Isotope Dilution:
- Where it works
- Where it doesn't work
- Where we should expand its use

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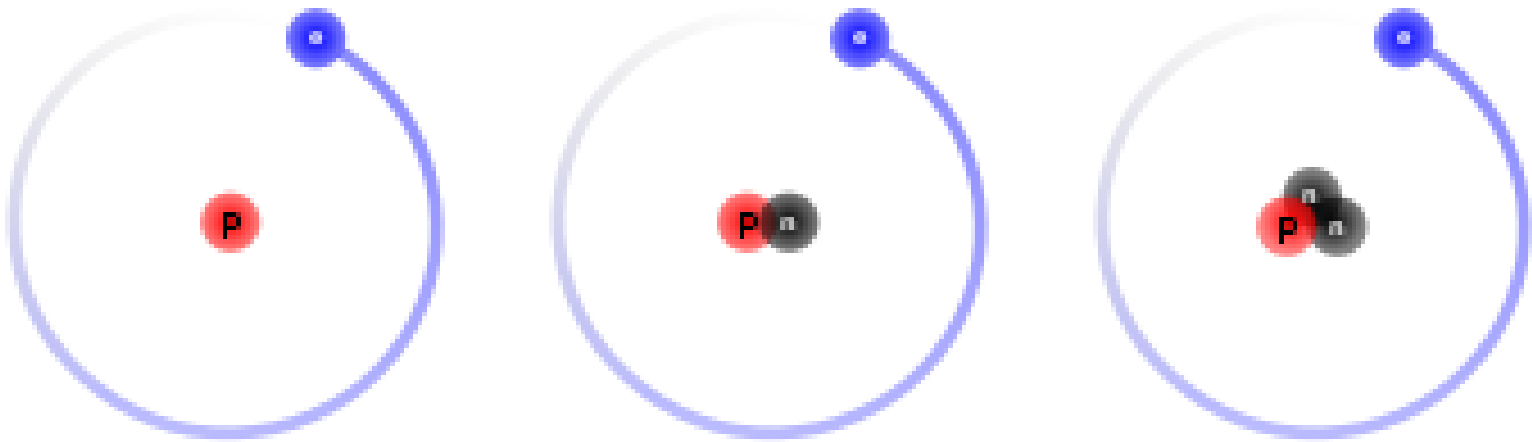
Isotope Dilution - Definitions

Isotope - any of two or more species of atoms of an element with the same atomic number and nearly identical chemical behavior but with differing atomic mass or mass number and different physical properties

Stable Isotopes do not decay into other elements. In contrast, radioactive isotopes (e.g., ^{14}C) are unstable and will decay into other elements.

Isotope Dilution: Spiking of stable isotope labeled analogs of the analyte to the SAMPLE before any processing; then adjusting analyte concentration for analog recovery

Addition of Neutrons – Conservation of Protons and Electrons



Stable Isotope Labeling vs Radioisotope Labeling

Adding one neutron tends to be a more stable configuration. Adding more or removing neutrons tends toward radioactivity:

Deuterium: Stable

Tritium: Radioactive

^{13}C: Stable

^{14}C Radioactive (along with 12 others)

^{15}N: Stable

^{16}N: Radioactive (along with 13 others)

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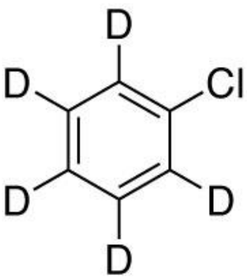
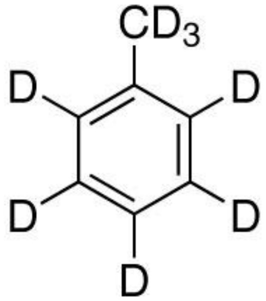
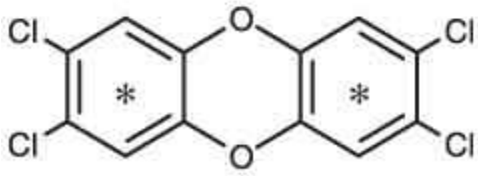
Familiar Examples

