Analytical Concerns Associated with Emerging Polyfluoroalkyl Substances

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WELLINGTON LABORATORIES INC.

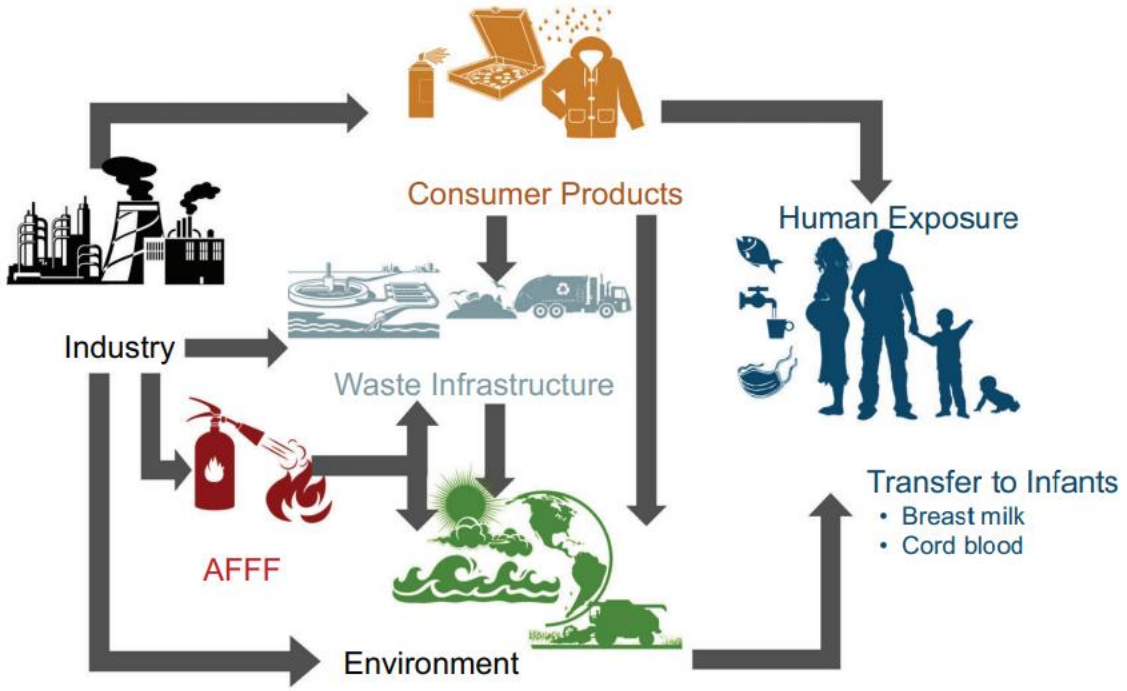
Per- and polyfluoroalkyl substances (PFAS)

- First synthesized in the 1930s

Common Uses:

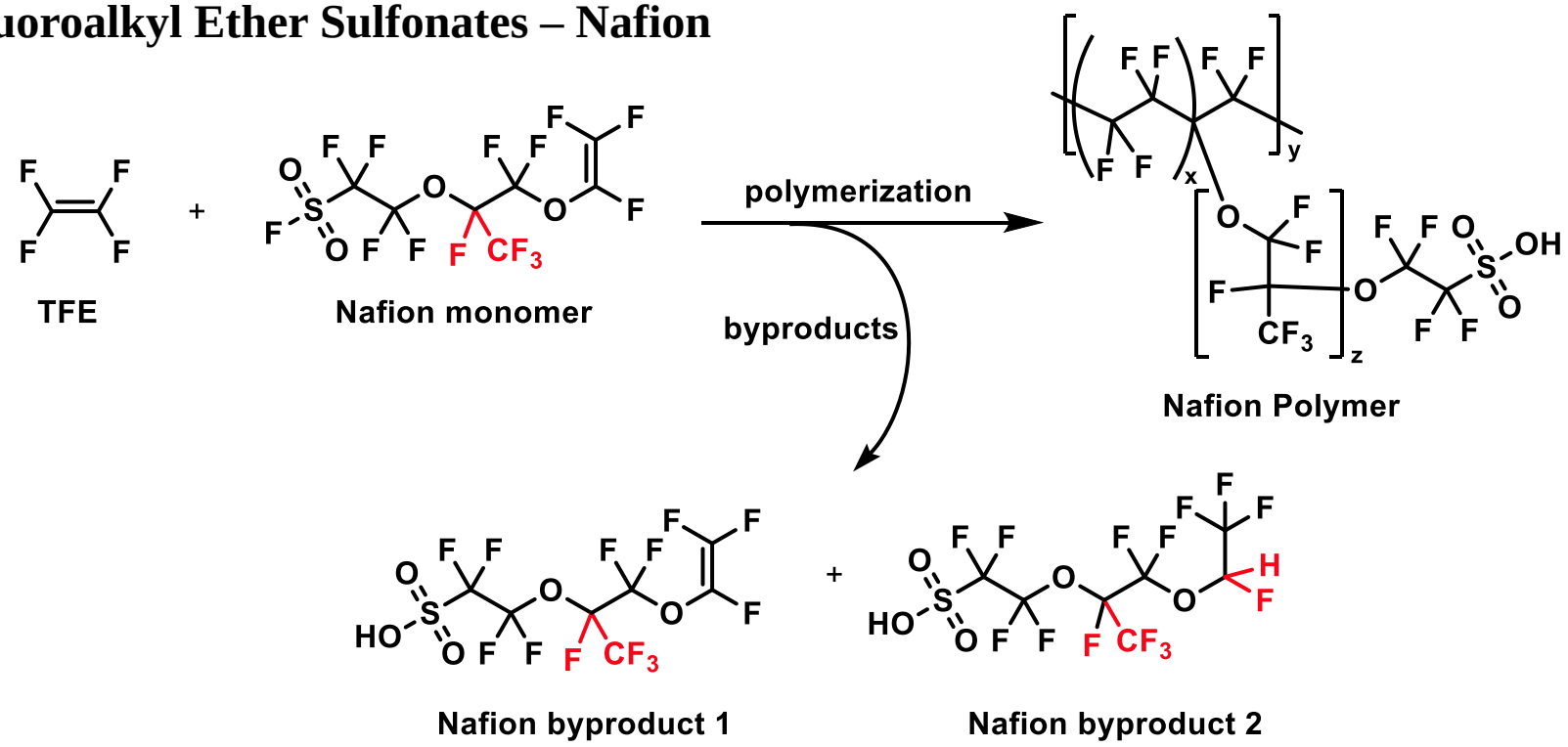
- Non-stick, grease/water resistant coatings
- Aqueous Film Forming Foams (AFFFs)
- Aids in polymerization
  - PFOA used in emulsion polymerization of fluoropolymers

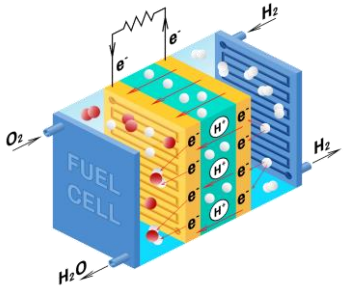
- Persistent & bio-accumulative
  - Found worldwide



Sunderland, E.M. *et al. J Expo Sci Environ Epidemiol* **29,** 131–147.

Perfluoroalkyl Ether Sulfonates – Nafion





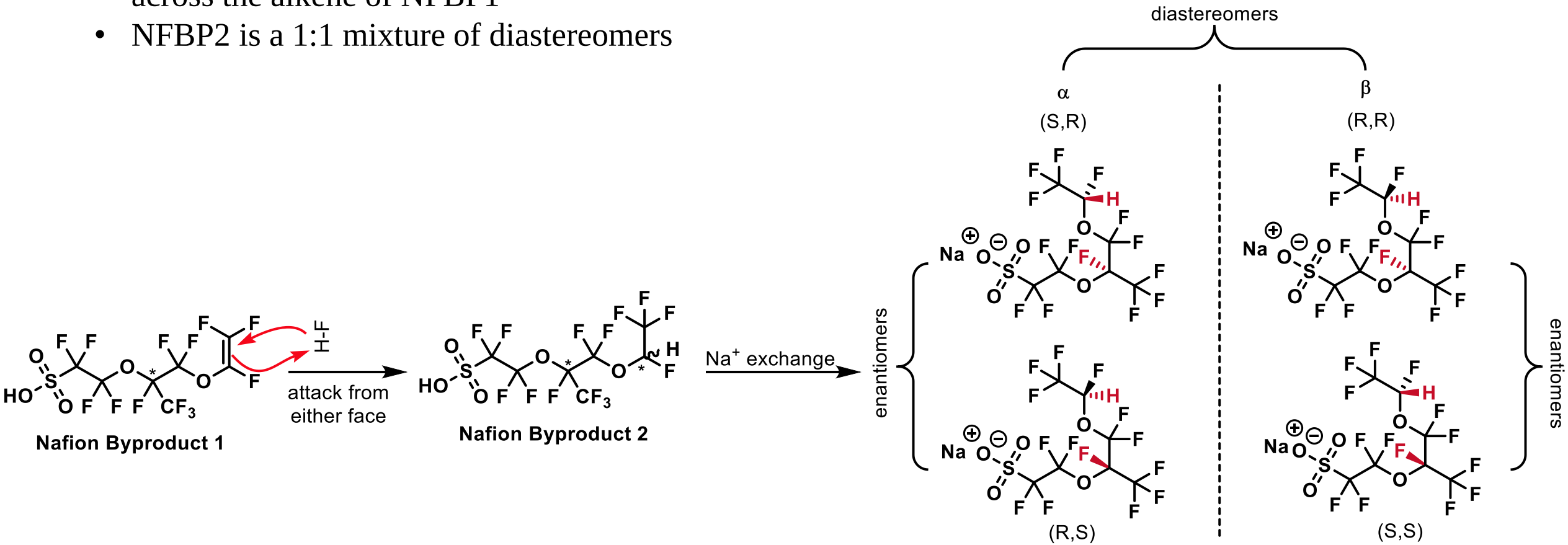
- Fuel cells [a]
- Metal ion recovery
- Plating

- Nafion byproducts were first detected in the Cape Fear River in 2012 [b]
- Mouse toxicology research reports liver enlargement + damage [c]
- NFBP2 has been found in seabirds in the CFR area [d]

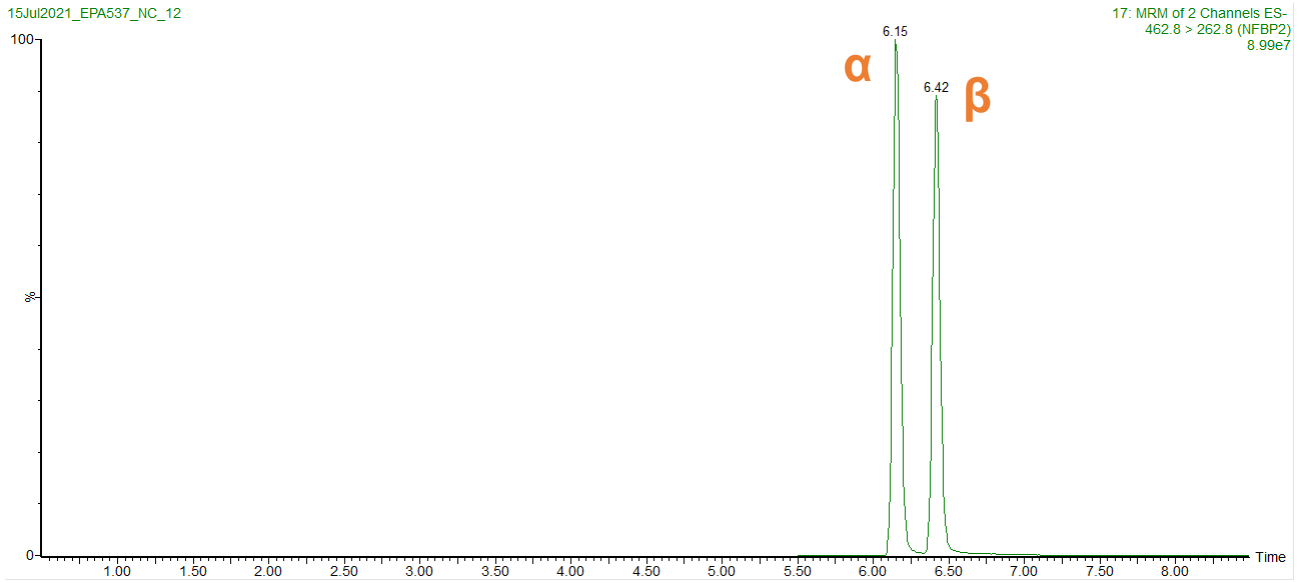
a.) Heitner-Wirguin, C. *J. Membr. Sci.* **1996**, *120*, 1-33 **b**.) Strynar, M. *Environ. Sci. Technol.* **2015**, *49*, 11622–11630
c.) Lang, J. R. *Toxicology* **2020**, *441*,152529 **d**.) Robuck, A.R., *Environ. Sci. Technol.* **2020**, *54*, 12938−12948

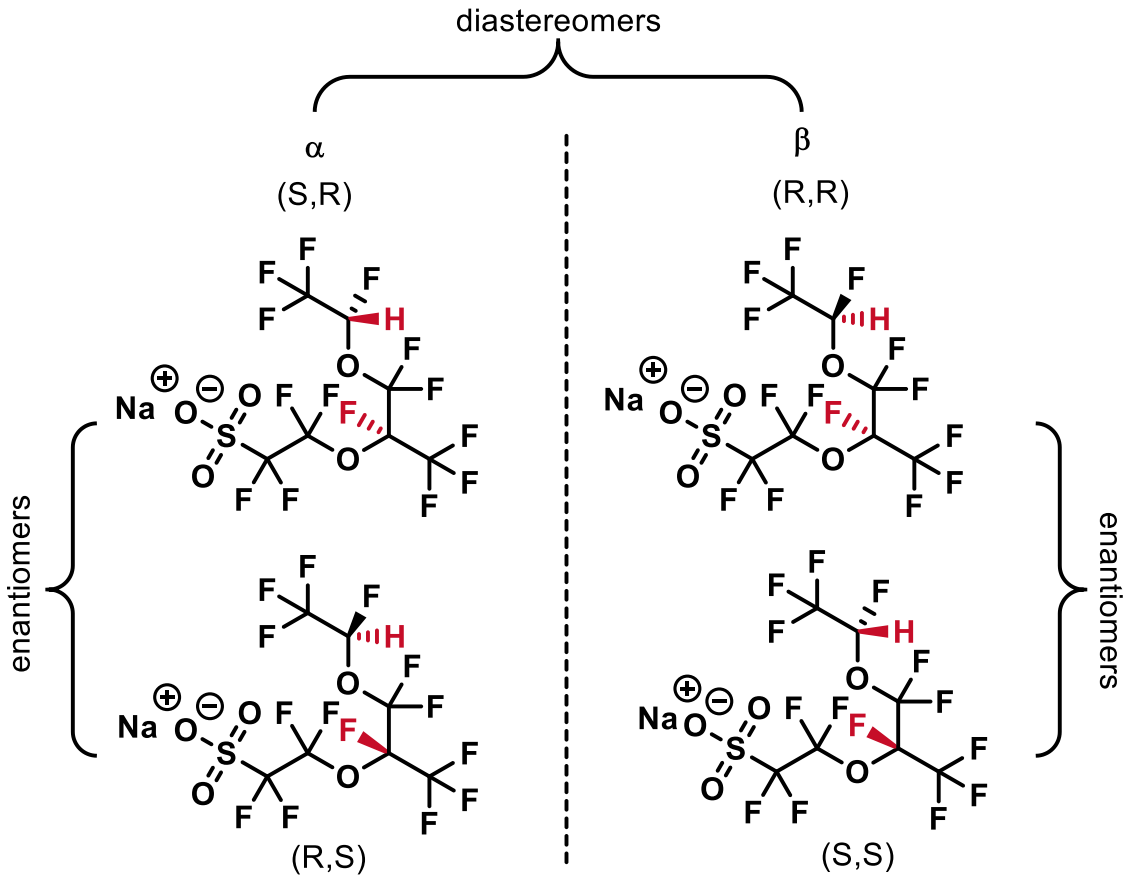
Nafion Byproduct 2 (NFBP2)

- Formation: Strynar *et al*. (2015) suggested H-F addition across the alkene of NFBP1
- NFBP2 is a 1:1 mixture of diastereomers



Characterization: Nafion Byproduct 2 (NFBP2)





MRM Transitions
m/z 463 > 263 (Quantitative Transition)
m/z 463 > 213 (Qualitative Transition)

