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A Strategy for the Extraction and Analysis of Perfluoroalkyl Substances (PFAS) in Human Serum Using Weak Anion Exchange Chemistry

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Senior Scientist
NEMC 2020

Perfluoroalkylated Substances (PFAS)

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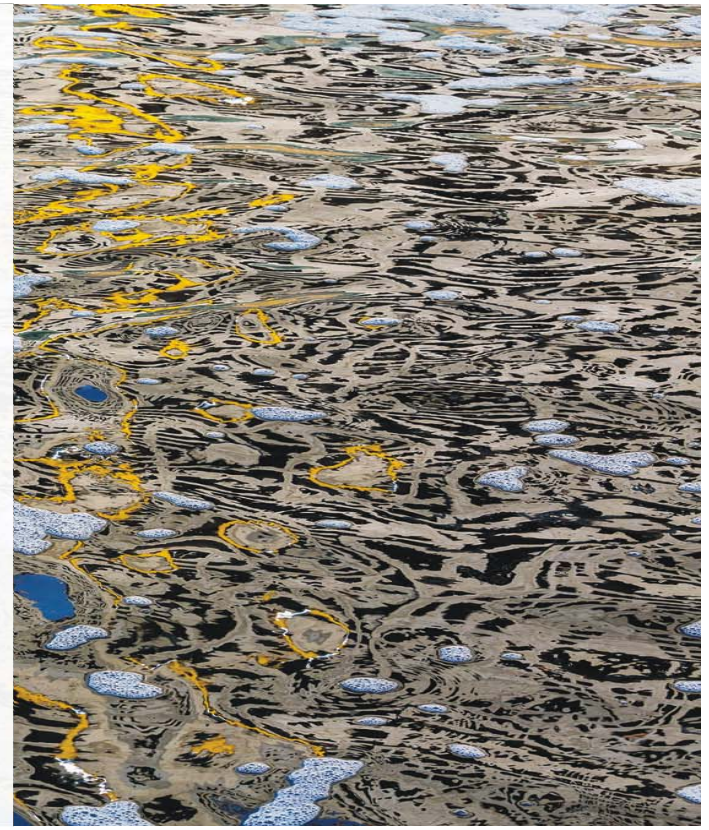
- PFAS = PFC = AFFF
- First **created** in the **1930s**
- **Widespread applications**
  - Non stick **coatings**, **surfactants**, food **packaging**, firefighting **foams**
  - **Polymerization aid** for polytetrafluoroethylene (PTFE) and other fluoropolymers – how PFOS and PFOA became famous
- **Stable** and **persistent** in the environment (POP)
  - Bio-accumulative
- **Identified** in environmental samples **worldwide**
  - Found in arctic **polar bears**



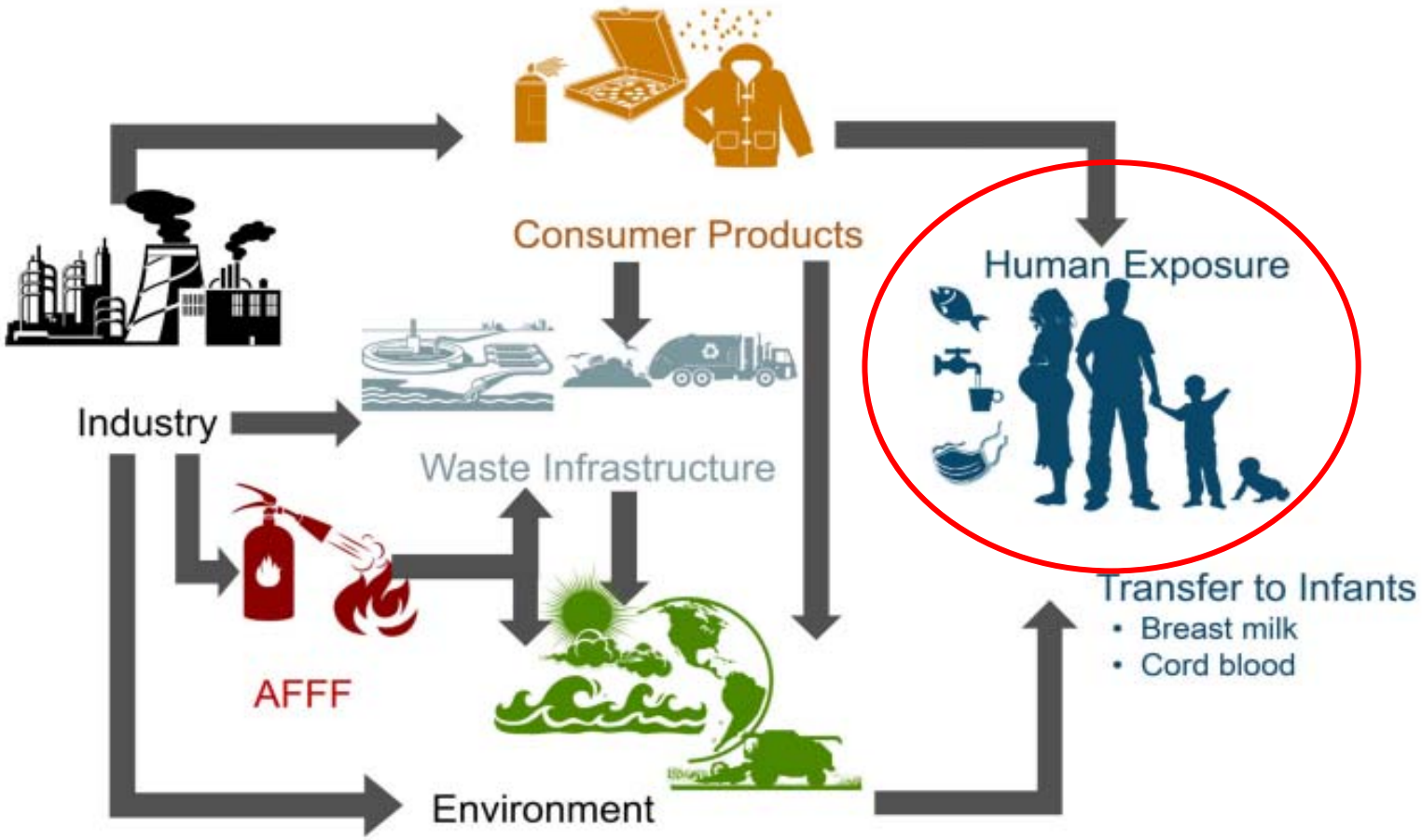
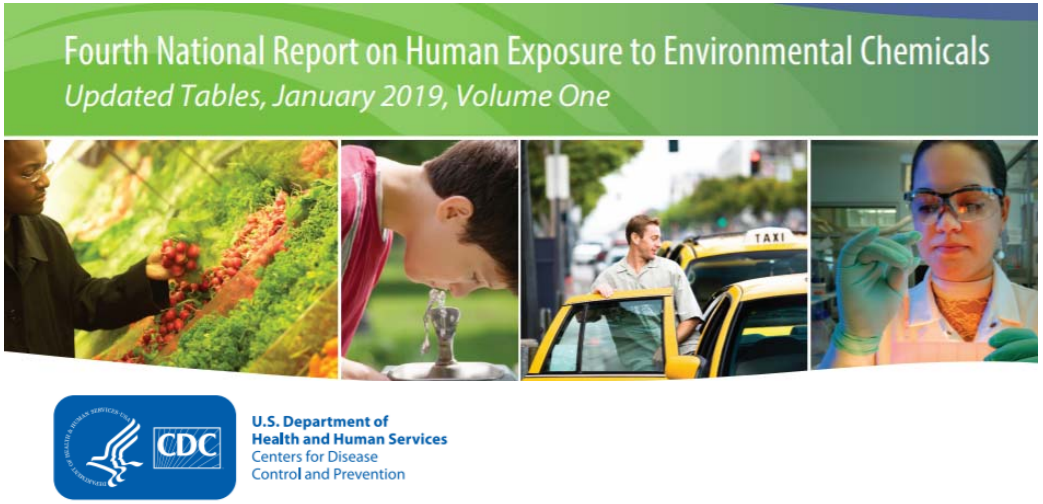


Image from doi: 10.1038/s41370-018-0094-1

NHANES Studies on PFAS in Human Serum

- 1999-2016
- Age, gender, and race
- 12 PFAS detected in human serum:
  - PFBS
  - PFDA
  - PFDoDA
  - PFHpA
  - PFHxS
  - PFNA
  - PFUnDA
  - PFOA (branched and linear)
  - PFOS (branched and linear)
  - PFOSA (aka FOSA)
  - NEtFOSAA
  - NMeFOSAA



Finding a measurable amount of PFCs in serum does not imply that the levels of PFCs cause an adverse health effect. Biomonitoring studies of serum PFCs can provide physicians and public health officials with reference values so that they can determine whether or not people have been exposed to higher levels of PFCs than are found in the general population. Biomonitoring data can also help scientists plan and conduct research on exposure and health effects.

https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf

https://www.cdc.gov/biomonitoring/PFAS_BiomonitoringSummary.html

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Transitioning from Water to Serum

Solid Phase Extraction Set Up - Cartridge

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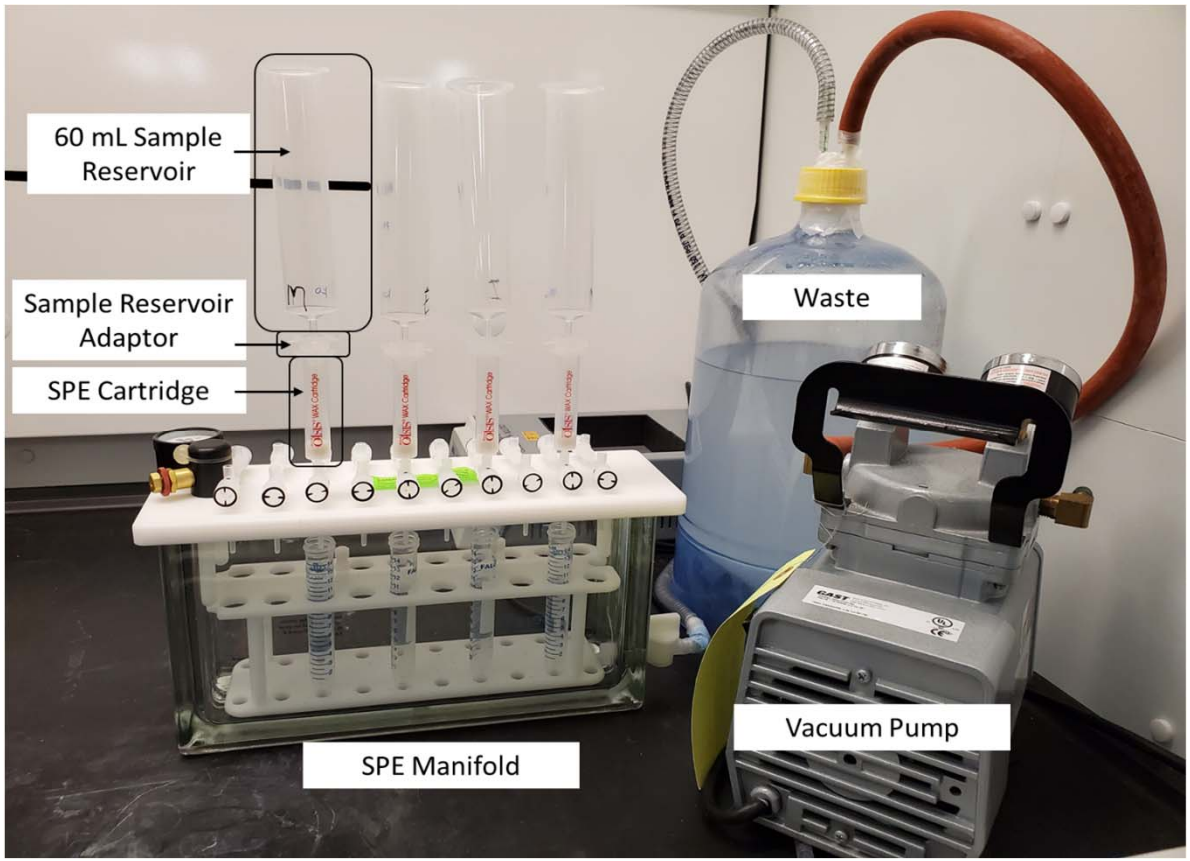

