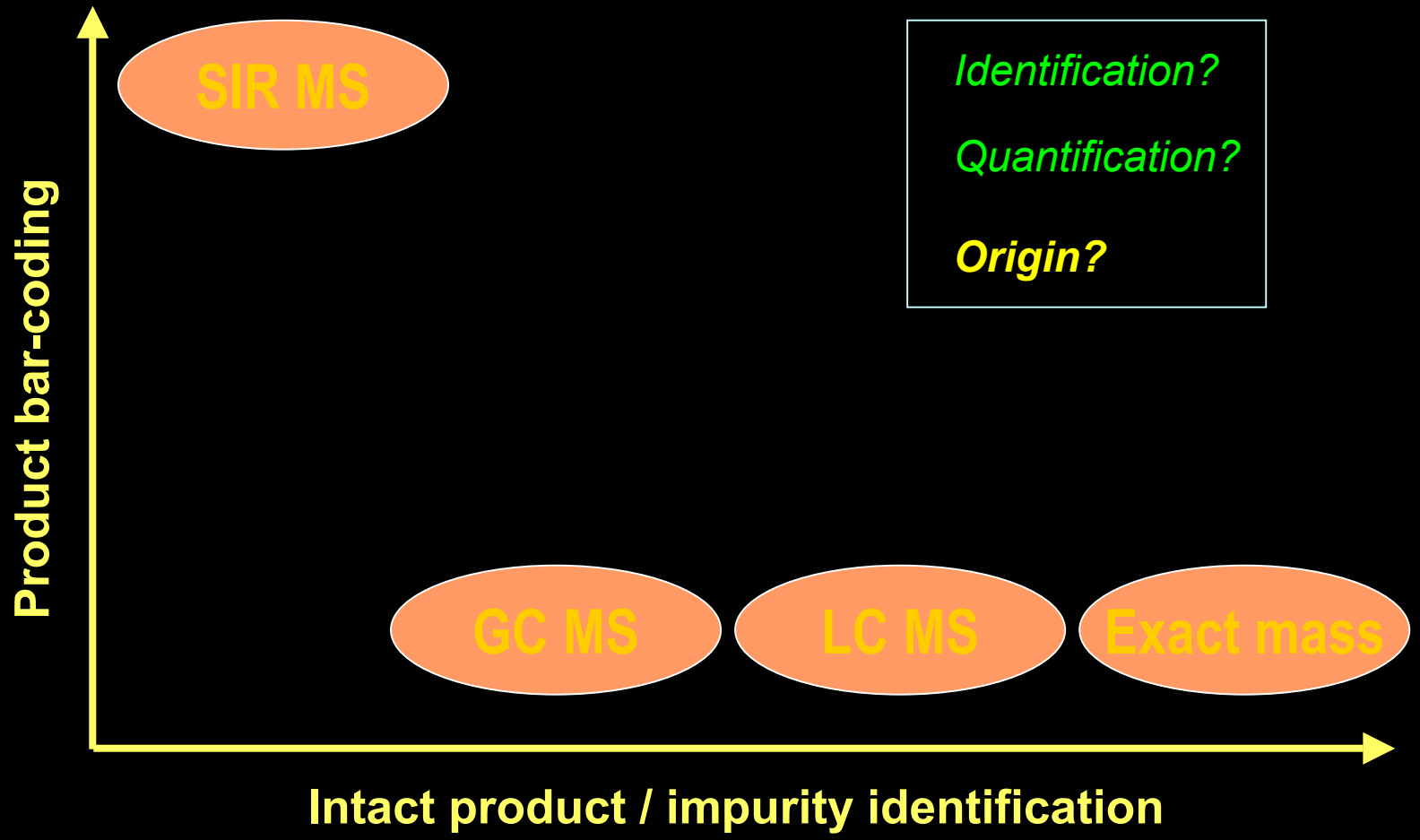
When the question triggered by a sample is not

"How Much?"

But "Where From?"

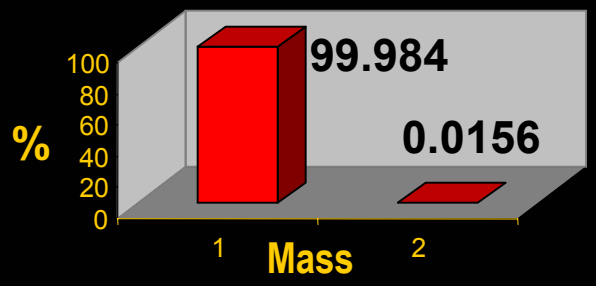
Stable Isotope Ratio MS may be the answer...

IRMS for Drug Product Authentication

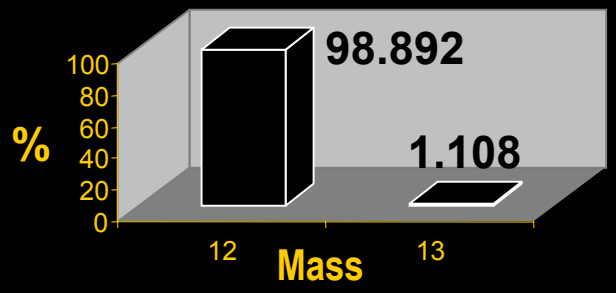


Stable Isotopes

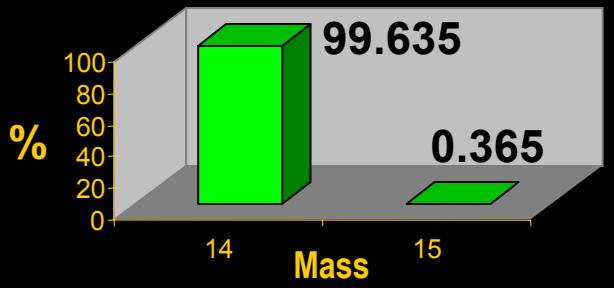
Hydrogen



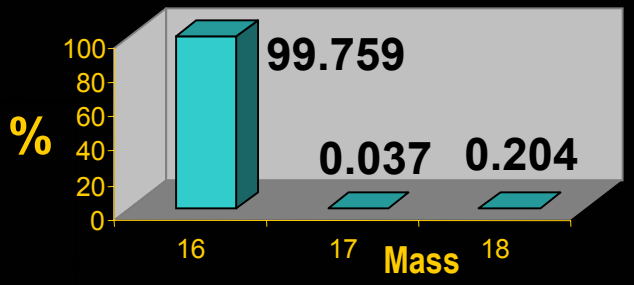
Carbon



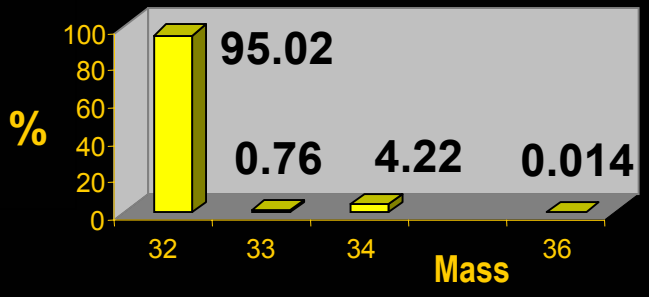
Nitrogen



Oxygen



Sulphur



Stable Isotope Ratio MS

The units used to express this relative difference are either Atom% or Delta (per mil, ‰).

At% = (No of minor Atoms /No of major atoms) x 100

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

Isotopic variations

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

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

Applications

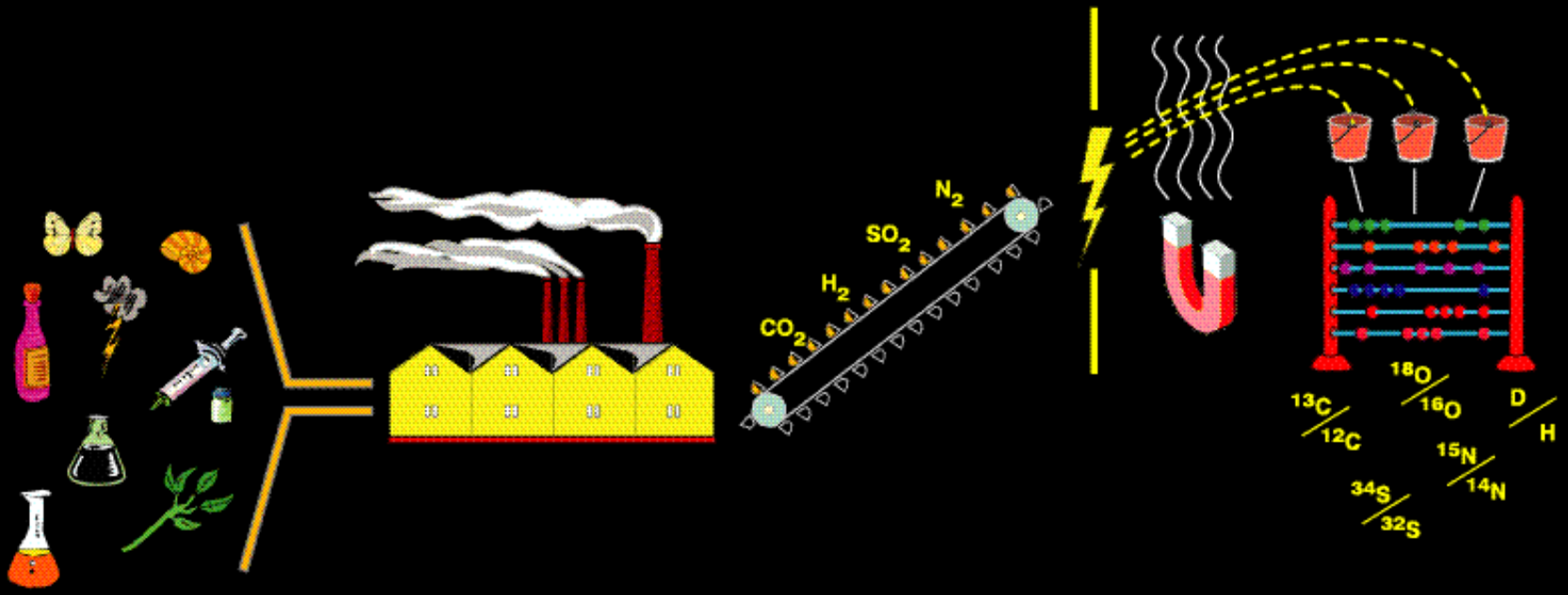
- **Natural Abundance**

  Natural variations in the isotopic content of substances occur due to the different physico-chemical properties of isotopes

- **Tracer Levels**

  A chemical containing a ‘high’ dose of a minor isotope is input into a system to enable a process to be followed. (Note: many agricultural and biomedical applications use enriched tracers)

What is Stable Isotope Ratio MS?



Evolution Of Stable Isotope Analyses

TECHNIQUES

Offline prep - Single Inlet	Water, Carbonates...
Offline prep - Dual Inlet	D/H, ^{18}O, ^{13}C, ^{15}N, ^{34}S
Online - Dual Inlet	
EA - Continuous Flow	Pure compounds EA - ^{13}C, ^{15}N EA - ^{18}O, ^{34}S, D
GC - Continuous flow	Organic mixtures GC - ^{13}C GC - ^{15}N GC - H/D
TraceGas	Atmospheric gases ^{13}C, ^{15}N, ^{18}O