MS Fragmentation Analysis: NFBP2

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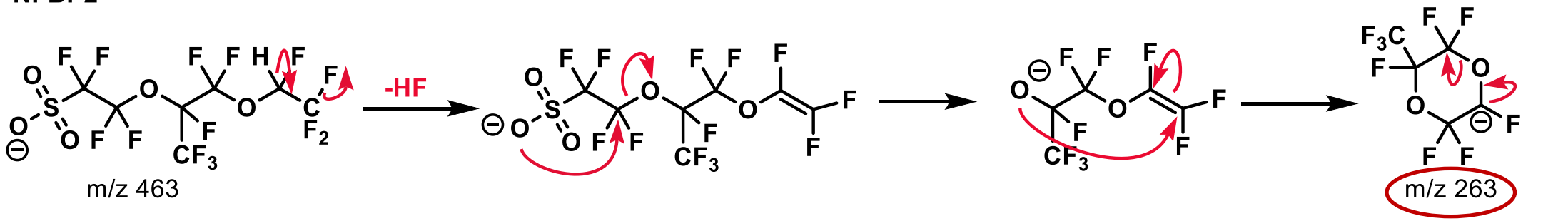
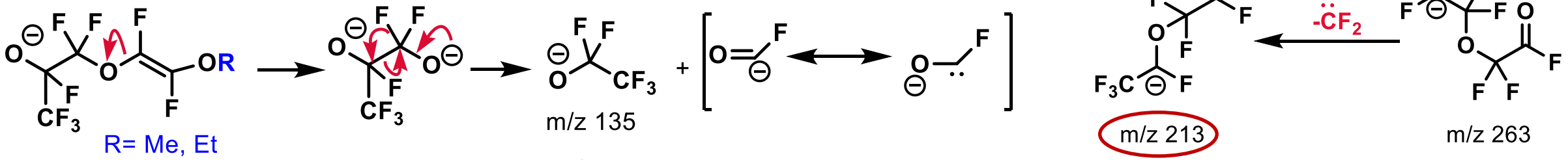
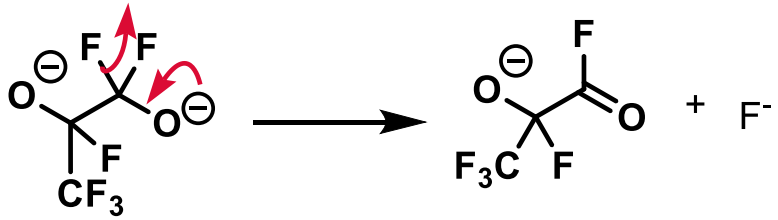
NFBP2



Common fragments m/z 135 and m/z 163

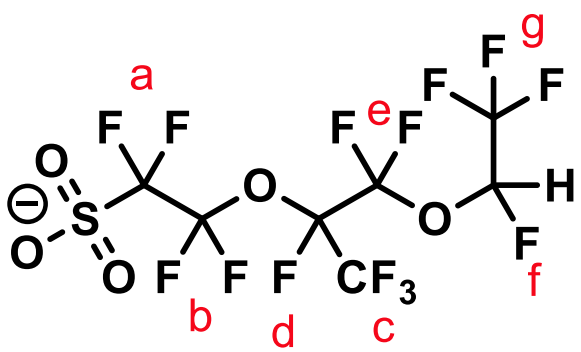


Fragments independent of alkoxy substitution.
Observed in both MeO- and EtO- products.

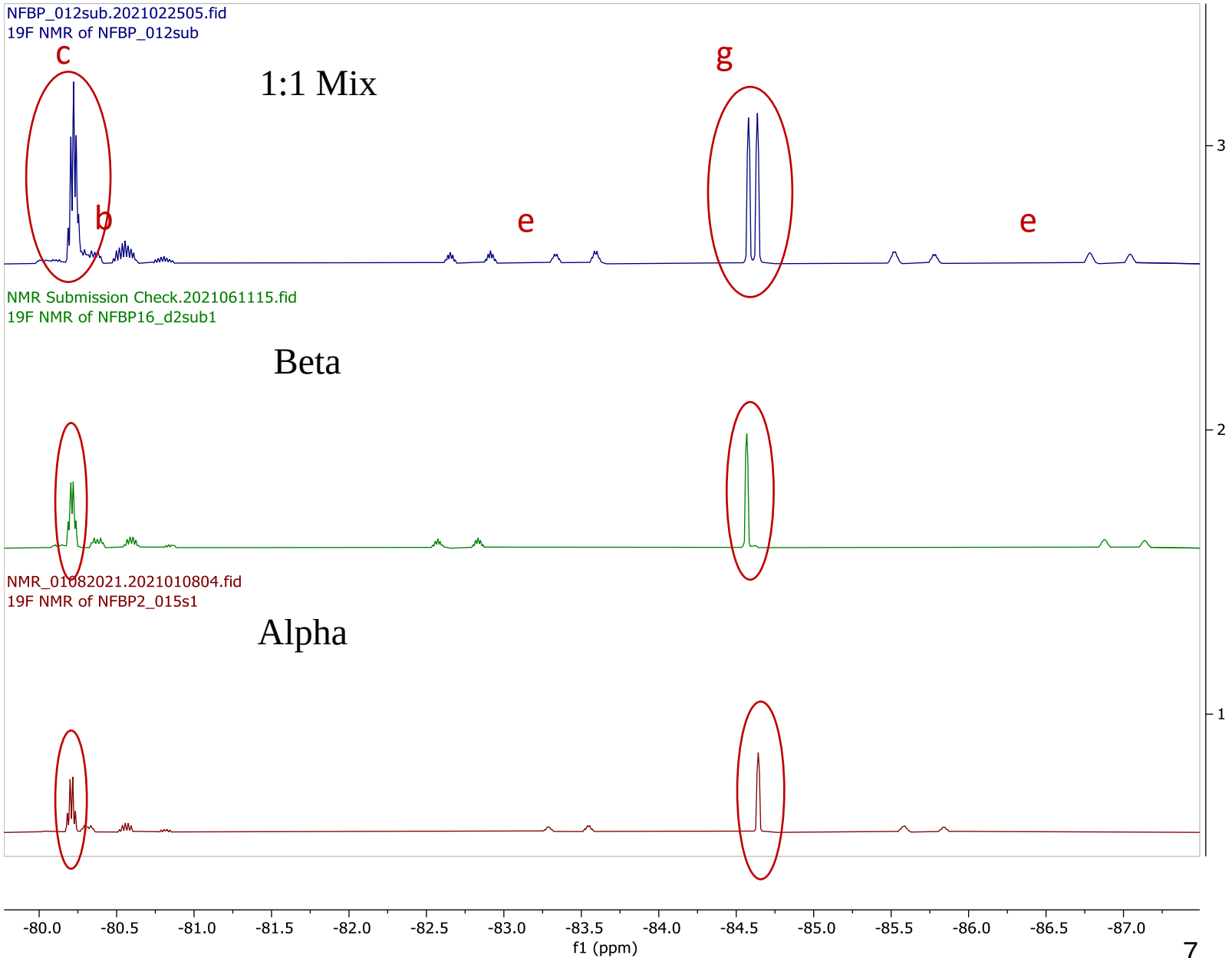


Characterization: NMR Analysis NFBP2

Constitutional or Stereoisomers?



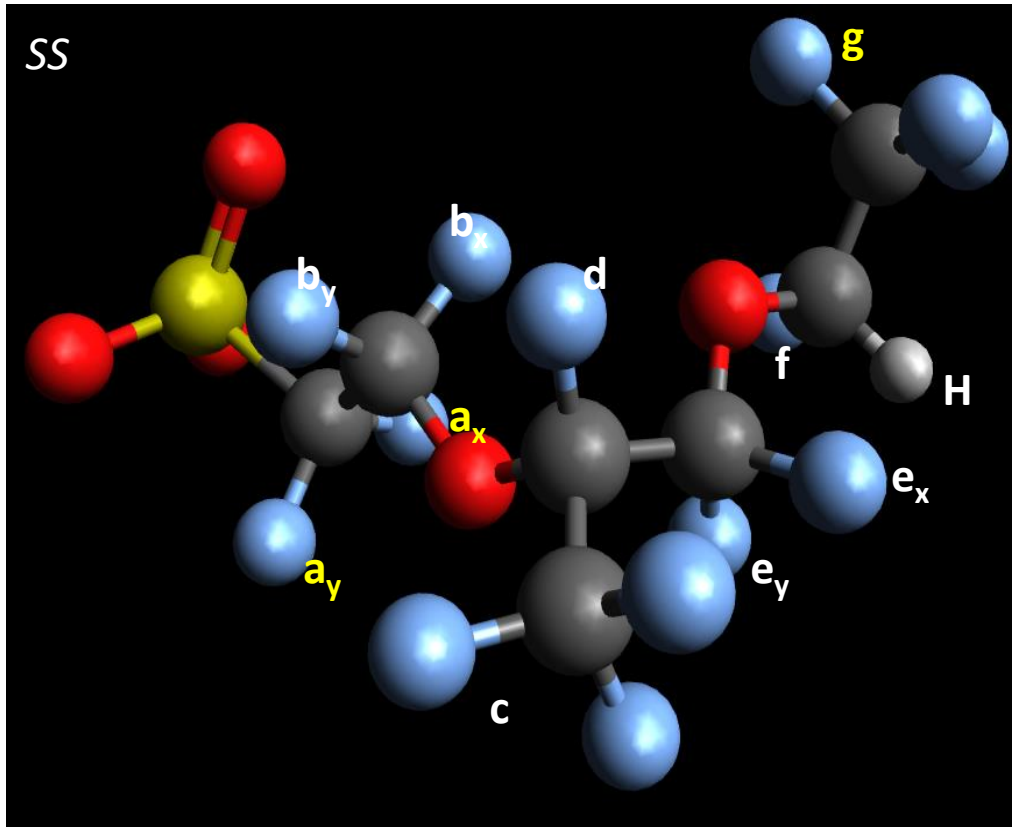
^{19}F NMR confirms diastereomers

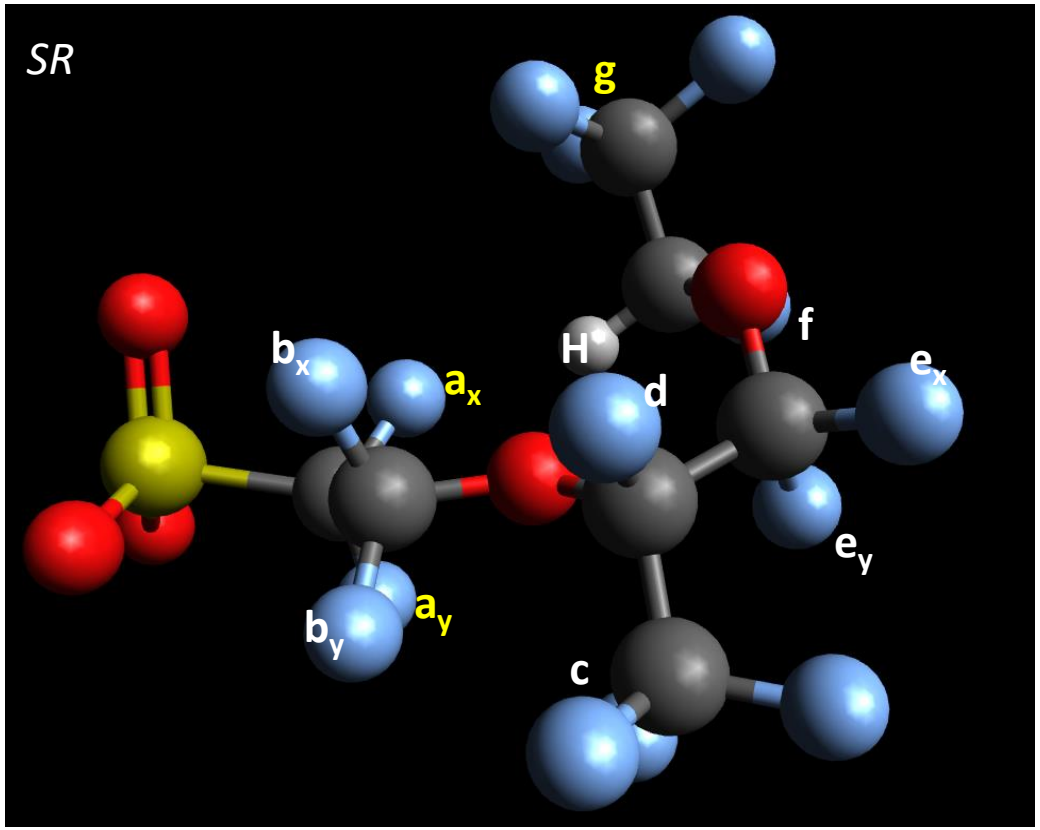


Computational Modeling NFBP2

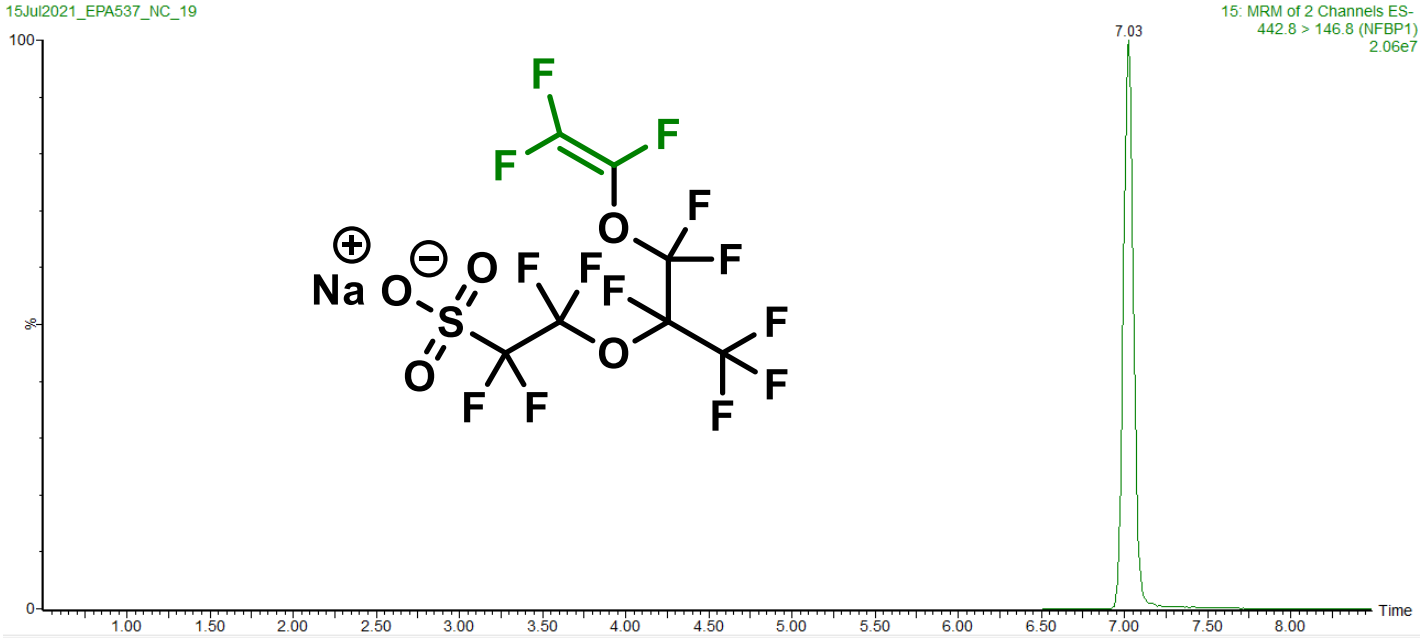
ΔE(SS-SR) = 0.015928105 Hartree
= 9.99 kcal/mol energy difference between isomers

SR has a slightly lower energy (believed to be the alpha isomer but 1D/2D NMR data is not 100% conclusive)



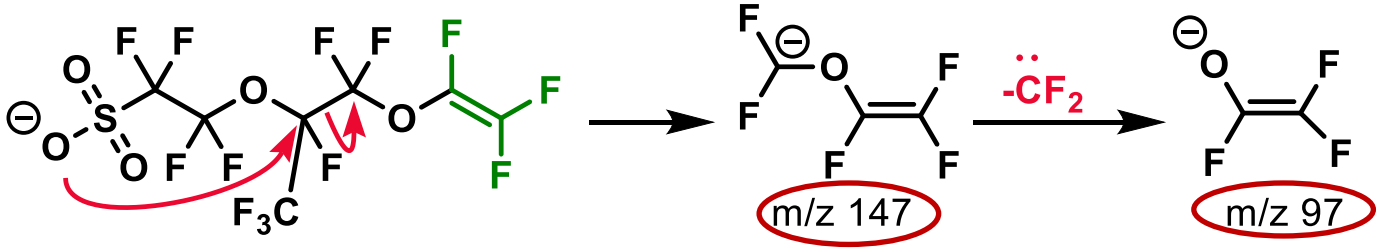


Characterization: Nafion Byproduct 1 (NFBP1)