Solid Phase Extraction Set Up – 96 well plate

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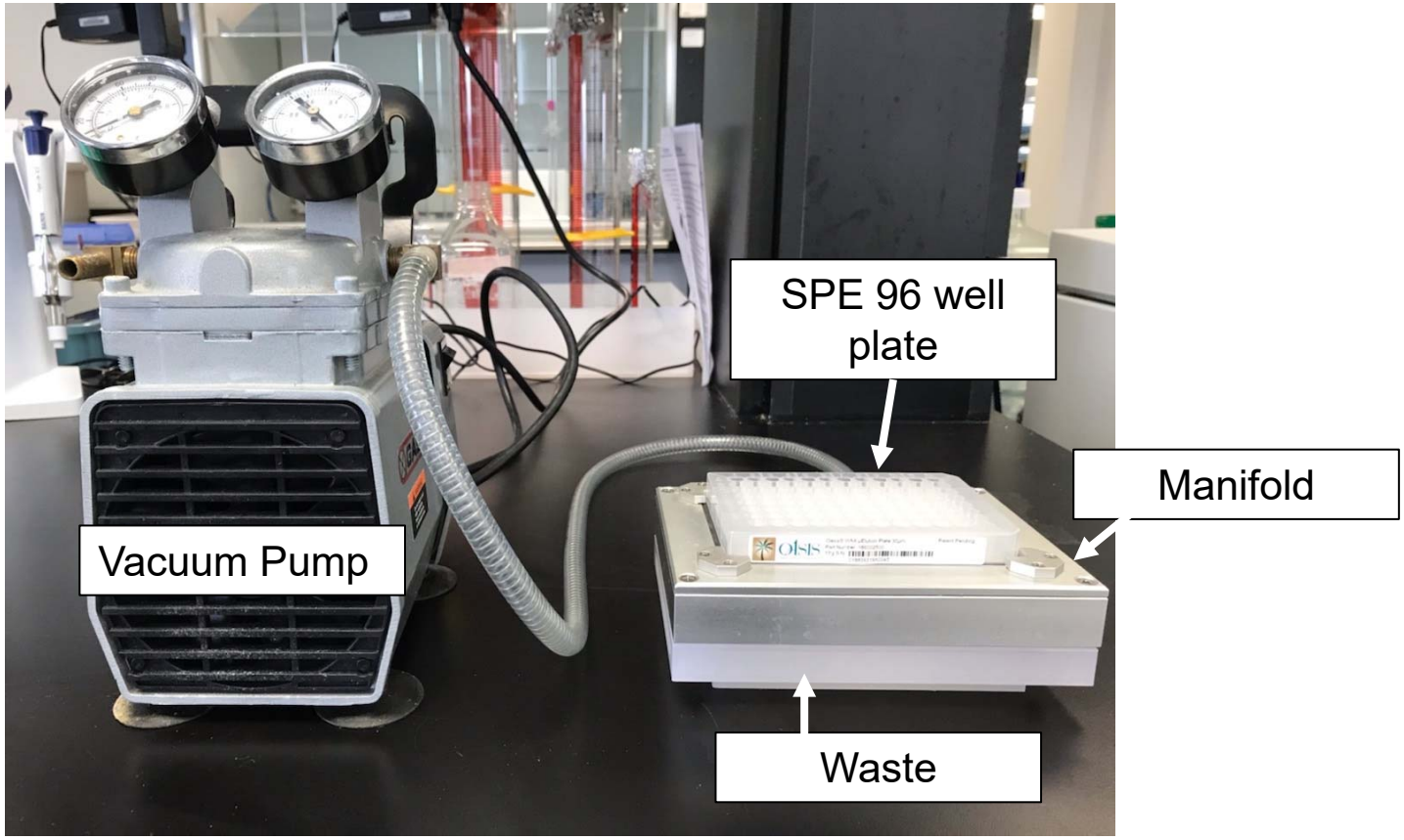

Wide variety of chemical properties

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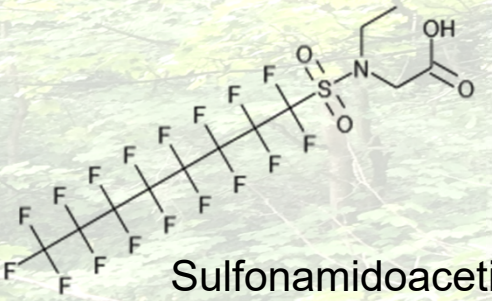
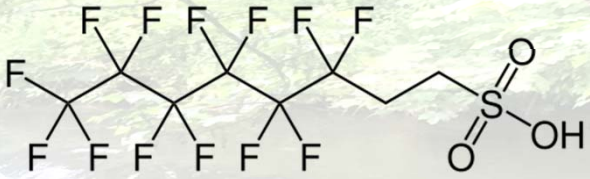
Perfluoro Carboxylic Acid
(PFOA)

Perfluoro Sulfonic Acid
(PFOS)

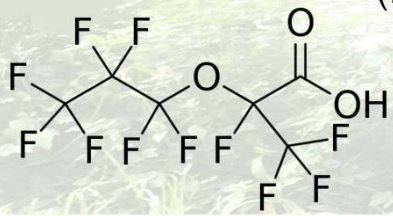


Sulfonamidoacetic
acid
(N-EtFOSAA)

Perfluoro Telomer Acid
(FHEA or 6:2 FTA)

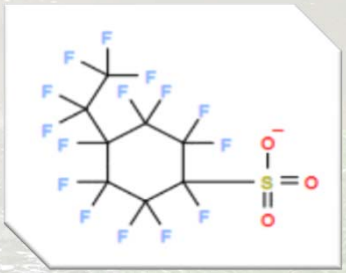


Perfluoro Telomer Sulfonate
(6:2 FTS)



Emerging PFAS
(GenX)

Cyclic
(PFecHS)



30 PFAS targeted in method

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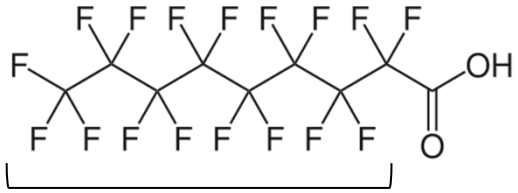
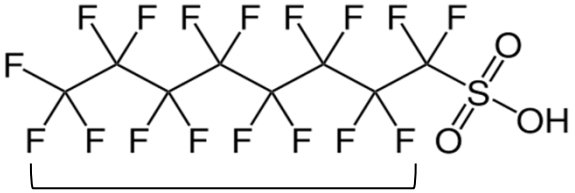
Carboxylates	Sulfonates
 C4 – C14	 C4 – C10

Emerging		Precursors
GenX	ADONA	4:2/6:2/8:2 FTS
11Cl-PF3OUdS	9Cl-PF3ONS	FOSA
FBSA	FHxSA	NMeFOSAA/NEtFOSAA

Instrument Methods

Source Parameters

- Instrument: Xevo TQ-S micro
- Ion Mode: ESI-
- Capillary Voltage: 0.5 kV
- Desolvation Temperature: 300° C
- Desolvation Flow: 900 L/hr
- Cone Flow: 100 L/hr

MS Method

- Developed using QuanOptimize
  - MRMs, CV, CE
- Divert flow to waste from 0 – 3 and 16 – 22 mins

LC Method

- Instrument: Acquity I Class PLUS **modified with PFAS Kit**
- Column: HSS T3 2.1mm x 100 mm, 1.8 µm
- Mobile Phase A: 95:5 H2O:MeOH + 2 mM ammonium acetate
- Mobile Phase B: MeOH + 2 mM ammonium acetate
- Injection Volume: 5 uL
- Gradient:

Time (min)	Flow (mL/min)	%A	%B
0	0.3	100	0
1	0.3	80	20
6	0.3	55	45
13	0.3	20	80
14	0.4	5	95
17	0.4	5	95
18	0.3	100	0
22	0.3	100	0

Introduction to Weak Anion eXchange (WAX)