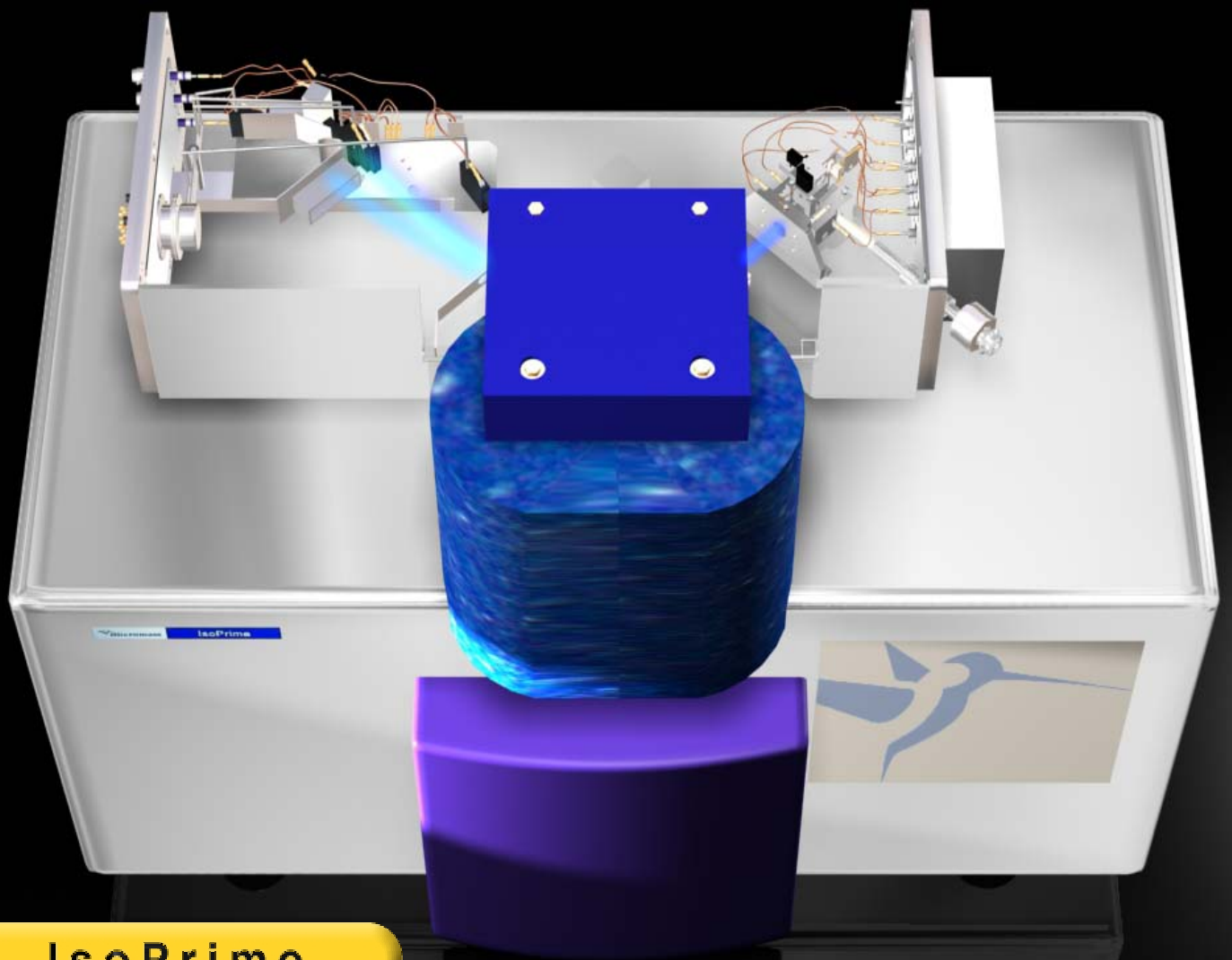
APPLICATIONS

- Food Adulteration
- Soil sciences
- Environment
- Geology
- Medical
- Hydrology

Key dates in development

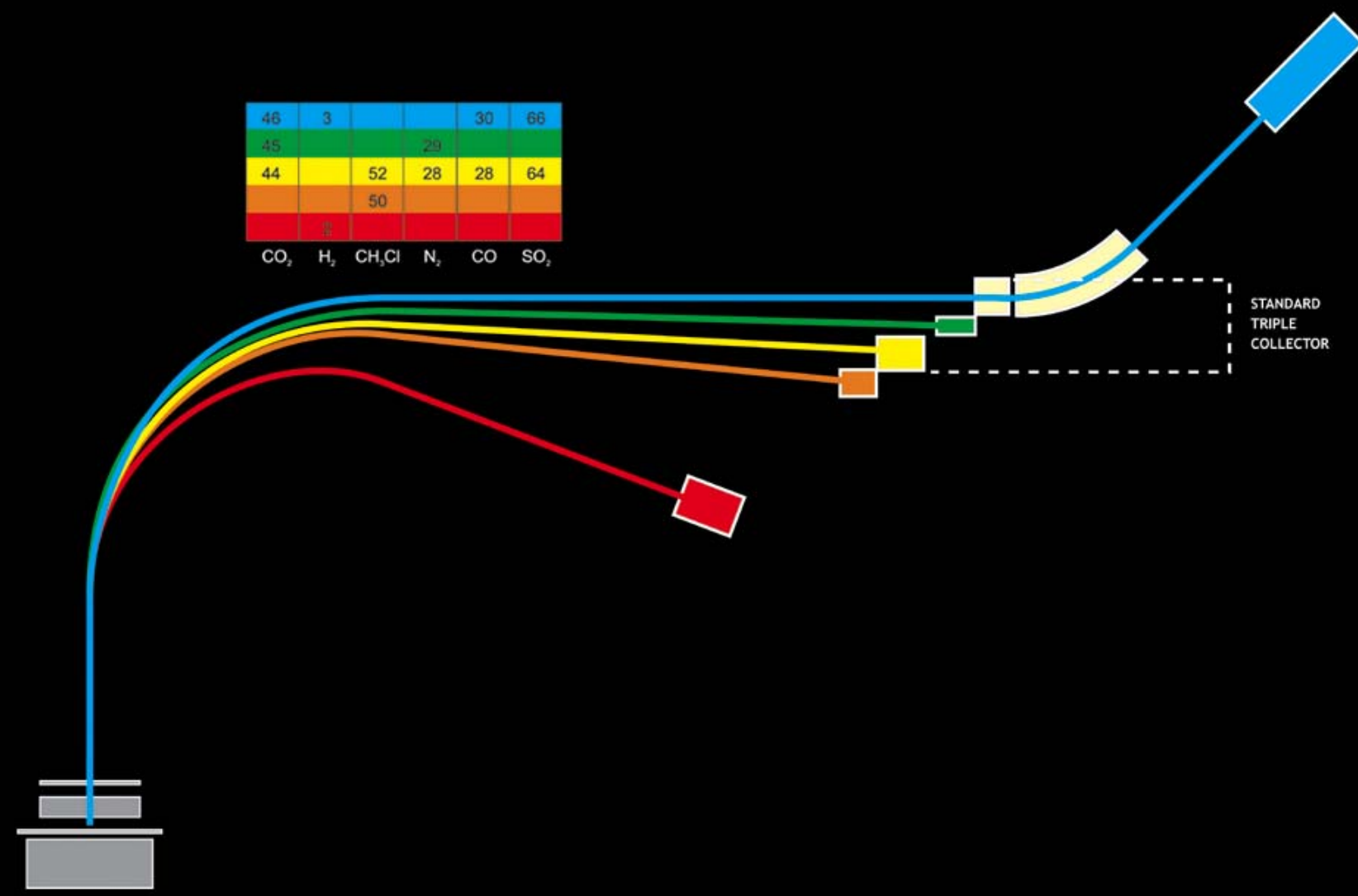
983	Preston and Owens	Interfaced EA to IRMS	C&N
986	Hall	GC-C&N	
987	Pichelmayer	Measured organic sulphur	
994	Giesemann	EA Inorganic sulphur	
995	Brockwell	GC-H	
997	Kornexl	EA Organic oxygen	
998	Merren	Hydrogen ESF	

Isotope Ratio Mass Spectrometer



IsoPrime

Collector configurations



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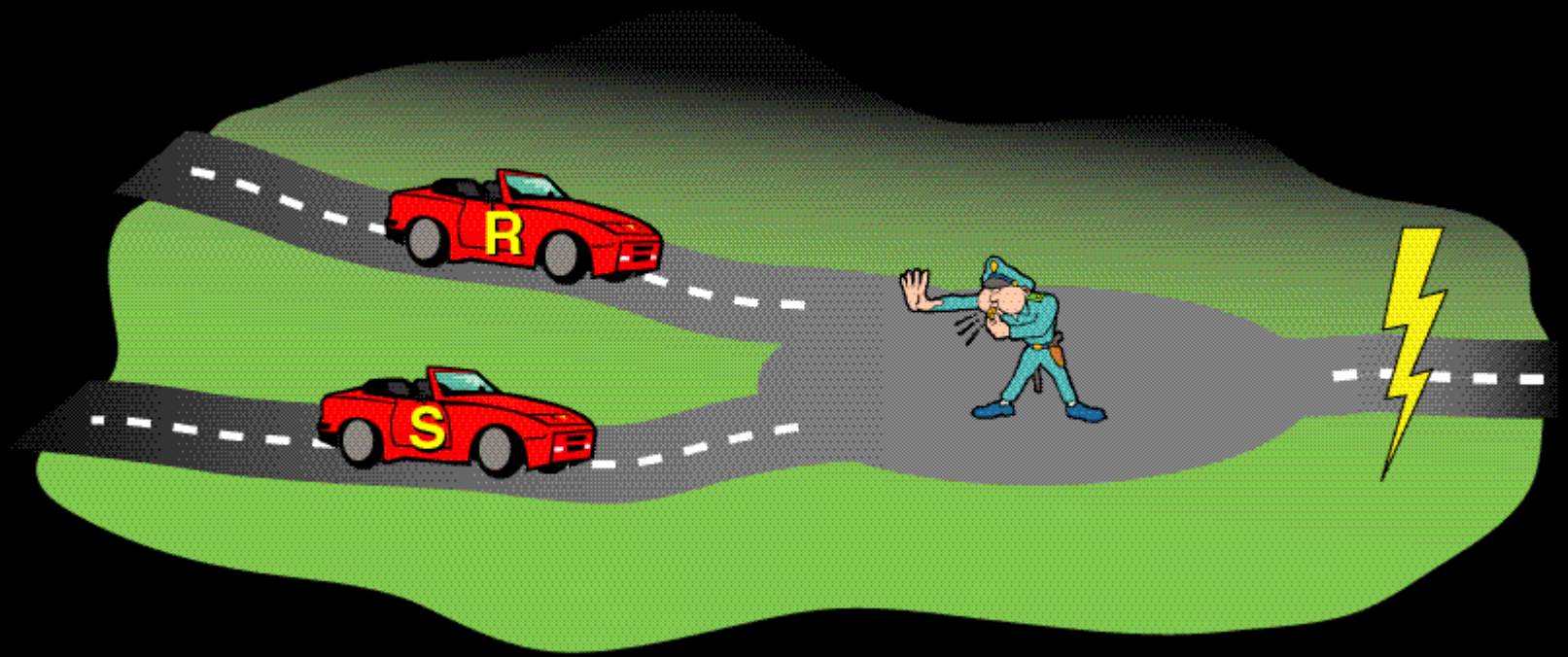
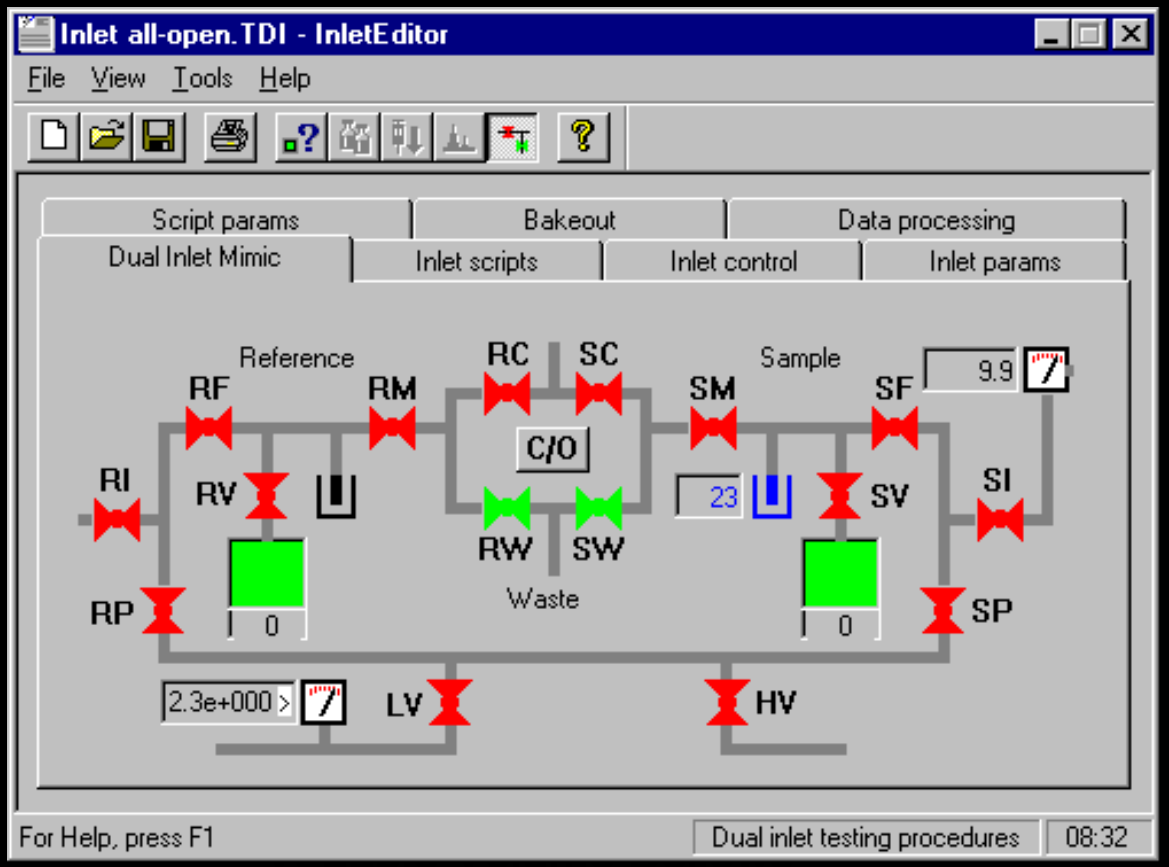
Dual Inlet

