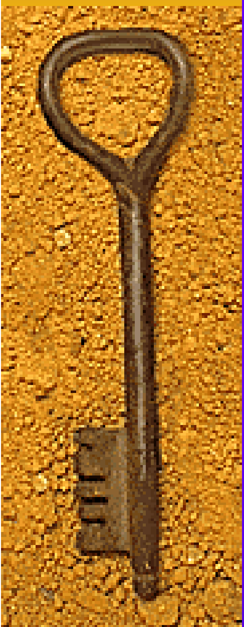

Stable Isotopic Characterization of Pharmaceutical Materials

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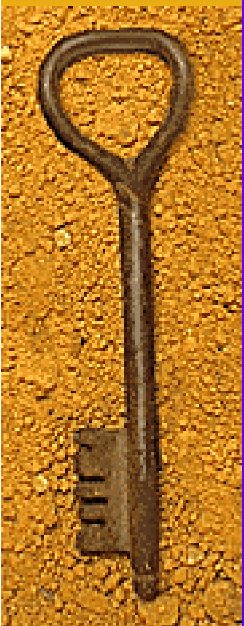
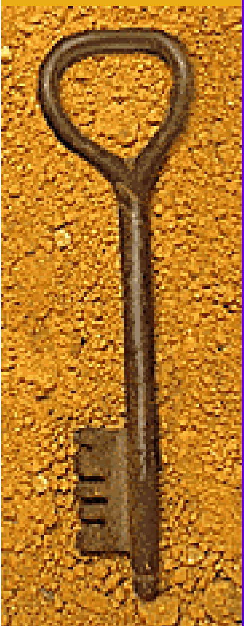


Isotopic Characterization of Drug Products and APIs

- Introduction
- Stable Isotopes
  - A. Natural Distribution
  - B. Instrumentation
    - 1. Dual-Inlet Mass Spectrometer (DIMS)
    - 2. Elemental Analyzer MS (EAMS)
- Isotopic Results ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$, and δD)
  - A. Four OTC Drug Products
  - B. Four APIs

IV. Summary

MIT
MOLECULAR ISOTOPE TECHNOLOGIES, LLC



Is Pharmaceutical Counterfeiting a Problem?

1999

"Counterfeit 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*."

(G. Power, Interpol *Intl. Crime Pol. Rev.* – No. 476-477)

2000

From The Subcommittee on Investigations' Hearings on Counterfeit Bulk Drugs

….Some have estimated that 50-70% of the drugs in some developing countries are counterfeit. The World Health Organization and some industry analysts estimate about 5-8% of drug products shipped to the U.S. are counterfeit, unapproved or substandard.

(Honorable Fred Upton, Chairman)

October 15, 2001

The proceeds from pharmaceutical and other product diversion have significantly underwritten recent terrorist events.

(Atty. D. deKieffer, *Pharm. Anti-Cnterftg Mtg*., Wash., DC)

2002

Counterfeiting is 'the crime of the 21st century accounting for losses of between $350B and $1T annually. Diversion, illegal gray market goods and organized retail theft fit neatly into a set of criminal attacks on the world's best-known brands.

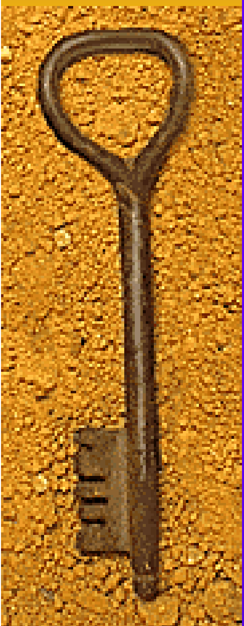
(FBI in *Reconnaissance International*)

Pharmaceutical Phases

- APIs (or, Drug Substances)

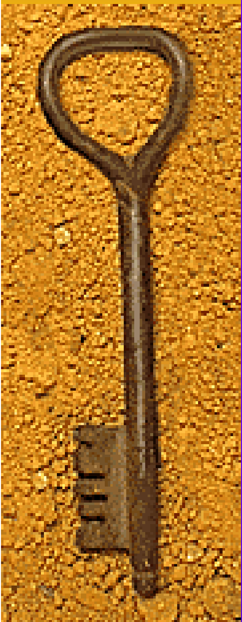
2. Excipients

3. Drug Products



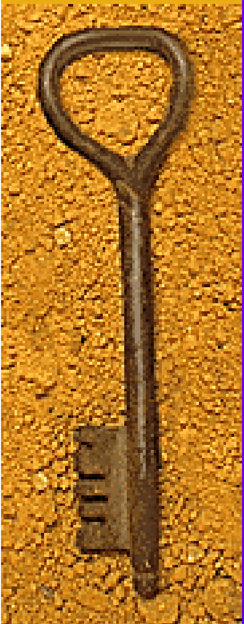
What Can Stable Isotopic "Fingerprinting" Mitigate?

1. Counterfeiting
2. Countertrading
3. Vicarious Liability
4. Theft



What are Stable Isotopes?

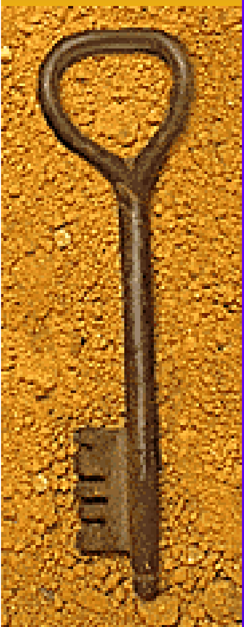
Simply put, they are all non-radioactive elements.