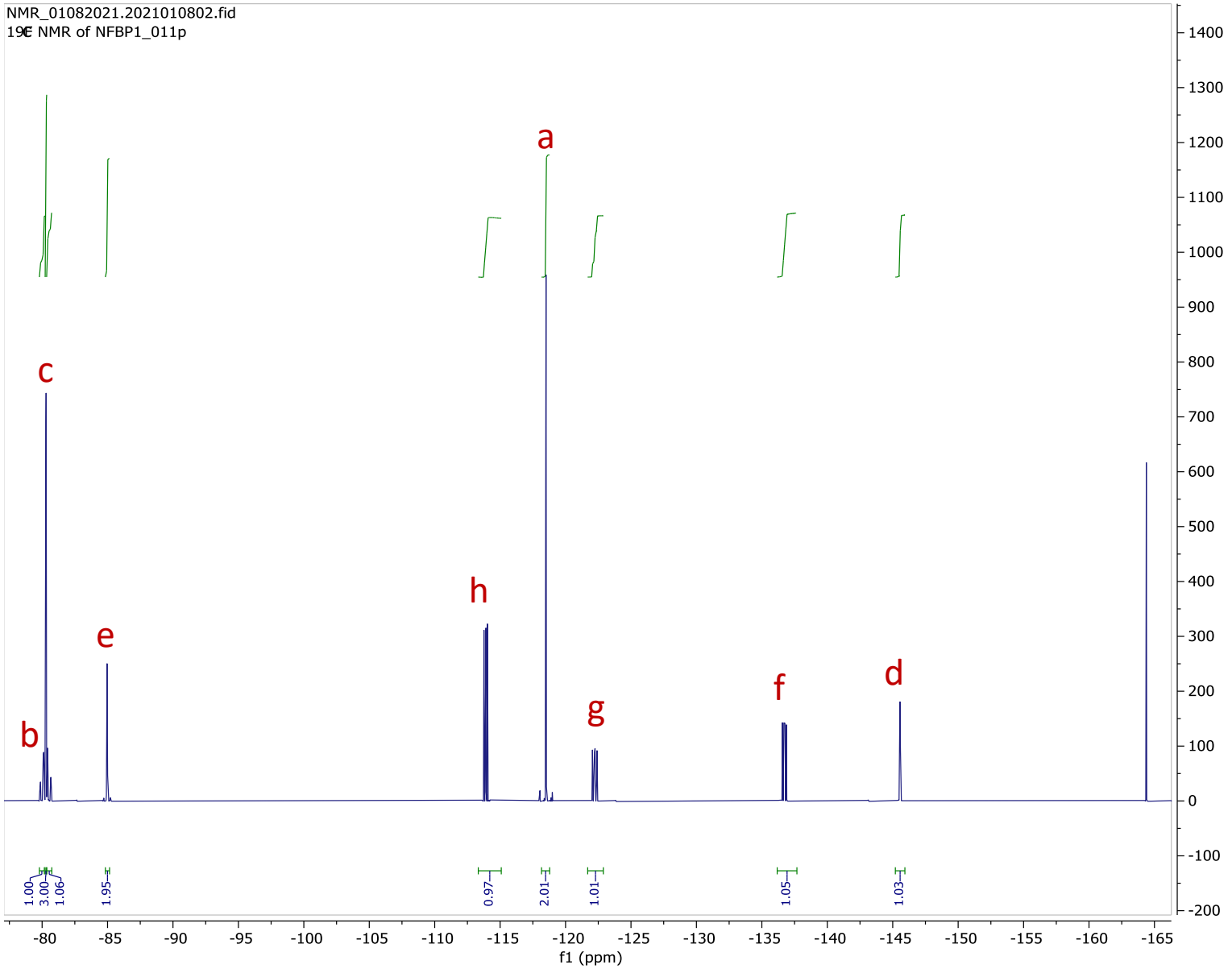


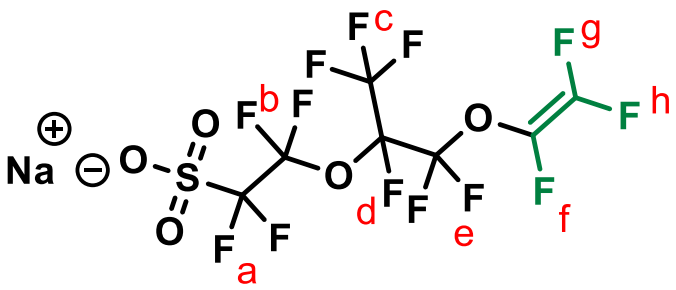
MRM Transitions
m/z 443 > 147 (Quantitative Transition)
m/z 443 > 97 (Qualitative Transition)

NFBP1



Characterization: NMR Analysis NFBP1

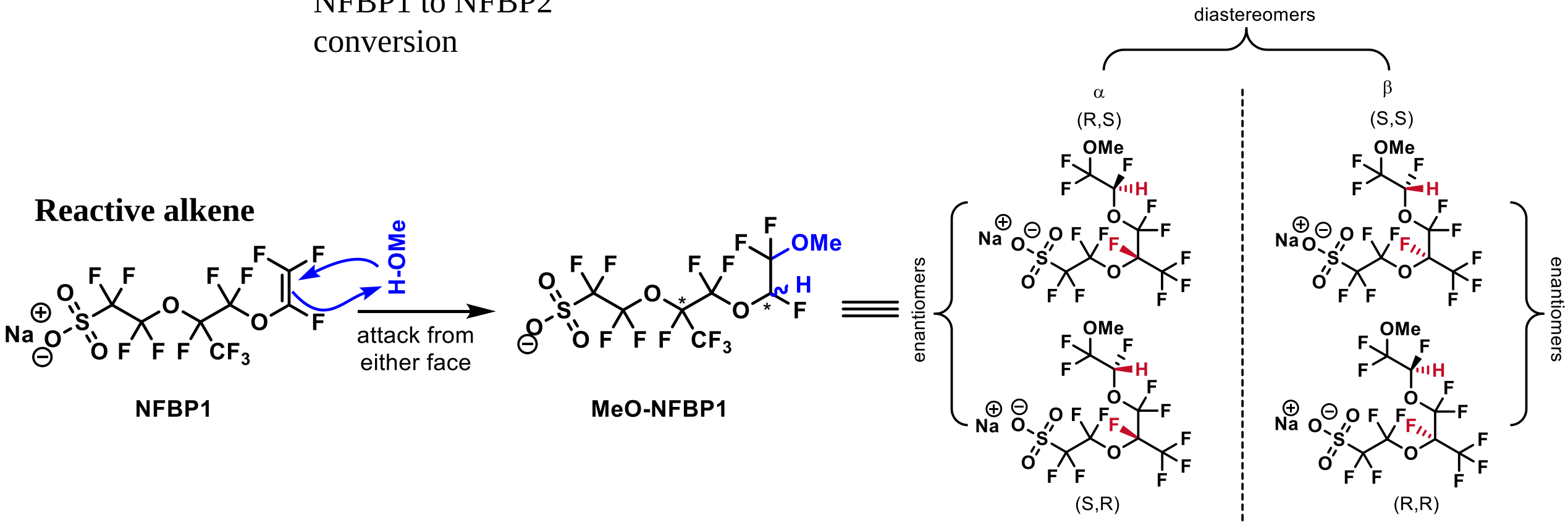




- ^{19}F NMR spectrum agrees with proposed structure

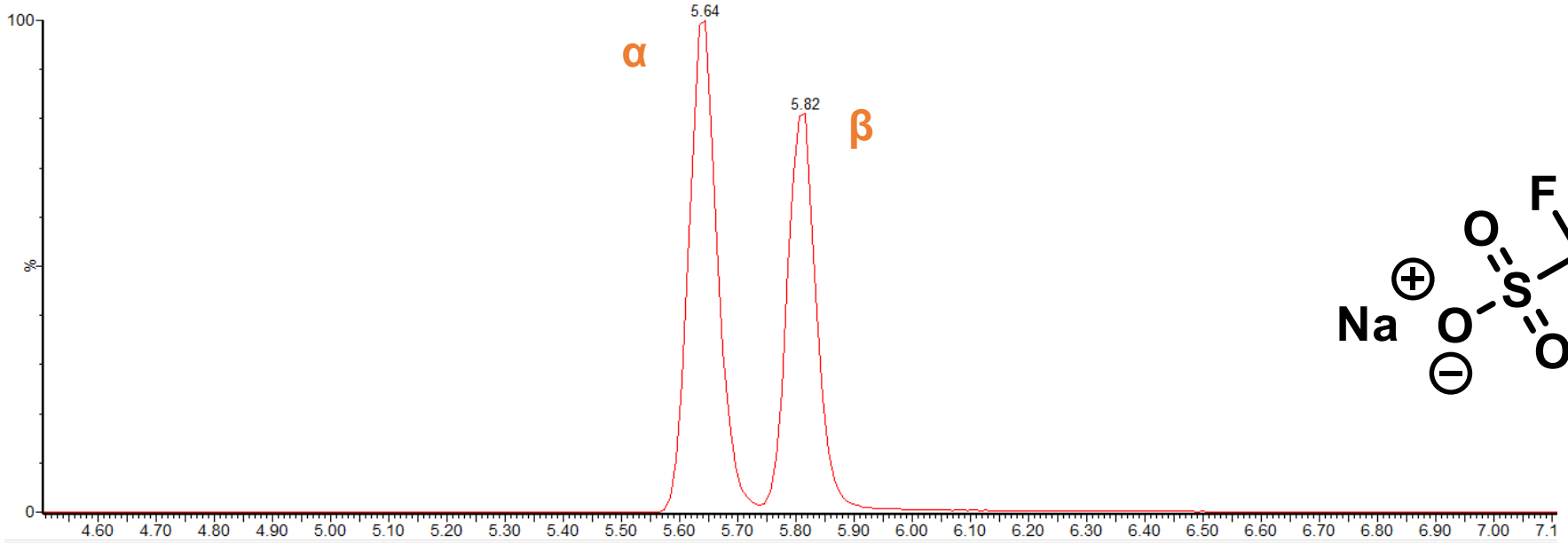
Reactivity of Nafion Byproduct 1 (MeO-NFBP1)

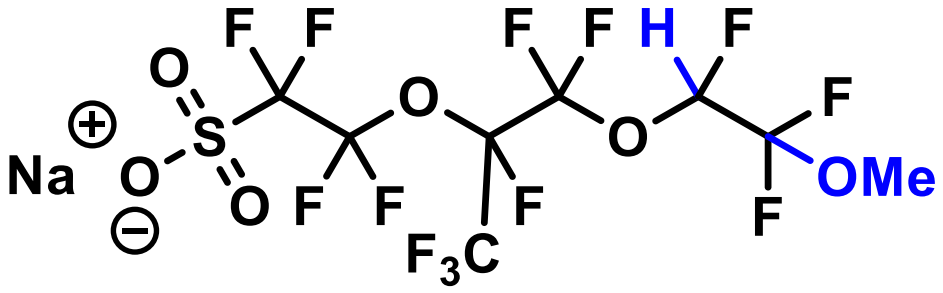
- Same addition mechanism as previously shown for NFBP1 to NFBP2 conversion



Mixture of diastereomers

Methanol Addition to NFBP1 (MeO-NFBP1)





MRM Transitions
m/z 475 > 275 (Quantitative Transition)
m/z 475 > 225 (Qualitative Transition)

MS Fragmentation Analysis: MeO-NFBP1

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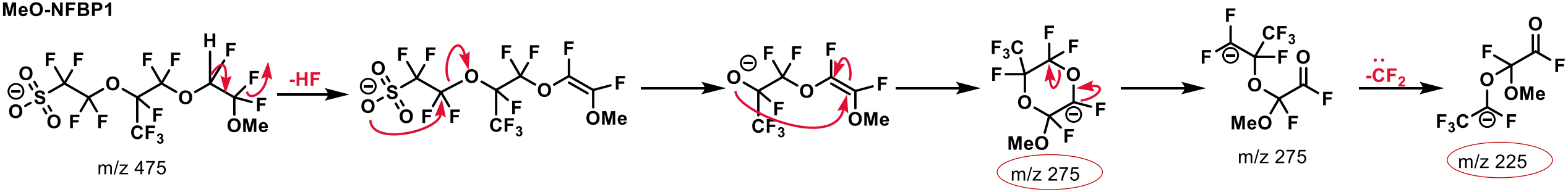
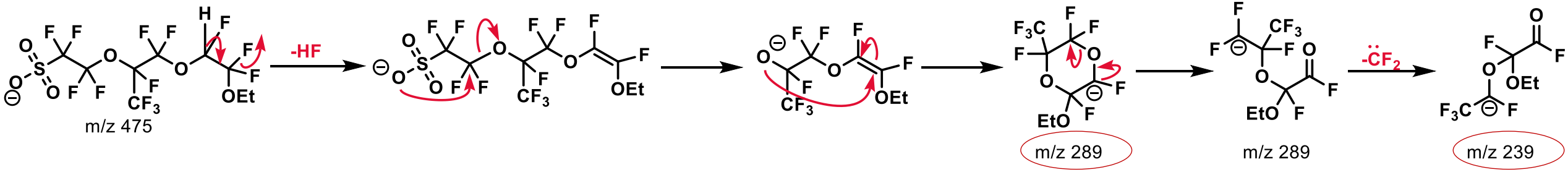
MeO-NFBP1



Fragmentation confirmed by synthesizing the EtO-NFBP1

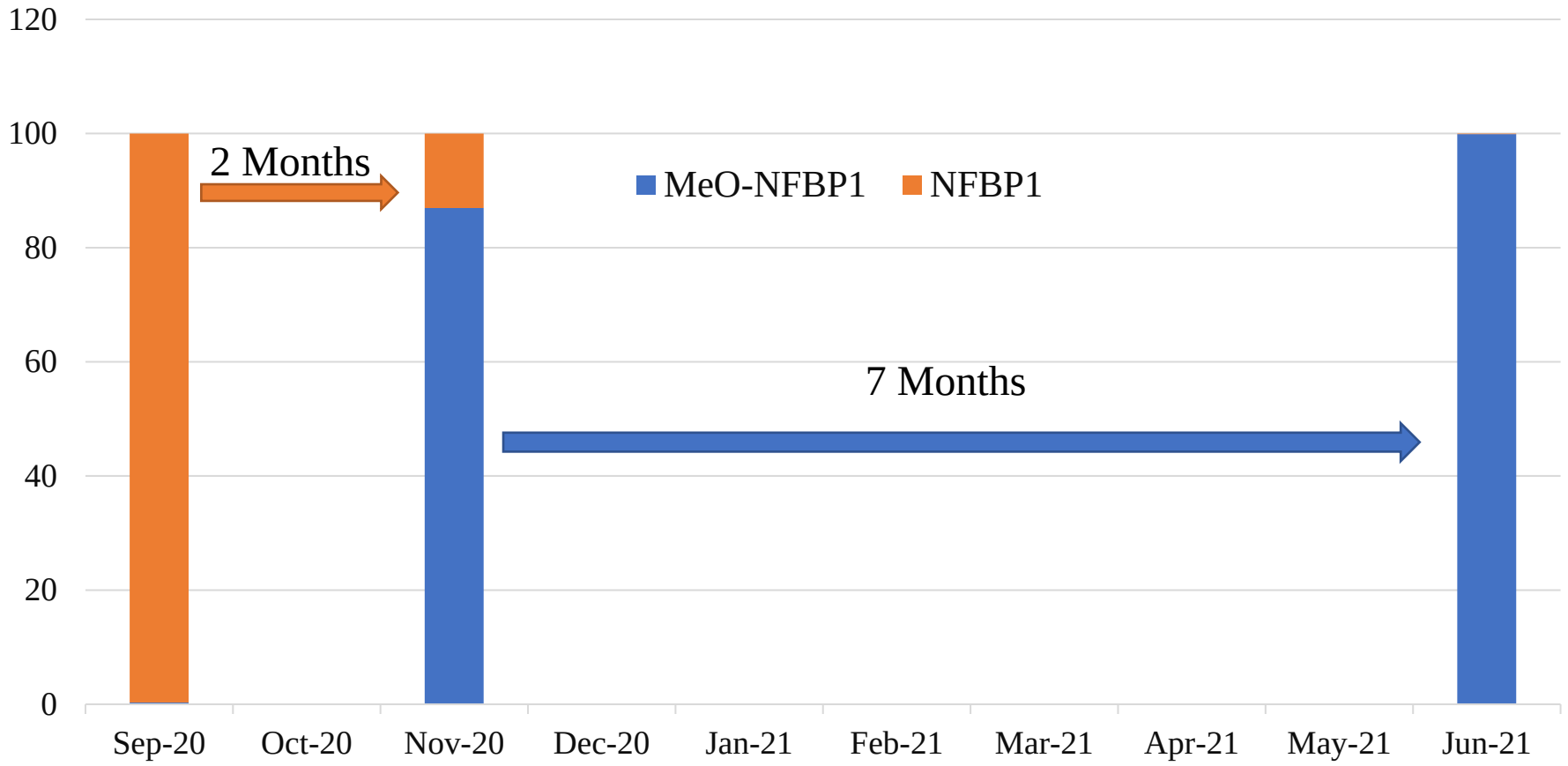
- m/z 239 observed

Check: EtO-NFBP1



Methanol Addition to NFBP1 (MeO-NFBP1)

5 mg of NFBP1 in 1 mL of methanol (19-24°C)
Ambient temp + pressure. Clear glass vial.