- chlorobenzene-d_5 (IS)
- 1,4-dichlorobenzene-d_4 (IS)
- 1,2-Dichlorobenzene-d_4 (Sur)
- Toluene-d_8 (Sur)
- naphthalene-d_8 (IS)
- phenanthrene-d_{10} (IS)
- Phenol-d_5
- Terphenyl-d_{14}
- $^{13}C_{12}$-2,3,7,8-TCDD
- $^{13}C_{12}$-PCB-126







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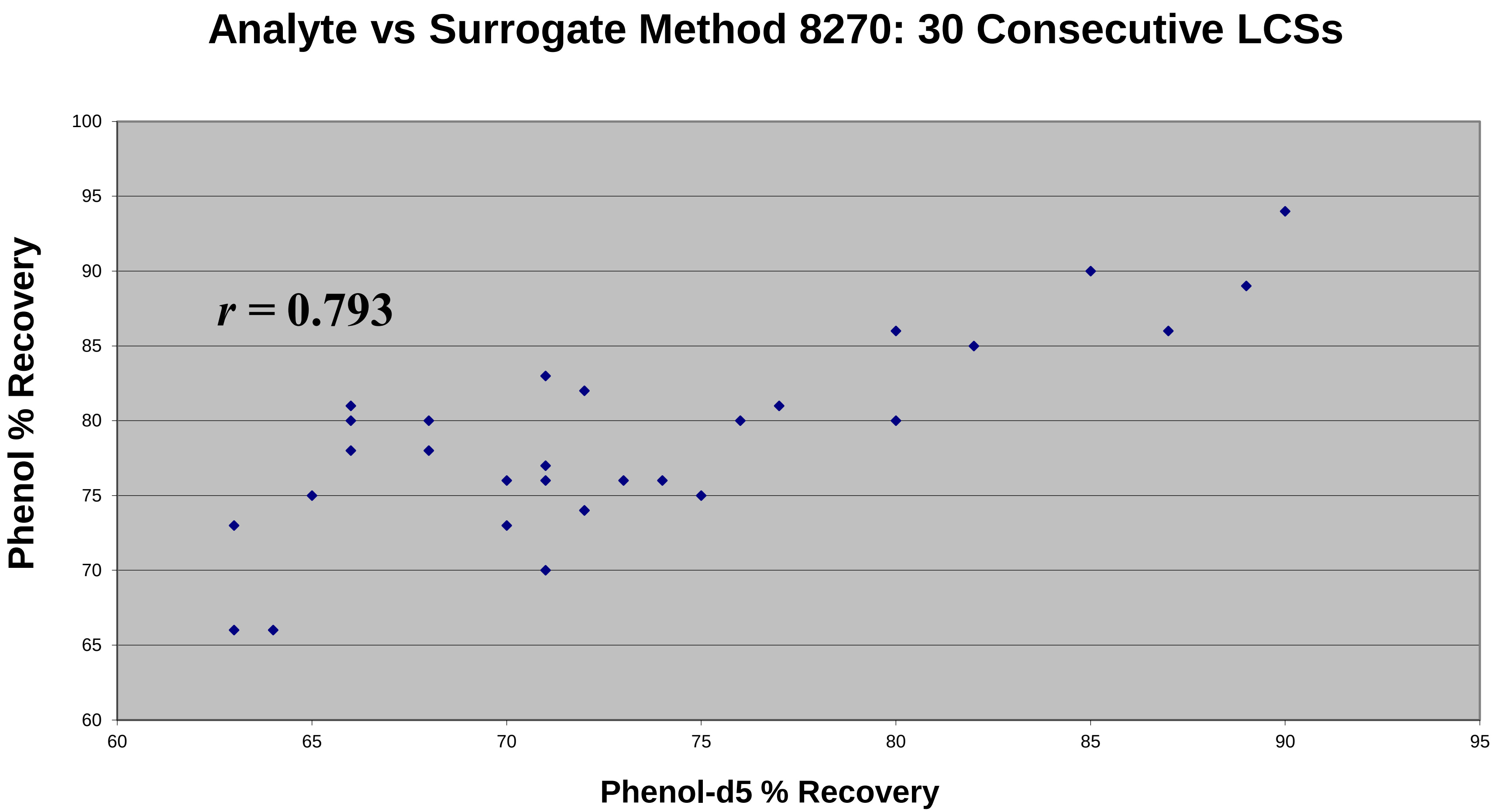