Direct comparison of pure sample gas to calibrated reference gas

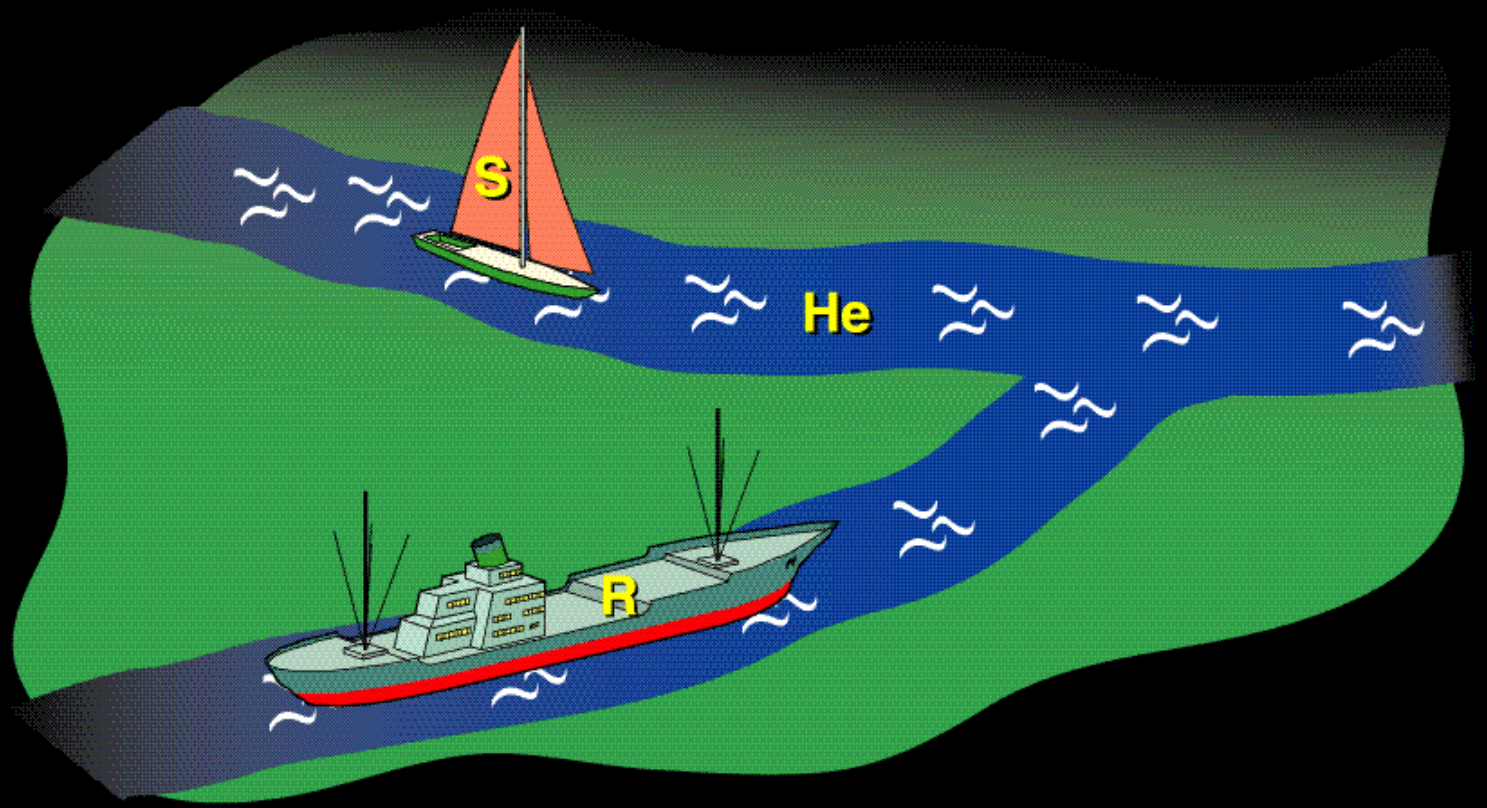


Dual Inlet Mode

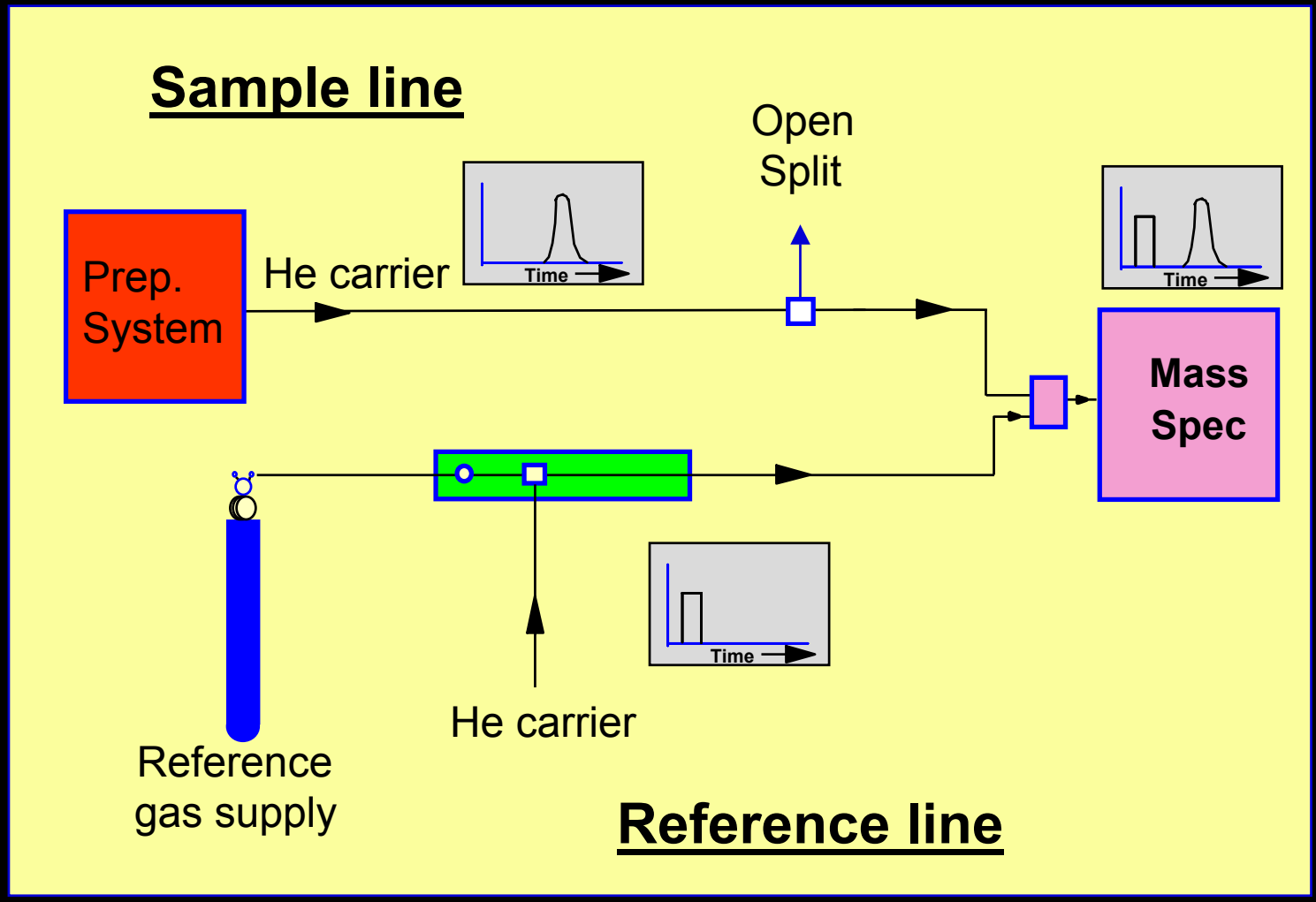


Continuous Flow

Sample as a transient signal
Reference gas injected as a gas pulse



Continuous Flow

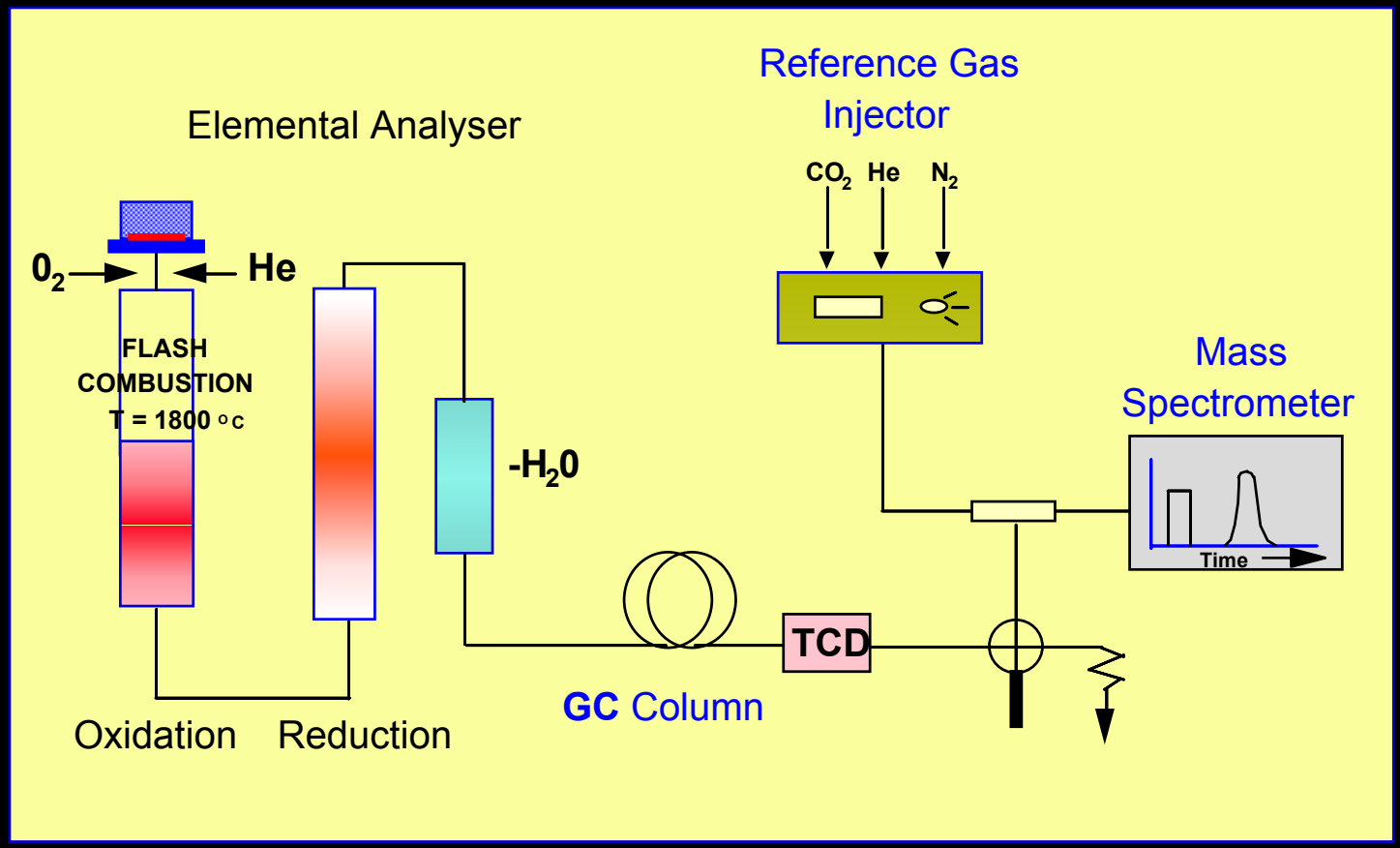


Continuous Flow

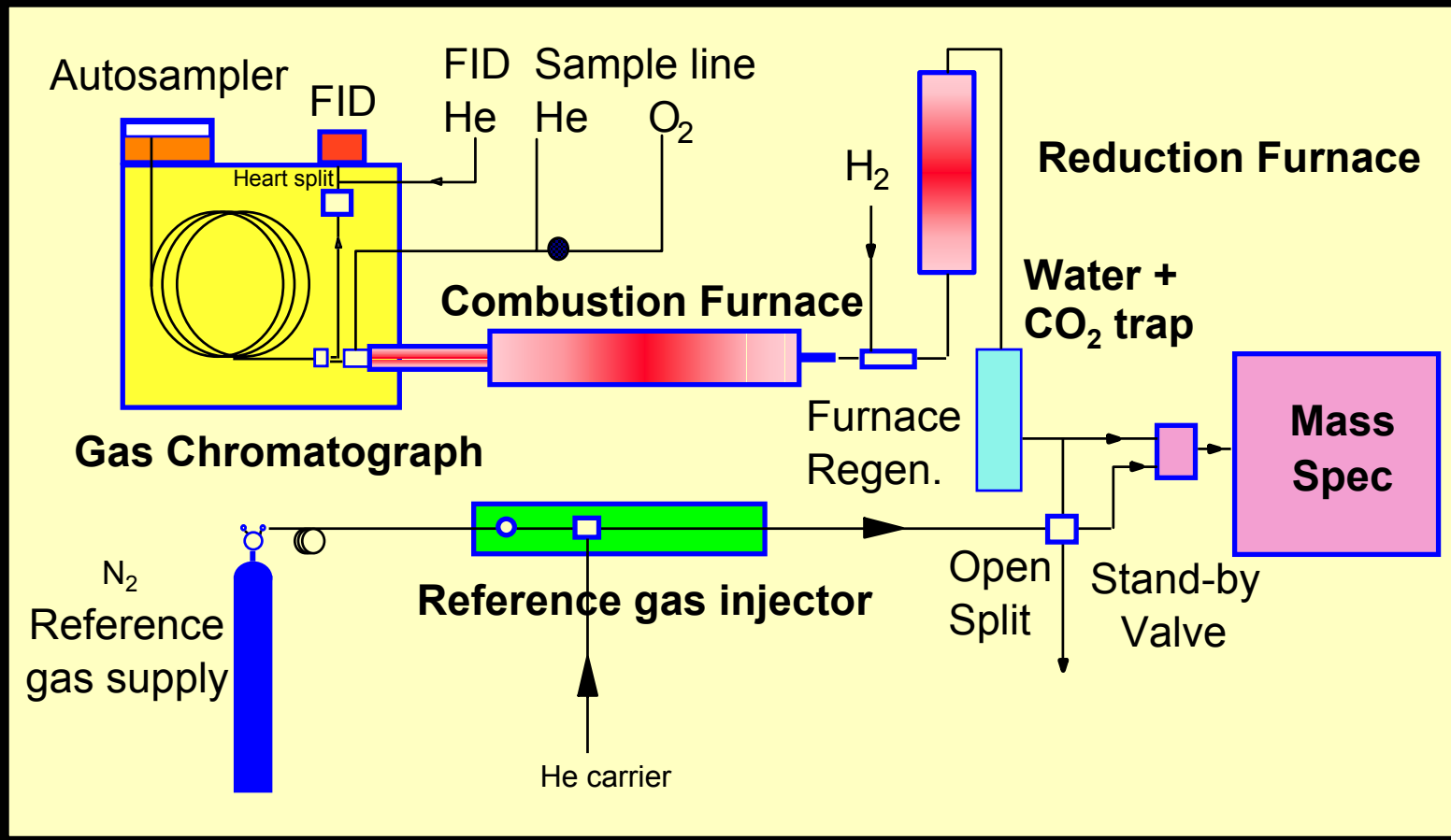
The advantages of the Continuous Flow (CF) technique are
- Simpler systems
- High throughput

Continuous Flow techniques offer an alternative, which is faster, simpler and more cost-effective than classical dual inlet preparation systems

Elemental Analyser



GC - Combustion (^{15}N)



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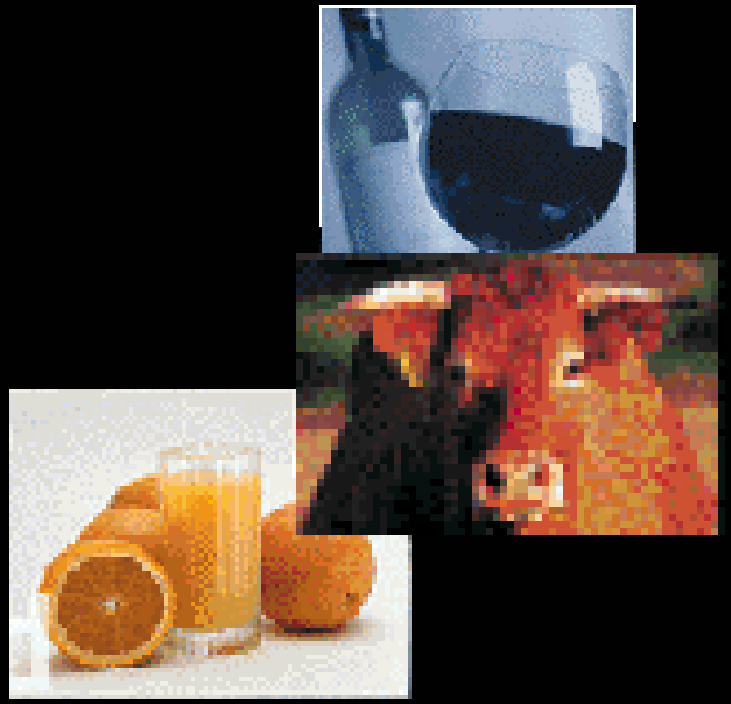
Food Adulteration



Isotopic ratio