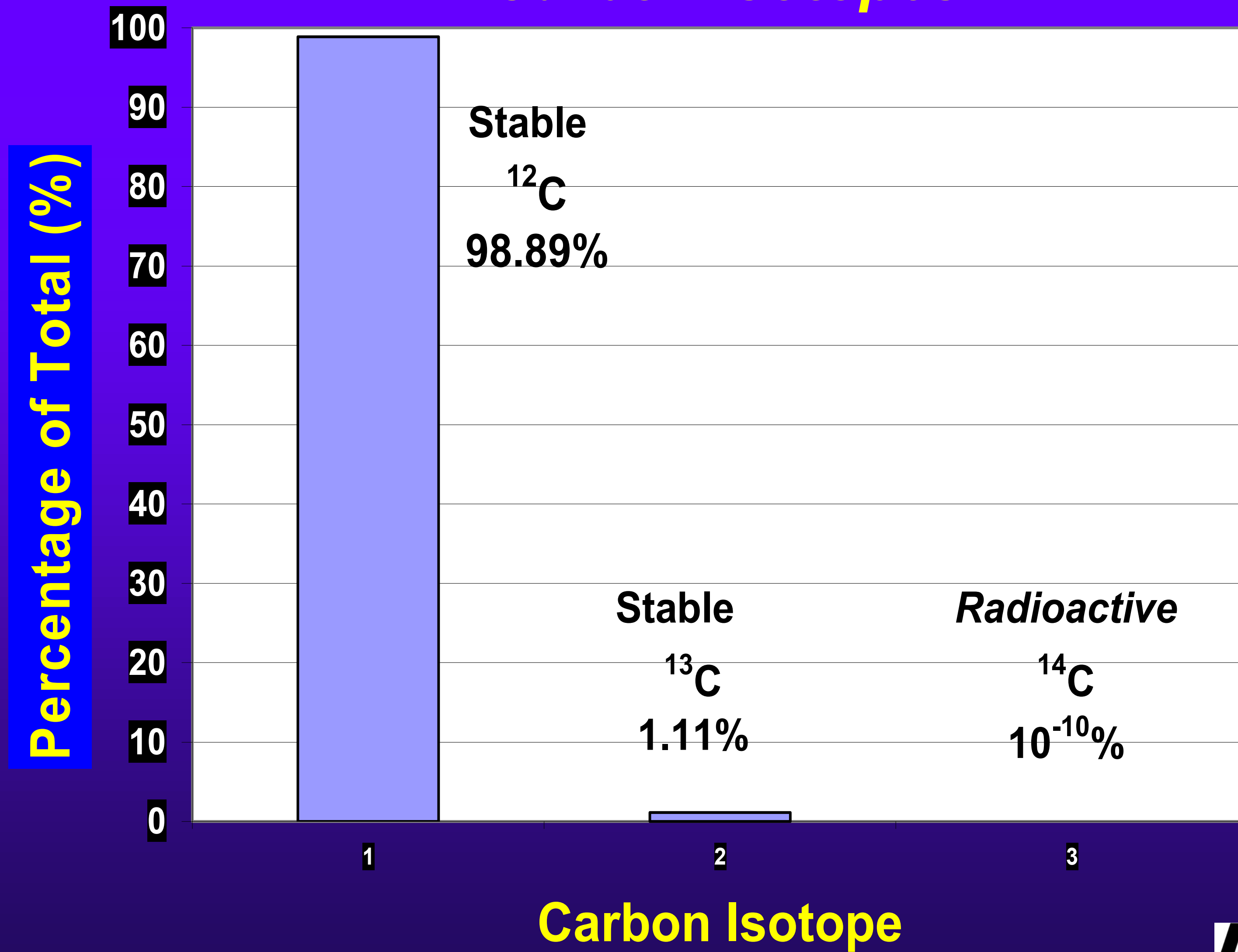
Stable Isotopes as Tracers

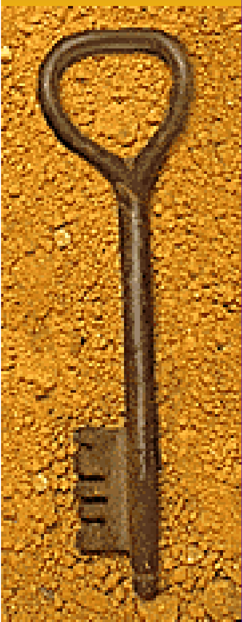
- Heavy-and-light pairs of isotopes of one element are used to form a “tracer.” For example, ‘heavy’ carbon and ‘light’ carbon can be used to form a tracer: $^{13}C/^{12}C$.

