False Positives, False Negatives, and Interferences:
A Deep Dive into the PFAS Data

January 21, 2026

Today's Presentation

"Level 2" versus "Level 4" Data Packages

False Positives/ Interferences

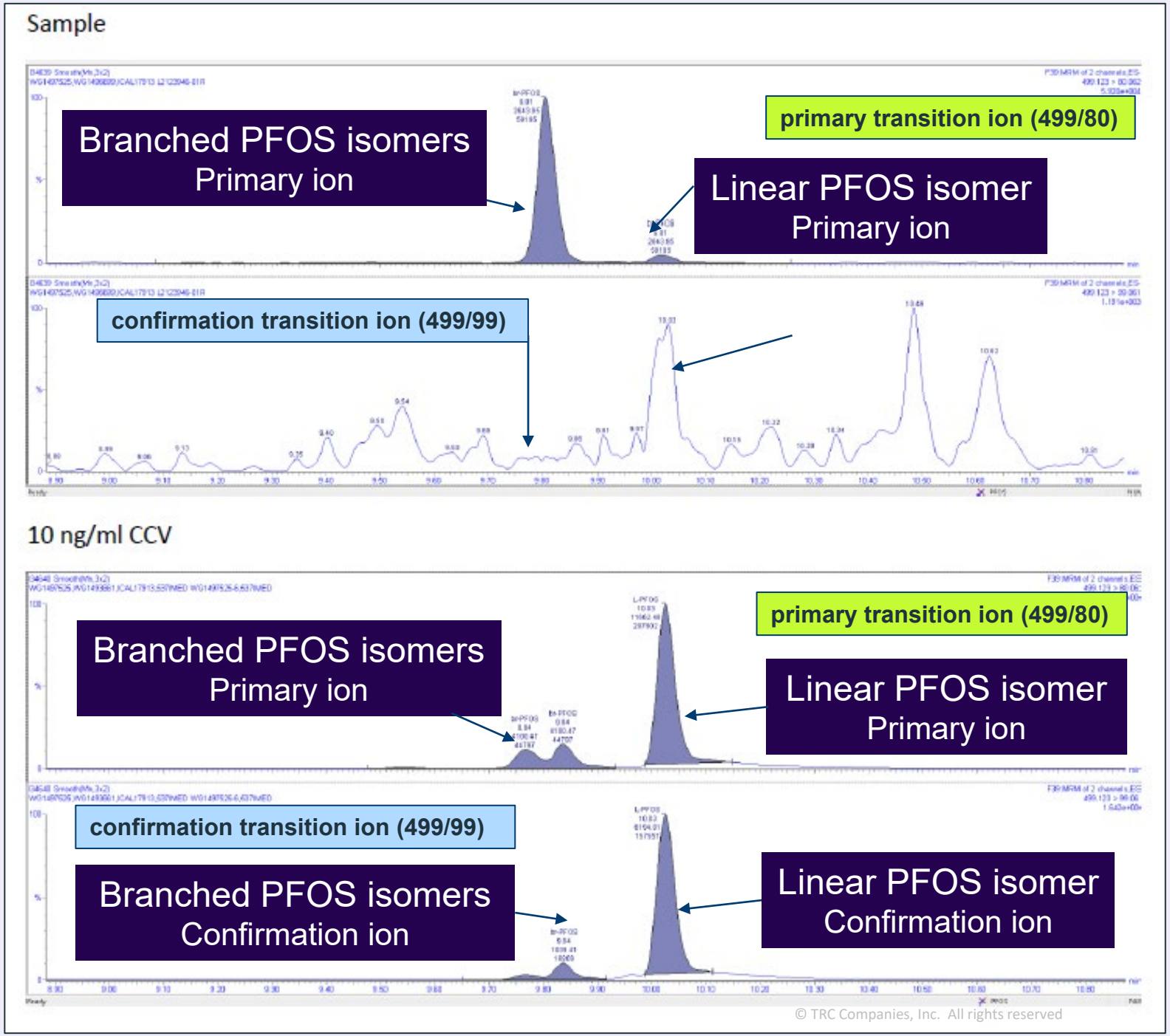
TRCCOMPANIES.COM

Confirmation Ions: Why Important?