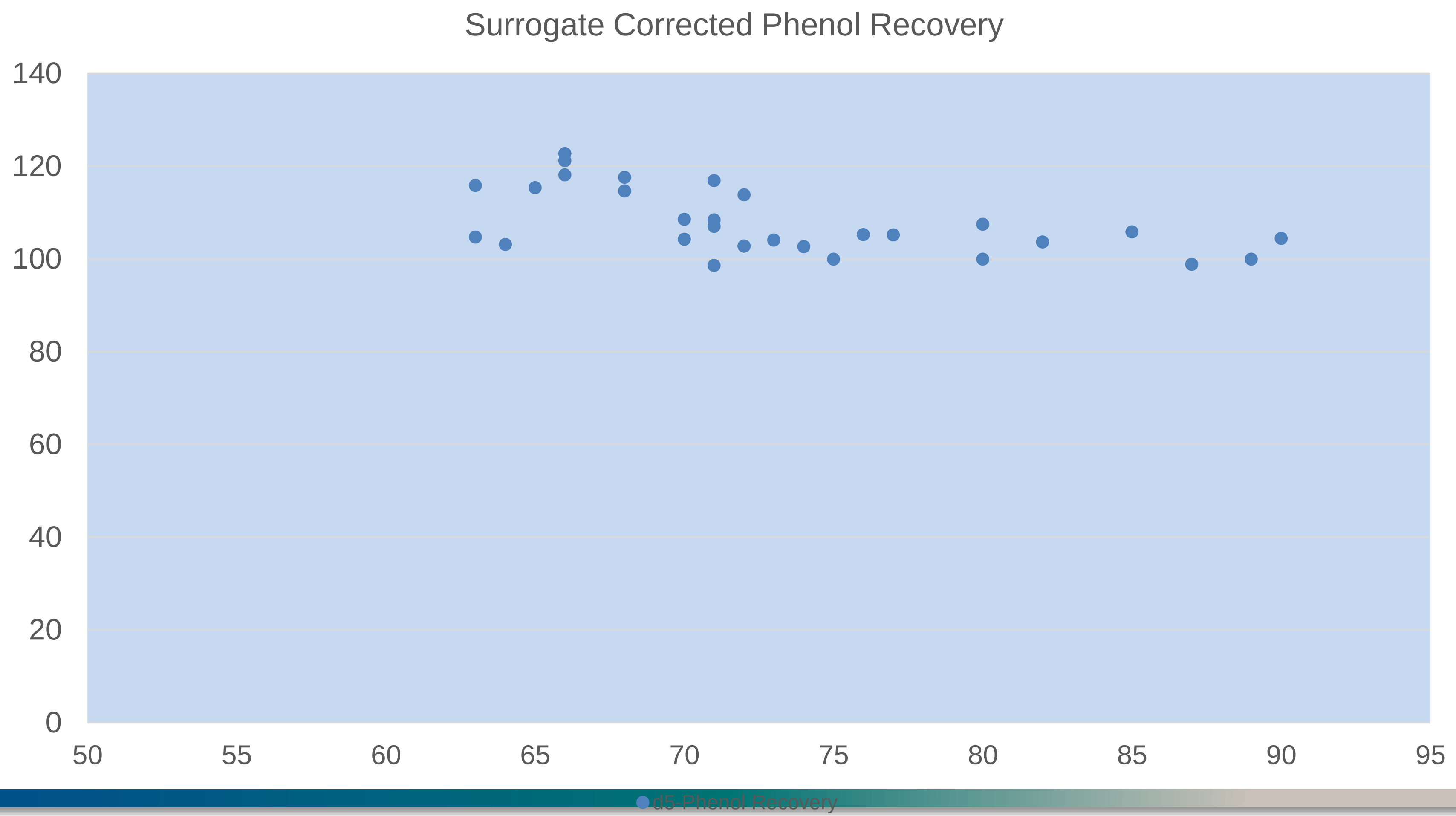
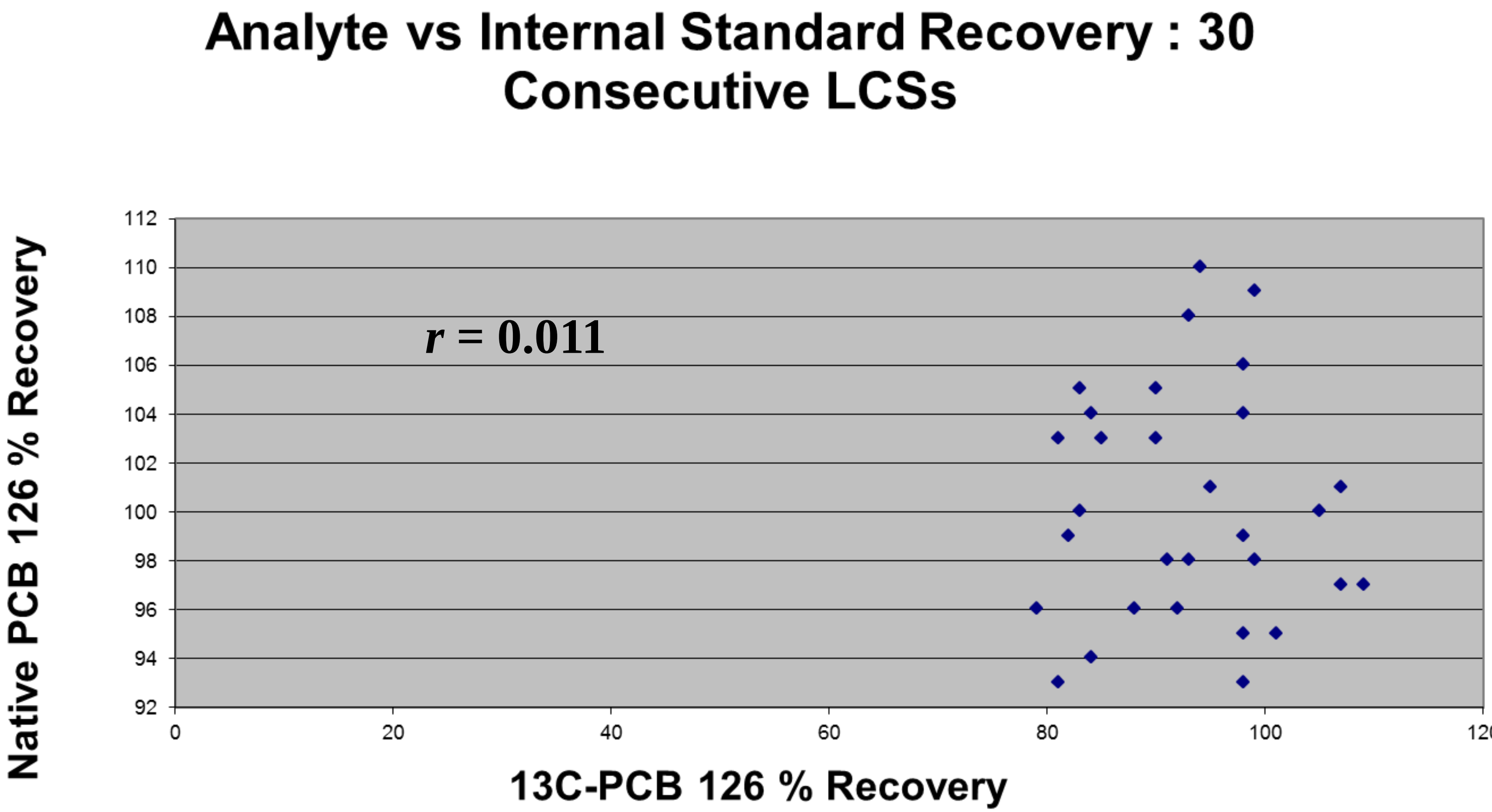
Isotope-labeled Surrogate and Native Analyte Covariance



