PFAS in Wild Fish Tissue: Development of a Simple and Robust Extraction Procedure Using Pass-Through Matrix Removal

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DE56928231

Background

Goal of pilot study

Goal of application note

Goal of new product development

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- Quantify PFAS in sediment and fish collected in Tampa Bay, FL, USA
- Estimate dietary exposure from fish consumption
- Publish in *Frontiers in Marine Science*[1]

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[1] Pulster, E. L., Rullo, K., Gilbert, S., Ash, T. M., Goetting, B., Campbell, K., Markham, S., and Murawski, S. A. (2022). Assessing per- and polyfluoroalkyl substances (PFAS) in sediments and fishes in a large, urbanized estuary and the potential human health implications. *Frontiers in Marine Science* 9, doi 10.3389/fmars.2022.1046667.

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Goal of new product development

- Can better sorbent chemistries be developed for reducing complex matrix interferences for PFAS analysis?

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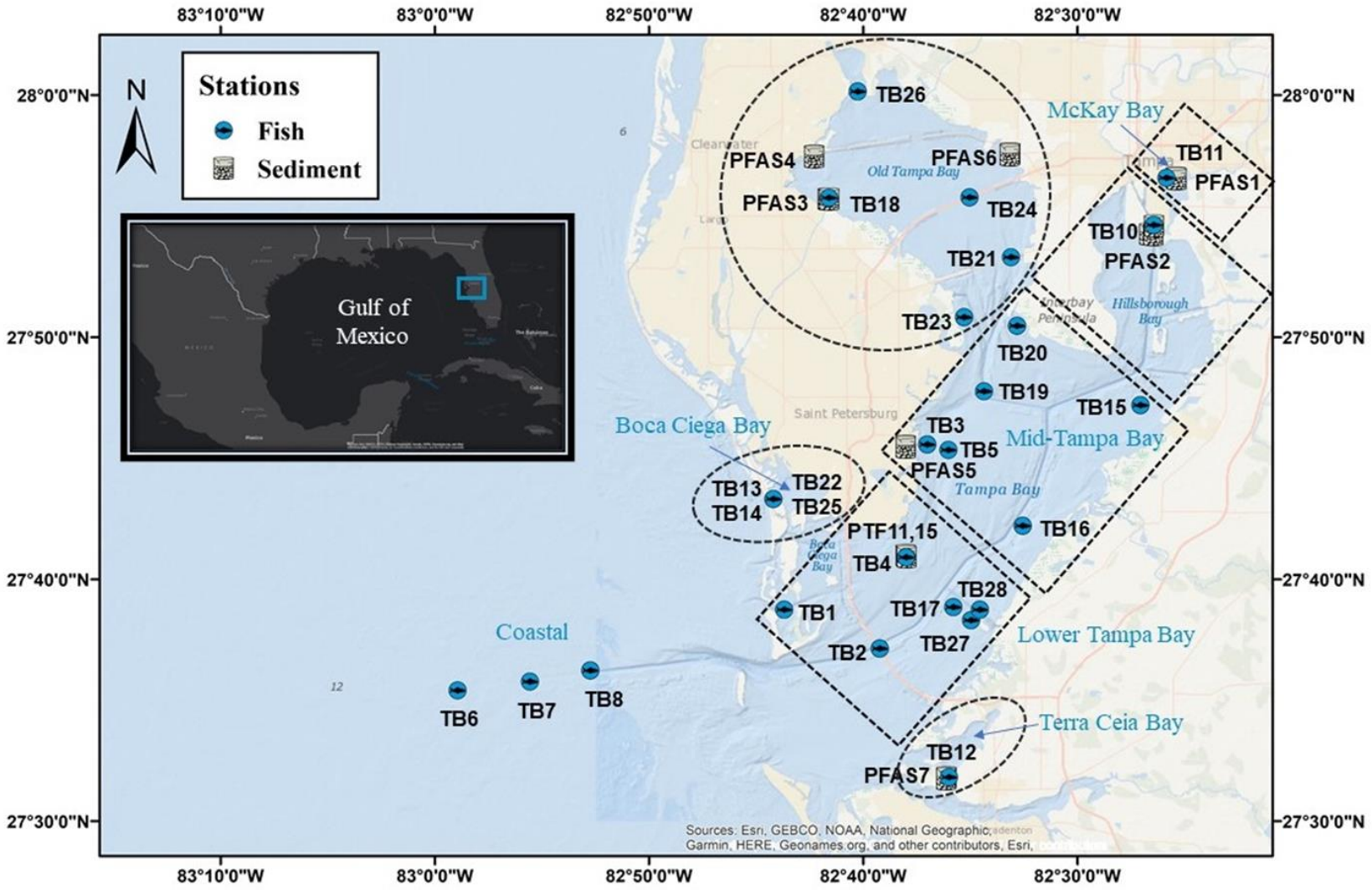
Sample Collection

- Fish collected from 28 locations throughout Tampa Bay
- A total of 140 individual fish were collected from 26 species
- Sediment samples collected from seven sites in duplicate and one site in triplicate (17 total)
- Sediment locations adjacent to wastewater treatment plants and airports



Quantitation

- LC-TQ (dMRM)
  - 25 native targets
  - 4 isotopically labeled surrogates
  - 15 isotopically labeled internal standards
- Quantitation based on ion ratios of targets to internal standards
- Five calibration levels
  - Quadratic least squares regression
  - 1 to 1000 ng/L targets and surrogates
- Surrogate recovery used quality control

Targets

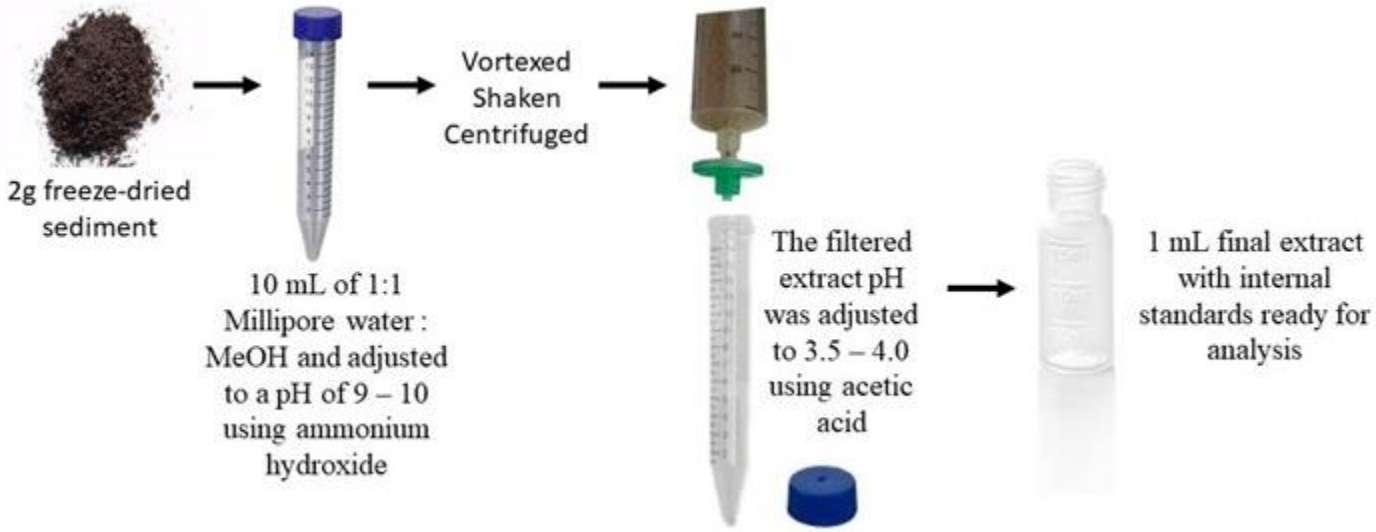
Compound	Internal Standard
PFBA	M4-PFBA
PF4OPeA	M5-PFPeA
PFPeA	M5-PFPeA
PF5OHxA	M2-4:2FTS
4:2FTS	M2-4:2FTS
3,6-OPFHpA	M5-PFHxA
PFHxA	M5-PFHxA
PFBS	M3-PFBS
HFPO-DA	M3-HFPO-DA
PFEESA	M4-PFHpA
PFHpA	M4-PFHpA
NaDONA	M3-HFPO-DA
6:2FTS	M2-6:2FTS
PFPeS	M2-6:2FTS
PFOA	M8-PFOA
PFHxS	M3-PFHxS
PFNA	M9-PFNA
8:2FTS	M2-8:2FTS
PFHpS	M6-PFDA
PFDA	M6-PFDA
PFOS	M8-PFOS
PFUnDA	M7-PFUdA
9Cl-PF3ONS	M7-PFUdA
PFDoA	M7-PFUdA
11Cl-PF3OUdS	M7-PFUdA

Surrogates

Compound	Internal Standard
M3-PFBA	M4-PFBA
M2-PFOA	M8-PFOA
M2-PFDA	M6-PFDA
M4-PFOS	M8-PFOS

Sediment Extraction Methodology

- Followed ASTM D7968-17a
- Important to use consumables which have been qualified for PFAS analysis*

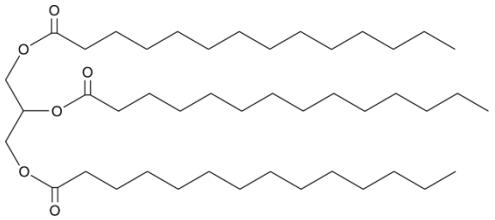


*Giardina, M. (2021). Analysis of Per- and Polyfluoroalkyl Substances in Soil Extracts: A workflow approach to sample preparation method development. Agilent Technologies, Inc., Wilmington, DE, USA. Application Note 5994-2999EN.