- **Definitive Identification of Compounds**
  - Retention time from HPLC separation
  - Transition to characteristic daughter ions (primary & confirmation ions)
  - Ion ratios
- **What happens when the ion ratios are outside limits?**
  - Potentially suspect positive result
  - Lab may qualify result
- **What if there is no confirmation ion?**
  - PFBA
  - PFPeA
  - NMeFOSE
  - NEtFOSE
  - PFMPA
  - PFMBA

Examples				
Analyte	**Retention Time (min)**	**Primary/ Confirmation Ions**	**Ion Ratio**	**Ion Ratio Limit**
PFBS	4.79	299/80 299/99	2.91	1.35-4.05
PFOS	7.59	499/80 499/99	4.19	2.04-6.12
PFOA	6.16	413/369 413/169	3.0	1.72-5.10

NOTE:
EPA Method 1633A and 537.1 require the use of confirmation ions.
EPA Method 533 does not require confirmation ions.



Issue:

- Sample first analyzed using EPA Method 533 (not pictured here). **PFOS = 2,680 ng/L**
- Asked lab to reanalyze using Modified 537

Observations:

- PFOS peaks in sample did not produce ion ratio signatures similar to standard
- Not all branched isomers of PFOS produce same confirmation ion: can make identification of branched PFOS isomers questionable since not monitoring all confirmation ions

How Should Lab Report This?

- If 533, report as is.
- If 1633 or 537 mod, may vary by lab:
  - ND due to lack of confirmation ion
  - As is with knowledge that not all branched PFOS isomers produce same conf ion
  - As is with ion ratio qualifier

Ion Ratios out: Detection or Nondetect?

Bile Acid Interferences

Compound	Parent Ion	Primary Ion	Confirmation Ion
PFOS	499	80	99
TDCA	498	80	107
TCDCA	498	80	107
TUDCA	498	80	107

- **PFOS reported as false positive in samples since Bile Acids have common transition ion (80)**
- **PFOS also measured using 499→99 allowing Interference to be eliminated**

NPDES Monitoring of Effluent: False PFOS Result?

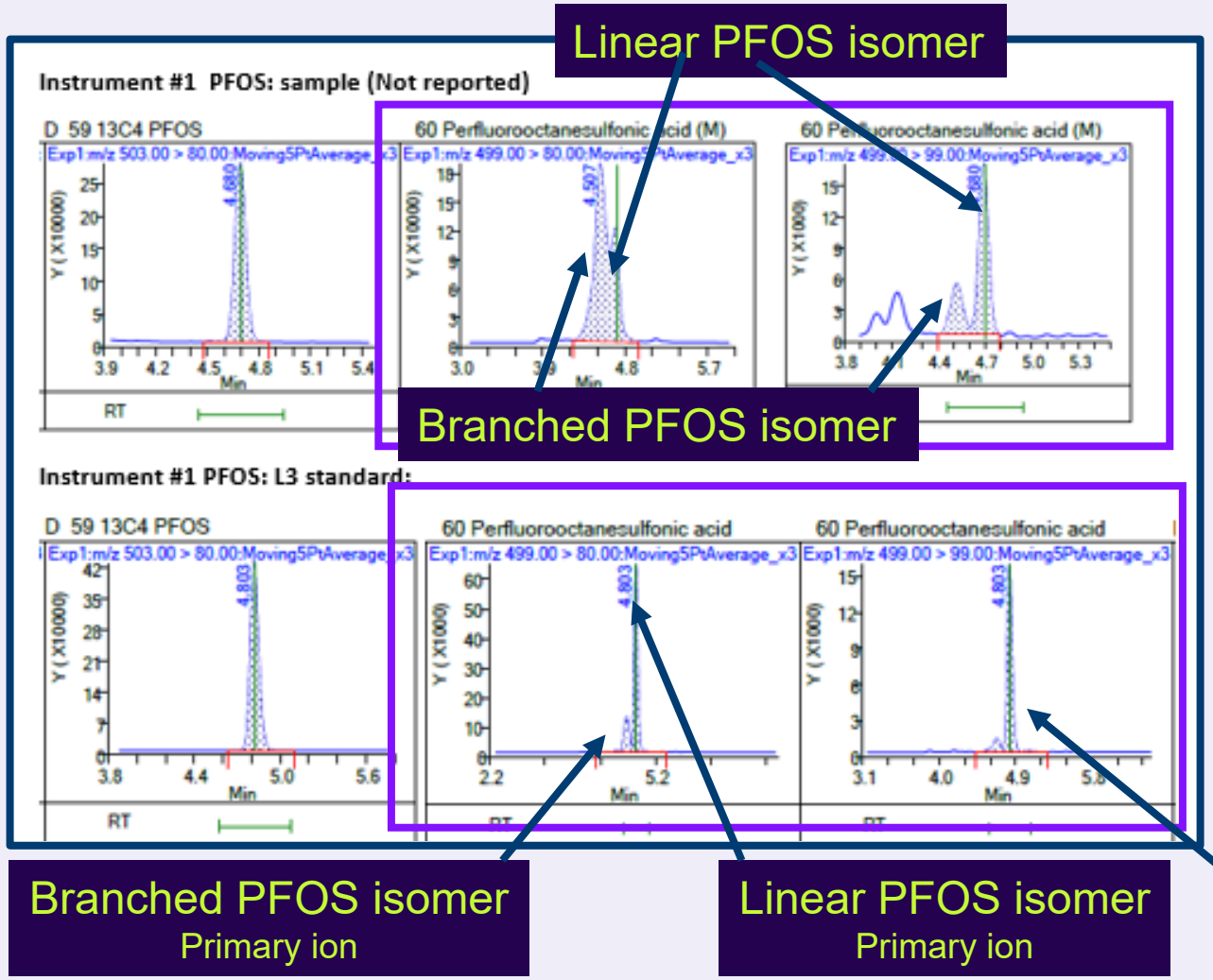
	Original Instrument #1	Confirmation Instrument #2	
PFOA (ng/L)	29	22	* ICAL & CCAL ok both instruments * EIS %Rs ok both instruments * Ion Ratios ok both instruments
PFOS (ng/L)	78	21	* ICAL & CCAL ok both instruments * EIS %Rs ok both instruments * Ion Ratios: outside limits instrument #1; ok instrument #2