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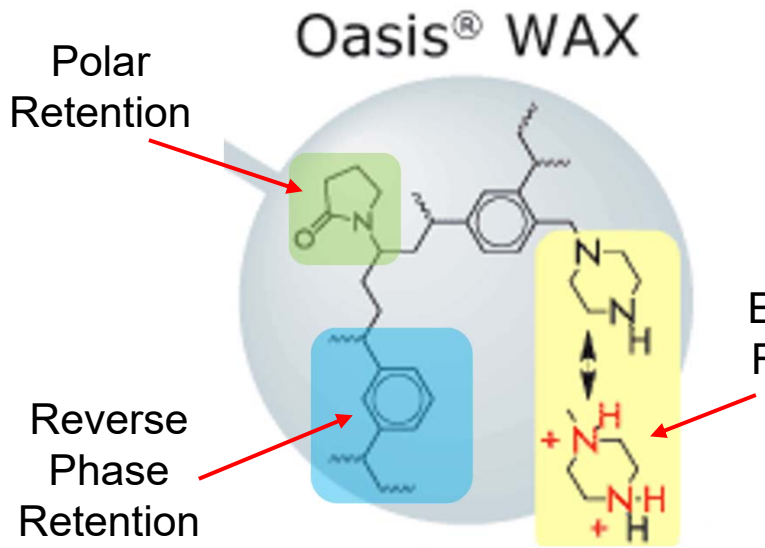
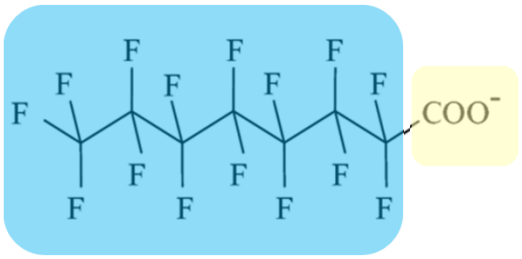
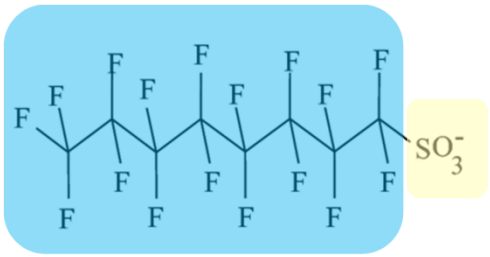
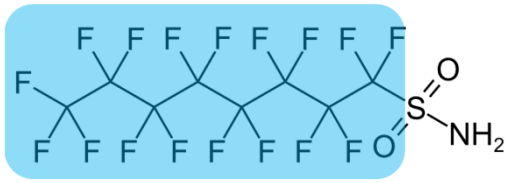




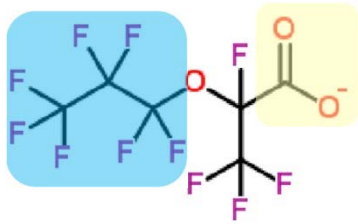
PFOA



PFOS



FOSA



GenX

SPE Sample Preparation

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Pretreat Samples (50 µL)

pH adjust to < 3 – **activates ion exchange sites in WAX sorbent when loaded**

Condition Plate

Load Sample

Wash Plate

Elute

Eluent with pH > 8 – **"turns off" ion exchange sites in WAX sorbent to release PFAS**

Oasis WAX 96 well µElution plate

2 mg per well

SPE Sample Preparation

Waters
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Pretreat Samples (50 µL) ★
pH adjust to < 3 – **activates ion exchange sites in WAX sorbent when loaded**

Condition Plate

Load Sample

Wash Plate ★

Elute
Eluent with pH > 8 – **"turns off" ion exchange sites in WAX sorbent to release PFAS**

Oasis WAX 96 well µElution plate

2 mg per well

SPE Sample Preparation

Waters
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Pretreat Samples (50 µL) ★
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Wash Plate ★

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Eluent with pH > 8 – **"turns off" ion exchange sites in WAX sorbent to release PFAS**

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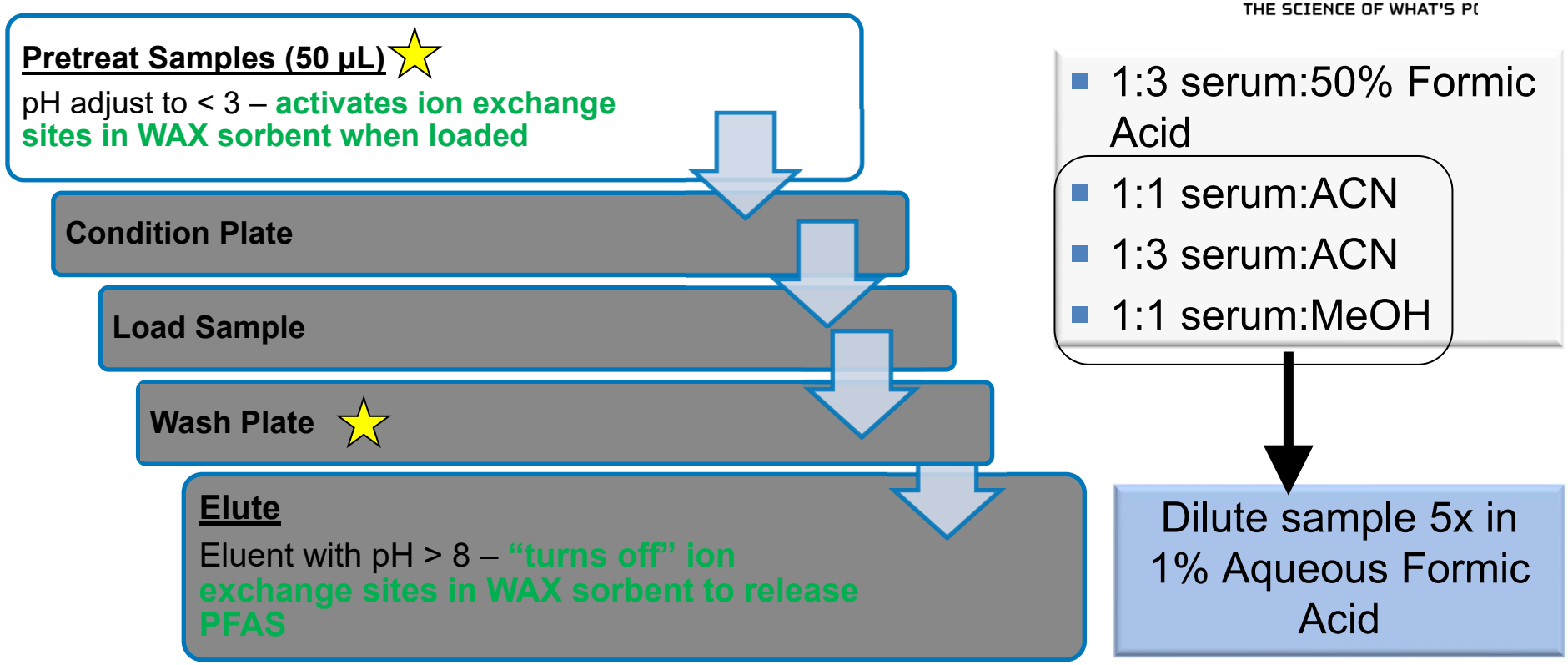
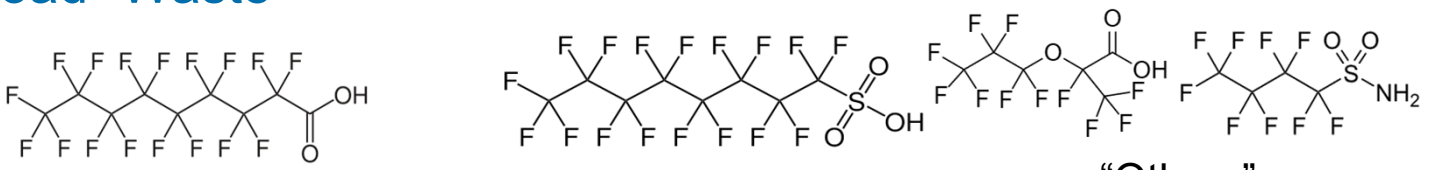
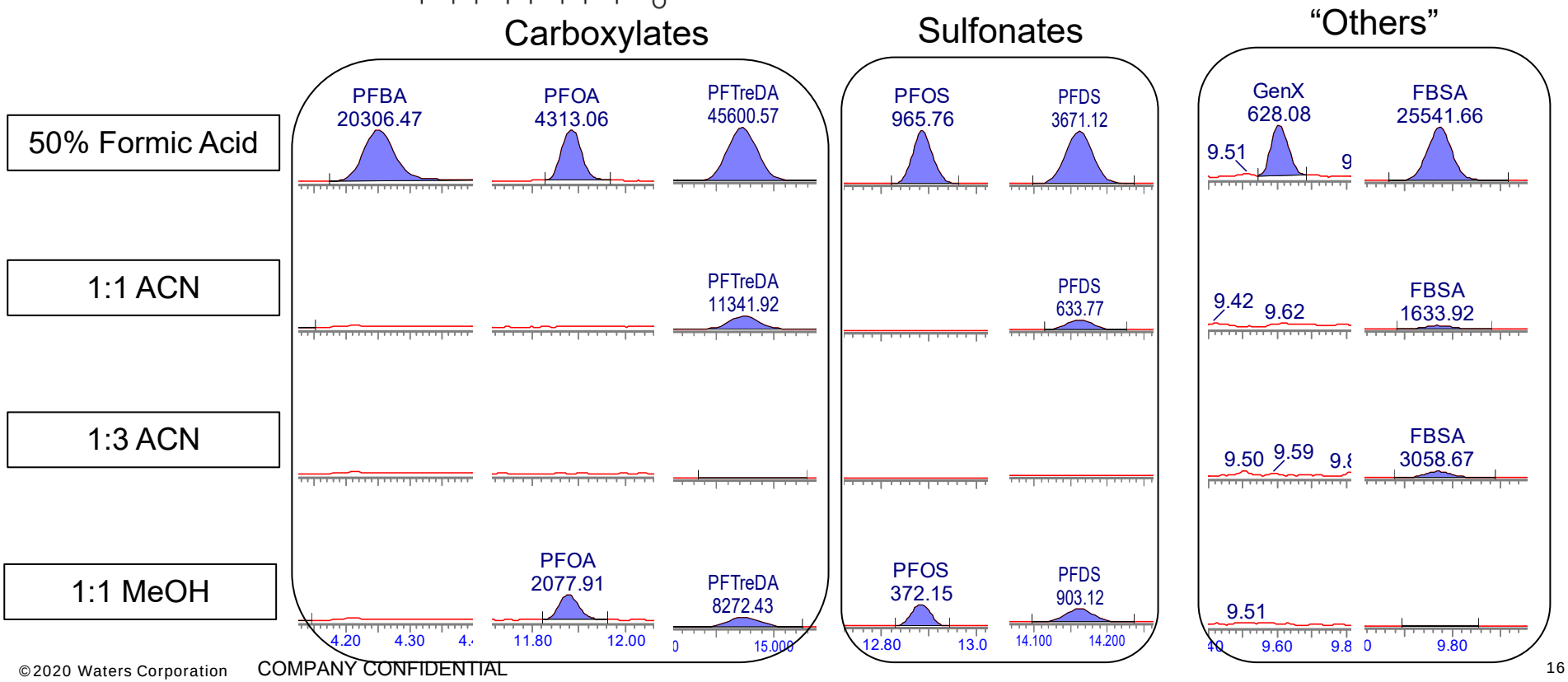
SPE Sample Preparation

