The First Rule of Isotope Dilution Club is that We *Do* Talk about Isotope Dilution Club

Harry McCarty, Mirna Alpizar, and Kevin Roberts

General Dynamics Information Technology

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And as always:

- Your mileage may vary
- May cause drowsiness
- Do not operate heavy machinery while viewing this presentation

The Bare Basics of Isotope Dilution

- A long-established analytical technique dating back over 100 years to the work of George de Hevesy and Friedrich Adolf Paneth using lead isotopes as radioactive tracers.
- Can be performed using either radioactive isotopes, as above, or stable (non-radioactive) isotopes or compounds enriched in one or more of those isotopes.
- Begins by adding a known amount of an isotopically enriched tracer to the sample.
- Generally, requires use of a mass-selective detector in order to distinguish the tracer from the non-enriched analyte of interest.

Used in Many Disciplines

- Biomedicine
- Geology
- Chemistry
- Nuclear science
- Oceanography
- Environmental science

To name just a few

History at EPA

EPA methods for environmental analyses began using stable isotopically labeled compounds in the early 1970s. Examples include:

- Deuterated compounds such as Phenol-d_5 used as surrogates and deuterated PAHs used as internal standards in methods for semivolatile organics in various matrices (*not isotope dilution*)
- ^{37}Cl- and ^{13}C-labeled analogs of 2,3,7,8-TCDD used in Method 613 and soil analysis protocols at Times Beach for isotope dilution quantification
- Dozens of deuterated and ^{13}C-labeled analogs of volatile and semivolatile organics on the Priority Pollutant List specifically used for isotope dilution quantification in Methods 1624 and 1625 as early as 1977
- EPA worked with vendors to promote synthesis of labeled analogs of many pollutants for use in EPA methods

Our point is – ***It's not new!***

Big Push at EPA in the 1980s and 1990s

- CLP and SW-846 dioxin methods used isotope dilution quantification for some analytes
- Office of Water isotope dilution methods included:
  - Those associated with the pulp and paper industry, such as Methods 1613 (dioxins) and 1653 (chlorinated phenols)
  - Method 1668 for PCB congeners
  - Method 1614 for PBDE congeners
  - Pharmaceutical industry methods for volatiles and semivolatiles, such as Methods 1665 and 1666

And Today

PFAS, PFAS, PFAS …

Quantification Schemes

The biggest difference between isotope dilution and internal standard methods is the associations of the target analytes and the standards used for quantification and how they are used:

- Traditional “internal standards” in EPA methods are introduced immediately before the sample or extract is injected into the GC/MS
- The target analytes are associated with the internal standards based solely on chromatographic retention times
- In isotope dilution methods, the labeled compounds are introduced into the sample at the very beginning of the analytical process
- Given the structural similarities of the target analyte and its labeled analog, they behave nearly identically in terms of extraction and cleanup and elute very close together during chromatography, but they can be separated by mass
- Labeled compounds used for isotope dilution are quantified against a non-extracted internal standard (NIS) added to the sample extract before injection.

Terminology Comparison

Term	Isotope Dilution Quant.	Internal Standard Quant.
Internal standard	Yes, but called non-extracted internal standard (NIS) or an injection internal standard	Yes
Labeled compound	Yes (also called labeled analog)	No
Extracted internal standard (EIS)	Yes	No
Surrogate	NO !	Yes
Non-extracted internal standard	Yes	Just called an internal standard
Recovery standard	Less-than-accurate term for the NIS	No

Isotope Dilution vs. Internal Standard Equations

Isotope Dilution	**Internal Standard**
$RR = \frac{Area_n\, C_l}{Area_l\, C_n}$	$RF = \frac{Area_s\, C_{is}}{Area_{is}\, C_s}$
$Area_n$ = The measured area of the *m/z* for the native (unlabeled) analyte $Area_l$ = The measured area at the *m/z* for the labeled analog used for isotope dilution quantitation C_l = The concentration of the labeled analog used for isotope dilution quantitation in the calibration standard (ng) C_n = The concentration of the native analyte in the calibration standard	$Area_s$ = Area of the characteristic m/z for the target analyte $Area_{is}$ = Area of the characteristic m/z for the internal standard C_{is} = Concentration of the internal standard C_s = Concentration of the target analyte

