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Comparison of Calibration Technique in Analysis of PFAS by Two ASTM Methods

William Lipps

Shimadzu Scientific Instruments, Inc.

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External Standard Calibration compares a reference standard response to unknown response to establish a linear relationship of concentration and response

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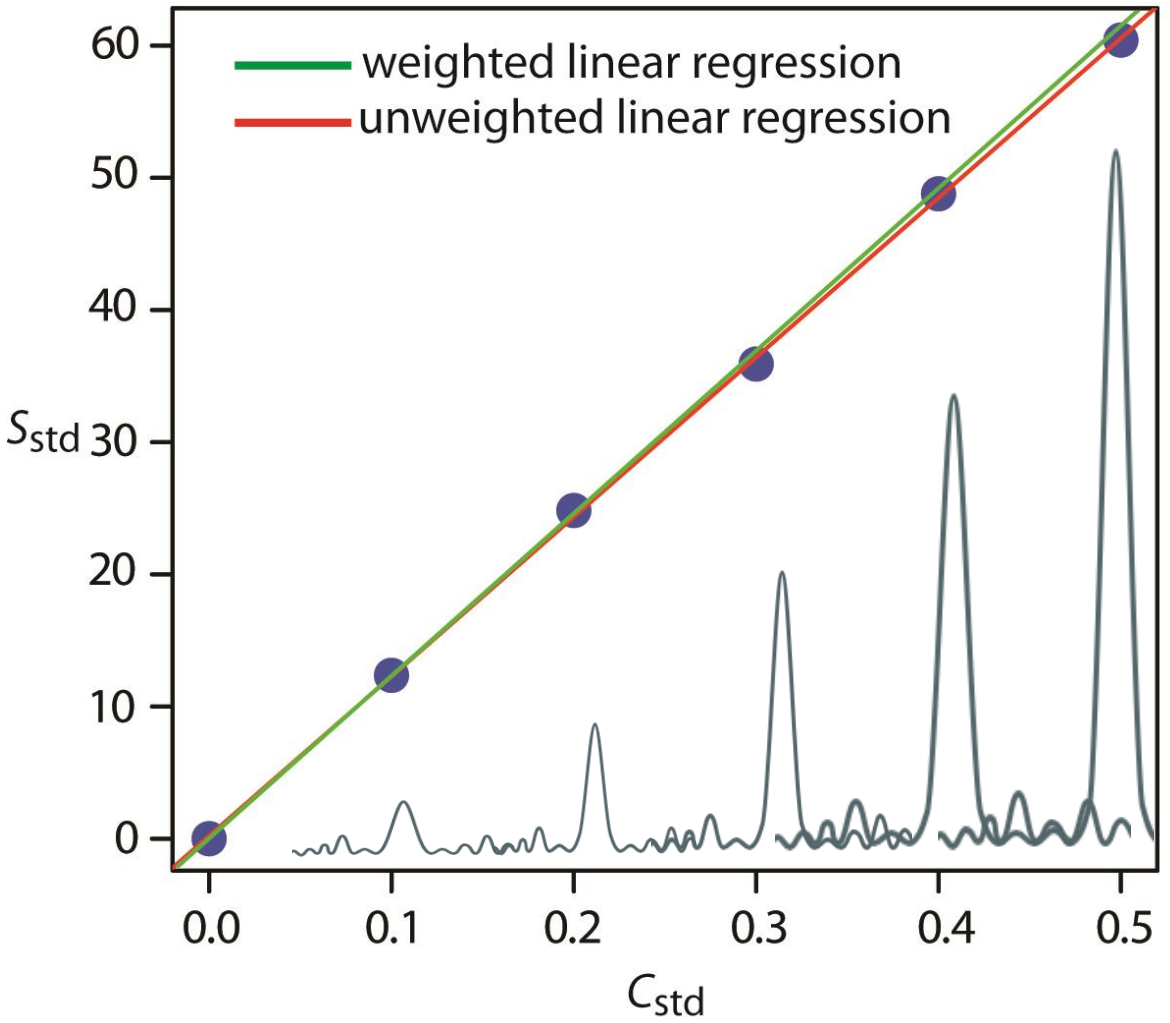
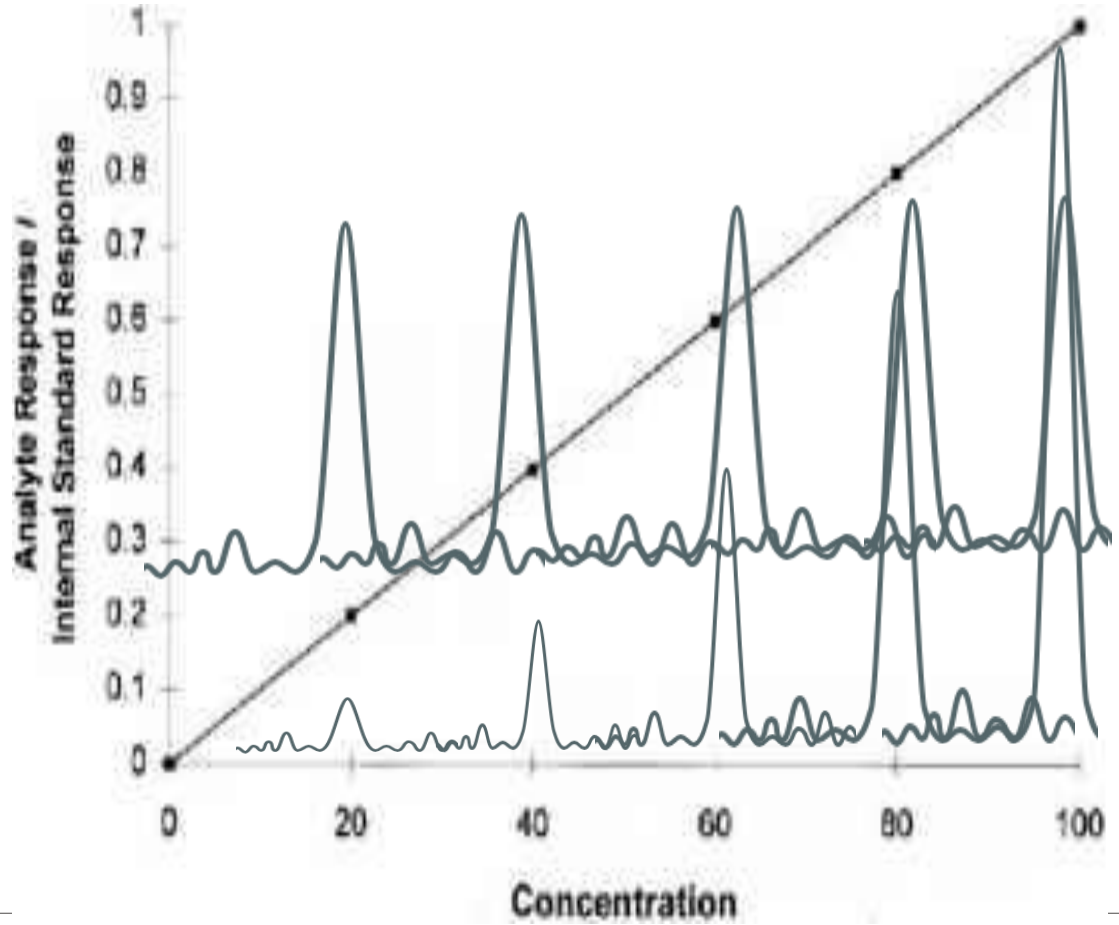
  - Standard mass or concentration versus response
    - Standards are not extracted
    - Standards are usually not added to the sample
    - Multiple point curves created