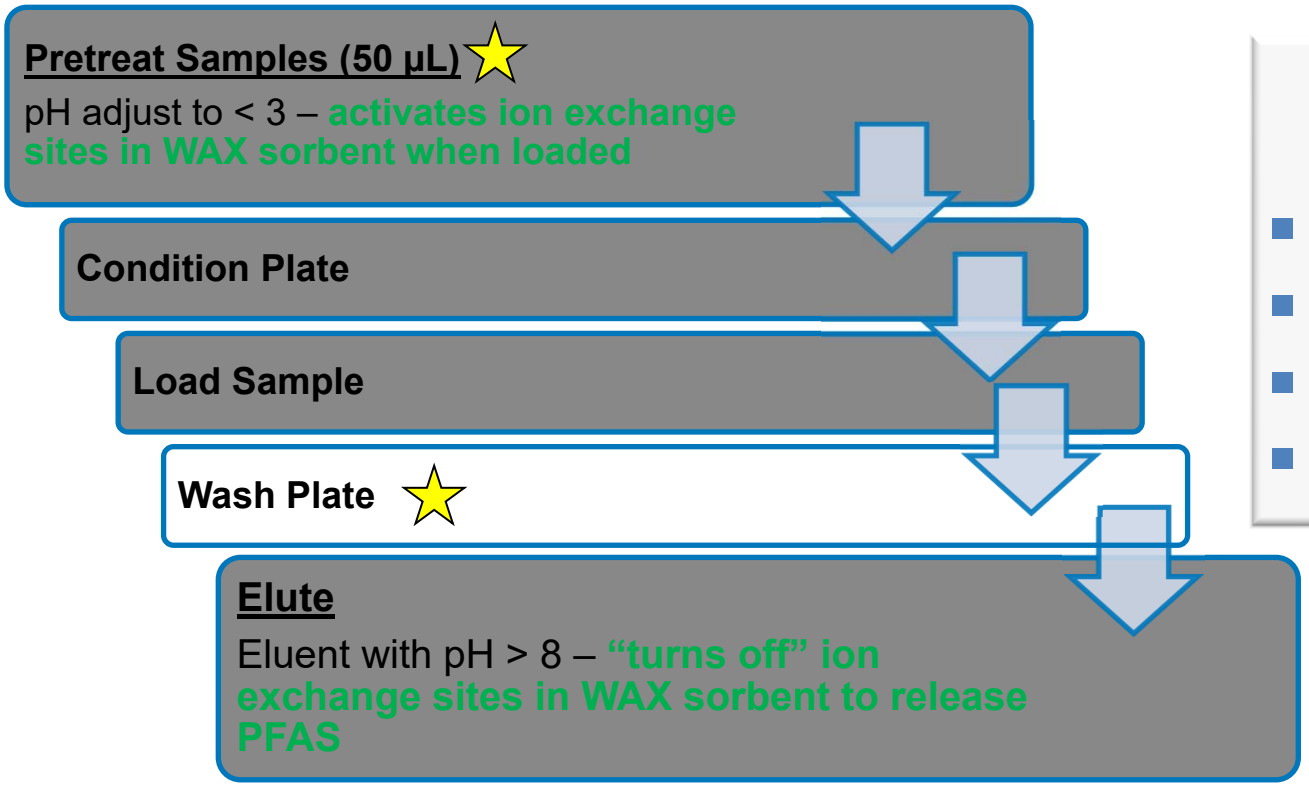


Injections of Plate Load “Waste”





SPE Sample Preparation

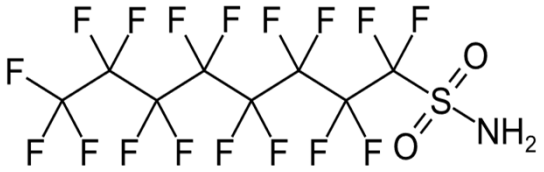


2% Formic Acid

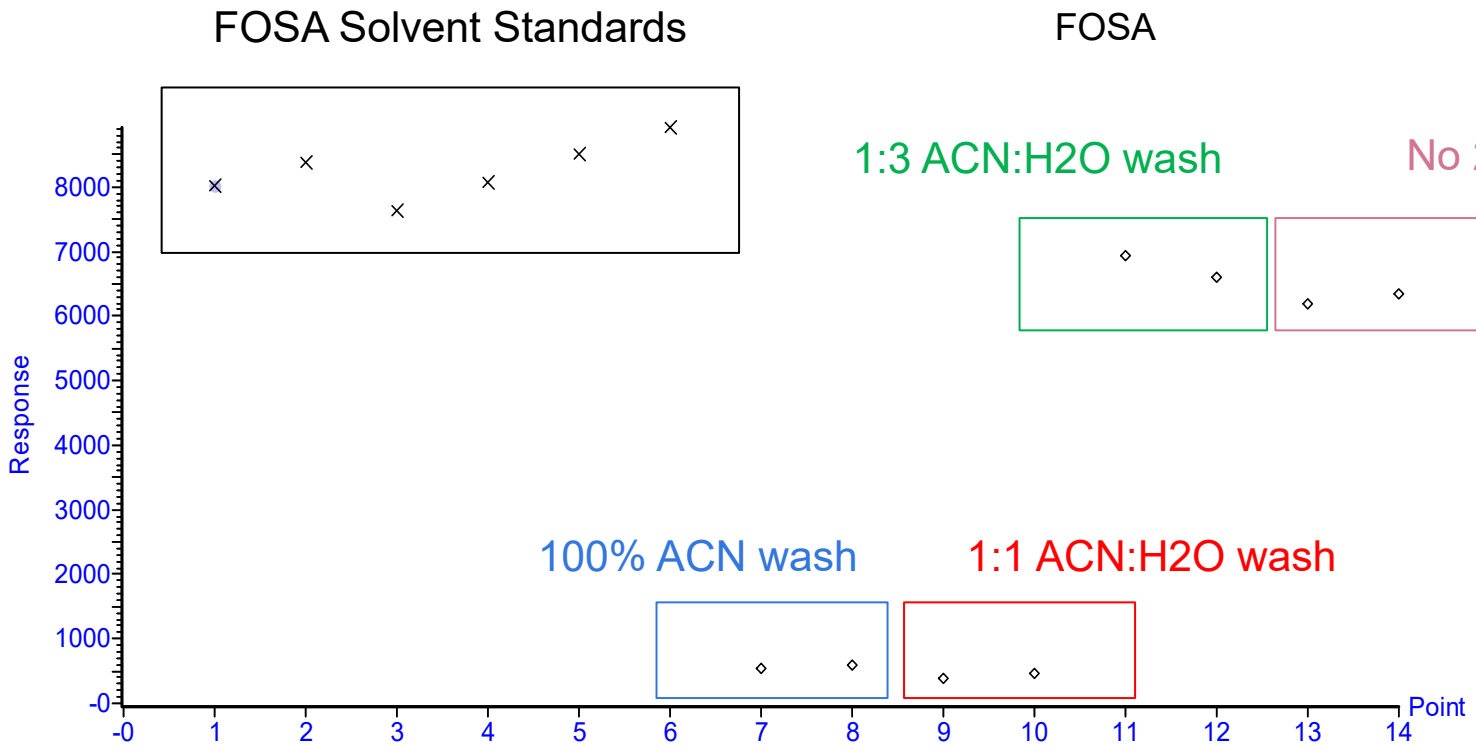
+

- 100% ACN
- 1:1 ACN:H2O
- 1:3 ACN:H2O
- No 2nd Wash

2nd Wash – Neutral Compounds



FOSA



SPE Sample Preparation – Final Method

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Pretreat Samples (50 µL) – spike isotope dilution standards, PPT with 3x ACN, dilute with 5x 1% Formic Acid

Condition Plate
200 µL 2% ammonia/methanol
200 µL methanol
200 µL 1% Formic Acid

Load Sample

Wash Plate
200 µL 1% Formic Acid
200 µL 1:3 ACN:Water

Elute
50 µL methanol
50 µL 2% (v/v) ammonia/methanol → 2x
Dilute with 50 µL 2% ammonium acetate containing Injection Standards

Oasis WAX 96 well µElution plate

2 mg per well

FBS Curves

Cal points of 0.05 – 20 ng/L

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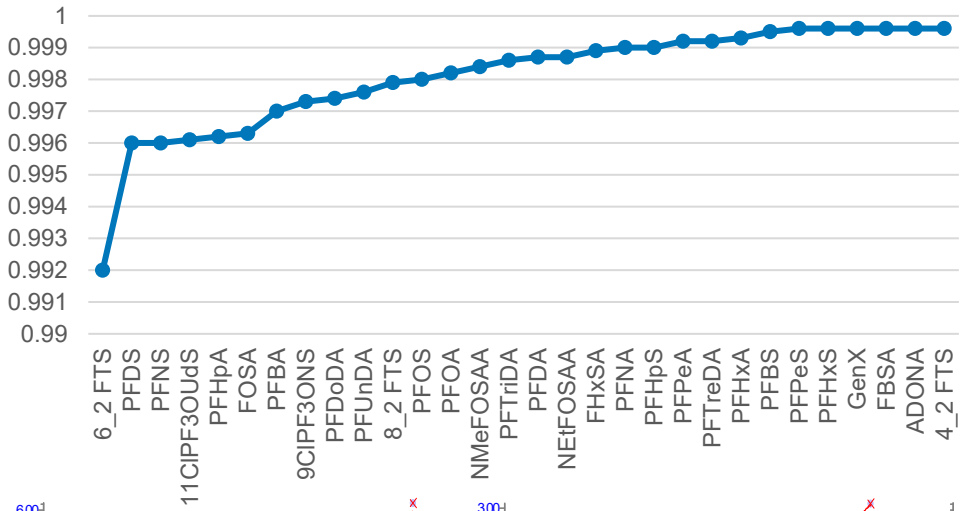

