Comparison of calibration models for SW-846 Methods 3512 and 8327 for Per- and Polyfluoroalkyl Substances (PFAS) by Liquid Chromatography Tandem Mass Spectrometry (LC/MS/MS)

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Outline

- Background, Motivation
- Post-hoc Analysis of Validation Data
- Data Transformations
  - Remodel by isotope dilution/extracted internal standard
  - Recovery-correct external standard calibration results
- Comparisons
  - Isotope dilution vs. recovery correction across 5 labs
  - Recovery correction vs. external standard
- Outcomes, Conclusions

Background, Motivation

SW-846 Method 3512: Solvent Dilution of Non-Potable Waters

- "Direct inject" sample preparation method
  - Dilute 1:1 with methanol, vortex, filter, add 0.1% acetic acid by volume
  - 5 mL recommended sample volume

SW-846 Method 8327: Per- and Polyfluoroalkyl Substances (PFAS) by Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS)

- Electrospray ionization negative mode
- Validated by external standard calibration

Developed by EPA Region 5 Laboratory, also method developer for ASTM D7979-20 and D8421-22

Background, Motivation

Multi-laboratory validation study design:

Matrices:	• Groundwater, Surface Water, Wastewater
Prepared Concentrations:	• Background (unspiked), 60 ng/L, 200 ng/L (nominal)
Replicates:	• 5 reps per matrix at each concentration – analyzed blind

Validation study outcomes: Met EPA's data quality objectives for precision (≤50% RSD), bias (70-130% recovery) and sensitivity (verified Lower Limits of Quantitation as low as 10 ng/L) across 8 laboratories

- Exception: 6:2 fluorotelomer sulfonate (half of labs had background problems)

Public comments on proposed methods: Most common theme was to include isotope dilution/extracted internal standard calibration in addition to or in lieu of external standard calibration

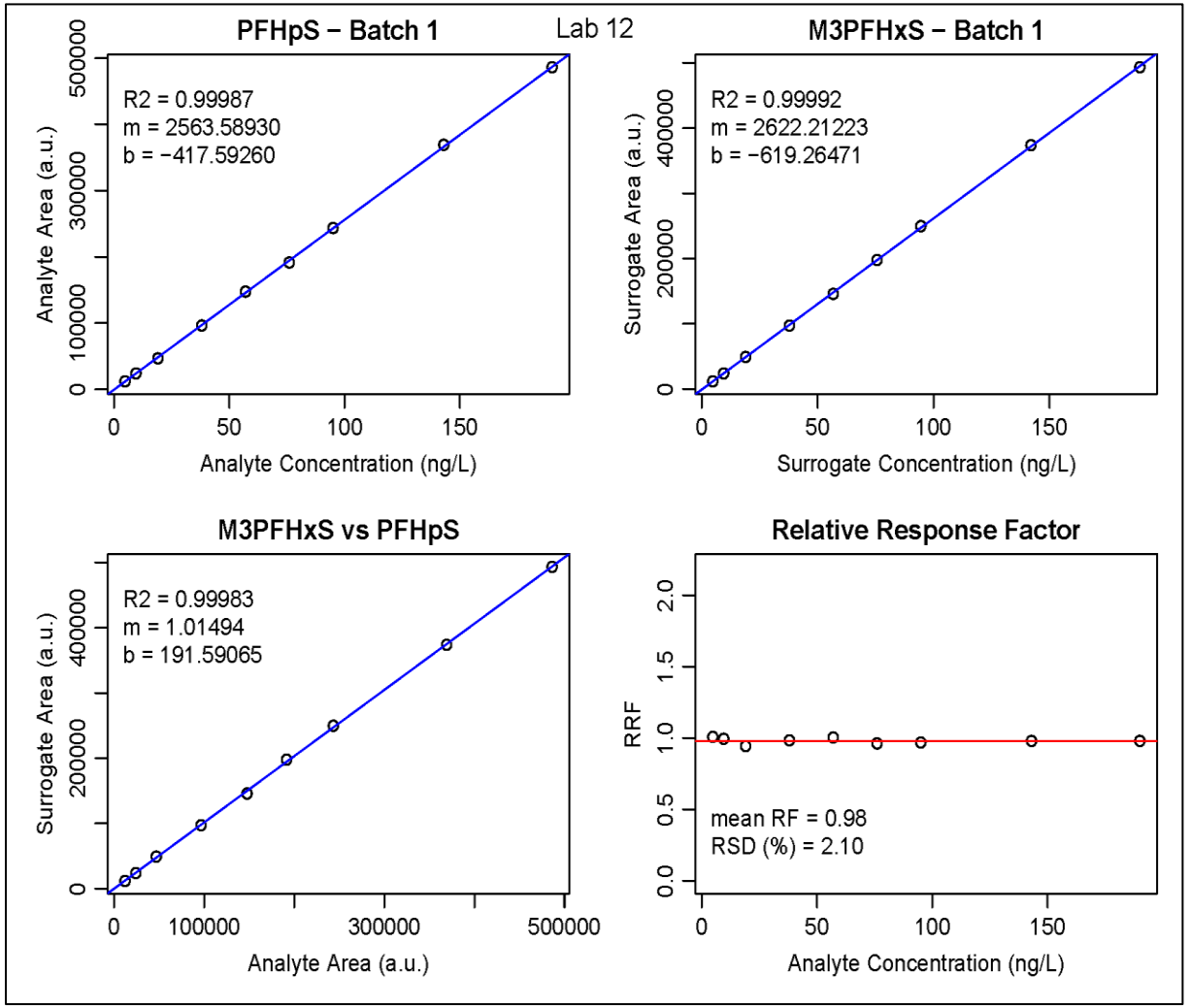
Post-Hoc Analysis of Validation Data

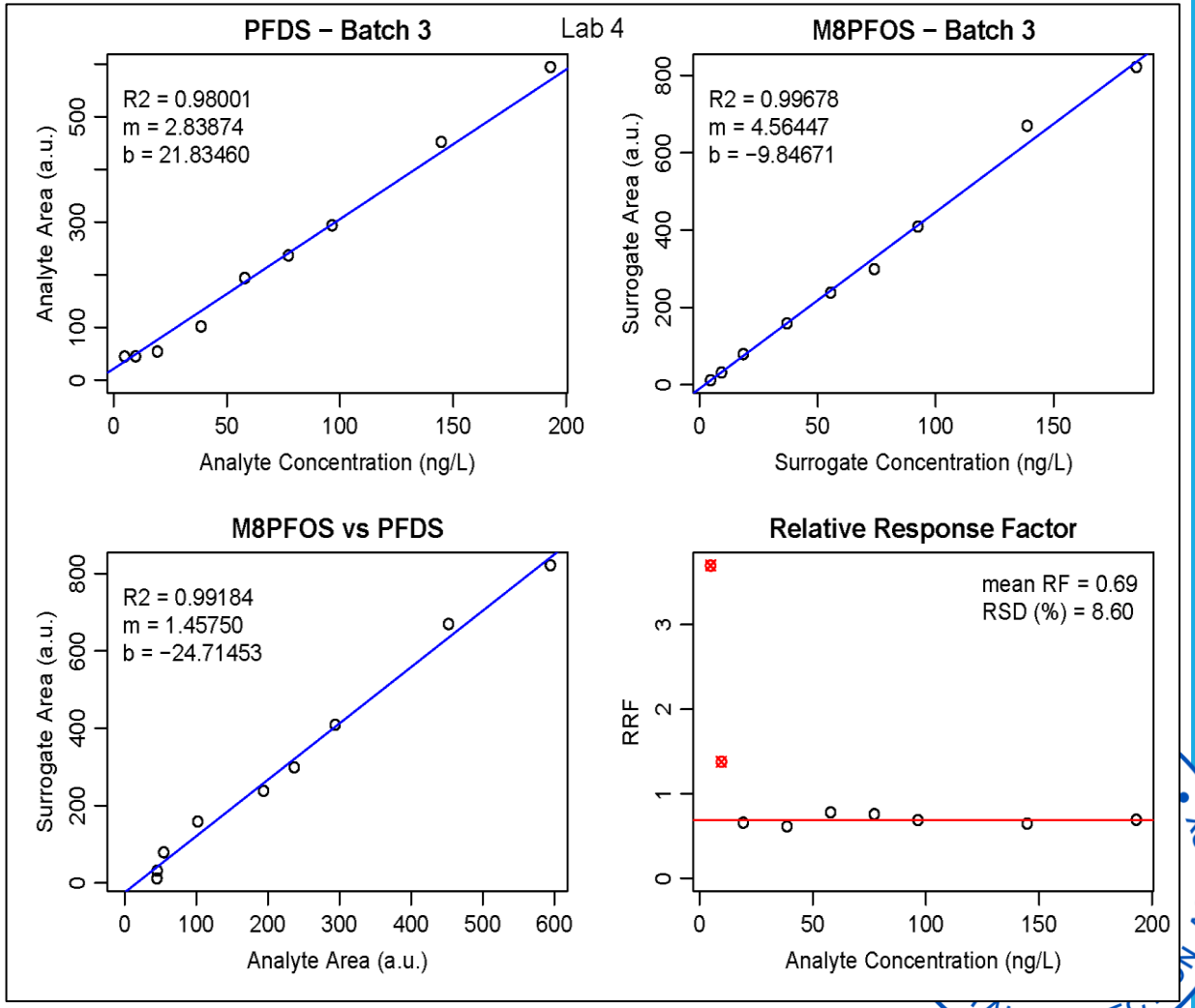
- This work: Evaluate effect of recovery correction on SW-846 methods 3512/8327 multi-laboratory validation data calculated by external standard calibration.
- Two types of comparisons:

1. Isotope dilution/extracted internal standard vs recovery corrected external standard concentrations – 5 labs
2. Recovery corrected external standard vs external standard concentrations – 8 labs