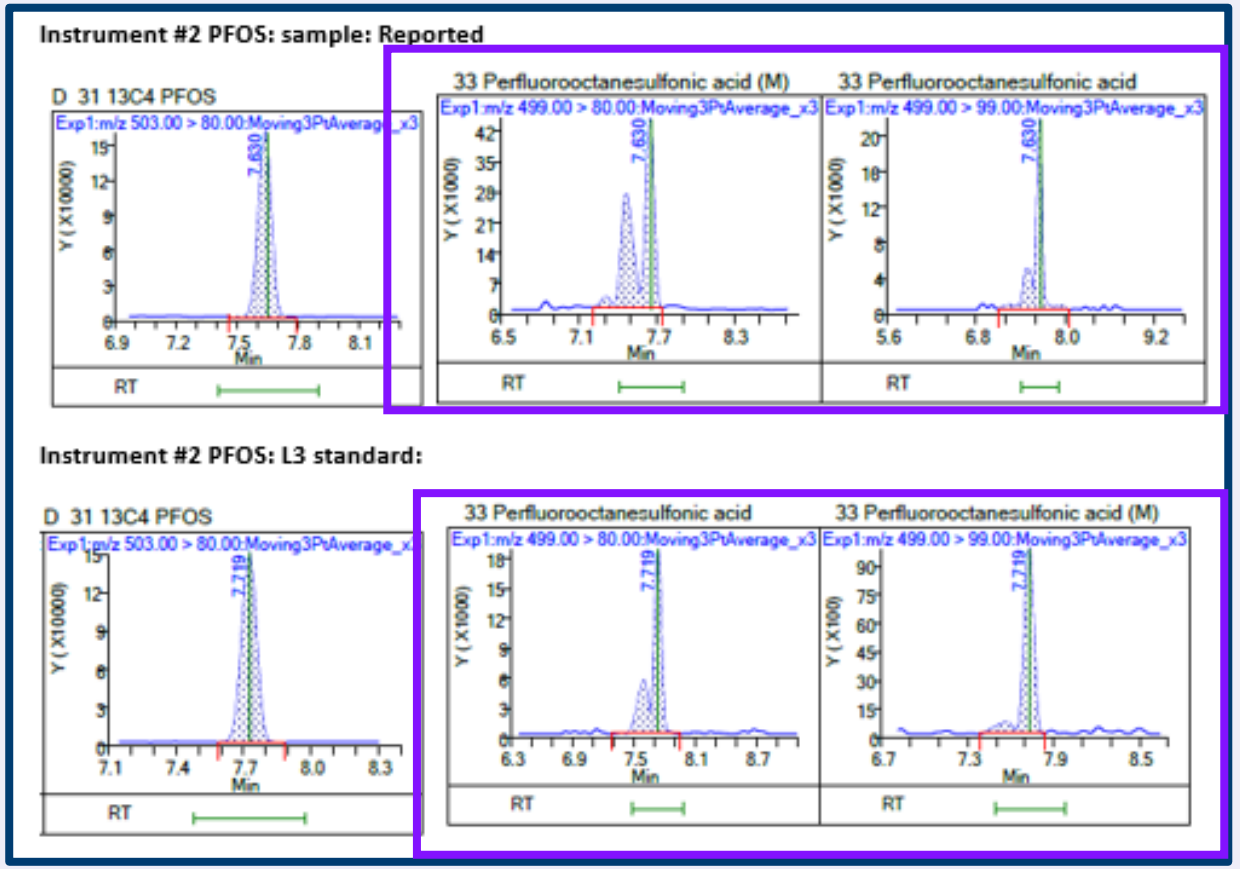
ICAL = Initial Calibration
CCAL = Continuing Calibration
EIS = Extracted Internal Standards
%R = Percent Recovery