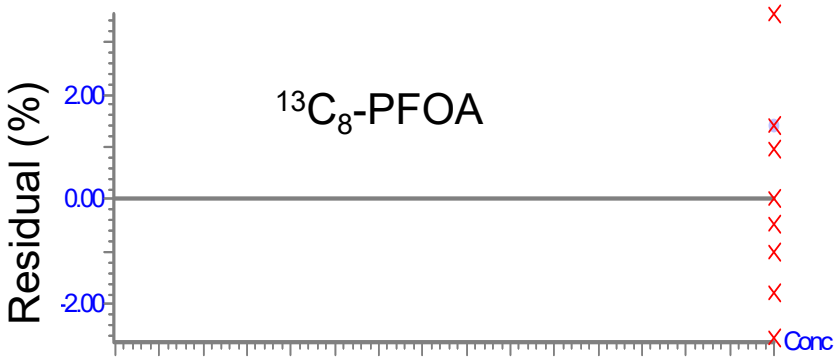

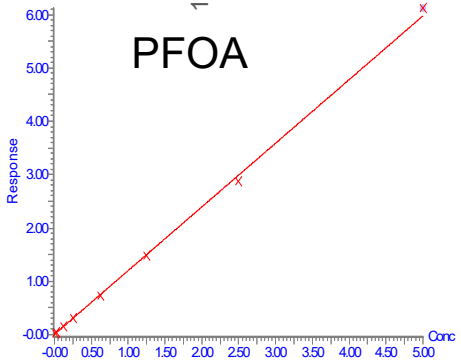

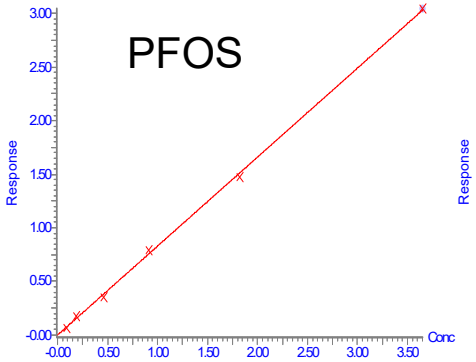

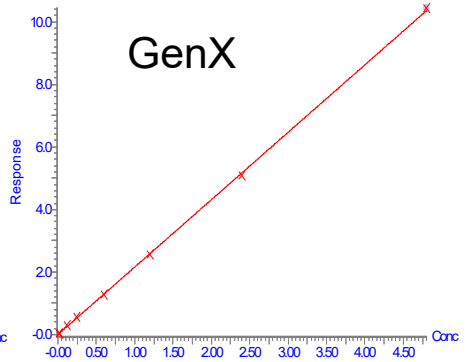

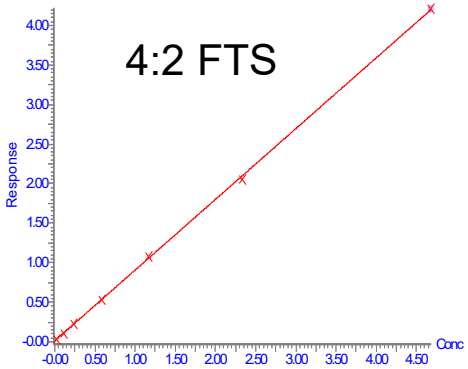

Recovery in Fetal Bovine Serum

Pre and Post Spike area over calibration range:
0.05, 0.1, 0.5, 1.0, 2.5, 5, 10 and 20 ng/L

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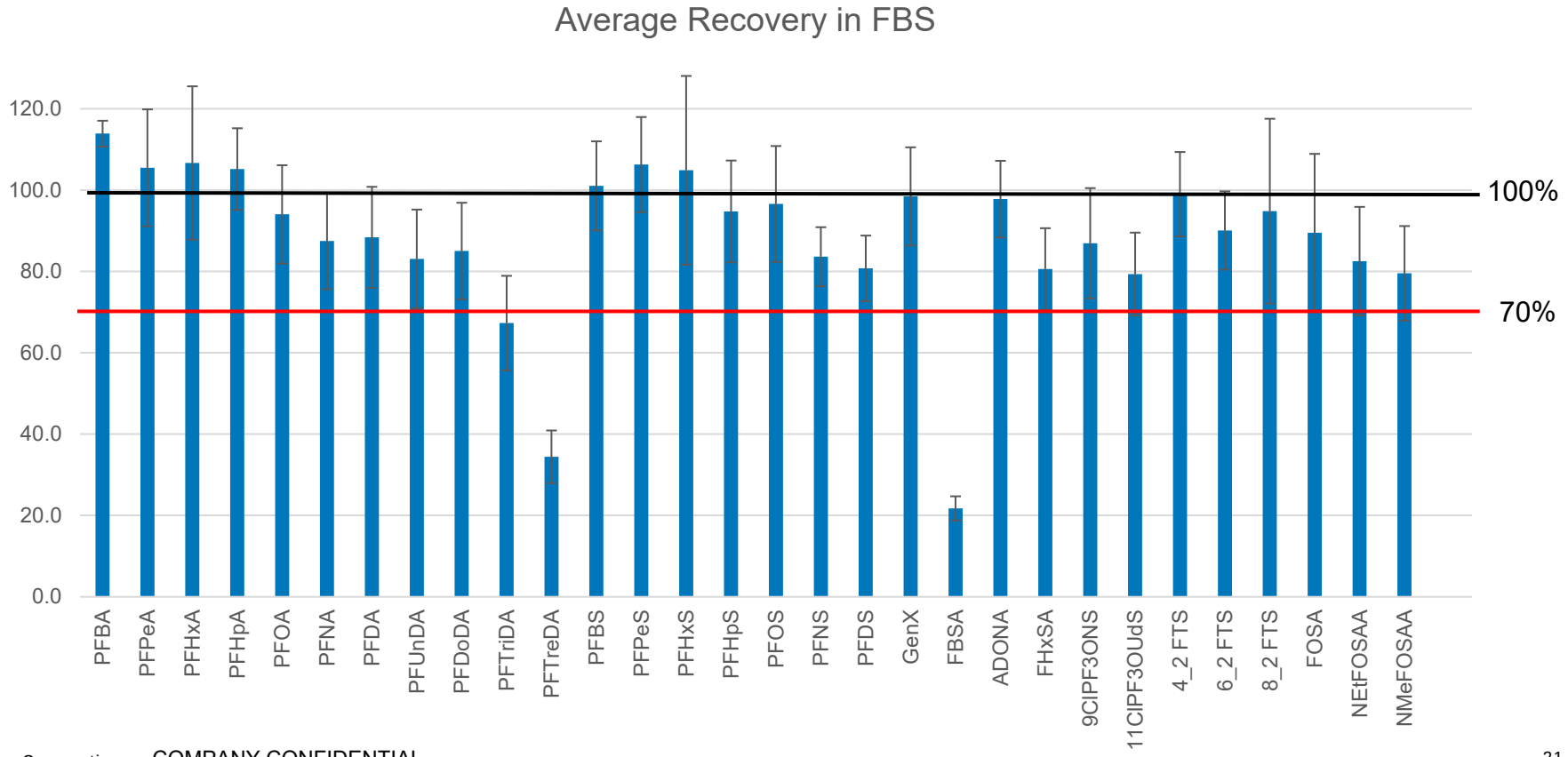
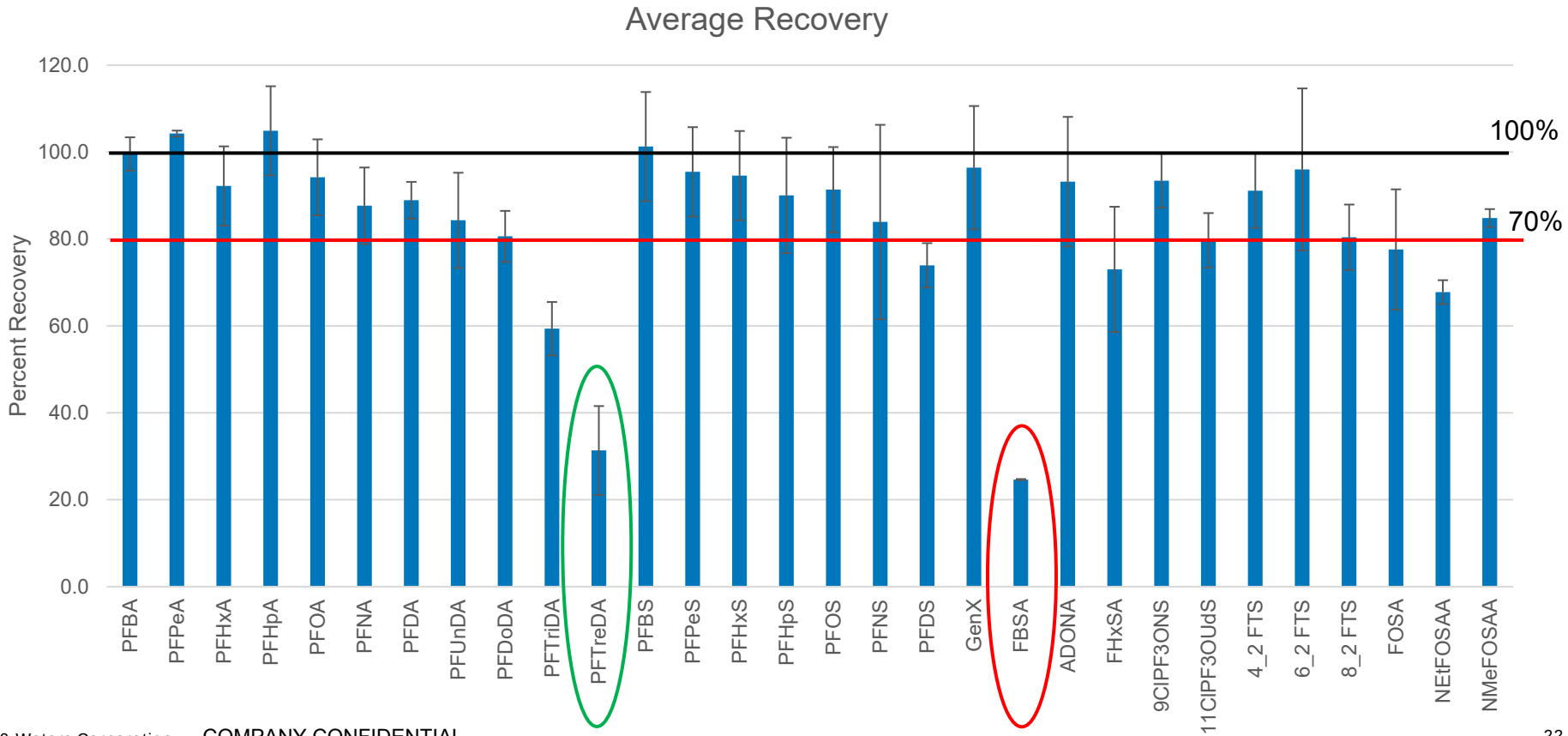


