

Overview of the Analysis of Perfluorinated Acids

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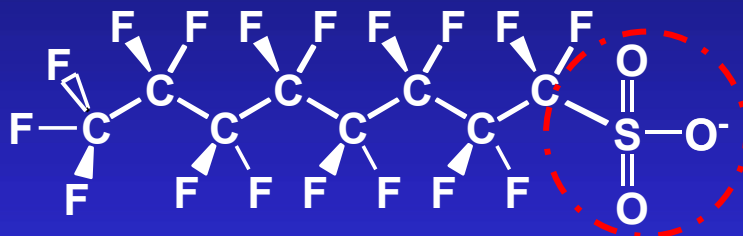
Main Points

- Perfluorinated Acids
- Historical Perfluorinated Acid Analytical Methods: Prior to 1996
 - ◆ Total Organic Fluorine
 - ◆ Derivatization-GC/MS
- Electrospray LC/MS-MS Methods
 - ◆ Analytical Challenges
 - ◆ EPA Method 537
 - ◆ Aqueous/Soil-Sediment-Sludge/Biological Extraction Methods
- Quality Control
 - ◆ Performanced-Based Method Criteria - Data Quality

Two Primary Classes of Perfluorinated Acids

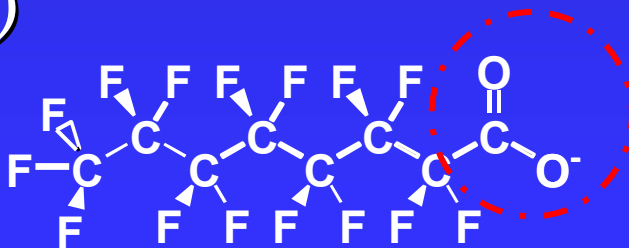
■ Perfluorinated Sulfonates: PFAS

- ◆ For example: *perfluorooctane sulfonate (PFOS)*



■ Perfluorinated Carboxylic Acids: PFCA's

- ◆ For example: *perfluorooctanoate (PFOA, C8 acid)*



Perfluorinated Acids – Target Analytes

Perfluoroalkyl sulfonates:

PFBS $\text{C}_4\text{F}_9\text{SO}_3^-$

PFHS $\text{C}_6\text{F}_{13}\text{SO}_3^-$

PFOS $\text{C}_8\text{F}_{17}\text{SO}_3^-$

PFDS $\text{C}_{10}\text{F}_{21}\text{SO}_3^-$

Perfluoroalkyl carboxylic acids:

PFBA $\text{C}_3\text{F}_7\text{COOH}$

PFPeA $\text{C}_4\text{F}_9\text{COOH}$

PFHxA $\text{C}_5\text{F}_{11}\text{COOH}$

PFHpA $\text{C}_6\text{F}_{13}\text{COOH}$

PFOA $\text{C}_7\text{F}_{15}\text{COOH}$

PFNA $\text{C}_8\text{F}_{17}\text{COOH}$

PFDA $\text{C}_9\text{F}_{19}\text{COOH}$

PFUnA $\text{C}_{10}\text{F}_{21}\text{COOH}$

PFDoA $\text{C}_{11}\text{F}_{23}\text{COOH}$

PFTrA $\text{C}_{12}\text{F}_{25}\text{COOH}$

PFTA $\text{C}_{13}\text{F}_{27}\text{COOH}$

Precursors

Fluorotelomer alcohols:

4:2 FTOH: $\text{C}_4\text{F}_9\text{CH}_2\text{CH}_2\text{OH}$

6:2 FTOH: $\text{C}_6\text{F}_{13}\text{CH}_2\text{CH}_2\text{OH}$

8:2 FTOH: $\text{C}_8\text{F}_{17}\text{CH}_2\text{CH}_2\text{OH}$

10:2 FTOH: $\text{C}_{10}\text{F}_{21}\text{CH}_2\text{CH}_2\text{OH}$

Sulfonamides :

FOSA:

$\text{C}_8\text{F}_{17}\text{SO}_2\text{NH}_2$

EtFOSEOH:

$\text{C}_8\text{F}_{17}\text{SO}_2\text{N}(\text{CH}_2\text{CH}_3)(\text{CH}_2\text{CH}_2\text{OH})$

MeFOSEOH:

$\text{C}_8\text{F}_{17}\text{SO}_2\text{N}(\text{CH}_2\text{CH}_3)(\text{CH}_2\text{CH}_2\text{OH})$

PFCs Analytical Challenges

PFOA and PFOS have unique chemical and physical properties which adversely affect analysis, including:

- No chromophores: prevents using conventional methodology such as UV-Vis spectroscopy or LC/UV
- Extremely low volatility: eliminating the possibility of using conventional methodology such as GC/MS, GC/ECD for analysis
- Not easily derivatized to volatile form for GC or GC/MS*:
 - ◆ PFOA difficult to derivatize for quantitative analysis
 - ◆ No quantitative derivatization available for PFOS
- Strong anions: PFOA and PFOS form strong anions which may adhere to various container surfaces, suspended particulates, and biomass, causing them to be unavailable for analysis

PFCs Analytical Challenges

There have not been:

- PFC Reference Test Methods (until 2008)
 - ◆ EPA Method 537
- Standard Reference Materials
 - ◆ e.g., NIST SRM Human Serum (10 ppb PFOS)
- Stable Isotope Internal Standards
 - ◆ [1,2,3,4 -¹³C₄]PFOS

Historical Perfluorinated Acid Analytical Methods: Prior to 1996

- ◆ Total Organic Fluorine
- ◆ Derivatization GC/MS

Electrospray LC/MS-MS

(quantitative analysis of semi- & non-volatile compounds, 1996 to present)

■ Advantages:

- ◆ First reliable quantitative technique and instrumentation for PFCs
- ◆ Measures individual PFCs
- ◆ Highly sensitive
 - ◆ PFC Practical Limit of Quantification: ppm → ppb → ppt



Electrospray LC/MS-MS Methods:

- ◆ Analytical Challenges
- ◆ Matrices: Water/Soil-Sediment-Sludge/Biological
 - ◆ Extraction Methods
 - ◆ Calibration Methods
- ◆ EPA Method 537 – Water SPE, published in 2008

LC/MS-MS Analytical Challenges: PFCs (1996-2005)