- 252 stable isotopes!



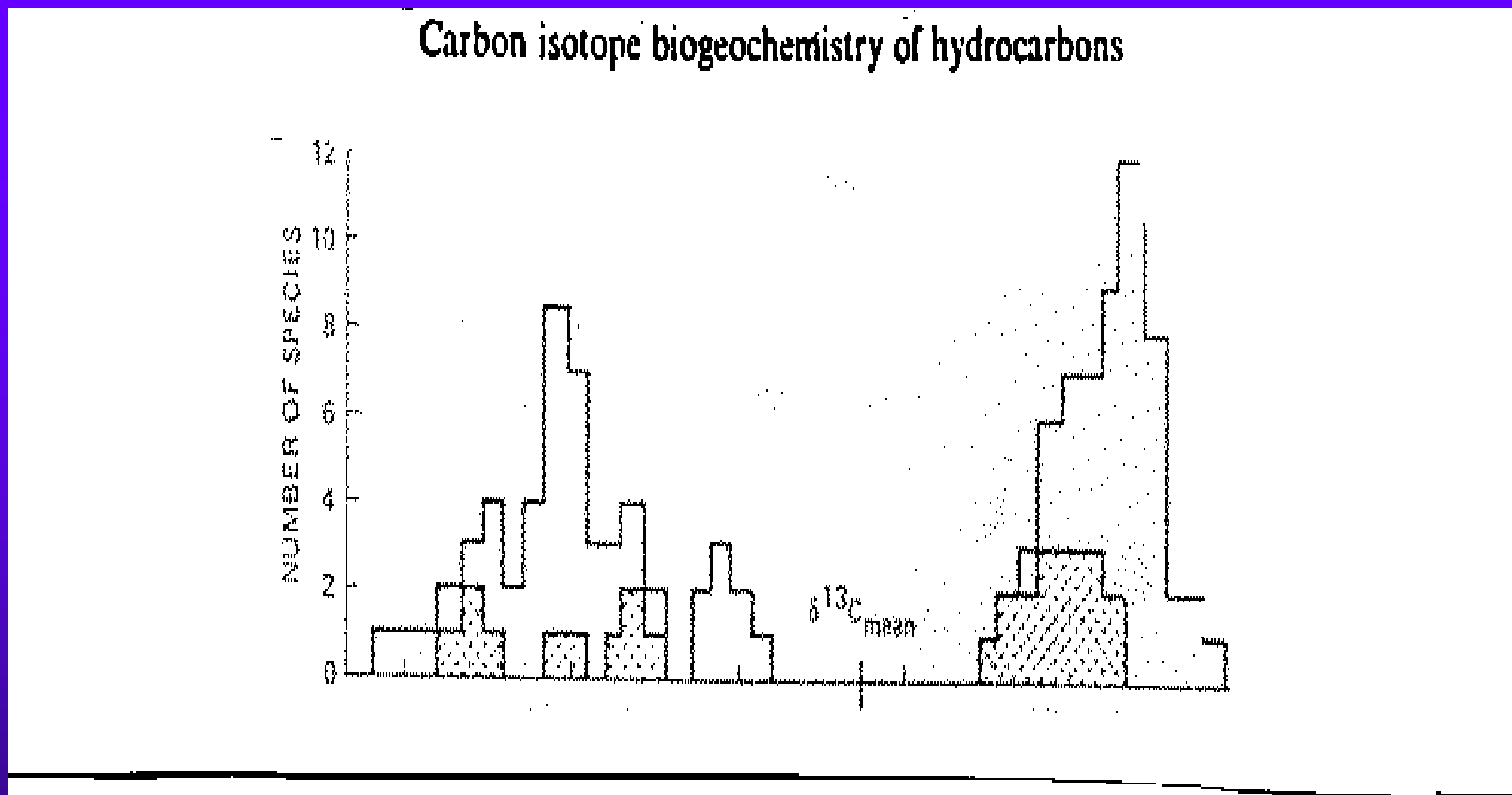
Natural Distribution of Carbon Isotopes





Major Classes of Organic Carbon

C3 **C4**

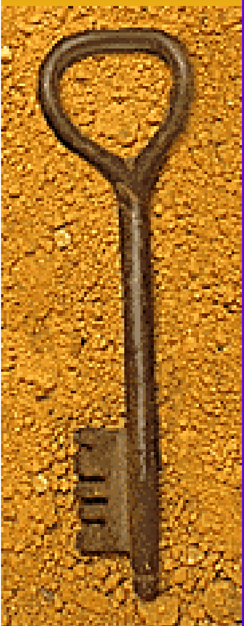


-27 **-12**

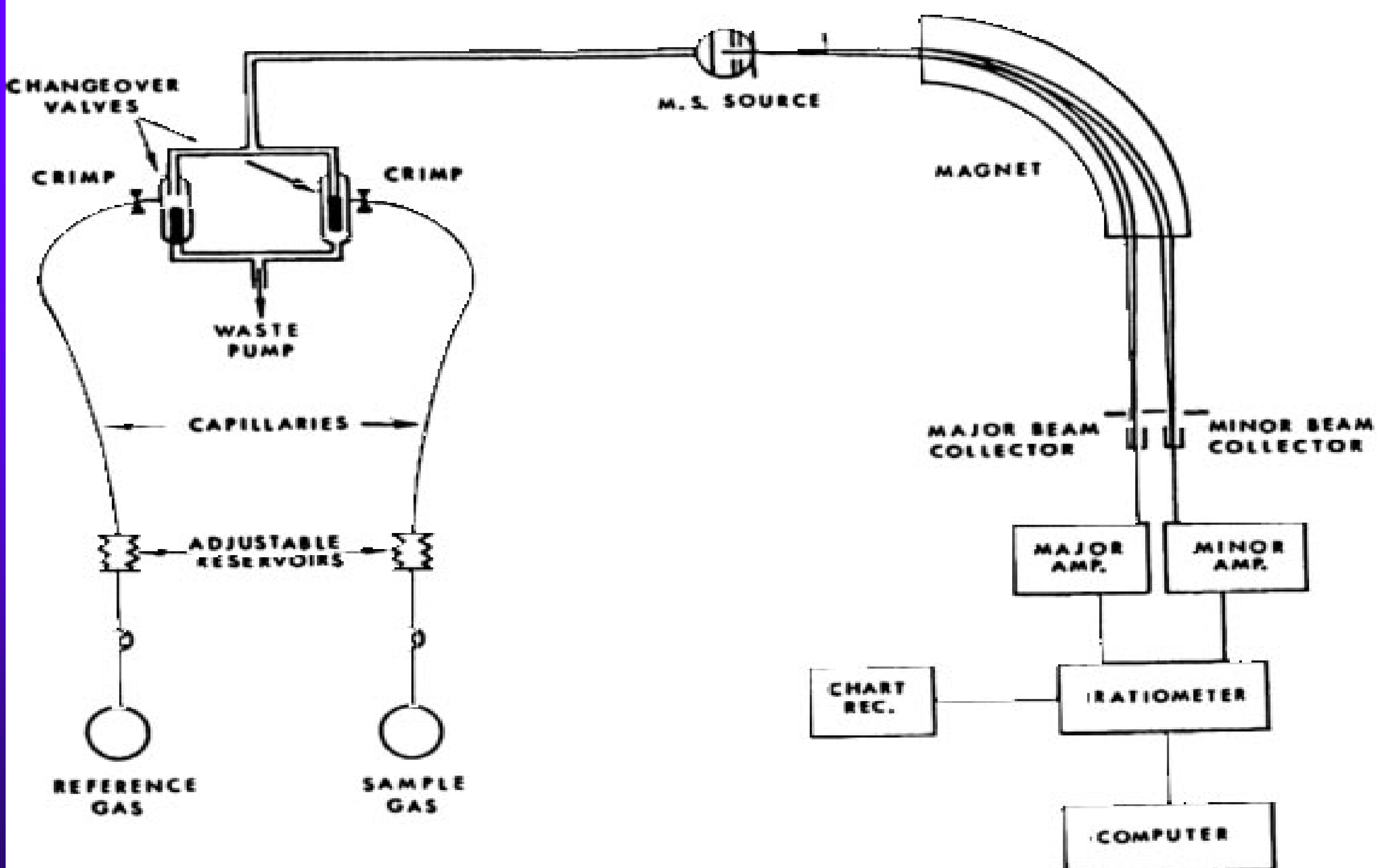
$\delta^{13}C$ (‰ vs VPBD)