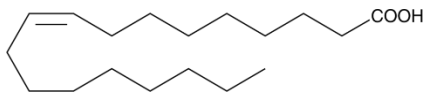
Fish Tissue Extraction Methodology

- Enhanced Matrix Removal (EMR) Lipid Technology – introduced in 2015
- Sorbent that effectively traps lipids through size/shape selectivity and hydrophobic interactions
- Captiva EMR-Lipid – pass through cleanup (2017)

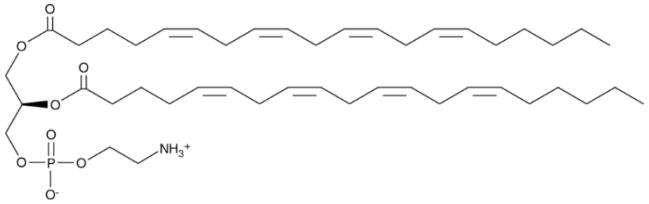
Triglycerides

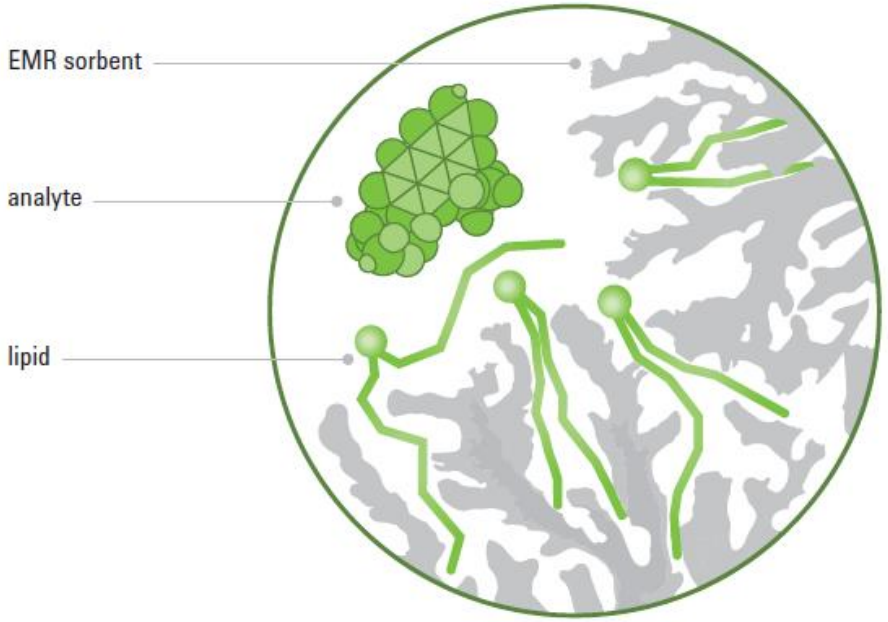


Free fatty acids



Phospholipids

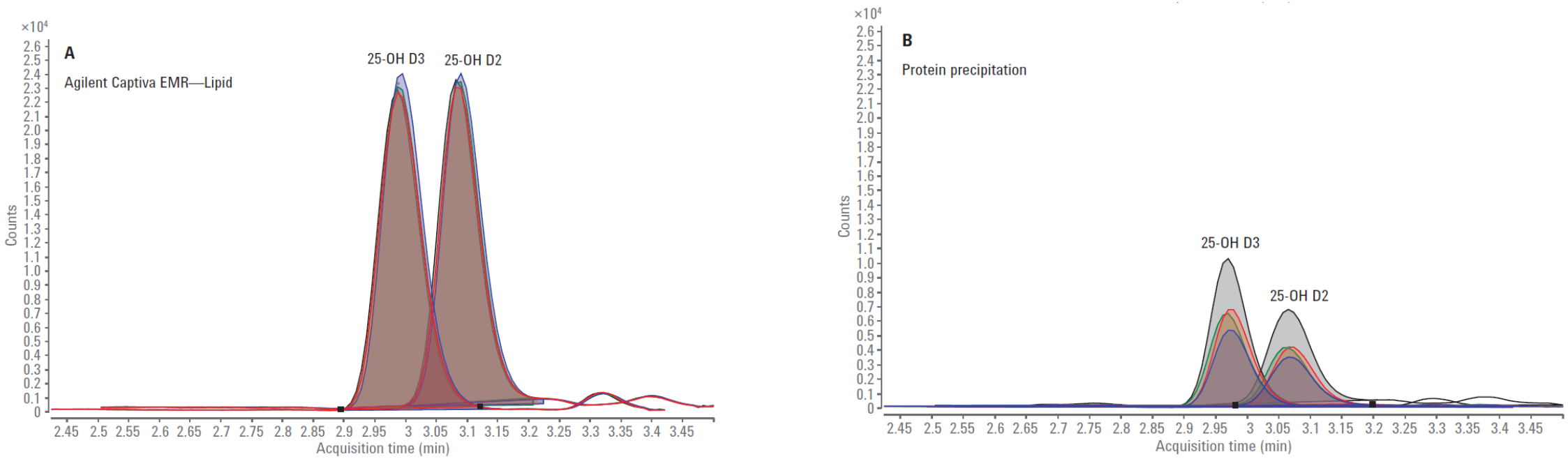




Fish Tissue Extraction Methodology

- Why is lipid removal important?
  - Reduce ion suppression, improve S/N, fewer interferences

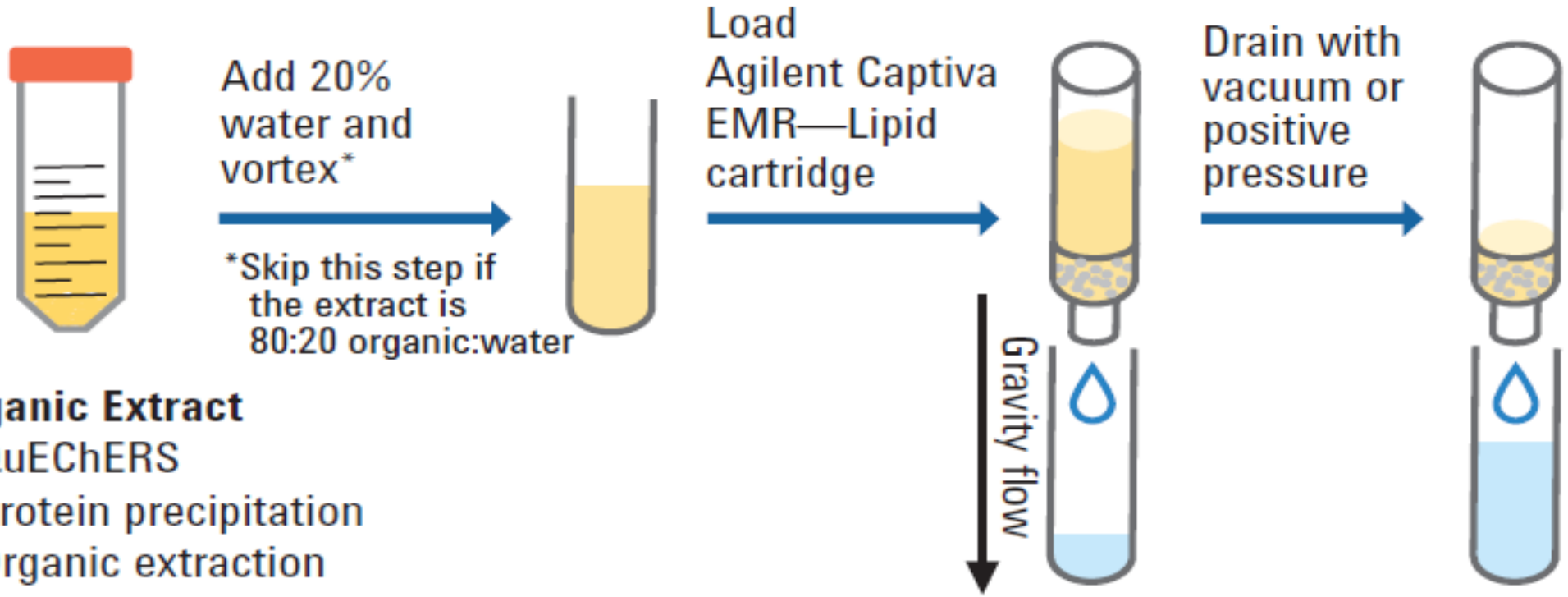
Vitamin D metabolites (25-OH D2, 25-OH D3) in human plasma with and without lipid removal*



*Lucas, D. and Zhao, L. (2017). Vitamin D Metabolites Analysis in Biological Samples Using Agilent Captiva EMR-Lipid. Agilent Technologies, Inc., Wilmington, DE, USA. Application Note 5994-7956EN.