	External standard	Recovery-corrected external standard	Isotope dilution, Extracted IS
Labeled compounds added:	Pre-extraction	Pre-extraction	Pre-extraction
Corrects for bias due to losses during preparation, instrument effects	No – monitoring only	Yes	Yes
Concentrations in calibration standards	Multi-point	Multi-point	Single point

Data Transformations: Remodel by Isotope dilution /extracted internal standard





Data transformations: Recovery-correction of external standard results

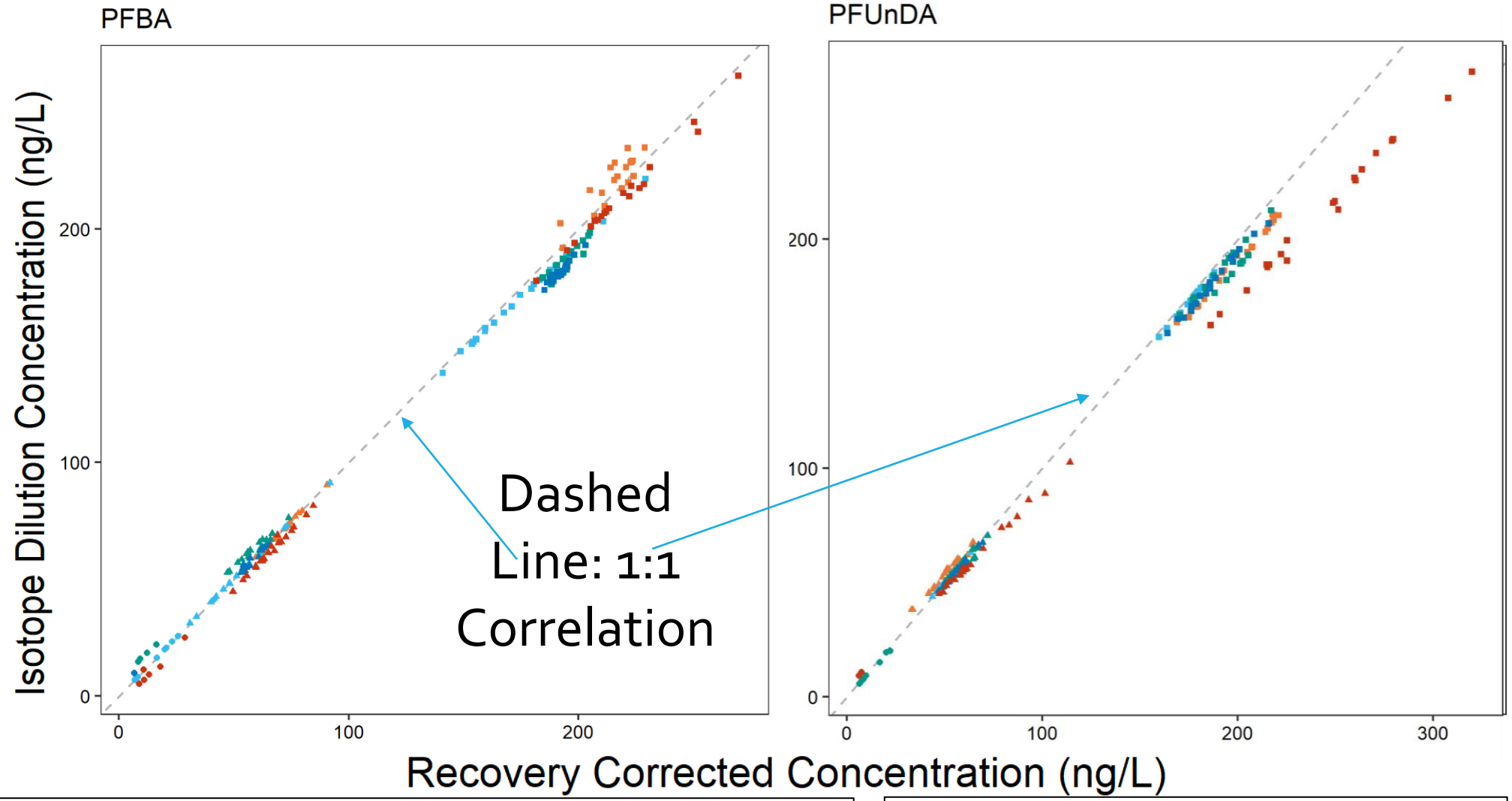
- Divide external target analyte concentration by recovery of labeled analog
  - Example:

    Sample concentration of PFOS: 20 ng/L (external standard)

    M8PFOS surrogate recovery in the same sample: 80% (external standard)

    Recovery-corrected external standard concentration = 20 ng/L / 0.80 = 25 ng/L

Isotope dilution vs Recovery Corrected External Standard Calibration: Correlation Plots



Recalculation of Data

Recovery Corrected External Standard

$$C_{RC} = \left(\frac{A}{\overline{CF}}\right) \times \left(\frac{C_{IS_e}}{C_{IS}}\right)$$

Isotope Dilution

$$C_{ID} = \left(\frac{A}{\overline{RF}}\right) \times \left(\frac{C_{IS_e}}{A_{IS}}\right)$$

$\boldsymbol{C_{RC}}$ = Recovery corrected concentration of analyte

$\boldsymbol{A}$ = Measured peak area of analyte

$\boldsymbol{C_{IS}}$ = Measured concentration of isotopically labeled analog

$\boldsymbol{C_{IS_e}}$ = Expected concentration of isotopically labeled analog

$\boldsymbol{\overline{CF}}$ = Average calibration factor (external standard)

$\boldsymbol{A_{IS}}$ = Measured peak area of isotopically labeled analog

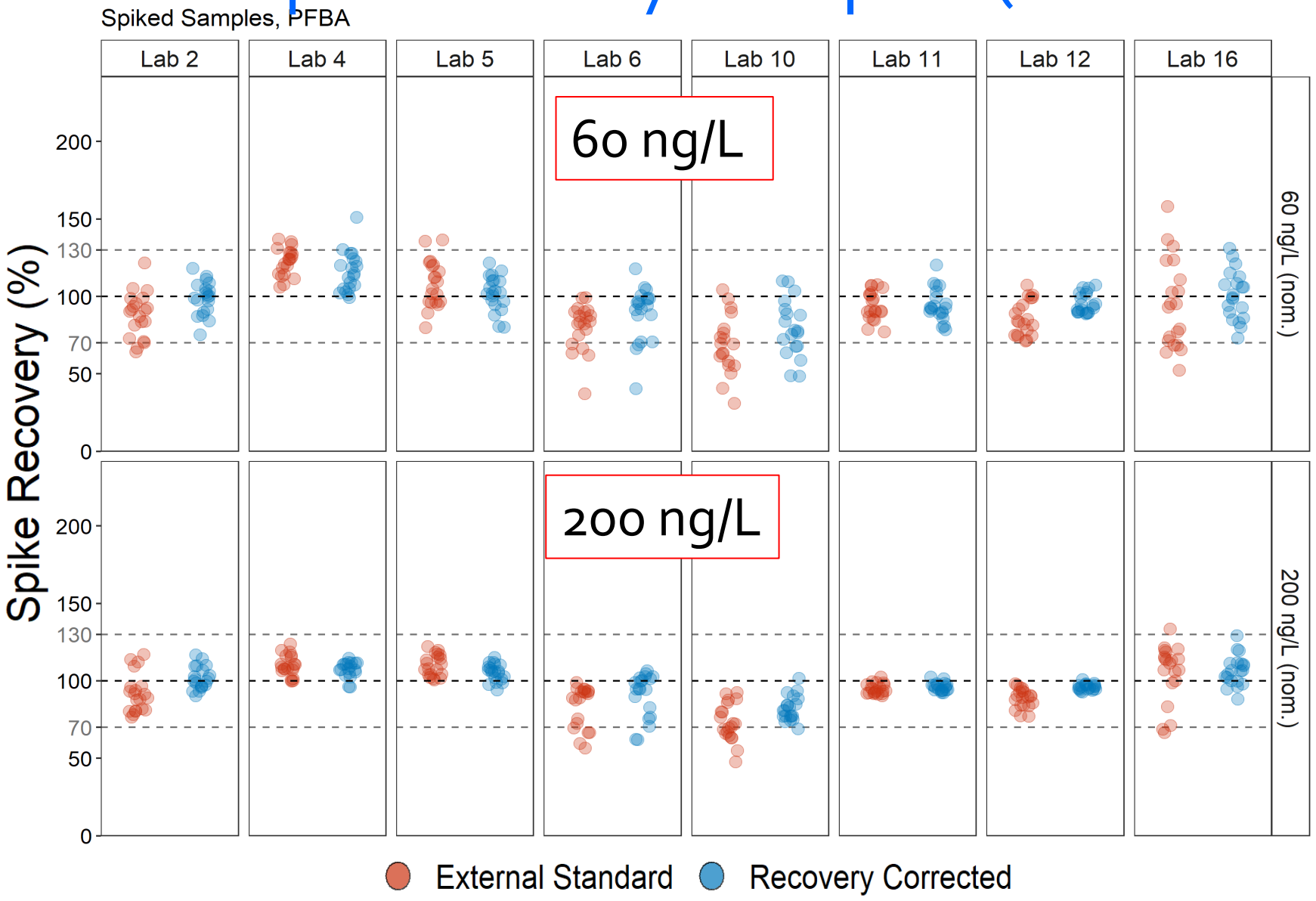
$\boldsymbol{C_{ID}}$ = Isotope dilution concentration of analyte

$\boldsymbol{\overline{RF}}$ = Average response factor (internal standard)