(Des Marais, D., 1980, *Geochim. Cosmochim.Acta*, **44**:2075-2086)

How Are Isotopes Measured?



Dual Inlet Mass Spectrometer



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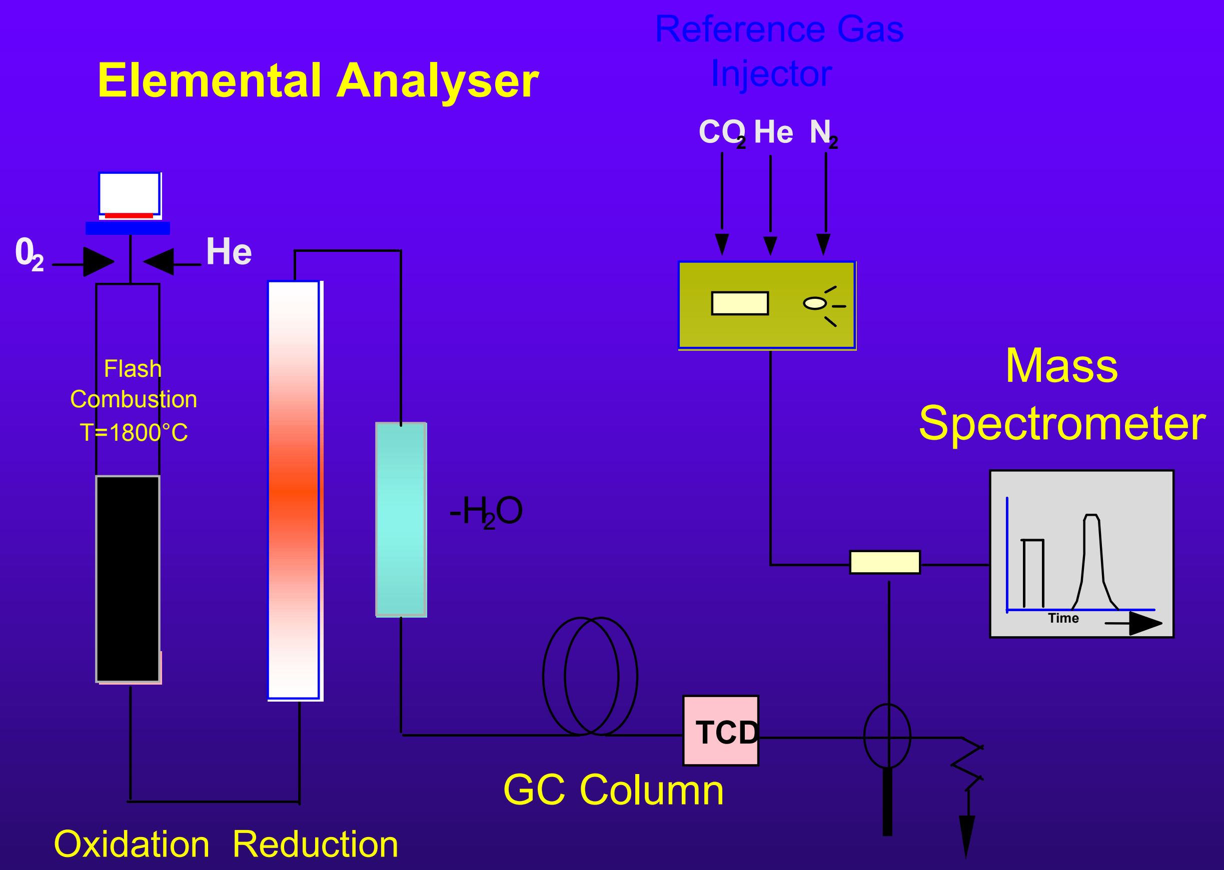
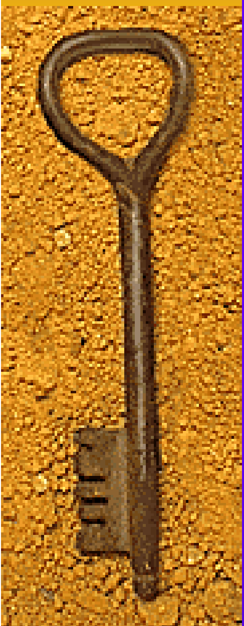
Elemental Analyzer/Mass Spectrometer (EAMS)





Units: Definition of δ

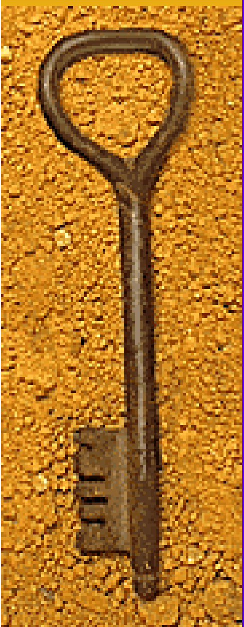
$$\delta^{13}C\ (‰) = \left(\frac{R_{smpl}}{R_{std}} - 1\right) * 1000$$

where,

R_{smpl} = $^{13}C/^{12}C$ of a sample, and

R_{std} = $^{13}C/^{12}C$ of an intl. standard (*e.g.*, VPDB).

(That is, the δ-notation represents a part-per-thousand difference from a standard.)