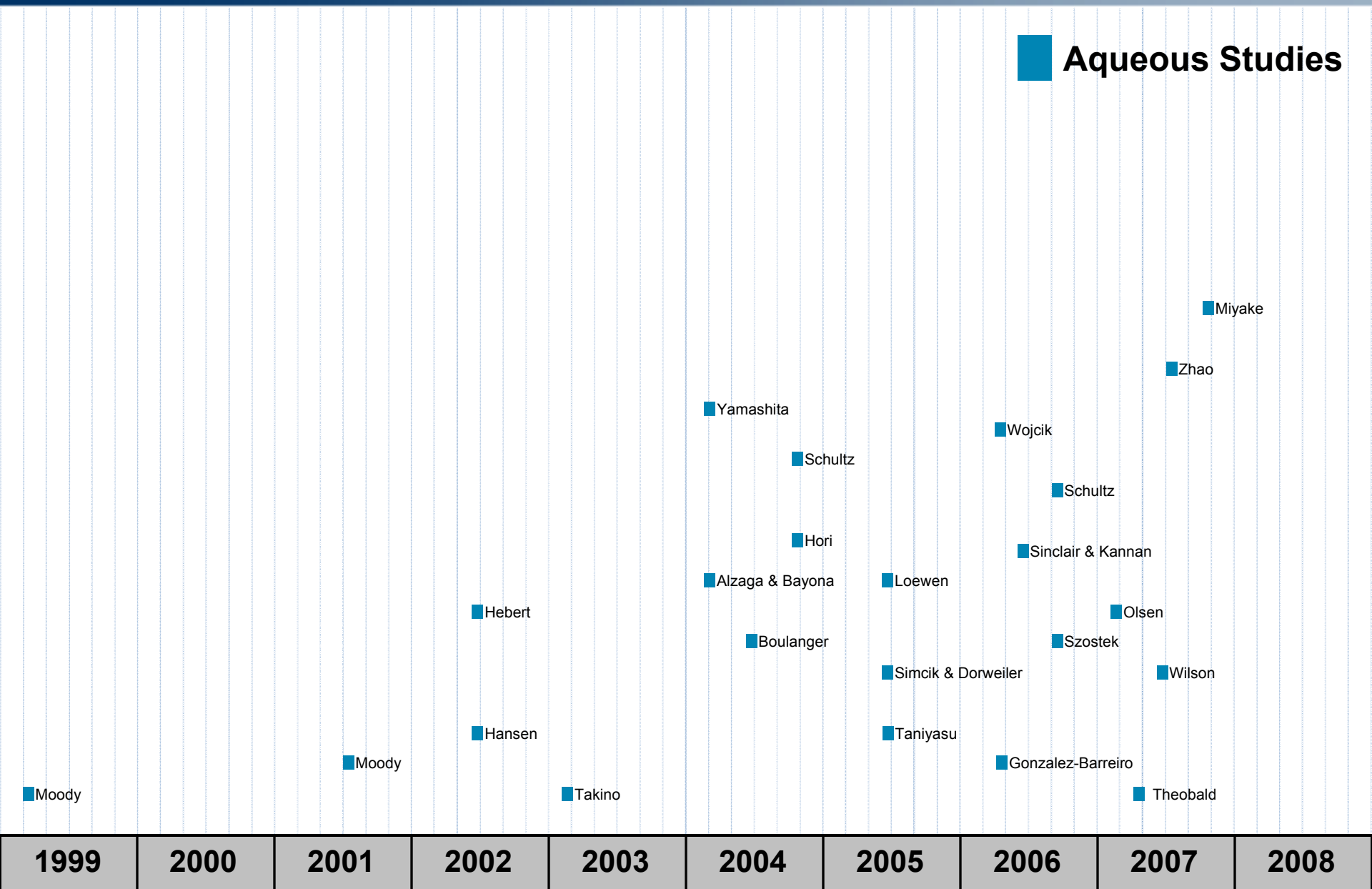
- Data *Accuracy* – Analyte & Matrix Specific Instrument Response Factors
- Multiple Laboratory Methods –
 - ◆ Non-standardized Data Quality Criteria
 - ◆ Non-validated test methods
 - ◆ Method Validation (defined selectivity, accuracy, precision, recovery, calibration, stability, sensitivity, reproducibility)

■ *No: Reference Test Methods, Standard Reference Materials, Stable Isotope Internal Standards**

*2005 Stable Isotope PFOA available

* “Guidance for Industry, Bioanalytical Method Validation”, U.S. Department of Health and Human Services, FDA, May 2001.

Analytical PFC Publications in the Open Scientific Literature



Analytical PFC Publications in the Open Scientific Literature



Wildlife Studies



Aqueous Studies



1999

2000

2001

2002

2003

2004

2005

2006

2007

2008

Analytical PFC Publications in the Open Scientific Literature*



Human Studies



Wildlife Studies



Aqueous Studies

Most data not based on validated methods.

Data accuracy?

Data not comparable?

2008 – First EPA reference method (LCMS) for PFCs in Water

2007 – Commercial Stable Isotope IS's

2001 – FDA Guidance
Bioanalytical Method Validation
(LC/MS-MS)

Method of Std Addition



Hansen

Moody

Hebert

Hansen

Moody

Takino

Tomy

Holm

Berger & Haukas

Kuklenyik

Taniyasu

So

Gonzalez-Barreiro

Ferdui

Theobald

Wilson

Olsen

Olsen

Henderson

Sinclair & Kannan

Verreault

Flaherty

DeSilva & Mabury

Schultz

Yamashita

Karman

Taniyasu

Inoue

Maestri

Ehresman

Olsen

Verreault

Karman

Olsen

Miyake

Miyake

Zhao

Powley

1999

2000

2001

2002

2003

2004

2005

2006

2007

2008

LC/MS-MS Analytical Test Methods

■ Primary Extraction (Sample Preparation) Techniques *

- ◆ Ion Pairing Extraction
 - ◆ liquid/liquid extraction with ion pairing reagent
- ◆ Solid Phase Extraction
 - ◆ C18 cartridge extraction with solvent elution
- ◆ Protein Precipitation Extraction (biota)
 - ◆ Solvent precipitation of biota homogenate
- ◆ Direct Injection (aqueous)

*Validated Published Methods – Standard Addition Calibration**

*a.) Environ Health Perspect, Vol 111, p1892-1901, 2003 b.) ES&T, 36, p 1681-1685, 2002 c.) J. Chrom. B, Vol 819, p 329-338, 2005

* “Guidance for Industry, Bioanalytical Method Validation”, U.S. Department of Health and Human Services, FDA, May 2001.

LC/MS/MS Analytical Test Methods

■ Primary Calibration (Quantitation) Techniques*

2001 FDA
Guidance
(Biota)

- ◆ **Method of Standard Addition (Matrix Matched Calibration)**
 - ◆ e.g., control human blood calibration matrix with known quantities of PFOA to quantify PFOA in human blood samples

EPA
Method
537
(Water)

- ◆ **External (Solvent) Stable Isotope Internal Standard**
 - ◆ e.g., methanol calibration solvent with known quantities of PFOA and [1,2,3,4- $^{13}\text{C}_4$]PFOA Internal Standard to quantify PFOA in samples ([1,2,3,4- $^{13}\text{C}_4$]PFOA added to samples)

Analytical
Bias ?

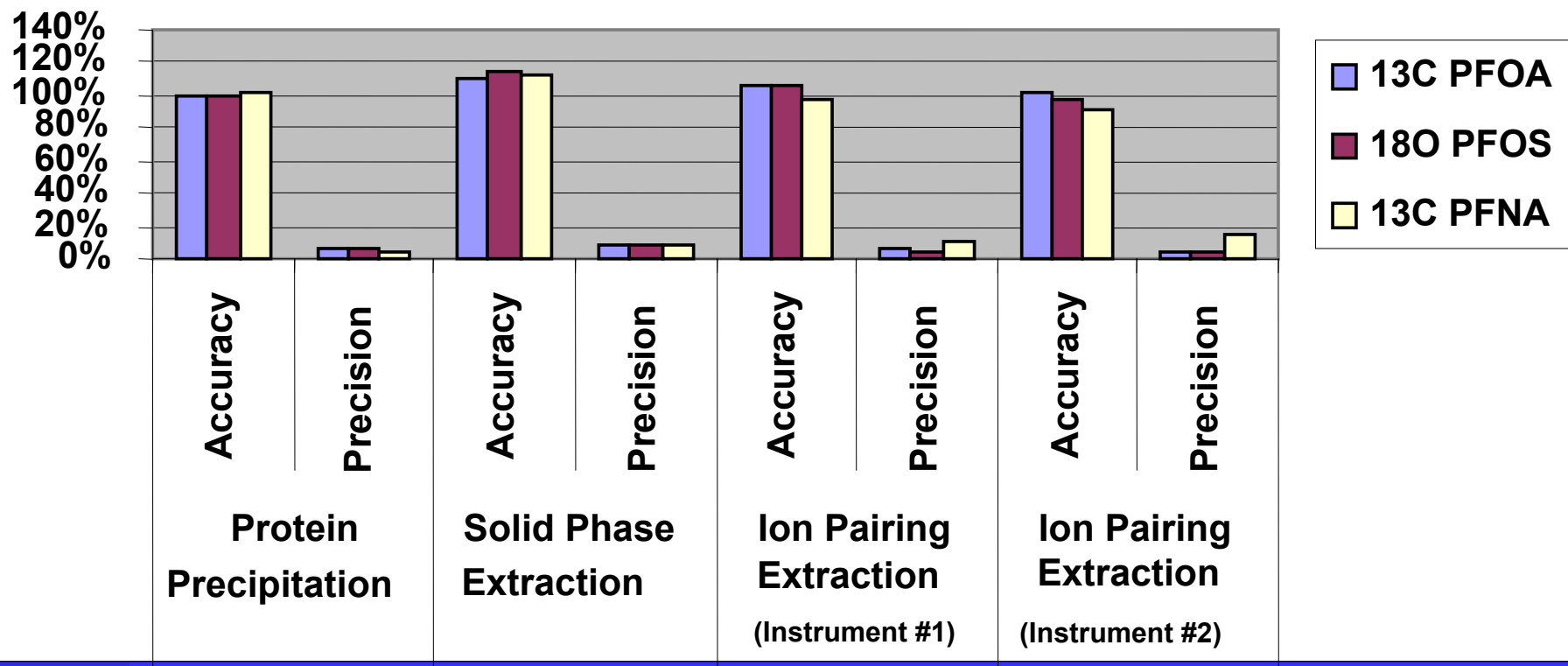
- ◆ **External (Solvent) Surrogate Internal Standard**
- ◆ **External (Solvent)**