NPDES Monitoring of Effluent: False PFOS Result? *(continued)*

Instrument #1: PFOS: Ion Ratio Out: PFOS: 78 ng/L

Instrument #2: PFOS: Ion Ratio ok: PFOS: 21 ng/L





Bile Acid Interferences

Compound	Parent Ion	Primary Ion	Confirmation Ion
PFOS	499	80	99
TDCA	498	80	107
TCDCA	498	80	107
TUDCA	498	80	107

- **PFOS reported as false positive in samples since Bile Acids have common transition ion (80)**
- **PFOS also measured using 499→99 allowing Interference to be eliminated**

Lessons Learned

- Ask the lab when something does not look right.
- Ion ratio anomalies and interference with PFOS can be common.
- Think about this issue for any historical PFOS data you are looking at.
- Remember EPA method 533 does not require the use of confirmation ions.

False Negatives

TRCCOMPANIES.COM

Field Duplicate Results from Level 2 Report

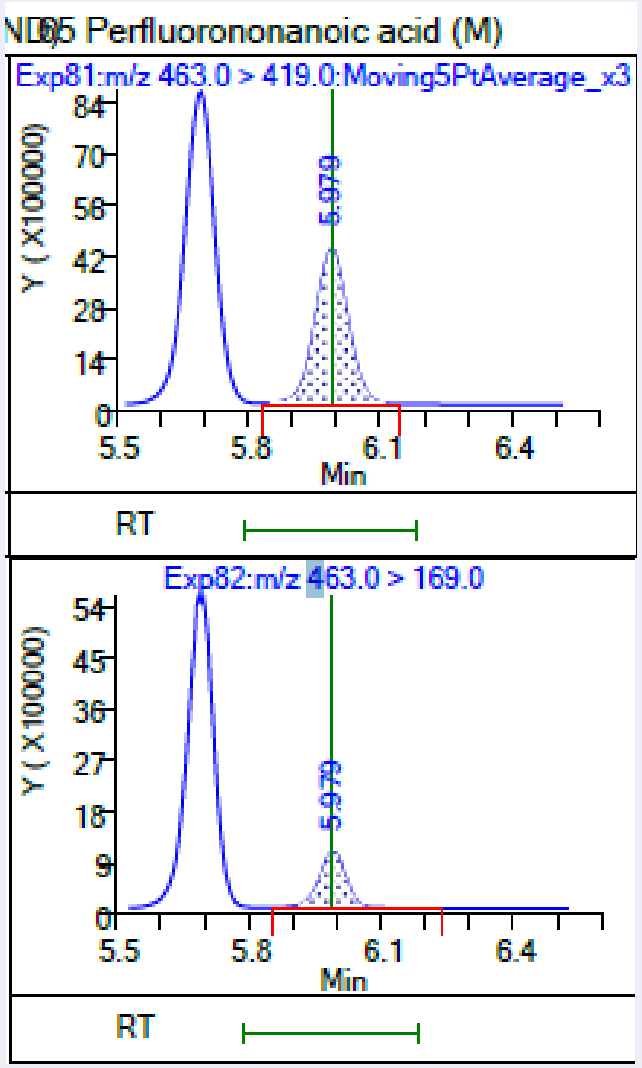
PFAS	GW Sample (ng/L)	Field Duplicate (ng/L)
PFOA	33	31
PFBS	1.4 J	1.3 J
PFHxS	0.96 J	0.82 J
PFOS	5.4	5.0
PFNA	290	1.8 U

