

Holy Cow – is that PFAS in my Milk?

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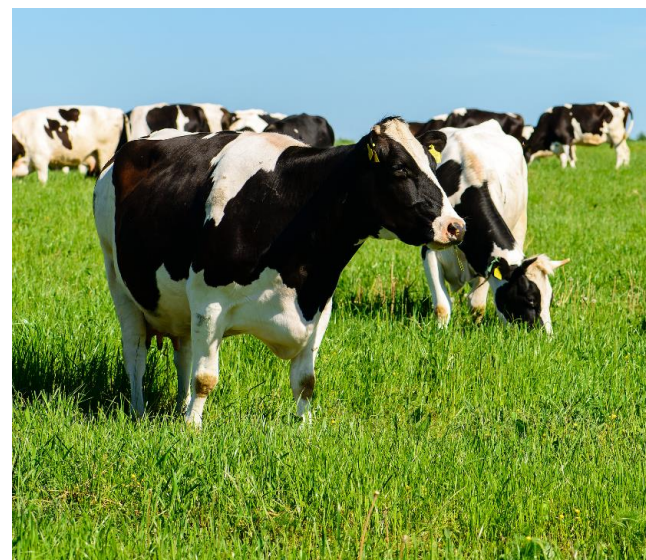
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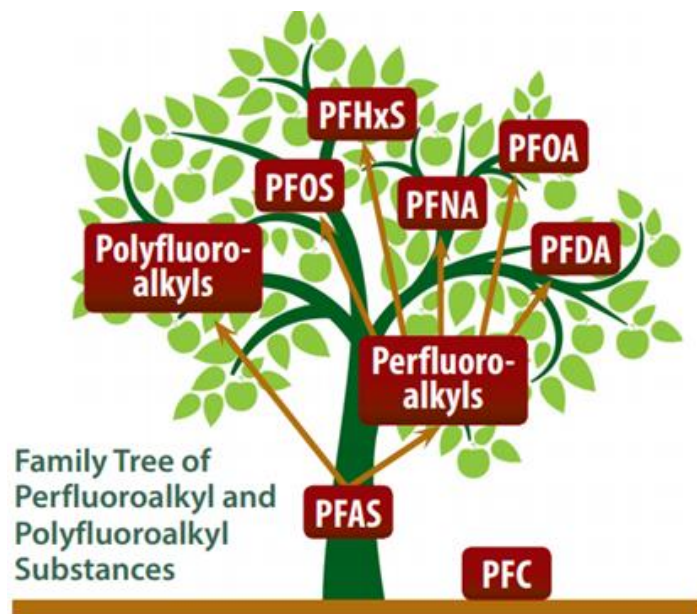
Objective

To develop

- a semi-quantitative screening method for measuring PFAS residues in milk using MALDI-TOF – Axima Confidence
- a quantitative confirmation method with LCMS/MS 8050



What are PFAS?