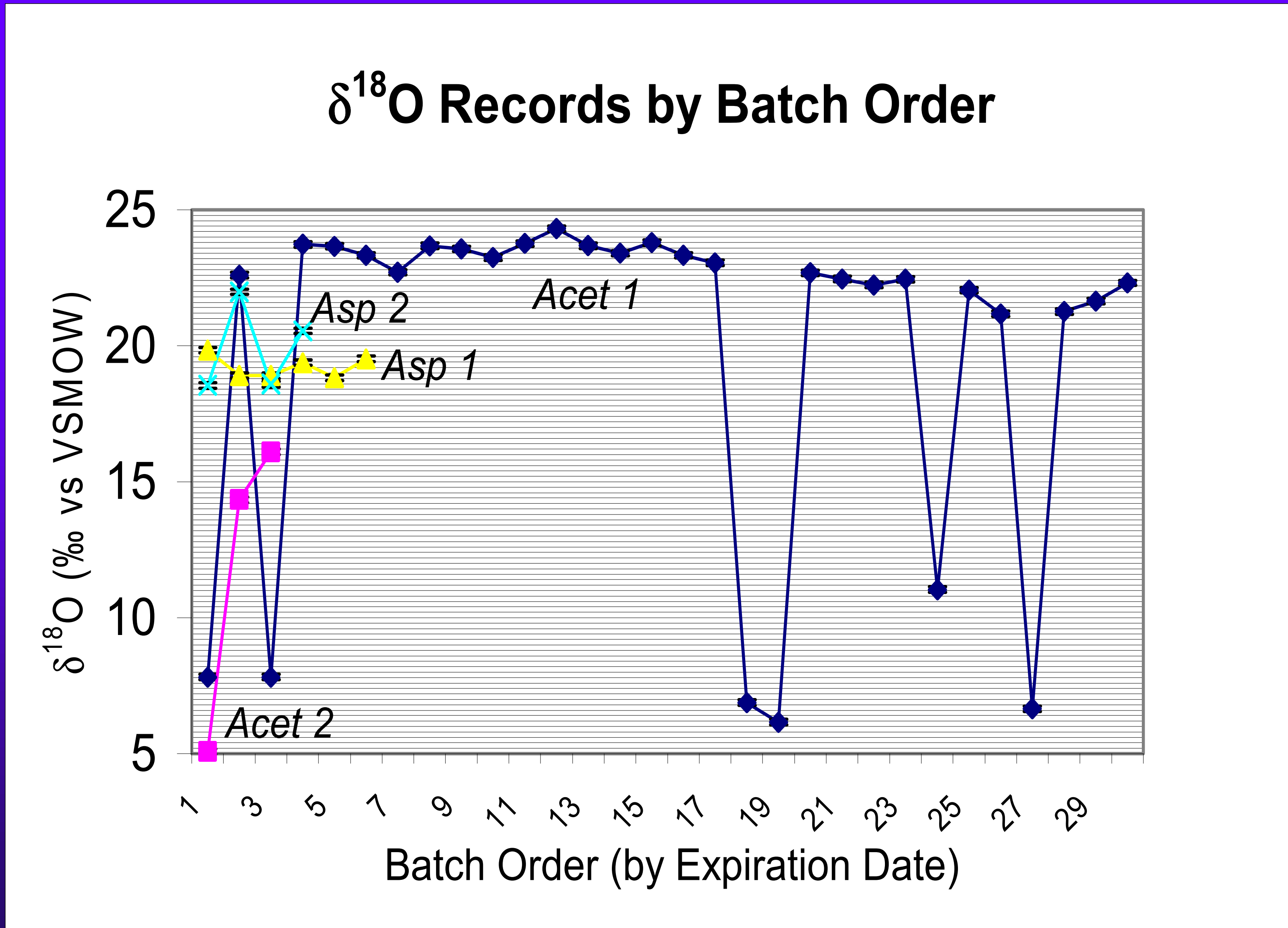
MIT-Micromass Analgesic-Isotope Study

I. Acetaminophen ($C_8H_9NO_2$)

A. Acetaminophen 1 (n = 30)
B. Acetaminophen 2 (n = 3)

II. Aspirin ($C_9H_8O_4$)

A. Aspirin 1 (n = 6)
B. Aspirin 2 (n = 4)



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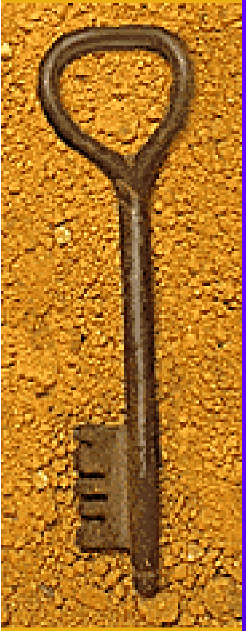
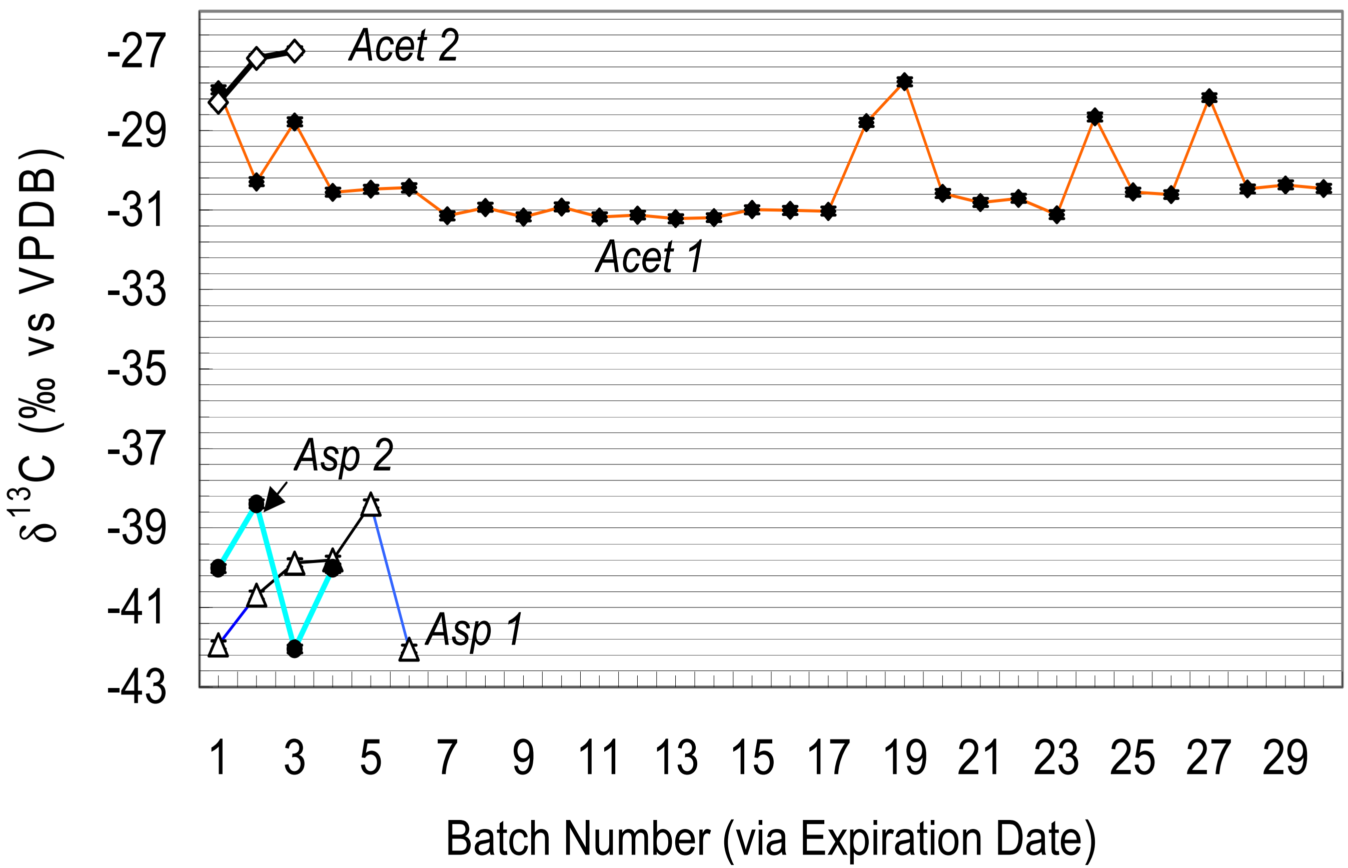
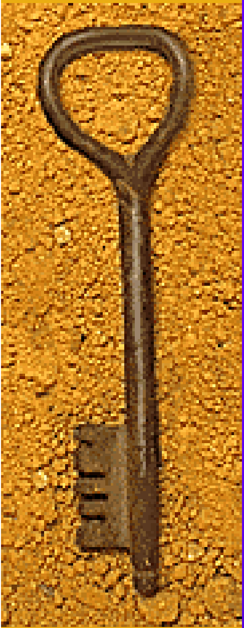
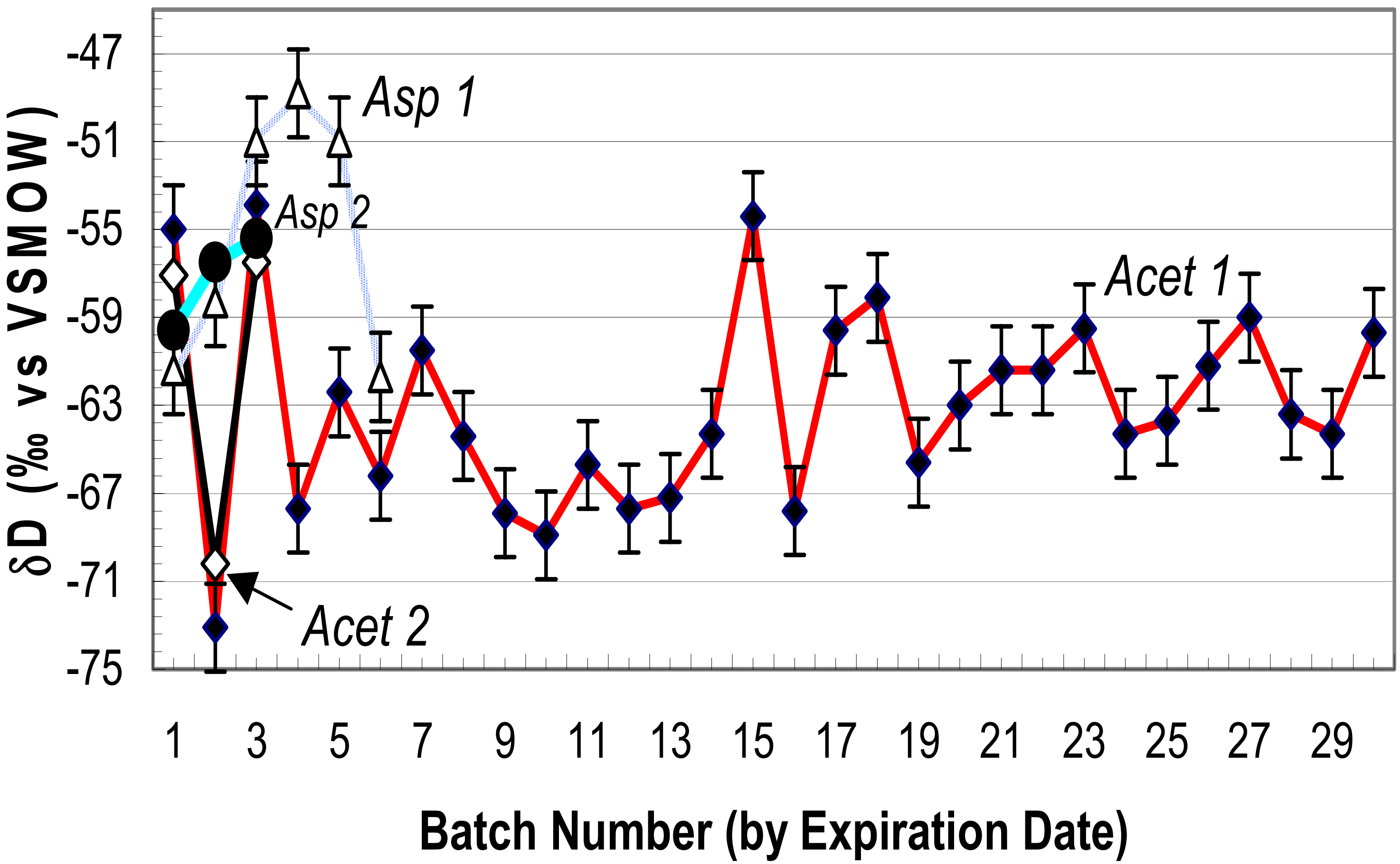