Issues:

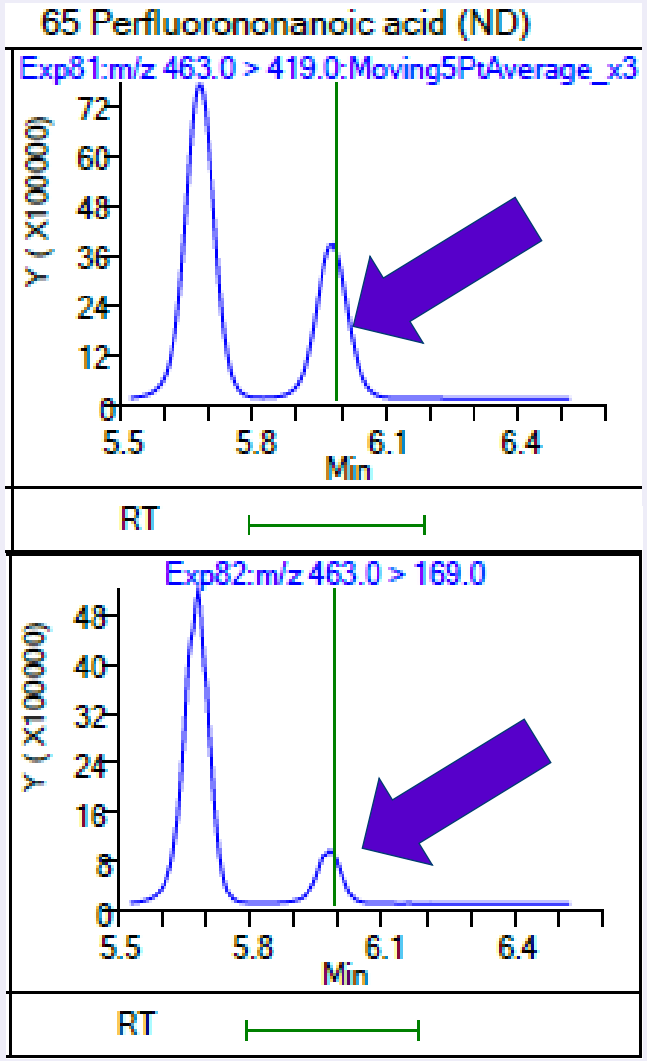
- Reviewing a Level 2 Report
- PFNA results did not look right

Missing Peak Integrations

GW Sample



Field Duplicate



- We happened to also have Level 4 reports.
- Upon review, noticed PFNA not integrated in field duplicate sample.
- Requested lab revise and review all other data generated for project.

PFNA (ng/L)	
Before	After
1.8 U	9.2
1.8 U	16
1.8 U	1.4 J
1.8 U	290
1.8 U	14
1.9 U	14
1.9 U	1700
1.9 U	350
1.9 U	620
1.9 U	350

Regulatory Criteria:
20 ng/L
(sum of 5 PFAS)

PFHpA (ng/L)	
Before	After
1.7 U	12
1.9 U	22
1.7 U	11
1.7 U	7.5
1.8 U	14
1.8 U	1.6 J
2.0 U	23
1.7 U	5.9
1.8 U	9.0
1.8 U	6.7

Regulatory Criteria:
20 ng/L
(sum of 5 PFAS)

After Lab Reviewed all Peaks for Missing Integration

- Revised data showed false negative results originally reported
- Some of revised data went from ND to causing a regulatory criteria exceedance

Why Did This Happen?