Stable Isotope Ratio Analysis (SIRA) by Isotope Ratio Mass Spectrometry (IRMS)

- ^{13}C: Plants forming sugar through the C3 pathway (beets and most fruits) do not incorporate ^{13}C in the same way as plants using the C4 pathway (cane, corn), e.g. addition of corn or cane sugar to fruit products.

Detection of adulterated honey using EA-IRMS

Pure honey

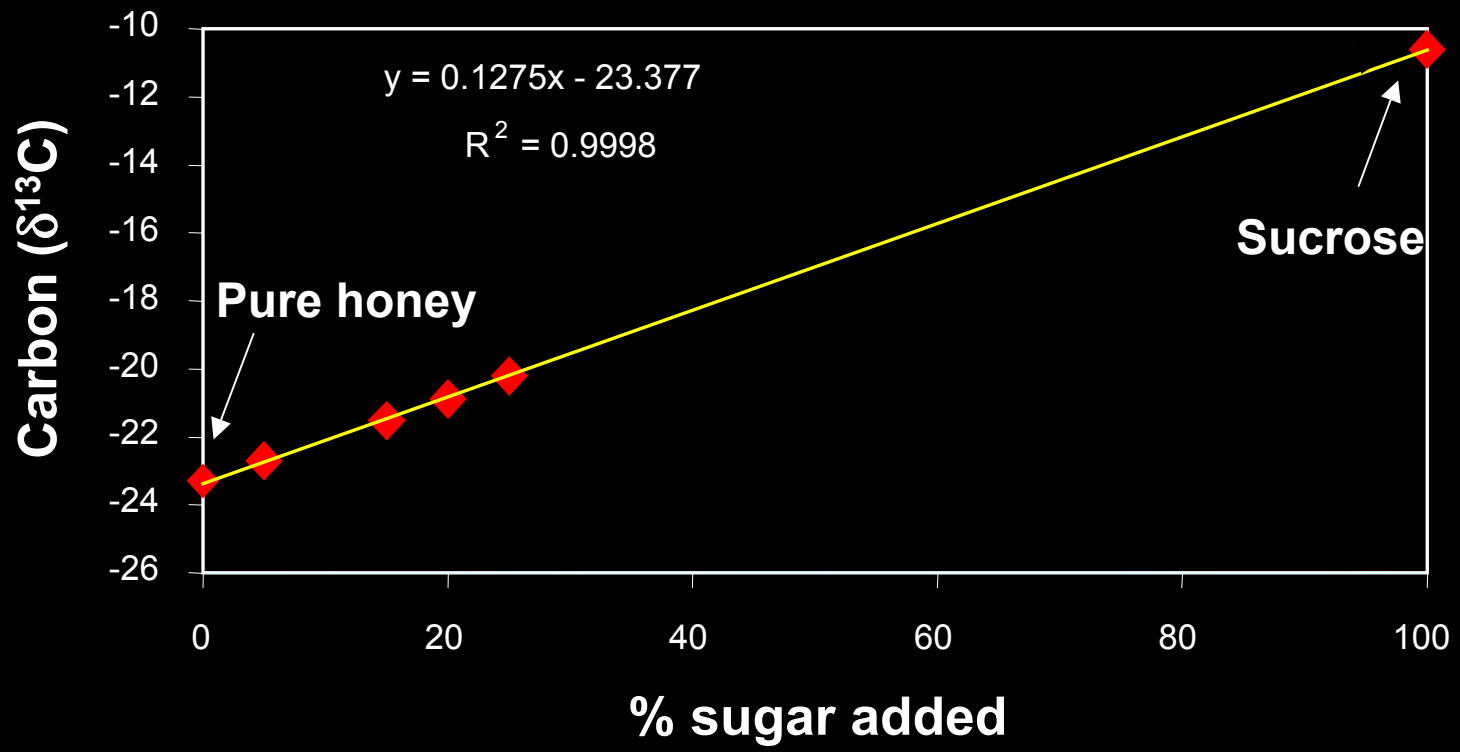
The principle of adulteration detection is to compare characteristics of a product with those of a similar product of known origin

High fructose corn syrup

Adulterated honey

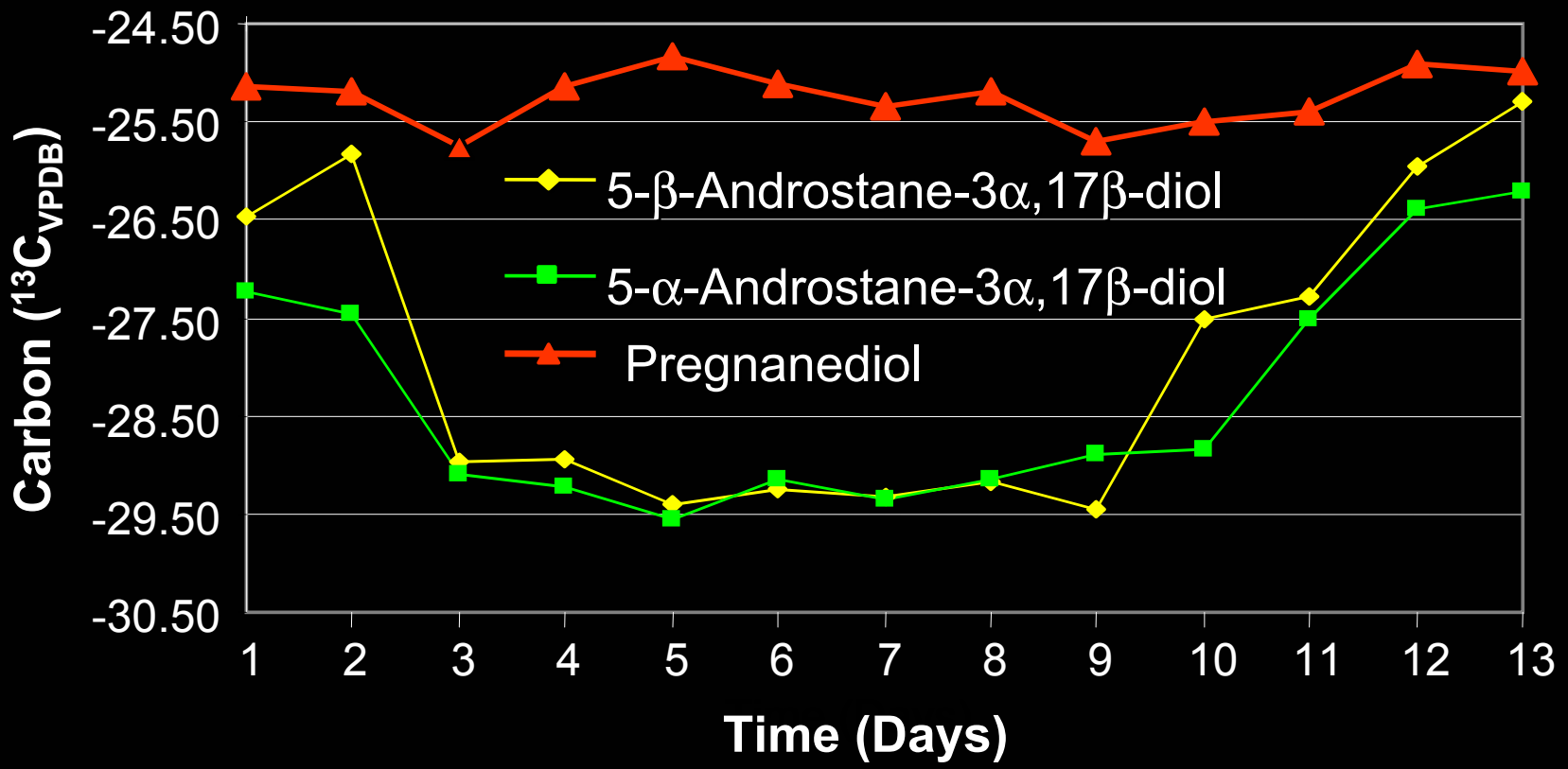
- Natural / Artificial
- Species
- Deliberate Adulteration
- Production Method
- Production Year
- Content
- Type, Variety
- Geographical origin
- Organic / Conventional