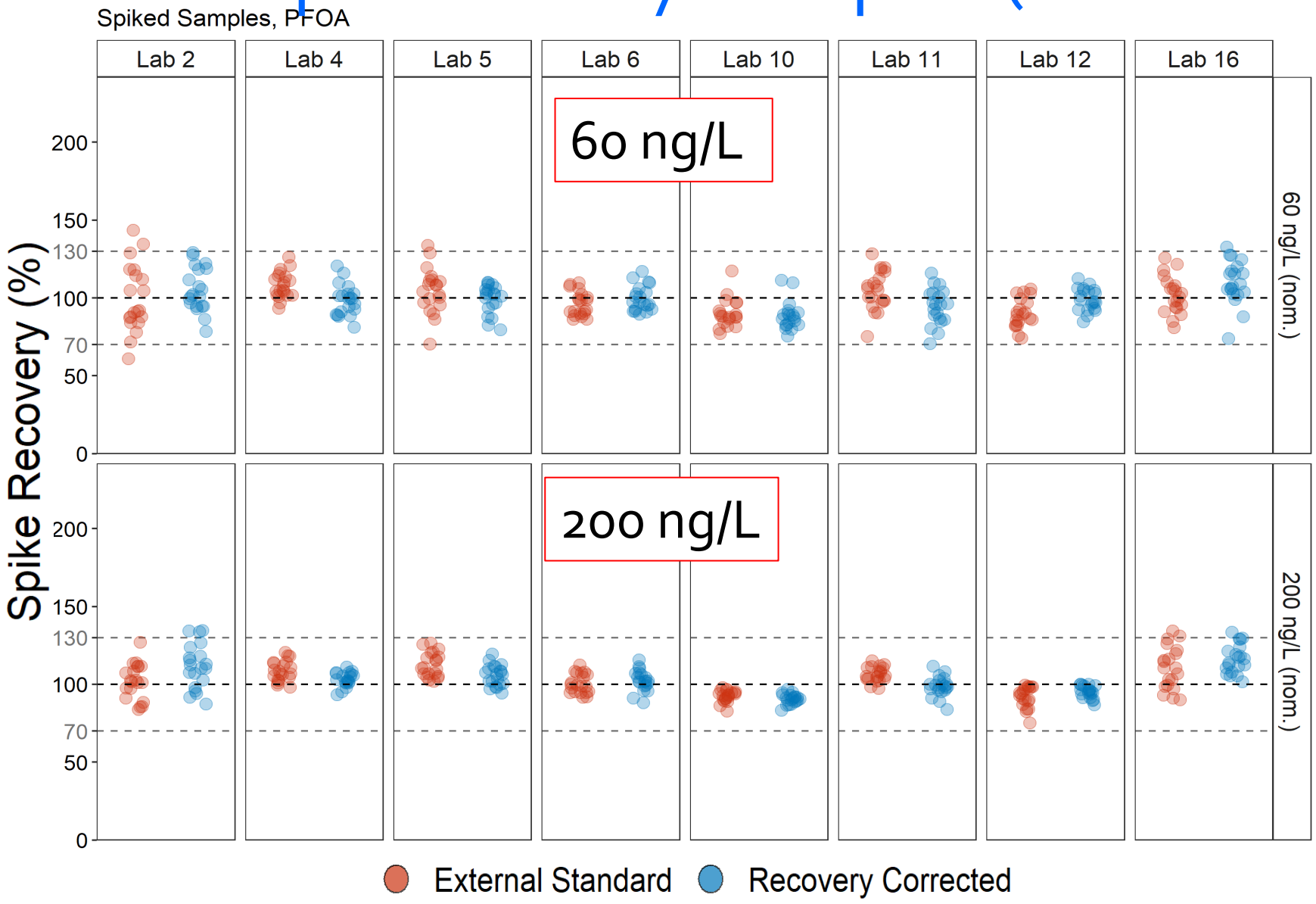
Compare Recovery Corrected External Standard to External Standard

- Jitter plots comparing distribution in measured concentrations by lab
- Bins for % recovery frequency distributions by target analyte across labs
- Distribution of measured concentrations across all target analytes, all labs

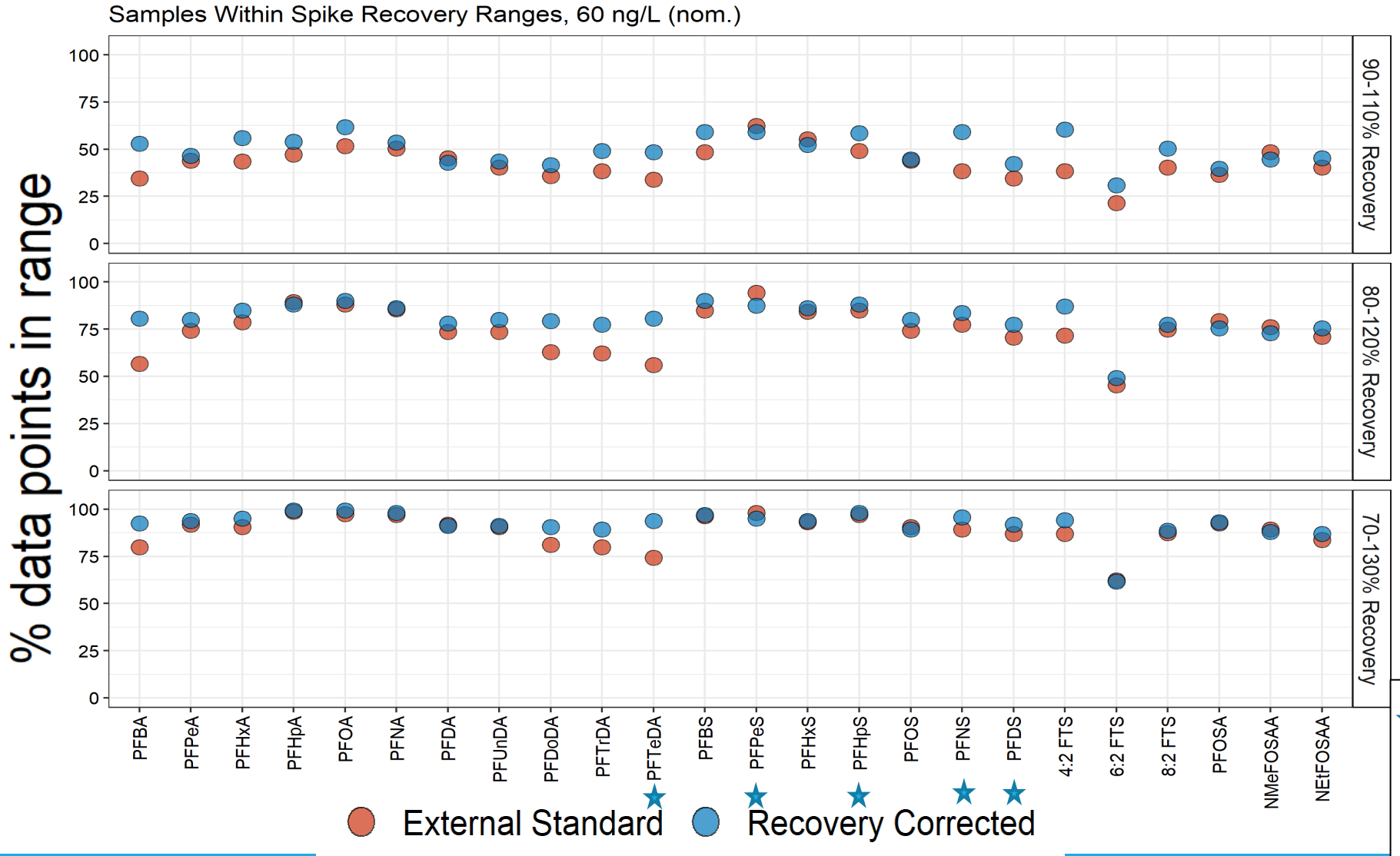
PFBA in Spiked Study Samples (All Matrices), by Lab



PFOA in Spiked Study Samples (All Matrices), by Lab



Compare External Standard & Recovery Correction 60 ng/L Spiked Samples – 8 labs, ~160 analyses