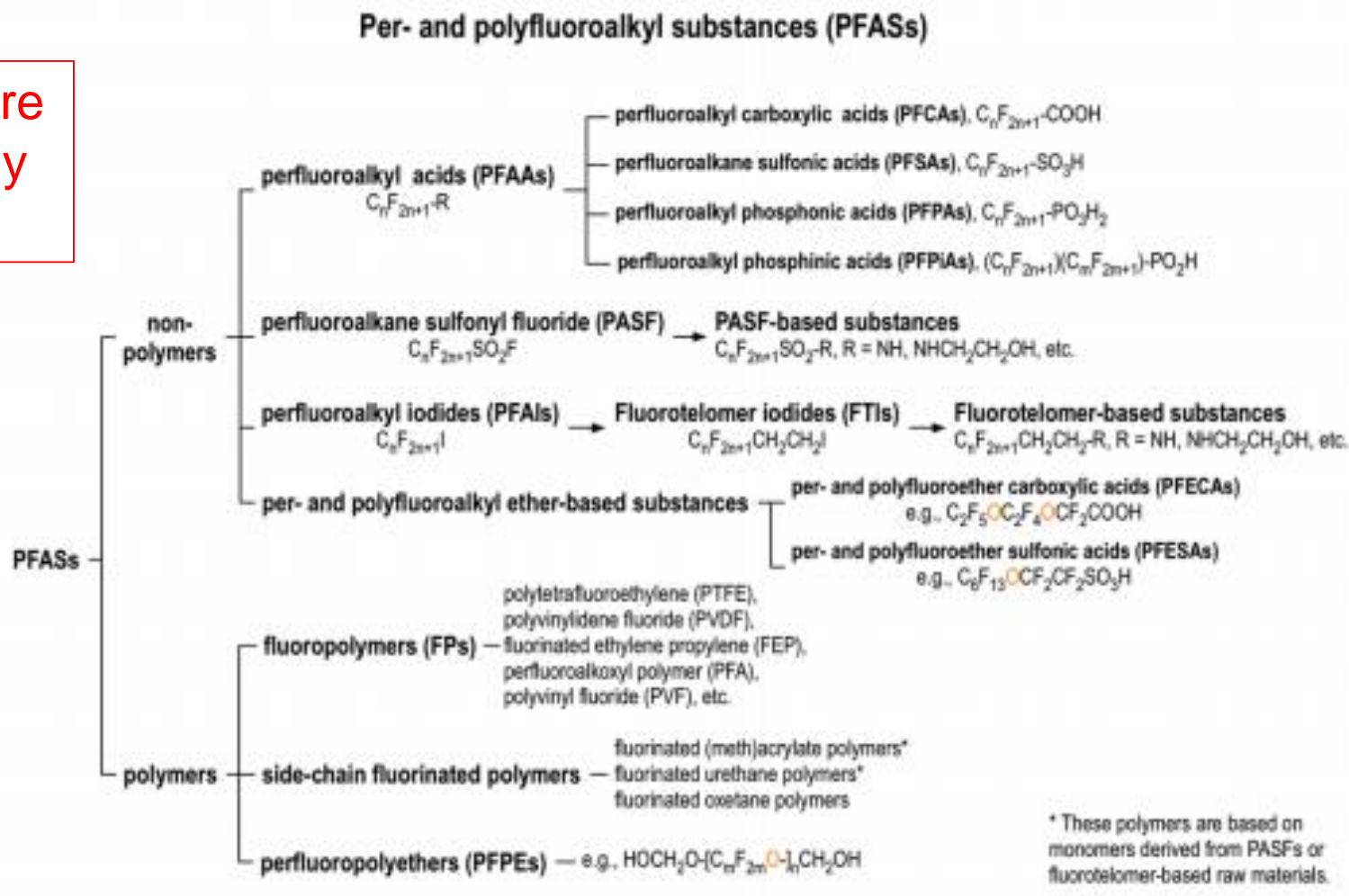


<https://www.atsdr.cdc.gov/pfas/PFAS-health-effects.html>

- *Per- and polyfluoroalkyl substances (PFAS)* are man-made chemicals that have been used in industry and consumer products worldwide since the 1950s.
- Previously, different organizations used the acronym **PFCs** (from *perfluorinated chemicals*) to refer to a subset of PFAS, including PFOA and PFOS.
- PFCs also refers to perfluorocarbons, the most potent and long lasting anthropogenic green-house gases.

PFAS... only PFOA and PFOS?

PFOA and PFOS are the most commonly known PFAS.



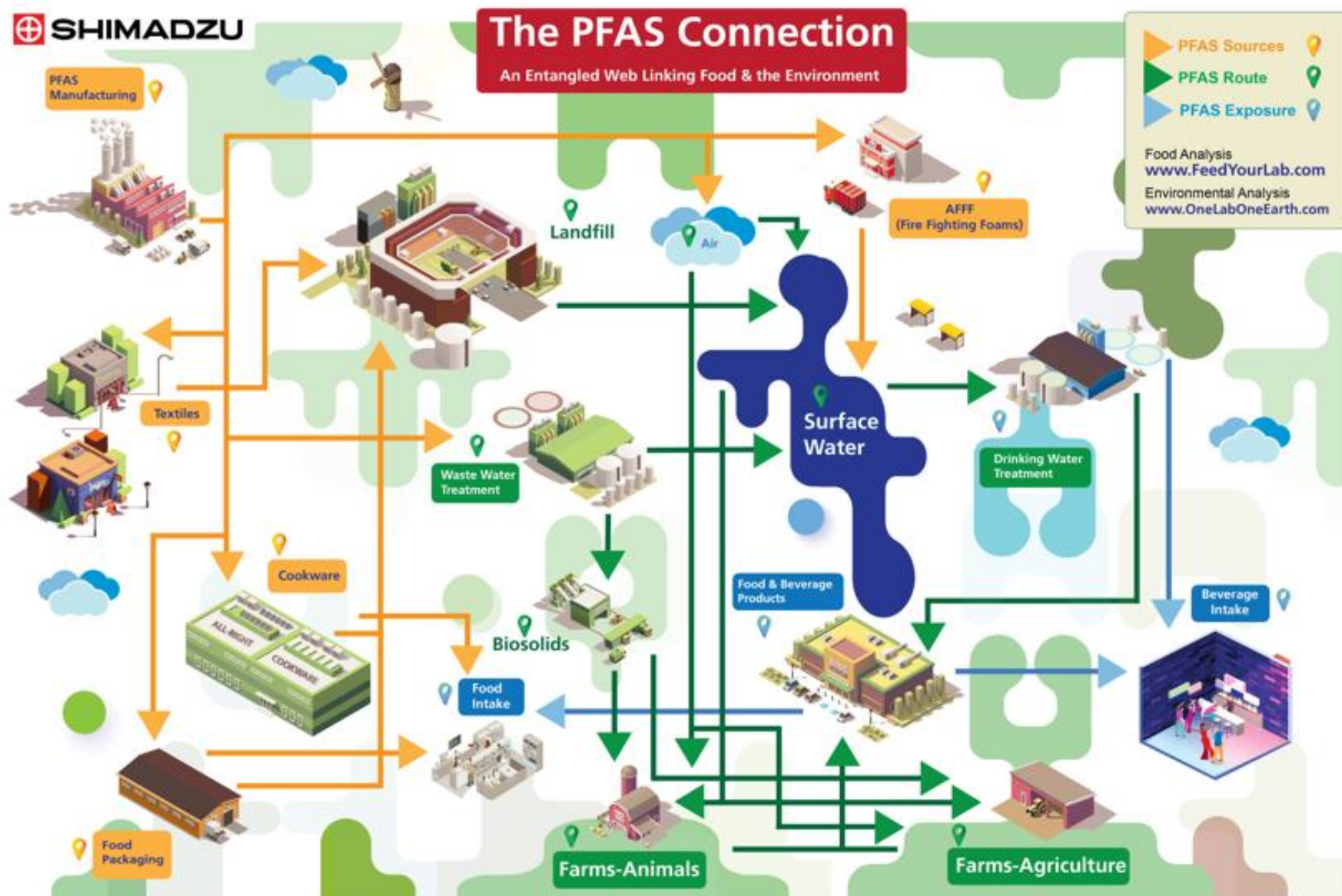
<https://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/aboutpfass/>
Figure1-classification-of-per-and-polyfluoroalkyl-substances%20-PFASs.pdf