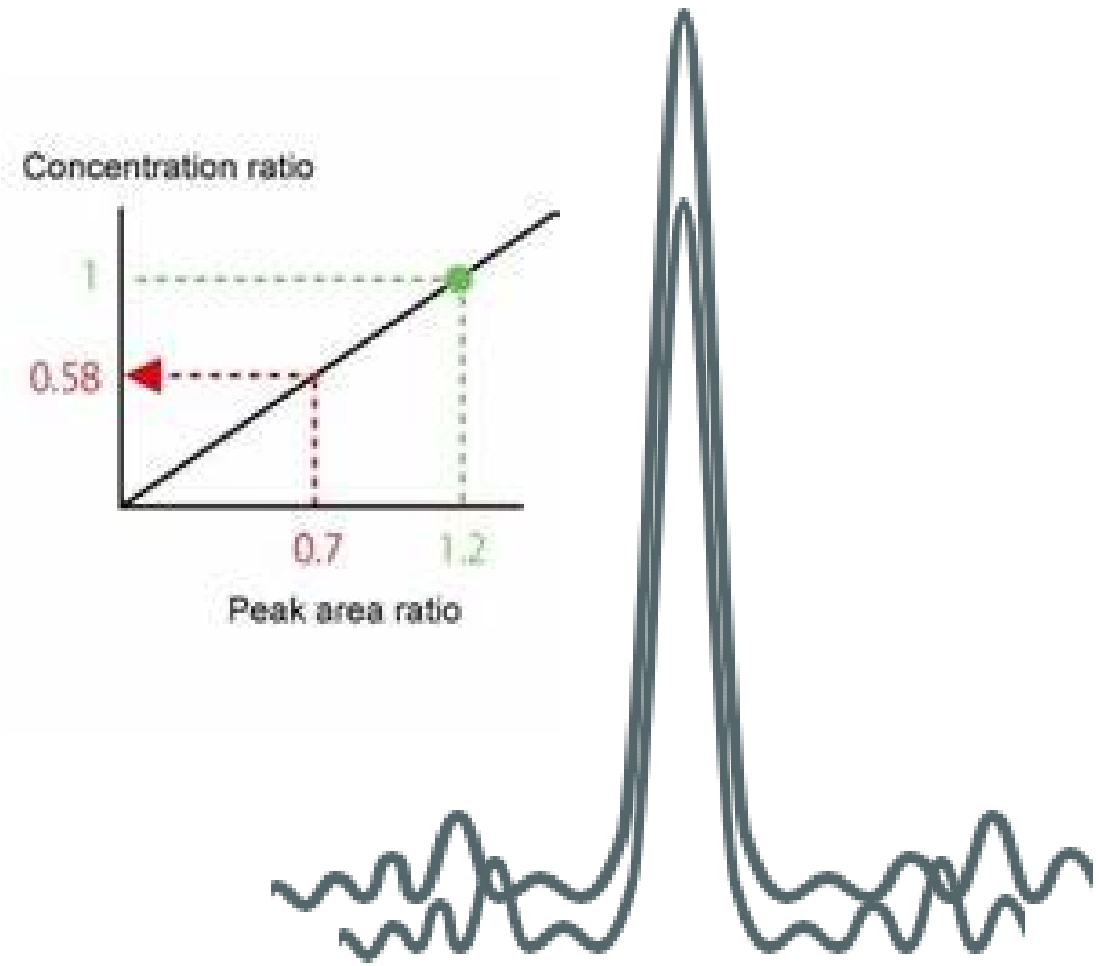
Internal Standard Calibrations ratio the response of unknowns with knowns that are added to the sample–

- Ratio of responses prepared by mixing one concentration of a standard with unknowns (internal)
- Concentration determined by ratio of known standard
- Standard is added at a single concentration for all calibrations and samples, after any extractions or digestions



Isotope dilution standards are added, at a single concentration, to all standards and samples prior to any extraction or digestion

- Extracted internal standard – ratio of extracted isotope with extracted analyte
- Standard should be an exact isotopic analogue of the analyte
- Assume analyte and standard behave the same during extraction and analysis



A calibration technique involves confirming that an instrument produces the correct result

How do you determine if a calibration technique produces the correct result?

- Set performance criteria goals based on matrix and concentration
- Optimize method for best instrument performance
- Optimize method for greatest precision
- Optimize method for ~ 100% recovery of spikes at different concentrations, within precision of the method.
- Extract different matrices in triplicate at increasing concentration and determine recovery using external standard calibration
- If acceptable ➔ Stop
- If not acceptable, modify method if possible and repeat
- If not acceptable, use internal standards to correct incomplete recovery or precision, or
- Use internal standards to improve precision and recovery to make BETTER than the external standard calibration.

Example of determination of calibration technique, from PFAS in fish oil

External Standard Calibration		Isotope Dilution Calibration	
ppb PFOA	% Recovery	ppb PFOA	% Recovery
0.25	142.5	0.25	105.5
0.25	126.9	0.25	111.6
0.25	125.8	0.25	108.5
0.5	129.8	0.5	90.6
0.5	152.1	0.5	95.6
0.5	116.2	0.5	95.2
1	117.1	1	100.7
1	118.9	1	101
1	120.9	1	102.7
5	181.2	5	110.7
5	133	5	101.6
5	142.8	5	101.9
10	91.8	10	104.9
10	116	10	104.8
10	101.1	10	102.1

Performance goals:

- ≤ 20% RSD
- 80 – 120 % Recovery

- Isotope Dilution necessary to meet performance goals

Determination of calibration model for ASTM D8421, determination of 44 PFAS in wastewater matrices

Performance goals:

1. Surrogate recovery no tighter than 70 – 130%, not greater than 60 -140% if possible
2. Analyte recovery no tighter than 70 – 130% , not greater than 60 – 140% if possible
3. RSD single operator ≤ 30%
4. RSD multiple operator ≤ 40%
5. Matrices = same wastewater matrices used for 1633 plus ground and surface water
6. Extraction = fast, minimal solvent, minimal sample volume, minimal labware

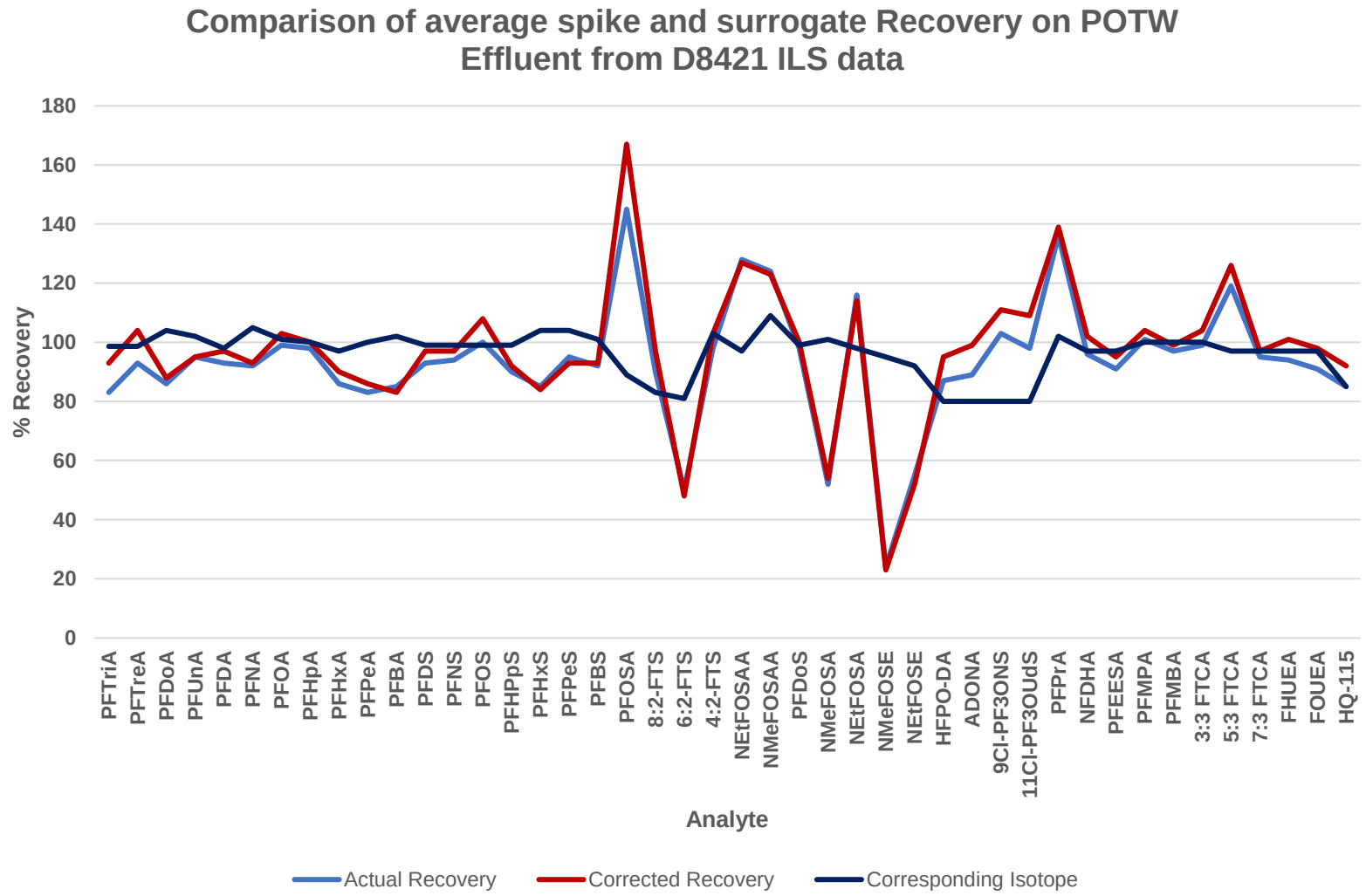
ILS Data for surrogates met criteria using External Standard Calibration, with very few exceptions