Where do we go from here?

PFBA
PFPeA
PFHxA
PFHpA
PFOA
PFNA
PFDA
PFUdA
PFDoA
PFTrDA
PFTeDA
FBSA
FHxSA
FOSA
HFPO-DA
N-MeFOSAA: linear isomer
N-MeFOSAA: ∑ branched isomers
N-EtFOSAA: linear isomer
N-EtFOSAA: ∑ branched isomers

Goal: Expand target list of PFAS analytes in drinking water and analyze real water samples

PFAC30PAR

+NFBP1

+αNFBP2
+βNFBP2

+αMeO-NFBP1
+βMeO-NFBP1

L-PFBS
L-PFPeS
PFHxSK: linear isomer
PFHxSK: ∑ branched isomers
L-PFHpS
PFOSK: linear isomer
PFOSK: ∑ branched isomers
L-PFNS
L-PFDS
4:2FTS
6:2FTS
8:2FTS
NaDONA
9Cl-PF3ONS
11Cl-PF3OUdS

Instrument Parameters

LC Method

- Instrument: Waters Acquity I Class with modified PFAS kit
- Column: Waters ACQUITY UPLC BEH Shield RP18
- Mobile Phase A: 10 mM ammonium acetate in water
- Mobile Phase B: 10 mM ammonium acetate in 80:20 methanol:acetonitrile
- Injection volume: 1.5 µL
- Gradient:

	Time (min)	Flow (mL/min)	%A	%B
1	Initial	0.300	60.0	40.0
2	3.50	0.300	45.0	55.0
3	10.00	0.300	30.0	70.0
4	15.00	0.300	15.0	85.0
5	18.00	0.300	15.0	85.0
6	18.50	0.300	60.0	40.0

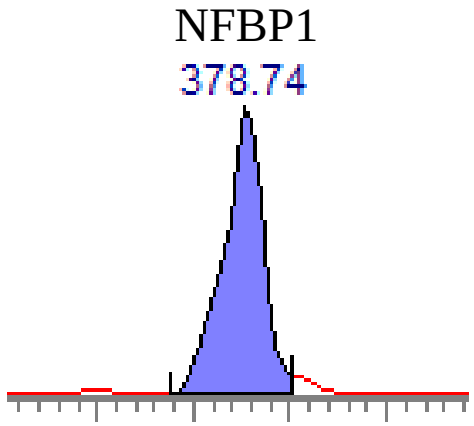
Source Parameters

- Instrument: Waters Xevo TQ-XS
- Ion mode: ESI-
- Capillary voltage: 1.50 kV
- Desolvation temperature: 450°C
- Desolvation gas flow: 1000 L/hr
- Cone gas flow: 150 L/hr

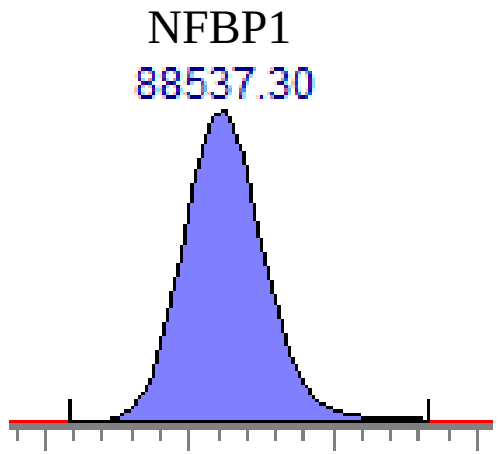
Reference Method Selection

Blank water samples spiked with PFAC30PAR, NFBP1 and NFBP2 (and associated surrogates)

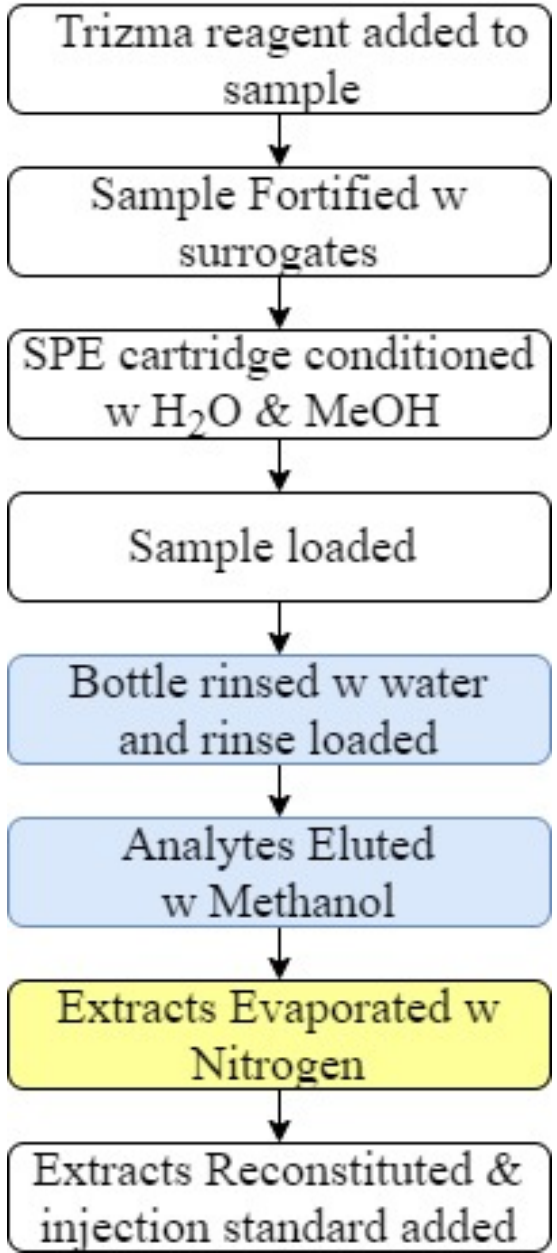
- EPA Method 533:
  - WAX SPE cartridge
  - PFAC30PAR and NFBP2 recoveries 80-120%
  - Lost majority of NFBP1, barely detectable (ND - 0.4%)
    - Possibly due to base exposure (NH_4OH)
  - Extraction repeated to ensure accuracy of results



- EPA Method 537:
  - SDVB SPE cartridge
  - Good recoveries of NFBP1 (>78%), NFBP2, and the majority of PFAC30PAR analytes
  - PFBA, PFPeA and FOSA recoveries were problematic at first due to SPE break-through and volatility issues



Method: Modified In-House Method

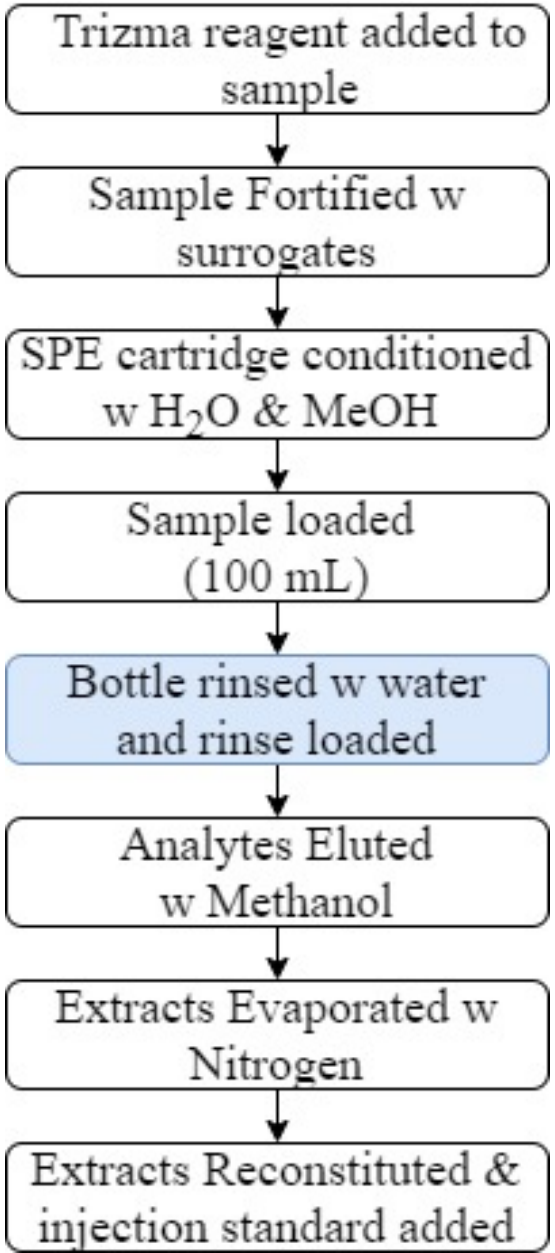


SDVB SPE Cartridge

$^{13}C_4$-PFBA, $^{13}C_5$-PFPeA and $^{13}C_8$-FOSA surrogate recoveries were problematic

Surrogate	Surrogate % Recovery
13C4-PFBA	3
13C5-PFPeA	43
13C5-PFHxA	125
13C4-PFHpA	112
13C8-PFOA	105
-	-
-	-
13C9-PFNA	110
13C6-PFDA	101
13C7-PFUdA	87
13C2-PFDoA	87
-	-
13C2-PFTeDA	93
-	-
-	-
13C8-FOSA	17

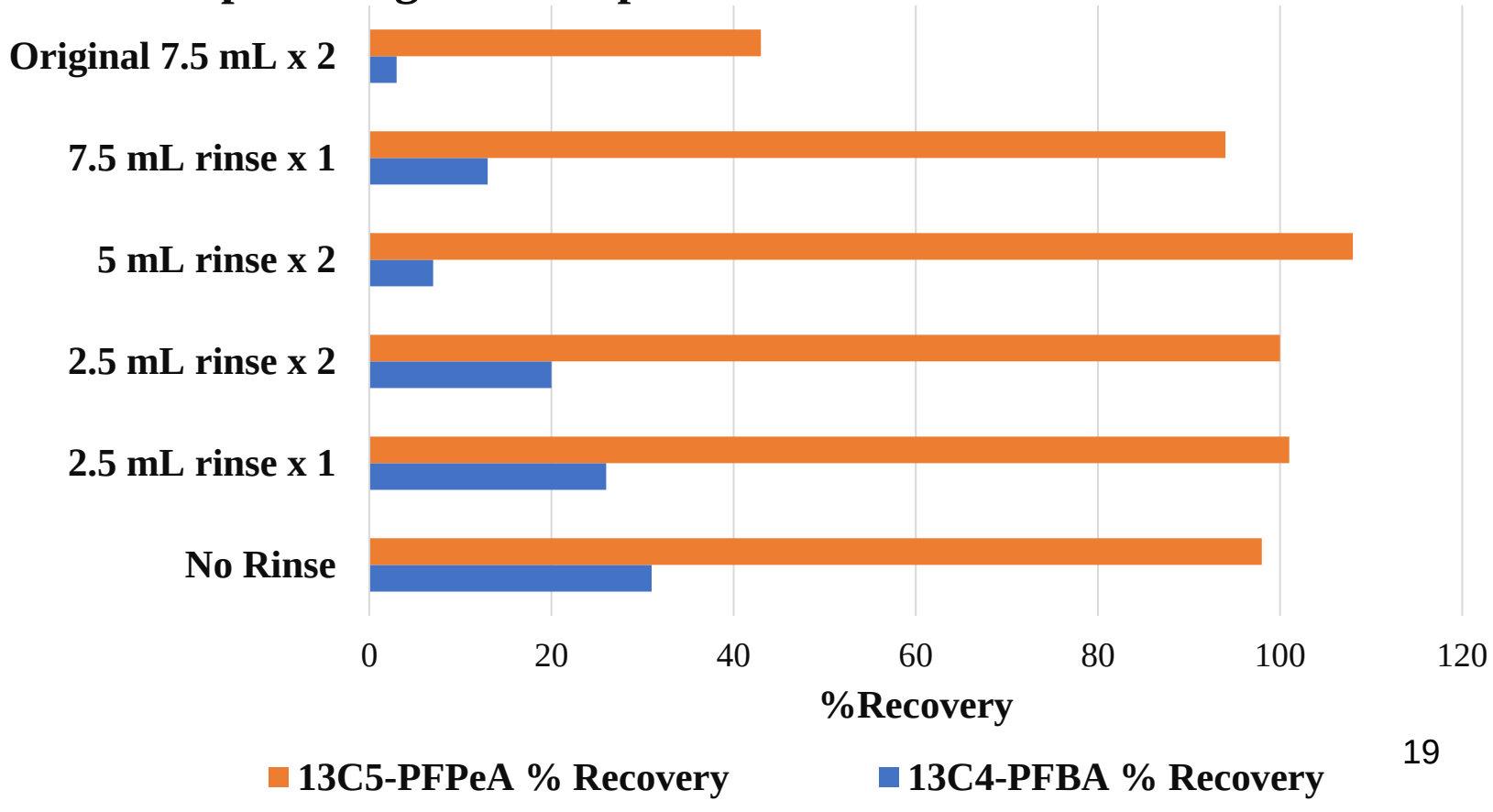
Rinse Step: Optimization of In-House Method



Optimized Rinse Step

- EPA 537 specifies a water rinse of 2 x 7.5 ml
- While maintaining a rinse step, one rinse of 2.5 mL allowed for surrogate recoveries of $^{13}C_4$-PFBA>25% and $^{13}C_5$-PFPeA ~100%