Recovery in Human Serum

Pre and Post Spike area at 0.1, 1.0, and 10 ng/L



Recovery in Human Serum

6 Lots of Pooled Human Serum
Pre and Post Spike area at 0.1, 1.0, and 10 ng/L

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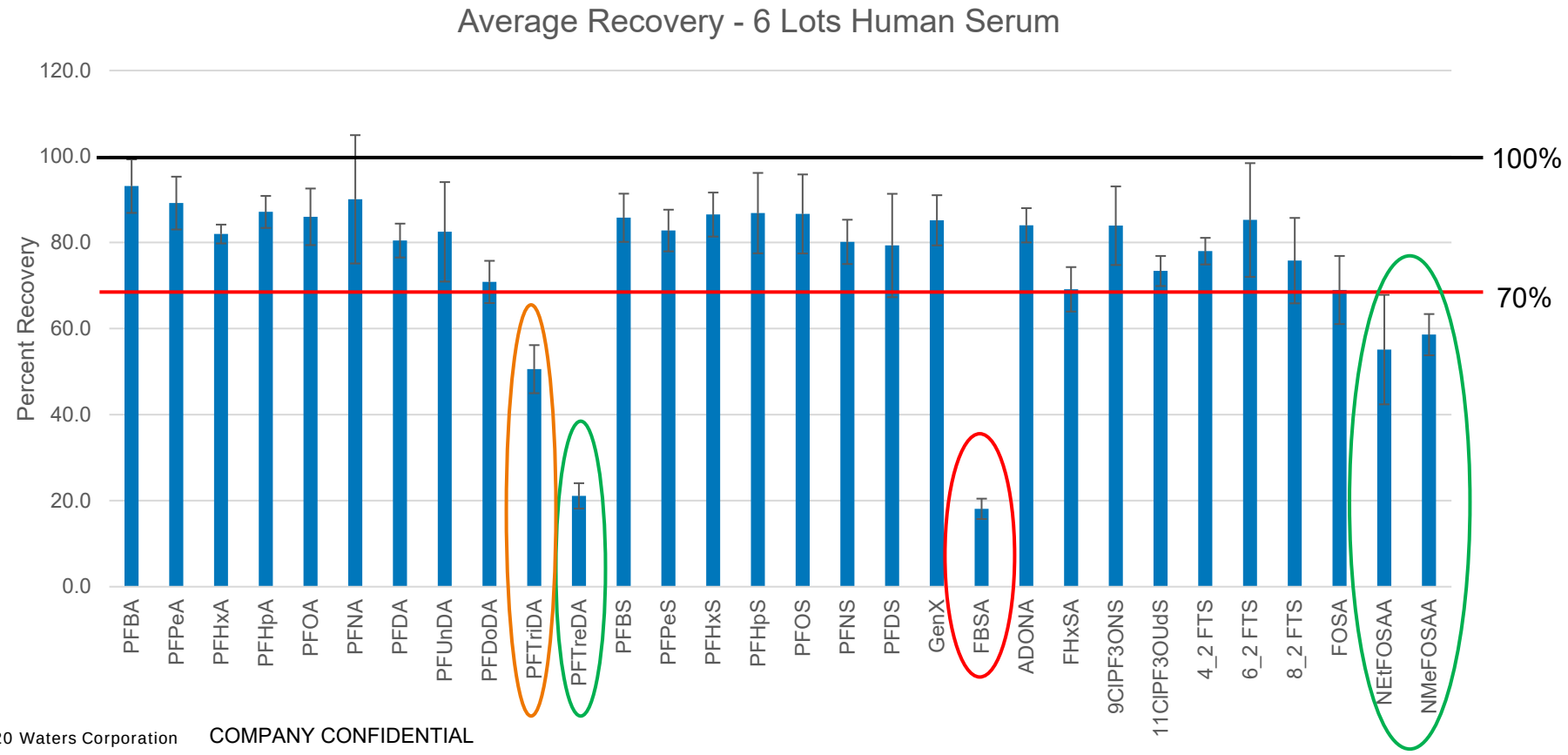
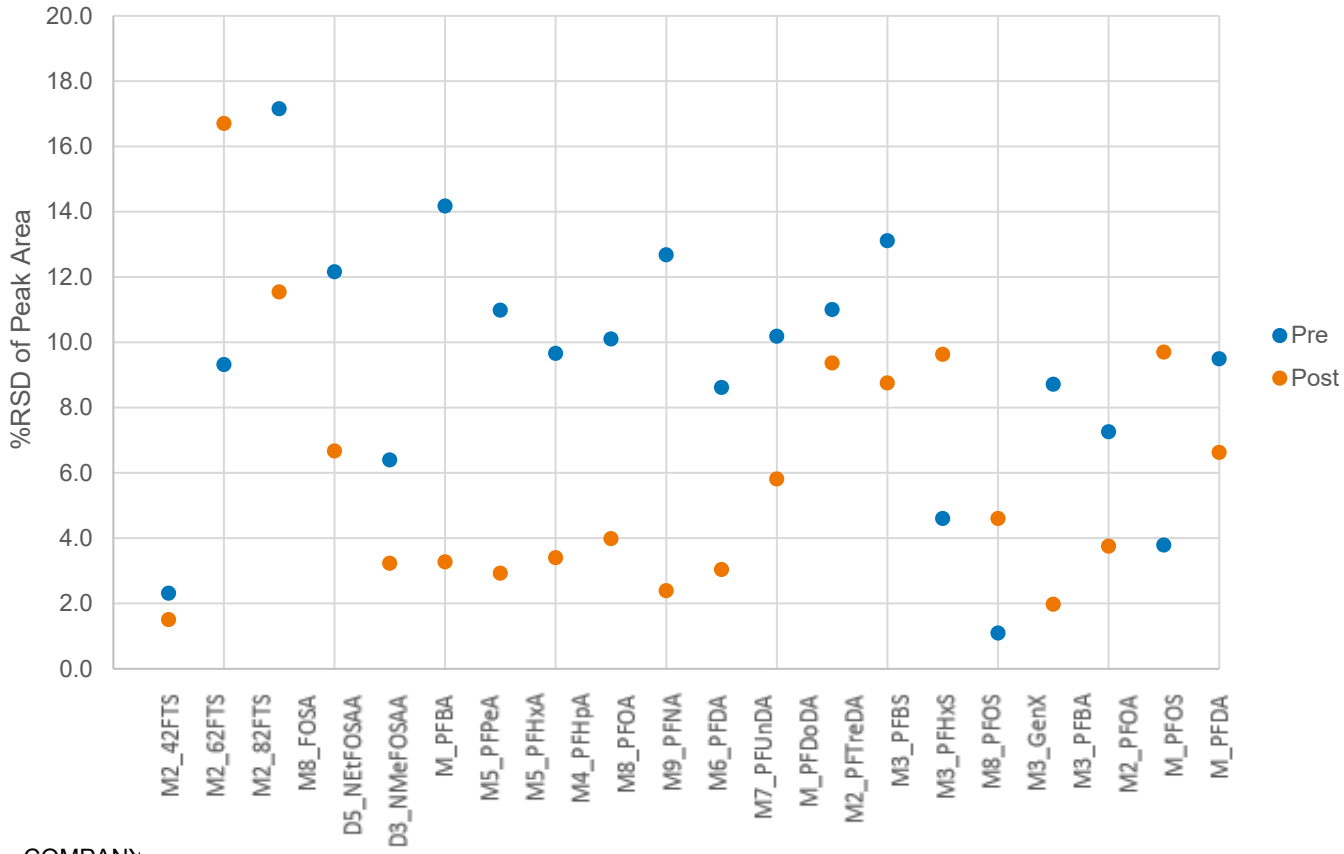
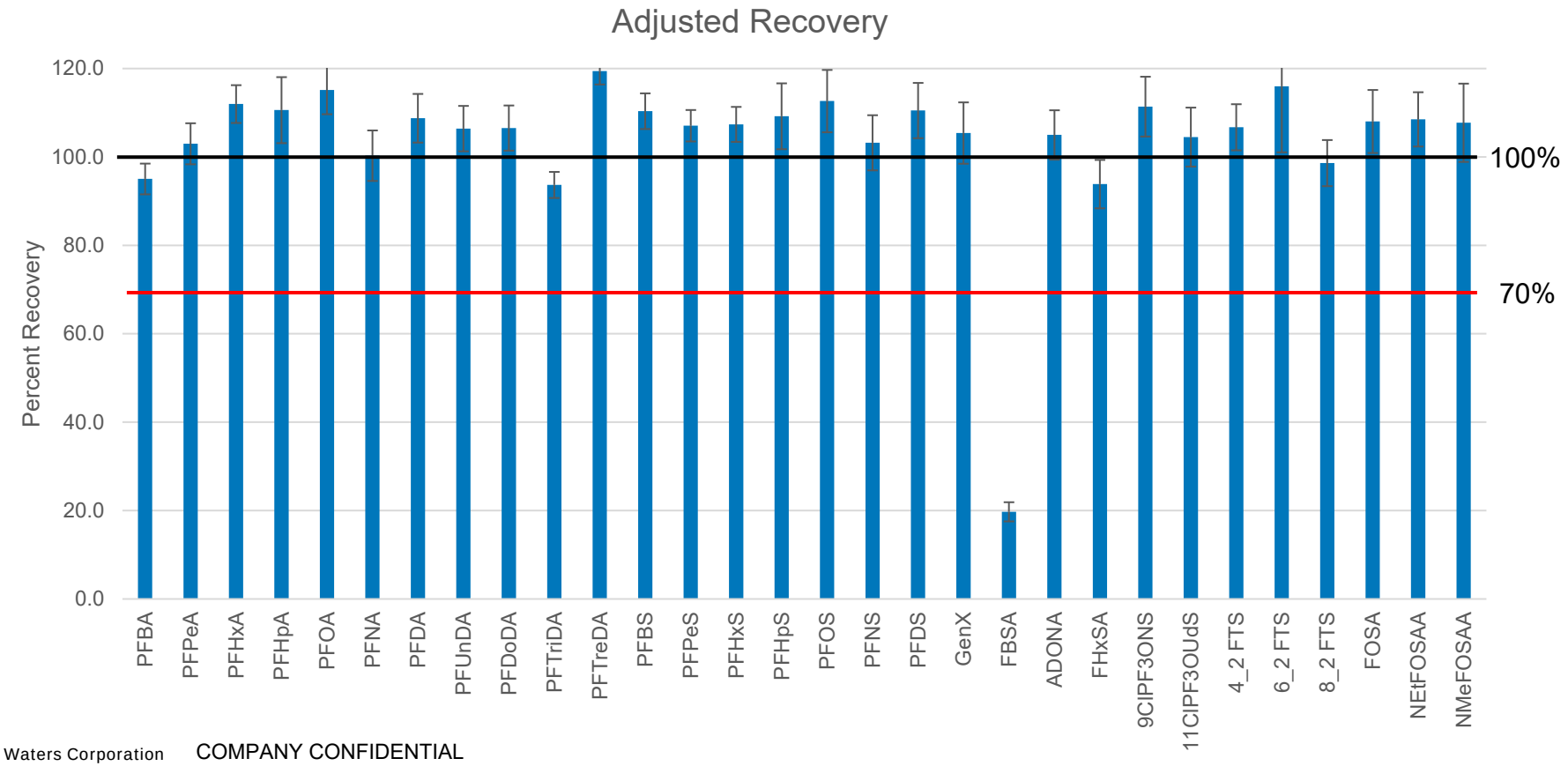