* "Guidance for Industry, Bioanalytical Method Validation", U.S. Department of Health and Human Services, FDA, May 2001.

*2008, EPA Method 537: DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

LC/MS-MS Method of Standard Addition*

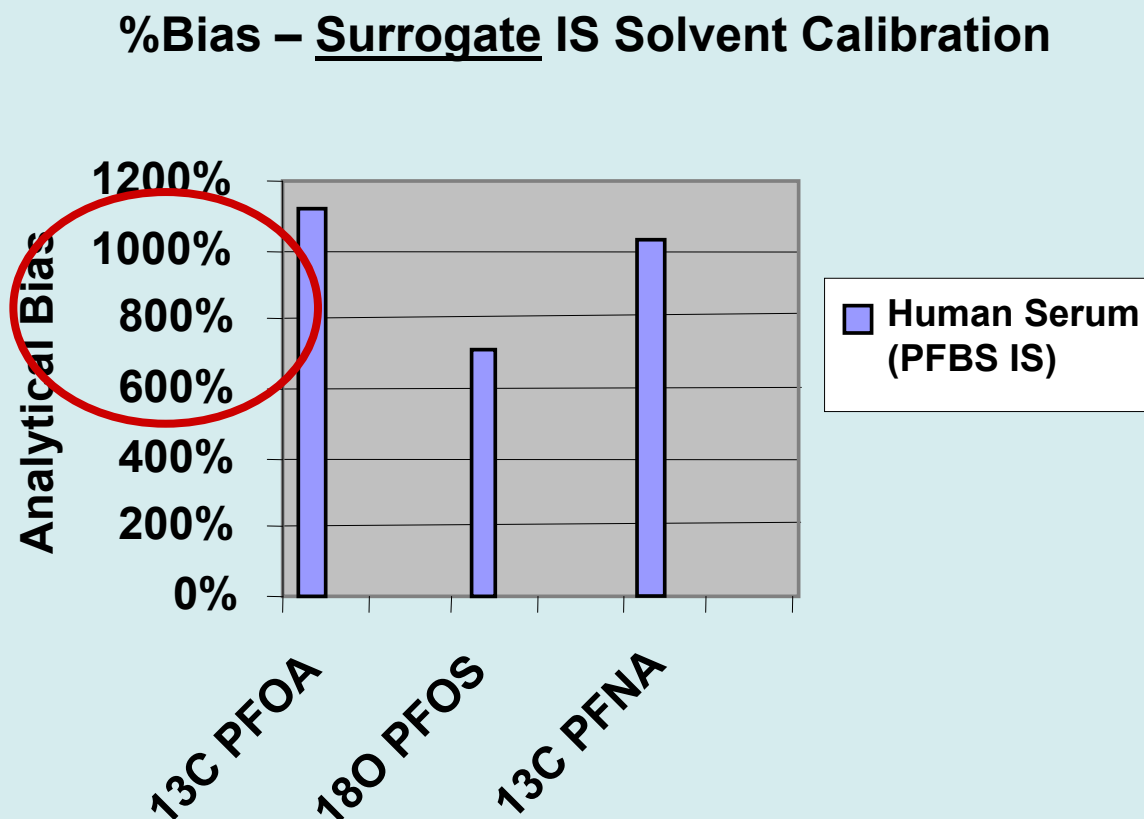
Validated Method Accuracy & Precision – Extraction & Calibration (Human Sera)



**Validated Calibration For All Three Extraction Methods – External Matrix Matched*

Ion Pairing Extraction – Human Serum

(*Surrogate Internal Standard Solvent Calibration*)



-Many publications present data on unvalidated methods with undefined accuracy and precision

-Need use of *either*:

- EPA ref method (available for certain analytes in water)
- Validated method (defined accuracy, precision, recovery, calibration, selectivity, stability, sensitivity, reproducibility)

-Need either the method of standard addition (matrix-matched calibration) or a stable isotope internal standard calibration

-Must consider these data reliability issues before using any study (tox, human, environmental)

LC/MS-MS Data Accuracy – Quality Control

- **2005 FLUOROS Conference (Mabury, University of Toronto)**
 - Data Accuracy & Comparability A Major Technical Issue
 - No Reference Test Methods, Standard Reference Materials, IS's

- **2005 PERFORCE - 1st Worldwide Inter-laboratory Study On Perfluorinated Compounds In Human And Biological Matrices (38 Labs, 13 countries)***
 - Data Accuracy A Major Technical Issue
 - Large % Labs Using Solvent Calibration Without Stable Isotope Internal Standards *

* Van Leeuwen et al, Environ Sci Technol, Vol 40, p7854-7860, 2006

Challenges in analysis of PFOA and PFOS in water

- **2005 PERFORCE 1st Worldwide Inter-laboratory Study On Perfluorinated Compounds In Environmental and Human Samples (38 Labs, 13 countries)**
 - Most Laboratories Used Solvent Based Calibration (half used ¹³C-labeled PFCA IS for PFOS water results)

| <u>Water Results</u> | PFOA | PFOS |
|---|------|----------|
| <i>Spiked concentration (ng/L)</i> | 19.4 | 19.5 |
| <u><i>Analytical results (ng/L)</i></u> | | |
| Minimum concentration | 3.4 | 4.7(6.6) |
| Median concentration | 23 | 25 |
| Maximum concentration | 190 | 112 |
| <u><i>Evaluation of results (16 Labs)</i></u> | | |
| %Satisfactory | 31 | 22 |
| %Questionable | 13 | 6 |
| %Unsatisfactory | 56 | 72 |

Study conclusions

- “The agreement between submitted water results (16 results for PFOS) is relatively poor. Although it may appear to be an easy matrix to extract (mainly using SPE.....”
- “The reported concentration range for PFOS is considerable: from 6.6 to 112 ng/L.”