Fish Tissue Extraction Methodology

- Simple pass-through cleanup



Organic Extract

- QuEChERS
- Protein precipitation
- Organic extraction

ACN preferred

Fish Tissue Extraction Procedure

Captiva EMR-Lipid, 3 mL, 300 mg sorbent (5190-1003)

1. Weigh 2 g of fish muscle into a 50 mL centrifuge tube
2. Add surrogate to the tissue and equilibrate at room temperature for 15 min
3. Add a ceramic homogenizer and 2 mL of 1% formic acid in water
4. Homogenize for 2 min at 1500 rpm
5. Add 8 mL of cold ACN with 2% formic acid and homogenize for an additional 5 min at 1500 rpm
6. Centrifuge for 5 min at 5,000 rpm
7. Transfer 2.4 mL of the supernatant to the 3 mL Captiva EMR-Lipid Cartridge and elute by gravity
8. Add 600 µl of 80:20 ACN:Water to the cartridge and elute by gravity. Apply pressure to transfer remaining solvent.
9. Mix the clean extract and transfer 500 µL of the to an autosampler vial
10. Add internal standard and 300 µL of water for a final volume of 800 µL
11. Analyze by LC/MS/MS

Instrument Conditions

- LC configured for PFAS - bypassed degasser, replaced pump seals, inline filters, PTFE tubing
- Delay column
- Large volume injected
- Keep sheath temperature low
- Method based on ASTM D7968-17a

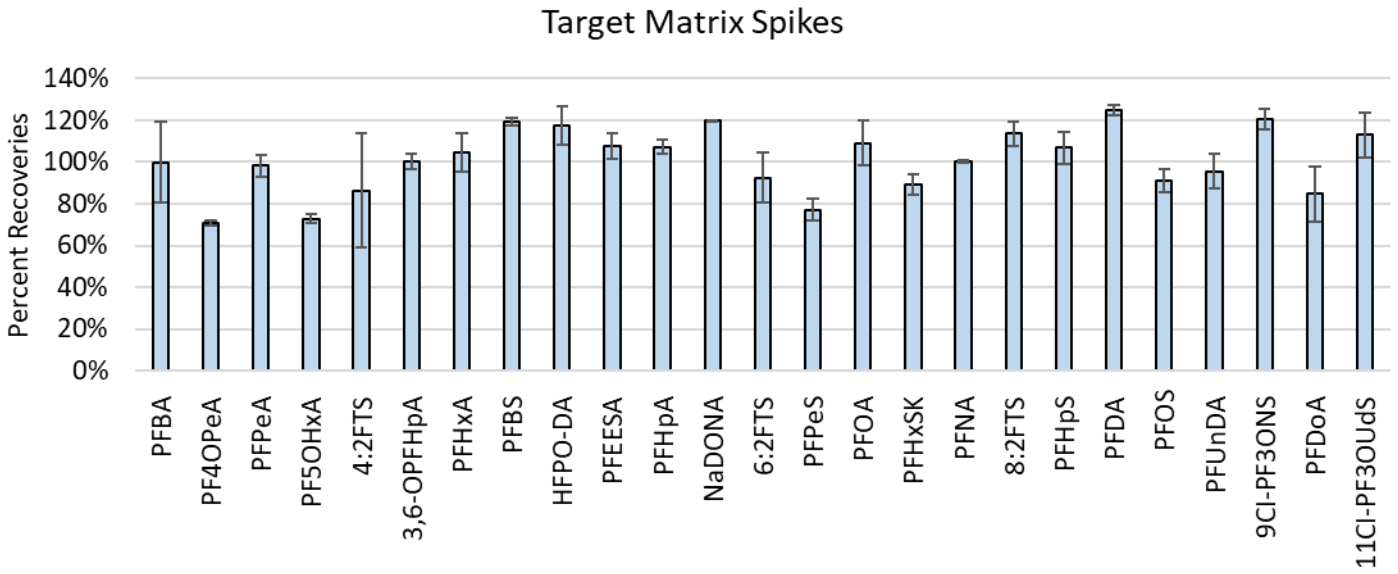
Parameter	Value
LC	Agilent 1290 Infinity II LC
Analytical Column	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 x 100 mm, 1.8 µm (p/n 959758-902) Agilent 1290 Infinity inline filter 0.3 µm (p/n 5067-6189)
Delay Column	Agilent ZORBAX Eclipse Plus 95Å C18, 4.6 x 50mm, 3.5µm (p/n 959943-9020)
Column Temperature	50°C
Injection Volume	20 µL
Mobile Phase	A: 20 mM ammonium acetate in 95:5 water : acetonitrile B: 10 mM ammonium acetate in 95:5 acetonitrile : water
Column Flow	0.30 mL/min

Gradient	Time (min)	% A	% B
	0	100	0
	6	70	30
	9	50	50
	16	15	85
	17	0	100
	20	0	100
	21	100	0
	31	100	0

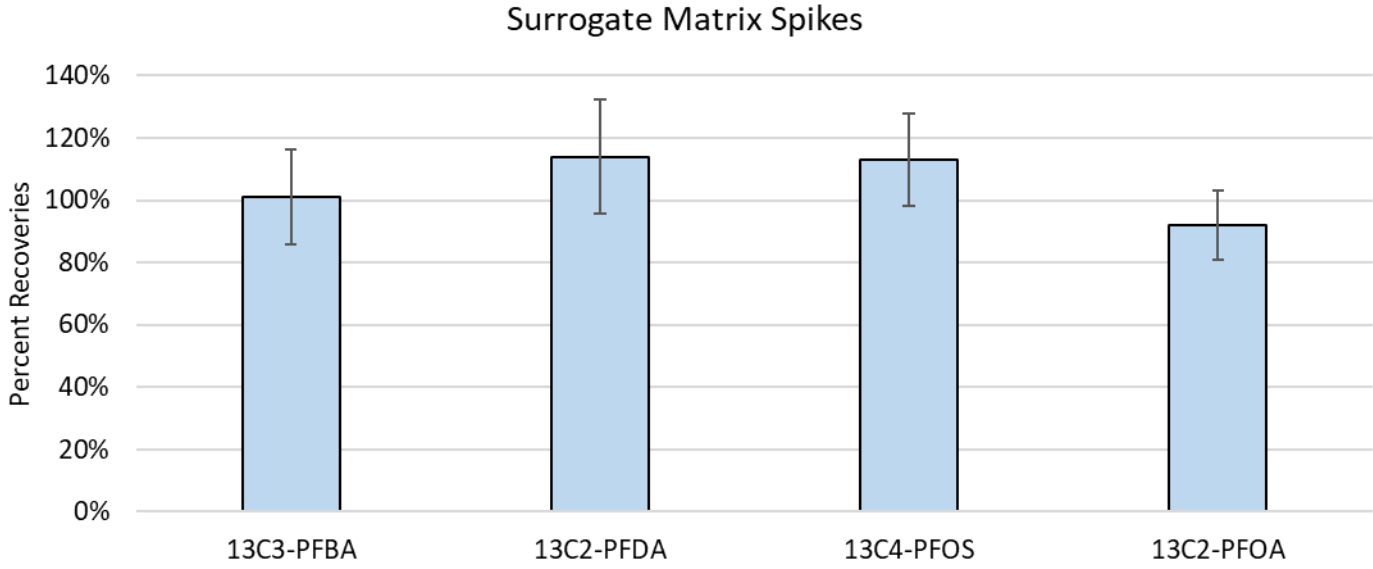
Parameter	Value
MS	Agilent 6470 triple quadrupole LC/MS with Agilent Jet Stream ESI source
Source Parameters	
Polarity	Negative
Drying Gas	230 °C, 4 L/min
Sheath Gas	250 °C, 12 L/min
Nebulizer Gas	15 psi
Capillary Voltage	2,500 V
Nozzle Voltage	0 V

Spike Recoveries Accuracy and Precision

- Target spikes
  - Duplicate fish tissue samples
  - Spiked with 25 native targets at 100 pg/g
  - Average recoveries ranged from 70 to 125% with an overall average of 102% and average range of 15%.