PFAS in our daily (food) life

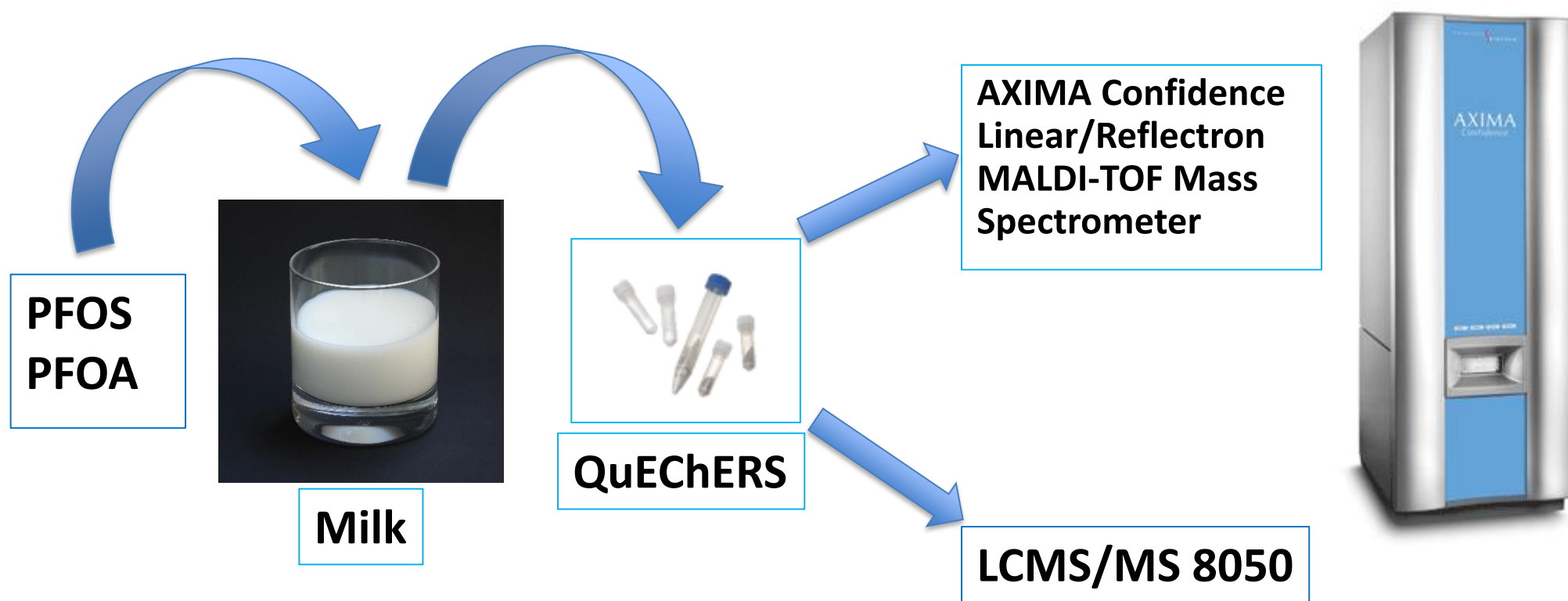
The “Forever” Chemical is also the “Wherever” Chemical



PFAS in our daily (food) life



Work Flow and Instrumentation



50th
MS anniversary
1970-2020



Combining Ultrafast
Technology with
Ultrahigh Sensitivity

Instrumentation

Why AXIMA Confidence Linear/Reflectron MALDI-TOF MS?

- Instrument used by collaborating customer in the dairy industry for screening of milk (e.g., proteins)

Why LCMS 8050 UFMS?