$\delta^{13}C$ Records by Batch Order

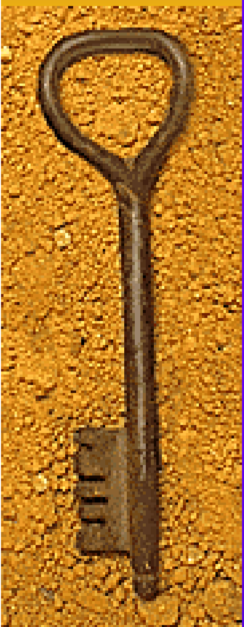


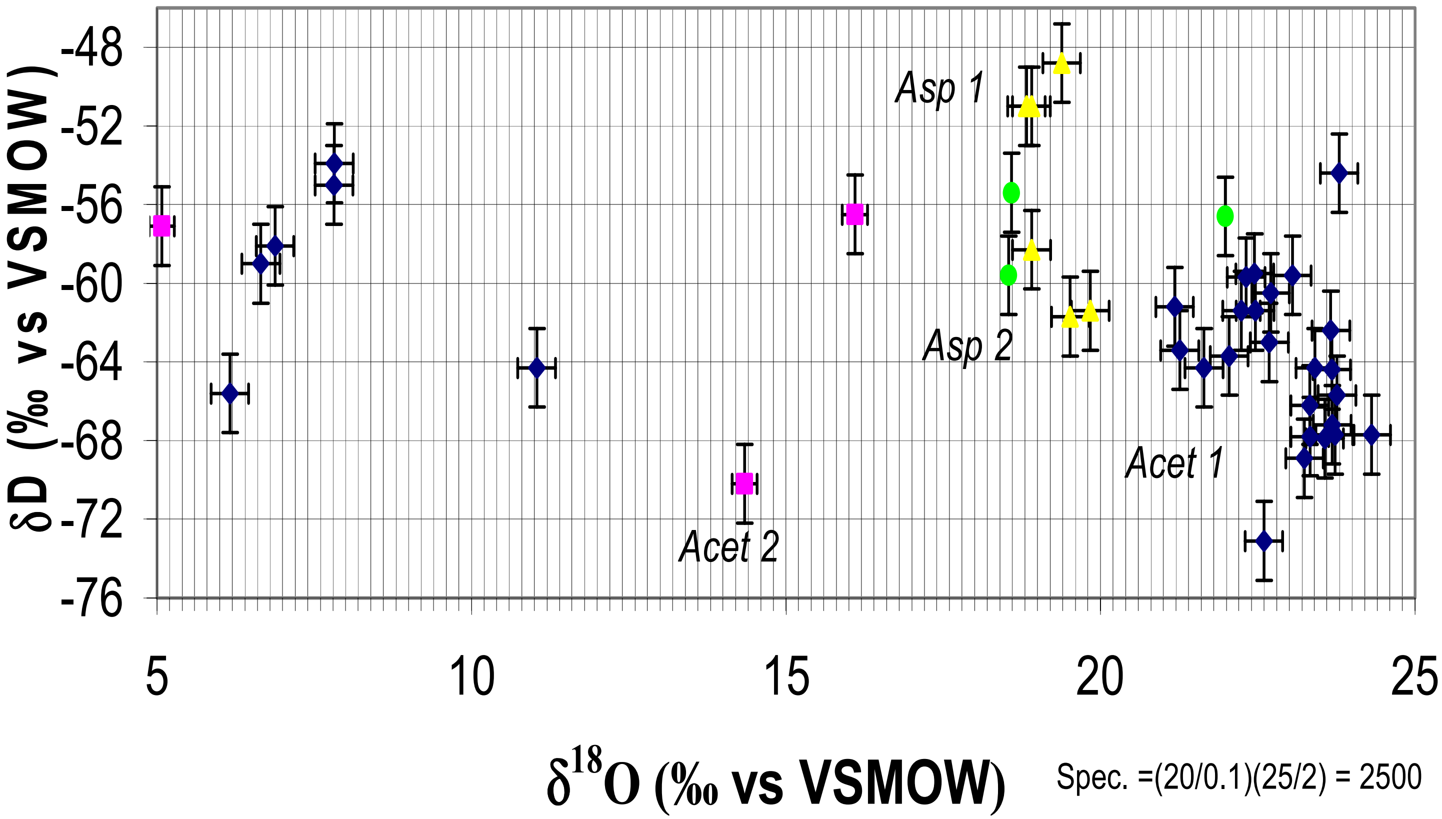
δD Records by Batch Order





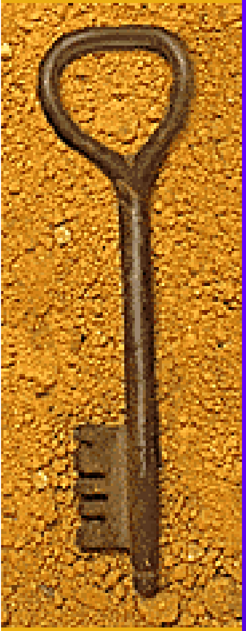
δD vs $\delta^{18}O$: Analgesics

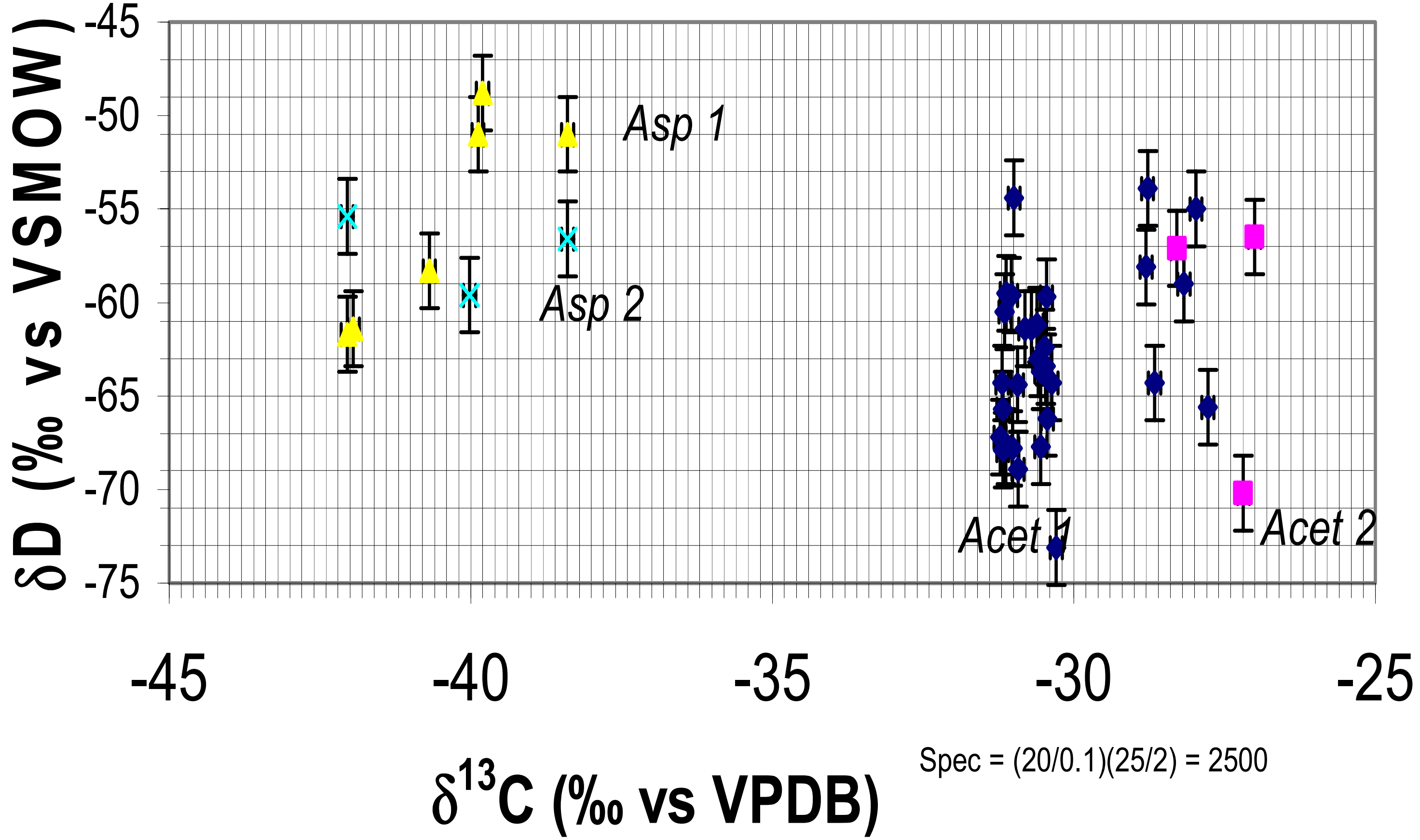


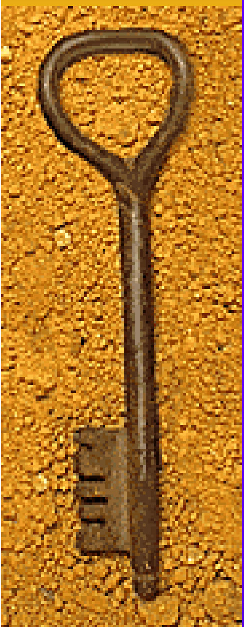


Spec. =(20/0.1)(25/2) = 2500

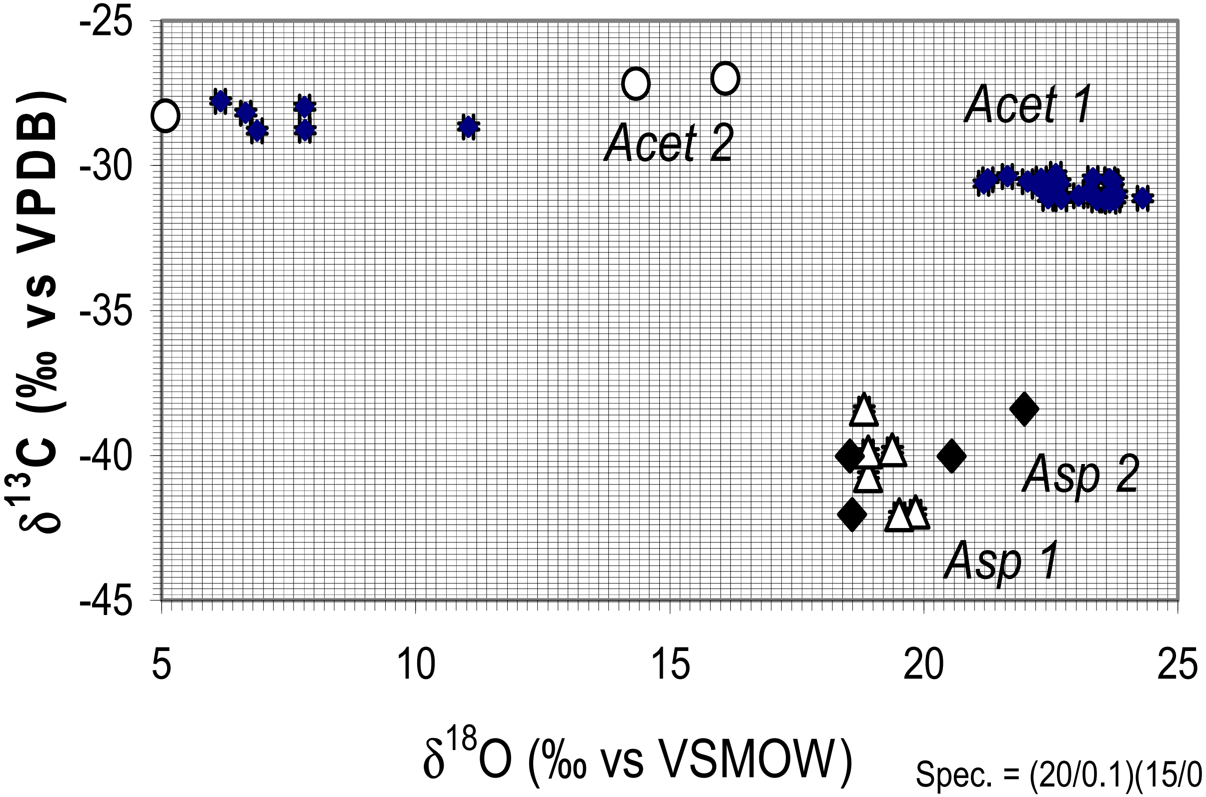
δD vs $\delta^{13}C$: Analgesics



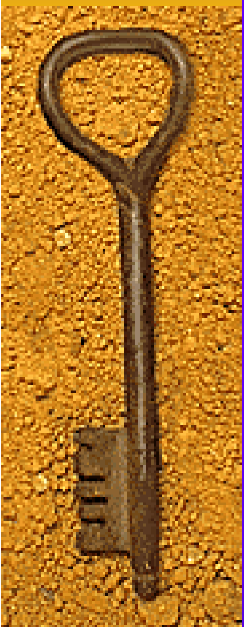




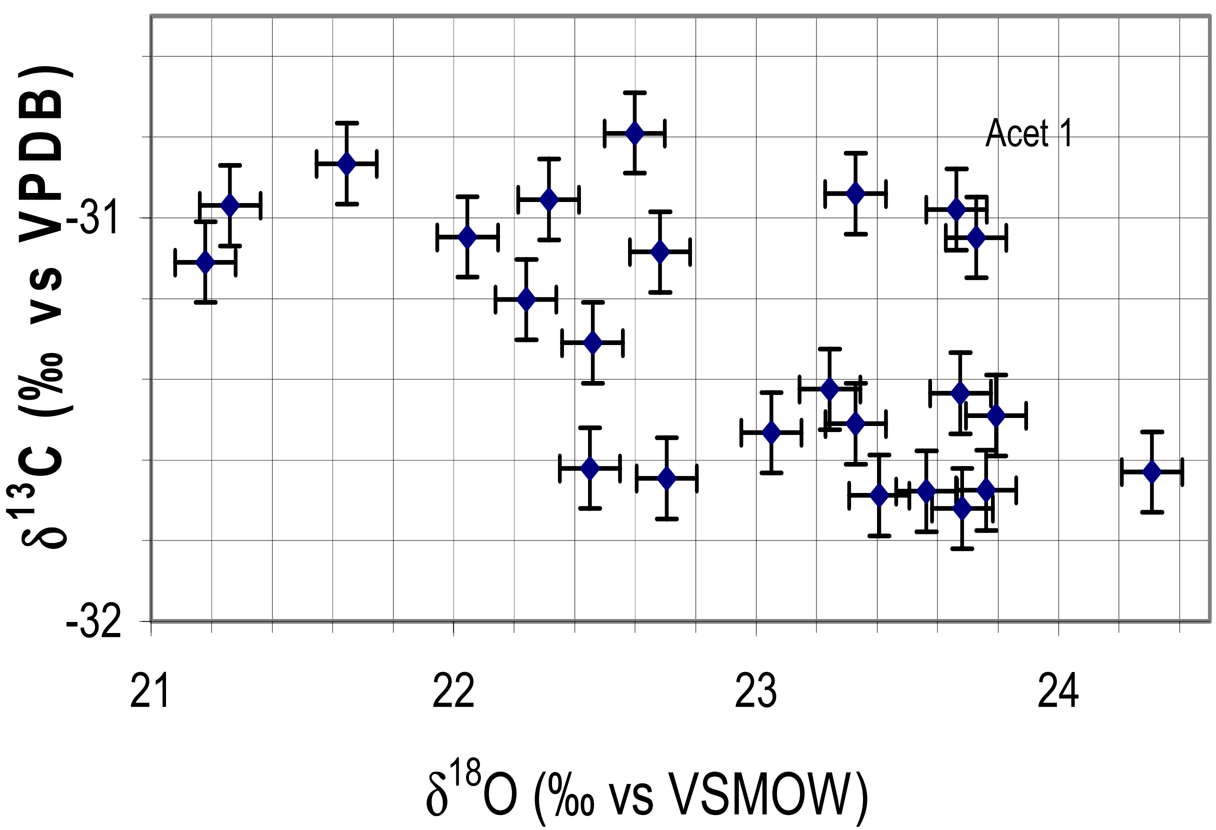
$\delta^{13}C$ vs $\delta^{18}O$: Analgesics

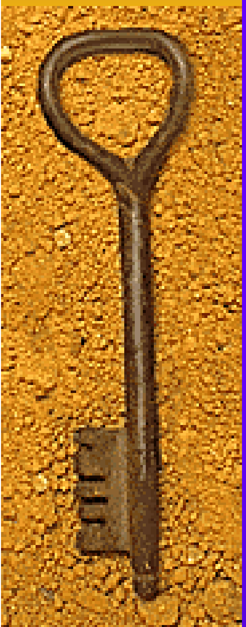


Spec. = (20/0.1)(15/0.1) = 30,000



$\delta^{13}C$ vs $\delta^{18}O$: Analgesics, Focused Plot





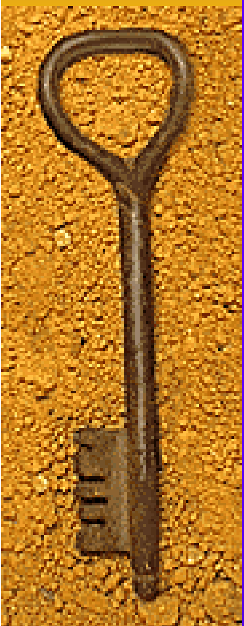
The Combination Lock Analogy For Dynamic Range and Specificity

Combination Lock:

A combination lock has 4 tumblers each with 10 numbers: Thus, the lock has 10^4 (=10,000) combinations.

Pharmaceutical Product Integrity:

Analogously, imagine a pharmaceutical which contains C, N, O, and H and each of their isotopic dynamic ranges is ~100. With that, there are $\sim100^4$ = 100 million possible unique "isotopic combinations" for that drug – via bulk isotopic analyses; many more via compound-specific isotope analysis!



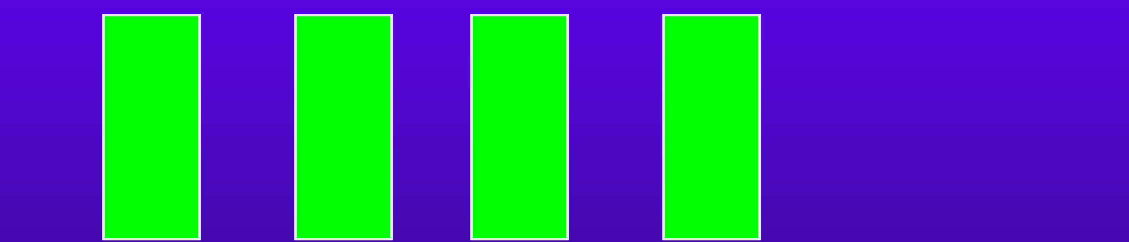
Specificity of the Isotopic Approach

Specificity
(~Upper Limit)

I. Bulk Isotopes (Dual Inlet)

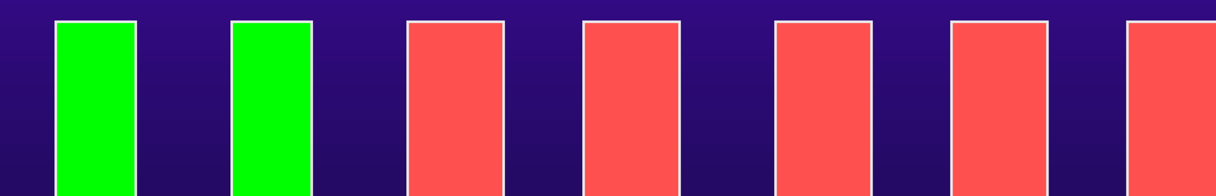
1:1000

II. Bulk Isotopes (Elemental Analyzer/MS)



1:100M

III. Bulk + Molecular Isotopes (irmGCMS)



1:5.0Tr

Perspective on Multi-Isotope Product Integrity

Isotopic Ranges (1s Precision)

Isotope	Typical $\Delta\delta$	Maximum $\Delta\delta$	Analgesic $\Delta\delta$
$\delta^{13}C$	15 (0.1)	80 (0.02)	20 (0.1)
δD	80 (2)	450 (1.5)	25 (2)
$\delta^{18}O$	20 (0.1)	100 (0.2)	20 (0.1)
Specificity	1.2 M	600 M	500 K

What Causes the Variations in ^{18}O? Partially, Natural Variations in Rain.