Internal Standards



Recoveries Corrected with Internal Standards



National Institute of Standards & Technology

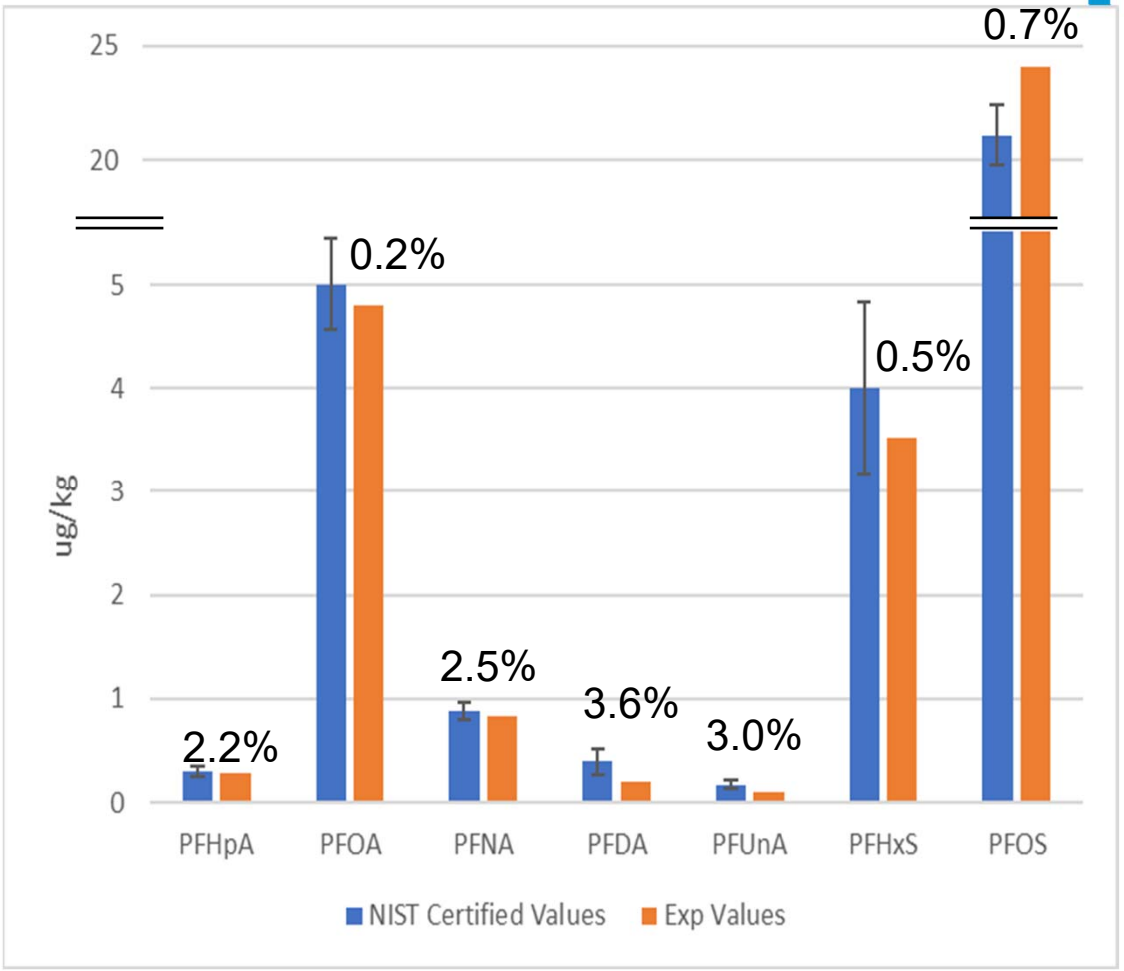
Certificate of Analysis

Standard Reference Material® 1957

Organic Contaminants in Non-Fortified Human Serum
(Freeze-Dried)

"Extra" Compounds Detected

Compound	ng/ml
NMeFOSAA	1.01
NEtFOSAA	0.19
PFHpS	0.30
PFBA	< MQL
PFPeA	< MQL



Human Pooled Samples

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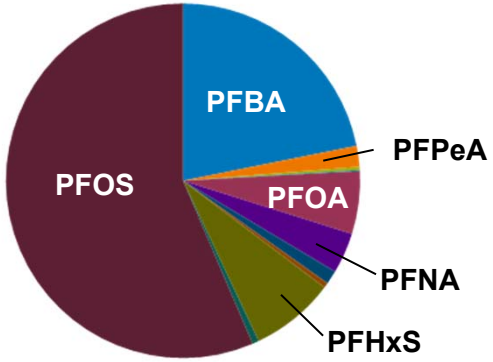
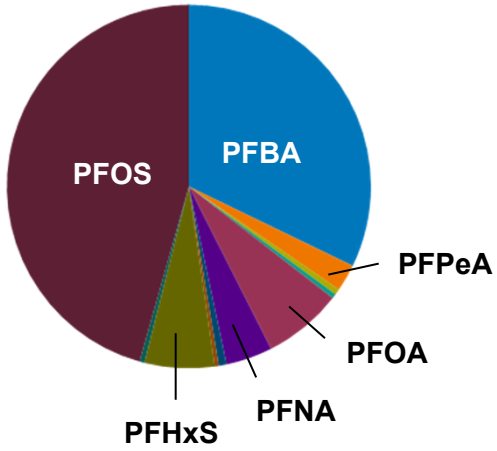
Lot 1



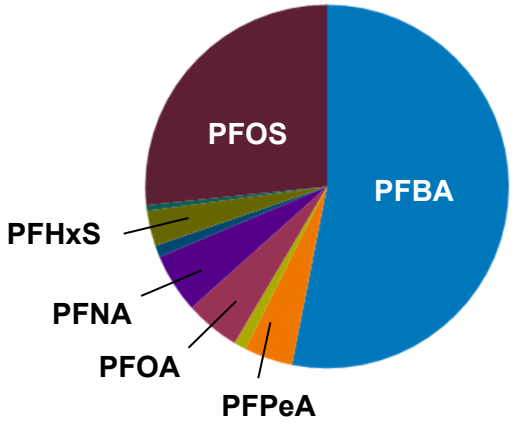
Total PFAS = 18.96 ng/L

Lot 2



Total PFAS = 14.44 ng/L

Lot 3



Total PFAS = 8.86 ng/L

Linear and Branched Isomers

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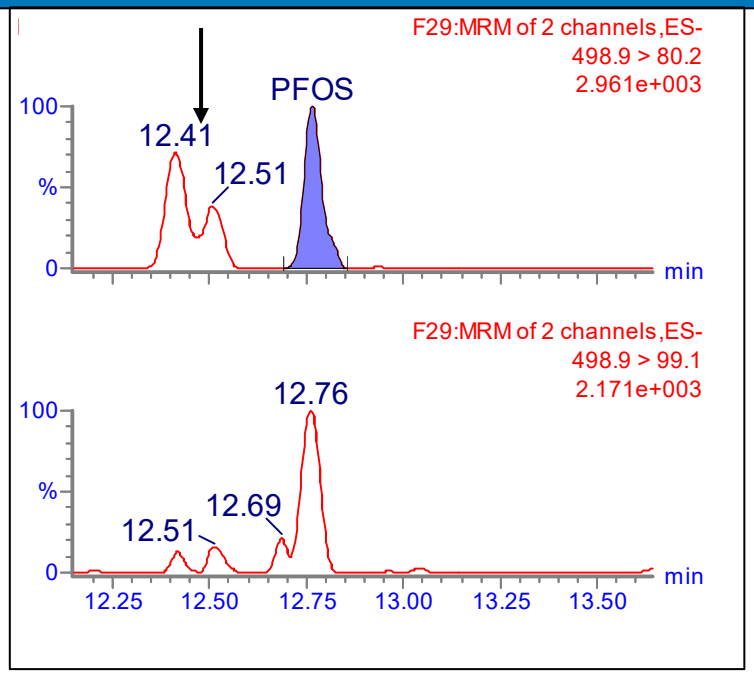
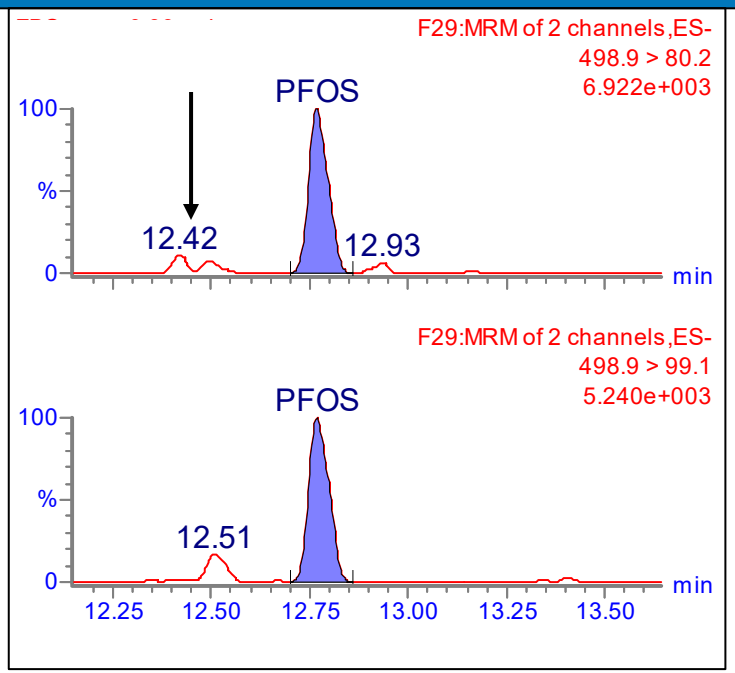
PFOS isomers in Human Serum



PFOS isomers in Standard



Sometimes SPE can't remove it all…

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Anal Bioanal Chem (2015) 407:3751–3761
DOI 10.1007/s00216-015-8601-x

RESEARCH PAPER

An automated high-throughput SPE micro-elution method for perfluoroalkyl substances in human serum

Sandra Huber · Jan Brox

Three MRM transitions were implemented for PFHxS and PFOS in order to monitor separation of the potentially interfering endogenous steroid sulphates [34, 39] and bile acids [39]. In addition, a chromatographic separation of the PFAS compounds and the mentioned interferences of 0.3 min at least were set as criteria (Figs. 1, 2 and Electronic Supplementary

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2009; **23**: 1405–1410
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/rcm.4012

RCM

Endogenous high-performance liquid chromatography/tandem mass spectrometry interferences and the case of perfluorohexane sulfonate (PFHxS) in human serum; are we overestimating exposure?