- Higher sensitivity for confirmation



QuEChERS Extraction Protocol

Extraction salts:

- **ECQUEU7-MP** (Mylar pouch containing 4g MgSO_4 , 1g NaCl, 1 g sodium citrate tribasic dehydrate and 0.5 g sodium citrate dibasic sesquihydrate)
- **ECQUEU750CT-MP** (Same salts as the above product but also includes empty 50 mL centrifuge tubes)

Dispersive-SPE cleanup tubes:

- **ECQUEU615CT** (15 mL dSPE tube with 900 mg MgSO_4 , 150 mg PSA and 45 mg GCB)

Sample extraction

1. Transfer 10 mL of milk sample into a 50 mL polypropylene centrifuge tube.
2. Add 10 mL acetonitrile.
3. Add the contents of the **ECQUEU7-MP** Mylar pouch and shake/vortex immediately for at least 1 minute (by hand or mechanically).
4. Centrifuge the samples at ≥ 3000 rpm for 5 min.

Sample clean-up

1. Transfer 5-6 mL of the acetonitrile supernatant to a 15 mL dSPE tube (**ECQUEU615CT**).
2. Vortex for 30 sec.
3. Centrifuge at ≥ 3000 rpm for 5 min.
4. Transfer 1 mL of purified supernatant into an autosampler vial(s) for analysis. Filter if desired. (NOTE: This procedure is scaled for increased sensitivity. If more sensitivity is required, the extract may be concentrated by evaporation.)

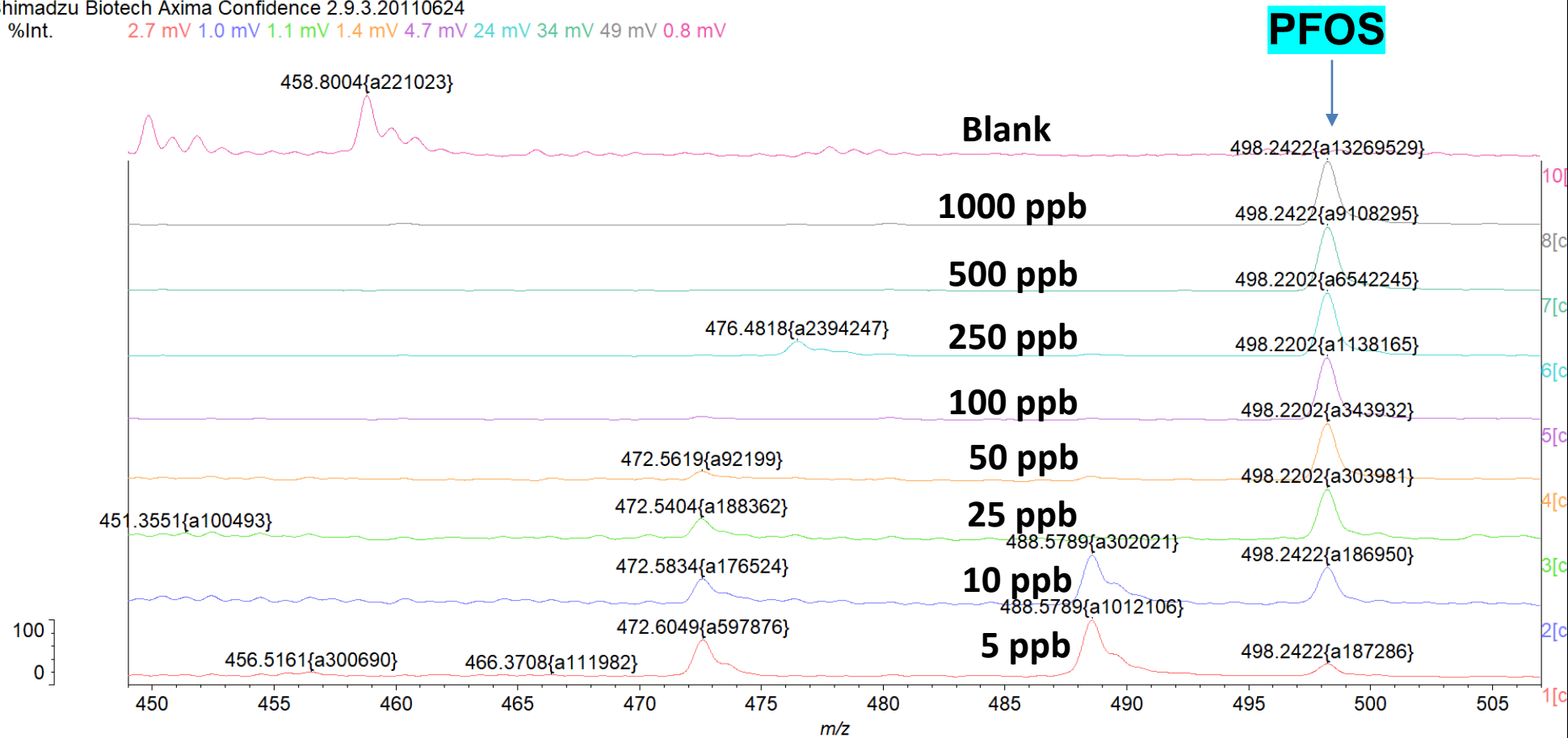
MALDI Chromatogram

Matrix - DHA

C1_20001, C2_30001, C3_30001, C4_30001, C5_20001, C6_30001, C7_30001, C8_30001, blank_A_30001