Detection of adulterated honey using EA-IRMS



Natural/synthetic: Doping in Sport

GC-IRMS for Doping Control



Testosterone administered at the end of day 2

Misuse = proven unambiguously using IRMS

Drug misuse: Testosterone

+ administered testosterone

	T/E ratio	IRMS
Volunteer 1	Negative	Positive
Volunteer 2	Positive	Positive
Volunteer 3	Positive	Positive
Volunteer 4	Negative	Positive
Volunteer 5	Negative	Positive

The affect of diet or time of administration can also be investigated by closely monitoring the ratio of the androstanediols to pregnanediols

IRMS: IOC Recognised Method in Doping Control

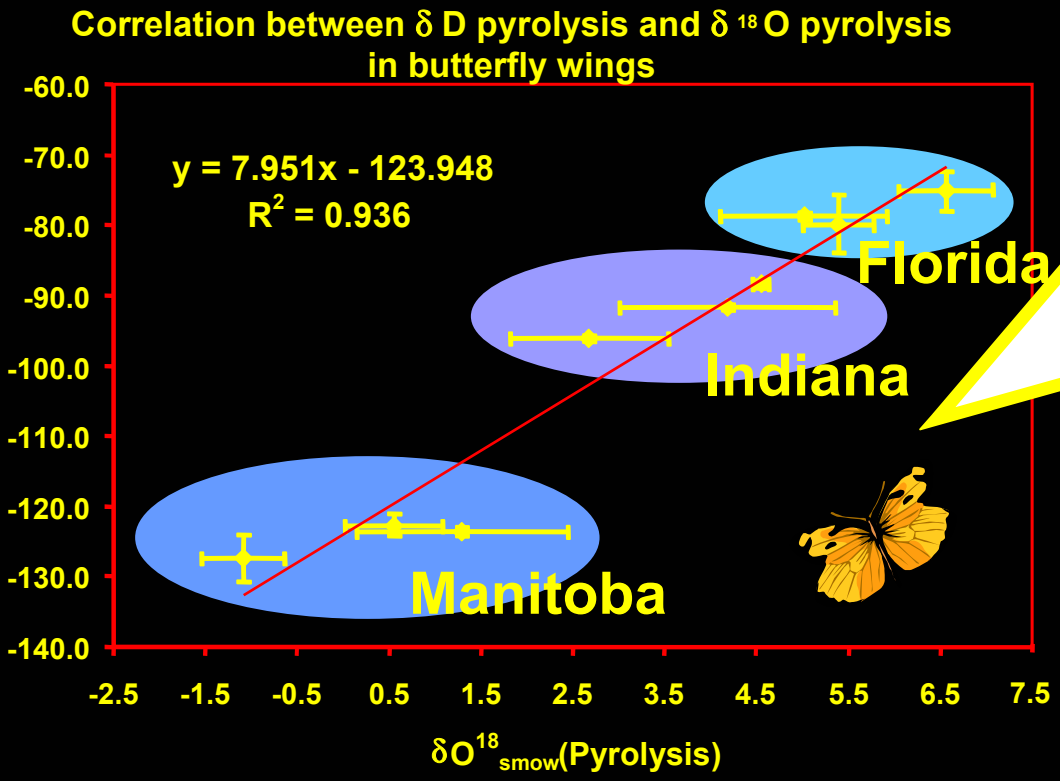
- GC-C-IRMS is the only technique which can unambiguously distinguish between endogenous and exogenous steroid metabolites
- Technique adopted by I.O.C.
- Micromass IsoPrime used for testing in International competitions: Winter Olympics in Nagano and Asian games in Thailand.

Monarch Butterfly Migration

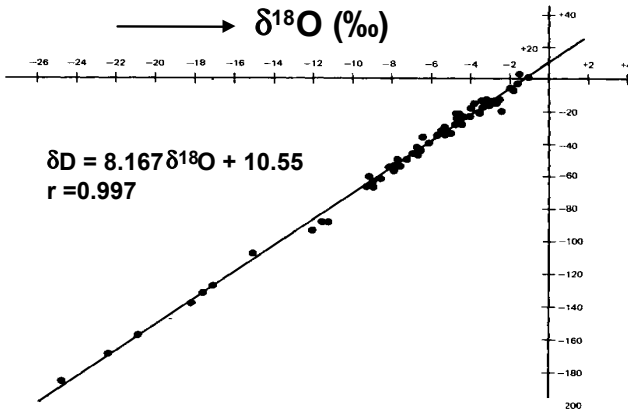
Butterfly Migration Patterns

Map of North America showing sample sites and contours of weighted average $\delta D‰/_{SMOW}$ in summer rainfall (from IAEA Network 1958-1997)

Example of Application



Atmospheric Waters



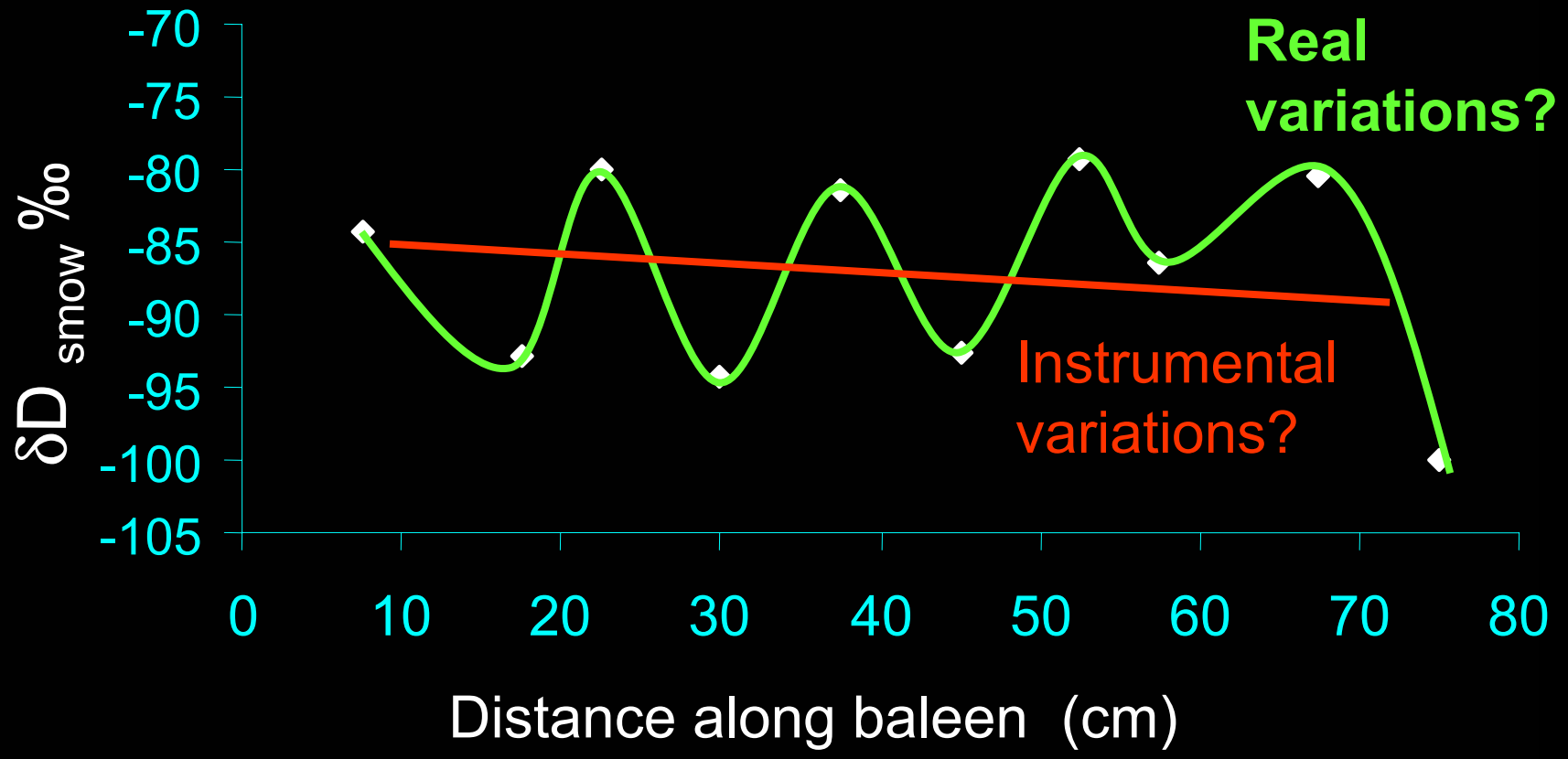
Relationship between weighted average δD and $\delta^{18}O$ in precipitation
Ref. Stable Isotope Hydrology. Deuterium and Oxygen -18 in the Water Cycle.
IAEA , Vienna, 1981, pp. 121.

Baleen Whale Migration Patterns
EA-Pyrolysis

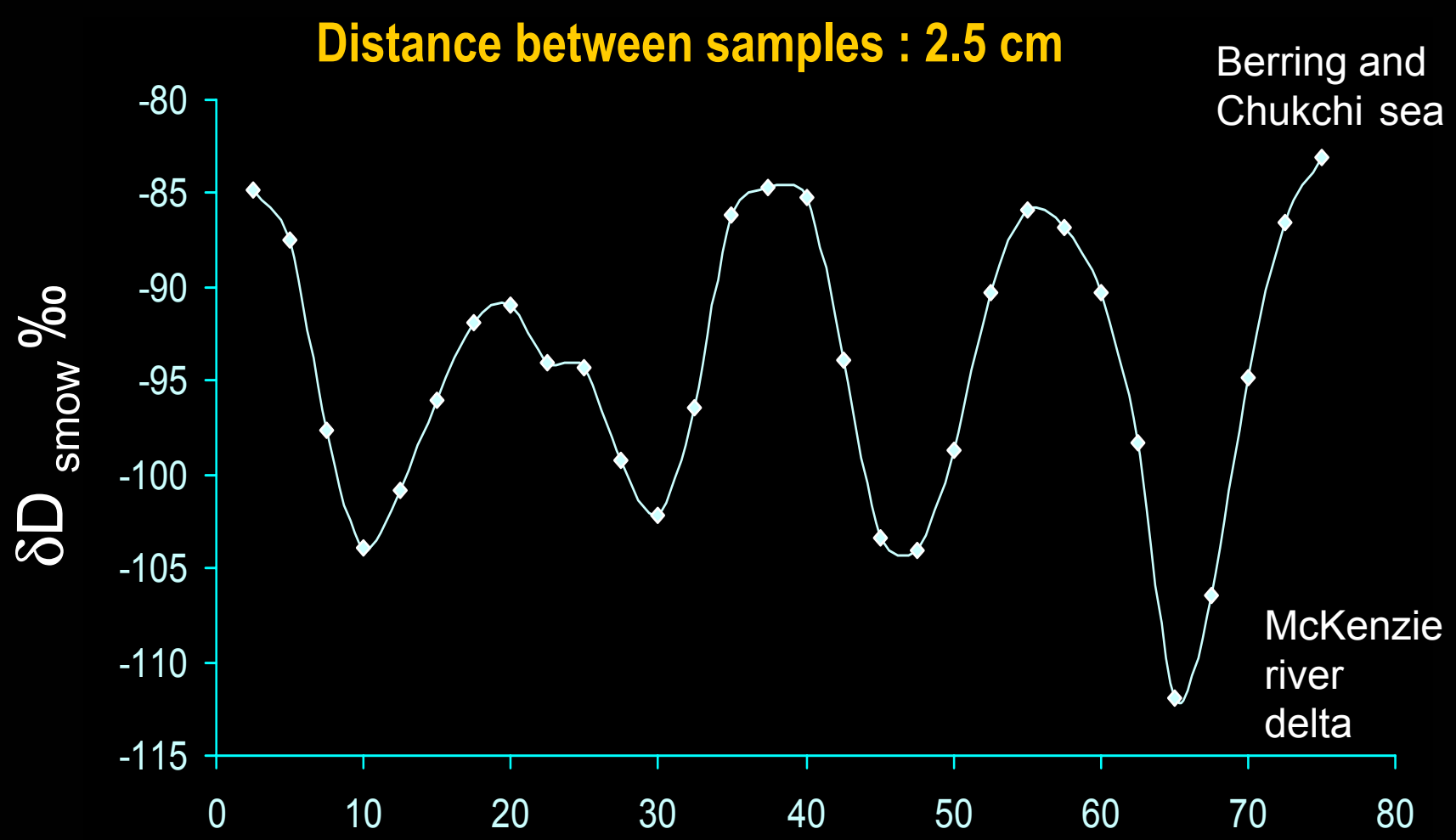
Baleen Whale

Migration Route

Variation in δD along the length of baleen



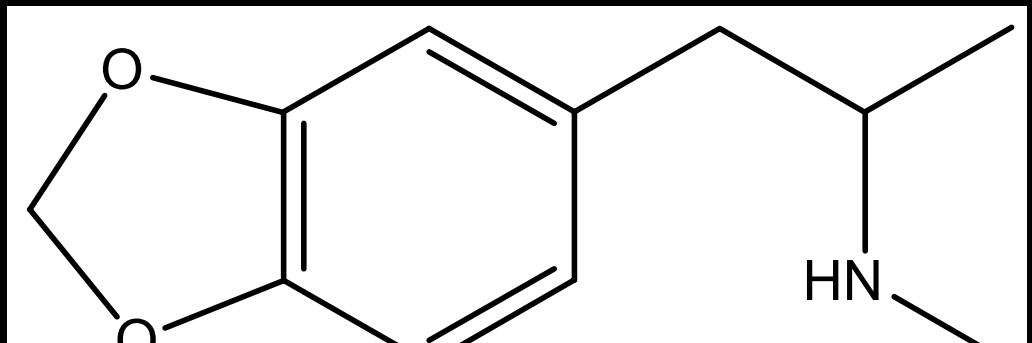
Variation in dD along the length of baleen