Emily Chan[1], Mandeep Sandhu[2], Jonathan P. Benskin[2], Milan Ralitsch[3], Nicole Thibault[3], Detlef Birkholz[3] and Jonathan W. Martin[1,2*]

MS).[10,11,13–27] The challenges of PFA analytical methods have been reviewed,[28] but, for PFOS in particular, the very first report of it in human serum[11] noted the presence of a co-eluting MS/MS interference in the most sensitive MS/MS transition (i.e. *m/z* 499/80) – later identified as taurodeoxycholate isomers[29] – which could cause significant over-reporting of concentrations. For PFHxS ($C_6F_{13}SO_3^-$; *m/z* 399), almost all existing data has been generated using its most sensitive MS/MS transitions: *m/z* 399/80 ($[SO_3]^-$) or *m/z* 399/99 ($[SO_3F]^-$).[11,19–21,23–25,27,30] However, it is now unclear whether the existing PFHxS data may be systematically biased as a result of co-eluting endogenous interferences in pooled maternal serum which share both these MS/MS transitions:[29] the major source being 5-pregnane-3,20-diol-2-sulfate, commonly known as pregnandiol sulfate, and the minor source being ^{34}S-3-hydroxy-5pregnan-20-one sulfate, commonly known as isopregnanalone sulfate.

Matrix Interference

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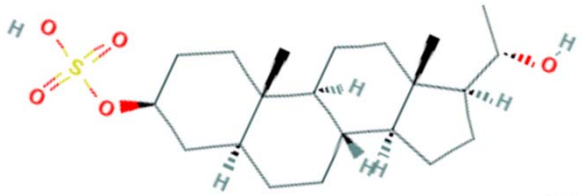
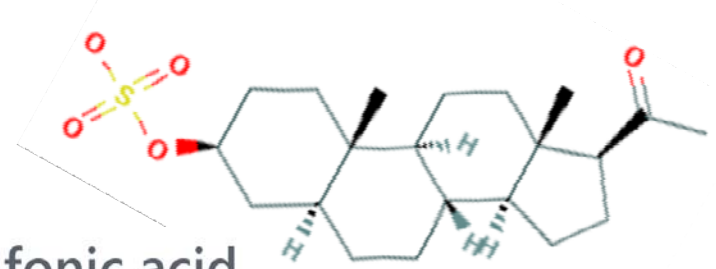
Pregnane-3,20-diol 3-sulfate

Molecular Weight: 400.6 g/mol



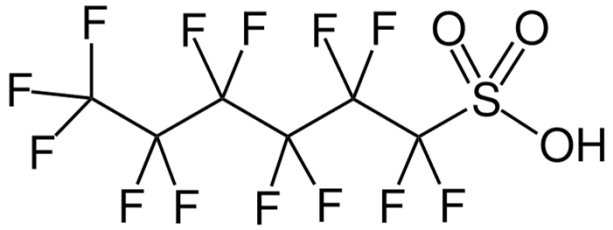
3alpha-Hydroxy-5alpha-pregnan-20-one sulfate

Molecular Weight: 398.6 g/mol



Perfluorohexanesulfonic acid

Molecular Weight: 400.12 g/mol



PFHxS Matrix Interference – BEH C18

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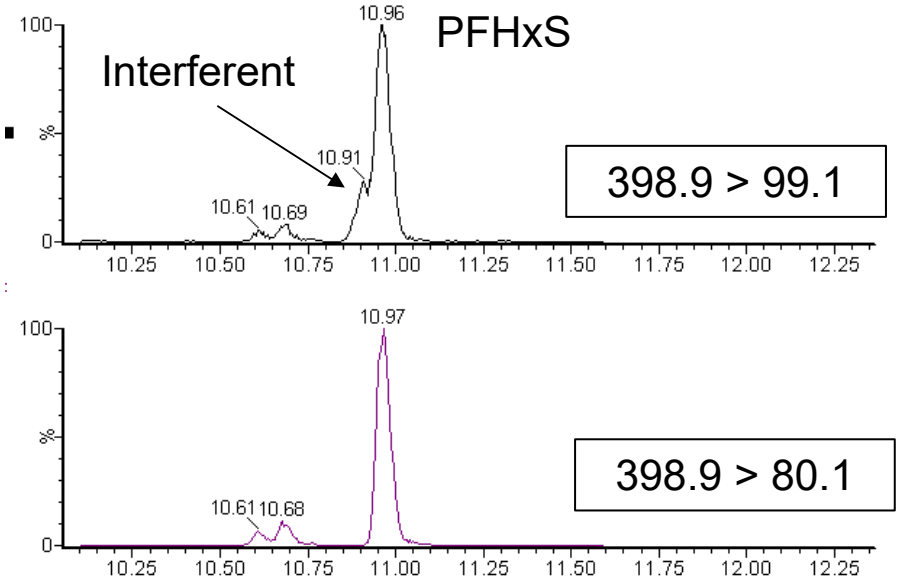
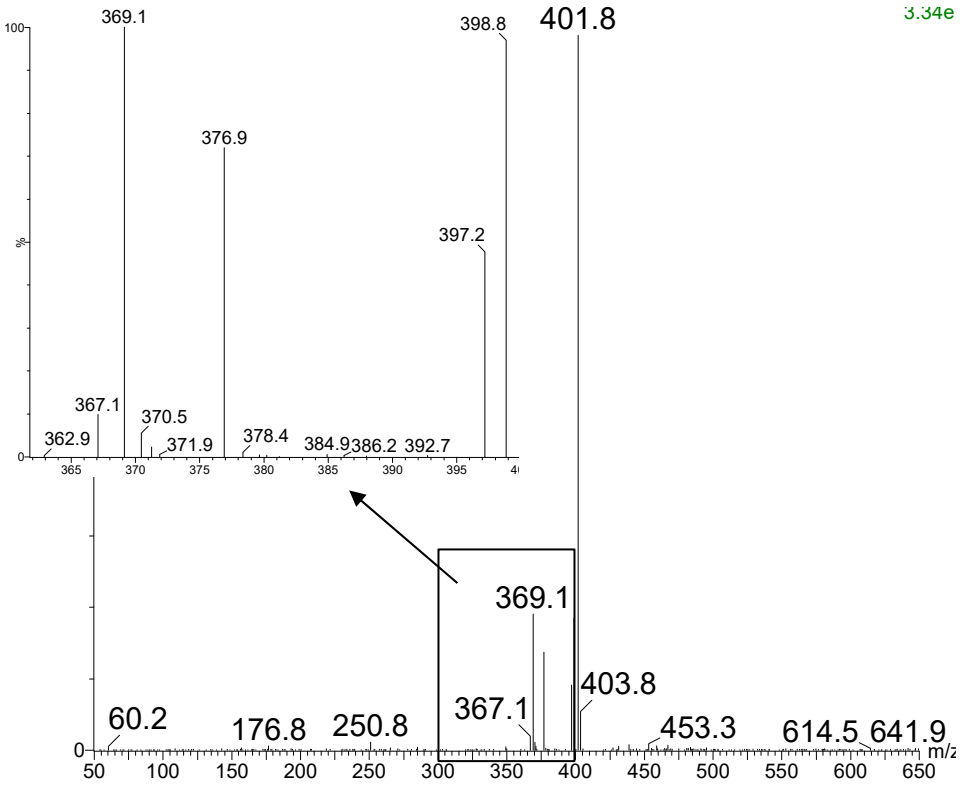
Acquity BEH C18





PFHxS Matrix Interference – HSS T3

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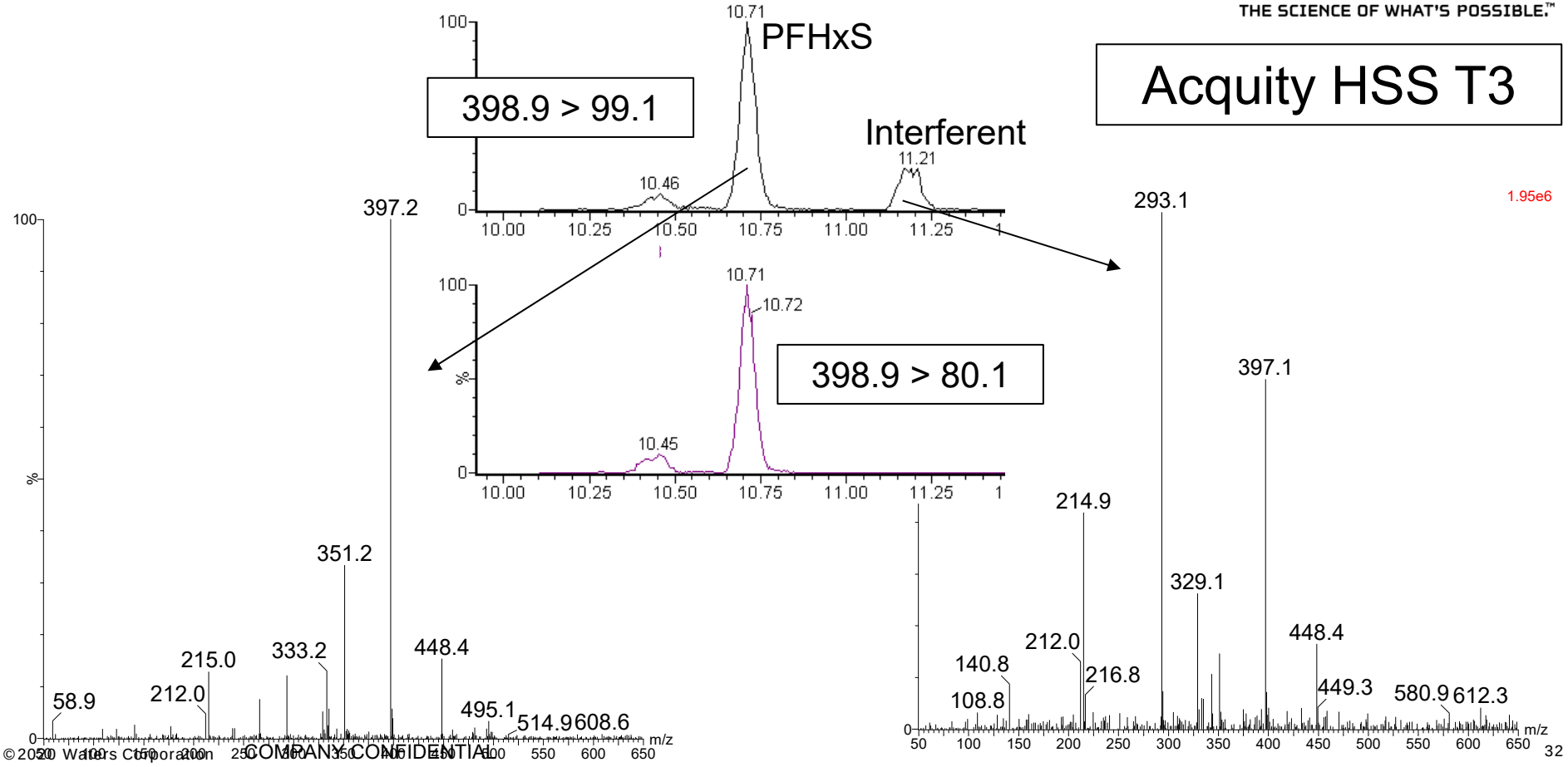
Acquity HSS T3



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2020 Proficiency Testing Scheme Schedule

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