Shimadzu Biotech Axima Confidence 2.9.3.20110624

%Int. 2.7 mV 1.0 mV 1.1 mV 1.4 mV 4.7 mV 24 mV 34 mV 49 mV 0.8 mV



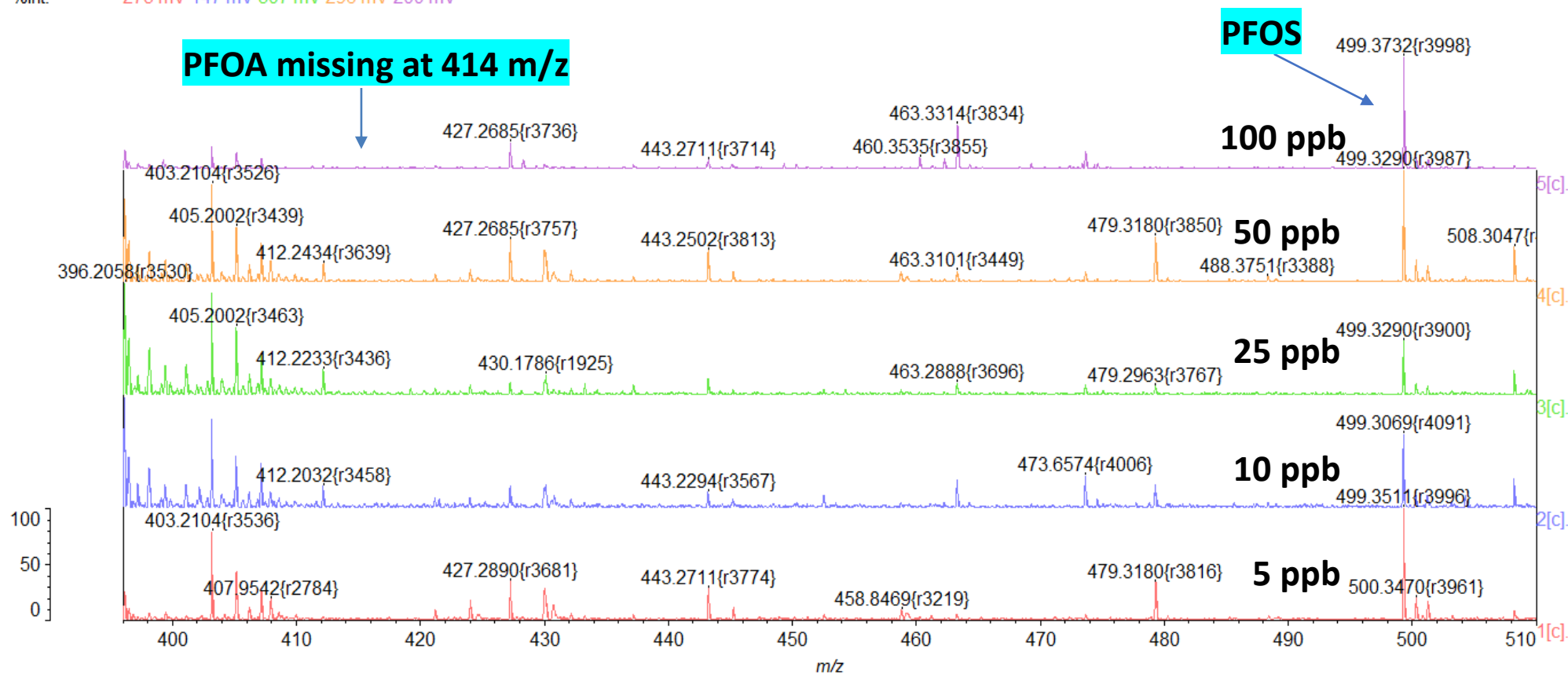
MALDI Chromatogram

Matrix – TMGN

Catchers 5 ppb PFOS B70001, Catchers 10 ppb PFOS B100001, Catchers 25 ppb PFOS B130001, Catchers 50 ppb PFOS B160001, Catchers 100 ppb PFOS B200001