Material	Landfill Leachate	Metal Finisher	POTW Effluent	Hospital Wastewater	POTW Influent	Bus Washing Station	Power Plant Effluent	Pulp and Paper Effluent	POTW Effluent	Groundwater	Surface water
	Surrogate										
	% Recovery										
MPFBA	**69.4**	94.1	99.7	103	103	102	98.1	87.3	104	103	102
M5PFPeA	86.9	91.7	101	101	97.4	97.6	96.2	91.1	99.0	98.9	97.9
M5PFHxA	87.0	90.2	99.9	99.3	97.3	97.7	95.7	89.9	97.7	96.6	96.1
M8PFOA	91.3	92.3	100	99.6	96.5	100	96.1	91.0	95.4	96.3	96.3
M9PFNA	92.8	93.4	102	99.4	97.1	75.0	97.9	91.8	95.1	95.1	96.6
M6PFDA	92.4	97.0	99.7	101	96.9	105	96.5	91.9	97.0	95.5	96.1
M7PFUnA	94.6	94.5	102	101	96.3	83.9	96.1	93.1	94.8	93.8	93.6
MPFDoA	92.2	95.2	105	99.5	95.4	116	97.5	94.9	93.4	93.7	94.7
M2PFTreA	80.1	89.7	102	79.8	80.6	113	95.3	96.1	82.8	67.1	78.6
M8FOSA	93.8	96.4	101	99.5	97.0	92.2	95.0	92.8	96.4	95.1	94.3
D3-N-MeFOSAA	87.8	93.7	105	99.4	93.2	108	99.2	92.5	92.5	94.1	96.3
D5-N-EtFOSAA	86.9	95.7	105	99.4	95.6	**65.8**	98.5	93.3	93.3	97.0	95.2
d-N-MeFOSA	96.4	100.6	92.8	95.2	95.0	135	96.9	94.3	94.5	94.4	102
d-N-EtFOSA	94.4	97.2	91.4	96.1	92.9	113	94.7	93.4	92.9	95.5	92.8
d7-N-MEFOSE	91.3	92.5	92.2	96.7	94.8	99.6	94.9	93.6	93.5	93.7	93.8
D9-N-EtFOSE	91.5	95.7	91.3	96.8	93.6	**132**	95.2	93.1	93.6	93.1	92.4
MHFPO-DA	85.2	89.5	90.2	94.9	96.2	92.7	96.4	88.9	93.2	95.7	92.6
M4:2 FTS	101	86	107	93.5	88.7	120	94.4	86.8	90.1	85.9	91.0
[1]M6:2 FTS	**169**	**739**	91.3	91.2	83.9	125	92.5	81.1	85.9	84.4	90.1
M8:2 FTS	84.3	91.8	97.7	98.8	93.4	129	100.0	90.3	92.8	91.5	95.1
M8PFOS	94.0	93.8	101	98.7	96.7	93.5	95.7	97.1	96.6	96.3	96.0
M3PFBS	92.9	92.1	99.1	99.9	97.7	98.4	96.6	92.2	99.0	95.5	96.5
M3PFHxS	94.1	92.9	101	99.8	97.7	109	96.5	92.7	96.3	95.5	96.1
M4PFHpA	91.5	92.8	101	99.1	97.2	97.9	95.4	91.4	96.3	96.1	96.9

The average recovery and % RSD of All analytes for 8 labs at 11 matrices met the performance criteria

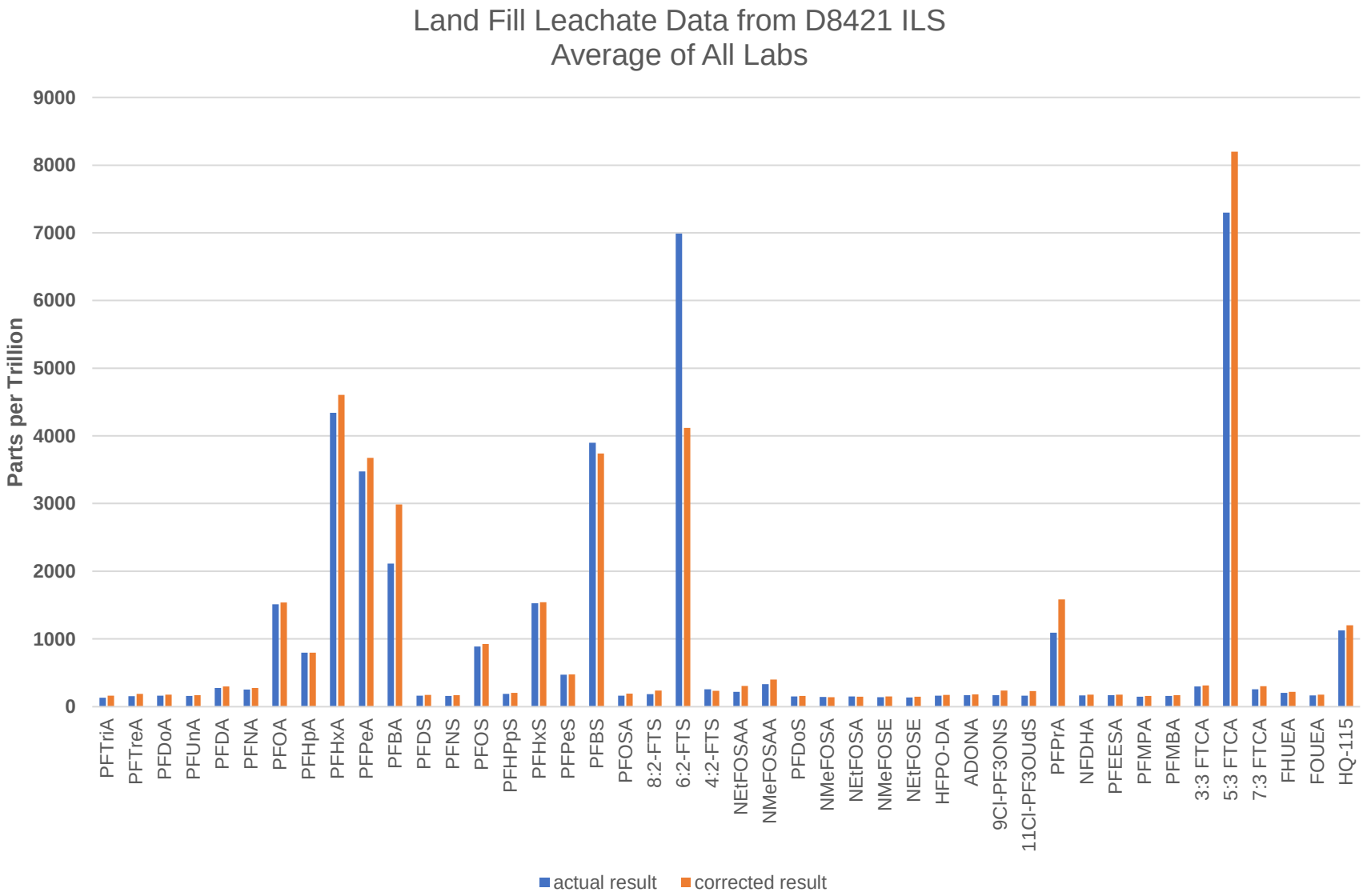
Analyte	Average	Std DEV	%RSD		Analyte	Average	Std DEV	%RSD
PFTreA	87.9	16.16198	18.4		NEtFOSAA	98.7	19.57896	19.8
PFTriA	96.0	10.39273	10.8		NMeFOSAA	101.0	23.66764	23.4
PFDoA	96.5	8.701534	9.0		PFDoS	85.7	12.71849	14.8
PFUnA	94.2	6.065846	6.4		NMeFOSA	80.7	25.6642	31.8
PFDA	97.0	8.201668	8.5		NEtFOSA	82.1	22.00351	26.8
PFNA	96.3	9.642531	10.0		NMeFOSE	76.8	17.0981	22.3
PFOA	107.1	11.30858	10.6		NEtFOSE	81.2	18.52559	22.8
PFHpA	99.3	8.032821	8.1		HFPO-DA	95.3	5.381097	5.6
PFHxA	103.4	14.71039	14.2		ADONA	98.6	5.934762	6.0
PFPeA	98.7	5.36818	5.4		9Cl-PF3ONS	98.6	6.510788	6.6
PFBA	102.6	7.858704	7.7		11Cl-PF3OUdS	97.4	5.115169	5.3
PFDS	93.0	7.80376	8.4		PFPrA	93.6	12.83553	13.7
PFNS	97.3	7.371524	7.6		NFDHA	97.5	4.702531	4.8
PFOS	99.1	10.74186	10.8		PFEESA	97.9	4.56108	4.7
PFHpS	98.7	8.309803	8.4		PFMPA	97.3	6.196714	6.4
PFHxS	101.8	7.262622	7.1		PFMBA	94.7	6.812989	7.2
PFPeS	95.2	10.5278	11.1		3:3 FTCA	96.3	8.296539	8.6
PFBS	104.8	10.35058	9.9		5:3 FTCA	100.6	21.38414	21.3
PFOSA	101.7	18.27127	18.0		7:3 FTCA	93.0	14.14273	15.2
8:2 FTS	95.3	19.63287	20.6		FHUEA	95.9	8.667336	9.0
6:2 FTS	93.8	21.42385	22.8		FOUEA	98.0	13.74043	14.0
4:2 FTS	97.2	16.84549	17.3		HQ-115	99.0	3.675254	3.7