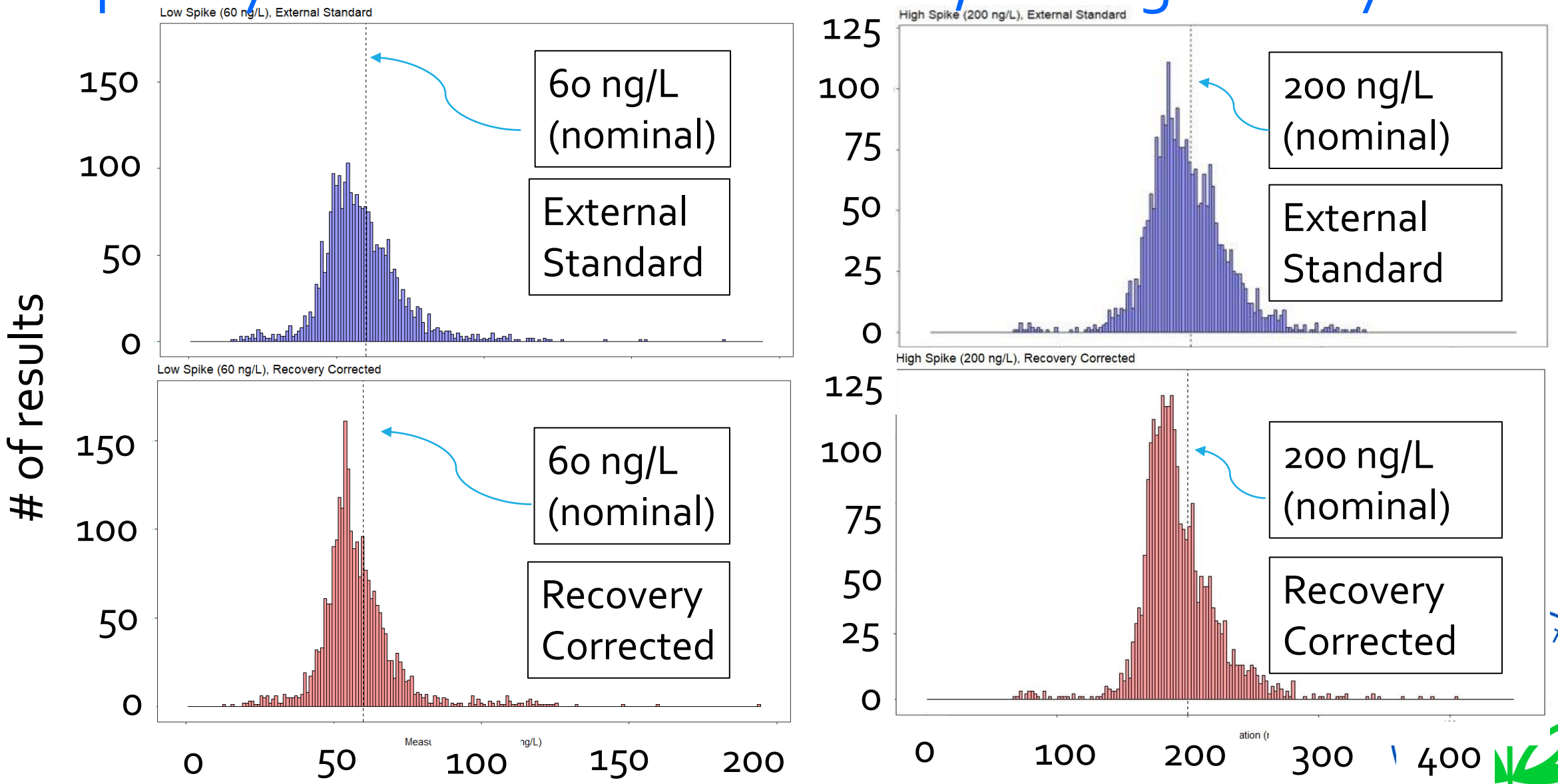
90-110%

80-120%

70-130%

★ = no structurally identical labeled analog

Frequency Distribution: All matrices, all target analytes



3512/8327 Isotopically Labeled Surrogate Recoveries: External Standard Calibration

Isotopically labeled surrogate	Average Recovery in Study Samples, $\bar{X}$[2] (%)	Within Laboratory Standard Deviation, S_w[3] (%)	Between Laboratory Standard Deviation , S_b[4] (%)
MPFBA	95.6	3.7	7.1
M5PFPeA	98.6	1.7	5.3
M5PFHxA	97.3	4.5	8.2
M4PFHpA	98.7	3.6	7.9
M8PFOA	101	2.6	6.2
M9PFNA	102	3.0	9.4
M6PFDA	104	2.5	9.0
M7PFUnDA	103	3.4	7.5
MPFDoDA	101	5.6	10.8
M2PFTeDA	96.8	5.8	14.2
M3PFBS	96.8	4.6	8.1
M3PFHxS	102	2.3	5.5
M8PFOS	104	2.9	9.0
M8PFOSA	101	3.0	6.7
M2-4:2FTS	97.8	13.6	4.5
M2-6:2FTS	100	6.2	6.5
M2-8:2FTS	106	3.3	6.2
d3-N-MeFOSAA	103	5.9	12.0
d5-N-EtFOSAA	104	4.7	11.7

From “Additional Performance Data from validation study for Methods 3512 and 8327 (pdf)”, available at: https://www.epa.gov/hw-sw846/sw-846-test-method-8327-and-polyfluoroalkyl-substances-pfas-liquid-chromatographytandem

Conclusions From Comparisons of 3512/8327 Validation Data Transformations

Isotope dilution/extracted Internal Standard vs Recovery-Corrected External Standard

- Results across labs were very similar, close to 1:1 correlation
  - Math is almost identical
  - Differences mainly attributable to bias in remodeled average RF for given laboratory/initial calibration curve

Recovery-Corrected External Standard vs External Standard

- Recovery-correction generally produced results in slightly tighter clusters, increased frequency of results within a% recovery range
  - Extent of any improvement varied by analyte, laboratory
  - At 70-130% recovery, increase in frequency evident for only a few PFAS
  - Did not improve 6:2 FTS performance - due to laboratory background

Take-Home Points:

- Comparison supports inclusion of isotope dilution/extracted internal standard calibration as an option in Method 8327
- Isotope dilution/extracted internal standard calibration can:
  - Account for losses during sample preparation
  - Account for changes in instrument performance, e.g., calibration drift, signal enhancement/suppression
    - As long as the same variables affect response of native chemical and labeled analog
- EPA met 3512/8327 validation study DQOs for precision and bias using external standard calibration
  - Recovery correction is small/negligible when recovery is near 100%
  - 50% methanol content was sufficient to recover PFAS at tested concentrations
- External standard calibration uses the same labeled chemicals to monitor for bias in samples – it just doesn't recovery-correct

SW-846 FAQ for Methods 3512 and 8327

- Q: Is isotope dilution calibration permitted to be used for quantitative analysis [for Method 8327]?
- A: "…**Appropriate modifications may be made… including the use of an alternate calibration model**, as long as the laboratory demonstrates it can generate data of appropriate quality for the intended application and the modifications are acceptable and transparent to the end data user (e.g., regulatory authority)"

  https://www.epa.gov/hw-sw846/sw-846-update-vii-announcements

- Methods 3512 and 8327 Response to comments document:
  - "**EPA will consider adding isotope dilution calibration as an option to this method** or to a future PFAS determinative method."

  https://www.epa.gov/hw-sw846/sw-846-test-method-8327-and-polyfluoroalkyl-substances-pfas-liquid-chromatographytandem

Acknowledgements

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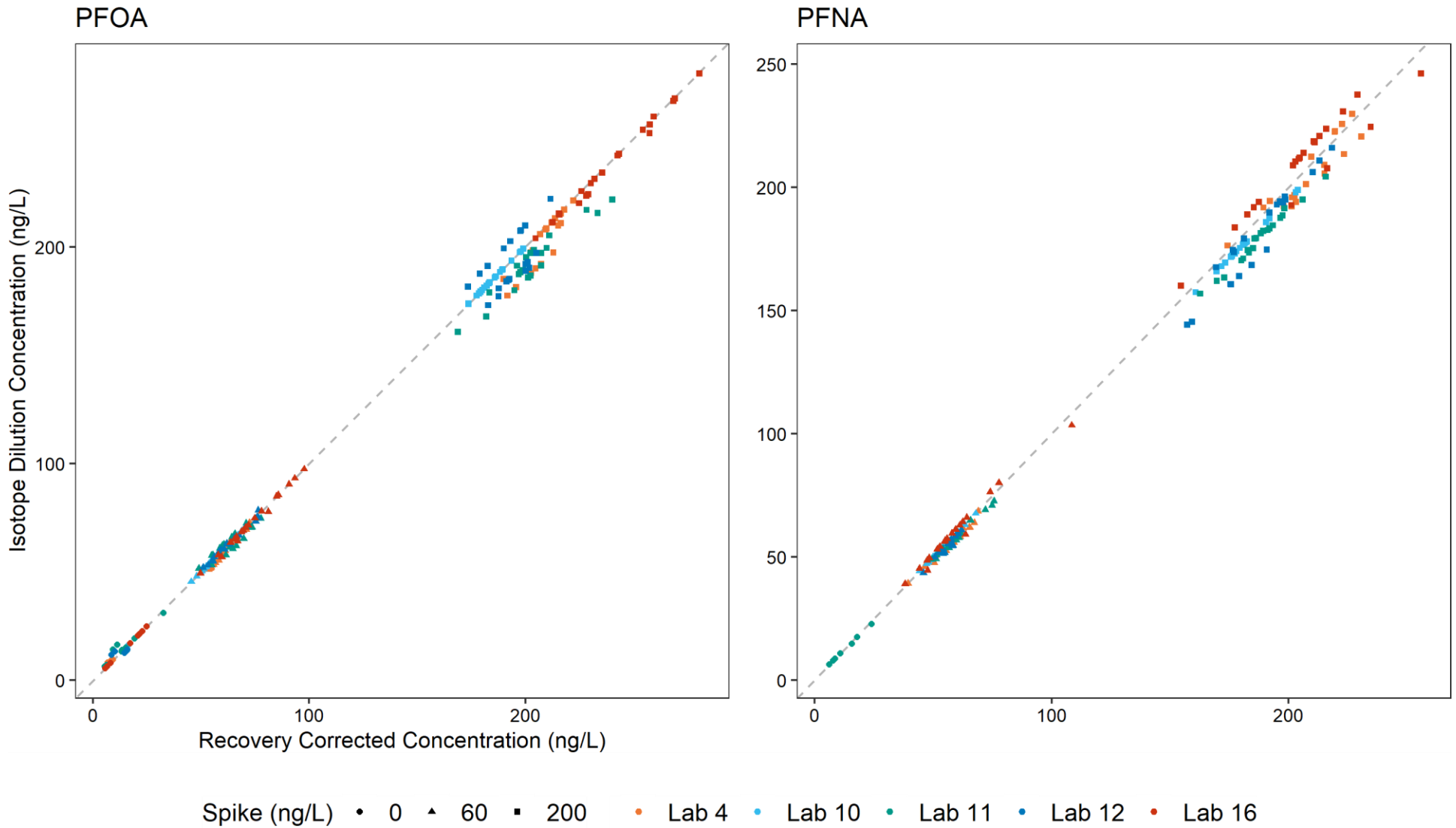
Supplemental Information

Types of Data Shown

- Samples (0, 60, 200 ng/L nominal spikes)
  - % recovery calculated after subtraction of average unspiked concentration from that matrix type and laboratory
- Lower Limit of Quantification = LLOQ (10-80 ng/L nominal concentrations)
  - Most at 10 or 20 ng/L; 10 ng/L was lowest tested
- Method Blanks = MB (0 ng/L), LCS = Lab Control Samples (160 ng/L)
- All isotope dilution result comparisons shown are 1:1, so cut to data only from the 5 labs that could be remodeled