Shimadzu Biotech Axima Performance 2.9.3.20110624

%Int. 273 mV 147 mV 307 mV 296 mV 200 mV

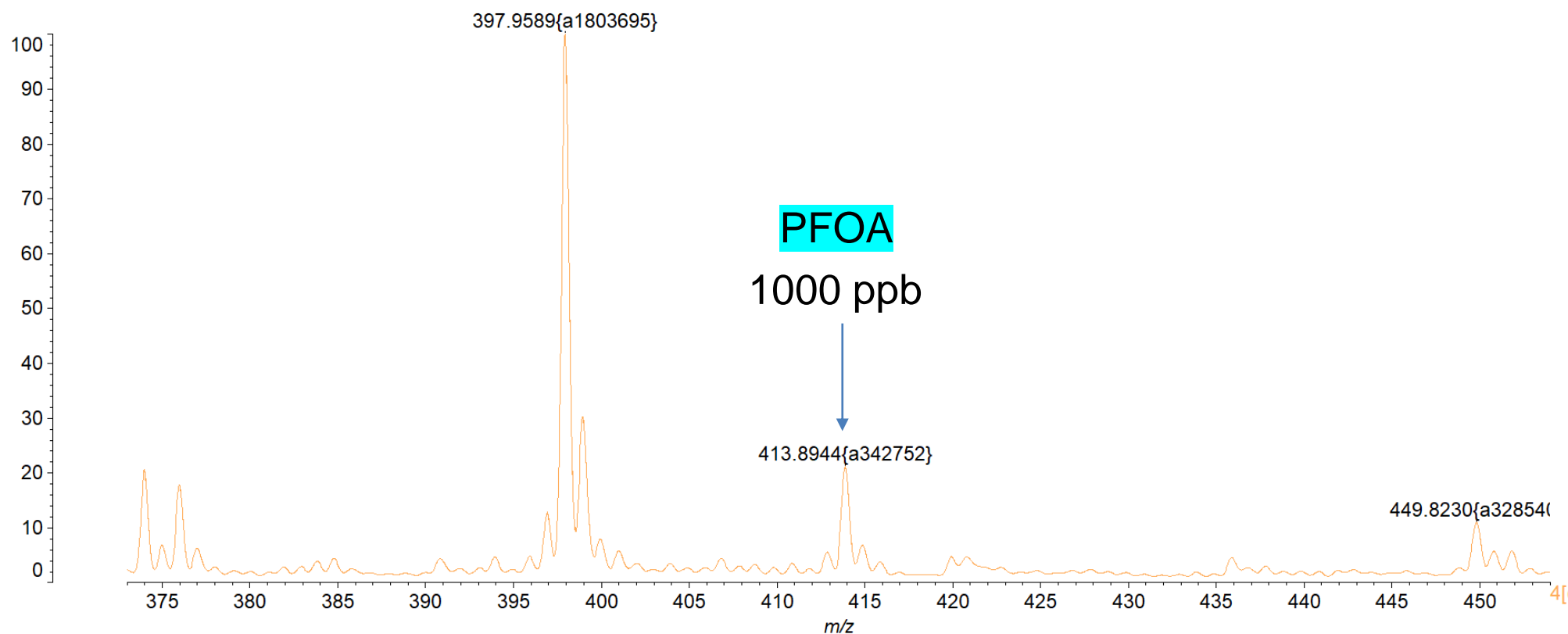


MALDI PFOA at 1000 ppb

Matrix – CHCA

Data: Cal8_20001.2E2[c] 6 Sep 2019 15:55 Cal: DH5_CHCA 11 Apr 2018 3:43
Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Reflectron_neg, Power: 50, Blanked, P.Ext. @ 413 (bin 57)