- All errors were due to one analyst who was not properly trained
- Proper secondary review had not been performed in the lab to catch this error prior to reporting

Isotope Dilution
(but not really)

TRCCOMPANIES.COM

Isotope Dilution

- **True isotope dilution:** response of target analyte is compared to response of its isotopically labeled analog (EIS). 24 target PFAS quantified in this way in EPA 1633.
  - 13C4-PFBA used to quantify PFBA
  - 13C8-PFOA used to quantify PFOA
  - 13C8-PFOS used to quantify PFOS
- **Extracted internal standard quantification:** response of target analyte is compared to response of isotopically labeled analog of another compound with chemical and retention time similarities. 16 target PFAS quantified in this way in EPA 1633.
  - 13C3-PFHxS used to quantify PFPeS
  - 13C8-PFOS used to quantify PFNS
  - 13C5-PFPeA used to quantify 3:3 FTCA

Table 10 in EPA 1633 defines which isotopically labeled analog (or EIS) is to be used for each target PFAS

Isotope Dilution: What is It?

- Sample spiked with KNOWN amount of extracted internal standards (EIS) (aka labeled surrogates)
- EIS match target analytes
  - $^{13}C_4$PFBA is EIS associated with PFBA
  - $^{13}C_4$PFOS is EIS associated with PFOS
- Target result corrected by proportional amount based on isotope
- **BENEFITS:**
  - Corrects for analytical error associated with matrix
  - Corrects for matrix interferences

EPA 537 and ASTM Method do NOT utilize isotope dilution

EPA 533, EPA 537 modified, and EPA 1633 use isotope dilution

$$\text{Concentration Target} = \frac{\text{Target Area} * \text{True Concentration Isotope}}{\text{Area EIS} * \text{Calibration Factor}}$$

How Did The Lab Quantify PFAS?

Remember: EIS used to quantify PFAS should have chemical and retention time similarities to target PFAS (it is supposed to mimic behavior of target PFAS)

PFHpS	5:3 FTCA	PFNS, PFDoS	PFTrDA	9Cl-PF3ONS
Quantified with: 13C6-PFDA	Quantified with: 13C8-PFOS	Quantified with: 13C7-PFUnA	Quantified with: D7-MeFOSE	Quantified with: 13C4-PFHpA
7-carbon sulfonic acid quantified using 10-carbon carboxylic acid	carboxylic acid quantified using sulfonic acid	9 and 10-carbon sulfonic acid quantified using 11-carbon carboxylic acid	carboxylic acid quantified using sulfonamido ethanol	ether sulfonate quantified using carboxylic acid

How Did This Impact Results?

Target PFAS	PFPeS	PFHpS	PFNS	PFDoS	PFDS	9Cl-PF3ONS	11Cl-PF3OUdS	3:3 FTCA	PFMPA	5:3 FTCA	7:3 FTCA
EIS Used by Lab	13C3-PFBS	13C6-PFDA	13C7-PFUnA	13C7-PFUnA	D5-EtFOSA	13C4-PFHpA	13C5-PFPeA	13C4-PFBA	13C4-PFBA	13C8-PFOS	13C8-PFOSA
EIS Required to be Used by Method	13C3-PFHxS	13C8-PFOS	13C8-PFOS	13C8-PFOS	13C8-PFOS	13C3-HFPO-DA	13C3-HFPO-DA	13C5-PFPeA	13C5-PFPeA	13C5-PFHxA	13C5-PFHxA
Sample 1											
%R of EIS Required to Be Used by Method	77%	51%	51%	51%	51%	55%	55%	10% *	10% *	43%	43%
%R of EIS Used by Lab	30%	49%	32%	32%	23%	63%	10% *	2% *	2% *	51%	63%
	Result biased high	No sig. effect	Result biased high	Result biased high	Result biased high	Result biased low	Result biased high	Result biased high	Result biased high	Result biased low	Result biased low