LC/MS-MS Data Accuracy – Quality Control

- **Year 2008: PERFORCE** – 3rd Worldwide Inter-laboratory Study On Perfluorinated Compounds In Water and Fish (21 Labs)
 - Method of Standard Addition**
 - Stable Isotope Internal Standard – Solvent Calibration
 - *Study Conclusion: “Analytical Methods For PFCs In Water And Fish Have Improved Considerably”*

- **Year 2008: EPA Method 537**
 - DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)
 - ◆ 250 mL Sample Volume, including 5.0 g/L Trizma (pH 7 buffer, free chlorine removal)
 - ◆ *Solvent/Stable Isotope IS Calibration*
 - ◆ SPE (SDVB Cartridge) including sample bottle rinse into SPE
 - ◆ MeOH Elution (two 4-mL aliquots used to rinse bottles)
 - ◆ Evaporate eluate to dryness (heated water bath, N₂ evaporation)
 - ◆ Add ISTD, adjust to 1-mL volume with 96:4% (v/v MeOH : H₂O)
 - ◆ 13C-PFOA
 - ◆ 13C-PFOS
 - ◆ D3-NMeFOSAA
 - ◆ Surrogate addition pre-extraction
 - ◆ 13C-PFHxA
 - ◆ 13C-PFDA
 - ◆ D5-NEtFOSAA
 - ◆ 14-Day Extraction Holding Time

EPA Method 537: Target Analytes Included/Target Analytes Evaluated*/Target Analytes Not Evaluated**

Perfluoroalkyl sulfonates :

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PFOS $\text{C}_8\text{F}_{17}\text{SO}_3^-$

****PFDS** $\text{C}_{10}\text{F}_{21}\text{SO}_3^-$

Additional Analytes

***NEtFOSA**

*** PFOSAA**

*** PFOSfn**

*** PFOF**

NEtFOSAA

NMeFOSAA

Perfluoroalkyl carboxylic acids:

***PFBA** $\text{C}_3\text{F}_7\text{COOH}$

***PFPeA** $\text{C}_4\text{F}_9\text{COOH}$

PFHxA $\text{C}_5\text{F}_{11}\text{COOH}$

PFHpA $\text{C}_6\text{F}_{13}\text{COOH}$

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***Sulfonamides :**

***FOSA:**

$\text{C}_8\text{F}_{17}\text{SO}_2\text{NH}_2$

***EtFOSEOH:**

$\text{C}_8\text{F}_{17}\text{SO}_2\text{N}(\text{CH}_2\text{CH}_3)(\text{CH}_2\text{CH}_2\text{OH})$

***MeFOSEOH:**

$\text{C}_8\text{F}_{17}\text{SO}_2\text{N}(\text{CH}_2\text{CH}_3)(\text{CH}_2\text{CH}_2\text{OH})$

LC/MS-MS Method (Stable Isotope IS Calibration)

EPA Method 537 Recommended Internal Standards (ISTD) and Surrogates*

| <i>Compound</i> | <i>Analyte Description</i> | <i>Compound</i> | <i>Analyte Description</i> |
|---|----------------------------|---|----------------------------|
| [1,2- ¹³ C ₄]PFOA | ISTD | [1,2- ¹³ C ₄]PFHxA | Surrogate |
| [1,2,3,4 - ¹³ C ₄]PFOS | ISTD | [1,2- ¹³ C ₄]PFDA | Surrogate |
| d ₃ -NMeFOSAA | ISTD | d ₅ -NEtFOSAA | Surrogate |

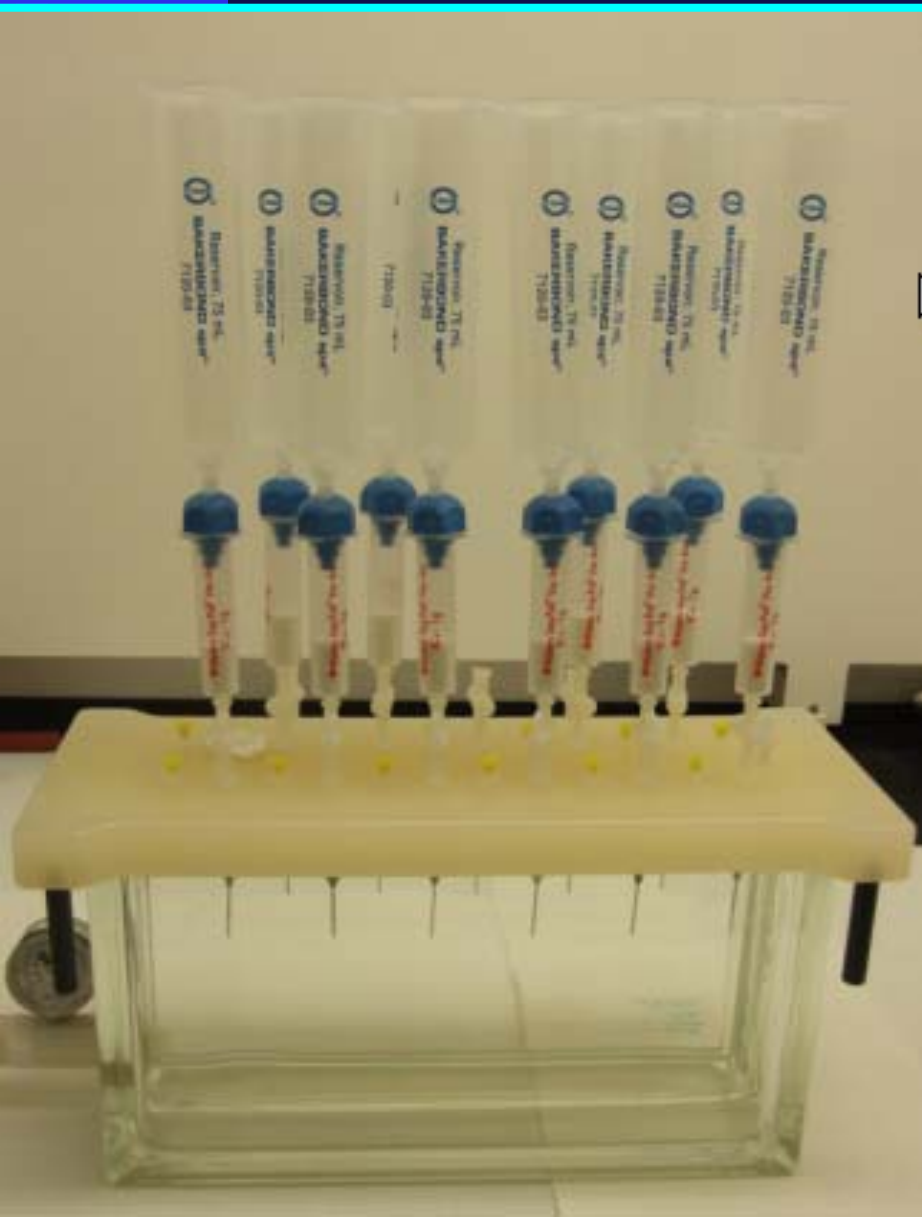
Internal Standards Options

| <u><i>Compound</i></u> | <u><i>Analyte Description</i></u> |
|--|-----------------------------------|
| [1,2,3,4 - ¹³ C ₄]PFBA | IS for PFBA |
| [1,2- ¹³ C ₂]PFHxA | IS for PFPeA, PFHxA |
| [1,2,3,4- ¹³ C ₄]PFOA | IS for PFHpA and PFOA |
| [1,2,3,4,5- ¹³ C ₅]PFNA | IS for PFNA |
| [1,2- ¹³ C ₂]PFDA | IS for PFDA |
| [1,2- ¹³ C ₂]PFUnA | IS for PFUnA |
| [1,2- ¹³ C ₂]PFDaA | IS for PFDaA |
| [¹⁸ O ₂]-PFBS | IS for PFBS |
| [¹⁸ O ₂]-PFHS | IS for PFHS |
| [1,2,3,4 - ¹³ C ₄]PFOS | IS for PFOS |

*Alternate isotopically labeled ISTD and Surrogates with similar functional groups may be used provided reasons are documented and also span solubility range of method and meet QC requirements.