After Surrogate Correction, Recovery of Phenol is Independent of “IS” Recovery



“When properly applied, results of isotope dilution analyses are not affected by internal standard recovery.”



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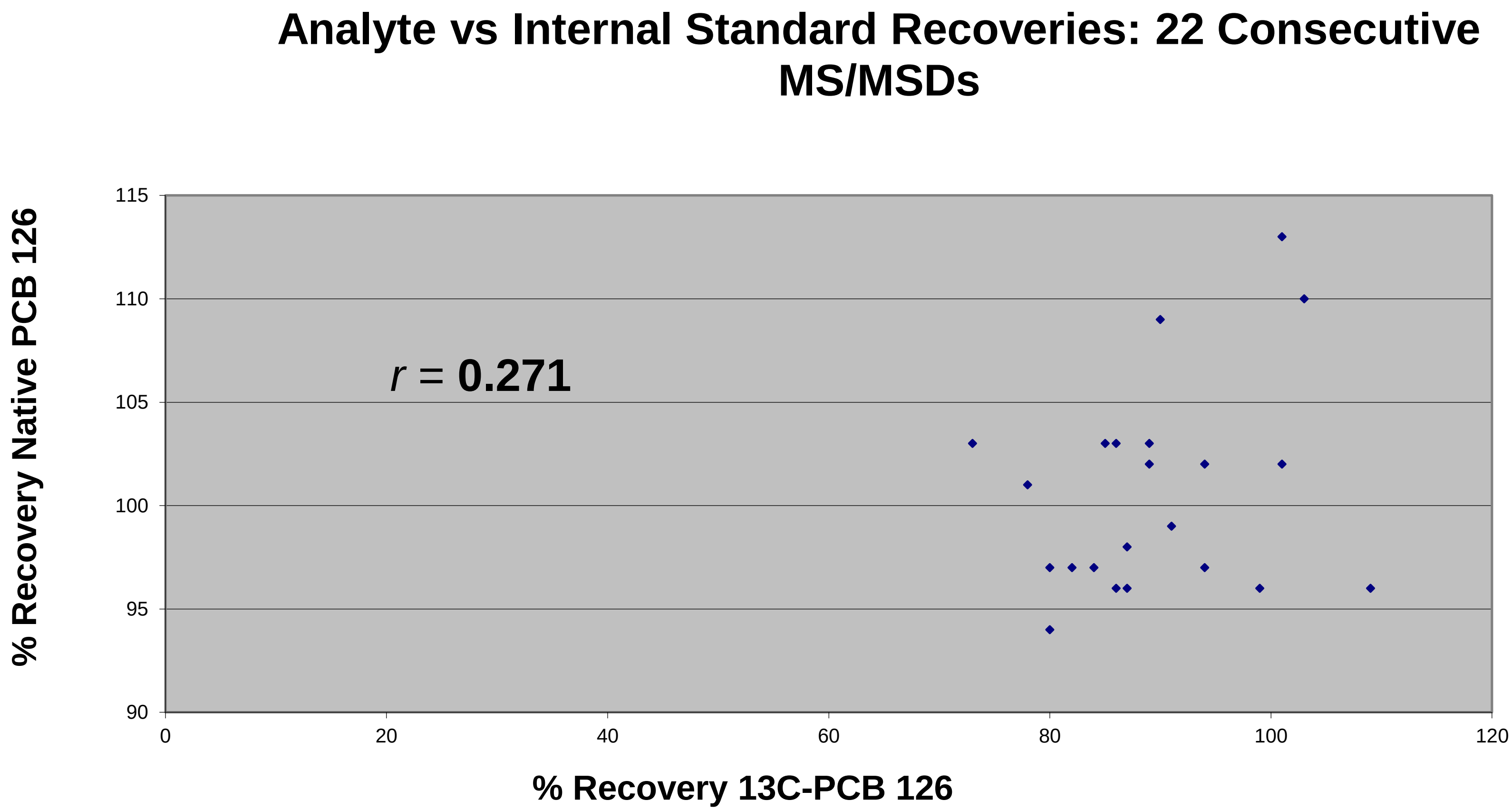