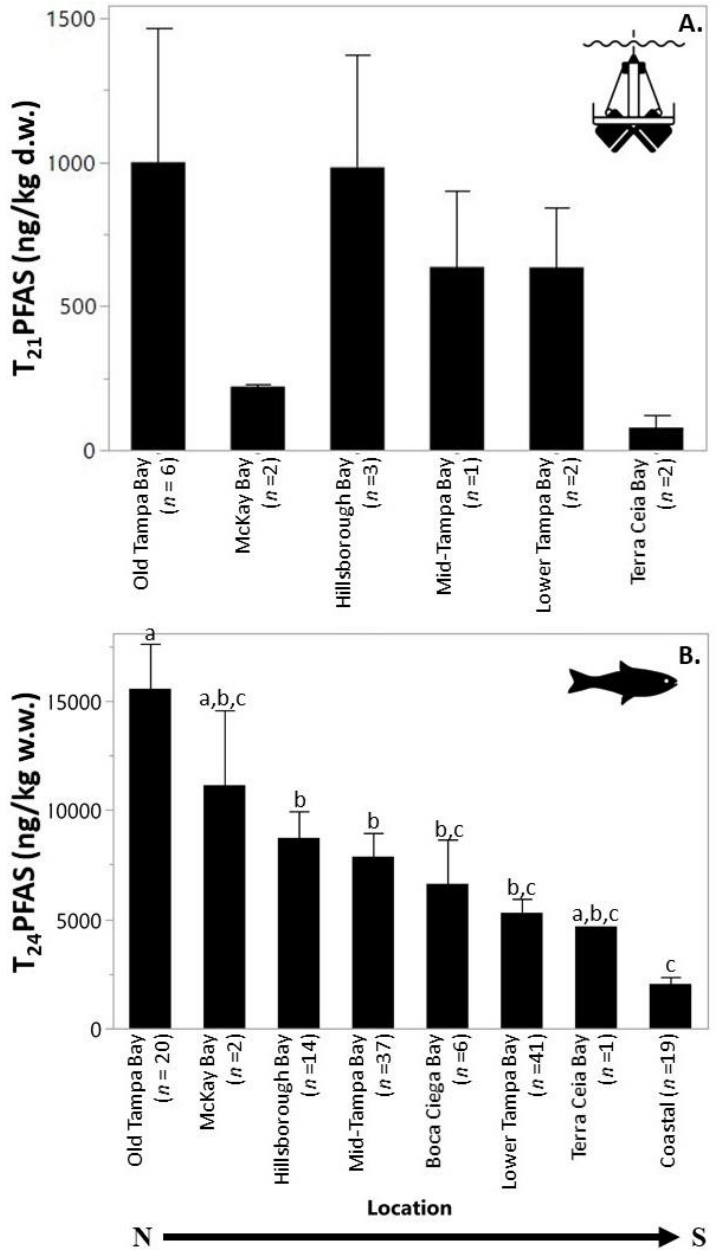


- Surrogate spikes
  - Total of 140 fish tissue samples (26 species)
  - Spiked with four isotopically labeled surrogates at 100 pg/g
  - Average recoveries ranged from 92 to 114% with an overall average of 105%. RSDs ranged from 12 to 16%.



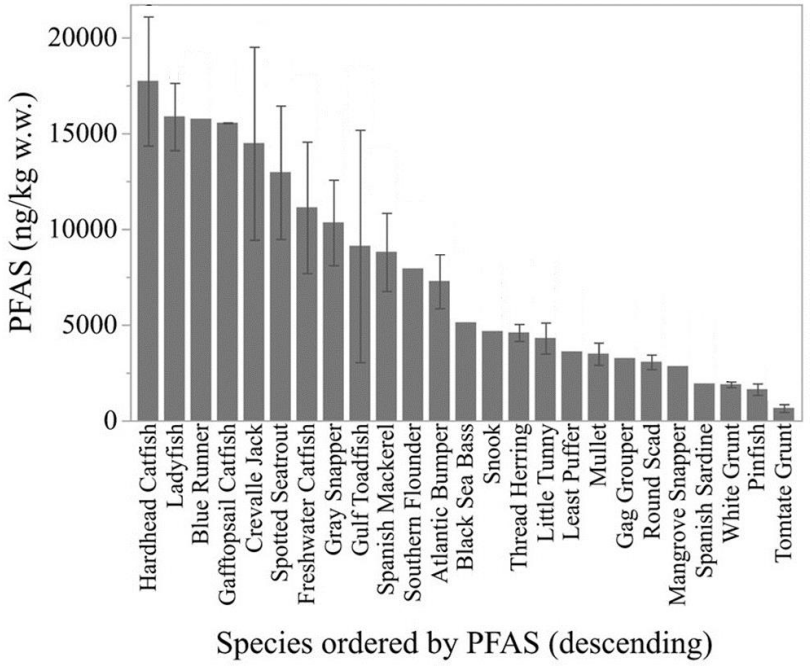
Selected Study Results

- Total PFAS
  - Sediment: 37 to 2,990 ng/kg (dw)
  - Fish: 307 to 33,600 ng/kg (ww)
- Highest levels in Old Tampa Bay
- Levels generally decreased from north to south toward the Gulf of Mexico