IRMS and Ecstasy

F. Pahlol Univ. Nantes in Press

3,4-MethyleneDioxyMethAmphetamine



Syntheses and precursors

- Many possible synthetic pathways ...
- 6 commonly used by clandestine laboratories

- Main precursors:
    - 3,4-methylendioxyphenyl-2-propanone (3,4-MDP-2-P)
    - safrole or isosafrole
- Main nitrogen sources
    - methylamine
    - formamide or N-methylformamide

GC-IRMS ^{13}C analyses

- Precursors :

Sassafras :	-28 to -27 ‰
Safrole :	-27 to -25,5 ‰
Isosafrole :	-28 to -25,5 ‰
3,4-MDP-2-P :	-28 to -27 ‰
Piperonal :	-28 to -26,5 ‰

- Seizures :

Ecstasy :	-29 to -23 ‰

GC-IRMS ^{15}N analyses

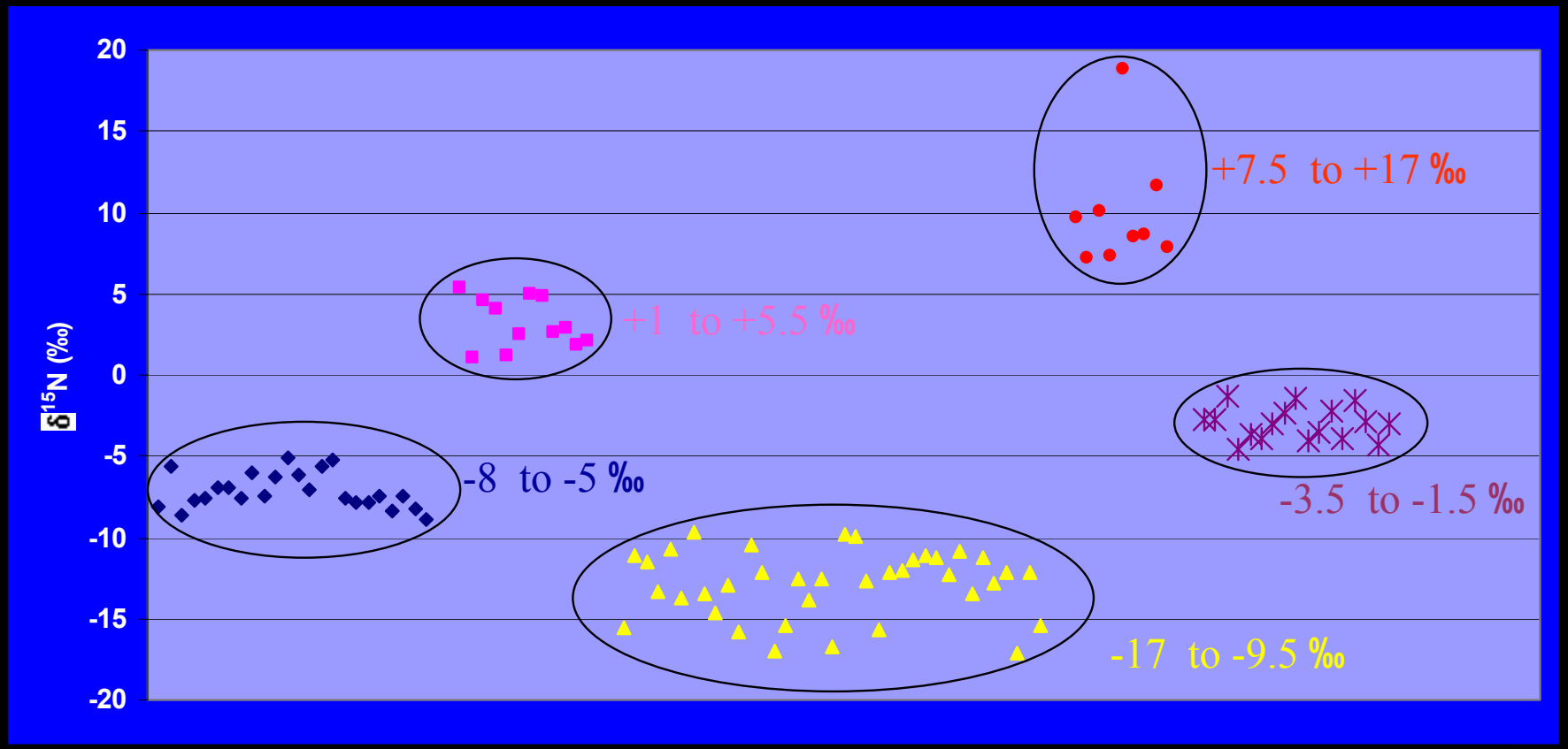
- Precursors :

Methylamine HCl :	2 groups: -5 and -0,5 ‰
Methylamine aq :	2,8 to 5,5 ‰
Formamide :	-1,5 to -0,5 ‰
N-methylformamide :	2 groups: -5 and -1 ‰

- Seizures :

Ecstasy :	between -17 and 19 ‰

Results for MDMA tablets ($\delta^{15}N$)



Ecstasy seizures

2 seizures in North of France, Dec. 01 and Jan. 02

$\delta^{15}N = -7,56$ and $-7,89$ ‰

8 seizures (27,000 tablets) in 2001...

5 seizures had $\delta^{15}N$ between -12,25 and -12,68 ‰

2 seizures (30,000 tablets): Paris and Spanish frontier

$\delta^{15}N = -8,61$ and $-8,67$ ‰

IRMS and heroin

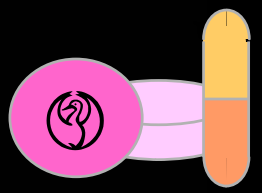
From Desage et al 1991
F. Besacier PhD thesis 1999

Drug classification

- Natural origin

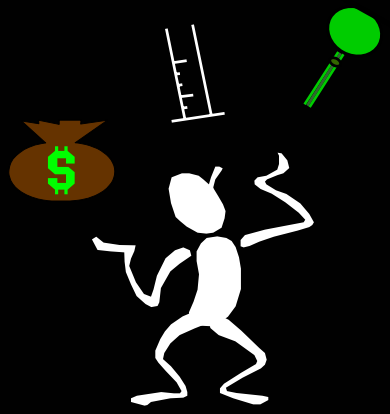
  - Plants (Cannabis)
  - Substances extracted from plants (Cocaine
  - Partially synthetic drugs (Heroine)

- Synthetic origin



  - Amphetamine and by-products

Heroin manufacturing



Papaver Somniferum L. var. *Album*

Opium

Morphine

Diacétylmorphine

Why IRMS?