But, using ILS data we compare recovery and precision for some analytes between external standard and isotope dilution



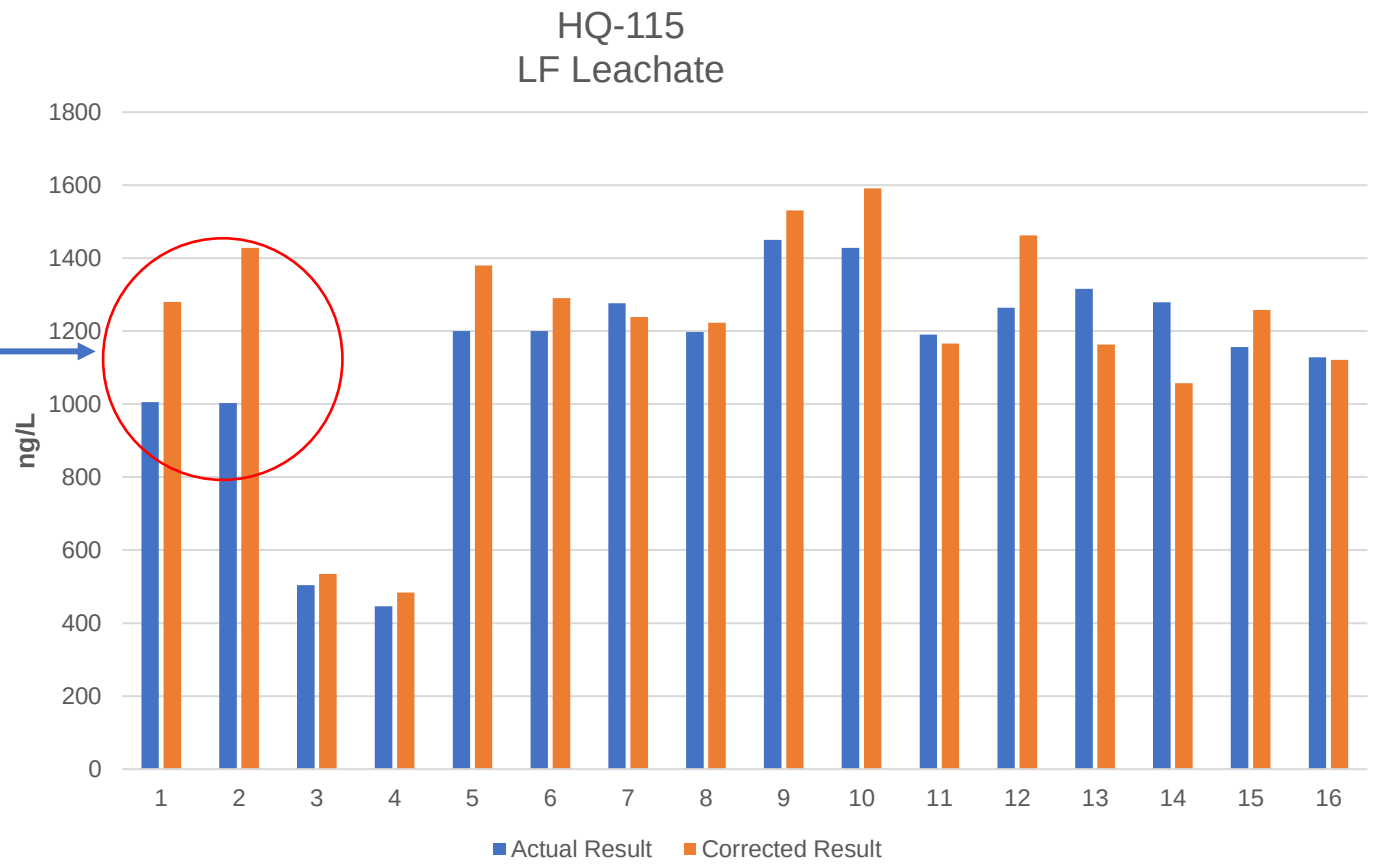
- For analytes with no isotopes, the same as named in 1633 are used
- Corrected result and external standard results almost the same
- Spike concentration is 20 ppt

All analytes from landfill leachate matrix, average of all labs, isotope dilution and external standard calibration data compared



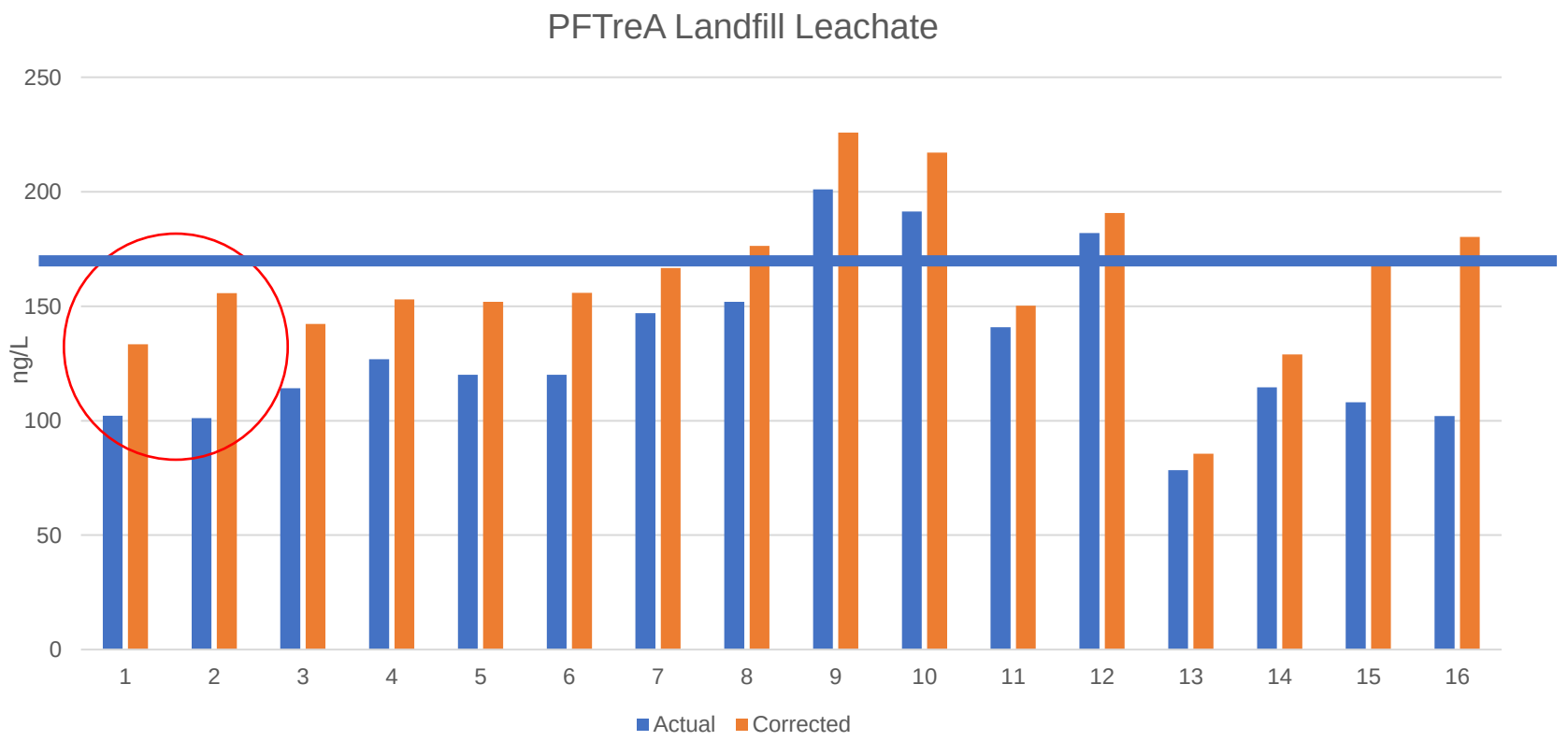
Isotope Dilution compared to External Standard results in Landfill Leachate