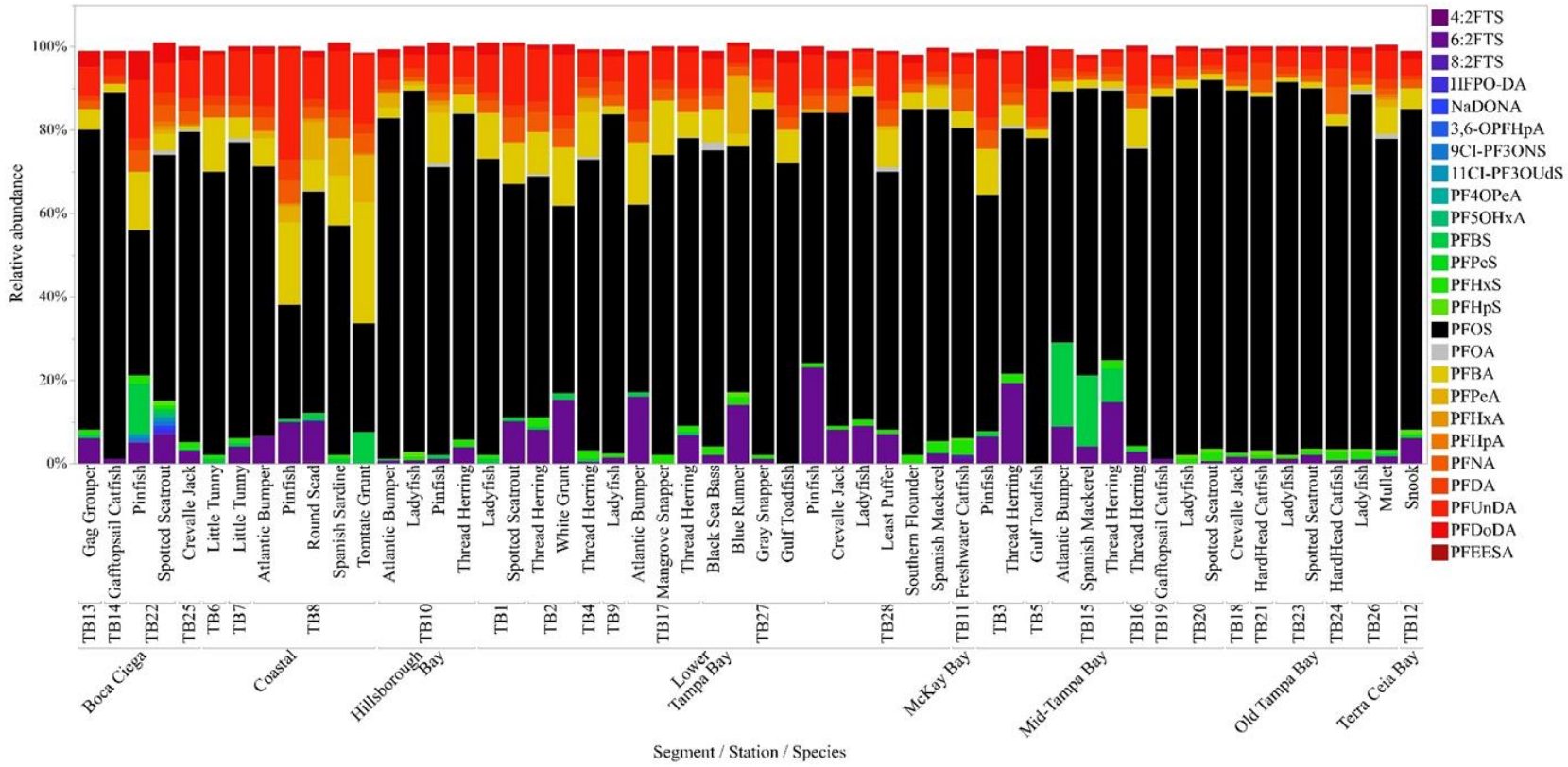


Selected Study Results

Total PFAS Concentration in Fish

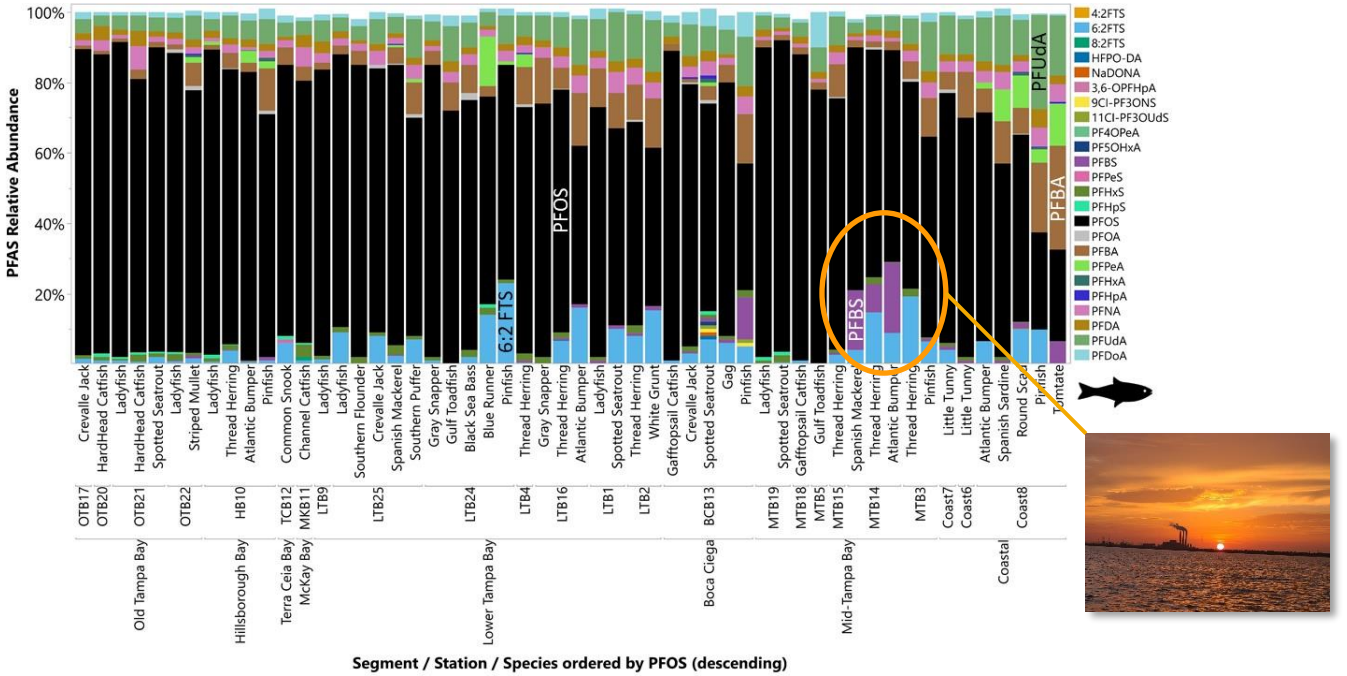


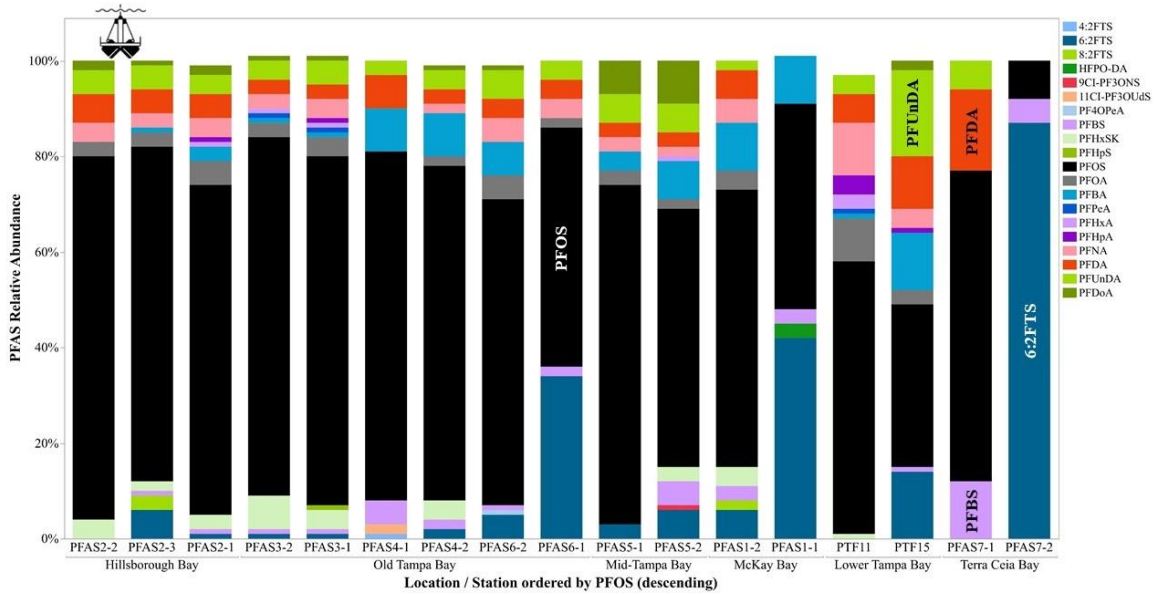
Relative Abundance of PFAS



Selected Study Results

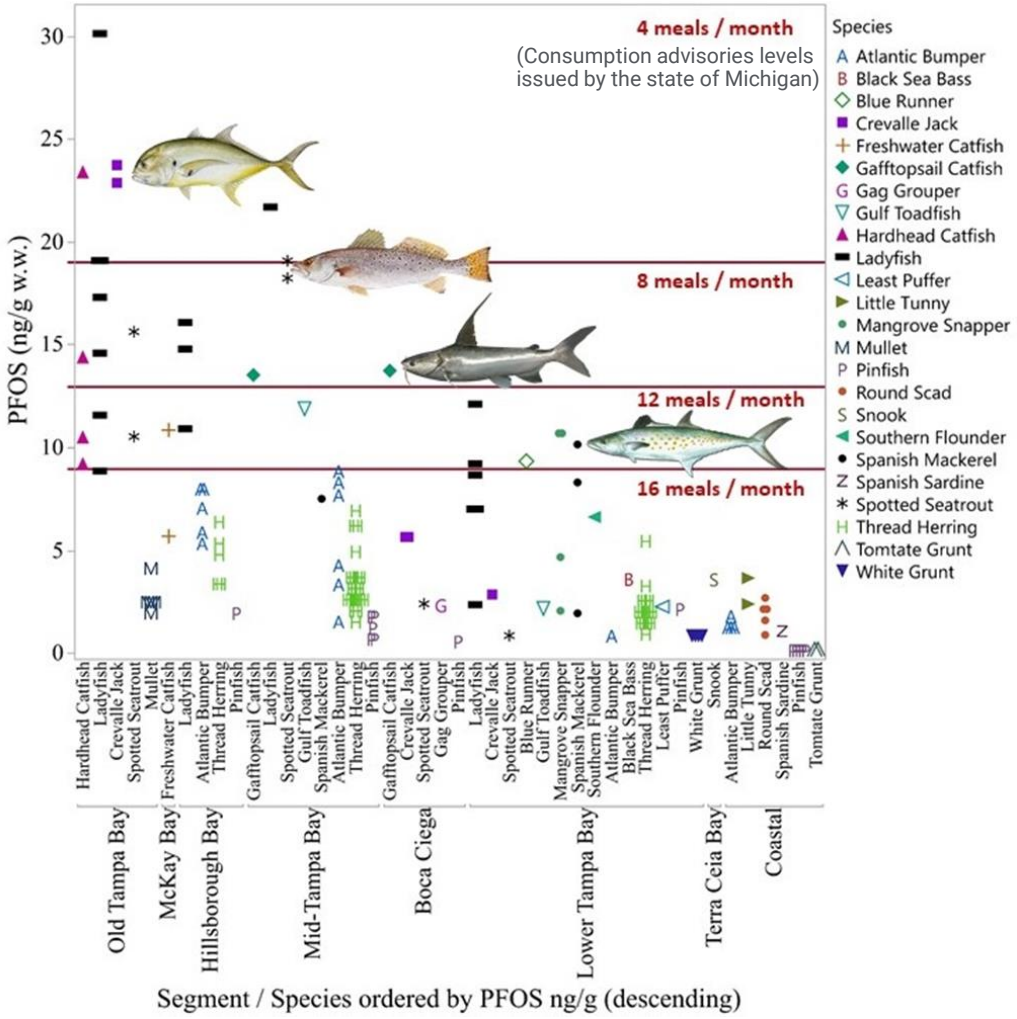
- PFOS predominated profiles in both sediment (59 ± 18%) and fish muscle tissue (59 ± 16%)
- PFOS detected in 100% of the samples
  - Sediment: 9 to 2,170 ng/kg dw
  - Fish: 92 to 30,100 ng/kg ww
- Site differences