Correlation Plots: Isotope dilution vs Recovery Corrected External Standard Calibration



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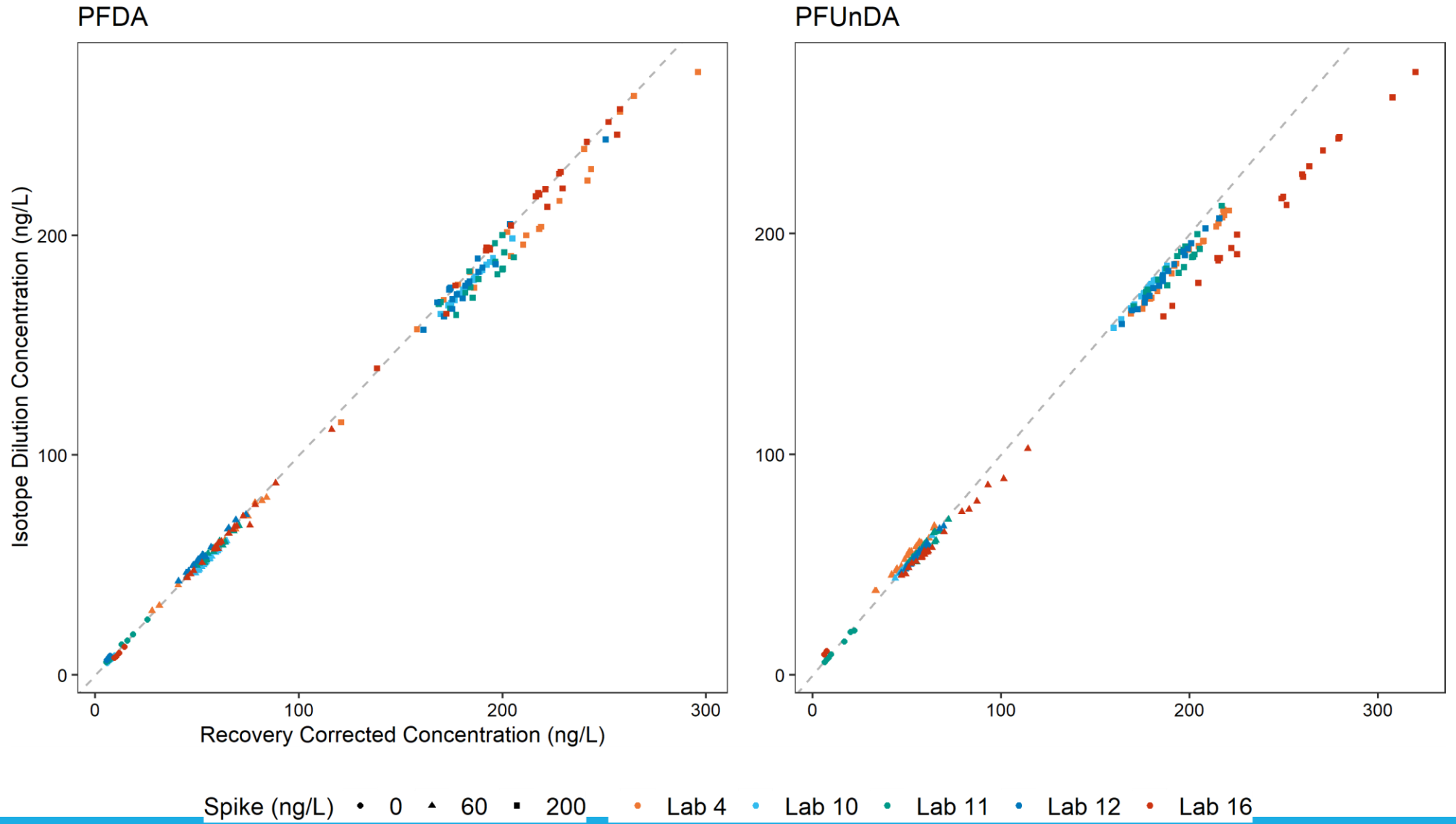

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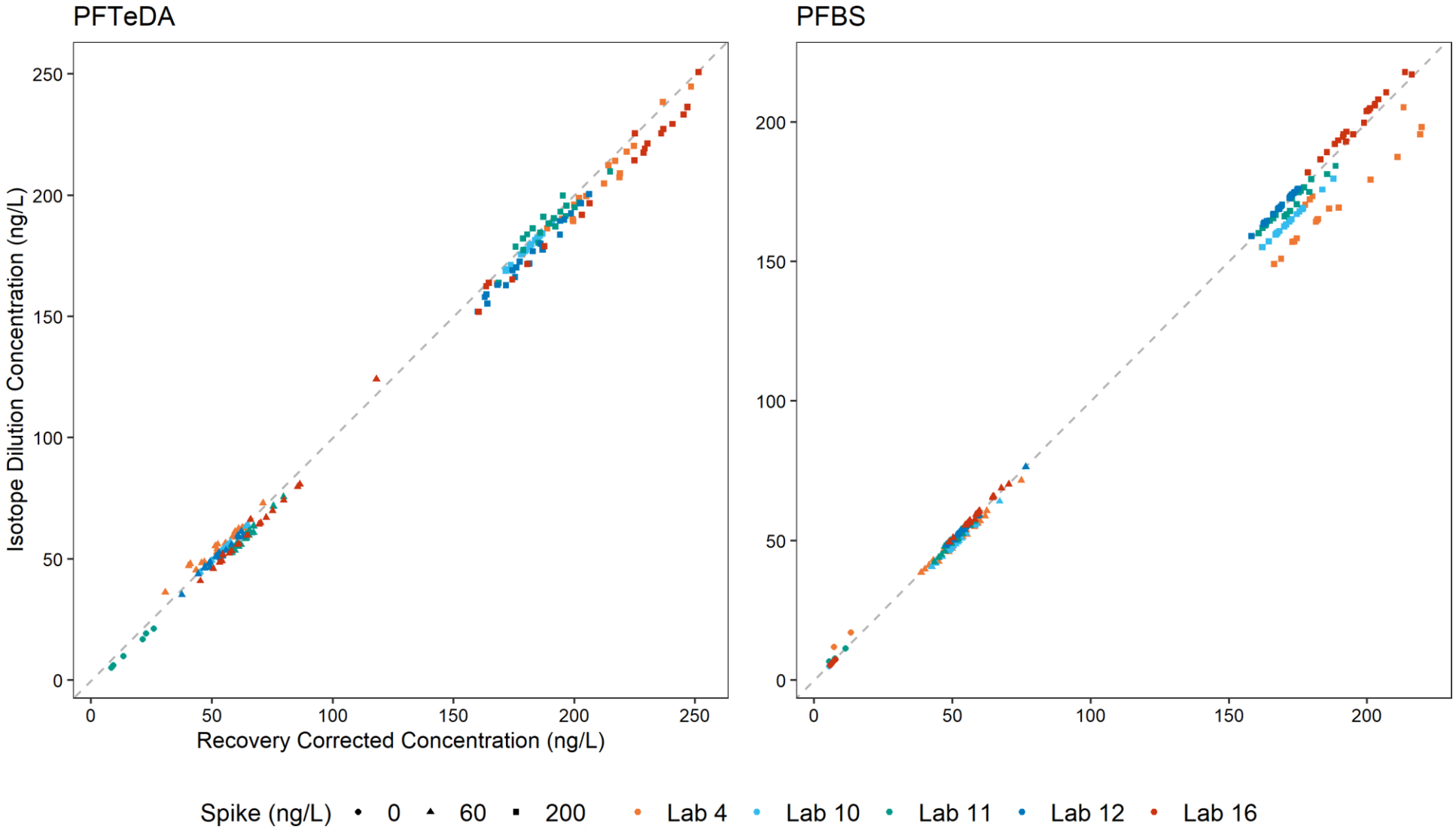