Optimizing Rinse step for 13C4-PFBA and 13C5-PFPeA



Elution: Optimization of In-House Method

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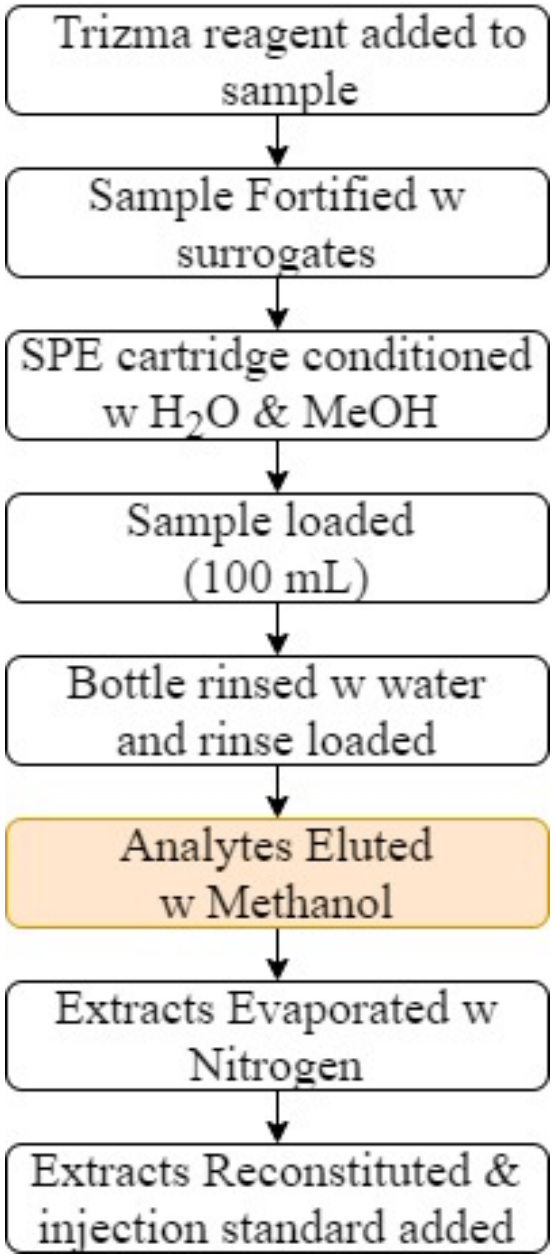
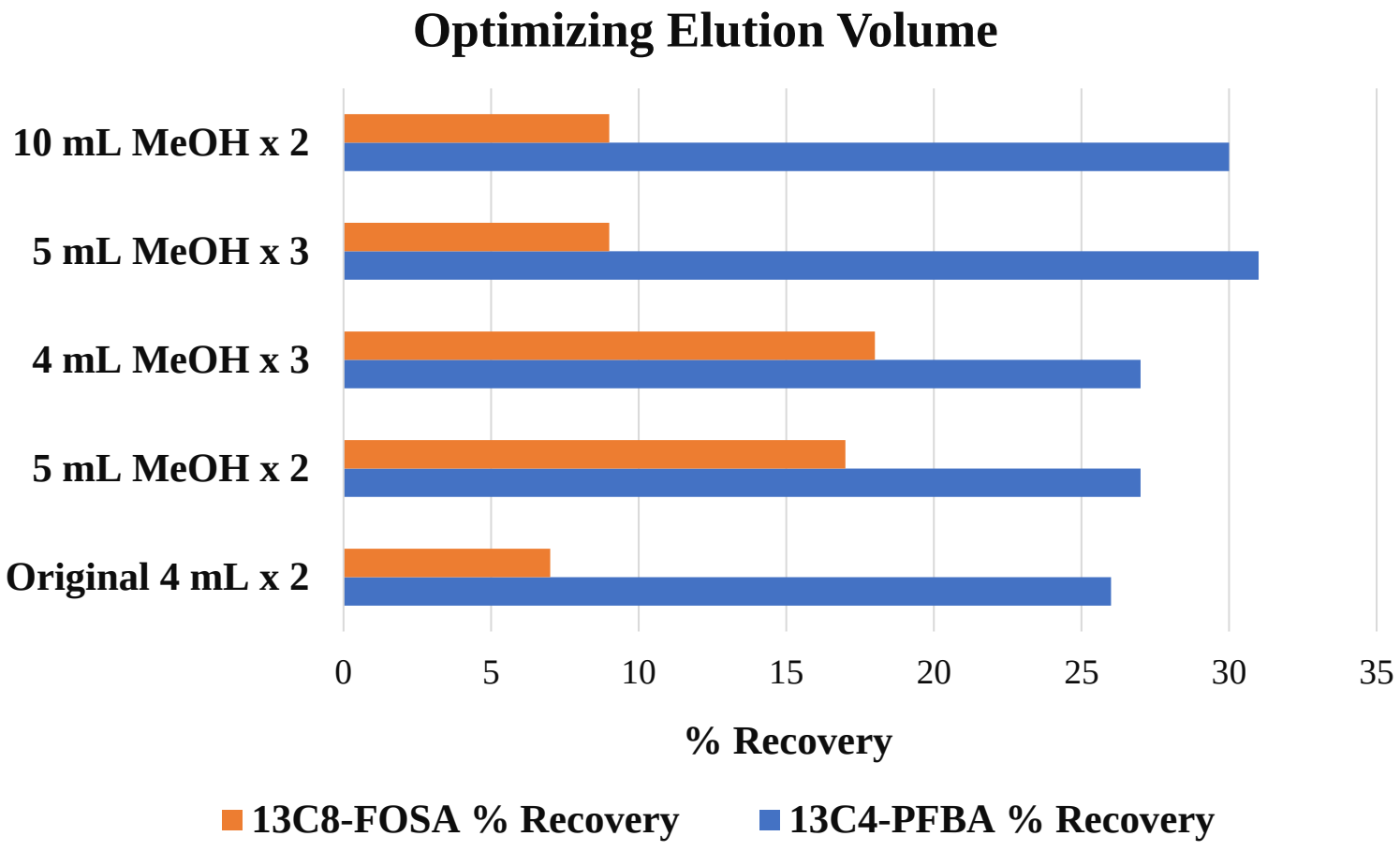


Optimized volume of elution methanol used:

- 5 mL methanol twice
  - Highest $^{13}C_4$-PFBA and $^{13}C_8$-FOSA recoveries while minimizing elution volume



Blow-Down: Optimization of In-House Method

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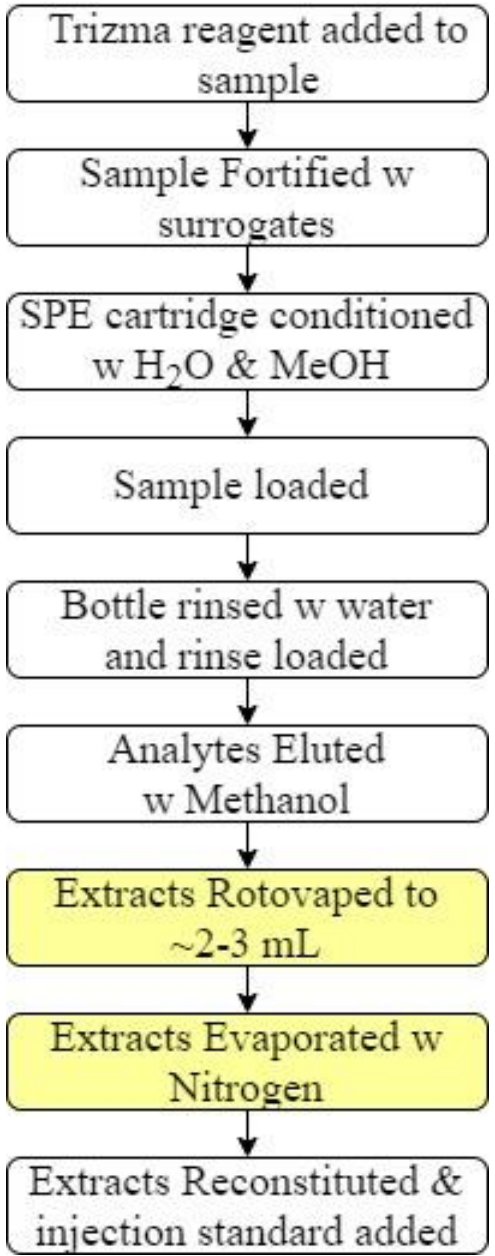
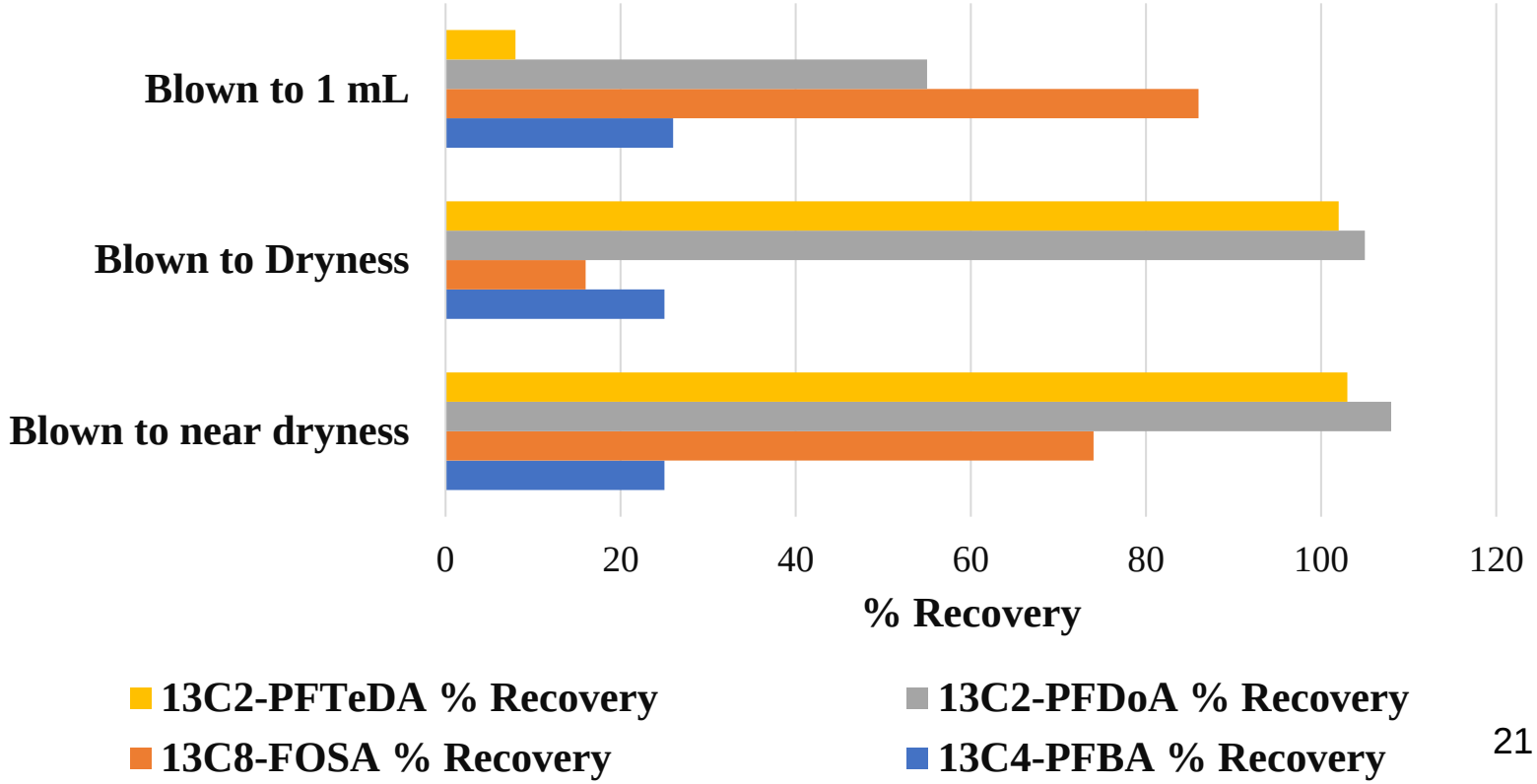


Optimized FOSA recovery to decrease losses in blow down step:

- Optimal = blown down to near dryness
- Blown to dryness: loss of sulfonamides (FOSA, FBSA, FHxSA)
- Insufficient drying: loss of long-chain carboxylic acids (PFDoA, PFTeDA)
- Too much heat = conversion of NFBP1 to MeO-NFBP1

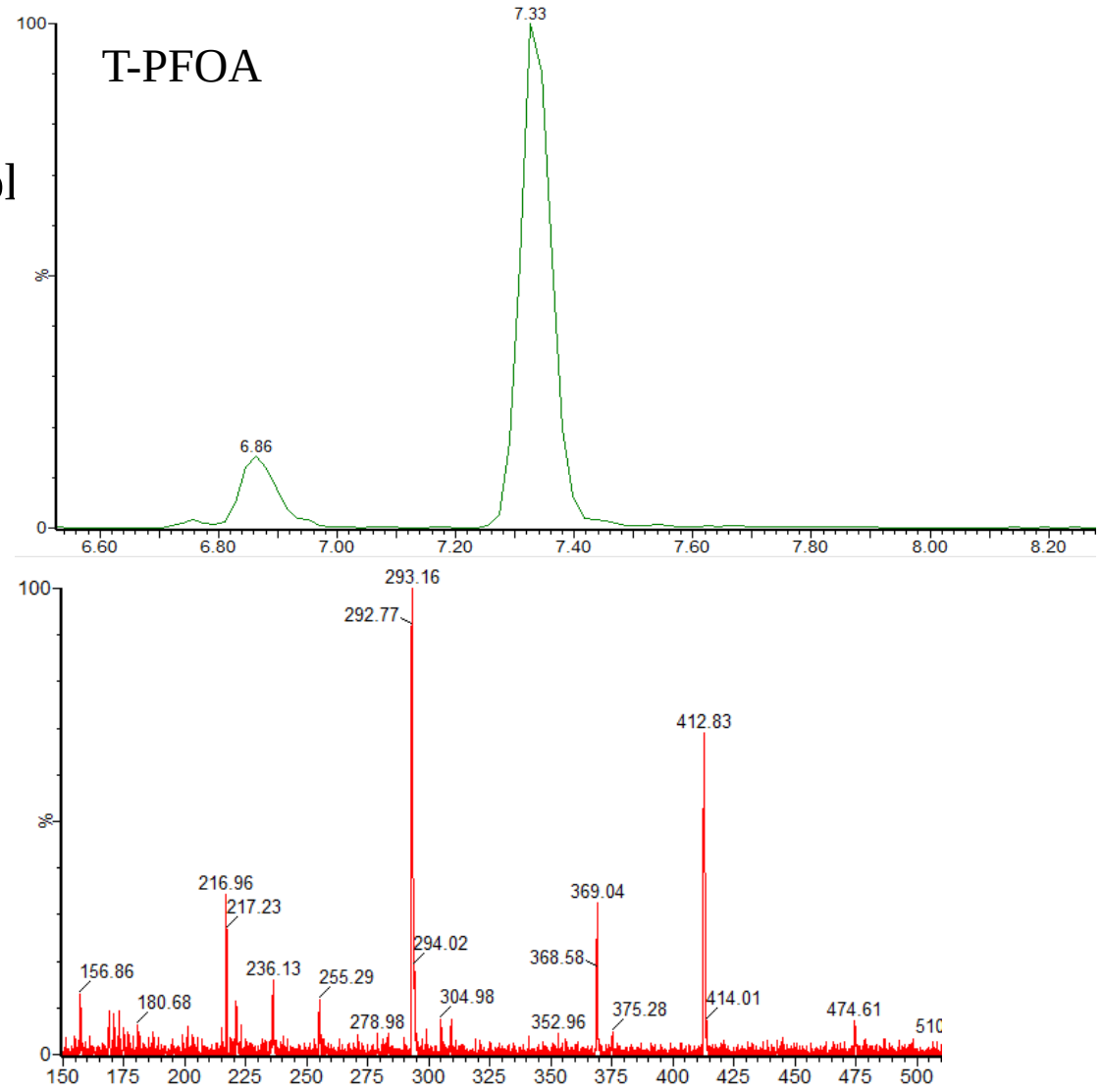
Optimizing the Blow-Down Step



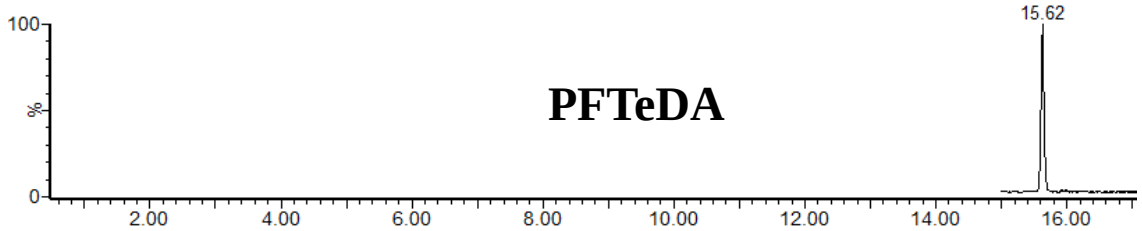
Modified In-House Method

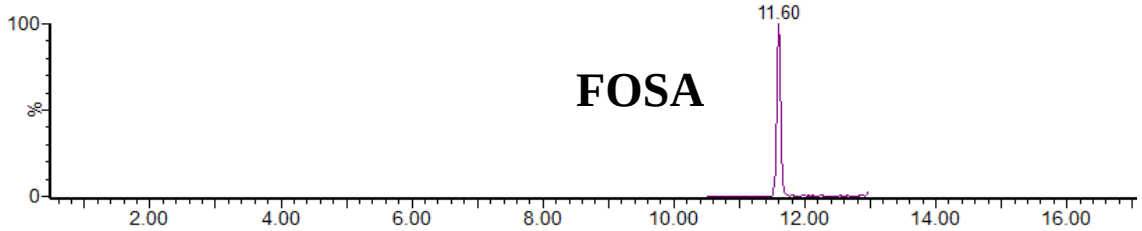
In-house method QA/QC requirements:

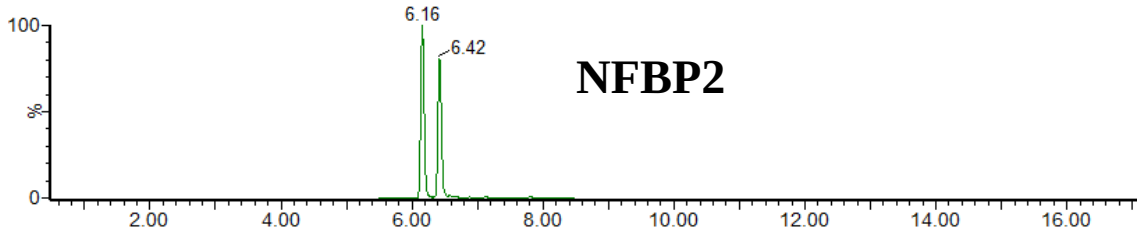
- Two Calibration Curves (0.0500 – 100 ng/mL)
  - PFAC30PAR + NFBP2 + MeO-NFBP1 in methanol
  - NFBP1 in acetonitrile
    - %RSD <25%
    - % Deviation <30% / <50%
- System Suitability
  - S/N requirement
  - MS Drift Check
    - T-PFOA
  - Sensitivity Control Charts
- Surrogate recoveries = 50-150%
  - $^{13}C_4$-PFBA and $^{13}C_8$-FOSA = 20-150%
- Acceptable Lab Blanks
- Acceptable recoveries for Fortified Lab Blank
- Samples prepared in duplicate
- Monitored for MeO-NFBP1