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EPA Uses of Isotope Dilution Strategies

- **Major Regulatory Method**
  - Polychlorinated Dioxins and Furans (8280, 8290, 1613)

- **Approved Methods (Specific Applications)**
  - Polychlorinated Biphenyl Congeners (1668)
  - Metals Speciation (6800)*
  - Volatile Organic Compounds (1624)
  - Semivolatile Organic Compounds (1625)
  - OC and OP Pesticides (1699)
    - **More on next slide**

Subregulatory Research and Applications

- Tetramine, VX, MTBE, TBA, 1,4-Dioxane, Sulfolane

EPA Isotope Dilution Methods Applied to Specific Industries

- Volatile Organic Compounds Specific to Pharma (1666)
- Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS (1694)
- Steroids and Hormones in Water, Soil, Sediment, and Biosolids by HRGC/HRMS (1698)
- Chlorophenolics – Specific to Pulp and Paper (1653)
- Chloroform – Pulp and Paper (1624)

Where it works well

- Overcoming adsorptive challenges (dioxins, PCBs, PAHs)
- Overcoming hydrophilic analytes (chlorophenols, 1,4-dioxane)
- Overcoming reactivity challenges (hexavalent chromium)
- Overcoming evaporative loss (1,4-dioxane, chloroform)

Where it does not work so well

- If mass spectrometry is not used (saturated hydrocarbons)
- If labeled standards are not available or are very expensive
- If the primary problem is extraction or digestion efficacy
- If deuterated standards are all that are available and the conditions are right for protic exchange. (protic solvents, acid, base or metal catalysts, coupled with conditions of increased temperature and/or pressure).
- Where exact analogues are not available for reactive analytes.

Volatile Organic Compounds

- Method 8260B recoveries of listed analytes in water range from 91% upwards. AOK.
- Method 624 recoveries of listed analytes in water range from 87% upwards. Again – method performs well for water.
- Method 8260B showed poor recovery of pyridine from oil (31%)
- More difficulty is seen in solid matrices.

VOAs - Garden Soil – a different story

Analyte	% Rec	Analyte	% Rec
1,2,3-Trichlorobenzene	11.4	Chlorobenzene	41.3
trans-1,2-Dichloroethene	13.6	Bromoform	41.4
1,4-Dichlorobenzene	15.0	2-Chlorotoluene	45.6
1,2,4-Trichlorobenzene	15.0	**Vinyl chloride**	48.7
1,3-Dichlorobenzene	17.2	Bromochloromethane	50.9
1,2-Dichlorobenzene	19.0	1,2,3-Trichloropropane	59.0
Bromobenzene	24.1		
4-Chlorotoluene	25.2		
Hexachlorobutadiene	26.4		
Naphthalene	26.5		
Dibromomethane	34.6		
cis-1,2-Dichloroethene	39.4		

Mean Recoveries – Water (3520C/8270D)

Compound	recovery, x’ (µg/L)	precision, sr’ (µg/L)
2-Nitrophenol	0.07C-1.15	0.16*mean+1.94
Dimethyl phthalate	0.20C+1.03	0.54*mean+0.19
δ-BHC	0.29C-1.09	0.34*mean+0.86
Endosulfan sulfate	0.39C+0.41	0.12*mean+2.47
Diethyl phthalate	0.43C+1.00	0.28*mean+1.44
Phenol	0.43C+1.26	0.26*mean+0.73
4,4'-DDD	0.56C-0.40	0.29*mean-0.32
Di-n-butyl phthalate	0.59C+0.71	0.13*mean+1.16