Undefensible Reporting Limits or LOQs

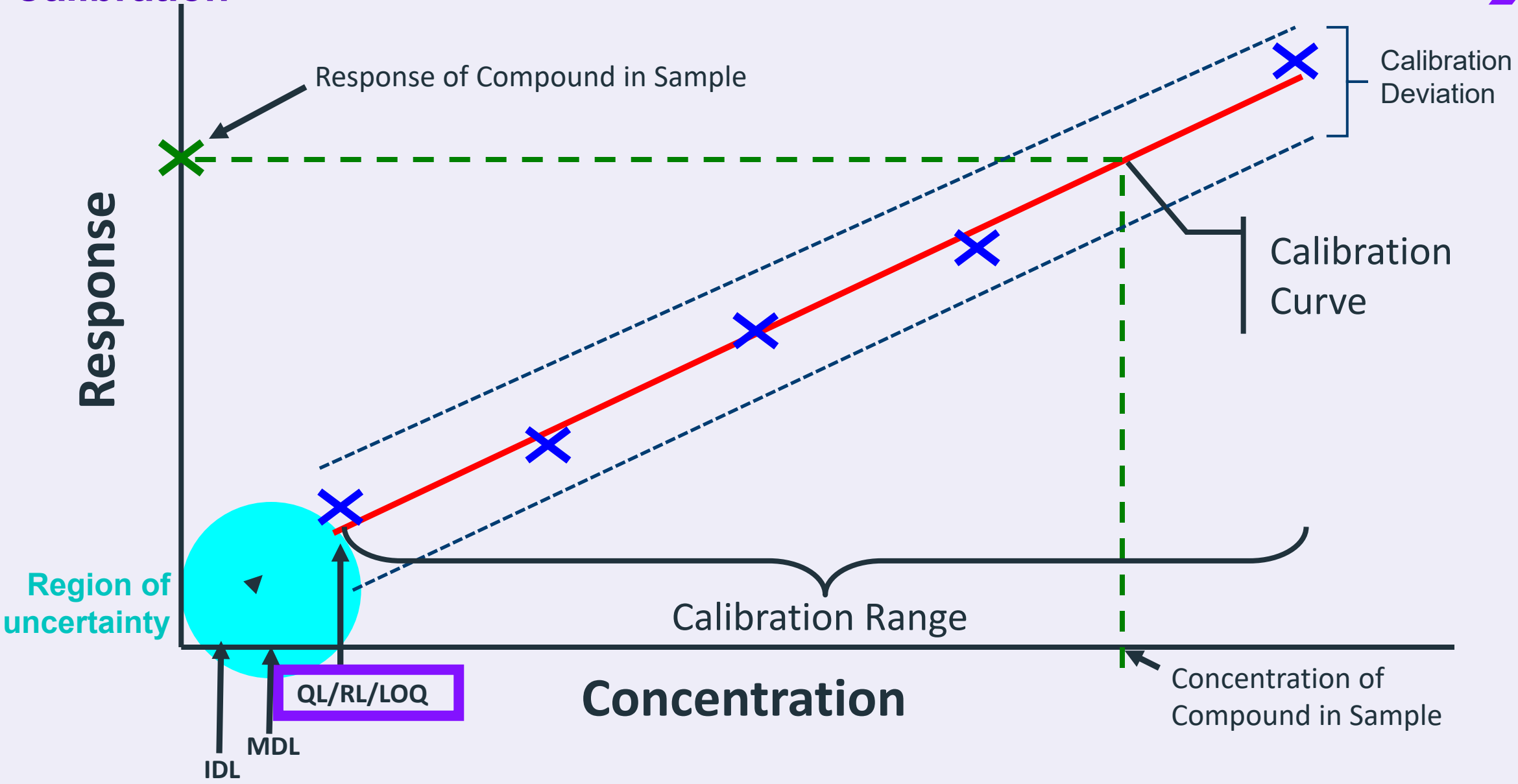
TRCCOMPANIES.COM

Reporting Limits by EPA 1633

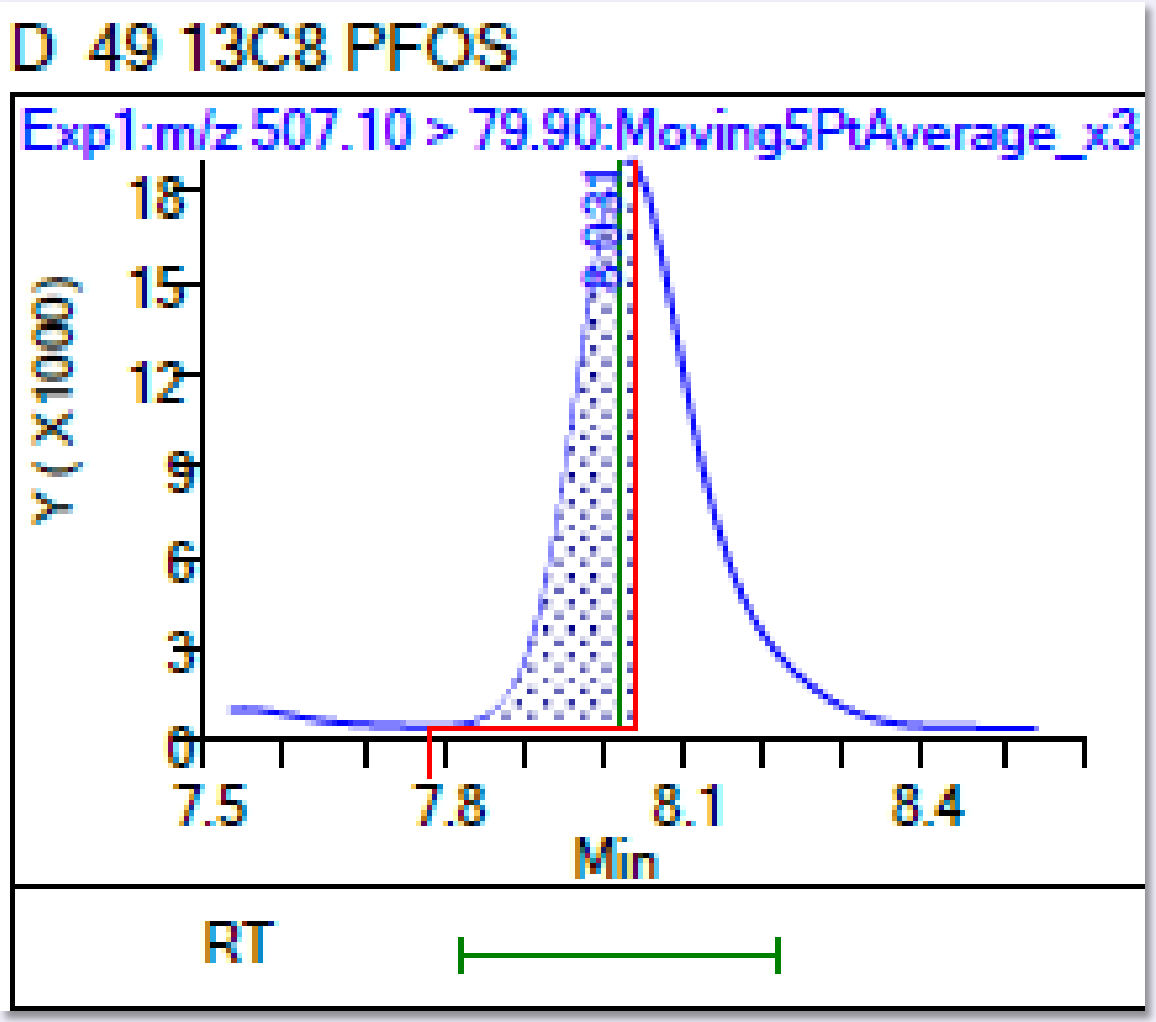
Reporting Limit or Limit of Quantitation (LOQ) requirements per EPA 1633: established by the laboratory through calibration of the instrument

The LOQ shall be set at or above the concentration of the lowest initial calibration standard (the lowest calibration standard must fall within the linear range).

Calibration



What Did We See?



- Low standard PFOS = 0.193 ng/mL
- Sample: 100.1 mL extracted to final volume of 5 mL
- LOQ/RL should be ≥ 9.6 ng/L (0.193 ng/mL*5 mL/0.1001 L)
- Reported LOQ/RL = 8 ng/L

- **Why was LOQ below lowest calibration standard concentration?**
- Lab dropped 2 lowest points in curve but did not raise LOQ.
- **Why did lab drop 2 lowest points in curve?**
  - They stated it was because %Rs of affected PFAS in Level 2 standard were >200% & outside criteria (70-130%)
- **Why were %Rs of affected PFAS in Level 2 standard >200%?**
  - Because EIS was not properly integrated; only 50% of peak was integrated
  - Lab did not see this during their own review
- **Resolution**: Lab revising 20 data packages to correctly report LOQs and target PFAS concentrations in each sample by adding 2 lowest points back into calibration curve

Takeaways

If the data do not make sense to you, ASK THE LAB!

In general, labs are doing a good job with PFAS analysis but errors or poor judgment can happen.

Reviewing raw data (Level 4 reports) can be more costly but can also give you more assurance in accuracy of your data.

Thanks!

Call Us:

Elizabeth Denly
PFAS Initiative Leader & Chemistry Director
P: (978) 328-2551

Email Us:

EDenly@TRCCompanies.com

Visit Us:

TRCcompanies.com