%Int. 11 mV[sum= 42186 mV] Profiles 1-4000 Smooth Gauss 20

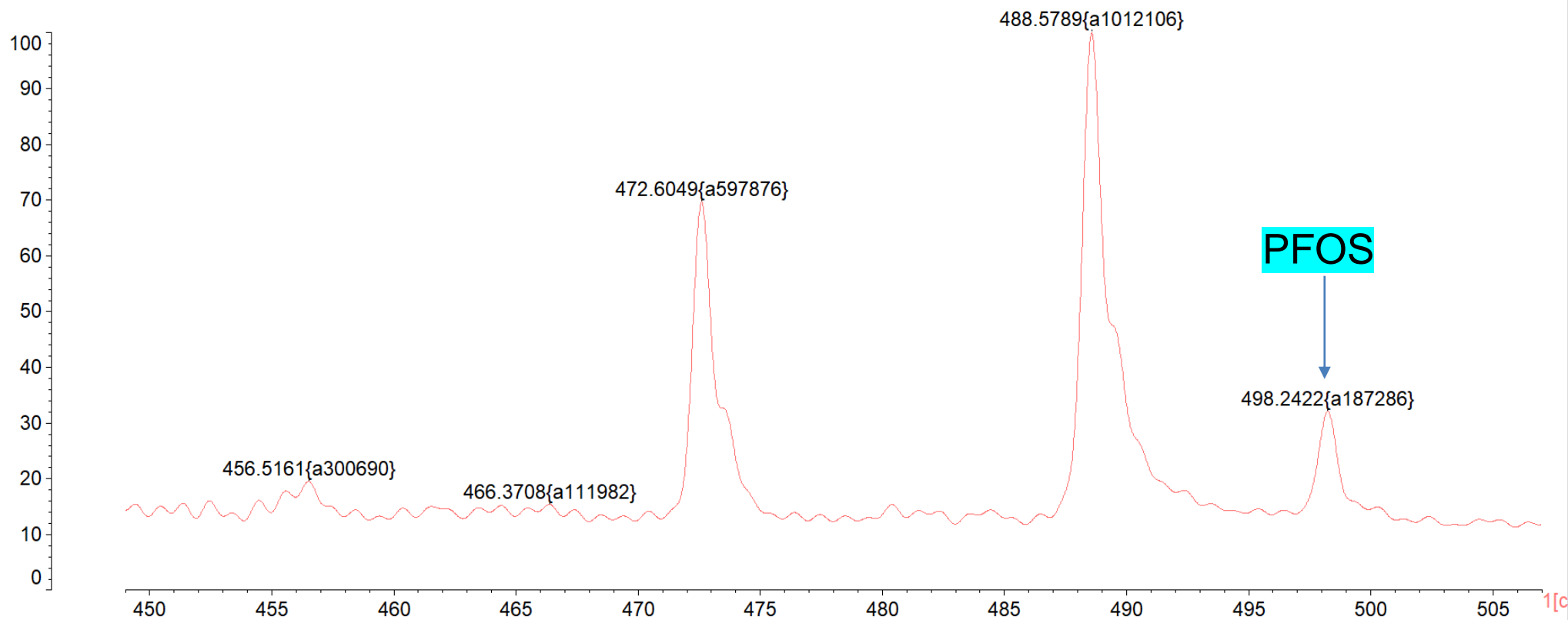


MALDI PFOS at 5 ppb

Matrix – DHA

Data: C1_20001.1L2[c] 23 Aug 2019 17:51 Cal: DH5_CHCA 11 Apr 2018 3:43
Shimadzu Biotech Axima Confidence 2.9.3.20110624: Mode Reflectron_neg, Power: 90, Blanked, P.Ext. @ 413 (bin 57)