Solid Phase Extraction/Direct Injection - Water

LC/MS-MS Method (Stable Isotope IS Calibration)



***C18 solid-phase extraction (SPE) cartridge (1 g, 6 cc)**

***40 mL of water sample is loaded onto the column using a vacuum manifold.**

***Loaded SPE cartridges eluted with 5 mL of methanol.**

(8x concentration of sample),

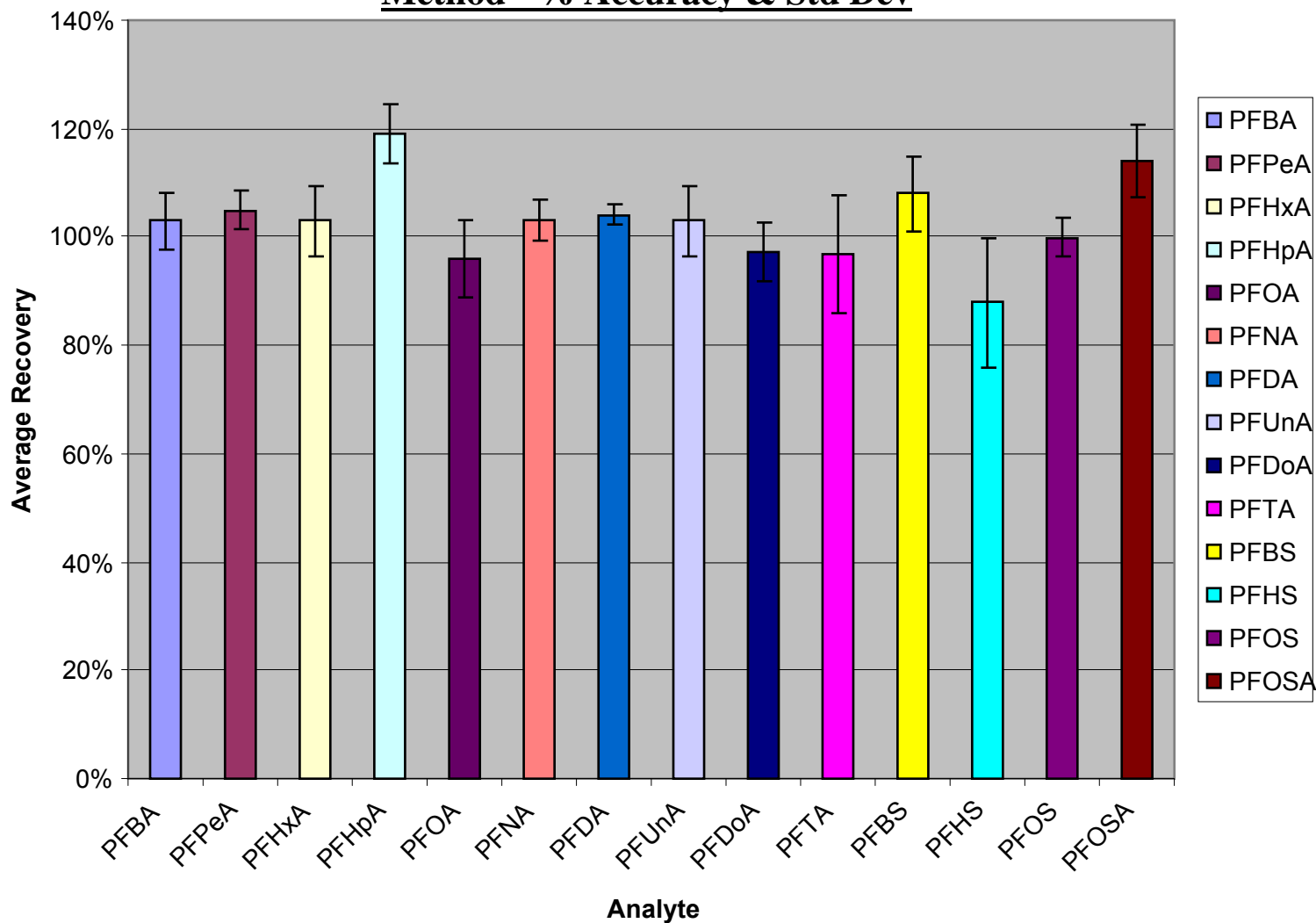
Direct Injection – Water Method Validation Summary

LC/MS-MS Method (Stable Isotope IS Calibration)

(2wk holding time assessment)

Direct Injection Water Analysis - Internal Standard Correction

Method - % Accuracy & Std Dev



0.25 & 2.5 ppb
Water (n=6)

Soil Method — solvent extraction/stable isotope IS calibration

■ Sample Preparation:

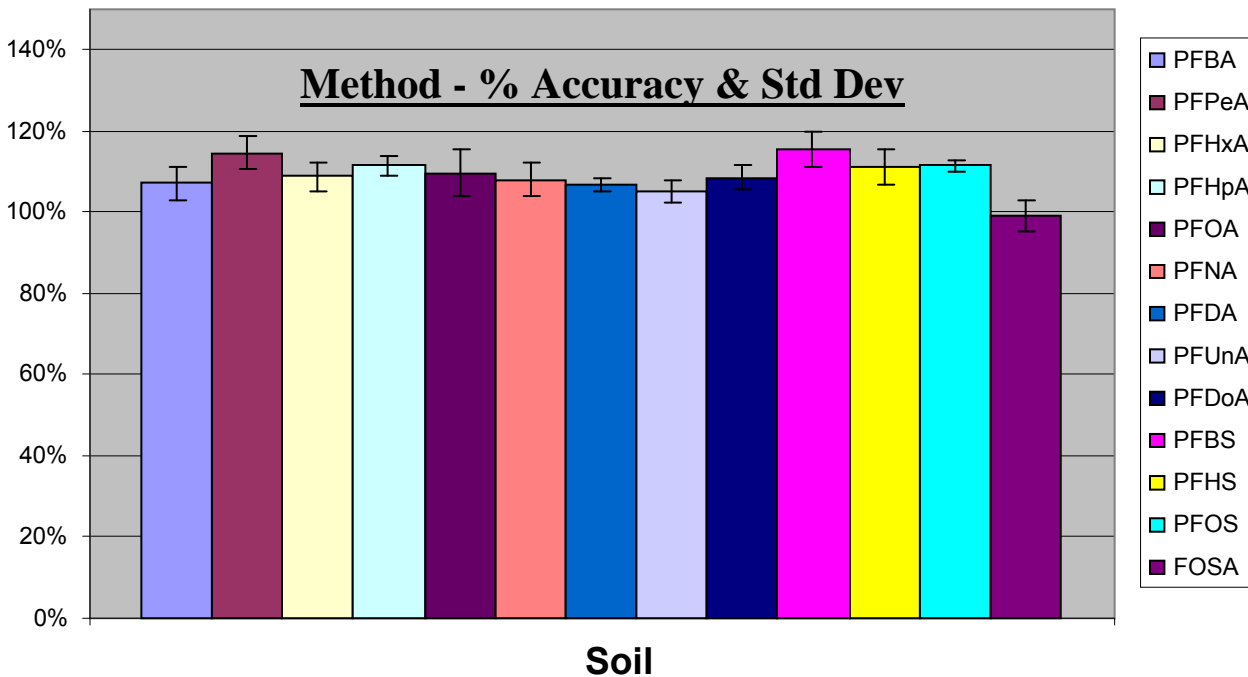
- ◆ Remove 1 cc of sample aliquot to tared 15 mL tube
- ◆ Accurately weigh the aliquot
- ◆ Fortify Lab Matrix Spikes (as necessary)
- ◆ Add Internal Standard Mix
- ◆ Add 7 mL of 4:1(v/v) acetonitrile:water
- ◆ Sonicate for ~ 1 hr
- ◆ Centrifuge
- ◆ Analyze supernatant