Duplicate results of one lab



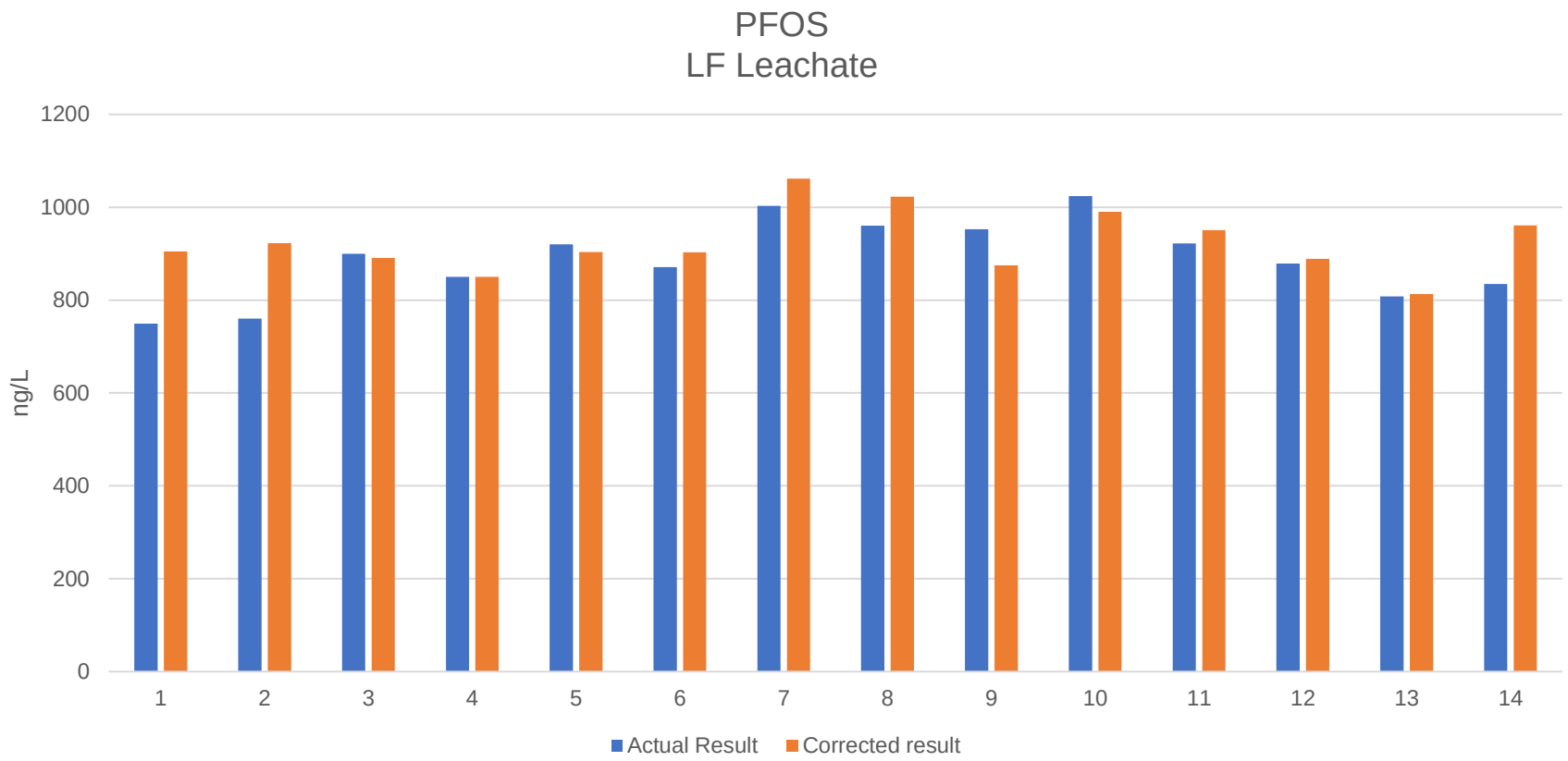
True value not known, no general pattern for correction.

Isotope Dilution compared to External Standard results in Landfill Leachate, spiked at 160 ng/L

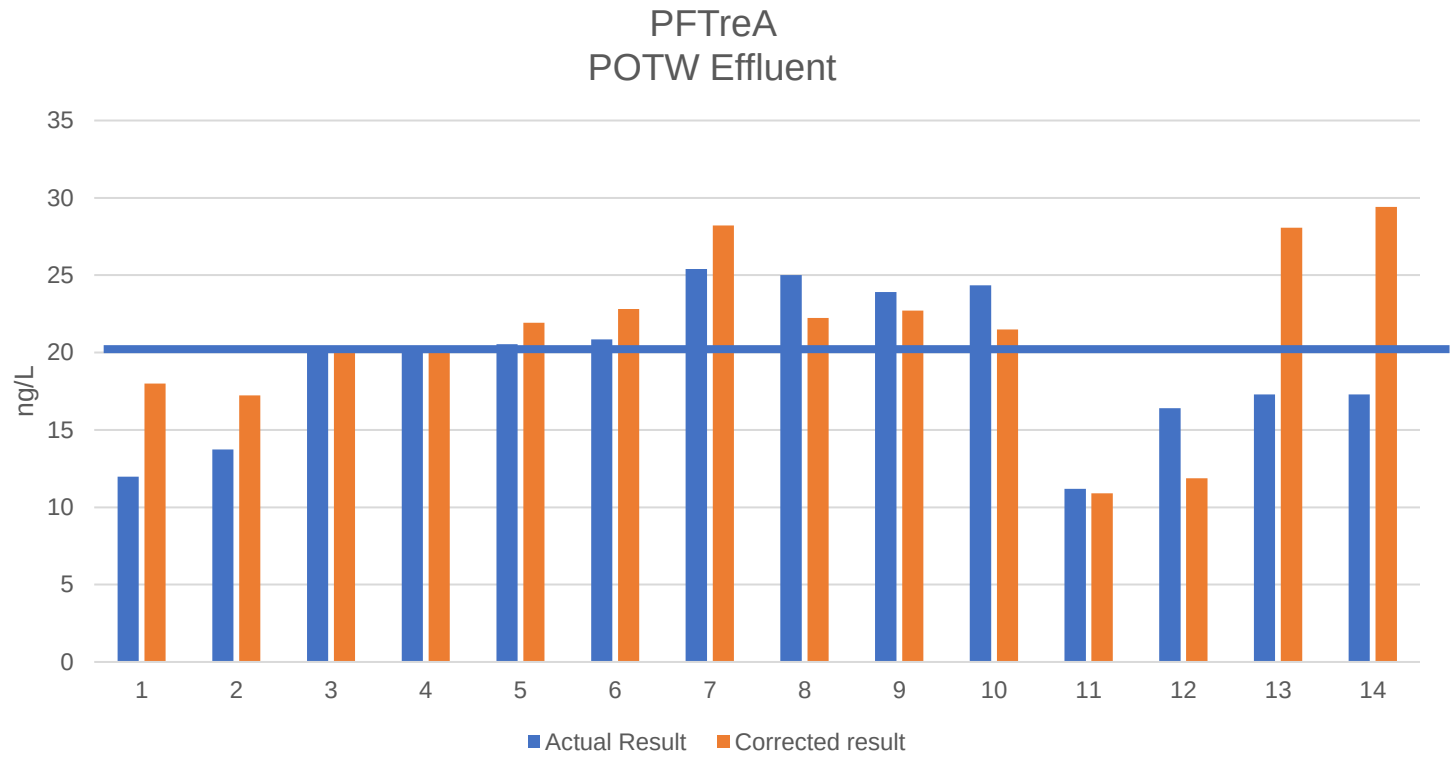


Generally, isotope dilution results are closer to known value

PFOS in Landfill Leachate, true value is not known



PFTreA (poor performer) in POTW effluent spiked at 20 ng/L



For some labs, correction makes result better and for some, the correction is worse. It's about extraction technique and not the calibration.