Challenges to Employing Isotope Dilution

- Limited number of elements with stable isotopes present in environmental contaminants
    - ^{13}C, ^{18}O, ^{15}N, ^{2}H (D), ^{37}Cl
    - Sadly, not Fluorine
- Someone needs to synthesize the standards from isotopically pure source materials
- The compounds need to be stable - Deuterium often will exchange with ^{1}H in aqueous samples
- Added costs for the labeled compounds (but not as great a challenge as some claim)

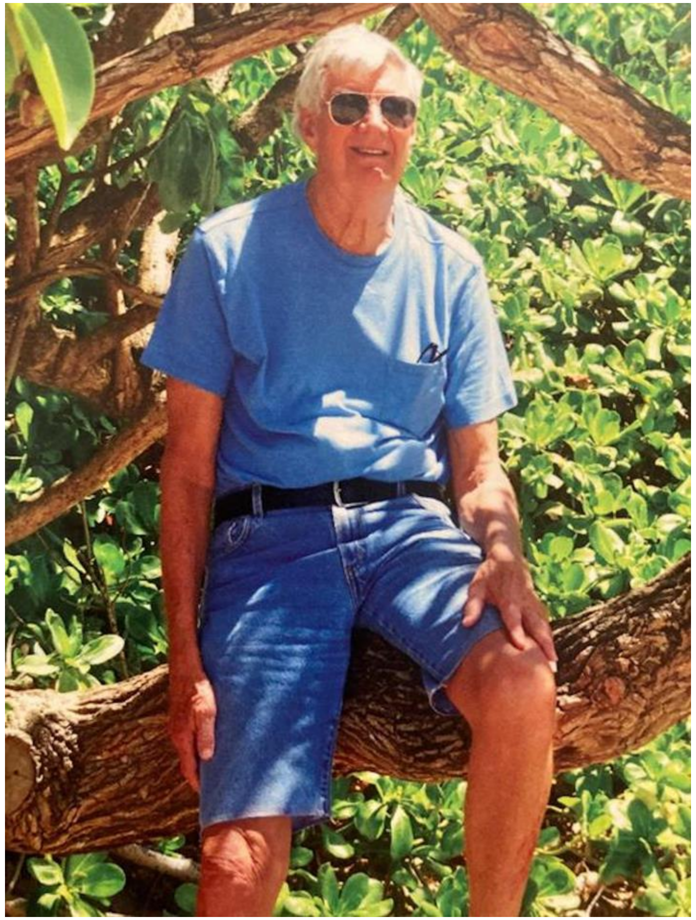
Challenges (continued)

- Software limitations in many instruments and data systems
  - Historically, isotope dilution quantification was beyond the capabilities of the Finnigan INCOS data system, so OW’s 4 contract laboratories running 1624 and 1625 sent 9-track tapes to the Sample Control Center and we uploaded the data to EPA’s mainframe and calculated the final results.
  - That’s not practical any longer
- Modern data systems have far more computing power, but laboratories may need vendor support to set up their systems for isotope dilution
  - Canned routines should not be difficult to provide
  - May require that laboratories and vendors work together to learn new terminology and routines consistent with newer methods
  - EPA may need to engage in outreach to vendors

Conclusions

- Isotope dilution analysis is not new
- It works, and it works well – During the validation of Methods 1624 and 1625, EPA showed that it can improve accuracy by a factor of 2, and precision by a factor of 4, compared to internal standard analyses
- It is most helpful for methods with many sample processing steps, where analytes may be lost along the way
- It is a minor paradigm shift for some laboratories and instrument vendors, but that’s not insurmountable.
- There are increased costs for the standards, but they are manageable.
- It is not a panacea for every GC/MS or LC/MS analyses, but the exceptions are rare.
- When in doubt, RTFM!

Remembering One of the Pioneers of EPA Isotope Dilution Methods …



Dale Richard Rushneck

January 5, 1938 - September 4, 2021

Worked at NASA's Jet Propulsion Laboratories on the original Mission to Mars as the Principal Technical Manager for the GC/MS on the first Viking lander sent to Mars.

Credited with finding krypton and xenon present on Mars, was awarded the American Association for the Advancement of Science 1977 Newcomb Cleveland Prize for an Outstanding Contribution to Science.

Pioneered the use of isotope dilution in EPA Methods 1624 and 1625 and contributed to most of the later OW isotope dilution methods.

Questions?