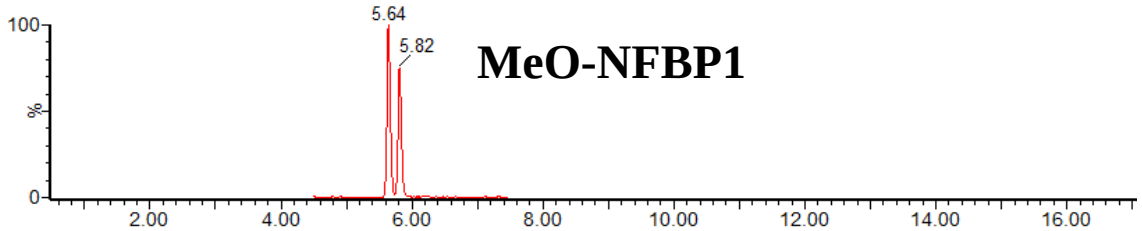


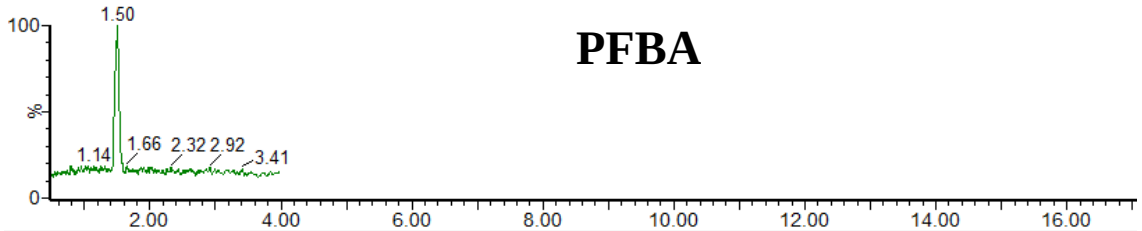
Calibration Set #1 (CS2 0.200 ng/mL)

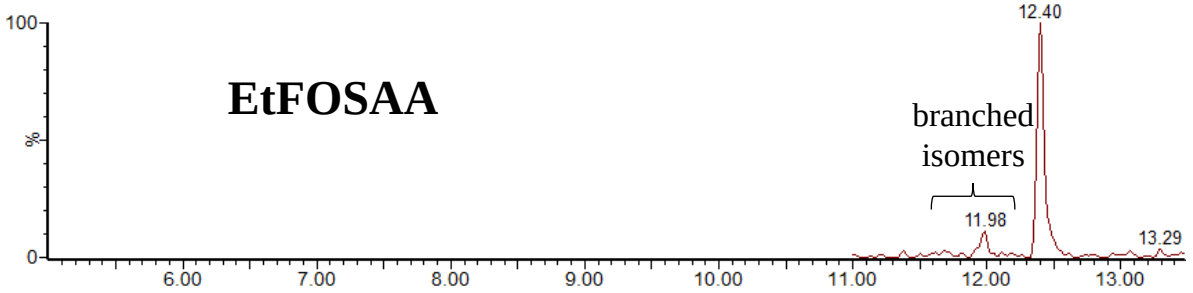


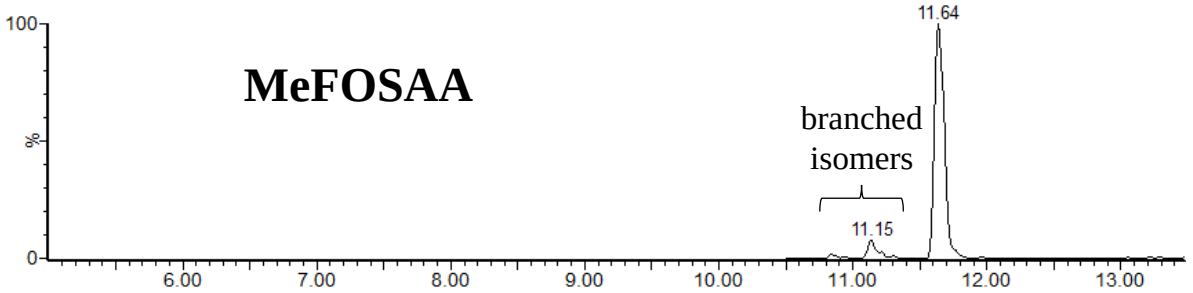


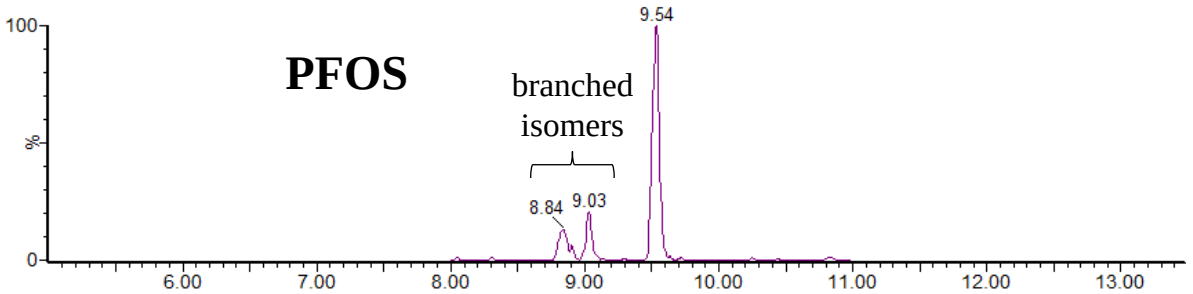


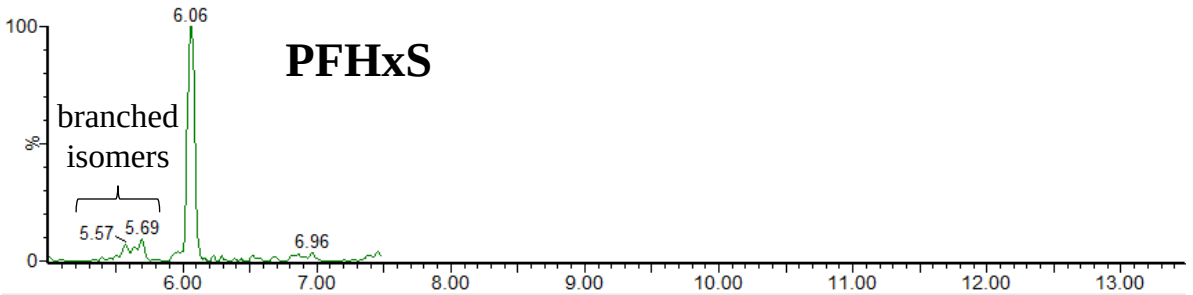












Results

Waters™

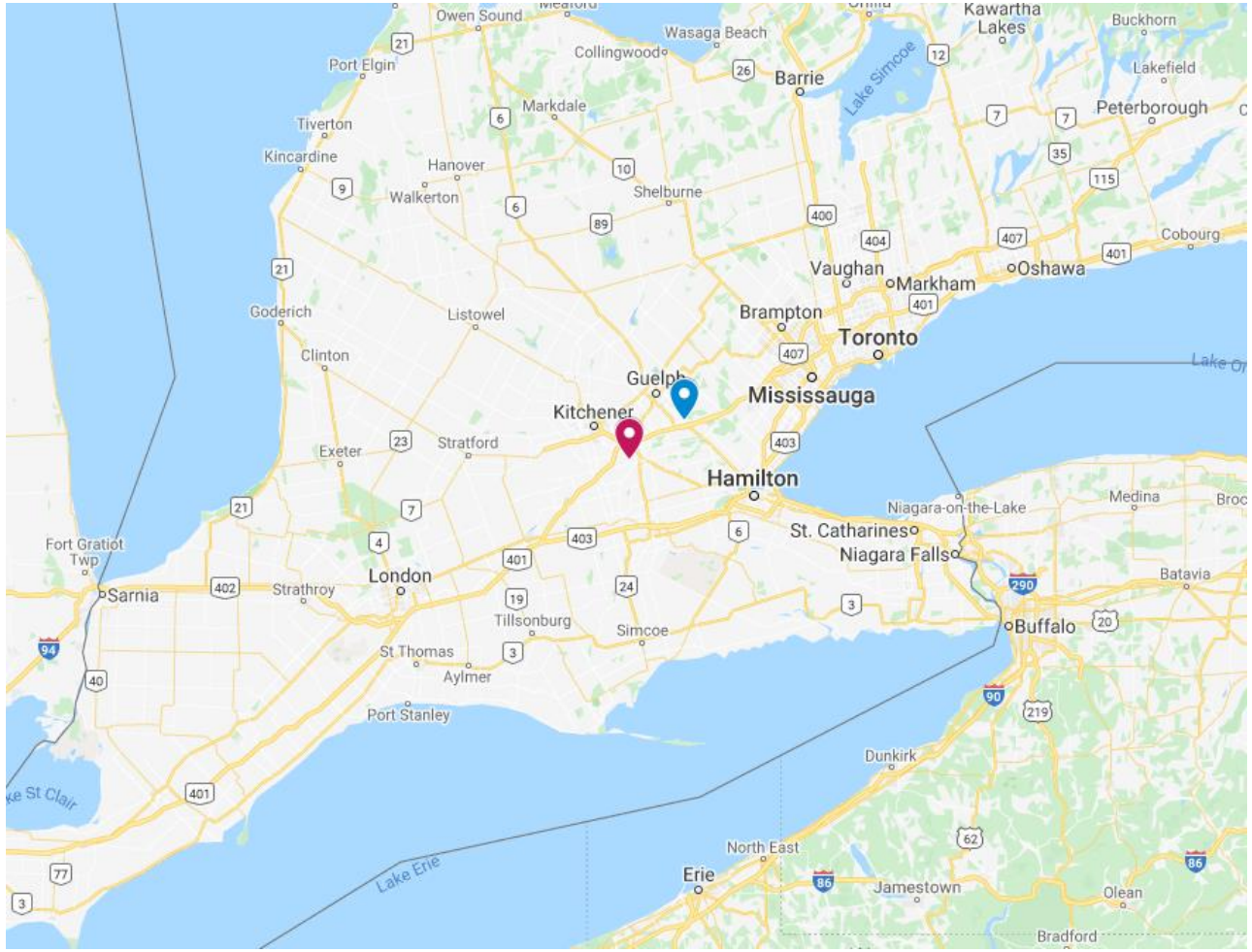
Confirmed Method Accuracy using a Waters ERA QA/QC Sample

	Replicate (ng/L)										
Instrument	**1**	**2**	**Mean**	**SD**	**%RSD**	**% of Target**	Category (Certified or Reference value)	Target value (ng/L)	Lower Limit	Upper Limit	Within Limit
L-PFHxA	395	416	405.5	15	3.7	101.38	ERA CofA Value	400	260	540	Yes
L-PFHpA	450	462	456.0	8.5	1.9	97.02	ERA CofA Value	470	306	635	Yes
L-PFOA	336	341	338.5	3.5	1.0	98.12	ERA CofA Value	345	224	466	Yes
L-PFNA	148	154	151.0	4.2	2.8	100.67	ERA CofA Value	150	97.5	203	Yes
L-PFDA	250	260	255.0	7.1	2.8	92.73	ERA CofA Value	275	179	371	Yes
L-PFDoA	424	430	427.0	4.2	1.0	98.16	ERA CofA Value	435	283	587	Yes
L-PFTrDA	158	166	162.0	5.7	3.5	101.25	ERA CofA Value	160	104	216	Yes
N-EtFOSAA	45.4	46.1	45.8	0.5	1.1	76.25	ERA CofA Value	60.0	39.0	81.0	Yes
HFPO-DA	621	649	635.0	20	3.1	97.69	ERA CofA Value	650	423	878	Yes
L-PFBS	749	800	774.5	36	4.7	103.13	ERA CofA Value	751	488	1014	Yes
L-PFHxS	146	154	150.0	5.7	3.8	80.21	ERA CofA Value	187	122	252	Yes
L-PFOS	124	132	128.0	5.7	4.4	79.01	ERA CofA Value	162	105	219	Yes
DONA	336	347	341.5	7.8	2.3	99.27	ERA CofA Value	344	224	464	Yes
9Cl-PF3ONS	177	171	174.0	4.2	2.4	95.60	ERA CofA Value	182	118	246	Yes

Real Water Samples - Ontario

New in-house method used to determine PFAS levels (including NFBP1 and NFBP2) in:

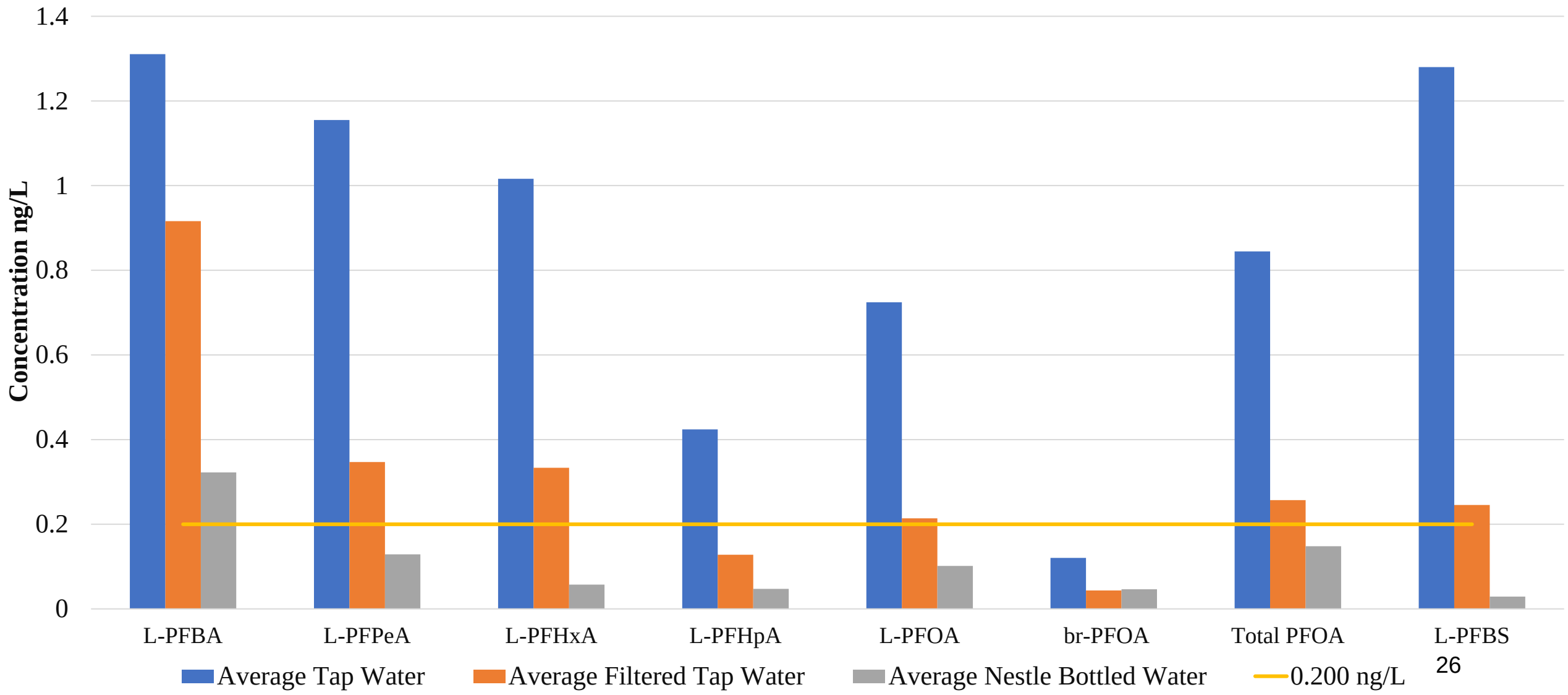
- Nestlé bottled water collected in Aberfoyle (**blue** marker)
- Cambridge tap water (**red** marker)
- Cambridge filtered tap water (**red** marker)
  - “Waterdrop” pitcher water with a carbon filter