Mean Recoveries – 3541/8270D – Spiked Clay

Analyte	**Avg % Rec**	RSD	Analyte	**Avg % Rec**	RSD
Hexachlorocyclopentadiene	**4.1**	15	Benzoic acid	**40.6**	7.7
Pentachlorobenzene	**13.7**	7.3	N-Nitroso-di-n-propylamine	**41.4**	6.2
Bis(2-chloroisopropyl)ether	**15.0**	15	2-Chlorophenol	**42.7**	4.3
2-Methylphenol	**17.6**	6.6	Bis(2-chloroethoxy)methane	**44.1**	3.0
1,2,4-Trichlorobenzene	**18.1**	31	**Heptachlor**	**46.9**	9.2
4-Methylphenol	**23.4**	19	2-Methylnaphthalene	**47.0**	8.6
Bis(2-chloroethyl)ether	**25.4**	6.7	Phenol	**47.8**	5.6
Naphthalene	**26.2**	13	2,4-Dimethylphenol	**50.1**	5.7
2,4,5-Trichlorophenol	**26.8**	15	2,4-Dichlorophenol	**55.6**	4.6
Hexachlorobenzene	**26.9**	2.9	4-Chloroaniline	**55.7**	12
Nitrobenzene	**28.2**	13	2,3,4,5-Tetrachloronitrobenzene	**55.9**	6.7
3,4-Dichloronitrobenzene	**34.9**	7.7	Benzyl alcohol	**55.9**	7.2
2,4-Dichloronitrobenzene	**35.2**	15	**Isophorone**	**56.1**	4.2
2-Nitrophenol	**36.0**	7.6	**alpha-BHC**	**58.2**	7.3

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Tentative Conclusions – Based on Performance Data Presented in Methods

- Volatiles/Water: No general need for isotope dilution for currently listed analytes. Exception: – 1,4-dioxane if using heated purge instead of SPE.
- Volatile/Solids: A need for isotope dilution is apparent for 6 toxic and one carcinogenic analyte. (7)
- Semivolatiles/Water: A need is apparent for isotope dilution techniques for some toxic and carcinogenic analytes (3-5).
- Semivolatiles/Solids: A need is apparent for isotope dilution techniques for some toxic and carcinogenic analytes. (17)

Perfluorinated Alkylated Compounds

- Current Methods Available
  - EPA 537 (UPLC-Tandem MS) – Drinking Water
  - ASTM 7968 – (Solid samples – UPLC-Tandem MS)
  - ASTM 7979 – (Aqueous samples – UPLC-Tandem MS)
- None of these methods are written as isotope dilution methods. For drinking water 537 is generally performed as written.
- However, for other matrices, because the laboratories and data users know from experience that adsorption/solvation issues are always a threat – isotope dilution is the current industry standard.
- State(s) and QSM 5.1 Appendix B stipulates isotope dilution

Perfluorinated Alkylated Compounds

- Summary for PFAS
  - No current regulatory or institutional method for more complex matrices. (Draft Biosolids Method does exist. Includes isotope dilution.)
  - Laboratories are practicing isotope dilution but each one is different.
  - The need for standardization is clear.

Other popular applications

- Polychlorinated Naphthalene Congeners
- Melamine
- Cyanuric Acid
- Pyrethroid Pesticides

Summary

- Value of isotope dilution for VOAs in water samples is negligible, except for purged1,4-dioxane
- Existing isotope dilution method for VOAs (1624) should be used more for solid samples. A list of analytes requiring ID should be developed based on more data.
- Existing isotope dilution method for SVOCs (1625) should be used more for both waters and solids. A list of analytes requiring ID should be developed based on more data.
- A standardized method is needed for PFASs using isotope dilution.
- Consider isotope dilution anywhere that you use MS, and have unpredictable recoveries.

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