%Int. 2.7 mV[sum= 10730 mV] Profiles 1-4000 Smooth Gauss 20



LCMS Calibration Chromatograms

5 ppb

10 ppb

25 ppb

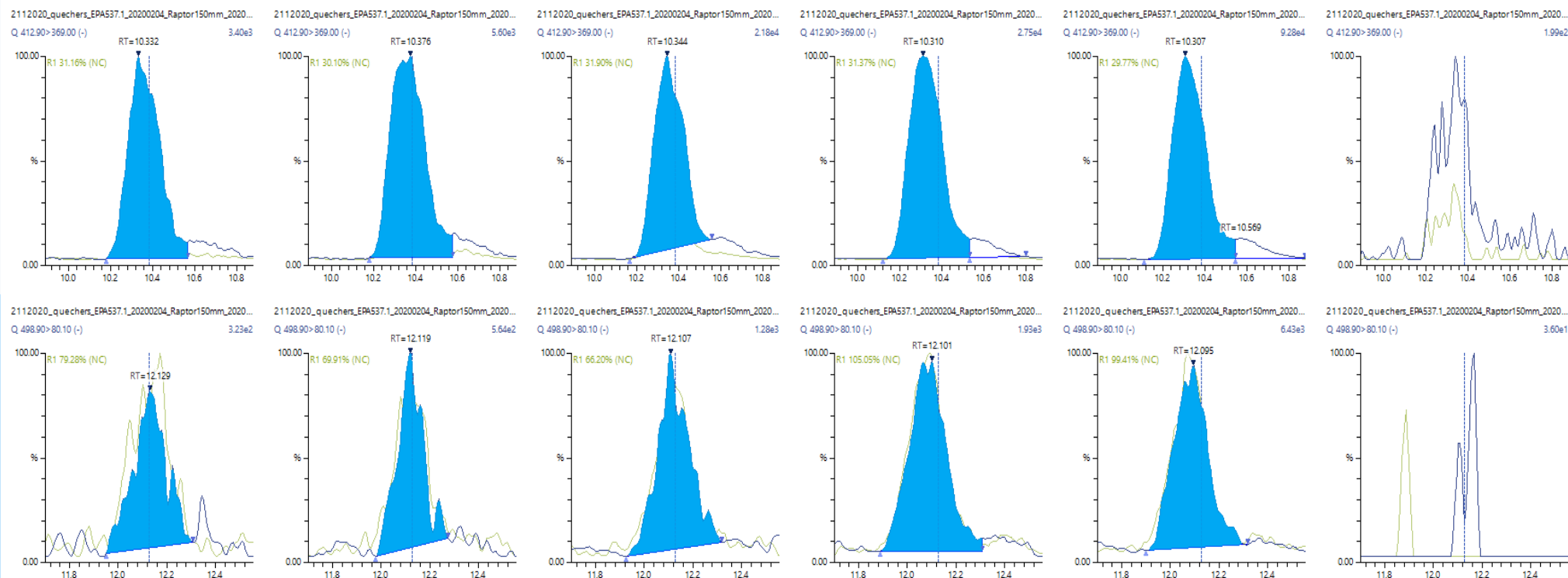
50 ppb

100 ppb

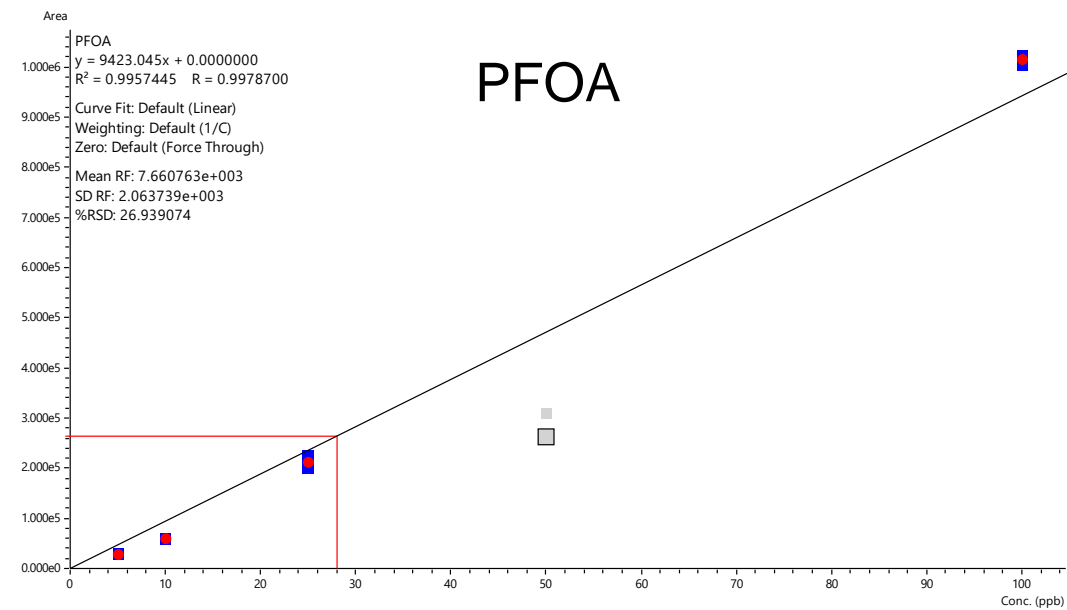
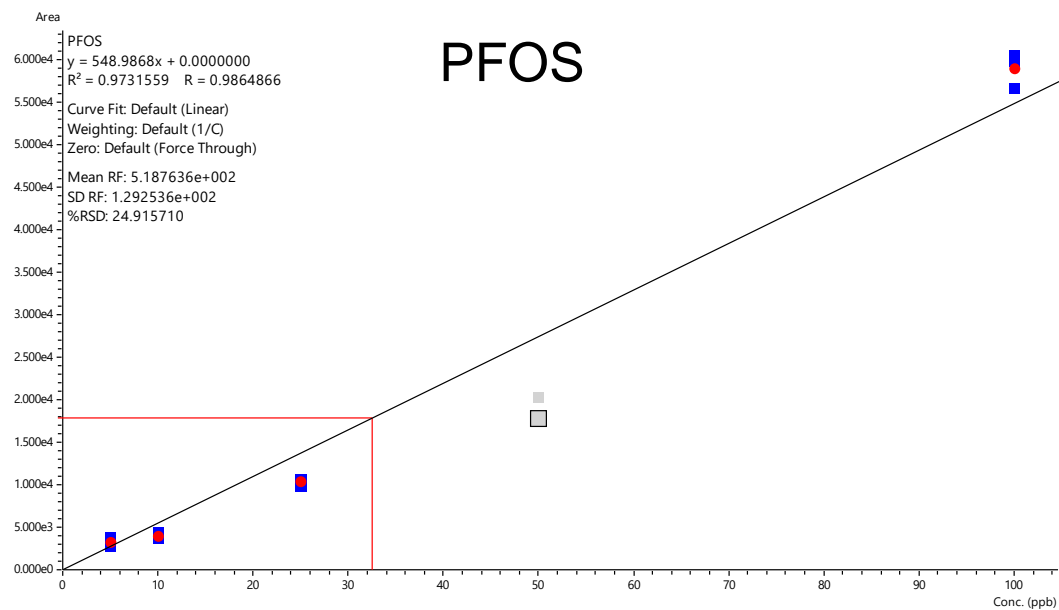
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#1 PFOA

#2 PFOS



LCMS Calibration Curve



LCMS Quantitative Results

	PFOS	PFOA	PFOS	PFOA
Standard Concentration	% Accuracy		% RSD	
5ppb	118	82	2.002	7.984
10ppb	80	81	6.766	9.09
25ppb	78	106	5.94	5.219
50ppb	74	73	7.97	7.097
100ppb	109	118	1.055	3.455

Conclusions

- Milk is a challenging matrix for both techniques
- **Sample preparation was the limiting factor in this study**
- QuEChERS Extraction did not yield samples that allowed for trace analysis of PFAS
- To fully utilize the detection power of both instruments, **cleaner samples must be extracted**
- MALDI-TOF – Axima Confidence
 - PFOS easily ionizable and visible at 5 ppb
 - PFOA only seen at higher concentrations with low intensity; not ionized as well
- LCMS-8050 could quantify both PFOA and PFOS at 5 ppb
- Continued efforts are required to develop a sensitive screening method for milk

The End

Thank you for Sticking Around



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