Results

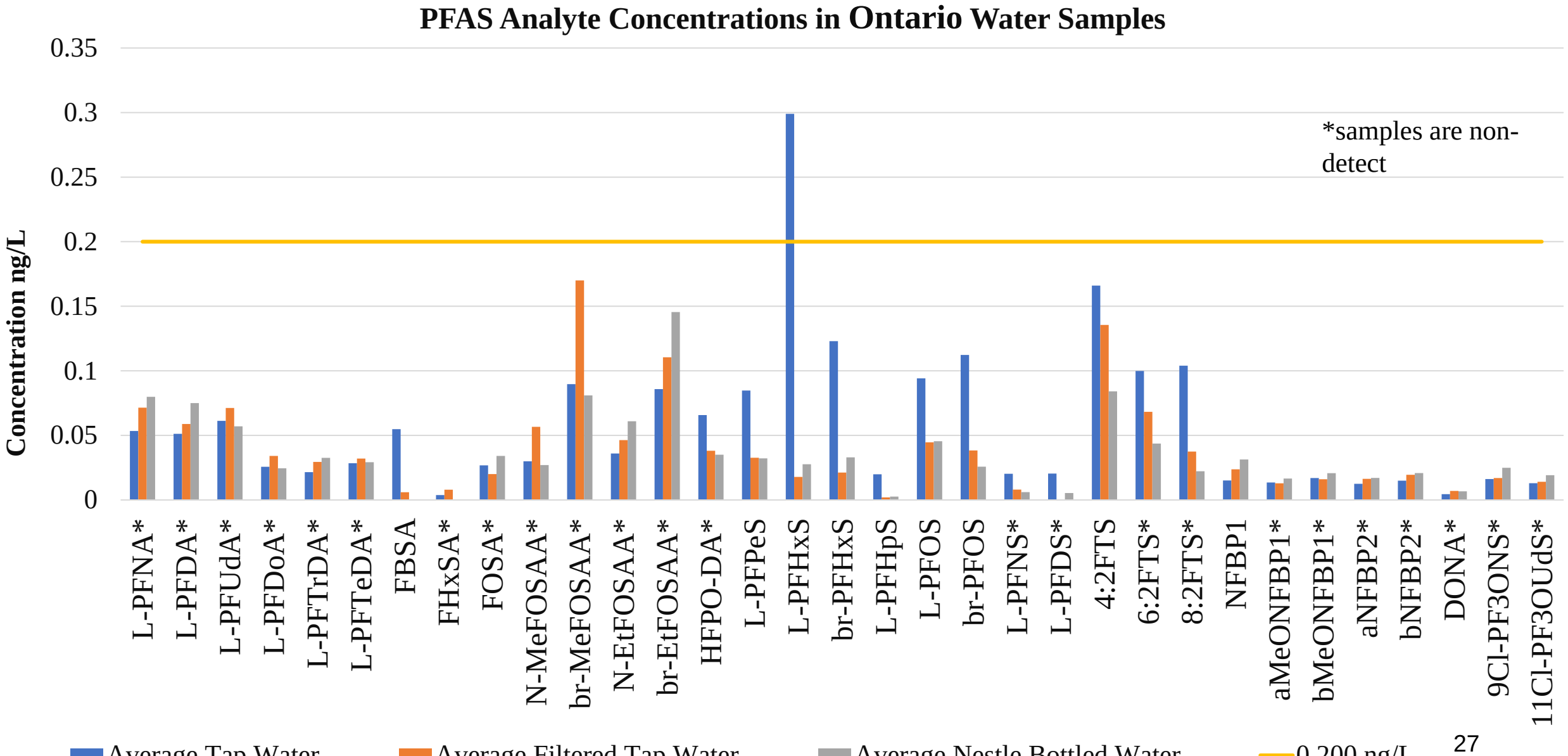
Waters™

Highest PFAS Analyte Concentrations in Ontario Water Samples



Results

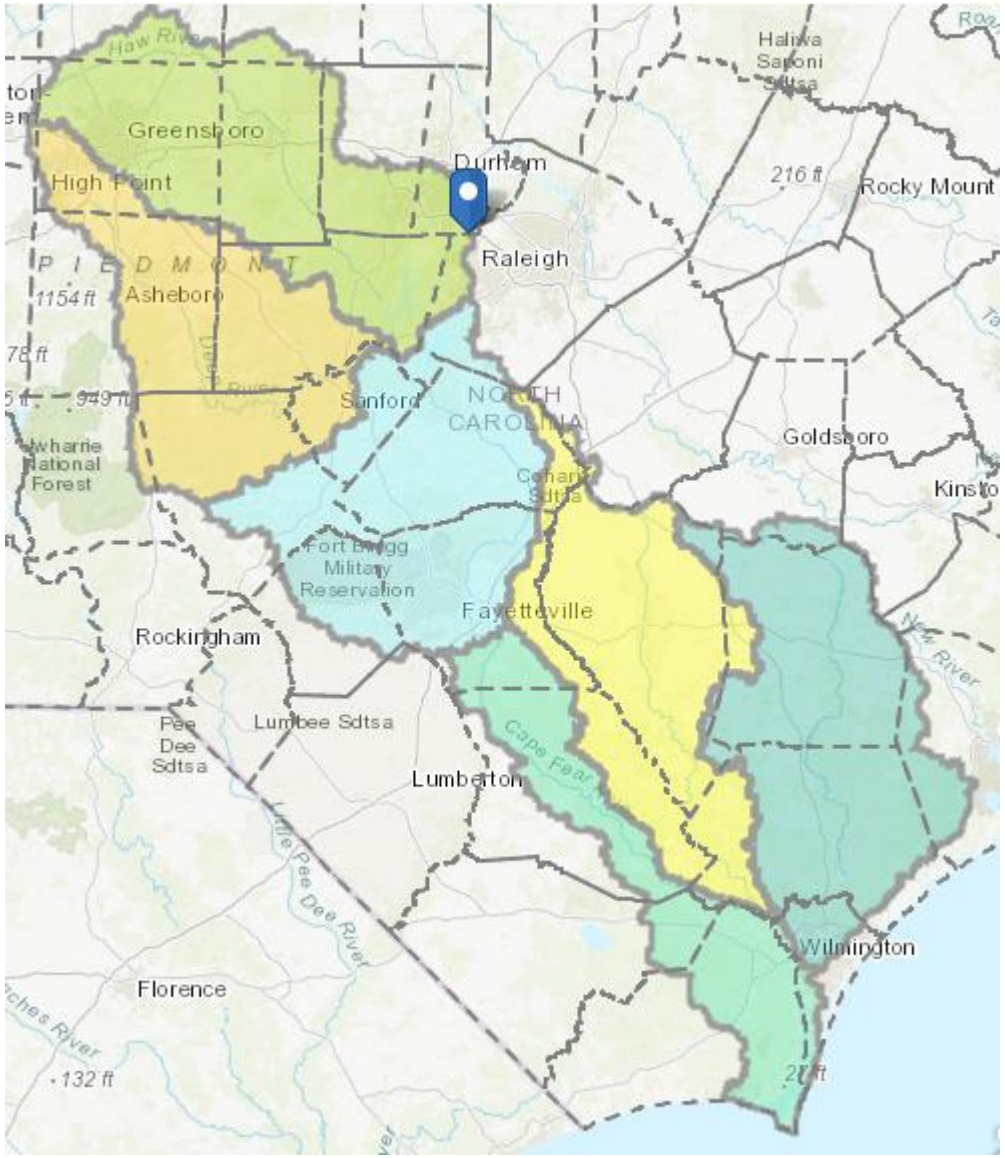
Waters™



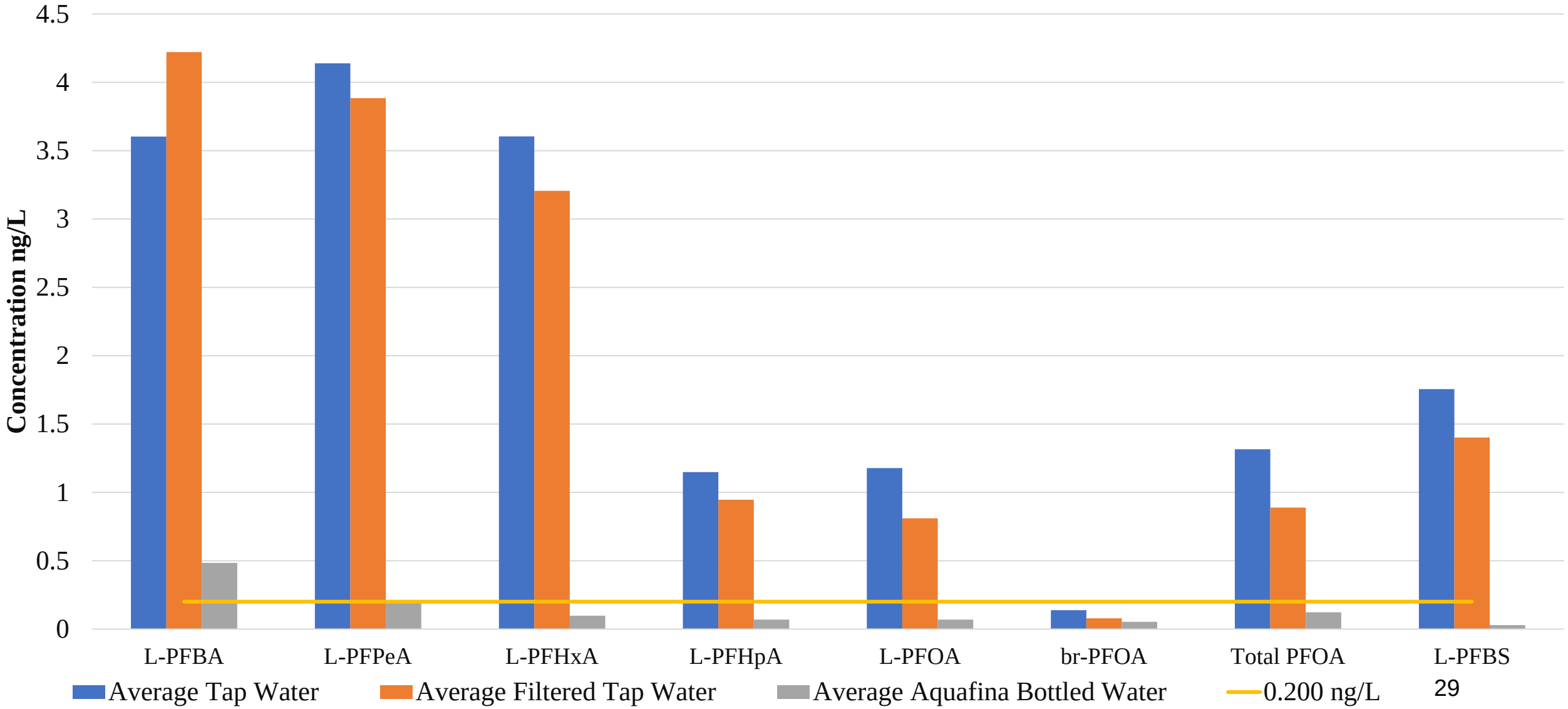
Real Water Samples – North Carolina

New in-house method used to determine PFAS levels (including NFBP1 and NFBP2) in:

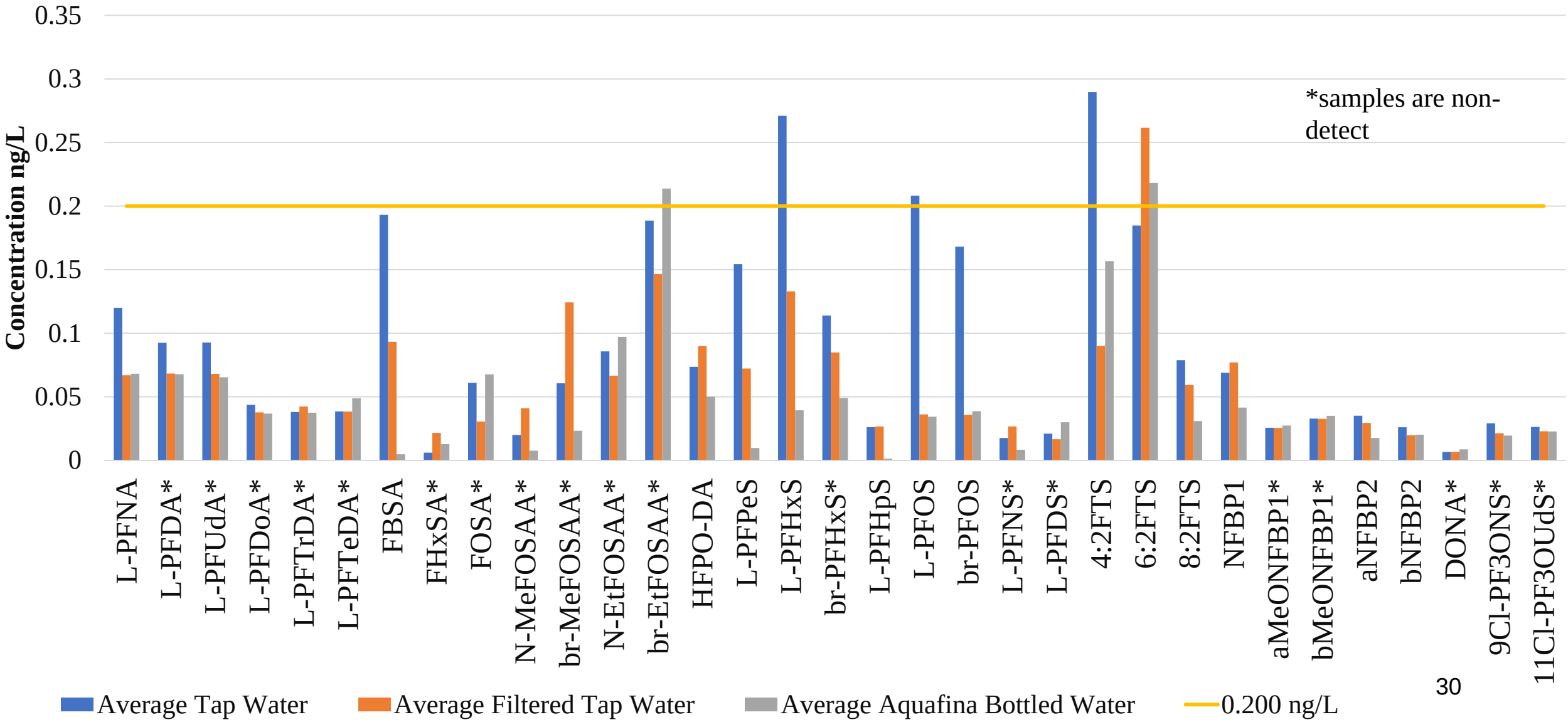
- Aquafina
  - reverse osmosis
- North Carolina tap water
- North Carolina filtered tap water
  - Samsung fridge filter (carbon)



Elevated PFAS Analyte Concentrations ion North Carolina Water Samples

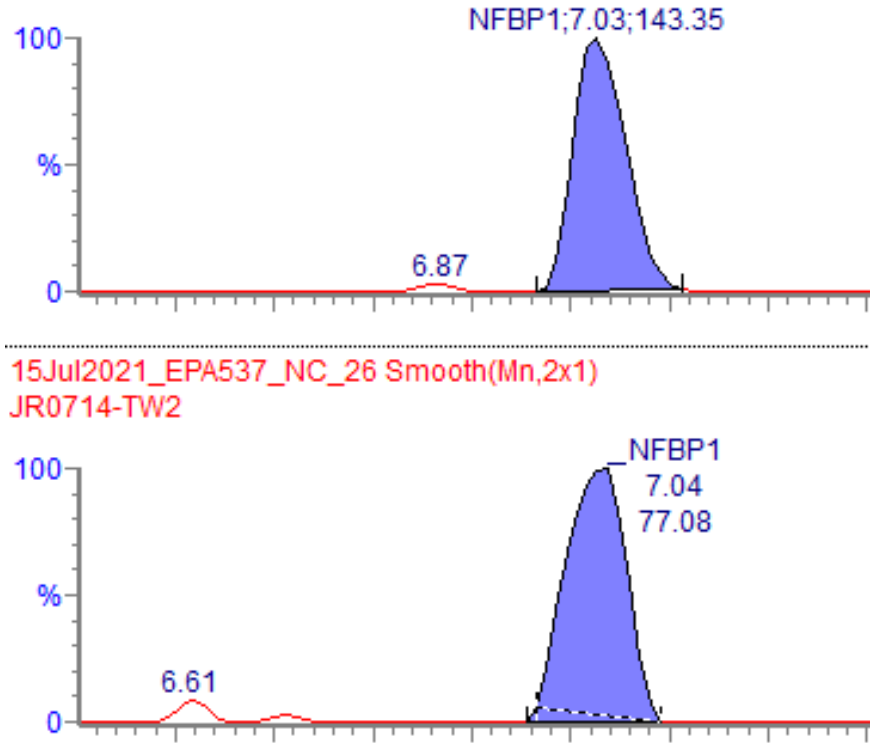


PFAS Analyte Concentration in North Carolina Water Samples

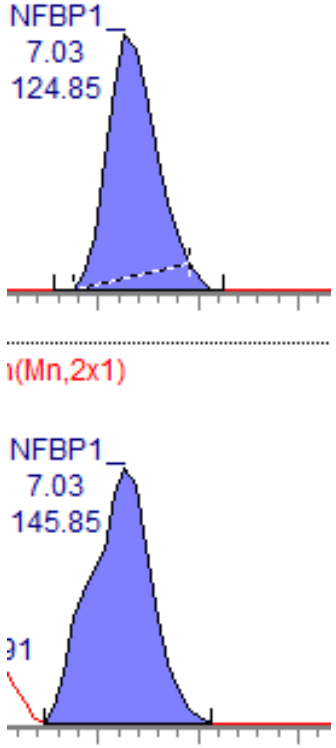


Nafion byproduct 1 in North Carolina Water:

Tap water
Concentration: 0.102 ng/L

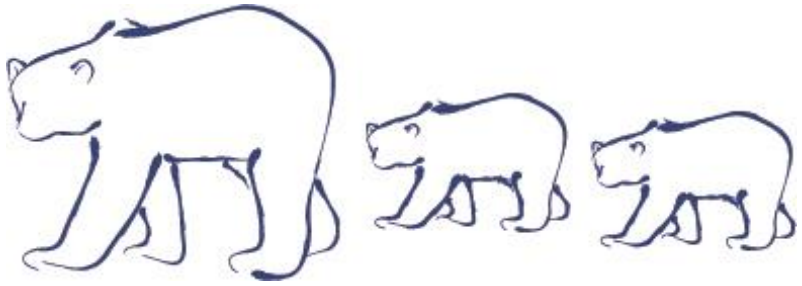


Filtered Tap water
Concentration: 0.0873 ng/L



CONCLUSIONS

- Nafion byproduct 1 was unstable when stored in methanol (especially when exposed to heat)
- Nafion byproduct 1 degraded in the presence of base (NH_4OH)
- Expansion and modification of the current in-house method allowed for the quantitation of NFBP1 and NFBP2 with legacy PFAS
- NFBP2 standards were stable in methanol
- NFBP1 standards were only stable in acetonitrile, therefore two calibration curves were required



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- Lauren Irwin

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- Kari Organtini

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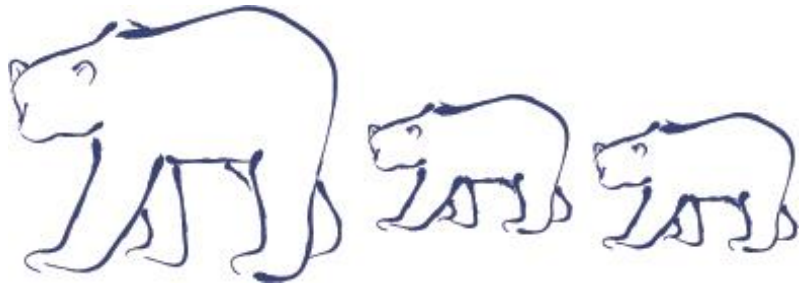
- Allison Brazeau and family
- Yadavan Varatharajah



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Questions??

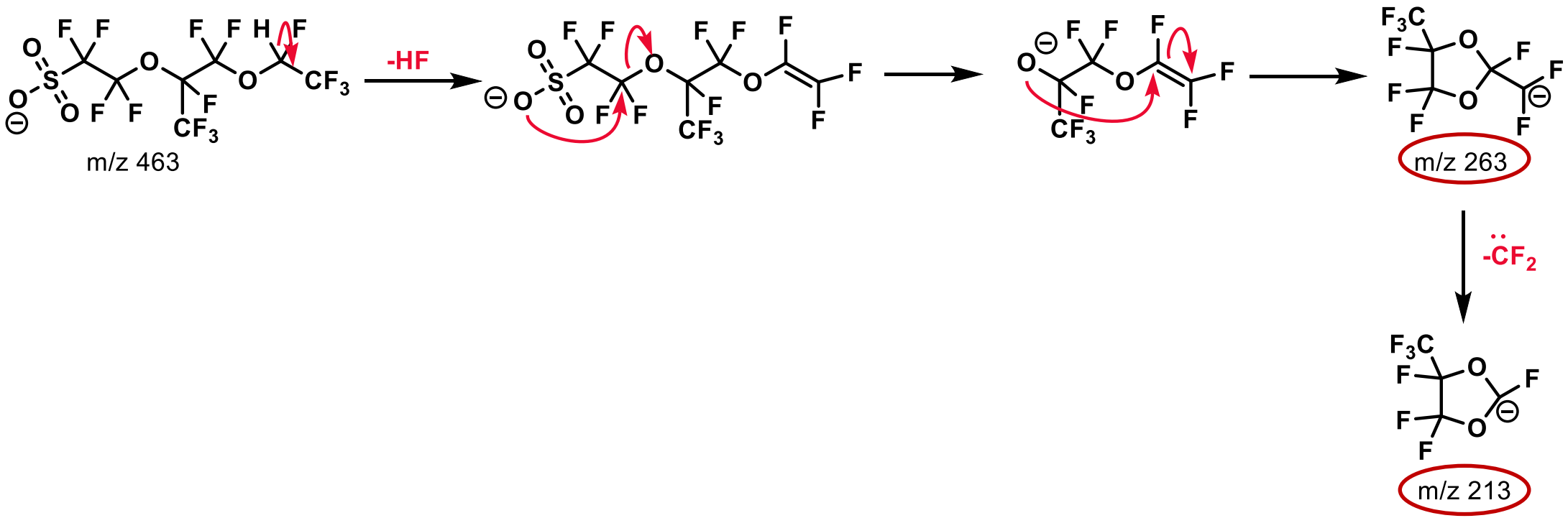
email: jennifer@well-labs.com

Extra Slides

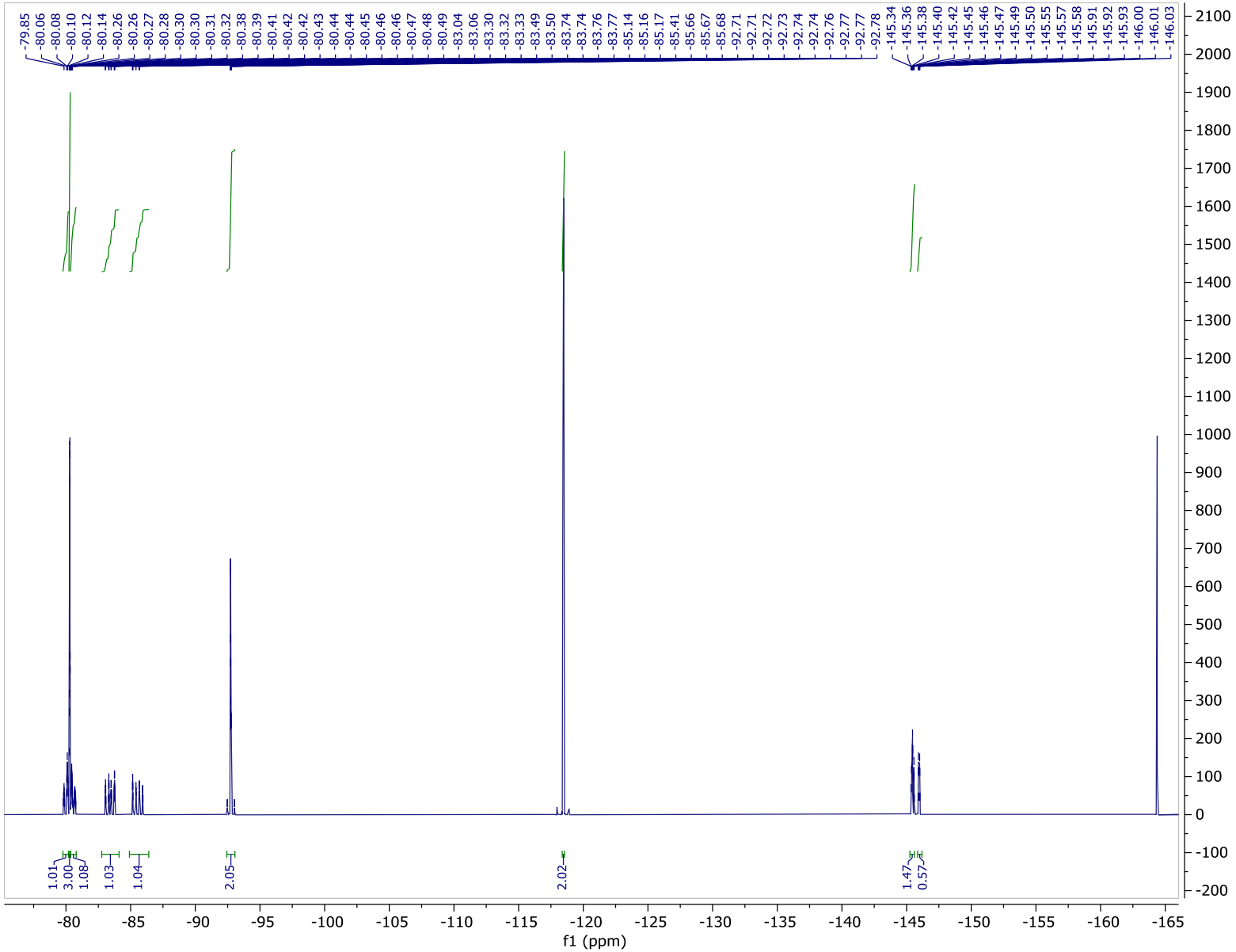
Alternate MS Fragmentation Analysis: NFBP2

Waters™

NFBP2

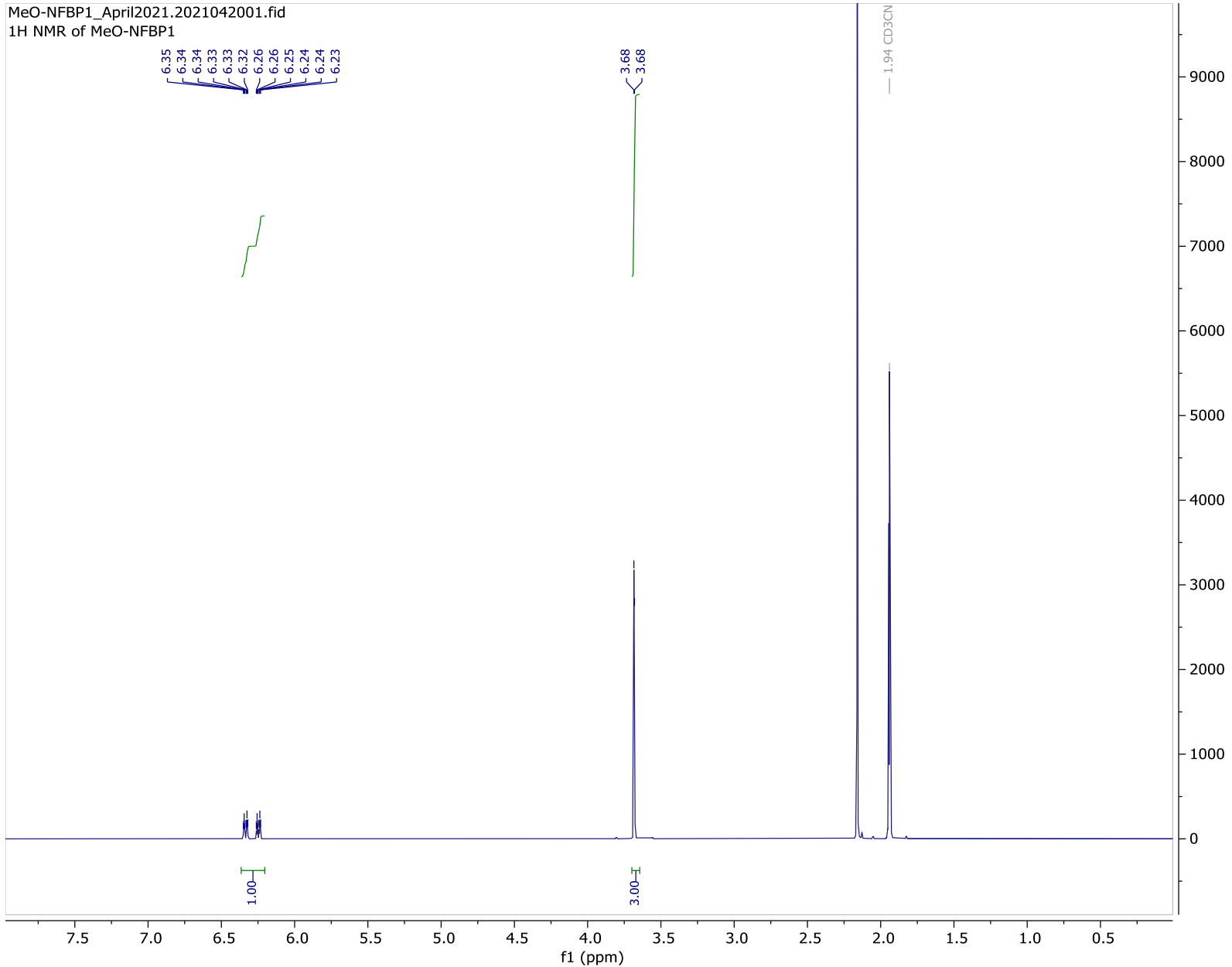


MeO-NFBP1 ^{19}F NMR



MeO-NFBP1_April2021.2021042002.fid — 19F NMR of MeO-NFBP1

MeO-NFBP1 ^{1}H NMR



Characterization Methods

NMR ANALYSIS

- 19F 565 MHz Bruker Avance III NMR
- 1H 600 MHz Bruker Avance III NMR

COMPUTATIONAL

- Firefly, DFT, B3LYP 6-311G(d,p)

Expansion of Targeted Analysis of Standard PFAS Analytes

Potassium perfluoro-1-butanesulfonate	L-PFBS
Sodium perfluoro-1-pentanesulfonate	L-PFPeS
Potassium perfluorohexanesulfonate [c]	PFHxSK: linear isomer
	PFHxSK: ∑ branched isomers
Sodium perfluoro-1-heptanesulfonate	L-PFHpS
Potassium perfluorooctanesulfonate [d]	PFOSK: linear isomer
	PFOSK: ∑ branched isomers
Sodium perfluoro-1-nonanesulfonate	L-PFNS
Sodium perfluoro-1-decanesulfonate	L-PFDS
Sodium 1H,1H,2H,2H-perfluorohexanesulfonate	4:2FTS
Sodium 1H,1H,2H,2H-perfluorooctanesulfonate	6:2FTS
Sodium 1H,1H,2H,2H-perfluorodecanesulfonate	8:2FTS
Sodium dodecafluoro-3H-4,8-dioxanonanoate	NaDONA
Potassium 9-chlorohexadecafluoro-3-oxanonane-1-sulfonate	9Cl-PF3ONS
Potassium 11-chloroeicosafluoro-3-oxaundecane-1-sulfonate	11Cl-PF3OUdS

Perfluoro-n-butanoic acid	PFBA
Perfluoro-n-pentanoic acid	PFPeA
Perfluoro-n-hexanoic acid	PFHxA
Perfluoro-n-heptanoic acid	PFHpA
Perfluoro-n-octanoic acid	PFOA
Perfluoro-n-nonanoic acid	PFNA
Perfluoro-n-decanoic acid	PFDA
Perfluoro-n-undecanoic acid	PFUdA
Perfluoro-n-dodecanoic acid	PFDoA
Perfluoro-n-tridecanoic acid	PFTrDA
Perfluoro-n-tetradecanoic acid	PFTeDA
Perfluoro-1-butanesulfonamide	FBSA
Perfluoro-1-hexanesulfonamide	FHxSA
Perfluoro-1-octanesulfonamide	FOSA
2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	HFPO-DA
N-methylperfluorooctanesulfonamidoacetic acid [a]	N-MeFOSAA: linear isomer
	N-MeFOSAA: ∑ branched isomers
N-ethylperfluorooctanesulfonamidoacetic acid [b]	N-EtFOSAA: linear isomer
	N-EtFOSAA: ∑ branched isomers

Results

Proficiency test performed to establish that the instrumentation method and cal curve are sufficient

- Compared to UPLC-TQ-S Micro

PFAC-PT-20

Waters Aquity UPLC/MS

TQ-XS MS/MS

Analytes	Concentration	Units
PFHxA	103	ng/ml
PFDA	202	ng/ml
PFBS	42.1	ng/ml
PFNS	76.1	ng/ml

Analytes	Concentration	Units
L-PFHxA	97.5	ng/ml
L-PFDA	206	ng/ml
L-PFBS	39	ng/ml
L-PFNS	82	ng/ml

Analytes	%RSD (Concentration)	Max % RSD Allowed
PFHxA	3.88	25.00
PFDA	1.39	25.00
L-PFBS	5.59	25.00
L-PFNS	4.85	25.00

Calibration Set #1 (CS2 0.200 ng/mL)