Geographical origin

- Natural drugs

Synthetic pathways

- Synthetic drugs
- Partially synthetic drugs

Manufacturing batch

- All drugs

Mixing

- All drugs except natural

Application to heroin

Pavot

Morphine
Codéine

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

geographical origin

Acétylation

Diacétylmorphine
Acétylcodéine

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

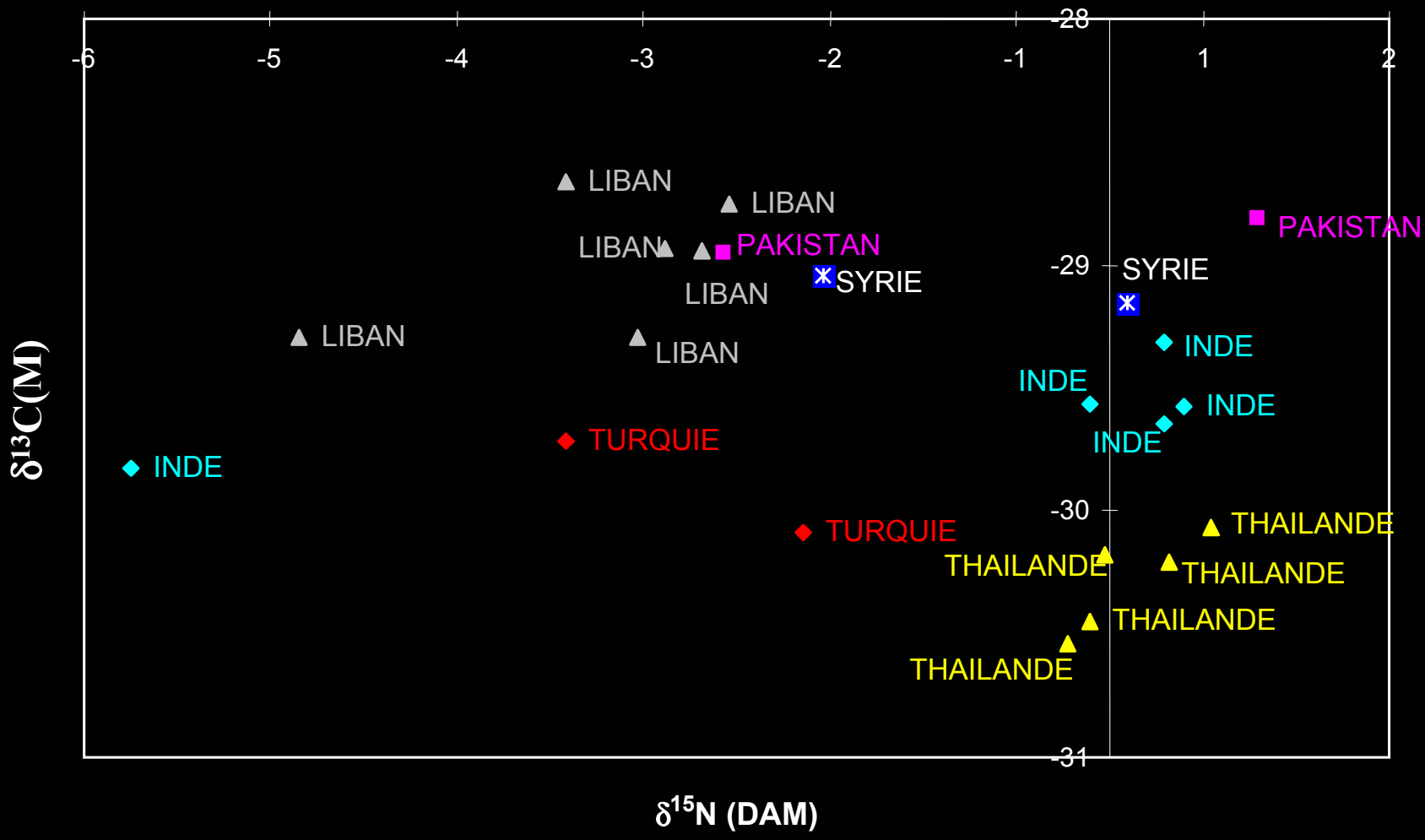
geographical origin
+
synthesis

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

geographical origin

Héroïne

Geographical origin



Synthetic pathway

Morphine
Codéine

$^{13}C/^{12}C$ ⟷ geographical origin

Hydrolysis ↑

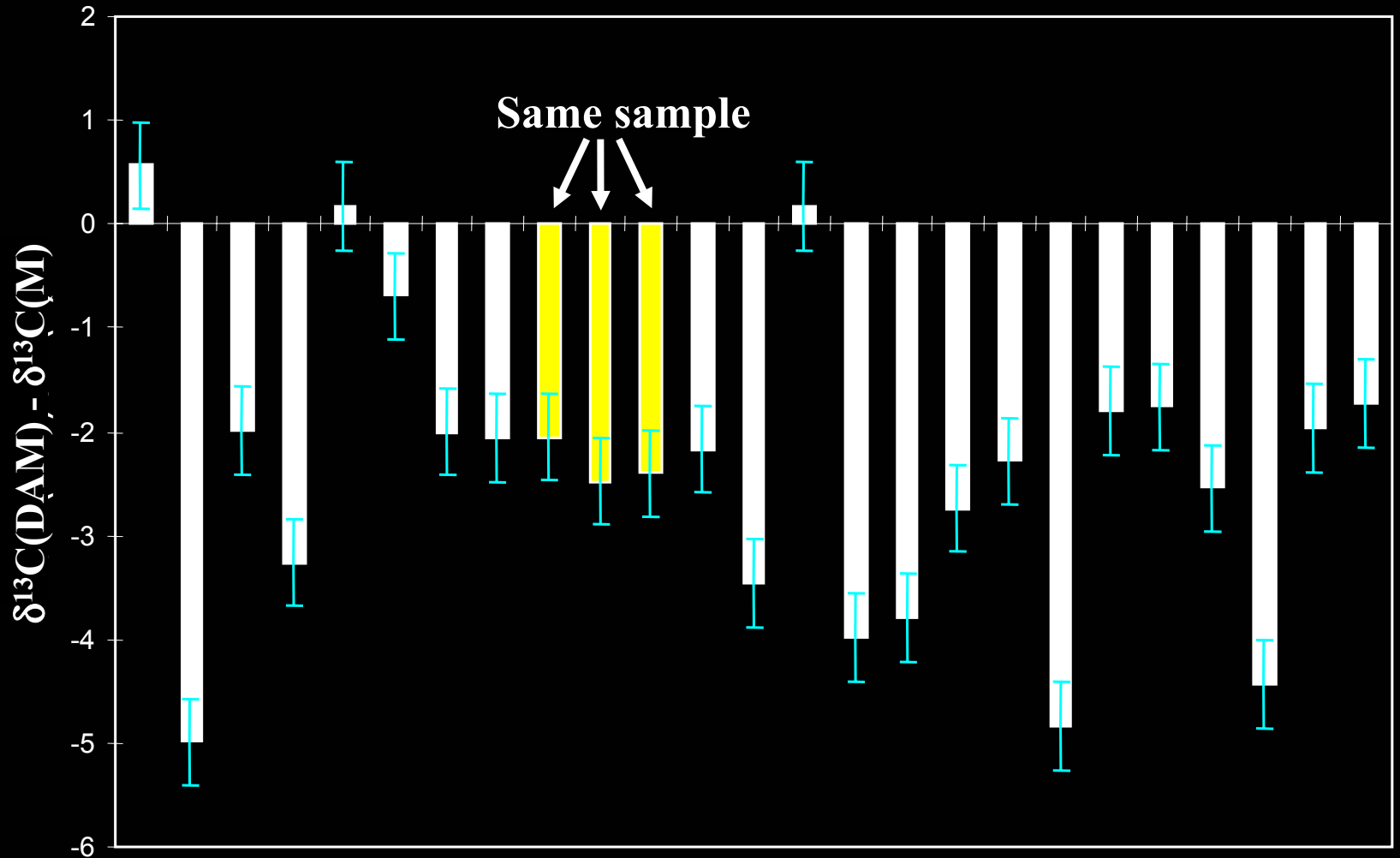
↕ différence
||
synthesis

Diacétylmorphine
Acétylcodéine

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

Héroïne

Synthetic pathway



IRMS and Drug Product Authentication

Drug Batch Characterisation Realised Through New Generation High Throughput IRMS

Collaboration with J. Jasper MIT.

The Cost of Counterfeiting

"Counterfeit paracetamol [acetaminophen] syrup cost the lives of 109 children in Nigeria, and whilst Third World countries are undoubtedly the most vulnerable to attack, there is clear evidence of highly organized criminal activity affecting most of the developed world."

From Geoff Power, 1999 ICPR No. 476 - 477

Why use Stable Isotopes?

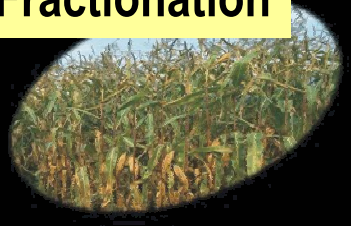
- Using natural abundance isotopic ratios, no spiking is required
- Unique signature, expected to be highly variable between batches of the same drug - thus forming the basis for the technique
- Using GC and EA IRMS we have an effective way of targeting active compounds and measuring, very precisely, their isotopic ratios
- Highly effective multidimensional isotopic approach: H, C, N, O, S

Factors involved in Inter-Batch Variability

Fractionation

Synthetic Compounds

Fractionation



Fractionation

Natural Products

Fractionation

Precursors

Fractionation

Synthesis

Fractionation

Excipients

Final Product

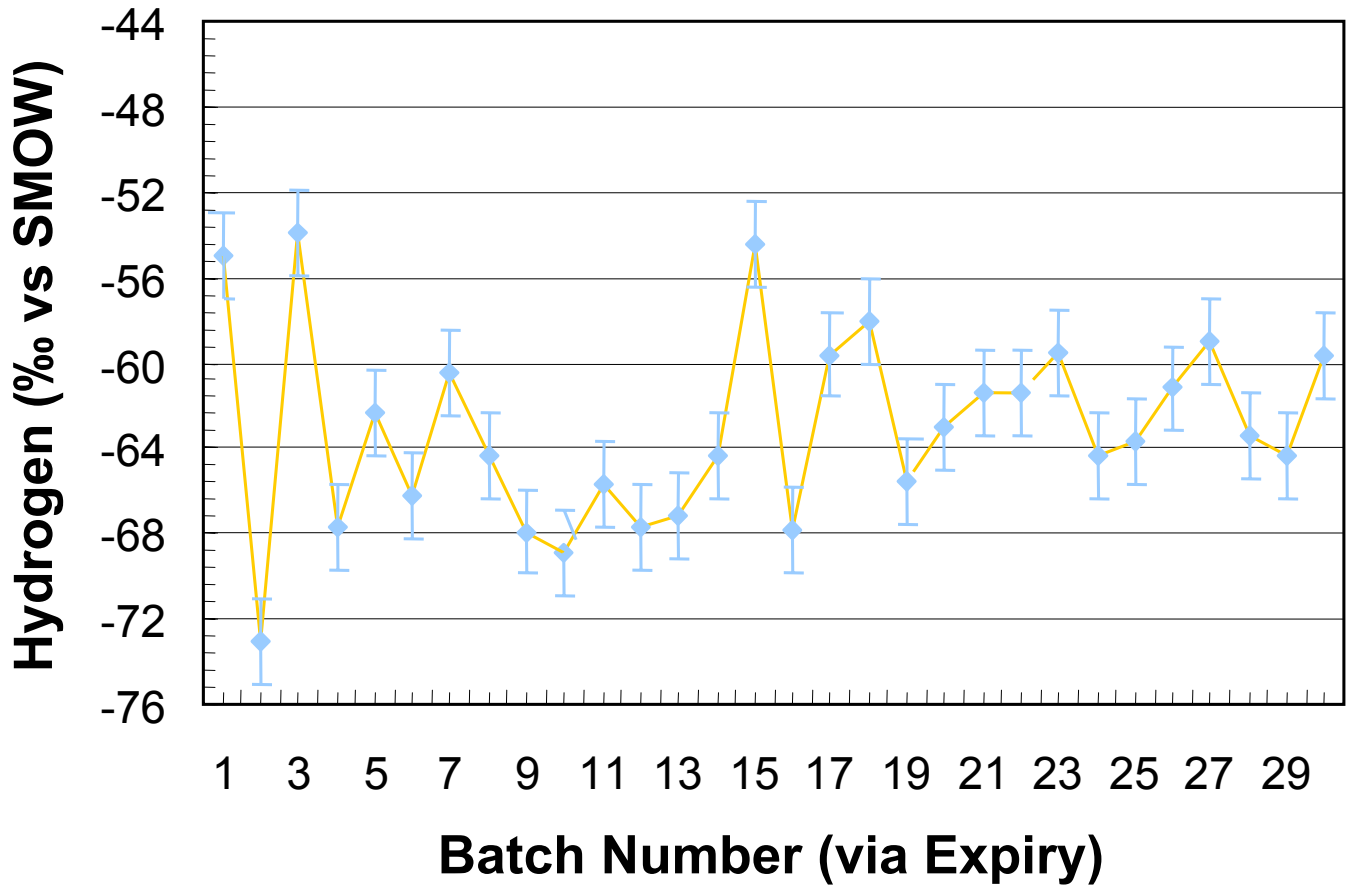
Fractionation

Formulation

Clear Temporal Variation in Analgesic Products

Hydrogen

Acetaminophen 1

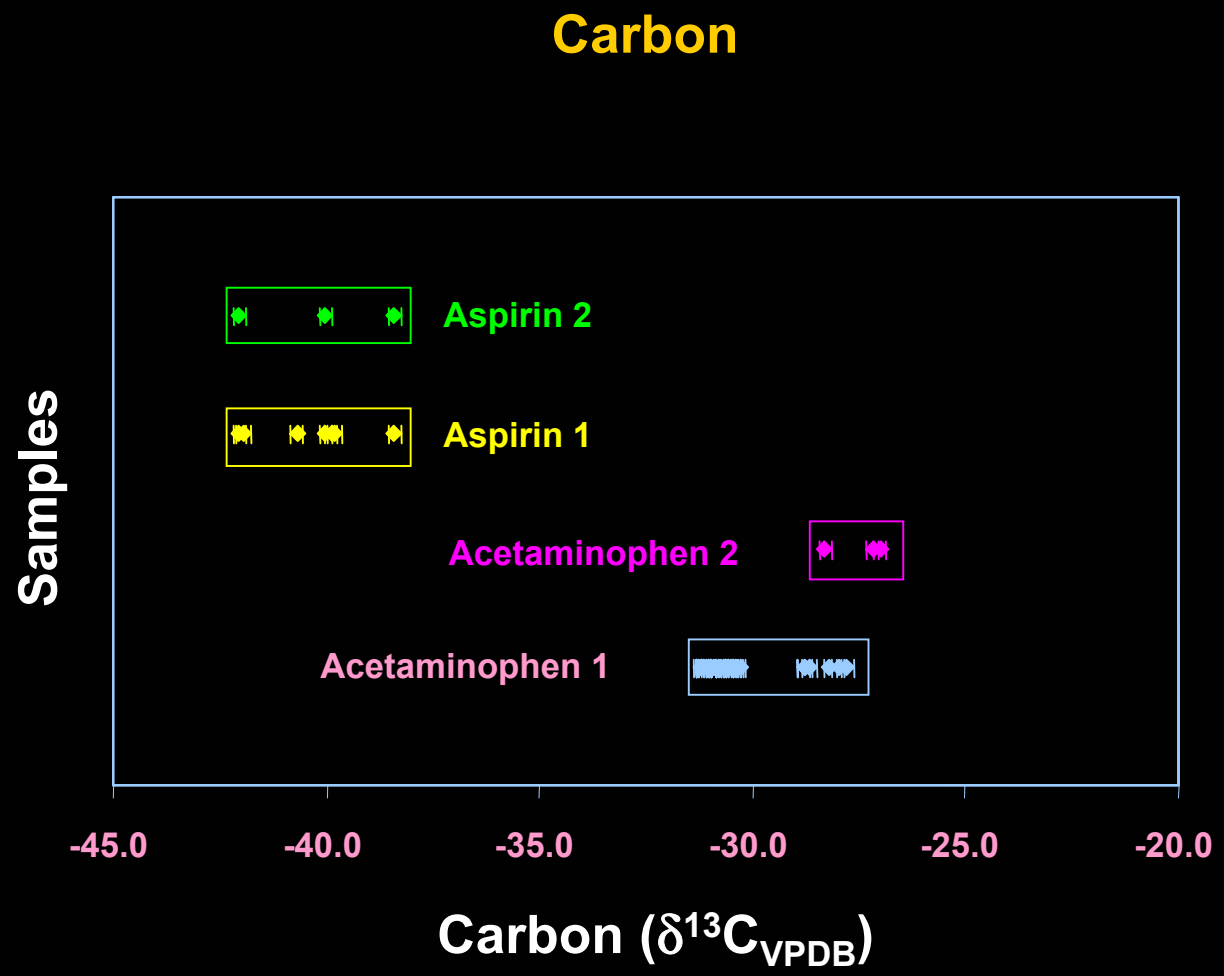


What can we detect?