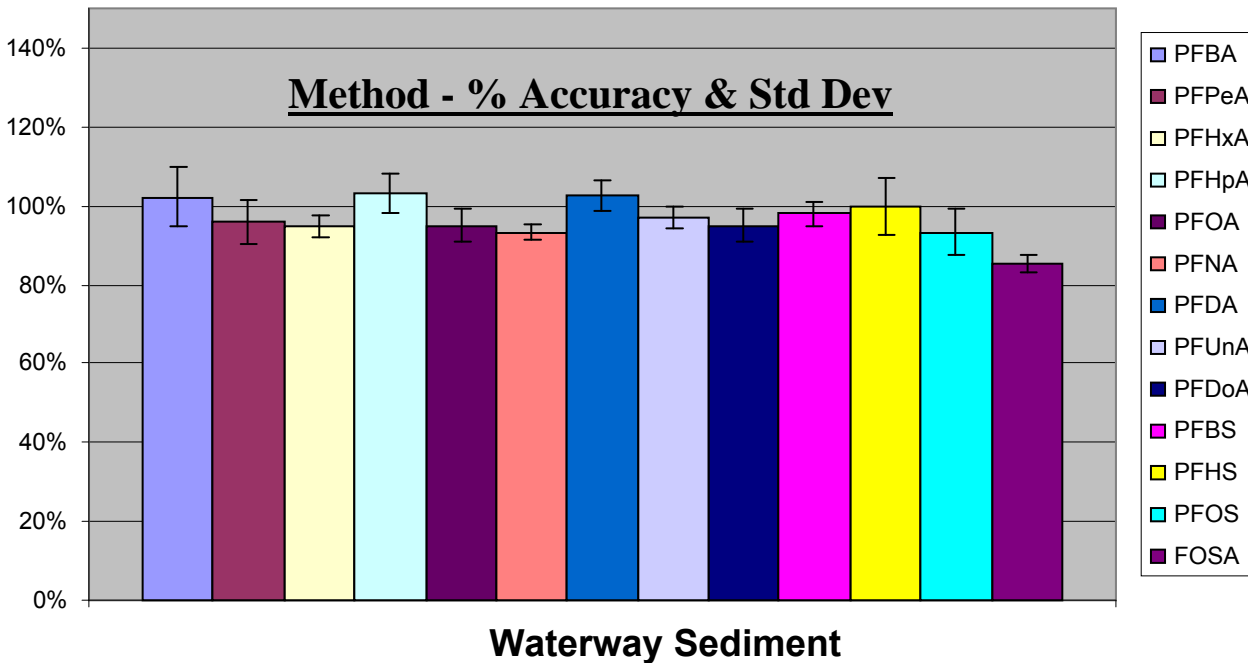
LC/MS-MS Method (Stable Isotope IS)

3 & 30 ppb (n=6)
1g soil

Method - % Accuracy & Std Dev



Method - % Accuracy & Std Dev



3 ppb (n=6)
1g sediment

Blood Tissue Method — protein precipitation/stable isotope IS calibration

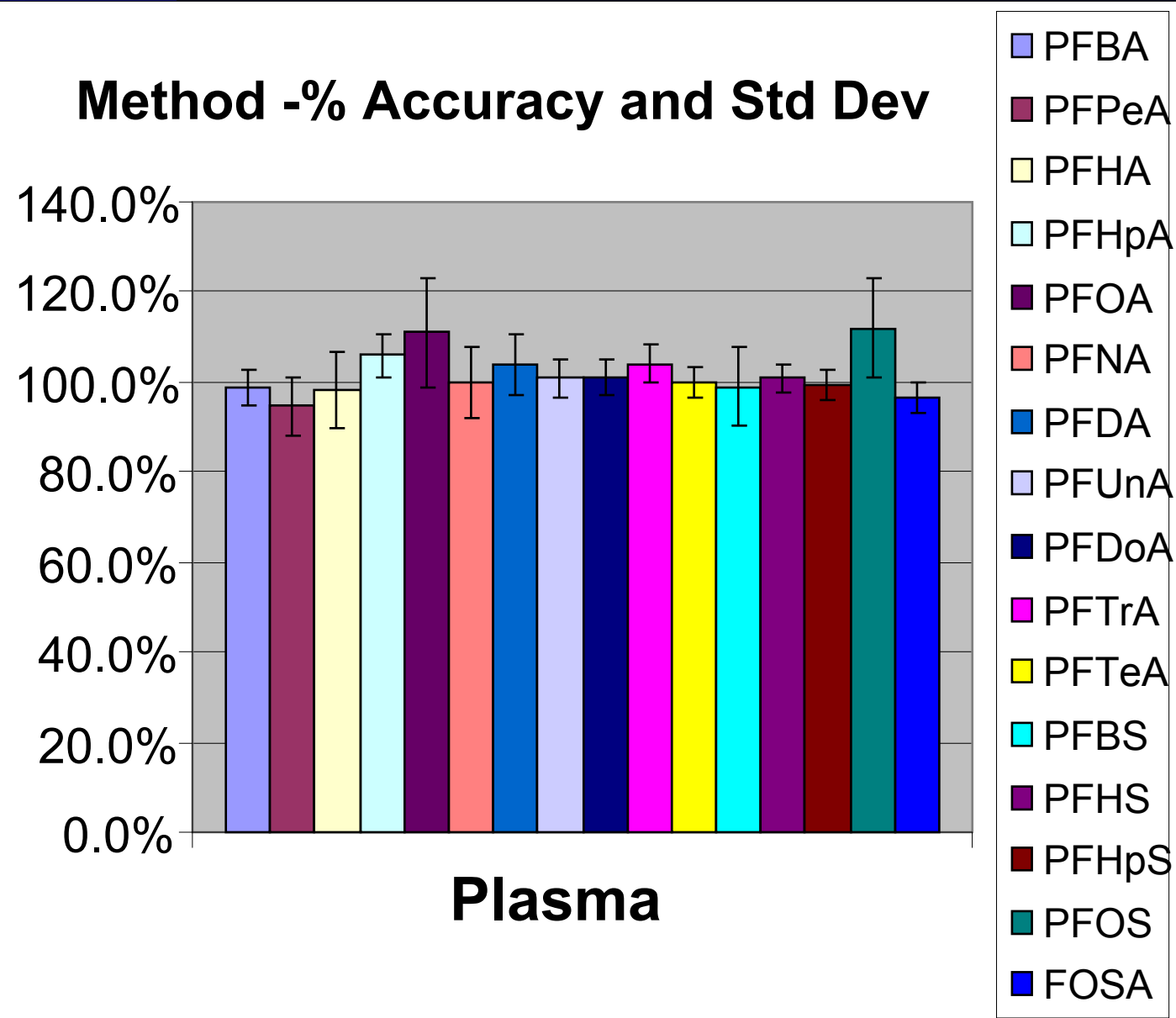
■ Sample Preparation:

- ◆ 200 μL sample in 1.5 mL centrifuge tubes
- ◆ Spike sample aliquots with 25 μL of acetonitrile solution containing surrogates/internal standards as appropriate
- ◆ Mix by aspirating and dispensing 100 μL volumes three times on the MultiPROBE II robotic pipetting station
- ◆ Add 775 μL of acetonitrile
- ◆ Vortex mix samples
- ◆ Centrifuge 20 minutes @ 10,000 rpm
- ◆ Transfer supernate to clean autovial
- ◆ Add 60 μL of aqueous 5% H_3PO_4 to each autovial
- ◆ Analyze using LC/MS/MS



Protein Precipitation – Blood Tissue Method Validation Summary

LC/MS-MS Method (Stable Isotope IS Calibration)