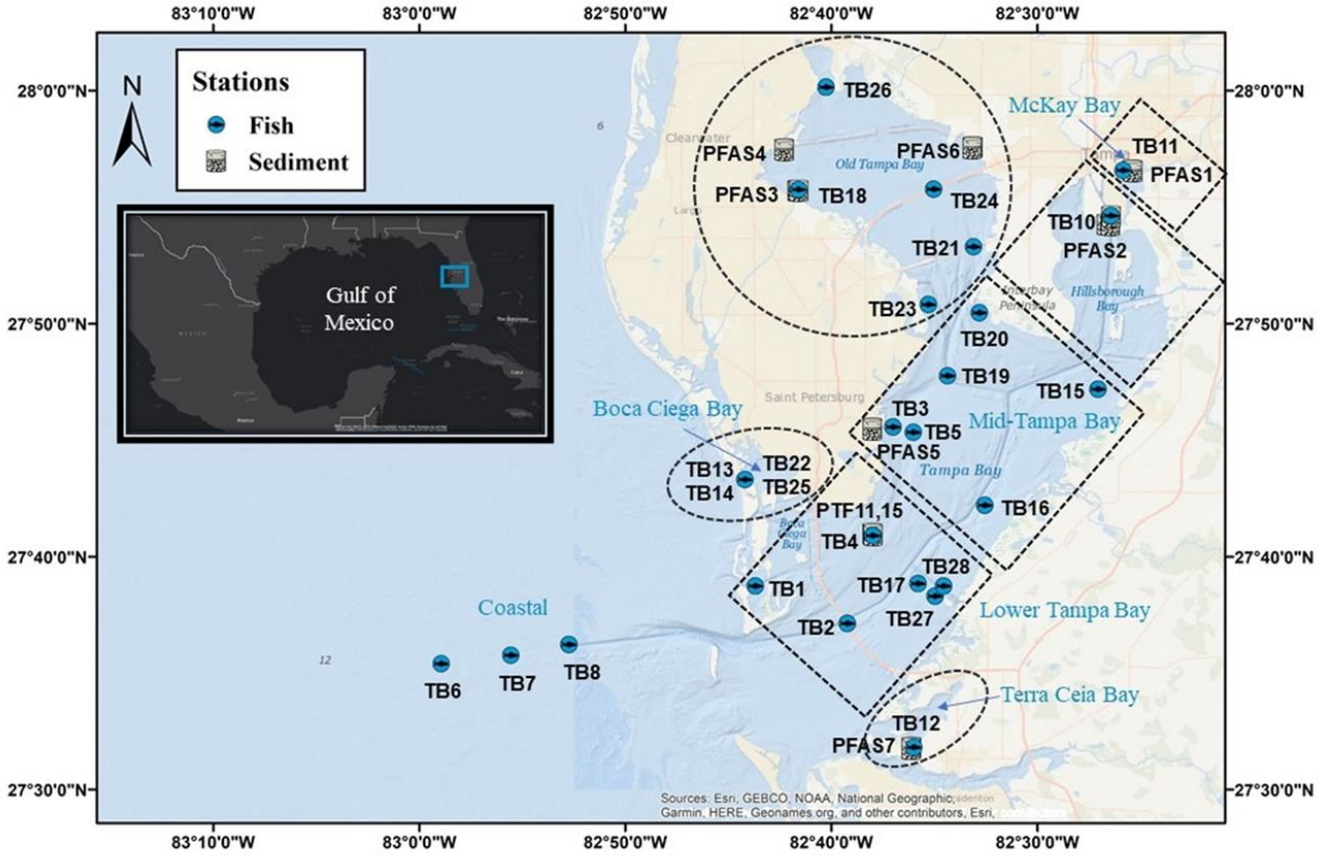


Selected Study Results

PFOS Concentration in Fish



Sampling Locations and Segments



Captiva EMR PFAS Food I & II

NEW!

Captiva EMR PFAS Food I

- For fresh produce and processed plant-origin food
- Used after QuEChERS extraction
- Removes sugars, salts, organic acids, pigments and other hydrophilic & hydrophobic interferences
- Two formats provide flexible sample crude extract loading capacity

Captiva EMR PFAS Food II

- For meats and complex dry food
- Used after QuEChERS extraction
- Removes sugars, salts, organic acids, fats and lipids, pigments and other hydrophobic interferences
- One format for multiple matrices

Captiva EMR PFAS Food I & II Certificate of Analysis (CoA)

NEW!

Agilent Product Name: Captiva EMR PFAS Food II, 6mL, 750mg, 30/pk
Agilent Part No.: 5610-2232
FG Lot No.: 6794012-01
Media Lot No.: 0006794012

Raw Materials Component Properties

Test	Method	Result
Tube Purity	GC-FID	Pass
Frit PFAS Cleanliness	LC-QQQ	Pass

Product Specifications/ Analysis

Test	Test description	Method	Result
PFAS Recovery	Recovery of a representative panel of PFAS compounds in food matrix using passthrough cleanup.	LC-QQQ	Pass
Matrix Removal	Matrix removal in representative food sample.	GC-FID	Pass
PFAS Cleanliness	Cartridge cleanliness for targeted PFAS background.	LC-QQQ	Pass
Flow Characteristics	Proprietary	Air Flow	Pass

AOAC SMPR® 2023.003

Standard Method Performance Requirements (SMPRs®) for Per- and Polyfluoroalkyl Substances (PFAS) in Produce, Beverages, Dairy Products, Eggs, Seafood, Meat Products, and Feed

Table 5. Target limits of quantification (LOQ) for PFOS, PFOA, PFNA, and PFHxS in other matrices.

Matrix Category	LOQ (µg/kg) [a]			
	PFOS	PFOA	PFNA	PFHxS
Produce	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Coffee	≤ 0.3	≤ 0.3	≤ 0.3	≤ 0.3
Milk (liquid)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Dairy powders and plant-based protein powders	≤ 0.08	≤ 0.08	≤ 0.08	≤ 0.08
Fish oil	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5
Food for infants and young children (baby food)	≤ 0.01	≤ 0.01	≤ 0.01	≤ 0.01
Feed	≤ 0.5	≤ 0.5	≤ 0.5	≤ 0.5

[a] The target LOQs are expressed on a w/w basis in samples as received for testing. These values may be revised in the future based on new toxicological studies and hazard assessments.

Table 7. Recovery, repeatability, and reproducibility.

Parameter	PFOS, PFOA, PFHxS, PFNA in regulated matrices (see Table 4)	PFOS, PFOA, PFHxS, PFNA in other matrices and all other analytes [a]
Recovery, %	80-120	65-135
Repeatability, RSD_r, %	≤ 20	≤ 25
Reproducibility, RSD_R, %	≤ 40	≤ 40

[a] For analytes without commercially available matching isotopically labelled standards, recoveries within 40-140 % and RSD_r ≤ 30% could be acceptable.

Table 6. Target limits of quantification (LOQ) for other PFAS.

Matrix Category	LOQ (µg/kg) [a,b]159	
	PFBA and PFPeA	Other PFAS
Eggs	≤ 3	≤ 3
Seafood (crustaceans and mollusks)	≤ 3	≤ 3
Fish meat and meat of terrestrial animals	≤ 1	≤ 1
Edible offal of terrestrial animals	≤ 4	≤ 4
Produce	≤ 1	≤ 0.1
Coffee	≤ 3	≤ 3
Milk (liquid)	≤ 1	≤ 0.1
Dairy powders	≤ 1	≤ 0.8
Fish oil	≤ 5	≤ 5
Food for infants and young children (baby food)	≤ 1	≤ 0.1
Feed	≤ 5	≤ 5

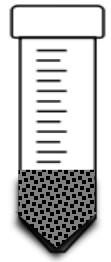
[a] The target LOQs are expressed on a w/w basis in samples as received for testing. These values may be revised in the future based on new toxicological studies and hazard assessments.
[b] Target LOQs were calculated by multiplying LOQs from Tables 4 and 5 by a factor of 10. The minimum LOQ for PFBA and PFPeA was set to 1 µg/kg.