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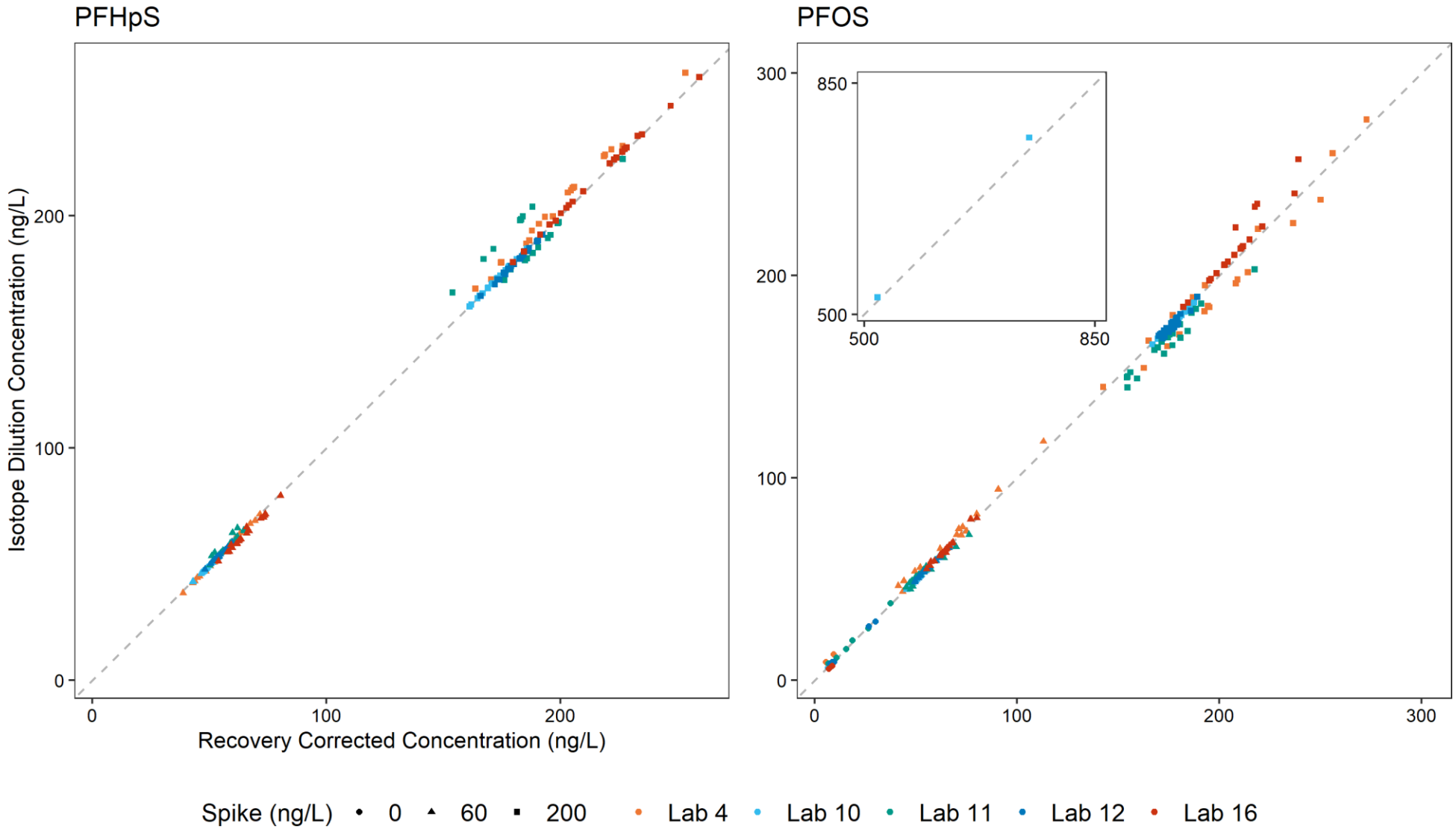
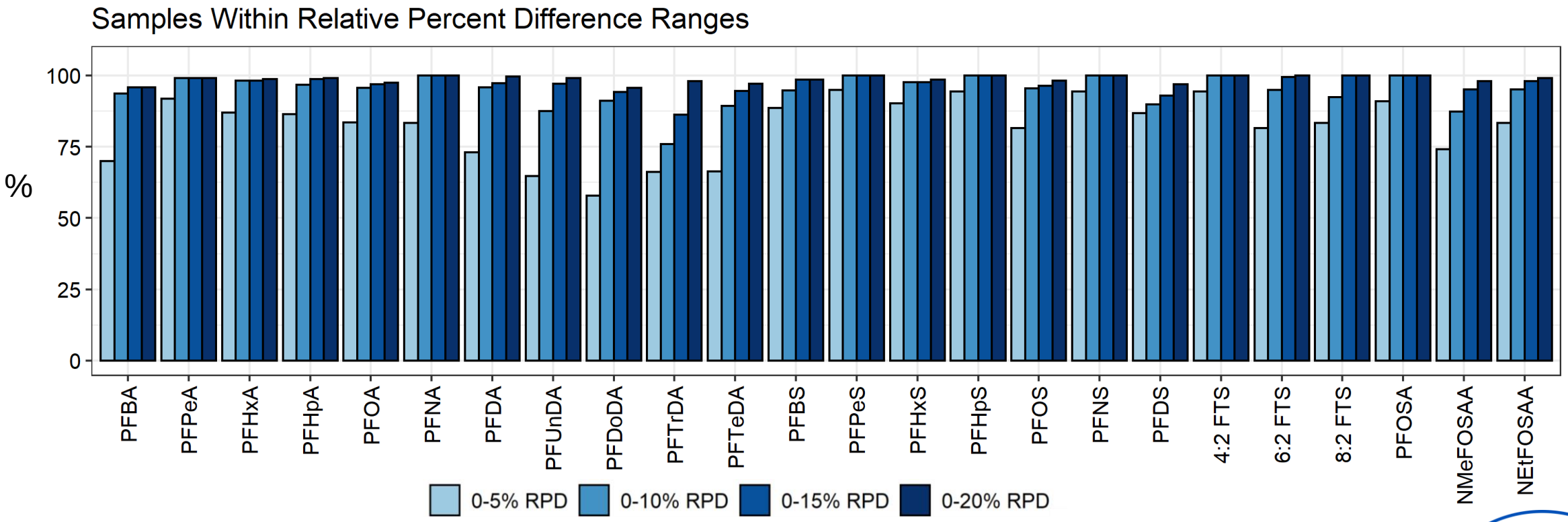
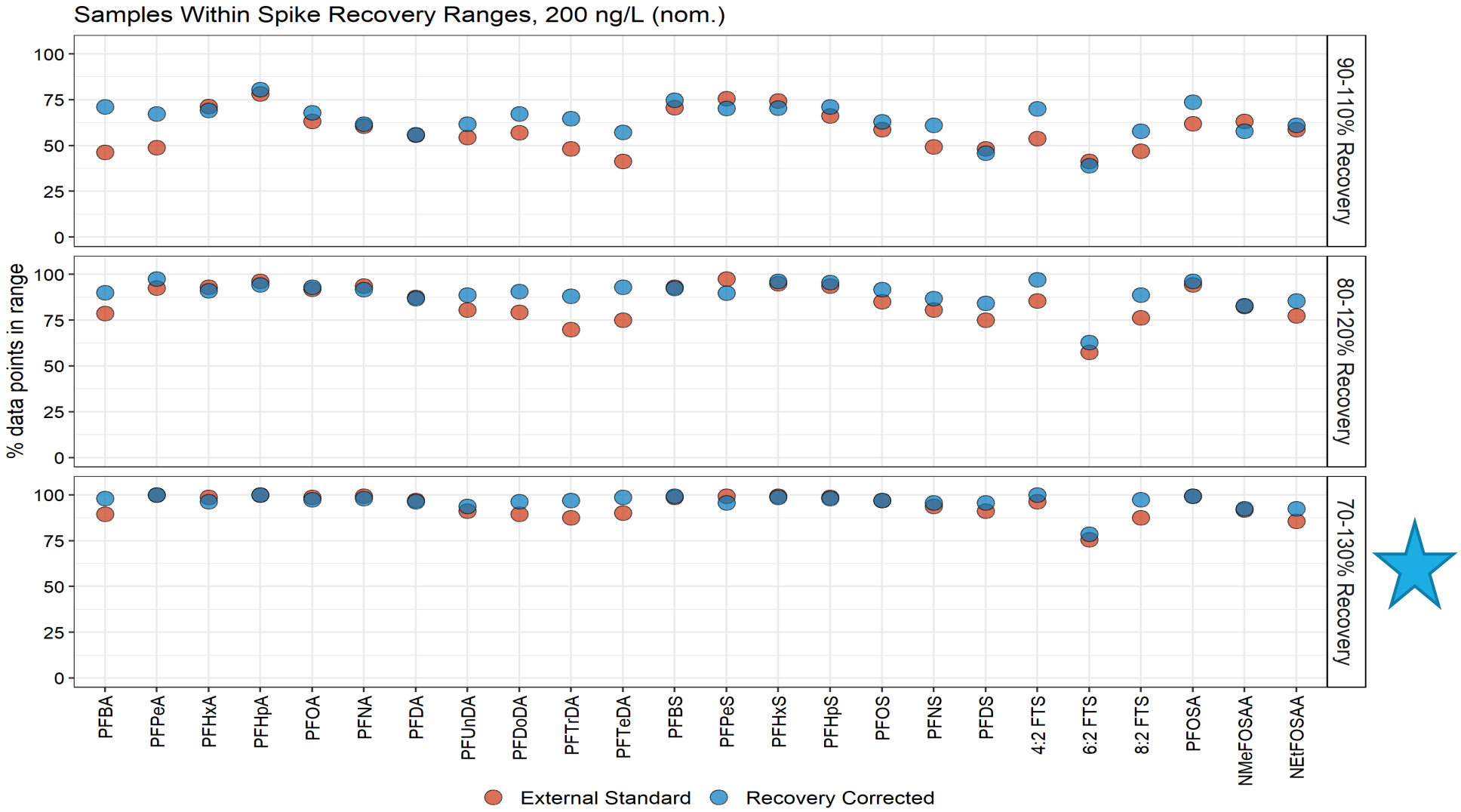