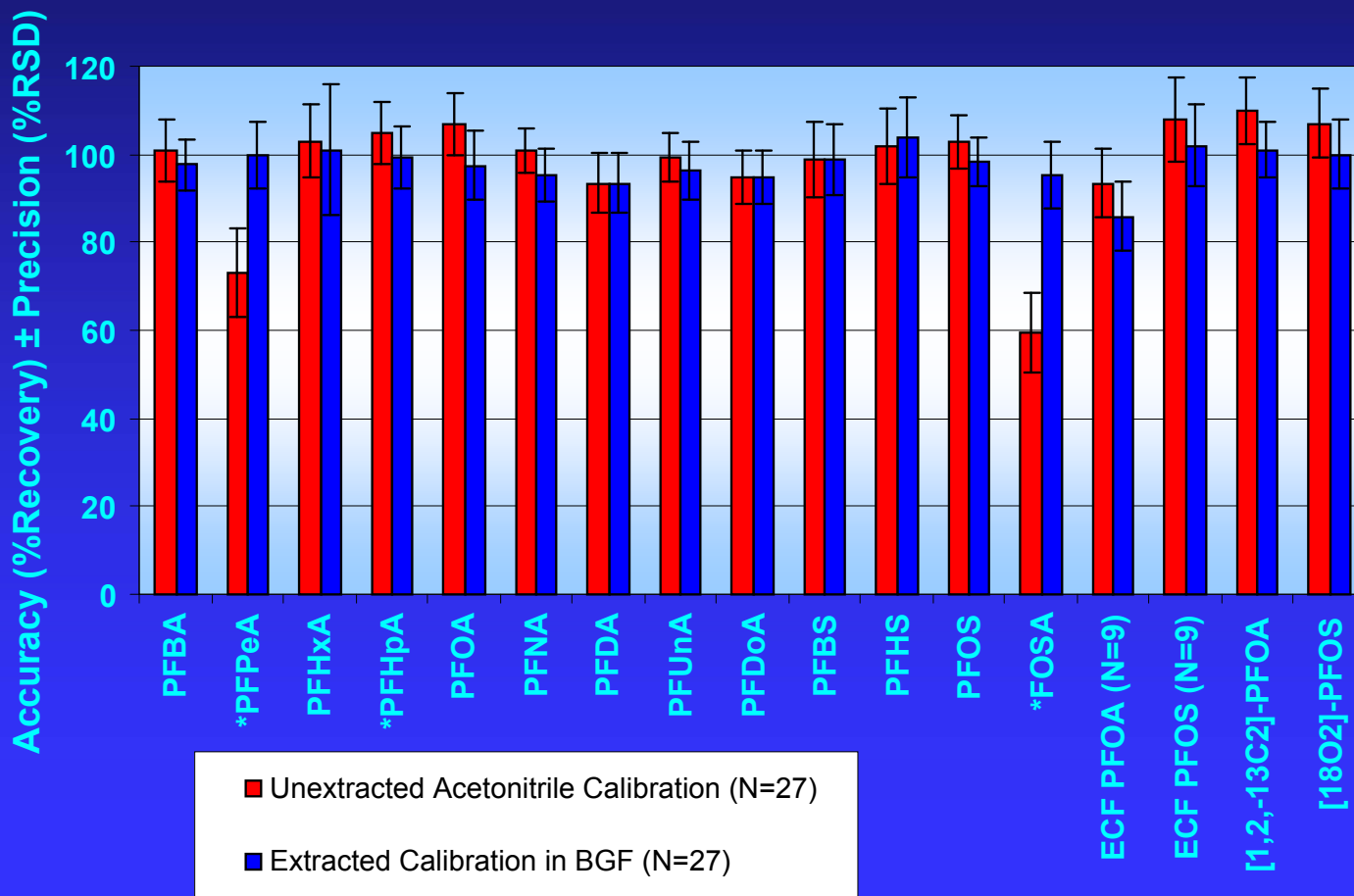
0.2, 1.0 & 18
ppb (n=9)
200 µL Plasma

Fish Method Validation Summary – Protein Precipitation

LC/MS-MS Method (Stable Isotope IS Calibration)

Bluegill Fillet:

Matrix-Matched Extracted IS Calibration vs. Unextracted IS Acetonitrile Calibration



*Surrogate IS used for target analyte



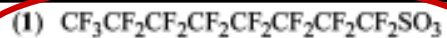
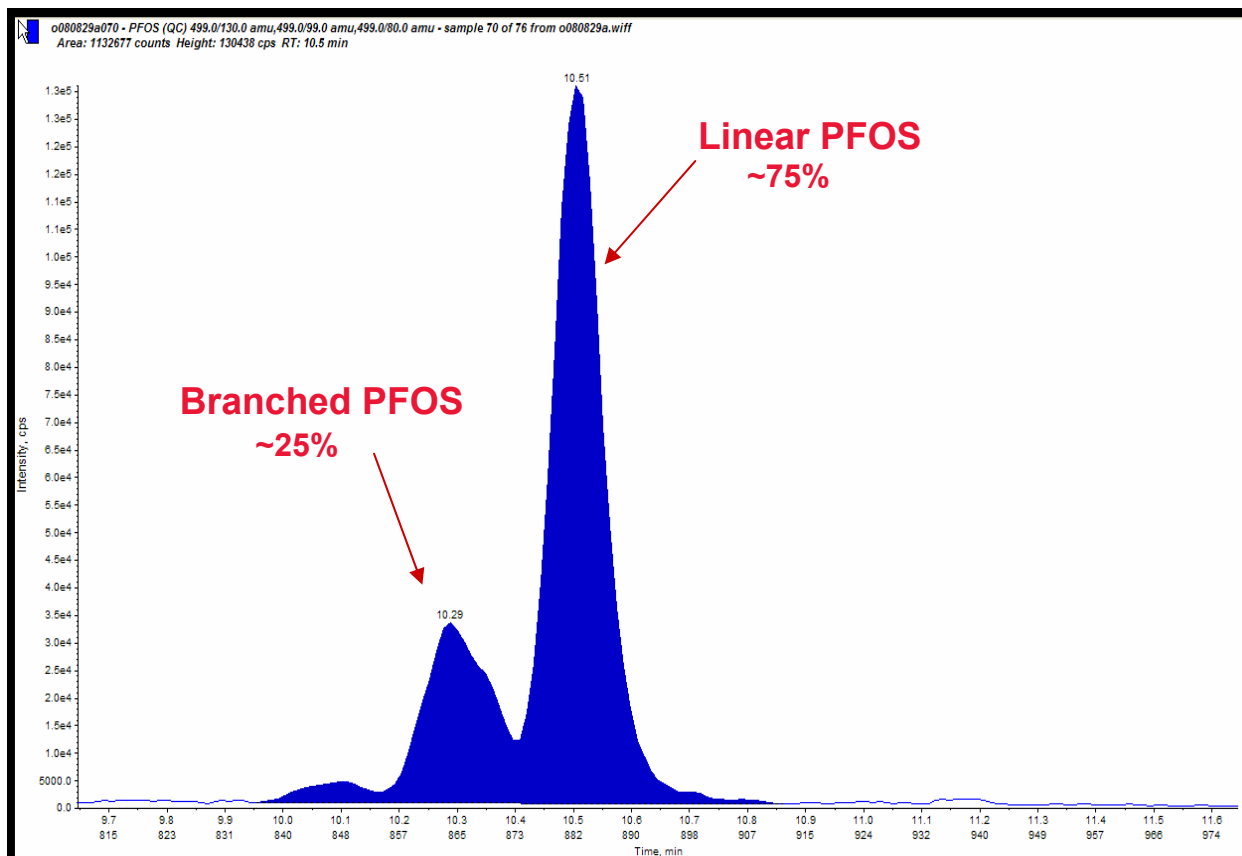
Quality Control Issues

- **No Reference Method = Immense analytical challenge**
 - ◆ (C4-C14 PFCA's, C4, C6, C8 PFSA's, Precursors)
 - ◆ Trace levels (ppm, ppb, ppt)
 - ◆ Many matrices/variations of matrices (water types, soil types, biological tissue types)
- **PFC Analytical Method Validation Criteria – Regulatory Guidance (?)**
- **PFC Performance-based Analytical Method Acceptance Criteria – Regulatory Guidance (?)**
- **Quantitation of Linear and Branched Isomers**

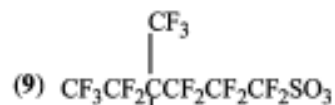
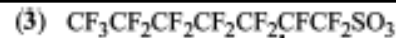
MS/MS relative response factors

| | | | | | | | | | | | |
|----------------------------------|-----|-----|----|-----|-----|-----|-----|-----|-----|-----|----|
| m/z 499 \rightarrow m/z 99 | 100 | 117 | 97 | 49 | 39 | 43 | 78 | 10 | 0 | 0 | 19 |
| m/z 499 \rightarrow m/z 80 | 100 | 0 | 78 | 135 | 241 | 142 | 123 | 113 | 118 | 220 | 90 |
| isomer | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |

PFOS Branched and Linear Isomers: Quantitation

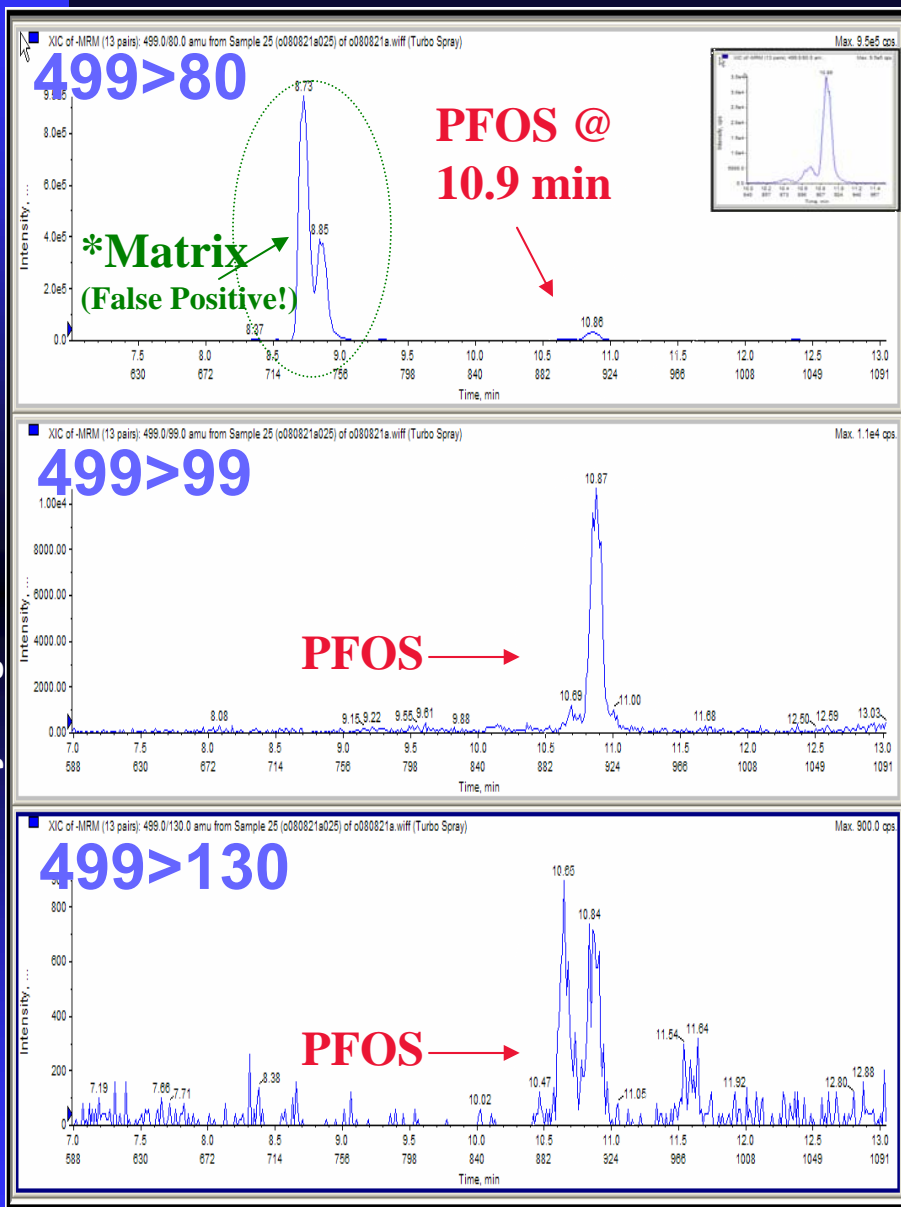


Linear PFOS Isomer

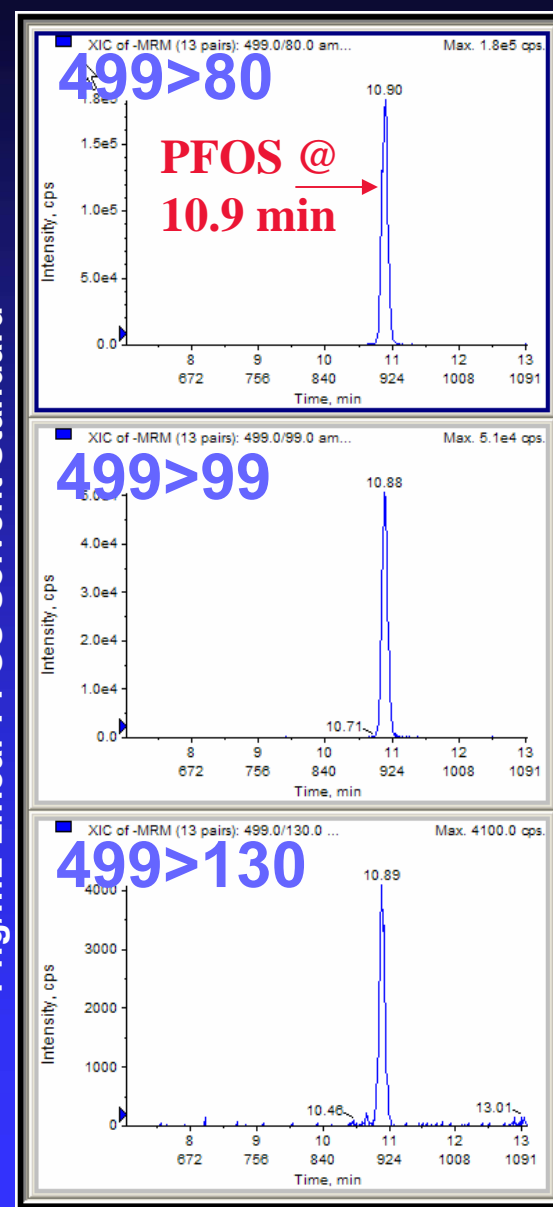


Importance of Multiple Transitions: Matrix vs. PFOS

Whole Body Largemouth Bass Matrix Blank



1 ng/mL Linear PFOS Solvent Standard



Minimum Quality Control Requirements – Performance Based Methods

- Procedures: Sample Collection-Handling-Analysis
- QC's: Field/Lab Matrix Spikes/Data Quality Acceptance Criteria
- Field Duplicates/Lab Duplicates
- Blanks/Blank Criteria
- Practical LOQs/MDLs – Defined Criteria
- Calibration (Method of Standard Addition/Stable Isotope IS)
- *Data Uncertainty (Accuracy & Precision)/Reporting Criteria*
- A Supporting Method Validation

Current Quantitation Issues: PFCs (LC/MS-MS)

- Quantitation Method of Branched Isomers*
 - ◆ Environmental Samples: PFOS, PFOA (branched/linear)
 - ◆ Calibration Standards/IS's: mostly linear standard applied
 - ◆ **EPA Method 537: PFOS standard must be Linear/Branched, m/z 499→m/z 80 required for quantitation**
- Quantitation Calibration**
 - ◆ Method of Standard Addition Calibration
 - ◆ *Stable Isotope IS Calibration*
 - ◆ EPA Method 537 – drinking water
 - ◆ Matrix Applications (waters/soils/sediments/sludge/biota)
 - ◆ Expanded List of Target Analytes
- Data Uncertainty (Accuracy/Precision) Reporting
 - ◆ Standardized QC's and Data Acceptance Criteria (with/without method validation)

* Riddel N., Environ Sci Tech A, **2009**; "Branched Perfluorooctane Sulfonate Isomer Quantification and Characterization in Blood Serum Samples by HPLC/ESI-MS(MS)

** "Guidance for Industry, Bioanalytical Method Validation", U.S. Department of Health and Human Services, FDA, May 2001.

**2008, EPA Method 537: DETERMINATION OF SELECTED PERFLUORINATED ALKYL ACIDS IN DRINKING WATER BY SOLID PHASE EXTRACTION AND LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY (LC/MS/MS)

**2008, PERFORCE Report, "Significant improvements in the analysis of perfluorinated compounds in water and fish, Results from an interlaboratory method evaluation study