Other PFAS = PFBS, 4:2 FTS, PFHxA, PFPeS, HFPO-DA, PFHpA, DONA, 6:2 FTS, PFHpS, 9Cl-PF3ONS, 8:2 FTS, PFDA, PFNS, PFDS, PFunDA, PFOSA, 11Cl-PF3OUdS, PFUnDS, PFDoDA, 10:2 FTS, PFDoS, PFTrDA, PFTrDS, PFTeDA

Captiva EMR PFAS Food I & II – Typical Extraction Method

- Sample weighing
- STD and ISTD spiking
- Optional: Dry sample hydration and equilibrium
- Sample QuEChERS extraction
- EMR mixed-mode passthrough cleanup
- Sample post-treatment by drying and reconstitution or direct dilution

Sample extraction



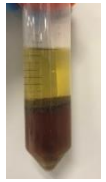
Sample

- Spike standard and ISTD
- Add 10 mL water (optional)

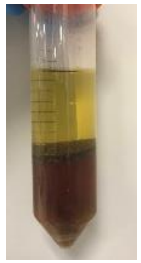
QuEChERS extraction

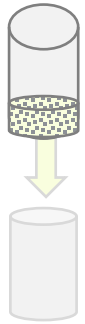
Mechanical shaker

Centrifuge



Sample cleanup





EMR mixed-mode passthrough cleanup

Post-treatment
Dry & reconstitution
or
Direct dilution

LC/MS/MS detection

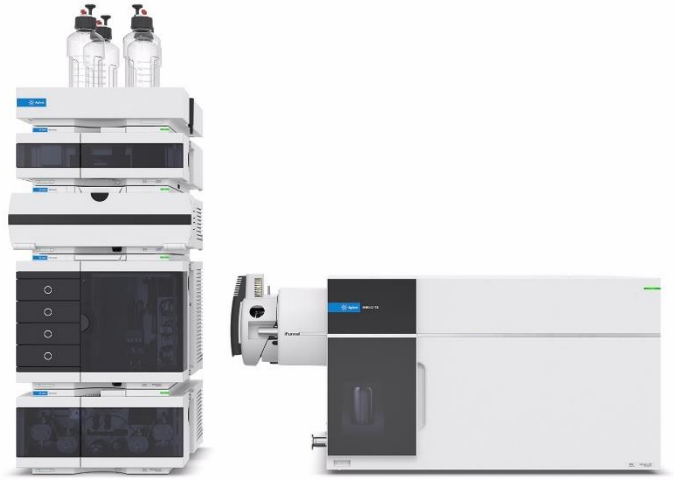
Instrumental Analysis by LC-MS/MS

LC method parameters			MS QQQ Parameters	
Solvent A	5 mM ammonium acetate in water		Ion Source	ESI
Solvent B	MeOH		Acquisition	dMRM
Flow		0.4 mL/min	Polarity	Negative
Pump Program	$T_{0.0}$	2% B		
	$T_{2.0}$	2% B	Source Parameters	
	$T_{2.5}$	55% B	Gas Temp	230 °C
	$T_{6.5}$	70% B	Gas Flow	4 L/min
	$T_{8.0}$	80% B	Nebulizer	15 psi
	$T_{14.2}$	100% B	Sheath Gas Heater	250 °C
	$T_{17.0}$	100% B	Sheath Gas Flow	12 L\min
	$T_{17.1}$	20% B	Capillary	2500 V (-), 0 V (+)
Stop Time		17.1 min	Nozzle Voltage	0 V
Post Time		7 min		
Injection Volume		10 µL		
Injection program		10 µL water + 10 µL sample + 50 µL water + 10 µL air	**Column and Guard**	
Needle Wash		Multi-wash program	Column	Eclipse Plus C18 RRHD 1.8 µm, 2.1 x 100 mm
Needle Wash Sol'n	1. IPA; 2. ACN; 3. H_2O		PN	959758-902
Needle Height		0 mm	Guard	Eclipse Plus C18, 1.8 µm, 2.1 x 5 mm
			PN	821725-901
Column temperature		55°C	Delay	InfinityLab PFC delay column, 4.6 x 30 mm
			PN	5062-8100

Poster P28

Enhanced Sensitivity for PFAS Using a Hybrid Autosampler Approach with LC-MSMS

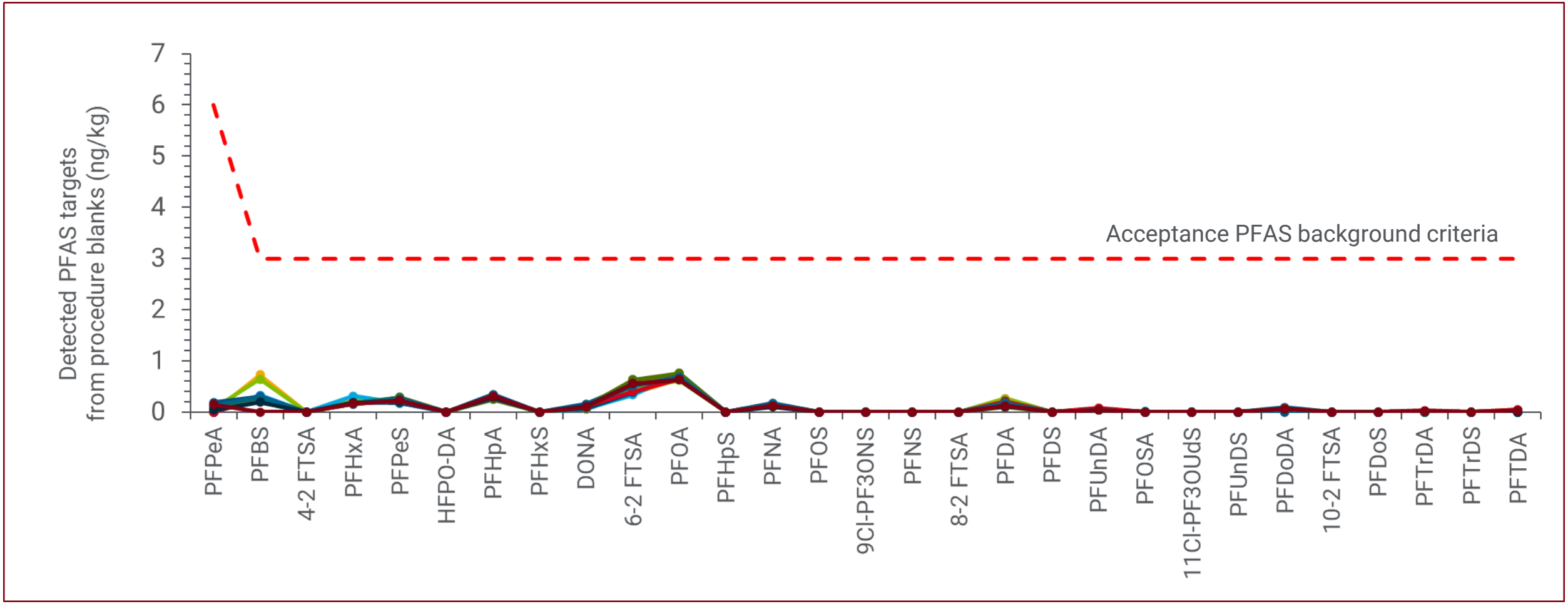
Emily Parry, Agilent Technologies



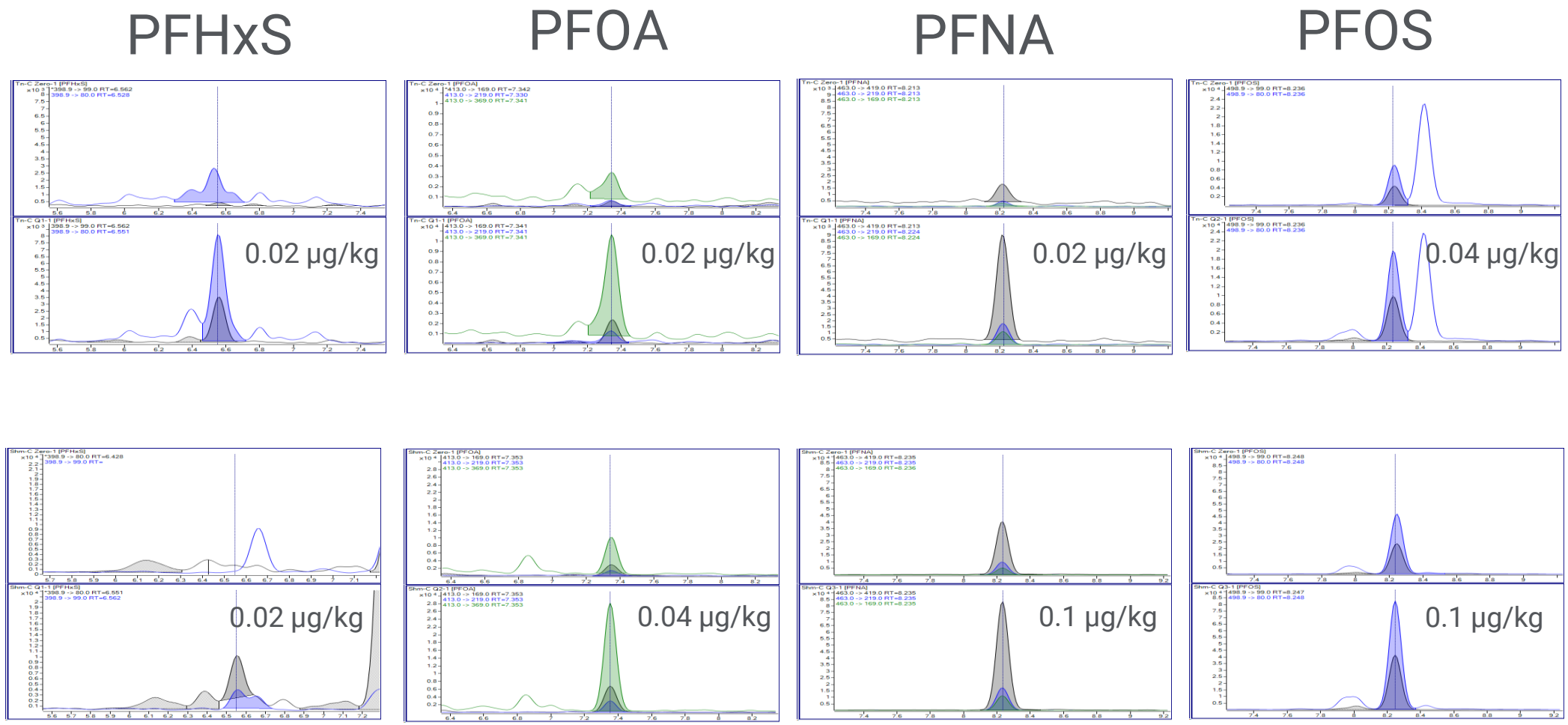
Agilent Triple quadrupole LC/MS system, 6495D mass spectrometer with PFC-free kit and delay column