$\Delta\delta^{18}O$ = ~18‰

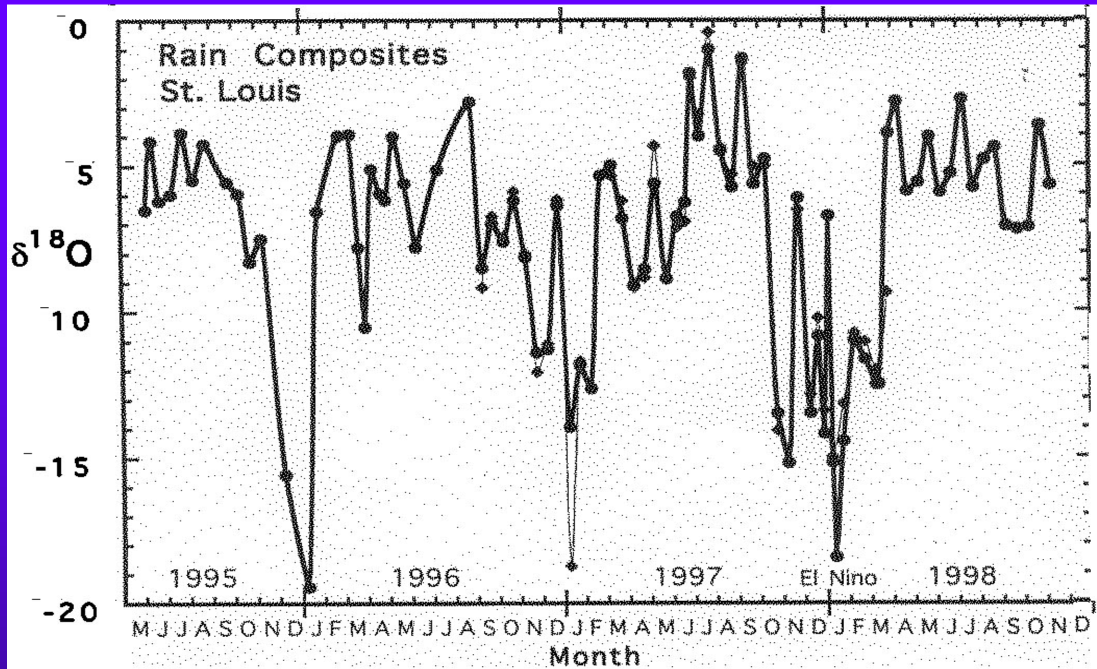


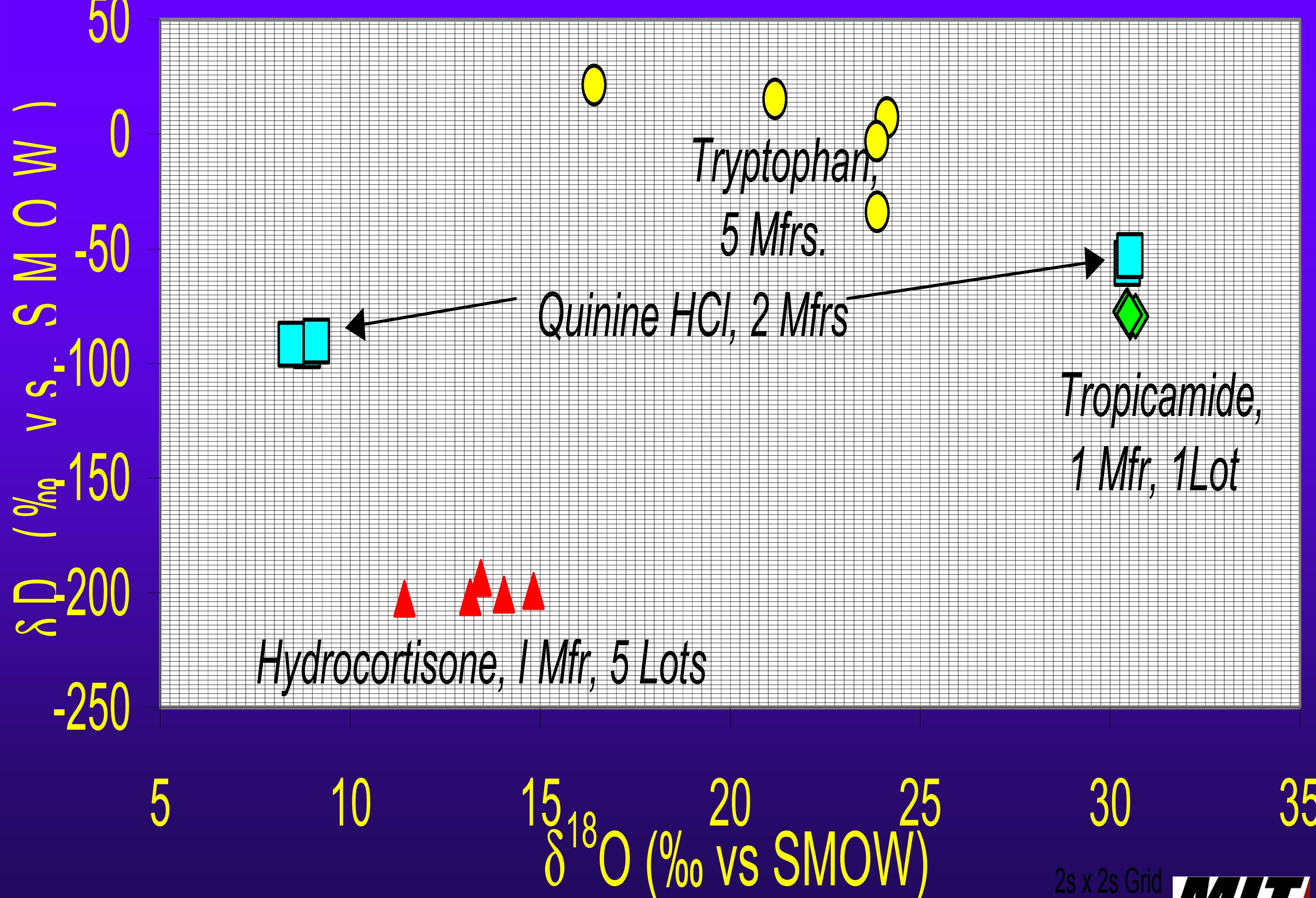
Figure 3.12 Graph of the $\delta^{18}O$ values of semi-monthly composites of meteoric precipitation for two rain gauges in St. Louis, Missouri, notably Ladue (circles) and Washington University (diamonds). For most of the year, the precipitation is within a few per mil of the yearly average $\delta^{18}O$ value of about −6.8. However, sharp negative deviations from this average occur in winter months, showing the influence of polar air masses. The large amount and duration of depleted precipitation in the El Nino winter of 1997–1998 is remarkable and unexpected because temperatures were well above normal for this period.

(Criss, R. E. 1999, *Principles of Stable Isotope Distribution*)

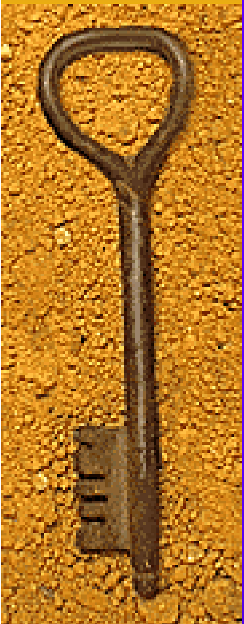
FDA-MIT Study of Four APIs:

1. Hydrocortisone
2. Quinine HCl
3. Tryptophan
4. Tropicamide

FDA-MIT Study of APIs: δD vs. δ^{18}O



2s x 2s Grid

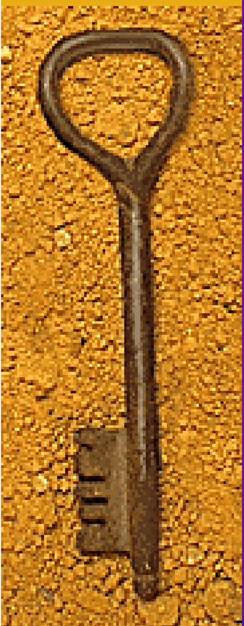


ANTHROPOGENIC PATHWAY OF ELEMENTS

NATURAL MATERIALS
Inorganic Elements
Natural Organics Materials

INDUSTRIAL MATERIALS
Industrial Raw Materials
Synthetic Intermediates
Manufactured Products

METABOLITES
In Vivo
Environmental



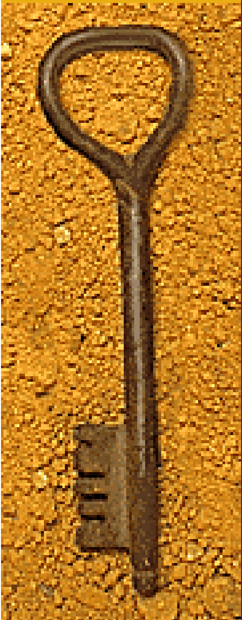
SUMMARY

1. **"Isotopic Fingerprinting"** is a highly-specific means of characterizing of industrial (*viz*., pharmaceutical) materials and thereby identifying and protecting these materials.

2. **"Scatter"** is good when establishing isotopic fingerprints.

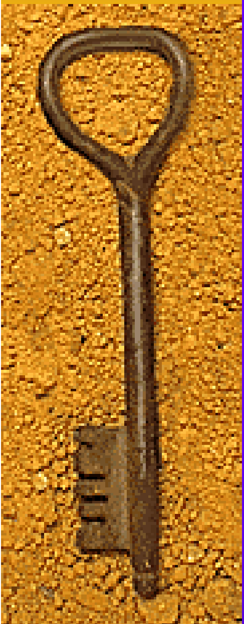
   A. **Dynamic Range Variations**: All sample suites of drug substances measured show significant DR variations for the isotopes analyzed (*viz*., ^{13}C, ^{15}N, ^{2}H, ^{18}O).

   B. **Batch-Series Variations** in δ-values were both continuous and episodic ("spikey").
   Dift. raw materials & variable synthesis cndtns.



3. **Specificity**: Isotopic crossplots show that the combined DRs of these analgesic drugs yield ~5*10^5 possibilities or "combinations." Can be much higher as seen in FDA APIs.

4. **Industrial Batch Products**: The present work on ethical pharmaceuticals focuses on multi-isotopic identification of batch-produced produced products (patents pending).



Acknowledgements

Isotech Laboratories, Inc.

Dennis Coleman   Todd Coleman   Steve Pelphrey

Patents Pending: G8 & Australia

Isotope Product Authenticity, "Natural Labeling"
(Multi-Isotopic Identification of Batch-Produced Industrial Products)

Literature Available at the Back of the Room.