Statistics test on POTW effluent 20 ng/L spike recovery all analytes

	Actual Recovery	Corrected Recovery				
Mean	93.25	97.15909				
Variance	434.192	525.811				
Observations	44	44				
df	43	43				
F	1.21					
P(F<=f) one-tail	0.266	0.533	Two-tail			
F Critical one-tail	1.66					
One-tail	Cannot Reject Null Hypothesis because p > 0.05 (Variances are not different)					
Two-tail	Cannot Reject Null Hypothesis because p > 0.05 (Variances are not different)					

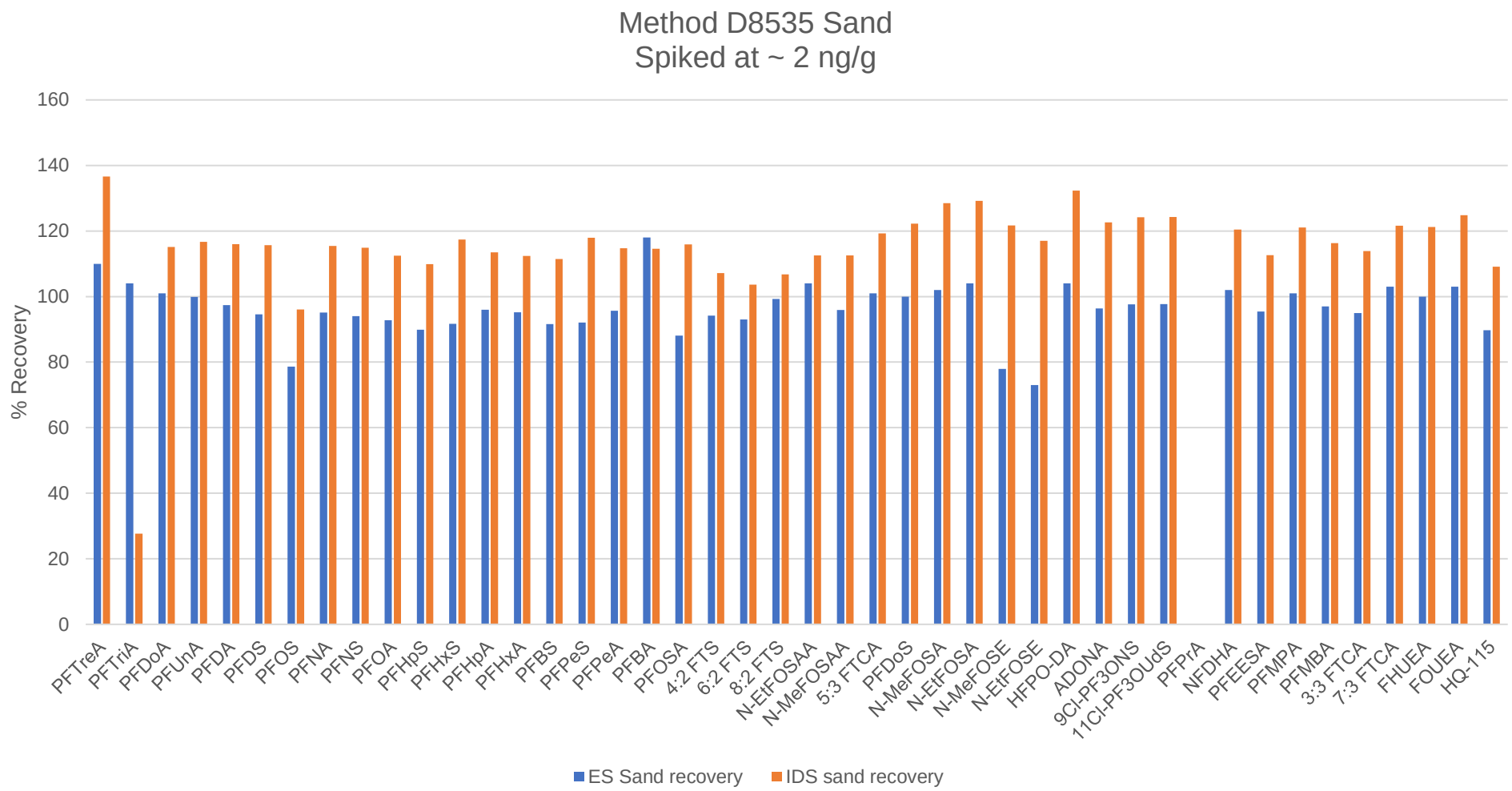
Isotope dilution corrects from 93% to 97% recovery

For method D8421, since the recovery and precision is near 100% and < 20% for most analytes regardless of matrix there is no correction needed.

Since surrogates are added prior to extraction, using isotope dilution could help with poor performers.

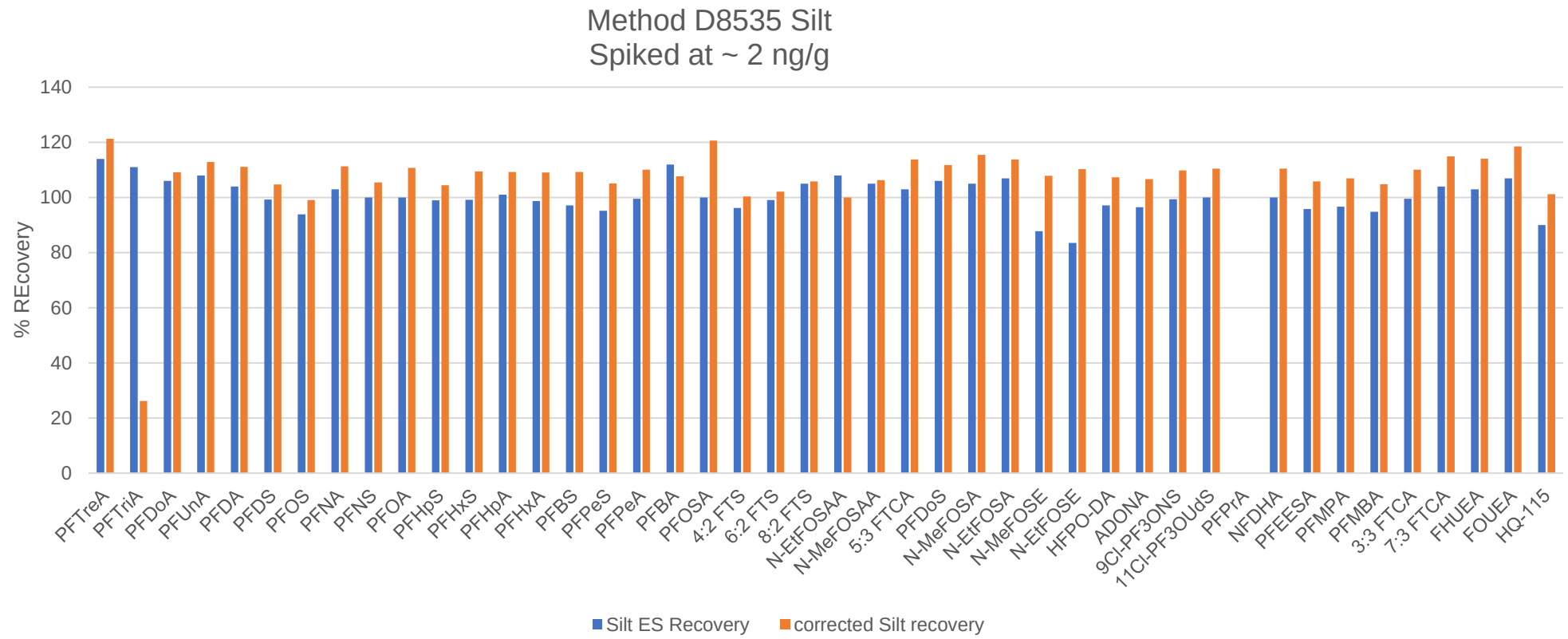
However, analysts should perfect the extraction procedure for recovery and precision before using isotope correction.

Comparison of Calibration Technique for D8535 soil and biosolids method in Sand



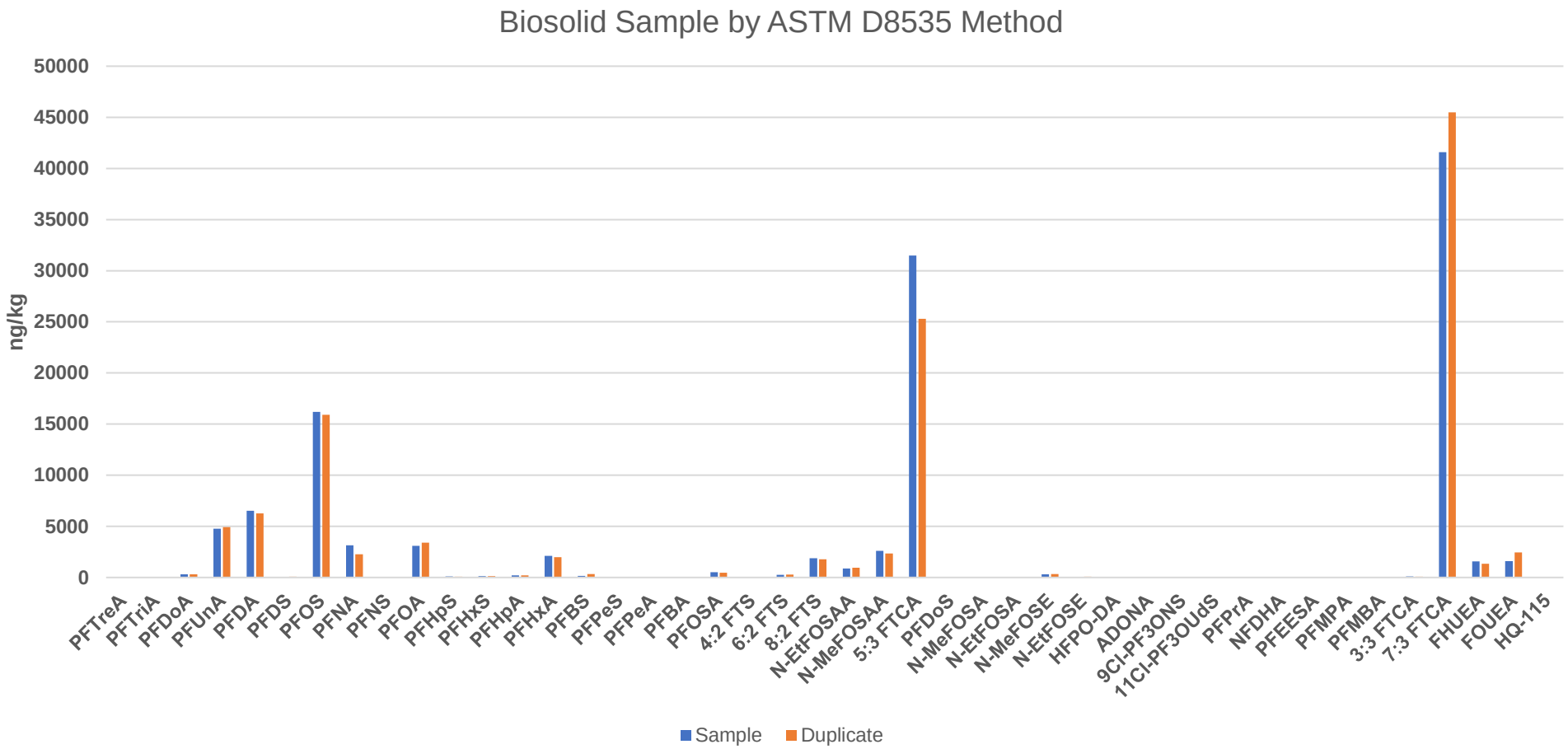
Isotope dilution over corrects, but both results still within 70 -130% recovery