PFAS in Food – Captiva EMR PFAS Food I & II

- Method limit of quantitation of 10 ng/kg for all compounds accept PFBA (100 ng/kg) and PFPeA (20 ng/kg)



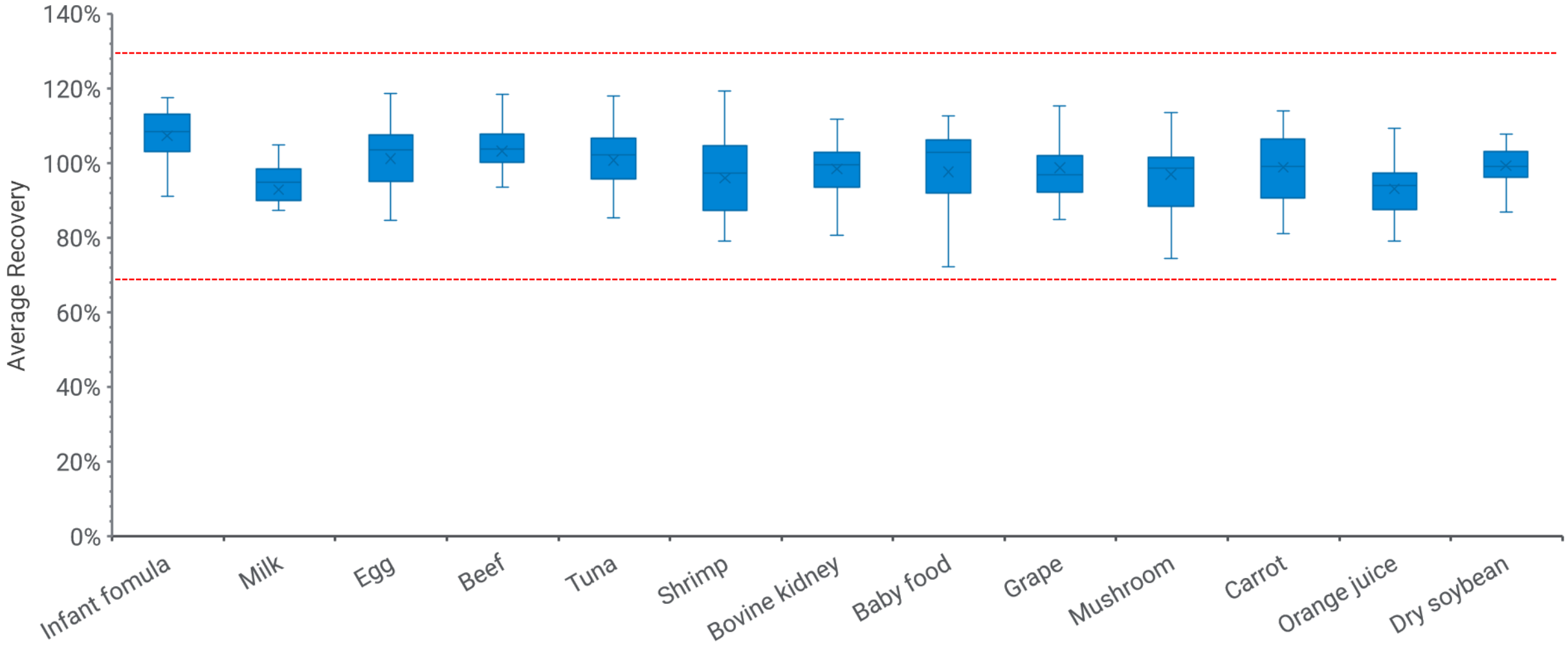
Lower Limit of Quantitation (Validated) – Captiva EMR PFAS Food II*



*Zhao, L.; Giardina, M.; Perry, E. (2024). Determination of 30 PFAS in Beef, Tuna, and Shrimp. Agilent Technologies, Inc., Wilmington, DE, USA. Application Note 5994-7368EN.

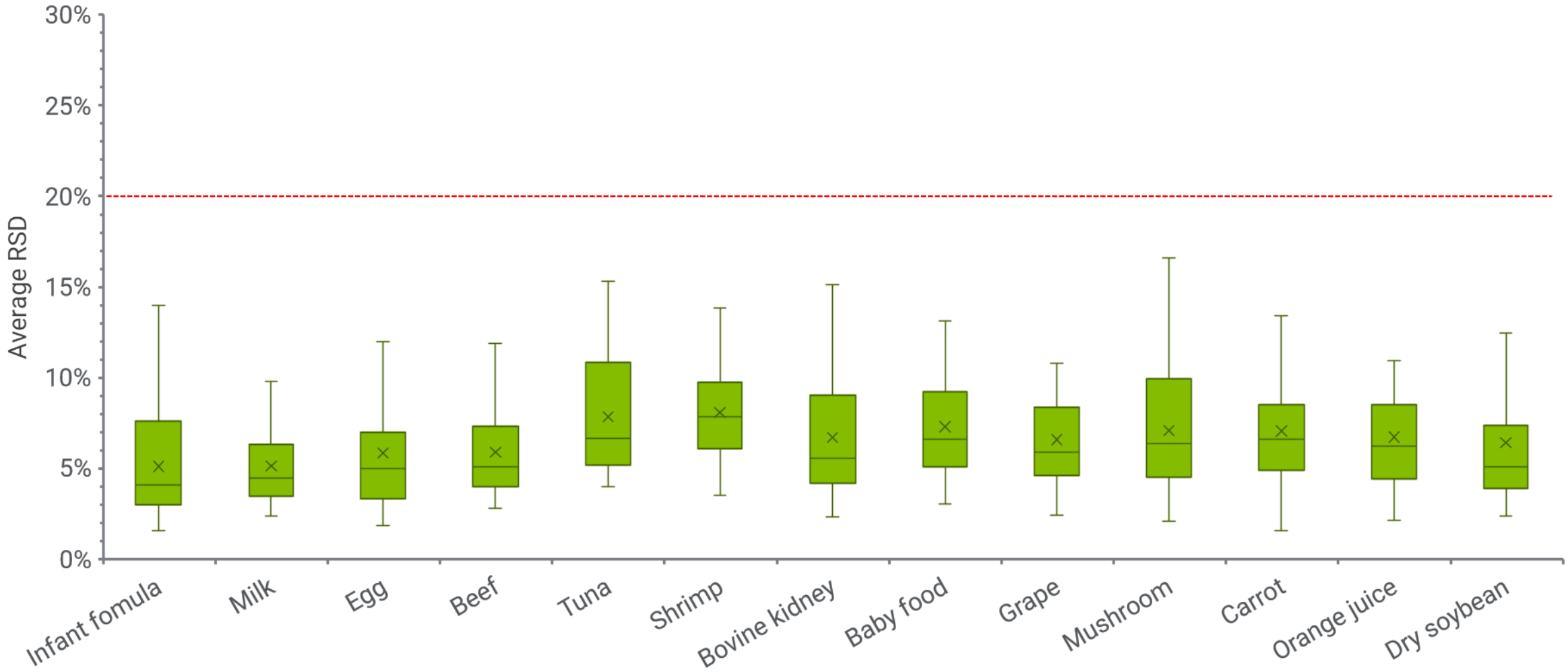
Method Accuracy

- Recovery accuracy of 30 PFAS targets at three spiking levels in 13 matrices



Method Precision

- Recovery precision of 30 PFAS targets at three spiking levels in 13 matrices



Conclusions and Summary

- Robust and reliable method for determination of PFAS in fish muscle using Captiva EMR-Lipid pass through cleanup
- Validated by a large-scale environmental study

- New PFAS matrix removal products developed with
  - ✓ Captiva EMR PFAS Food I (*flora*) and EMR PFAS Food II (*fauna*)
  - ✓ Rigorous QC testing - PFAS background, recovery, matrix removal
  - ✓ Fully validated method for 30 PFAS in multiple food matrices
  - ✓ Simplified pass-though cleanup with high recovery
  - ✓ https://www.agilent.com/en/product/sample-preparation/filtration/captiva-emr-pfas-food-cartridges