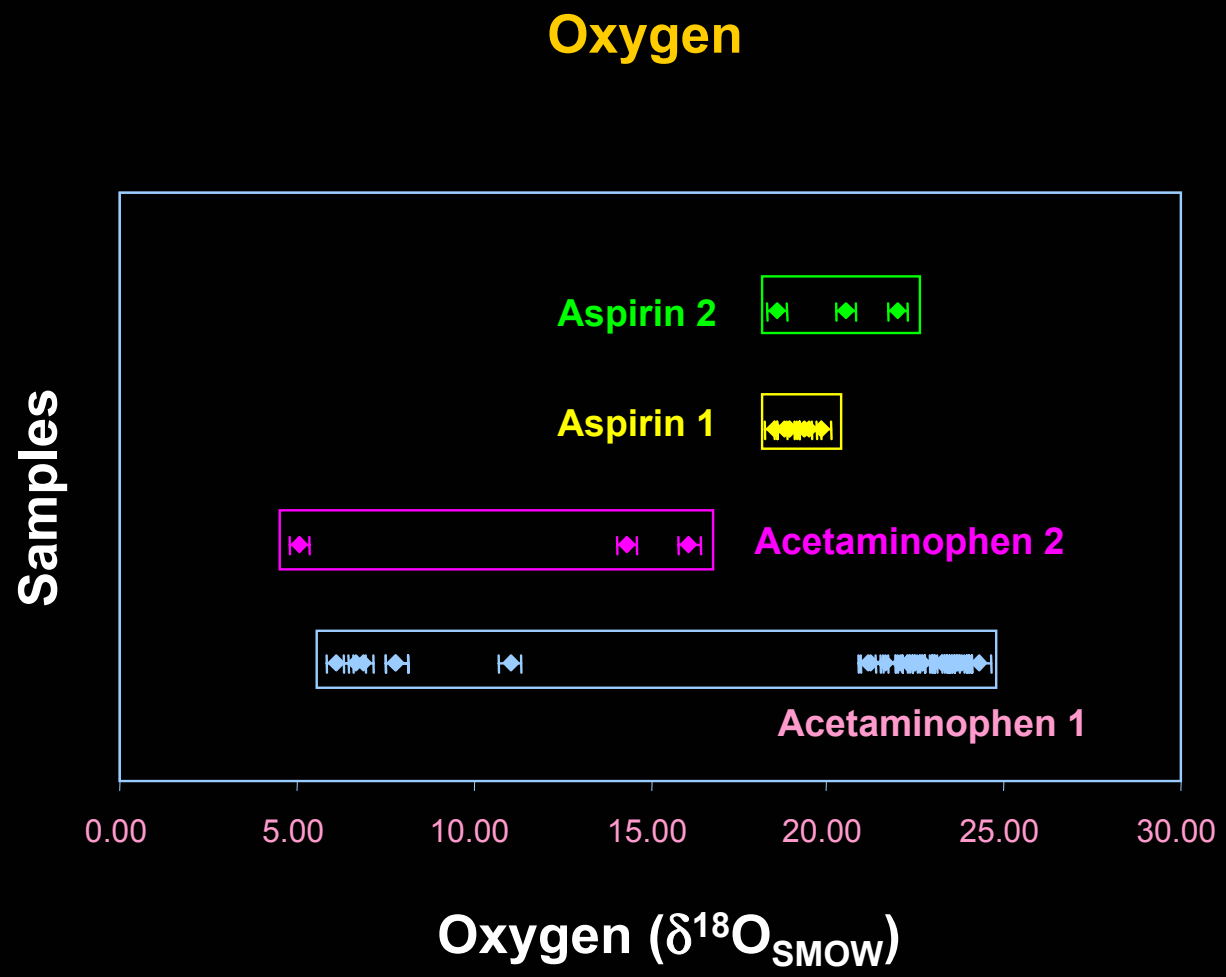
- Ext. Reproducibility ==> ^{13}C 0.2 ‰
- Ext. Reproducibility ==> ^{18}O 0.3 ‰
- Ext. Reproducibility ==> D/H 2 ‰

- Within batch variations in the order of Ext. reproducibility of the methods

Significant Inter-Batch Isotopic Variability



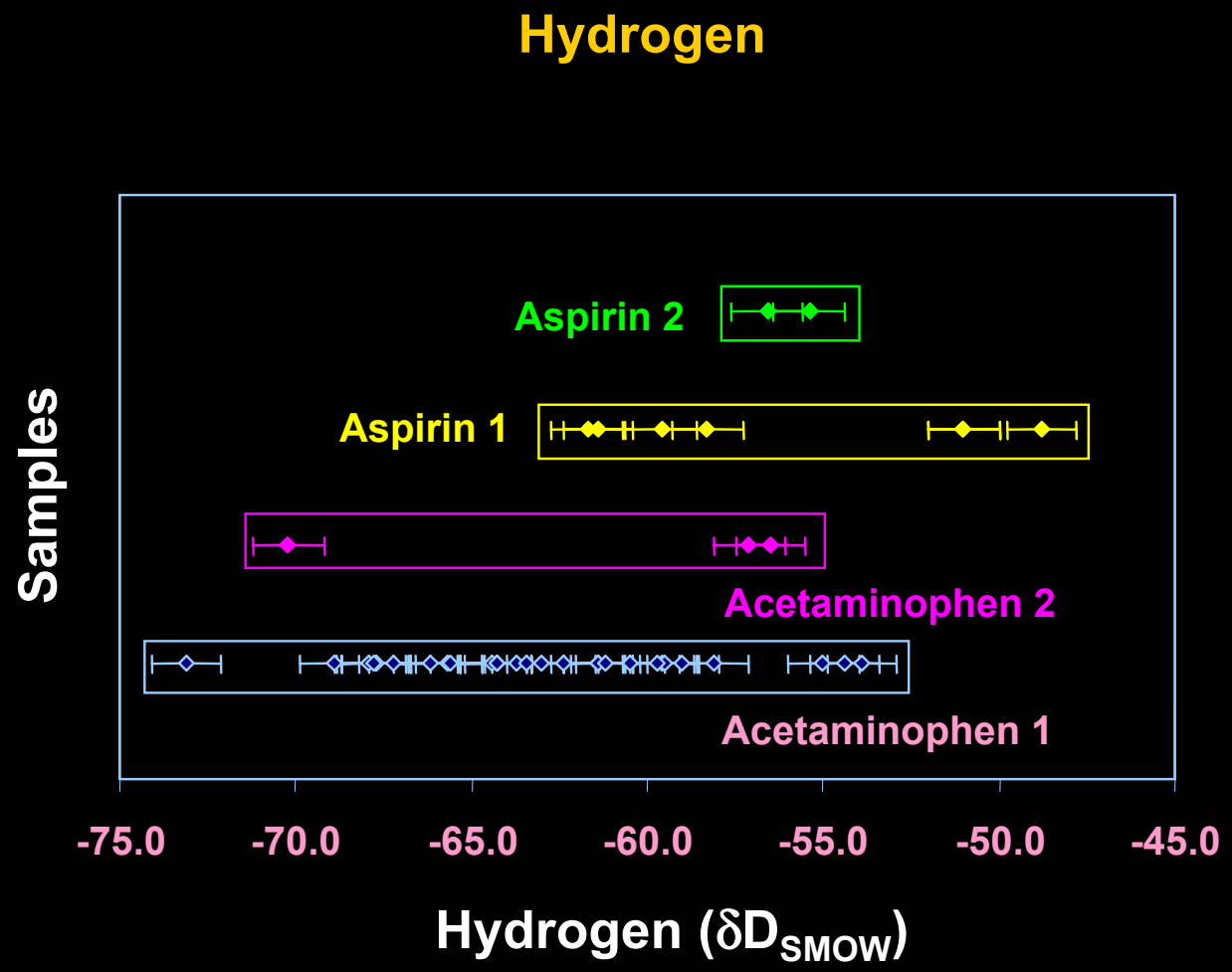
Significant Inter-Batch Isotopic Variability



Analyses of 3 different samples from the same batch

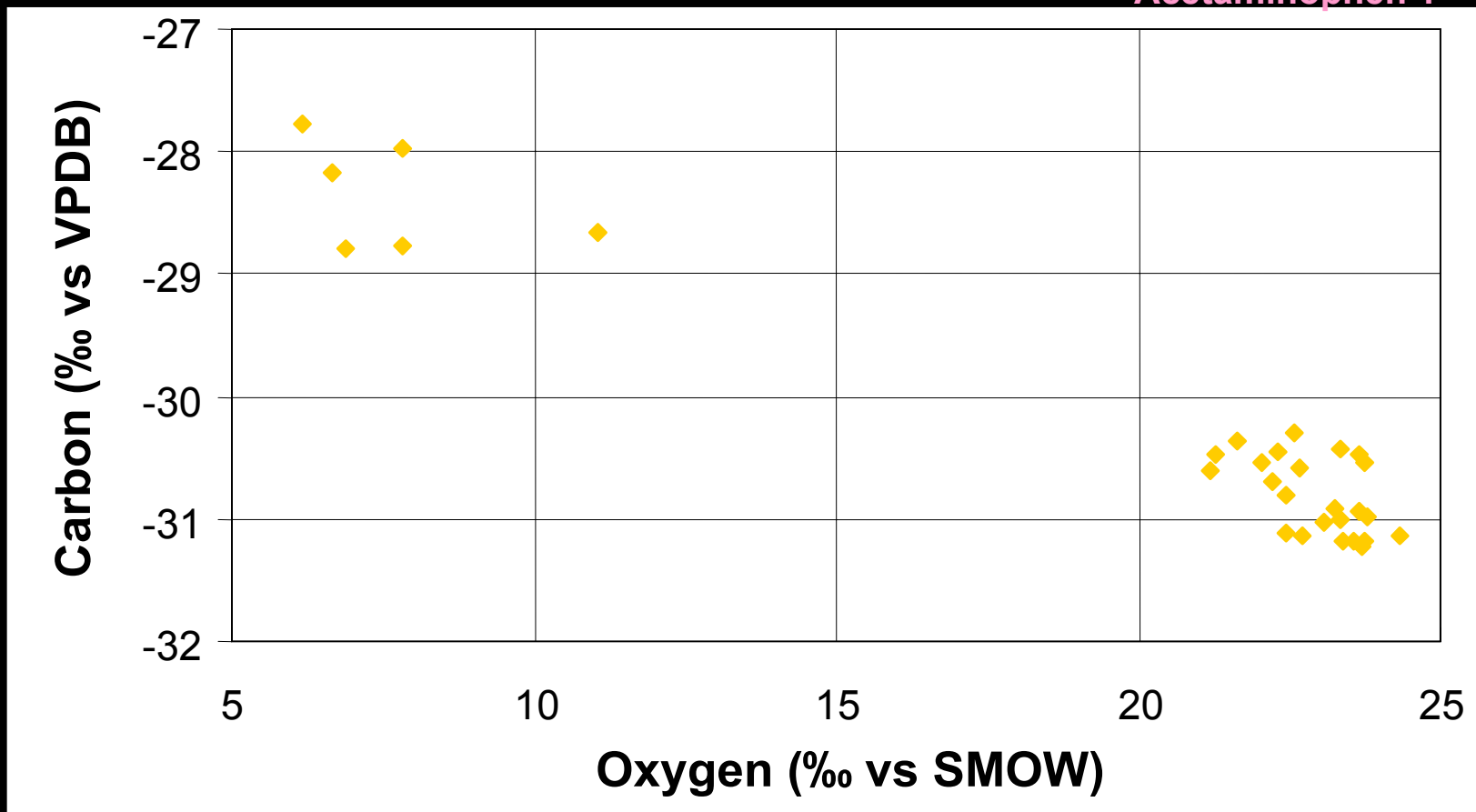
$\delta^{18}O_{SMOW}$ **= 7.91,** StDev = 0.21

Significant Inter-Batch Isotopic Variability



One Product, Different Batches, Large Variation

Oxygen *vs* Carbon



Product Authentication

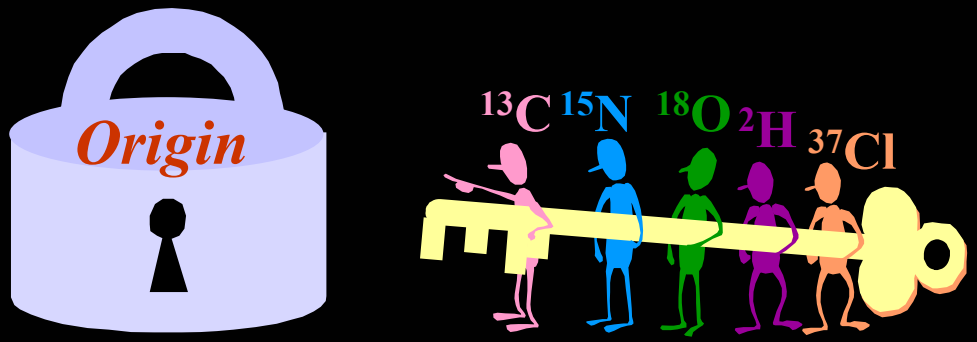
- Characterisation of the ambient batch to batch stable isotopic variation of such products represents a means by which to ‘fingerprint’ individual drug batches
- By bar-coding products with their specific isotopic ‘fingerprint’ we make them very difficult/impossible to counterfeit
- Active compound authentication ultimately aids control of the supply, use and disposal of drugs, saving money, reputation and lives

CONCLUSION

Isotopic ratios (EA-IRMS, GC-IRMS)

→ Precious tool for police and justice
- geographical origin
- synthetic pathway
- Natural/sysnthetic origin
- batch identification

Origin determination :



Used in combination with other methods