Comparison of Calibration Technique for D8535 soil and biosolids method in Sand

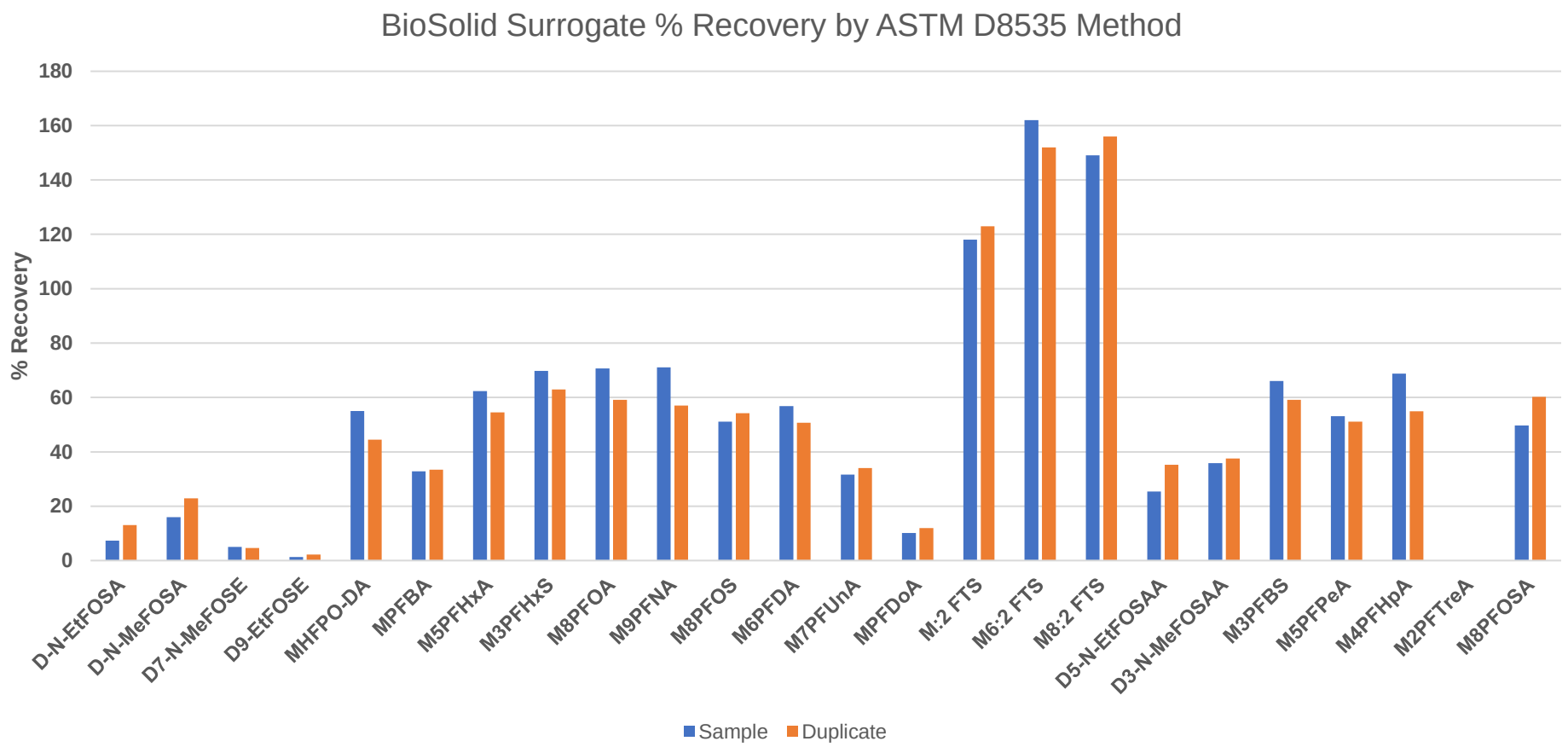


Isotope dilution over corrects, but both results still within 70 -130% recovery

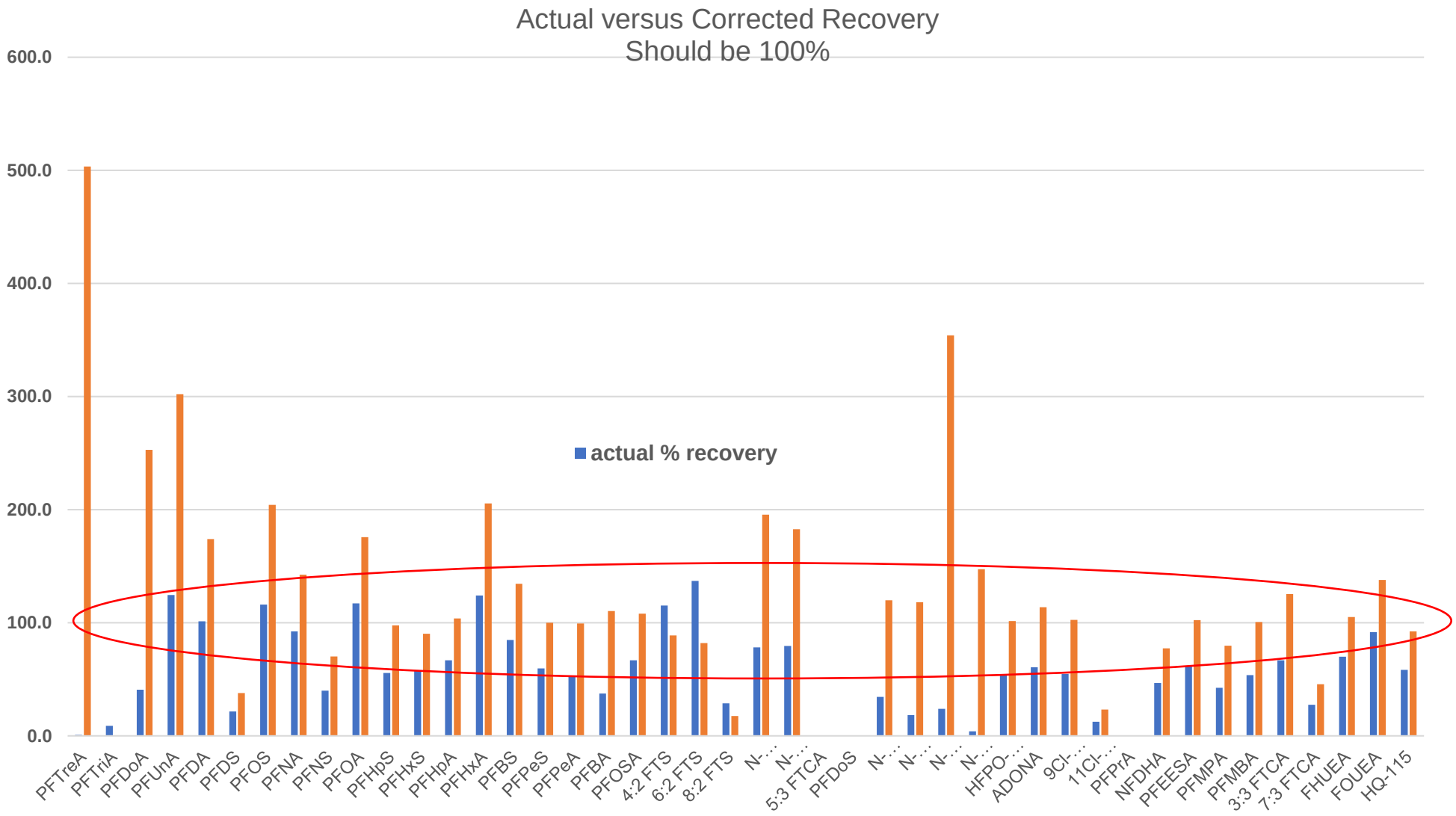
Analysis of Biosolids by ASTM D8535 → very high in some analytes