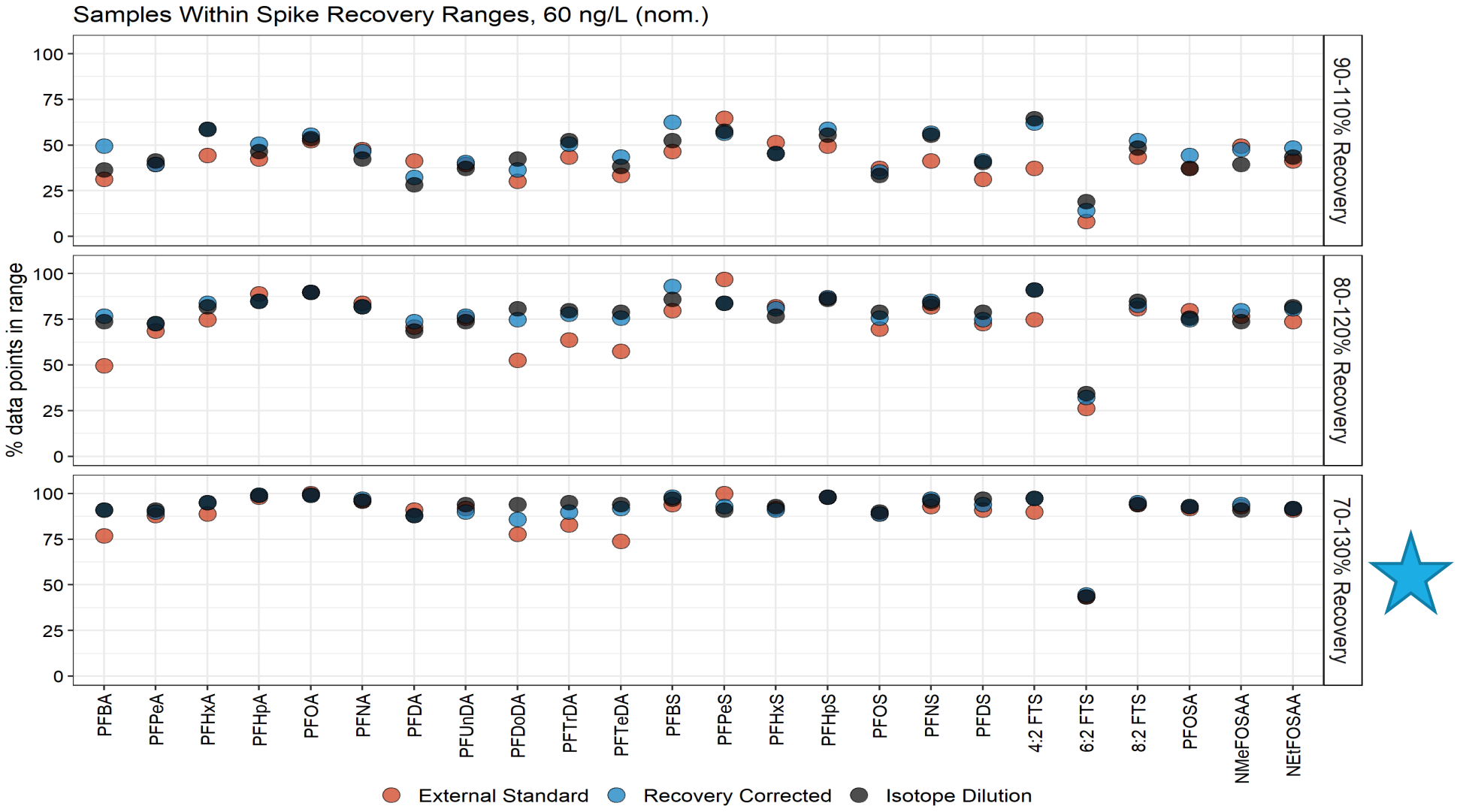
Isotope dilution vs Recovery Corrected External Standard Calibration: Relative percent difference



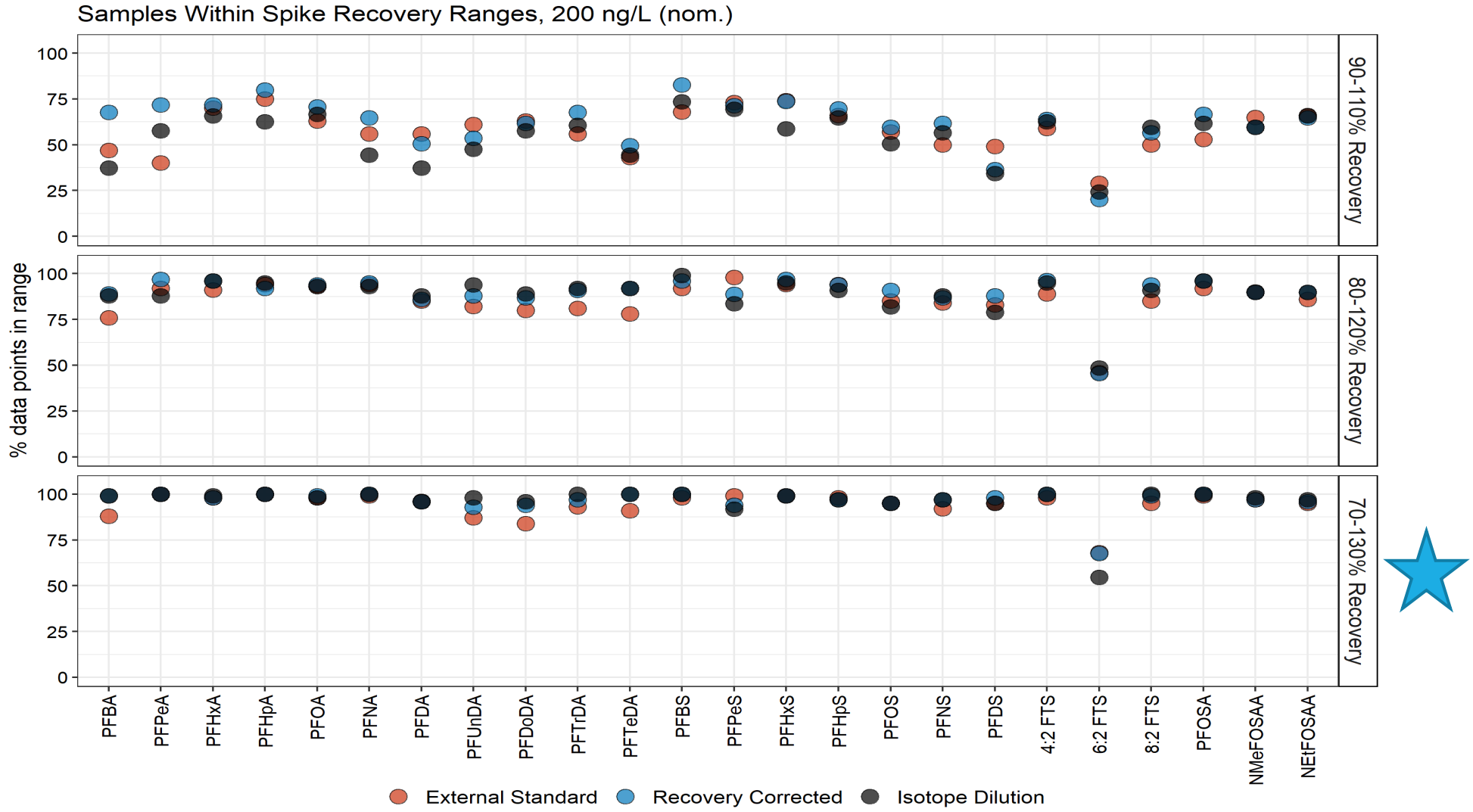
Compare External Standard & Recovery Correction 200 ng/L Spiked Samples – 8 labs, 160 analyses



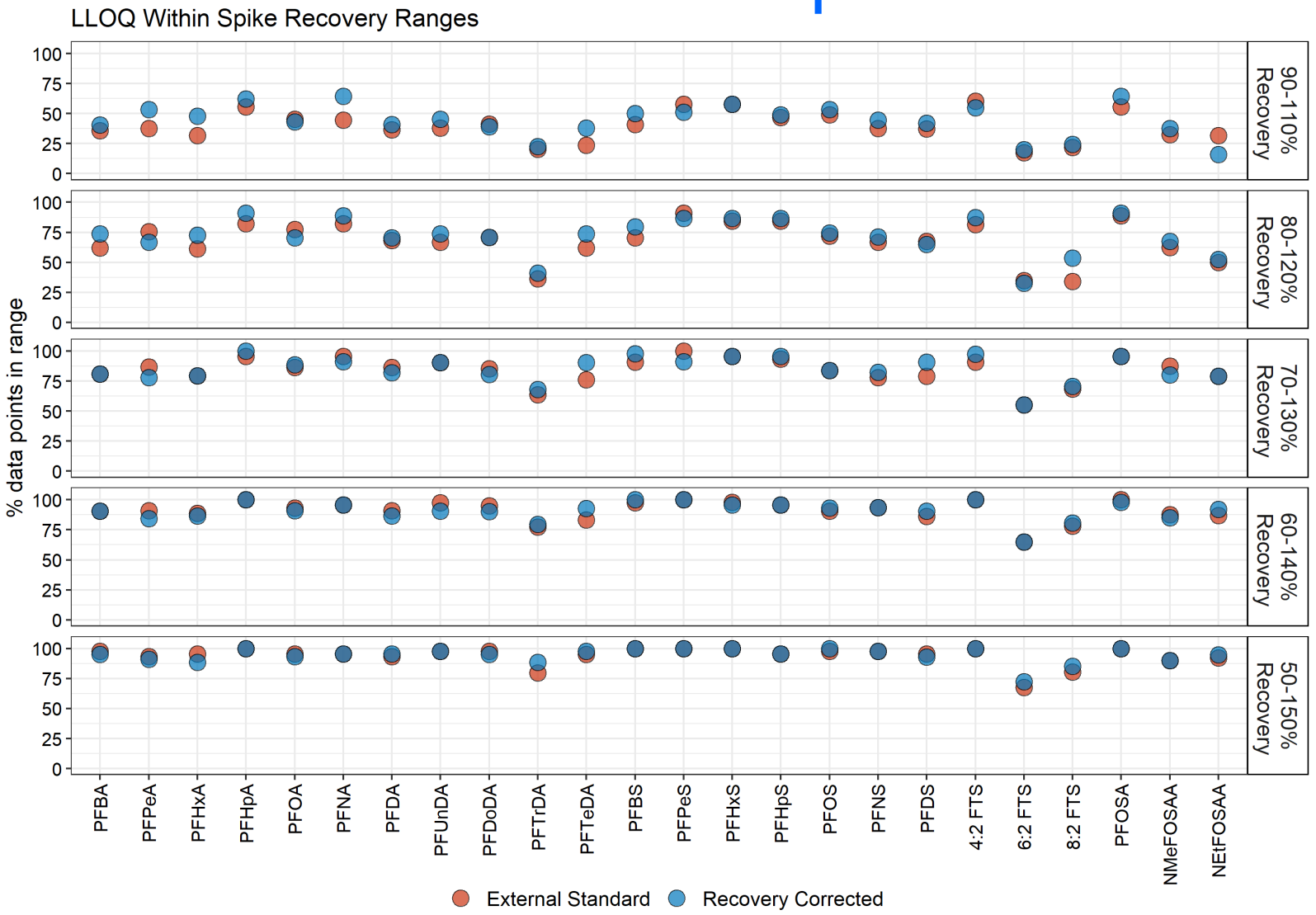
Compare External Std, Recovery Correction, and ID 60 ng/L Spiked Samples – 5 labs, ~100 analyses



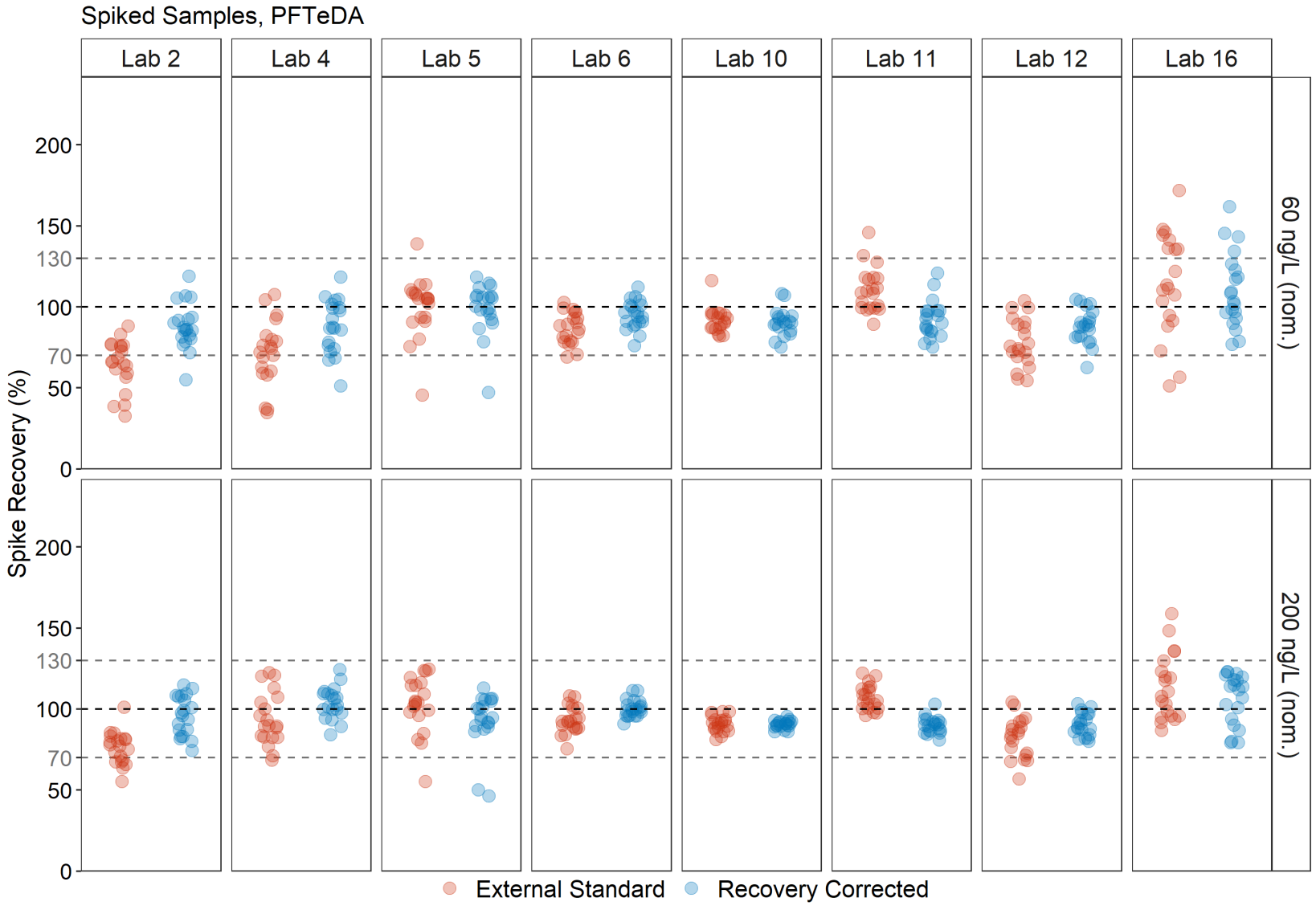
Compare Recovery Correction, External Standard and ID 200 ng/L Spiked Samples – 5 labs, ~100 analyses



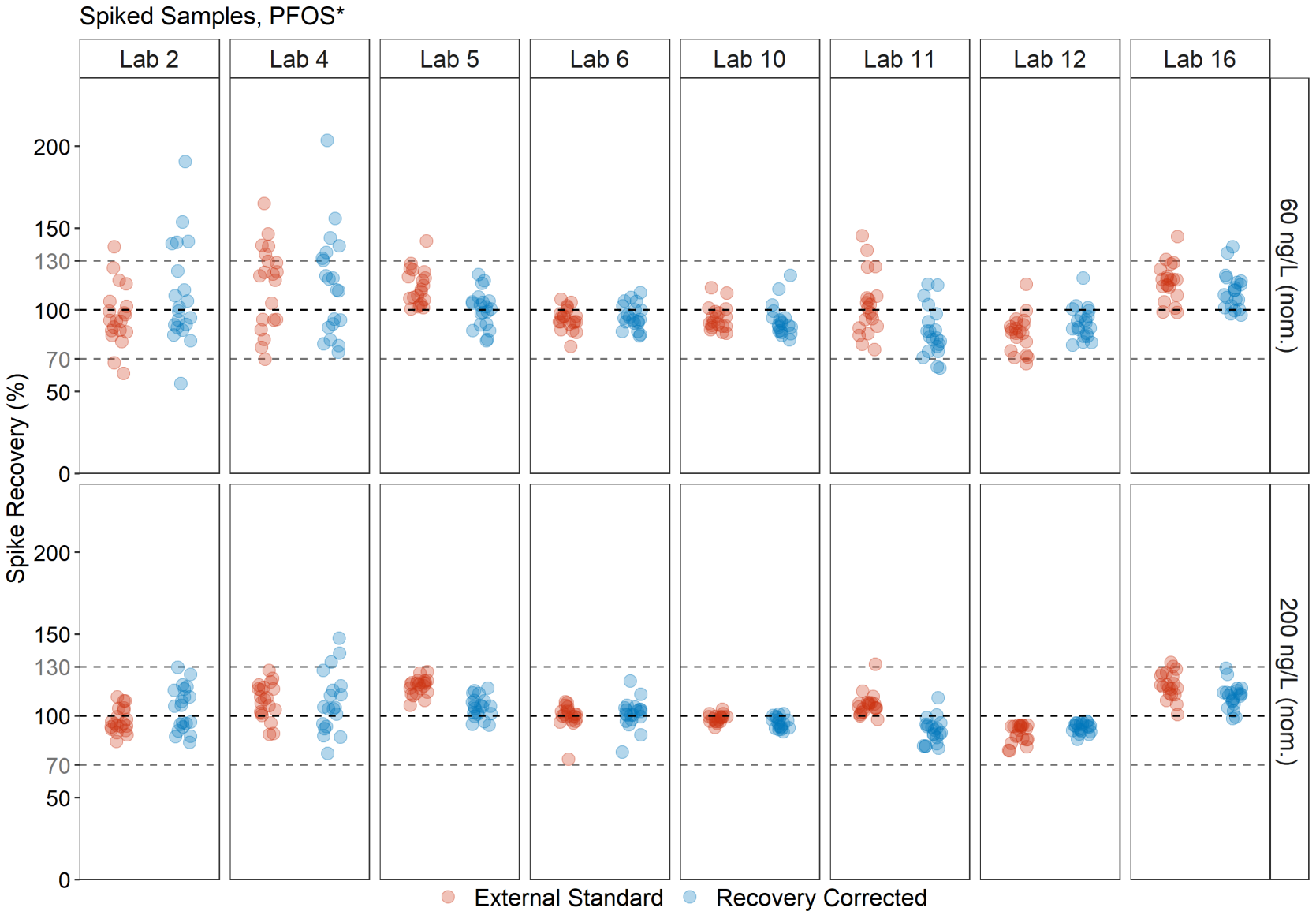
Compare External Standard & Recovery Correction LLOQ Verification QC Samples – 8 labs



PFTeDA in Spiked Study Samples, by Lab



PFOS in Spiked Study Samples, by Lab



Background: Validation study

Multi-laboratory validation study design:

Matrices:	• Groundwater, Surface Water, Wastewater
Prepared Concentrations:	• Background (unspiked), 60 ng/L, 200 ng/L (nominal)
Replicates:	• 5 reps per matrix at each concentration – analyzed blind

Data Quality Objectives:

Precision	• Average 70-130% recovery (95% CI of median) for each matrix and spike level combination
Bias	• ≤ 50% RSD in each matrix, spike level, laboratory combination
Sensitivity	• Lower Limits of Quantitation (LLOQ) verification QC samples within 50-150% - lowest tested 10 ng/L

For more detail, see: https://www.regulations.gov/document/EPA-HQ-OLEM-2018-0846-0111

Validation Study Design

- 8/12 labs' data used for final statistical analysis
- 4 excluded labs:
  - Subsampled prior to adding solvent - low recovery of longer-chain target analytes in study samples
  - Prepared spiking solutions in 1:1 MeOH-water+0.1% acetic acid and stored in glass
    - Resulted in high or variable recovery of longer-chain target analytes in study samples, likely due to loss of chemicals from spiking solutions
  - One lab identified having instrument stability problems
- This recalculation: 8/8 labs for recovery correction, 5/8 labs for isotope dilution (required raw data)
  - 4:2 FTS excluded for one lab because of contamination affecting labeled analogue