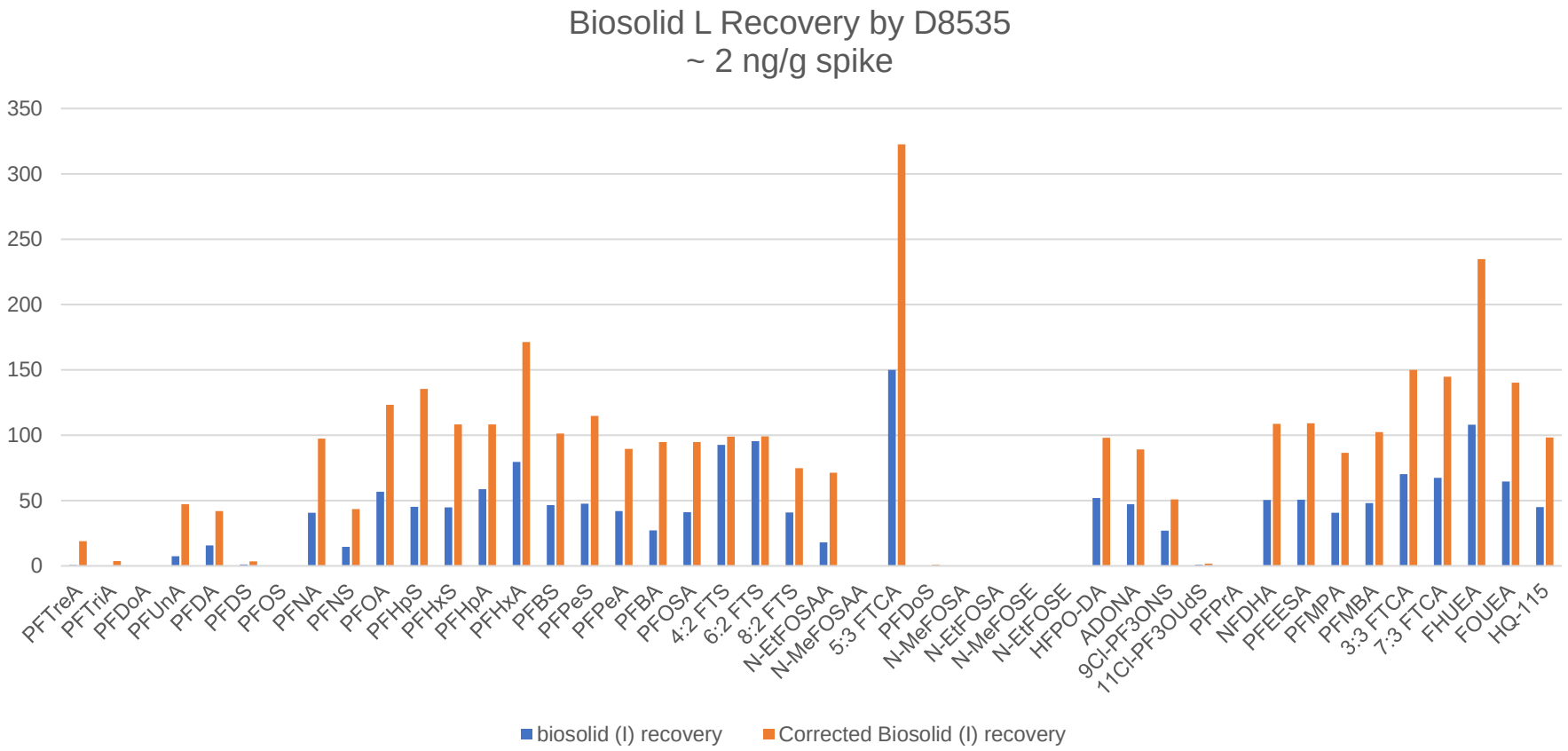
Recovery of surrogates (or isotope dilution standards) in biosolid sample



About ½ of analytes are better with isotope dilution and other over correct by a lot. Low level (~ 0.5 ppb) in biosolids

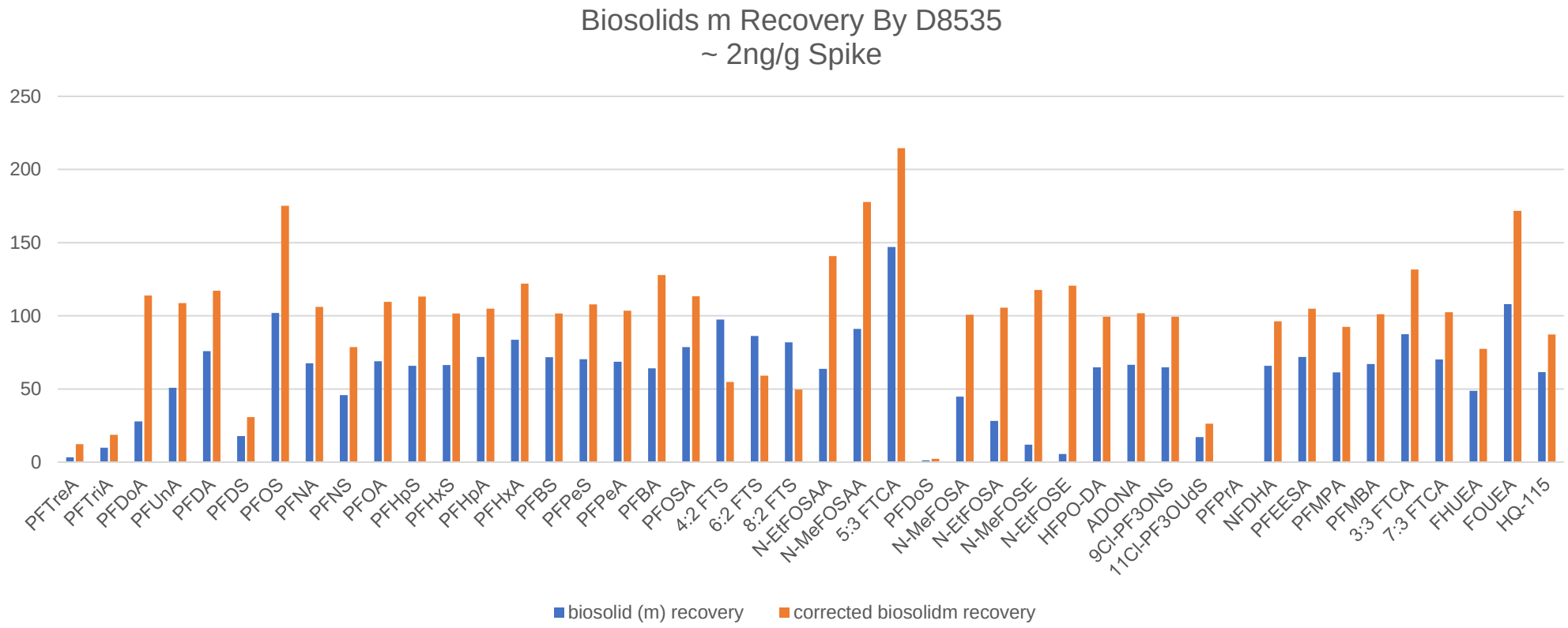


Comparison of Recovery in another biosolid sample.



Generally, the corrected data is better but some analytes over correct.

Comparison of recovery in another biosolid sample



With a few exceptions, the corrected results are better than the non-corrected results.

Conclusions

- The calibration technique involves confirming that an instrument produces the correct result.
- Correct results can only be assumed by analyzing spiked samples, or samples of known concentration.
- The method extraction, digestion, and instrument operation needs to be optimized and recovery determined with external standard calibration first, before applying corrections.
- ASTM Method D8421 obtains equivalent recovery in all matrices, therefore equivalent results in all matrices tested by either external or internal standard calibration.
- ASTM D8535 obtains equivalent recovery in sand and silt matrices (other similar with data not shown) regardless of internal or external calibration.
- For complex matrices like biosolids, internal standard calibration (isotope dilution) may be necessary.

Questions?

wclipps@shimadzu.com